FIBRE METABOLISM IN THE RAT

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I hereby declare that this thesis was composed by myself, and that the work it contains is my own.

For Gordon.

"Time it was and what a time it was,

It was a time of innocence,

A time of confidences.

Long ago it must be,

I have a photograph,

Preserve your memories,

They're all that's left you".

OLD FRIENDS
(PAUL SIMON)

ABSTRACT

Dietary Fibre is metabolised in the human colon. The inaccessibility of the human colon necessitates the use of indirect methods to study the effects of fibre upon metabolism and stool weight. The time duration required for dietary trials, to insure adaptation to the new regime is completed, is not known. This thesis describes an animal model which could be of relevance to human nutrition for the evaluation of dietary fibres and their action upon stool bulking and caecal metabolism. At present there is no simple method of predicting quantitatively the effect of dietary fibre upon metabolic and physiological functions.

Adult male Albino Wistar rats, were fed for periods of 4-, 8- and 12 weeks with low fibre containing diets, either of plant origin, animal origin or an elemental diet, unsupplemented or supplemented with 100 g/kg of gum arabic (readily fermented polysaccharide) or coarse Canadian Red Spring Wheat Bran (non-fermented fibre). The effects on live-weight, liver weight, dry stool weight, and caecal content weight, wet caecal sac weight, faecal- and caecal short chain fatty acids, average bile acids, expired hydrogen and methane were recorded. Bacterial mass was measured indirectly using 2,6-Diaminopimelic acid.

Wheat bran alone significantly increased dry stool weight, irrespective of the basal diet. As with all the parameters measured, the absolute values, and magnitude of the changes, were related to basal diet. Gum arabic had no influence on stool weight. The addition of gum arabic to a diet increased the caecal sac wet weight and dry caecal content weight. Total, and concentrations of caecal and faecal bacterial mass and short chain fatty acids increased with gum arabic. The addition of gum arabic increased the major anion, acetate, in caecal and faecal material. The presence of wheat bran increased the molar proportion of caecal butyrate. Isomeric forms appeared with the animal origin and elemental diets. Methane production in rats on gum arabic diminished with time. Wheat bran abolished methane production. Gum arabic increased total caecal bile acids, in particular, the muricholic acids. The effect of bran was less clear cut and varied with the basal diet given.

The various interactions between basal diets, supplements and time were recorded. The basal diets influenced the way in which the two fibre

supplements behaved. The differences were both quantitative and temporal.

Basal diet, duration of feeding and supplement have noticeable effects upon all the measurements made. For the initial assessment of dietary fibre, a 4-week stool collection may be adequate. For metabolic analysis a longer period is required. Gum arabic was found to alter all metabolic events, but not stool weight. Wheat bran influenced stool weight, indicative of the caecal degradation of these fibres. These results are in agreement with previous work and confirm that the rat is a suitable animal model for the routine evaluation of dietary fibres.

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ABBREVIATIONS

GI tract	gastrointestinal tract
W.H.C.	water holding capacity
C.E.C.	cation exchange capacity
I.T.T.	intestinal transit time
p W.H.C.	potential water holding capacity
DAPA	diaminopimelic acid
RNA	ribonucleic acid
^{35}S	35 sulphur
H_2	hydrogen
CH_4	methane
CO_2	carbon dioxide
N_2	nitrogen
O_2	oxygen
H^+	hydrogen ions
H_2O	water
SCFA's	short chain fatty acids
FBA	faecal bile acids
LBC	large bowel cancer
O.B.D.	oxoid breeders diet
S.D.S.	special diet services
ADF	acid detergent fibre
NDF	neutral detergent fibre
Anal R.	analytical reagent
B.D.H.	British Drug Houses
I.D.	internal diameter
R.B.F.	round bottom flask
NaCl	sodium chloride
NaOH	sodium hydroxide
HCl (conc. HCl)	hydrochloric acid (concentrated HCl)
H_2SO_4	sulphuric acid
0.2N, 4N and 6N	0.2-, 4-and 6 normal
M	molar
KOH	potassium hydroxide
Mg	magnesium
Ca	calcium
P	phosphorus
K	potassium
C.S.W.W.	caecal sac wet weight (voided of contents)
C.C.	caecal contents (dry)
W-AW	washed-acid washed
C.S.	chromatography services
0	no supplement
G	gum arabic
B	Canadian Red Spring wheat bran (coarse)
PF	plant origin diet
PG	plant origin diet plus 10% gum arabic
PB	plant origin diet plus 10% wheat bran
AF	animal origin diet
AG	animal origin diet plus 10% gum arabic
AB	animal origin diet plus 10% wheat bran

:F	elemental diet
:G	elemental diet plus 10% gum arabic
:B	elemental diet plus 10% wheat bran
:	supplement
,	time
)XT	the interaction between diet and time
;XT	the interaction between supplement&time
;.A.D.	small animal diet
nols	moles
nmols	millimoles
umols	micromoles
v/v	weight per volume
v/w	weight per weight
:/v	volume per volume
-im	nanometre
:m	centimetre
-nm	millimetre
ug	microgram
mg	milligram
g	gram
kg	kilogram
ul	microlitre
ml	millilitre
l	litre
vlms	volumes
MJ	megajoule
kJ	kilojoule
rpm	revs per minute
ppm	parts per million
min	minute
hr	hour
ANOVA	analysis of variance
L.S.D.	least significance difference
S.E.D.	standard error of the difference
S.E.M.	standard error of the mean
R.M.S.	residual mean square
NA	not available
NS	not significant
p	probability
*	p<0.05
**	p<0.01
***	p<0.001
>	greater than
<	less than

CHAPTER 1

AN INTRODUCTION

INTRODUCTION

Fibre in the diet has attracted renewed interest over the past 10-20 years. The best selling book in 1982 was entitled F-plan diet by A. Eyton. (Eastwood and Passmore, 1983). And yet fibre has been a recognised entity since the turn of the 19th century (Trowell, 1977; Van Soest, 1978).

The precedent for any scientific investigation must be an understanding of the subject, preceeding work and a working definition. With fibre studies it is important to define what is meant by "fibre", as there is much conflict over its definition (McCance and Lawrence, 1929; Trowell, 1972 and 1974; Trowell et al, 1976; Spiller, 1977). As Trowell (1977) succinctly wrote "Words alter only slightly: it is their meaning which becomes more precise."

Fibre is a variable material with respect to its nutritional properties depending upon its composition and physical properties. The biological properties possessed by one fibre may not be exhibited by another thus requiring separate methods to describe quantity, composition and quality of fibre in foods (Van Soest, 1978).

The first recognised and accepted method for fibre determination and definition arose out of the analysis of Henneberg and Stohman, (1860) commonly known as the Weende proximate system for the analysis of foods. This is the currently approved method of crude fibre determination (Association of Official Analytical Chemists (AOAC)) and is used in any legal contest over the fibre component of human or animal feed (Van Soest, 1978). The procedure involves the measurement of carbohydrate by difference after deducting the moisture, protein, fat and ash from the total, whilst indigestible material is measured after extraction with hot acid and alkali (Southgate, 1976; Van Soest, 1978).

The main disadvantage of the method is that it was considered to be too empirical and not specific enough: it does not measure any specific class of chemical substance, merely the indigestible residues in a food (Southgate, 1976). Similarly the error in measurement through loss is variable and can be as high as 700% (Van Soest, 1978). For over a century, the inadequacies of the crude fibre method have been known and attempts have been made to develop improved fibre methods. Methodology, however can only be developed, if there is a standard, acceptable working definition.

As the clinical importance of fibre began to grow and its beneficial effects came to light (Painter and Burkitt, 1971; Burkitt et al, 1972; Cleave, 1974), the century old definition of crude fibre (AOAC), the portion of the plant food resistant to hydrolysis by acid and alkali, was no longer adequate, and there was a need to refine the definition. McCance and Lawrence, (1929) proposed the term "unavailable carbohydrate" which was widely used until 1970. But not all the polymers that constitute fibre, are carbohydrate, nor are they entirely unavailable. Some are metabolised in the colon, by bacteria, yielding short chain fatty acids (SCFA's) which can then be used as a source of energy.

Trowell, (1972 and 1974) then revived the term "dietary fibre", defined as "the skeletal remains of plant cells that are resistant to digestion by the enzymes of man: the group of substrates remaining in the ileum, but partially hydrolysed by the colonic bacteria". This did not account for the other constituents of the plant cell (Trowell, 1976), i.e. the plant polysaccharides, both storage and structural. Consequently the definition was extended to include those indigestible plant materials associated with the cell wall (Trowell et al, 1976). Even this definition has its drawbacks. It precludes those plant polysaccharides which are in no way dietary eg isphagula husk fibre; nor does it include polysaccharide derivatives (methyl cellulose) or biosynthetic polysaccharides (Godding, 1976). Even the words "fibre" and "plant" come under attack: fibre is a word used in the textile industry and by nutritional and nerve pathologists (Spiller, 1977). The use of the word plant denies the action of the animal polysaccharides (Godding 1976). Godding (1976) proposed the phrase "edible fibre" thereby encompassing those polysaccharides, related polymers and lignin, which are resistant to the digestive enzymes of man. Spiller, (1977) proposed the term "plantix", which then assumes that all fibres are of plant origin. There is really little to commend this hybrid neologism.

Whatever the definition, fibre is a mixture of different substances with properties that will vary according to age, species, processing and digestion (Van Soest, 1978; Royal College of Physicians, 1980). The effects of these undigested residues, are more important than the undue attention given to perfecting a definition. To ruminant nutritionists it is alien to think of gums or pectins as dietary fibres, yet their role in the diet is to exert influences generally associated with fibre eg bran. Thus indicating that one definition would probably be inadequate and that at least for human or ruminant nutritionists two would be necessary.

It is apparent that the definition of dietary fibre is in a state of flux and will probably remain so, for years to come.

DIETARY FIBRE COMPONENTS

Fibre is not a single entity, but a complex heterogeneous matrix comprising substances which are largely found in the plant cell wall and to a lesser degree in the plant cell (celluloses, lignins, pectins, gums, mucillages, structural and non-structural plant polysaccharides). These are thought not to be digested in the upper gastrointestinal tract (Cummings, 1976). It is this definition that will be referred to throughout the coming text when dietary fibre is talked about.

None of the components that constitute dietary fibre are nutrients and their absence from a diet does not lead to any specific deficiency disease; their effects on health are secondary to their effects on alimentary functions (Eastwood and Passmore, 1983).

Polysaccharides and lignins are the two major constituents of fibre (Table 1a). The group of polysaccharides can be further divided into (i) structural and (ii) non-structural, (Table 1b).

a) **STRUCTURAL**

This group includes cellulose, non-cellulosic polysaccharides, pectic substances and lignin.

i) Cellulose is considered the most abundant organic substance on earth and consists of a linear array of glucopyranose units linked with α (1-4) glycosidic bonds possibly 4000-6000 nm long and 4 nm in diameter (Mahler and Cordes, 1971; Eastwood and Passmore, 1983). It is the residue insoluble in strong alkali (Southgate, 1976) and of the individual components of dietary fibre, it has been the most studied.

TABLE 1a **CHEMICAL CLASSIFICATION OF DIETARY FIBRE**
(Kay and Strasberg, 1978)

Fibre	Description	Major chemical components	
		Main chain	Side chains
Polysaccharides Cellulose	Linear homoglycan* main structural component in plant cell wall; insoluble in concentrated alkali; soluble in concentrated sulphuric acid.	Glucose (1 4)	None
Noncellulose Hemicellulose	Diverse group of heteroglycans† occurring in cell wall; vary in degree of branching and uronic acid content; soluble in dilute alkali.	Xylose Mannose Galactose Glucose	Arabinose Galactose Glucuronic acid
Pectic substances	Occur in primary cell wall and middle lamella (intercellular cementing substance); vary in degree of methylation.	Galacturonic acid	Rhamnose Arabinose Xylose Fucose
Gums	Formed by secretory cells and released at site of injury to plants.	Galactose Glucuronic acid–mannose Galactouronic acid-rhamnose	Xylose Fucose Galactose
Mucilages	Synthesized by plant secretory cells; occur in endosperm of plant seeds where they act to prevent excessive dessication, vary in uronic acid content.	Galactose-mannose Glucose-mannose Arabinose-xylose Galacturonic acid-rhamnose	
Algal polysaccharides	Derived from algae and seaweed which differ in that cellulose in cell wall may be replaced by xylose and mannose; may be linear or branched; some types contain sulfated residues.	Mannose Xylose Guluronic acid Glucose	Galactose

TABLE 1a Continued

Fibre	Description	Major chemical components	
		Main chain	Side chains
Lignin	Non-carbohydrate; complex cross-linked phenyl propane polymer; 3 broad classifications corresponding to predominance of sinapyl, coniferyl, or coumaryl alcohols; probably has three dimensional structure; infiltrates mature cell wall; resists bacterial degradation insoluble in concentrated sulphuric acid.	Sinapyl alcohol Coniferyl alcohol Coumaryl alcohol	

*Containing one type of sugar residue
≠Containing more than one type of sugar residue

TABLE 1b **A CLASSICIATION OF THE UNAVAILABLE CARBOHYDRATES IN FOODS**
(Southgate,1976)

Principal Sources in the diet	Description	Classical Nomenclature
Structural materials of the plant cell wall	Structural polysaccharides	Pectic Substances Hemicelluloses Cellulose
	Non-carbohydrate constituents	Lignin Minor constituents
Non-structural materials, either found naturally or used as food additives	Polysaccharides from a variety of sources	Pectic Substances Gums Mucilages Algal polysaccharides Chemically modified polysaccharides

ii) The Non-cellulosic polysaccharide group contains a large number of different polymers frequently branched and containing a mixture of pentose and hexose sugars and uronic acids (Kay and Strasberg, 1978).

Hemicelluloses are branched polymers of pentose and hexose sugars, and are usually smaller in size (150-200 sugar residues) than cellulose. (Eastwood and Passmore, 1983; Kay and Strasberg, 1978). They can be further classified into acidic or neutral forms based on the content of the uronic acids (Southgate, 1976; Kay and Strasberg, 1978).

Pectic substances: are common to all cell walls. They are a complex mixture of colloidal polysaccharides that are partly esterified rhamnogalacturonans with α-(1-4) linked D-galacturonan chain, interspersed with L-rhamnopyranosyl residues with side chains including D-glucuronic and galacturonic acids. Some acidic groups are methylated. Some pectic substances are extractable from plant tissues by means of hot aqueous chelating agents, but 20-30% is closely bound to other cell wall constituents, particularly cellulose (Southgate, 1976; Eastwood and Passmore, 1983).

iii) Lignin: is not a carbohydrate but a complex, cross linked polymer based on oxygenated phenylpropane units. Three broad classes may be distinguished based upon the predominance of sinapyl, coniferyl and coumaryl alcohols. It is a highly branched polymer containing about 40 phenyl propane units and encrusts the cellulose and hemicellulose during secondary thickening. It is extremely resistant to both chemical and enzymatic degradation and is possibly the most resistant substance found in nature, thus making it one of the most difficult components to measure adequately. It is isolated as the residue insoluble in 72% (w/ w) H_2SO_4 (Southgate, 1976 ; Kay and Strasberg, 1978; Eastwood and Passmore, 1983).

b) **NON-STRUCTURAL**

i) Gums: form a very heterogeneous group of complex, branched, viscous, water soluble heteropolysaccharides, of 10 000 - 30 000 units. These are mainly glucose, galactose, mannose, arabinose, rhamnose and their uronic acids, which may or may not be methylated or acetylated (Southgate, 1976;

Eastwood and Passmore, 1983). There are many gums: gum arabic, gum tragacanth, gum karaya, (gum Stercularia), carob bean gum and guar gum. The former two are widely used in food processing.

ii) Algal polysaccharide these are polysaccharides extracted from algae, usually marine forms, and widely used in food processing. They are distinctive in that cellulose is often replaced by xylans and mannans. Chemically they consist of linear or branched molecules containing mannose, xylose, guluronic acid, glucose and galactose. Some also have highly sulphated galactose residues (Kay and Strasberg, 1978).

iii)Mucilages polysaccharides obtained from seeds and seaweeds used in small amounts in the food industry as thickening and stabilising agents, by virtue of their water holding capacities and viscous properties. (Eastwood and Passmore, 1983). They can be divided into two groups: neutral and acidic. The former include galactomannans, glucomannan and highly branched arabinoxylans The latter group are mainly rhamnogalacturonans. They do not occur in the cell wall but are found in the endosperm of seeds where they act to retain water and prevent excess dehydration. (Kay and Strasberg, 1978).

ANALYTICAL TECHNIQUES

With the realisation that fibre was not a single entity (Crampton and Maynard, 1938; Kay and Strasberg, 1978; Royal College of Physicians, 1980; Eastwood and Passmore, 1983) grew the necessity for the development of more refined techniques to measure the specific components and their different physiological properties. Williams and Olmsted, (1934) developed methods for estimating the amounts of hemicelluloses and cellulosic components of the diet, but it was the work of Van Soest and his colleagues and the use of detergents that provided the greatest advances in this field (Van Soest, 1963(a), (b); Van Soest and Wine, 1967; Van Soest and McQueen, 1973). These techniques provide the best available method for measuring fibre in foodstuffs (Goering and Van Soest, 1970). The method requires extraction with neutral detergent (NDF) followed by further extraction with acid detergent (ADF). The method is quick and can be used for screening at the food laboratory level. But as fibre in the digestive tract is probably specific and not only a function of the nature of the fibre but also of other luminal materials present, it is necessary to/....

analyse individual foodstuffs in individual species to obtain accurate and more useful information. What occurs in vitro may not necessarily happen in vivo; and methods available for determining the fibre components of an animal feedstuff may not be suitable for human feedstuffs. Southgate (1969) developed a method for the estimation of vegetable fibre in foodstuffs more commonly associated with human nutrition. The method is time consuming and requires the sequential removal of food fractions by selective reagents until only lignin and inorganic ash are left. Morrison (1972) developed a method to measure the amount of lignin in plant materials by dissolving the lignin in acetyl bromide. There is a wide range of techniques available for the determination and evaluation of food carbohydrates, which are detailed in the literature, (McCance and Lawrence, 1929; Williams and Olmsted, 1934; Van Soest, 1963, (a), (b); Southgate, 1969; Goering and Van Soest, 1970, Schweizer and Wursch, 1979), and are also critically compared and reviewed (Southgate, 1973; Van Soest and McQueen, 1973; McConnell and Eastwood, 1974; Morrison, 1980).

In summary dietary fibre is a complex heterogeneous material, comprising those constituents of the plant cell and wall that are undigested by the gastric enzymes in man. In the plant these components form a matrix. In man this matrix will be altered by eating, gastrointestinal secretions and interactions with other dietary components. Evaluating the chemical and physical properties of the individual fibre components has its limitations. They are not ingested as discrete molecules, but as a piece of plant tissue resulting in numerous complicating interactions. The same weight of estimated fibre eg cereal bran, apple, pectin or guar gum will have quite different effects along the gastrointestinal tract (McConnell and Eastwood, 1974). Whilst a systematic approach to fibre requires the isolation and study of individual fibre components, the chemical extraction from the plant produces states of undefined chemical and physical natures which may have altered physiological properties (Eastwood and Kay, 1979).

FIBRE IN THE GASTROINTESTINAL TRACT

Until the beginning of the nineteenth century it was believed that fibre passed through the gastrointestinal tract (G I tract) undigested and unaltered. In the review "The digestion and utilisation of crude fibre", Mangold (1934) chronologically details the history of fibre and its digestion: Sprengel(1832) and ruminant digestion., Hoffmeister(1880) and fibre degradation in the caecum

and colon. According to this review Weiske (1870) was the first worker to have studied the digestion of fibre in man, although accurate digestibility figures were few due to the use of single subjects in experiments. Even then the macroscopic examination of faeces was important to determine the extent of degradation within the GI tract, and that undigested fibre caused an increase in stool weight (Mangold 1934).

It was not until 1936 that the first systematic investigation into the effects of dietary fibre in the G I tract was made by Williams and Olmsted. They found that dietary fibre (bran) increased stool weight and that lignin affected the digestibility of the other components. Since then subsequent studies have shown the effect of fibre on stool weight (Calloway, 1966; Eastwood et al; 1973; Southgate et al, 1976; Cummings et al 1978) and the presence of interactions between the fibre polymers, gel formation, the surrounding solutes pH and the intestinal bacteria (Eastwood 1975; Southgate 1978).

Whilst the fate of fibrous foods has been thoroughly investigated in the ruminant (Czerkwaski et al 1966; Blaxter 1967; Ballard et al 1972; Ullyatt et al 1975; L'estrange and Mulvihill 1975; Kaufman 1976; Czerkwaski 1976; Czerkwaski and Breckenbridge 1979), this is not the case in man. Ruminant studies are facilitated by the use of cannulation techniques allowing for the ready removal of gastrointestinal contents, or the implantation of foodstuffs. This is not possible in the human, and there is a reliance on detailed in vitro techniques, animal models and population studies to assess what happens to ingested food within the human GI tract.

a) FATE OF INGESTED FIBRE

i) Fore and mid gut: Food is ingested through mouth, the motor functions of which are mastication and swallowing. (Royal College of Physicians, 1980). Mastication also increases the flow of saliva and therefore adds a liquid phase to the gastric contents. Once ingested the food bolus is passed, by peristalsis, through the cardiac sphincter acting as a valve between the oesophagus and stomach, to the stomach where storage, mixing, acidification and proteolytic digestion of swallowed food occurs. The nature of the ingested food affects the volume of the stomach contents and rate of gastric emptying.

As the contents of the stomach become liquified they pass through the pylorus, a sphincter at the lower end of the stomach, into the small intestine. It appears that the liquid contents of the stomach have a quicker gastric emptying rate than solids. Gastric emptying rate seems to be different after wholemeal and white bread meals (Royal College of Physicians,1980). Grimes and Goddard (1977) found that the liquid phase emptied more rapidly after white bread, while the solid phase emptied at the same rate. As a consequence, any nutrients associated with a solid phase will tend to be retained longer within the stomach than any of their refined counterparts such as fruit juices or vegetable extracts. The overall effect of fibre could therefore be to slow down the entrance of soluble nutrients into the intestine.

ii) Small Bowel: The contents of the stomach are expressed into the small intestine, where such properties as pH, osmotic conditions and electrolyte concentrations can modify the physical and chemical properties of the fibre (Eastwood and Kay, 1979), although this is unlikely as fibre is resistant to degradation by small intestinal enzymes. The physiological actions of dietary fibre in the small intestine are a result of gel formation, water holding capacity (W.H.C), cation exchange capacity (C.E.C), (McConnell et al, 1974; Eastwood, 1975; Eastwood and Kay, 1979; Van Soest and Robertson, 1976), and bile acid adsorption (Pomare and Heaton, 1973). Fibre can also influence the time taken for food to leave the mouth and reach the caecum (intestinal transit time, I.T.T.). The alteration in I.T.T has been related to the nature of the fibre source. Bran has been shown to decrease I.T.T. (Burkitt et al, 1972; Kirwan et al, 1974; Cummings et al, 1978; Heller et al, 1980); as have fruit and vegetables (Kelsay et al, 1981; StasseWolthius, (1980). Pectin has been found to have little effect on I.T.T. (Kay and Truswell, 1977 (a)) as do more refined diets (Burkitt et al, 1972; Spiller et al, 1980).

The nature of the preparation of the dietary source will also affect function. Kirwan et al, (1974) demonstrated that whilst coarse bran decreased I.T.T, fine bran produced no such change. The importance of this is reflected in cholesterol metabolism and the propensity for gallstones. By altering I.T.T, various fibres can alter the bile acid pool size, by their ability to adsorb bile acids, and thus the propensity for cholesterol gallstones (Eastwood and Kay, 1979). The influence of fibre on bile acids will be described later.

The terminal small intestine then enters the large intestine. The large intestine in adults is approximately 1.2 m long and includes the caecum, ascending, transverse descending and sigmoid colon, rectum and anal canal. The large intestine can be divided into right and left sides: the right side and caecum are the sides of most fermentation, absorptive and secretary activities while the left side is mainly for the storage of food residues and excretion products. The luminal content of the colon consists of bacteria, undigested food residue, water and electrolytes, shed mucosal cells, secreted and excreted substances. In the colon fibre provides an absorptive surface for both bacteria and isolates. The most important property of fibre could be considered its ability to serve as a substrate for microbial function within the GI tract, as it is through this mechanism that energy, from that carbohydrate resistant to the digestive enzymes, becomes available for metabolism (Van Soest, 1978).

b) PHYSIOLOGICAL EFFECTS AND PHYSICAL PROPERTIES OF FIBRE

The action of fibre in passing along the G I tract has been likened to that of a sponge (Eastwood, 1975). It is able to hold water, bind metabolites, influence bulk density and alter C.E.C. properties, all of which are of nutritional importance. (Table 1c) The swelling power of fibre was originally shown by Gray and Tainter, (1941). Dietary fibres vary in their W.H.C. Whilst potato fibre has a capacity of 2g H_2O/g fibre, carrot fibre has a W.H.C. of 23.4g/g (McConnell et al, 1974.). This variation in W.H.C. may modify the absorptive and secretary activities of the colon. Stool weight and consistency are affected by W.H.C. (Stephen and Cummings, 1979). Cation exchange properties of plant fibres are also widely variable depending on source and preparation (Eastwood and Mitchell, 1976., McConnell et al, 1974.) Pear has a C.E.C. of 0.6 mmol/g whilst turnip has a C.E.C. of 2.3 mmol/g. Cellulose has no binding ability whatsoever hence its use as filter paper in chemical separations, (Van Soest, 1978), and yet it takes up water well. Fibres can also be regarded with respect to their ability for binding, (adsorbing) bile acids. Pectin may have a strong monofunctional C.E.C. but it is a poor binder of bile acids. Lignin is a strong bile acid binder and a weak polyfunctional cation-exchanger. (Eastwood and Mitchell, 1976). As a consequence of these varied physical properties, different dietary fibre sources could be expected to modify colonfunction by changing the constituent entering the colon and

TABLE 1c **PHYSIOLOGICAL ACTIONS OF FIBRE AS IT PASSES ALONG THE GASTROINTESTINAL TRACT**

(Eastwood and Kay, 1979)

PHYSIOCHEMICAL PROPERTIES	TYPE OF FIBRE	MODIFYING
Gel formation	Pectin Mucilages	Gastric emptying Mouth to Caecum transit small intestinal absorption
Water holding capacity	Polysaccharides Lignins	Mouth to rectum transit time Faecal weight Intraluminal pressure Faecal electrolytes
Matrix formation		Caecal bacterial metabolism
Bile acid adsorption	Lignin Pectin	Faecal Steroids Cholesterol turnover
Cation exchange	Acidic Polysaccharides	Faecal minerals
Anti oxidant	Lignin	Free radical formation + action
Digestibility	Polysaccharides	Energy availability Chemical environment of colon Other physiochemical properties

thereby altering the milieu of the lumen. For example the binding of bile acids would present them to the colon. This could then have consequences upon bacterial growth (Floch et al, 1971) and alter the enterohepatic circulation with consequences upon bile acid synthesis in the liver (Hofmann, 1976).

In summary, the colon filled with dietary fibre could be acting as a chromatography column with adsorptive, ion exchange and gel filtration properties (Eastwood, 1973).

i) Stool weight

The most recognised, associated effect of dietary fibre is to increase stool weight and volume. The importance of a normal bowel function is evident in problems of constipation, a painful and stressful condition. In a given 'normal' individual, stool weight has been proven to be altered by dietary means (Williams and Olmsted, 1936(b)). Certain "Western Diseases" have been attributed to the lack of dietary fibre: diverticular disease and heart disease. (Painter and Burkitt, 1971; Burkitt et al., 1972; Painter et al, 1972), these problems being rare or absent in countries little affected by civilisation, where the diet is mainly fibrous. Cereal fibres are known to be the best stool bulking agents Southgate et al, 1976; Cummings et al, 1978; Eastwood et al, 1983), whilst fruit and vegetables have been shown to have moderate influences on stool. (Cummings et al, 1978; Stasse-Wolthuis, 1980). Gums and pectins have not been shown to have any significant influence on stool weight (Kay and Truswell, 1977(a); Cummings et al, 1978). In their work Cummings et al (1978) found a relationship between the pentose fraction of foods eaten and stool weight. Stasse-Wolthuis (1980) found good correlations between pectin and stool weight ($r = 0.94$) for group averages, but not for the individual data. Pectin, which is amorphous rather than fibrous, may not increase stool weight, but it has been shown to cause a fall in serum cholesterol. (Jenkins et al, 1975; Durrington et al, 1976). Jenkins et al (1975) showed cholesterol to increase slightly after the ingestion of wheat bran. Heaton and Pomare (1974) found no change in serum cholesterol after the ingestion of wheat bran as did Eastwood et al (1973) and Truswell and Kay (1976). Bran could be considered as a stool bulking agent and pectin a hypocholesterolemic agent. This/....

demonstrates that different dietary fibre types are of importance in the human diet and that they may have different beneficial properties, (Kay and Truswell, 1977a), associated with their different structural makeups.

ii) Water holding capacity

Experiments to measure the influence of fibre on stool weight are difficult and lack precision. One important property of fibre which influences the ability of fibre to increase stool weight is its W.H.C. (Robertson et al, 1980; Eastwood et al, 1983). Water can be held as either trapped or bound water. Methods have been used to study W.H.C. and the different phases in which water is held. These include centrifugation, filtration and osmotic pressure (Eastwood et al, 1983). Some polysaccharides swell more than others and swell differently when as an individual as when constrained within a cell wall along with less hydrophobic substances (Eastwood and Robertson 1978). A fibre with a high W.H.C. may have a different internal structure from one which has a modest W.H.C., which in the small intestine could affect the entrapment of compounds within the lumen and consequently their absorption. Increased W.H.C. of cereal bran has been associated with increases in stool weight, (Robertson et al, 1979(a)), indicating that the capacity to hold water is important (Eastwood et al, 1973). Stephen and Cummings (1979) indicated an inverse relationship between W.H.C. and stool: the more water a fibre held there was a decrease in stool bulk. Bran may have a low W.H.C. but will increase stool bulk (McConnell et al, 1974; Durrington et al, 1976). Particle size may influence W.H.C. Kirwan et al (1974) showed that coarse bran was associated with a more watery stool, assumed to be associated with the ability to bind water.

However the measurement of W.H.C. and preparation of the sample can influence results, and careful interpretation is needed. In two studies Robertson et al (1979(a)) and Robertson et al (1980), to investigate the effect of age, species, variety and stage of development of prepared fibre from certain carrots with respect to W.H.C., it was apparent that the method chosen influenced W.H.C., and that the difference in W.H.C. was more pronounced when centrifugation was one of the methods chosen.

Variety and developmental age could be as important as the method chosen to study W.H.C. In their studies, Robertson and colleagues demonstrated that W.H.C. differences occurred between varieties and was dependent upon the method used. A mean W.H.C. of all varieties at all ages sampled was 23.4 g/g. But differences were found between and within varieties suggesting that variety and developmental age could be as important as the species when formulating high fibre diets (Robertson et al, 1980). Conversely the absence of change in W.H.C./100 g fresh carrot because of the decrease in fibre content with increasing developmental age, would suggest that age and variety are relatively unimportant when formulating high fibre diets (Robertson et al, 1980).

When W.H.C. was measured by filtration, no differences were found between varieties suggesting that centrifugation positively influences W.H.C. results. This apparent difference could be a reflection of the structural differences in fibre and highlights the problems associated with assessing the properties of fibre. If the combined results of centrifugation and filtration are studied, it appears that the W.H.C. can change with increasing developmental age and variety. Stephen and Cummings (1979), did a comparative study with dialysis tubing and centrifugation, and the results showed particle size to be the determining factor.

W.H.C. appears to be of little value for fruit and vegetable fibre, and the comments of Stephen and Cummings, (1979) apply. Nevertheless, W.H.C. is a reasonably accurate predictor of stool bulking ability for wheat fibre, (Robertson and Eastwood 1981(a)) and can be of value if there is no bacterial fermentation of a cereal bran. Where fermentation can occur, eg with pectin, then W.H.C. appears to be of little value Robertson and Eastwood, 1981(b); Eastwood et al, 1983).

Recent work by McBurney et al (1985) suggests that the potential water holding capacity (pW.H.C), a function of the extend of fermentability and the W.H.C. of the fermentation residues of fibres, is a better predictor of the effect of fibre on the rate passage of fibre and faecal mass in humans. They took four fibres and fermented them anaerobically, in vitro with human faecal innoculum. The results showed that pW.H.C was highest for lucerne followed by cellulose, cabbage and pectin. These ranked the four fibres in the same order as in vivo values, thus reversing the relationship seen by Stephen and Cummings (1979).

Until further work has been done to assess pW.H.C, the W.H.C. of fibres as yet cannot be used as an indicator of *in vivo* effects due to the absence of a reliable representative method. To date, the relationship between W.H.C. and stool weight can be summarised as follows:

1. Different fibres have different W.H.C.

2. W.H.C. can vary within a fibre source depending upon the method of preparation and that chemical composition within a fibre source that have been prepared differently is unimportant.

3. Differences in W.H.C. are a function of fibre structure, age, variety and preparation (Robertson *et al* 1979 (a)) and 1980).

4. Measurement of 'trapped' water within a fibre would be of little benefit to predict the effects of fibre in the gut unless it remained associated with fibre in the gut.

5. The amount of water held may not be as important as the nature in which it is held.

6. Centrifugation and filtration have little value in predicting the biological efficacy of a fibre of vegetable or fruit origin. (Robertson and Eastwood, 1981(a), (b);Eastwood *et al*, 1983).

iii) Bile acids and their adsorption to fibre

All bile acids are formed in the liver primarily from cholesterol. If cholesterol, labelled in the ring system, is injected into the circulation, then over 90% of it is eventually recovered from Bile acids (Physiology and Biophysics III). Bile acids are involved in fat absorption in the duodenum and jejunum (Eastwood and Girdwood, 1968.) Bile and bile salts are produced in the liver. Bile acids exist in bile as sodium salts of either glycine or taurine. The most important bile salts in man are the two primary bile acids cholic-and chenodeoxycholic acid and the secondary bile acids deoxycholic-and lithocholic acid. Adding a fibre to the diet can alter the bacterial metabolism of bile salts, by reducing

the amount of dehydroxylation of the bile salts (Pomare and Heaton, 1973). Normally the effect of bile acid synthesis is to keep the pool size constant by adding an equal amount to that lost. In man 5-10% is lost in the faeces with each circuit of the enterohepatic circulation. The products of extensive bacterial action are not absorbed and appear in the faeces. Enterohepatic circulation of bile acids depends on the active transport of bile salts from the terminal ileum (Hill, 1980). Bile salts have been shown to be distributed along the rat small intestine in such a manner that the maximum amount is found in the proximal ileum (Boyd et al, 1966). Eastwood and Mowbry (1976) in their study examined bile salt binding from mixed micelles, to show how the changing form of micelles along the intestine would effect bile salt binding. The amount of reabsorption occurring in the ileum decreased, as a result of the binding of bile salts to the fibre. Eastwood and Mitchell (1976) also demonstrated that fibre could bind bile acids, which could then change the colonic function. The adsorption of bile acids would be expected to change the enterohepatic circulation. A reduction in cycling frequency due to presence of mucilagenous polysaccharides may affect the bile acid pool size, (Eastwood and Kay, 1979), resulting in a negative feedback on cholesterol synthesis. Similarly adsorption of bile acids may prevent degradation by bacteria and reabsorption from the colon. Kritchevsky (1978) postulated that with a fibre free diet bile acids are not excreted at a rapid rate; therefore they accumulate, bile acid synthesis shuts off and cholesterol accumulates, resulting in a reduction of the synthesis of bile acids and continued degradation of primary bile acids. The relevance of this is that secondary bile acids may be toxic and act as cocarcinogens (Heaton 1982). Pomare and Heaton (1973) suggested that the high proportion of dietary fibre consumed by peoples of Africa and Asia protected against colon cancer, because of this lack of degradation. Similarly, Sian and Greenhalgh (1984) found that total faecal steroid excretion was higher in a group of subjects on a mixed diet compared to a group on a non-meat vegetarian diet. Comparison of faecal bile acids profiles showed that the bile acids were extensively degraded on the former diet compared with the latter.

Various components of dietary fibre have been shown to have differing binding properties of bile acids. Lignin has been shown to act as an acid binding agent (Eastwood and Girdwood, 1968; Eastwood and Hamilton, 1968; Hill, 1980).

By adding wheat bran to the diet the ratio of primary to secondary bile acids can be altered (Pomare and Heaton, 1973; Tarpila et al, 1978; Ullrich et al, 1981). These results suggested that bacterial degradation of primary to secondary bile acids was inhibited by fibre in the colon. The influence of dietary fibre, upon the total and concentration of bile acids in the stool is not clear cut. Whilst bagasse has been shown to increase the total loss of acid steroids this was not true for bran; the concentrations of bile acids within the stool decreased on the bran diet but remained unaltered with the bagasse diet (Walters et al, 1975); McLean Baird et al (1977) in a similar study found that 10 g bagasse resulted in an increase in stool weight and faecal acid steroids. Bran also increased faecal weight but not excretion of acid steroids, indicating a decrease in concentration Tarpila et al (1978) found bran to increase stool weight and to cause a definite decrease in total excretion and concentration of bile acids. Kritchevsky and Story (1974) demonstrated that a natural non nutritive fibre was better than a synthetic non-nutritive fibre at binding bile acids. It has been suggested that the amount of fibre in the diet may influence the faecal excretion of bile acids. Between 30 g and 60 g has been shown to cause an increase in faecal steroids (Cummings et al, 1976; Cummings et al, 1979(a)), whereas less than 20 g was ineffective (Kay and Truswell 1979(b); Tarpila et al, 1978). However McLean Baird et al (1977) used 39 g of wheat bran bran fibre and 10.5 g of bagasse (containing 3.7 g crude fibre) and reported that there was an increase in faecal acid steroids with bagasse, but not with bran over a 9 month period, with concomitant increases in stool weight. Stasse Wolthius et al (1979) reported the excretion of bile acids to be greater on a high fibre diet than on a low fibre diet in groups with both high and low senum cholesterol concentration with no consistent changes in the ratio of primary to secondary bile acids.

Carrot fibre on the other hand has quite different effects to that of bran. 200 g of raw carrot (3 g of dietary fibre) has been shown to increase total bile acid excretion with an increase in the concentration of bile acids expressed per g of dry weight of carrot (Robertson et al, 1979(b)). The effect of pectin has also been studied. Kay and Truswell (1977(a)) observed that 15 g of pectin fed for 3 weeks resulted in a decrease in plasma cholsterol concentration and increased steroid output. Miettinen and Tarpila (1977) observed that 50 g/day of pectin resulted in an increase in bile acid excretion with a concomitant reduction in serum cholesterol, and no change in faecal bulk

i.e. concentration of faecal steroids must also have increased. In a switch back design, Ross and Lecklem (1981) compared the effects of a pectin and non-pectin diet. The data tends to be confusing as they look at both 'end of period' data and 'mean values', perhaps in an attempt to look at the effect of time. When comparing mean values no difference was observed between the effects of the basal diet and pectin diet on bile acid concentration, although there was an 11% increase in excretion of bile acids on the pectin diet. But when looking at the mean end of period data, a 3.5% decrease in bile acid concentration on the pectin diet was observed concomitant with a 6% increase in excretion of bile acids. The results suggest that the groups of people studied either behaved differently or the time period (18 days) was not long enough.

There is growing awareness that there may be a correlation between large bowel cancer (LBC) and the concentration of faecal bile acids (FBA). Thompson (1982), describes studies where patients predisposed to LBC had increased FBA. In a series of short term studies he recorded that the addition of selected fibres to the diets of volunteers resulted in an increased concentration of FBA, but dependent on stool bulk there was a variable dilution effect with wheat bran.

Dietary fibre thus appears to influence the adsorption of bile acids, subsequently effecting excretion, concentration and the constituents. Effects appear to vary with fibre type and in terms of carcinogenic potential, the concentration of bile acids within the G I tract is more important (Kay, 1981). This could be as a result of more bile acids coming into contact with the mucosal surface, and the presence of more bile acids for bacterial degradation. Some dietary fibres can increase faecal bile acid output either due to adsorption (lignin) or partitioning of bile acids into an intraluminal gel phase (pectin, guar: Kay and Truswell, 1977(a)). Other fibre rich foods such as oat bran (Kirby et al 1981) can enhance faecal bile acid excretion and increase stool weight. In which case concentration is little affected. Others, such as bran, do not significantly alter total steroid output but as a consequence of an increase in stool output, decrease faecal bile acid concentration.

iv) Mineral binding

As dietary fibre has been linked with bile acid adsorption and cancer, so dietary fibre can cause mineral deficiencies as a result of the binding of minerals.

(McCance and Widdowson, 1942; Dunnigan et al, 1976; Reinhold, 1976; James et al, 1978).

In healthy men and women, calcium and magnesium have been shown to be absorbed much less readily from brown bread than white (McCance and Widdowson, 1942). This reduced absorption was attributed to the phytic acid content of brown bread, and could be reproduced by adding sodium phytate to white bread. Wheat bran contains the main proportion of total phosphorus of the whole wheat kernel, mostly in the form of phytate. Bagheri and Guegen (1982), showed that wheat bran did not have an unfavourable influence on the metabolism of calcium and zinc in the rat, which may be specific to the rat.

Mineral malabsorption can be a serious problem amongst children, different dietary trends of races and populations influence the risks. Rickets among rachitic Asian children, and non-rachitic asian children was shown to be as a result of the ingestion of chapatti flour (Dunnigan et al, 1976), because of the phytate content of the flour (Reinhold et al, 1973). Phytate can cause late rickets and osteromalacia in Indian communities (Ford et al, 1972). Whilst high phytate intakes can be the cause of disturbances of zinc and calcium metabolism, it can be removed by the presence of phytases in the diet, therefore there must be some other inhibitor in the gut (Reinhold et al, 1973). When Tanok (high in fibre and phytate) and Bazari (less fibre and phytate) replaced white bread, there was an increased faecal loss of calcium magnesium and zinc (Reinhold, 1976) suggesting that the intake of fibre is more potent than the phytate content, although a close association in wholemeal bread, results in a reinforcement of fibre action by phytate.

Calcium adsorbed to dietary fibre may be unavailable for absorption. The effect of dietary fibre on mineral absorption has been demonstrated in the growing rat. Harmuth-Hoene and Schelenz (1980) fed rats a semi-synthetic diet and 10% indigestible polysaccharides: carrageenan and agar-agar decreased the absorption of all minerals (Calcium, Iron, Zinc, Copper, Chromium, Cobalt). At the end of the 21 day trial there was no significant differences between the controls and animals fed carrageenan or agar-agar, suggesting that the rat was able to compensate for faecal losses, but Zinc, Chromium and Cobalt were significantly decreased over the entire period.

With the increase in dietary fibre by the public the hazardous effects of ion binding capacity should be emphasised because of its sequestrating effect

and possible inhibition of Calcium, Magnesium, Zinc and Iron absorption.

c) **BACTERIAL FLORA**

The physical and chemical effects of fibre are modified by the bacterial metabolites of fibre fermentation. Only in the lower ileum and large intestine is there sufficient stasis to permit bacterial proliferation. Any discussion of the human intestinal flora involves a discussion of the flora of the large intestine. The normal colonic flora is usually inferred from the composition of the faecal flora (Hill and Drasar, 1975).

The caecum environment differs from that of the rumen in as much as that all the readily digestible material has already been removed by the animal. Otherwise the rumen and caecum serve similar functions (McBee, 1970). The acidic pH of the stomach and small intestine is bactericidal thus ensuring that organisms do not spend long enough to colonise and proliferate.

Bacteria play an essential role because of their diversity and the wide range of metabolic activities they perform. The number of bacteria found in the colon is 10^{11} bacteria/g wet weight of sigmoid colon and rectum while in small intestine they number less than 10^{4}/g. (Drasar and Hill, 1974), (Table 1d). In the normal adult colon the resident bacterial population consists 96-99% anaerobes of which approximately 45% are reported to be Bacteroides fragilis (Moore et al, 1969; Hill and Drasar, 1975). The other major species found inhabiting the human intestine are well documented in the "Human Intestinal Flora" (Drasar and Hill, 1974). The bacterial flora is predominantly non-sporing, strictly anaerobic, with most species belonging to the two genera Bacteroides and Bifidobacterium species from these genera have been shown to ferment the widest range of substrates (Salyers et al 1978). Human digestive enzymes have little effect, if any, on cellulose, hemicellulose, pectins and polyuronic acid substances. Bowel bacteria are therefore supplied with a variety of fermentable substrates. About 20% of hemicellulose is degraded during transit through the gut.

Bacterial degradative activities have been implicated in large bowel cancer (LBC) (Aries et al, 1969; Hill and Drasar, 1975): the degradation of bile acids (Pomare and Heaton, 1973.) and the production of toxic substances from certain

TABLE 1d **THE BACTERIAL FLORA OF THE LOWER INTESTINE OF MAN**

(Hill and Drasar, 1975)

SPECIES	Mean Log_{10} Viable count/gram Intestinal material		
	Terminal ileum	Caecum	Faeces
Enterobacteria	3.3	6.2	7.4
Enterococci	2.2	3.6	5.6
Lactobacilli	<2	6.4	6.5
Clostridia	<2	3.0	5.4
Bacteroides	5.7	7.8	9.8
Gram +ve non-sporing anaerobes (Eubacteria and Bifidobacteria)	5.8	8.4	10.0

food additives (Rowland, 1981). Many plant products are glucosides of toxic compounds and it is the ability of the colonic fibre to produce enzymes that can hydrolyse these compounds and mediate the reactions, to release the toxic glycone or mutagenic substance, that renders them dangerous (Goldin and Gorbach, 1976; Mallett et al, 1983(b); Rowland et al, 1983; (a), (b); Mallett et al, 1984;(a), (b); These workers have comprehensively studied the antagonistic effect of the gut flora on the hepatic metabolism by the hydrolysis of biliary conjugates eg glucuronides and sulphides synthesised by the liver in an attempt to excrete toxic or non-polar drugs and nutrients : for example the toxicological potential of azo dyes (Rowland 1981). Aromatic and heterocyclic compounds are used widely in the industrial and medical fields; reduction of the nitro group is required for expression of their toxicological properties. Mallett et al (1983(b) demonstrated the debilitating effect of the inclusion of >10% cellulose in the diet on the activities of azoreductase, nitroreductase glucosidase and nitrate reductase. Rowland et al (1983(a)) demonstrated that carrageenan gum has a similar reducing effect on microbial nitro reduction, whilst pectin increased the rate of reduction. These studies demonstrate how the metabolic activity of the gut flora may be modified by certain dietary components including fibre. Any factor instrumental in altering the gut flora or its metabolic properties, could then induce profound alterations in the ability to produce and metabolize carcinogens. (Simon and Gorbach, 1981).

Most studies of human microflora utilise faeces in bacterial studies, as this is thought to be representative of the caecal flora (Drasar and Hill, 1974; Hill and Drasar, 1975, Moore et al, 1978). The composition of the intestinal flora is well documented (Moore et al, 1969; Savage 1977, Moore et al, 1978).

Studies to investigate the influence of diet on bacterial numbers have shown diversity in their results. Aries et al (1969) found a change in species composition associated with the high fibre diet of a Ugandan population compared with the high carbohydrate diet of an English population. Moore et al (1969) showed that a high carbohydrate diet decreased the number of Bacteroides and increased the Enterococci and Eubacterium species. Goldin and Gorbach (1976) reported changes in type and numerical concentration of bacteria in the colonic flora of rats, as influenced by a grain and meat diet. Thus change in enzyme synthesis may not just be as a result of inducement,

but of a change in bacterial populations. i.e. microbes and enzymes are substrate dependent. Drasar and Jenkins (1976) found no appreciable change in the relative number of faecal bacteria as fibre content of the diet increased. Similarly Bornside (1978) reported no changes in the major bacterial groups or their concentration in the stool, on both fibre supplemented or fibre depleted diets. One report, Winitz et al(1970), detailed an alteration of intestinal diet associated with chemically defined diets. But as Bornside (1978) reported there is more data to suggest that faecal flora was unaltered during dietary manipulations.

The literature indicates that the degradation of polysaccharides by intestinal enzymes is due to inducible degrading enzymes rather than constitutive, thus the metabolic activity of the flora could be changed by the diet, but not the composition of the flora itself, (Salyers, 1979; Bornside, 1978; Salyers et al, 1978; Mallett et al, 1984(b)) and that the effects of diet may be slow to manifest themselves (Hill and Drasar, 1975). In a series of studies, Salyers and her colleagues reported the ability of certain bacterial strains to metabolize certain dietary fibre components using substrates chosen to reflect the wide variety of linkages and carbohydrate components found in dietary polysaccharides and intestinal mucus. (Salyers et al, 1977(a), (b); Salyers et al, 1978; Salyers et al, 1983). Bacteroides strains isolated from the human colon can degrade and ferment both mucins and plant polysaccharides. Mucin degradation could impair the protective layer of the mucosa making it vulnerable to the action of noxious substances. It could influence the charge and hydration of colonic contents, thereby influencing bile acid absorption and excretion (Eastwood and Hamilton, 1968). The diversity within species was illustrated by the ability of Bifidobacterium longum to ferment gum arabic which was not fermented by Bacteroides strains (Salyers et al, 1977(b)). Even when the distribution of bacteria is the same, the overall metabolic activity could be different.

It appears, then, that diet influences the metabolic activity of bacteria, and consequently their ability to produce enzymes important in the metabolism of potentially toxicological components. It does not appear that diet influences numbers or type of the flora, but that these degrading enzymes are inducible, particularly those of the Bacteroides genera. Colonic bacteria must possess considerable diversity to recognise and degrade a particular substrate.

The human colon resembles a continuous system culture more closely than a batch culture (Salyers, 1979) and as such it is difficult to measure bacterial numbers and record accurately the species present. Enzyme assays described in the preceeding papers may provide a useful, relatively simple method for monitoring the responses of intestinal flora to dietary changes.

(i) 2-6, Diaminopimelic acid

It is difficult to sample the colonic flora of a human and so the faecal flora is taken as being representative. Despite their limitations, animal models are required. Extensive work has been reported from studies in the ruminant field showing the influence of diet on flow rates of digesta, bacterial alterations and biomass. Techniques used in this field must be the basis for current and future work in the human field. One of the parameters of the study described in this thesis has been to investigate the effect of diet on bacterial mass within the caecum and colon. Bearing in mind that this was not a microbiological study, the Diaminopimelic acid (DAPA) method of Czerkawski (1974) was chosen. DAPA is an amino acid found in nearly all bacteria with the exception of the gram positive cocci, (Bacteroides and Bifidobacterium are gram negative and gram positive rods respectively Lactobacillus is a gram positive rod), Streptomyces-and Actinomyces spp. It is also found in blue green algae, but not in other algae, fungi,yeasts, plant viruses or protozoa (Work and Dewey, 1953) and as such it can be used as a bacterial marker (Czerkawski,1974). DAPA appears to be a cell wall constituent which differentiates between bacteria and other microorganisms (Work and Dewey, 1953). Methods have been described for the determination of DAPA (El-shazyl and Hungate, 1966; Mason and White, 1971) but these are relatively slow, requiring the collection of fractions from large chromatographic columns. DAPA has been used as an estimator of the bacterial contribution of nitrogen in the ruminant. (El-shazyl and Hungate, 1966; Mason, 1969; Hutton et al, 1971; Mason and White 1971). It is necessary to be able to quantify the efficiency of nitrogen utilization by the ruminant, and the contributions of the various components that comprise duodenal nitrogen i.e., the contribution of undegraded dietary, bacterial, protozoal and endogenous fractions (Ling and Buttery, 1978). Quantifying the endogenous fraction is difficult and is reliant upon the measurement of the concentration of some microbial marker. The choice of which marker method to use is a problem, but in a comparative study of RNA,

^{35}S, 2-6 DAPA and 2 aminoethyl phosphoric acid, markers of microbial nitrogen, whilst ^{35}S was considered to be the best marker, DAPA was equally useful, depending on the precision required. Since the majority of microbial nitrogen is of bacterial origin, it could seem that the DAPA method would be widely used (Ling and Buttery, 1978).

Despite its inherent problems, variable concentration between bacterial species (Ling and Buttery, 1978), mutually exclusive with lysine (it may act as a precursor of lysine, (Work and Dewey 1953)) (Czerkwaski, 1974), and not present in all bacteria (Work and Dewey, 1953), this method was chosen as an indicator of bacterial mass.

d) CAECAL FERMENTATION

Material entering the caecum and colon has been believed to be mainly fibrous, or undigested residue, due to the efficient absorption of nutrients in the small bowel. In rabbits and birds caecal fermentation can make a significant contribution to energy requirements, and in some species the microbial activity within the caecum may provide as much as 25-30% of an animals nutritional needs (McBee 1970). A caecum has been defined as a blind sac extending from the side of an otherwise straight canal, between the small and large bowel. (Savage,1977).

(i) Hydrogen and Methane

The primary products of bacterial fermentation are hydrogen (H_2), methane (CH_4) carbon dioxide (CO_2) nitrogen (N_2) oxygen (O_2) and short chain fatty acids (SCFA's). In aerobic situations hydrogen ions (H^+) combine with O_2 to form water. Under anaerobic conditions H^+ are converted to molecular H_2 in the presence of the catalyst hydrogenase. The formation of CH_4 is confined to a specific group of bacteria, the methanogens, which are strictly anaerobic (Smith and Hungate, 1958). The production of CH_4 is a unique property of methanogens, concomitant with their narrow range of substrates, requirements for a low redox potential and the presence of seven co-enzymes or factors (Wolfe, 1979), although there is some evidence to suggest that small amounts of CH_4 may be produced from other gut organisms such as Clostridia (McKay et al, 1982). H_2 and CH_4 can be used as indicators of bacterial fermentation, as influenced by diet, (Calloway, 1966; Bond and Levitt, 1978) as there are no known human cellular metabolic pathways that liberate H_2 and CH_4. CO_2, N_2 and O_2 conversely are less suitable because

of their ubiquitous nature within the cell and surrounding environment. H_2 and CH_4 have been proven to be produced in the intestine (Levitt and Ingelfinger, 1968). The colon was the major site of production. Concentration of these gases is highest in the intestinal lumen, with a resultant diffusion of gases outward to the capillary blood to be removed to the atmosphere from the lungs (McIver et al, 1926; Bond et al, 1971.) Gases produced in the bowel as a result of microbial catabolism are excreted in expired air. Upon the stimulation of intestinal motility any gas resident in the lumen will be expressed as flatus.

The amount and composition of the diffused gas has been shown to vary with type and abundance of microorganisms present and with the substrate provided for their metabolism. Calloway (1966) showed an increase in H_2 levels in subjects 3-4 hours after the ingestion of beans in the meal. A similar pattern, but of smaller magnitude, was shown with the ingestion of onions. After the ingestion of bran, H_2 production was shown to decrease (Bond and Levitt, 1978) compared to the baseline H_2 exhibited with lactulose. With 30 g bran H_2 increased above baseline but was not significantly different from the level exhibited with 10 g of lactulose. H_2 production is reliant on the presentation of exogenous, non-absorbable materials to the colonic bacteria, as there was extremely low rate of H_2 production after a 24 hours fast (Bond and Levitt, 1978; Tadesse et al, 1980). Levitt and Donaldson (1977) reported an excessive amount of H_2 production as a result of the malabsorption of a readily digested carbohydrate being fermented in the caecum.

CH_4 production has been extensively studied in the ruminant, but has been reported as not being a characteristic component of the lower gut of man (Levitt and Ingelfinger, 1968). Formation of CH_4 is wasteful, resulting in approximately 8-10% of the gross energy of the animal diet being excreted. A large ruminant (cow) may produce >200* litres per day of CH_4 by belching. This production of CH_4 can be used as indicator of fermentation rates and how they are affected by type and amount of diet (Hungate, 1968). The ruminant can be considered as a far easier animal to study: it facilitates the use of fistulas to take internal samples and to study the effectof time and feeding on CH_4 production. Clapperton and Czerkawski (1969) found that in the absence of food, CH_4 production decreased, and that the infusion of unsaturated fatty acids into sheep given basal diets of grass also depressed CH_4 production (Czerkawski et al, 1966). Czerkawski and Breckenridge (1969) demonstrated

*> = more than

that the amount of CH_4 produced was not dependent on the amount of carbohydrate fermented, and that methanogens appear to utilize some common product of the fermentation of the carbohydrate by the non-methanogenic microbes. The majority of studies has been done in ruminants, but CH_4 production also occurs in other mammals: caeca of birds (Gasaway, 1976); caeca of rats (Rodkey et al, 1972). Gasaway concluded that the stoichiometry of CH_4 formation in avian caeca may differ from that in ruminants. McKay and Eastwood (1983) found no detectable H_2 in rats fed 3 different diets; nor was there any difference between the sexes. There was no CH_4 detected at any time from rats fed on bran or meat diet, but measurable quantities were recorded at 12 weeks of age and thereafter give the control diet.

In man interest in CH_4 arose out of the development of space travel: the possible danger potential of the accumulation of flatus within a confined space (McKay, 1981). The CH_4 content of flatus can vary from 0-54% (Calloway 1968). Levitt and Bond (1970) reported the actual composition of flatus to be also variable: N_2: 23%-80%., O_2 : 0.1%-2.3%., H_2 O.O6%-47%., CO_2 5.1%-2.9%., CH_4 : O-26%.

The variable proportion that can be excreted in the breath has also been the subject of investigation. Levitt and Ingelfinger (1968) reported that 14% of H_2 produced was being excreted in the breath, whilst Levitt (1969), and Levitt and Levitt (1973) investigated the intestinal absorption of gases as an indicator of the intestinal blood circulation.

Reports of the proportion of healthy subjects excreting CH_4 ranges from 30% to 58% (Calloway, 1968; Bond et al, 1971; Levitt and Bond, 1970; Pitt et al, 1980), and that production and subsequent excretion may be dependent on multiple factors. Bond et al (1971), suggested that age, sex, lactulose ingestion, familial relationships, dietary differences, differences in gut flora and genetic makeup may also have a part to play in the formation and production of CH_4. Age, and lactulose were shown to be ineffective prerequisites. There was evidence to suggest a familial relationship. Tadesse and Eastwood (1978) and Tadesse et al (1980) demonstrated the absence of the effect of substrate on CH_4 production and also to be unrelated to H_2 formation. Sex has been shown to be associated with CH_4 production. Bond et al (1971) observed that CH_4 production was greatest in females. Pitt et al (1980) reported that out of a sample of 256 subjects 41% were CH_4 producers

of which 49% (67/138) were female and 33% (39/118) male. Bjørnklett and Jensen (1982) observed that, whilst 48% of women and 46% of men were methane producers, this was not significant. H_2 and CH_4 are related in the ruminant (Hungate, 1968). This does not appear to be the case in man (Tadesse, et al 1980), suggesting that there are more complex factors involved in CH_4 formation in man. As yet the presence of CH_4 has not been used as a diagnostic value. Levey and Balchum (1963) found no difference between the excretion values for CH_4 of patients with constipation, liver disease, patients receiving intravenus alimentation or post operative patients. Haines et al (1977) did find that out of 30 patients with large bowel cancer (LBC) 80% had detectable methane in their breath; out of 64 with non malignent LBC only 39% had detectable CH_4 and only 40% registered detectable CH_4 out of 208 patients. The results do not satisfactorily indicate whether

1) there is a high proportion of CH_4 producers among LBC patients;
2) increased CH_4 is a result of LBC;
3) whether non CH_4 producers become CH_4 producers before the development of LBC.

They suggest that, because CH_4 producers and CH_4 production are highest in LBC subjects as compared with non malignant LBC group and non LBC group, between which there is no difference, CH_4 could be used as a diagnostic aid in the prediction of LBC. The variability of the data casts some doubt upon the conclusion.

The picture is confusing as to the factors that influence CH_4 production and its relationship with H_2 production. H_2 production will increase if there is malabsorption of an easily fermentable sugar (Levitt, 1969) or if there is the presence of an indigestible fermentable polysaccharide within the caecum (Calloway, 1966) but that this response will vary with dietary substrate (Bond and Levitt, 1978). Factors affecting CH_4 production are more complex and diverse. Differences of opinion exist over its relationship with H_2; sex has been shown to be of importance as has diet, but as yet no pattern has emerged. In the ruminant CH_4 production is definite and ruminant anatomy and physiology predisposes it to the production of CH_4. The ruminant has possibly evolved through its habitat, environment and its daily diet of fibrous foods, - hay, straw cereal grains etc, the ability to produce copious amounts of CH_4. Man however has a more varied diet comprising more refined products. As a

consequence his metabolism and physiology are altered. His voluntary food intake is smaller and his bacterial flora is different. These appear to be factors which relate to the absence of CH_4 production in a major proportion of the populus. Where CH_4 is produced it may be an artefact of the individual, and as yet of no clinical importance. McKay et al (1985), have suggested that CH_4 production is the norm, and that absence of excretion in the breath may possibly be due to the insufficiently high concentrations generated in the colon. Only when the production reaches a certain threshold does it materialise in the breath. H_2, however is of clinical importance as indicated by the following studies (Calloway and Murphy, 1968; Bond and Levitt, 1975; Metz et al, 1976,(a), (b), (c);Newcomer et al, 1977; Tadesse and Eastwood 1978).

(ii) Short chain fatty acids

SCFA's are the major end product of the microbial fermentation of carbohydrate, or malabsorbed material from the small bowel, within the caecum and colon. It is likely that SCFA's are generated by a series of pathways similar to those found in the rumen i.e. the degradation to simple sugars via the glycolytic and pentose phosphate pathways. The physiology, sources and metabolism of SCFA's, their daily production, absorption and measurement, mucosal absorption and the clinical implications, of SCFA's in man are detailed in a comprehensive review by Cummings (1981).

SCFA's are the predominant ions in several species: ruminant (Prins, 1977); kangaroo (Henning and Hird, 1970); rabbit (Henning and Hird, 1972); rodents (Yang et al, 1970); dog, pig and pony (Stevens, 1978; Ralston,1983), and there appears to be a close similarity between the molar ratios of colonic SCFA's amongst the various species. In the ruminant, diet has been shown to influence the molar proportions of SCFA's (Schambye and Philipson, 1949; Philipson, 1952; Opstvedt et al 1967; Judson et al 1968; Duncan et al, 1974; Orskov et al, 1974; Schwarz and Gilchrist, 1975).

The normal proportions of acetic; propionic; butyric acids are usually 70 : 20 : 10. A diet rich in concentrates effects an increase in propionate with a concomitant decrease in acetate, with accompanying changes in the metabolic activitiy of the microbial flora (Judson et al, 1968; Kaufmann, 1976.). Similarly, fasting can reduce the production and concentration of SCFA's in the non-ruminant (Argenzio and Southworth, 1974). In human dietary studies the picture is less clear. Rubenstein et al (1969) demonstrated a fall in the concentration of total faecal SCFA's with an accompanying change in the

molar ratios of acetic : propionic : and butyric acids, when subjects were switched from an ad-lib diet to a carbohydrate rich diet. Cummings et al (1979(b)) found no alteration in faecal SCFA's when their subjects were switched from a low protein to high protein diet, or when a substantial amount (29.8 g/day) of wheat bran was added to the high protein diet. Spiller et al (1980) reported a decrease I.T.T. and an increase in stool weight and SCFA's with the incorporation of cellulose in the diet, whilst pectin decreased stool weight but caused an increase in total faecal SCFA. Subjects on a low residue diet showed a steady decrease in both total and concentration of faecal SCFA's and stool weight.

McLean Ross et al (1983) observed the lack of effect of gum arabic on both total and concentration of faecal SCFA's in men fed gum arabic for 3 weeks. In a subsequent study rats were fed either an oxoid breeders diet (O.B.D.) with/without increasing doses of gum arabic or an elemental diet with/without gum arabic. Faecal SCFA's increased with increasing dosage of gum arabic and O.B.D, with concomitant increases in acetate and decreases in butyrate. Faecal SCFA's (mg/g) were greater on the elemental diet with gum arabic compared to unsupplemented elemental diet and caecal SCFA concentration was higher in the elemental group with gum arabic, when caecal size was accounted for, but otherwise gum arabic resulted in a decrease in caecal SCFA concentration. Dietary influences were also associated with changes in the concentration of faecal SCFA's and the proportions of individual acids Caecal contents of rats given a bran diet showed higher SCFA's than controls and caecal SCFA's, from rats given a meat diet were significantly lower than the controls and were lower than values exhibited by the bran diet (McKay & Eastwood, (1983)). Other dietary influences on faecal SCFA's have been reported in other species: pig (Cranwell, 1968) rodents (McBee, 1970), and dog, pig and pony (Stevens, 1978). All these studies indicate how the effects of dietary fibres cannot be generalised, and that what is fed along with the dietary fibre may be important.

Up until the early 1960's little work had been done to study the SCFA's in man, particularly colonic studies. The relative absorption of SCFA's in man was shown by Dawson et al (1964). The longer the chain length the faster the absorption rate, which probably accounted for the appearance of faecal acetic acid. The faecal SCFA's were shown to contain 10-20 mmol/100 g net weight of which at least 50% was acetic acid. The exact mechanism

of absorption still remains unclear but it is thought to be associated with the presence of sodium and bicarbonate (Cummings, 1981). Difficulties arise in the ability to study the colon and caecum of man in-vivo, and consequently reliance is put on in vitro techniques and include the study of faecal composition, tissue and blood studies, colonic perfusion and dialysis methods all of which are reviewed by Cummings (1981). Recent studies have shown that SCFA's are absorbed in man, although the overall contribution of colonic fermentation to the overall energy balance is unclear (Elsden et al, 1946; Cummings, 1975, McNeil et al 1978; Cummings et al 1979(b);Stevens, 1978; Smith and Bryant, 1979; Bond et al 1980; McNeil, 1984). Such reports dispel the considered opinion of many years standing that SCFA's are poorly absorbed if at all (Williams and Olmsted 1936(b). Rates of absorption from the human rectum using the rectal infusion technique of Edmonds (1971) (7.7 - 8.9 μmol/cm^2/hr) were found to be similar to those obtained from animal studies: 8.6 μmol/cm^2/hr for equine large intestine (Argenzio et al, 1974; McNeil et al, 1978). Therefore if SCFA's are absorbed then their role in faecal bulking is unlikely (Williams and Olmsted, 1936(b). In view of their ready absorption, high concentration in the stool could be a result of entrapment within the solid material in the colon and their inability to diffuse readily into the mucosa, while being continually produced by colonic bacteria (McNeil, et al 1978).

In rats the contribution of caecal SCFA's to the energy metabolism as shown by the recovery of labelled CO_2 in air expired following the introduction of radioactive SCFA into the caecum, is 4.7% of the energy requirement (Yang et al, 1970). In man the fermentation of 50-60 g of carbohydrate can yield 500-600 mmols of SCFA's - a total energy value of 600 - 750 kJ. This represents, approximately, 75% of the original energy content of the carbohydrate: 25% is utilised by the microflora and contributes 6-9% of the energy requirements (Rubenstein, et al 1969; McNeil, 1984). In the Western world up to 10% of daily energy needs may therefore be met by large intestinal function, being increased in the Third World, due to higher intakes of fibre and consequently greater metabolism of carbohydrate, and in the presence of malabsorption (McNeil, 1984.) In contrast ruminants derive 70% - 80% of their energy requirements from the metabolism of SCFA's. (Annison and Armstrong, 1970).

In summary, SCFA's are major end products of colonic carbohydrate

fermentation. As such they can contribute at least 10% of the energy requirements of man through absorption and metabolism of the mucosal and epithelial surfaces.

THE NEED FOR ANIMAL STUDIES

Dietary fibre is not a single entity but a heterogeneous material encompassing many forms of polysaccharides. As such these all have differing influences on gastrointestinal function, which will be subject to change depending on the colonic environment, the presence of other substances and the ensuing interactions. The effects of dietary fibre on the colon can be monitored by breath H_2,CH_4,SCFA's, bile acids, stool weight and bacterial mass. Different fibres will have different modes of action depending upon source i.e. cereal, fruit or vegetable and its treatment (Eastwood, 1975; McLean Baird et al, 1978; Cummings et al, 1978; Stephen and Cummings, 1980(a); Eastwood et al, 1983; Cummings, 1984).Chemical techniques have been developed to study the four major classes of fibre in foods (Van Soest, 1963(a) and (b); Southgate, 1969), but they are of limited value because of the heterogeneity of fibre and its alteration within the G I tract. Feeding trials in humans are time consuming, demanding on laboratory personnel and the subjects carry a risk of uncertainty (Eastwood et al, 1973; Findlay et al, 1974; Eastwood et al, 1983) and if a sufficient time period is not allowed between assays then there is a risk of carry over effects (Eastwood et al, 1973).

If the reliance upon human studies is to be reduced, another means of assessing the potential of dietary fibre sources, in conjunction with chemical analysis, is required. Animal experiments may be regarded as attempts to elucidate the human intestinal environment. These too have their problems: suitability of the animal; the influence of environment, eating of bedding , and lifestyles eg: the exercise, the inherent differences of gastrointestinal structure: the stomach of the rat is incompletely divided into two compartments, the epithelial linings of which maybe colonized by bacteria and or yeasts (Savage, 1977). Substantial research has been carried out to study the effect of dietary components on gastrointestinal growth, function and metabolism in the rat. The rat is a useful animal to study. It is easy to maintain and large numbers can be used without too much expense. Weanling rats, mature rats, male and female rats have all been studied. (Jelinek et al, 1952; Mitchell and Bert, 1954; Bright-Gaertener and Carroll, 1967; Christensen et al 1967; Dowling et al, 1967; Mason and Palmer, 1973; Leegwater et al, 1974; Moinuddin and Lee, 1959; Philbrick and Hill, 1974).

Certain food starches such as raw potato starch have been shown to be poorly utilised by the rat, causing distension of the caecum and intestines. (Jelinek et al, 1952). In a similar study Leegwater et al (1974) using mature rats, found raw potato starch to increase caecal size, with a return to normal size 4 weeks after the animals had reverted to a control diet. Moinuddin and Lee (1959) further investigated the effect of raw potato starch, cornstarch, lactose, agar and cellulose on physical parameters including caecal weight and noted similar results to those of Leegwater and Jelinek. i.e. undigestible residues cause caecal distension and increased caecal weight. Jacobs and White (1983), demonstrated that the chronic feeding of a 20% wheat bran supplement to rats led to an increase in large intestinal, but not proximal, jejunal or mucosal cell growth. Other studies have indicated that rats respond to bulky diets by eating a greater volume. (Dowling et al, 1967; Moinuddin and Lee, 1959). Attempts have even been made to reproduce the symptoms of malnutrition in man in the rat, and to determine the cause of the condition (Philbrick and Hill, 1974).

Caecal enlargement has been studied by Walker (1978) to ascertain whether it was an abnormal or an adaptive response. He determined that 3 weeks was long enough to cause caecal distension and that it was only justified to attach toxicological significance to this phenomena, when gross effects were accompanied by secondary nutritional and toxicological changes.

The products of caecal metabolism have been investigated in the rat, (Remesy and Demigne, 1976; Gordon et al, 1979; Nyman and Asp 1982; Gyory and Chang, 1983; McKay and Eastwood, 1983; Mallett et al, 1983(b); McLean Ross et al, 1984; Mallett et al, 1984(b)) as have H_2 and CH_4 (Rodkey, 1972; Bond and Levitt, 1976; McKay, 1981; McKay and Eastwood, 1983).

SUMMARY

This introduction has presented some of the literature concerned with dietary fibre, definitions, chemical and physical properties, methods of analysis, and physiology (Cummings 1973, Eastwood, 1975; Cummings, 1978; Trowell, 1978; Eastwood et al, 1980; Jenkins, 1980; Royal College of Physicians 1980; Smith and Eastwood, 1980; Story, 1980; Tasman-Jones, 1980; Cummings, 1981; Cummings 1984). Because of the slowness of human trials, and the inherent problems associated with the use of humans, there is a need for an animal model, (McLean Ross, 1983, and 1984) which also describes a suitable

time period. The rat is the oft used animal for dietary studies, but such trials have been of varied durations from 9 days (Schneeman and Gallaher, 1980; Ullrich et al, 1981); to 9 and 12 months (McLean Baird et al, 1977; Tarpilla et al, 1978). A few workers have considered the "short" and "long" term effects of time. Ullrich et al (1981) concluded that 4 days was adequate to cause changes in bile acids. Few have discussed their results with reference to time, or considered the changes that may occur as a result of time. Elsenhans et al (1981), did so in their studies of the long term feeding of carbohydrate gelling agents on the anatomy of the rat G I tract. Subsequently a period of 52 days was chosen as optimal. McLean Ross et al (1984) monitored the excretion of H_2 and CH_4 of rats over 14 and 28 days fed either a low or high fibre diet.

The study contained within this thesis describes the development of an animal model, with the rat as the chosen animal.

In the development of this model the influence of

1) three different, low fibre diets, on chosen indices of metabolism has been examined

2) two fibre supplements, one a relatively non-fermented fibre (Wheat bran) one a fermented fibre (gum arabic), upon chosen indices of metabolism has been studied

3) any interaction that may occur between the basal diet and the supplement being fed along with it i.e. the two are not independent of each other

4) the time of feeding has been examined.

It is hoped that this model will be used routinely for the evaluation of novel dietary fibres and the prediction of these effects on the colon and its function. This may eventually be of some clinical use in the assessment of nutritionally related disorders of the G I tract.

CHAPTER 2

MATERIALS, METHODS, STATISTICAL ANALYSIS AND DATA PRESENTATION

AIM

The aim of this thesis has been to develop an animal model which could be of relevance to human nutrition for the evaluation of dietary fibres, and their action upon stool bulking and caecal metabolism. This model may also lead to a better understanding of the mechanisms of the action of dietary fibre. At present there is no simple method of predicting, quantitatively, the effect of a dietary fibre upon stool weight and other physiological measurements. The present methods rely upon human volunteers to supplement their diet with a selected fibre source. This can be difficult, expensive and complicated by limited willingness to be subjected to dietary control and repeated faecal collections. There are prolonged effects of each experiment which results in subjects being suitable for only 2-3 experiments each year (Eastwood et al 1973).

Dietary fibre can be measured chemically (e.g. cellulose, hemicellulose, pectin, lignins) or physically (W.H.C.) but each has its limitations with regard to the prediction of stool weight. Similarly it is not known how good these indicators predict the effect of human caecal metabolism. It is hoped that, from this work, one or two indicators of fibre metabolism will be identified thus reducing time consuming current routine analysis and human trials. This would give a quick and accurate way of assessing the ability of new dietary fibre sources.

In developing this animal model three low fibre diets (plant origin, animal origin and Flexical) and two fibre supplements, (one a complex readily fermentable polysaccharide , gum arabic, the other a less readily fermented polysaccharide, Canadian Red Spring Wheat Bran), were chosen. These were fed to rats for 4-, 8-and 12 weeks periods, to study the effect of diet upon caecal metabolism and stool bulking. The basal diets were fed unsupplemented and supplemented to evaluate the effect, if any, that cojeners may have on the efficacy and physiological actions of each of the fibre supplements. The effect of the duration of feeding was considered, and the aforementioned time periods were chosen. This would result in a well controlled, well defined animal model system.

The parameters chosen as indicators of fibre fermentation and the physiological

consequences were:

(i) live-weight
(ii) liver wet weight
(iii) dry stool weight
(iv) wet caecal sac wet (voided of contents)
(v) dry caecal content weight (excluding the caecum sac)
(vi) 2-6 Diaminopimelic acid
(vii) hydrogen and methane
(viii)short chain fatty acids
(ix) bile acids

Dry stool weight is the most used indicator of dietary metabolism in the human. The caecum and its contents were studied because of its role as a fermenter, and its capacity for a large, metabolising bacterial mass. These have all been well documented in human and animal dietary studies.

MATERIALS

a) Animals

The experimental animal used was the adult male rat, 10-12 weeks of age of the Albino Wistar Strain. Whilst an animal model is desirable, the choice of animal is difficult. The rat is an omnivore, and this seemed a suitable and practical choice. Forty-five rats were allocated to each of the three dietary trials. In addition, concomitant with each of the three dietary trials, 10 rats were fed a small animal diet (S.A.D.) used routinely in the animal unit at the time. The animals that remained on the S.A.D., acted as an index of the inherent variability of the different measurements. These results are presented in Chapter 7. All rats were caged in solid bottomed cages in eleven groups of five with white wood shavings for bedding. They were maintained within the facilities of the animal unit of the Western General Hospital, Edinburgh. These animals were kept on a normal 12 hour light/dark cycle with constant recirculation of air. Room temperature was kept between 21-21.5°C. Animals were identified by indelible ink tail numbering.

b) The diets and supplements

(i) The basal low-fibre diet

The three low fibre diets chosen were:

Plant origin (0.8% fibre): - the fat and protein were of plant origin (Special

Diets Services Ltd (SDS), Witham, Essex) table 2a.

Animal origin (0.6% fibre): the fat and protein were of animal origin (SDS, Witham, Essex) Table 2 a (The methodsof fibre determination (ADFandNDF) used for both these diets is given in appendix 1a).

Flexical : a low residue, nutritionally complete diet (Mead-Johnson Nutritionals, Langley, Slough) table 2b. This is an elemental diet with amino acids and simple sugars requiring no digestion.

Table 2c gives the calculated crude analysis of the supplemented diets.

(ii) The supplements

Gum arabic: this was of confectionary grade (Rowntree McIntosh Ltd, York) supplied by Dr. D.M.W. Anderson, Department of Chemistry, University of Edinburgh.

Gum arabic is generally regarded as the dried gummy exudate from trees of the genus Acacia leguminosae which contains at least 900 species, and has a long history of use as an emulsifier and stabiliser at concentrations of upto 2% in commercial food products and pharmaceuticals, and at much higher levels as a confectionary ingredient, (Anderson et al 1982). It is a complex acidic heteropolysaccharide of very high molecular weight (circa 0.5-1.4 x 10^6), composed of a highly branched array of galactose, arabinose, rhamnose and glucuronic acids, is highly water soluble (Anderson et al 1966), and has been assessed toxicologically as a safe foodstuffs additive (Joint FAO/WHO Expert Committee on Food Additives 1982; Street and Anderson 1983). The gum arabic used in these studies was a sub-sample of the test article used for previous metabolic and toxicological studies within the Department of Chemistry, University of Edinburgh, Kings Buildings, and the Wolfson Gastrointestinal laboratory, Western General Hospital, Edinburgh. (Anderson et al 1981; Anderson et al, 1982; Anderson et al, 1983; McLean-Ross et al, 1982; McLean-Ross et al, 1983 McLean-Ross et al, 1984; Street and Anderson 1983). Table 2d gives the analytical data for the gum arabic specimen used in these studies. A comparison of the analytical parameters associated with the gum arabic used and that of authenticated specimens of Acacia senegal is given by Anderson et al (1983) in their paper entitled. "The chemical characterization of the test article used in toxicological studies of gum arabic (Acacia senegal (L.) WILLD)."

Canadian Red Spring Wheat Bran (coarse): this was supplied by Chancelot Mills, Edinburgh. Protein was estimated as nitrogen (Kjeldahl; N x 6.25) and starch colorimetrically as glucose after amylase (EC 3.2.1.1 and 3.2.1.2)

TABLE 2a The composition of the plant origin and animal origin diets

Plant Origin		Animal Origin	
Soya Concentrate	20%	Meat + Bone Meal	28%
Soya Oil	3%		
Cornflour	72%	Cornflour	67%
Supplement	5%	Supplement	5%
Calculated Analysis			
Oil	3.1%		2.5%
Protein	13.0%		12.9%
Fibre	0.8%		0.6%
Vitamin + Mineral Premix	- contributes the following/kg of diet		
Vitamin A	5000 iu		5000 iu
D_2	1000 iu		1000 iu
E	50 mg		50 mg
B_1	5 mg		5 mg
	5 mg		5 mg
B_6	5 mg		5 mg
	5 µg		5 µg
Nicotinic Acid	10 mg		10 mg
Pathothenic Acid	10 mg		10 mg
FolicAcid	0.5 mg		0.5 mg
Biotin	20 µg		20 µg
Choline Chloride	200 mg		200 mg
Vitamin K	10 mg		10 mg
Lysine	1000 mg		-
Methionine	1000 mg		-
Iron	50 mg		50 mg
Cobalt	0.5 mg		.5 mg
Manganese	25 mg		25 mg
Copper	5 mg		5 mg
Zinc	10 mg		10 mg
Iodine	0.5 mg		0.5 mg

TABLE 2a continued

	Plant Origin	Animal Origin
Magnesium	100 mg	1000 mg
Sodium Chloride	5000 mg	2500 mg
Calcium	6000 mg	-
Phosphorus	4000 mg	2000 mg
Potassium	-	

Energy values

Gross energy	15.4 MJ/kg	15.5 MJ/kg
Digestible energy	13.9 MJ/kg	14.0 MJ/kg
Metabolisable energy	12.5 MJ/kg	12.6 MJ/kg

Information supplied by S.D.S.
Witham, Essex

TABLE 2b The composition of Flexical, a complete elemental diet

PRODUCT CONSTITUENT VALUES (average)		
FLEXICAL	**PER 100 g POWDER**	
Energy	441 kcal	1850 kJ
Protein	9.9 g	
Fat		
Carbohydrate		
Corn Syrup Solids	61.7 g	
Modified Tapioca Starch	5.2 g	
Citric acid	0.4 g	
Total	67.3 g	
Mineral Salts		
Calcium	264 mg	6.61 mmol
Chloride	441 mg	12.4 mmol
Magnesium	88.1 mg	3.67 mmol
Phosphorus	220.0 mg	7.11 mmol
Potassium	557.0 mg	14.1 mmol
Sodium	154.0 mg	6.7 mmol
Trace Elements		
Copper	0.441 mg	6.91 µmol
Iodine	33.0 µg	0.26 µmol
Iron	3.96 mg	70.8 µmol
Manganese	1.1 mg	20.0 µmol
Zinc	4.41 mg	67.4 µmol
Vitamins		
A Retinol	330 µg	
B_1 Thiamine	0.837 mg	
B_2 Riboflavine	0.946 mg	
Pantothenic Acid	5.81 mg	
B_6 Pyridoxine	1.10 mg	
B_{12} Cyanocobalamin	3.3 µg	
C Ascorbic Acid	66.1 mg	
D Cholecalciferol	2.2 µg	
E d- α - Tocopherol	7.24 mg	
K Phytomenadione	55.1 µg	
Biotin	66.1 µg	
Folic Acid	88.1 µg	
Nicotinic Acid	11.0 µg	
Choline	110 mg	
Amino acid content		
Isoleucine	0.62 g	
Leucine	1.06 g	
Lysine	0.89 g	
Methionine	0.47 g	
Phenylalanine	0.50 g	
Threonine	0.49 g	
Tryptophan	0.16 g	
Valine	0.78 g	
Arginine	0.42 g	
Histidine	0.31 g	

TABLE 2b continued

	PER 100 g POWDER
Alanine	0.38 g
Aspartic acid	0.83 g
Cystine	0.04 g
Glutamic acid	2.42 g
Glycine	0.25 g
Proline	1.13 g
Serine	0.65 g
Tyrosine	0.26 g

Energy	% of total energy supply
Protein	9.0
Fat	30.0
Carbohydrate	61.0

100 g Flexical powder supplies approximately 1785kJ (425kcal).

Information supplied by Mead Johnson, Slough.

TABLE 2c The calculated crude analysis of the supplemented diets (g/100 g).

	Oil	Protein	Fibre
Plant origin + bran	3.2	13.2	1.92
Plant origin + gum arabic	2.8	11.7	0.7
Animal origin + bran	2.65	13.1	1.74
Animal origin + gum arabic	2.25	11.6	0.54
Flexical + bran	13.9	10.4	1.2
Flexical + gum arabic	13.5	8.9	0

Information supplied by M.J. Rickett, S.D.S., Witham, Essex (pers. comm.).

TABLE 2d Analytical data for gum arabic (Anderson et al, 1983).

Total ash, 550°C, %[a]	3.0
Protein (NX6.25), %[a]	1.9
Methoxyl, %[b]	0.26
Sp. rotation, $[x]_D$, degrees[b]	-30
Molec. weight (Mw)x10^5[a]	5.8
Uronic anhydride, %[b, c]	17.
Sugar composition after hydrolysis %	
4-0-methylglucuronic acid[d]	1.5
Glucuronic acid	15.5
Galactose	45
Arabinose	24
Rhamnose	14

a = corrected for moisture content

b = corrected for moisture and protein content

c = if all acidity arises from uronic acids

d = if all methoxyl groups located in this acid

treatment. Particle size distribution of the preparations was investigated by passage through sieves and the residue held by each sieving weighed. Table 2e gives the chemical composition of the bran. The coarse bran used in these studies was from the same batch used in a study within the Wolfson Gastrointestinal laboratories, Western General Hospital, Edinburgh to measure the W.H.C. of bran and its relationship to stool bulking ability (Eastwood et al, 1983).

These two supplements were chosen because of their different fermentabilities. They have also been studied in man (Robertson and Eastwood 1981 (a), (b); Eastwood et al, 1983; McLean-Ross et al 1982; McLean-Ross et al, 1983). Gum arabic and coarse bran were incorporated into the basal diets, upto a concentration of 10% of the dry weight of any one of the three basal low fibre diets.

Due to the very fine powdery texture of the plant-origin and animal-origin diets, a known volume of water was added to each weighed diet to form a stiff paste that was acceptable by the rats. The elemental diet had to be mixed with a known volume of water and a known quantity of gelatine (Anal R). Without the latter, the resultant paste was too sticky. Table 2f shows the exact preparation of diets. Each diet was mixed thoroughly. The elemental diets were mixed, inverted at intervals to discourage separation, and allowed to set. These latter diets were prepared by first allowing the gelatine to dissolve in the warm water and then adding to the dry mix. Each diet when made up was enough to feed 5 rats per day. All diets could be made up one week in advance if kept in a fridge. The food was presented to each cage of five rats in a stainless steel bowl in the corner of each cage. Rats had ad-lib access to water through a feeder. Although water consumption was not measured, there was no apparent change in daily intake on any of the diets.

All rats were weighed once each week. Food residues were recorded daily as an estimate of food intake. Accurate estimates were hindered by the occurrence of scattering.

TABLE 2e The chemical composition of Canadian Red Spring Wheat Bran (coarse) (Eastwood et al, 1983).

	g/kg
Starch	175
pectin	4.4
hemicellulose	228
cellulose	97
lignin	4.7
Protein	148

Particle size

	% passing through sieve						
Sieving tests (nm)	....	0.25	0.40	0.63	0.74	1.50	2.8	3.55+
Bran	-	-	17	31	46	4.1	-	

TABLE 2f Diet preparation for the dietary trials

	PLANT ORIGIN			ANIMAL ORIGIN			ELEMENTAL		
	O	+G	+B	O	+G	+B	O	+G	+B
DIET (g)	150	135	135	150	135	135	150	134	133.8
GUM (g)	O	15	O	O	15	O	O	17	O
BRAN (g)	O	O	15	O	O	15	O	O	15 g
WATER (g)	130	100	125	90	70	100	240	250	240
GELATINE (g)	0	0	0	0	0	0	12	17	12

Where O = the unsupplemented basal diet

+G = basal diet + 10% gum arabic

+B = basal diet + 10% coarse bran

c) Feeding plan and the duration of feeding

Figure 2a illustrates diagramatically the feeding plan adopted for each trial, using the plant origin diet as an example. The 45 rats to be fed were split into 3 groups of 15 and given either unsupplemented plant origin diet (PF), plant origin + 10% gum arabic (PG) or plant origin + 10% coarse bran (PB). The total feeding duration for each diet group (PF, PG and PB) was 12 weeks. This allowed killings at 4-, 8-and 12 weeks within each dietary group. A staggered time start was used to distribute the workload, in order that 15 rats were not being killed at one time. In effect group PG were started one week after group PF and group PB were started 2 weeks after group PG.

This exact format was used for the animal origin-(AF, AG and AB) and elemental trials (EF, EG and EB). The three dietary trials were run consecutively.

Rats were killed by diethyl-anasthaesia. Killings took place the same time each week.

Of the 165 rats only one had to be killed prematurely. This was a rat from the PF regime whose overlong teeth had caused a severe gum infection. As a consequence all rats' teeth were subsequently checked for length every two weeks and clipped where necessary. Tooth length was a function of the mash diet as was proved by a repeat of the plant origin trial at Inveresk Research Institute, where the same diet was fed in pelleted form. Another rat, from the diet PB, was found, at post mortem, to have hydronephrosis with numerous ureteric and bladder stones (Appendix 1b).

No such problems occurred with the animal origin diets. The only problem was that the rats did not like this diet which was reflected in liveweight. Further details are given in chapter 4. Flavouring the diet was ineffective.

No problems were incurred with the elemental diet.

METHODS

a) Stool collection

Prior to killing each rat was placed in individual broad spaced gridded cages, which prevented copraphagy, to facilitate three-day collections. At the end

FIGURE 2a The feeding plan used in all three dietary trials using the plant origin dietary trial as an example

WEEKS

	0	4	8	12
DIET	S.A.D.	PF	PF	PF
n=	5	5	5	5
	DIET	PG	PG	PG
	n=	5	5	5
	DIET	PB	PB	PB
	n=	5	5	5
				S.A.D.
			n=	5

S.A.D. = Small animal diet

PF = Plant origin diet

PG = PF + 10% gum arabic

PB = PF + 10% Coarse bran

of the three days the total wet stool weight was recorded prior to the addition of a known volume of water to make a slurry. The pH was then checked (pH8), the stool sample was frozen and freeze-dried to determine the dry weight of stools. Dry stools were then ground to a fine particle size.

Stools dried in an unpredictable manner overnight and dry weight was considered a better mode of comparison. Consequently, wet weights have been omitted.

b) The liver, caecal sac and caecal contents

At killing, livers were removed, washed in 0.9% w/v NaCl blotted dry, weighed and frozen for future sterol analysis. Entire caecums were removed and opened by cutting along the lesser curvature, pinning back the walls of the caecum and scraping the contents into a pre-weighed tube. The empty caecum sac was then washed with 0.9% NaCl blotted dry and put in another pre-weighed tube, weight recorded before freezing. Caecal contents were weighed, pH checked (pH8), freeze dried and re-weighed to give dry weight.

c) Hydrogen and methane

(i) The sampling technique

The method used in all of these studies was a modified method of Gumbann and Williams,(1971) and used in previous trials in this laboratory (McKay, PhD Thesis 1981; McKay and Eastwood, 1983; McLean-Ross et al, 1984).

Production of hydrogen (H_2) and methane (CH_4) was estimated from the change in gas composition of a sealed perspex box (30 x 20 x 20 cm) capacity 12 litres, containing a single animal (figure 2b) for 15 minutes after which time a 50 ml sample of gas was withdrawn, the animal removed and the box cleaned.

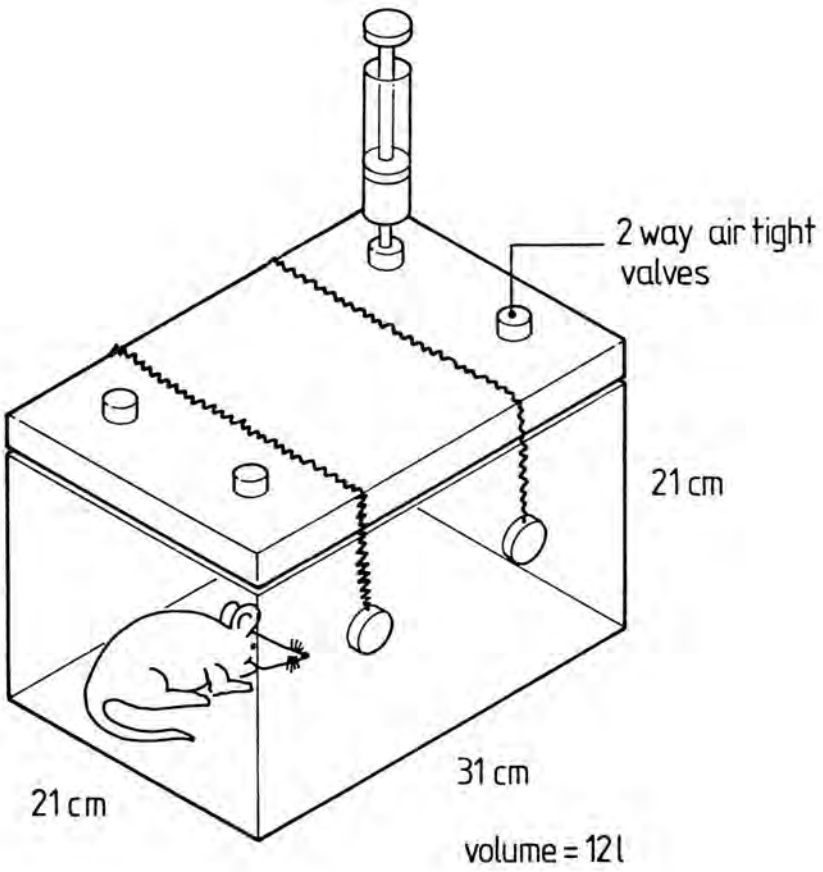
All joints of the chamber were recessed and airtight. In the lid of the box were 4 airtight holes, one of which was plugged with a rubber bung; the other three were fitted with two-way valves. A rubber seal smeared with grease around the lid ensured a tight fit. There were also two handles with springs to keep the lid firmly down. Excretion rate was calculated from the concentration of methane in the chamber. (1200 mls) minus the volume of the rat (density assumed to be 1.00.) –

$$\text{mls/hr/kg} = \frac{\text{ppm} \times 4 \times 1000}{1000\,000 \times \text{rat Weight (g)}}$$

The chamber was tested for airtightness and leaks by

(i) filling with water and leaving overnight

FIGURE 2b The diagramatic representation of the gas sampling technique used for the collection of H_2 and CH_4.



(ii) flushing with 20 ppm standard gas mixture of hydrogen/methane for 30 minutes. The box was then sealed and filled with 20 ppm, through a two-way valve for 30 minutes. Samples of gas were then taken immediately and thereafter at 15 minutes, 30 minutes, 60 minutes and 17 hours (overnight). There was no substantial loss, table 2 g. Standard curves were made on each day of analysis The relationship between different concentrations of standard gases and the deflection on the recorder (mv) was linear. The coefficient of variation for the repeat analysis of a standard 20 ppm sample (n = 77) was 11% for hydrogen and 14% for methane. All gas samples were analysed the same day as sampling.

(ii) The analysis of H_2 and CH_4

Gas analysis was made by gas-solid-chromatographic technique using the method of Tadesse et al (1979.) The instrument used was a gas-solid chromatograph (Pye Unicam, Series 140) with a Katherometer detector and a lmv pen recorder for display purposes. Chromatographic working conditions are given in Table 2h. Sample was injected by means of a sampling loop, after passage through a tube containing soda lime and silica gel (separated by glass wool) in series to remove CO_2 and H_2O vapour respectively.

d) Analytical Techniques

Aliquots of faecal material and caecal material were used for the estimation of 2-6 Diaminopimelic acid (DAPA) based on the method of Czerkawski (1974,) and short chain fatty acids (SCFA's) based on the method of Spiller et al (1980). A pooled sample of each group of faecal material and caecal material was used for bile acid analysis using the method of Brydon et al (1979).

(i) 2-6 Diaminopimelic acid

Diaminopimelic acid was determined as an indication of the change in bacterial mass as influenced by diet. 2-6 Diaminopimelic acid can be found in nearly all bacterial cell walls, excepting Streptomyces spp and Actinomyces spp, and blue green algae, but not in fungi, yeasts plant viruses or protozoa, (Work and Dewey, 1953). The presence of DAPA in the bacterial cell wall can be used as a marker of bacterial mass. DAPA presence varies from species to species and can be missing altogether being mutually exclusive with lysine (Czerkawski, 1974). The analysis of DAPA comprises the hydrolysis of the sample, its purification on charcoal columns, separation of DAPA from proline

TABLE 2g The air tightness of the perspex box used in the collection of H_2 and CH_4 from the rats

(i) 100 ppm Standard Gas

	H_2 (ppm)	CH_4 (ppm)
0 mins	42	45
15 mins	42	45
30 mins	42	4
60 mins	40	4
17 hours	40	4

(ii) 20 ppm Standard Gas

	H_2 (ppm)	CH_4 (ppm)
O mins	6.5	6
15 mins	6.5	6
30 mins	6.5	6
45 mins	6.5	6
60 mins	6.5	6
17 hours	6	6

TABLE 2h Chromatographic working conditions

	KATHAROMETER
Column type	Glass
size	2.6 mm (4 mm I.D.)
Mesh type	M.S. 5A
size	60/85
Column temp	50°
Detector temp	100°
Current	142 mA
Carrier gas	Oxygen free Nitrogen
Flow rate	60 ml/min
Sampling type	loop 10 ml
temp	Room temp
Attenuation	2 (x 500)
Display	Pen recorder
Chart speed	5 mm/min

on columns of an anionic resin and the determination of DAPA with acid ninhydrin (Czerkawski, 1974).

The columns: A charcoal/celite mix of 0.4 g acid washed charcoal [(Norit GSX) low in chloride, BDH] and 0.6 g celite (Chromosorb W-AW 100/120 mesh, C.S) were mixed together with a little water to make a slurry. This was then transferred to column A (figure 2c) the tip of which had been plugged with glass wool and a little celite. Pressure was added quickly to prevent separation of the mix. Finally another small layer of celite topped the celite/charcoal mix. This column was then washed with 3 mls of 4N NaCl followed by 2 x 5 ml 20% v/v ethanol and water and finally 5 mls 20% v/v ethanol.

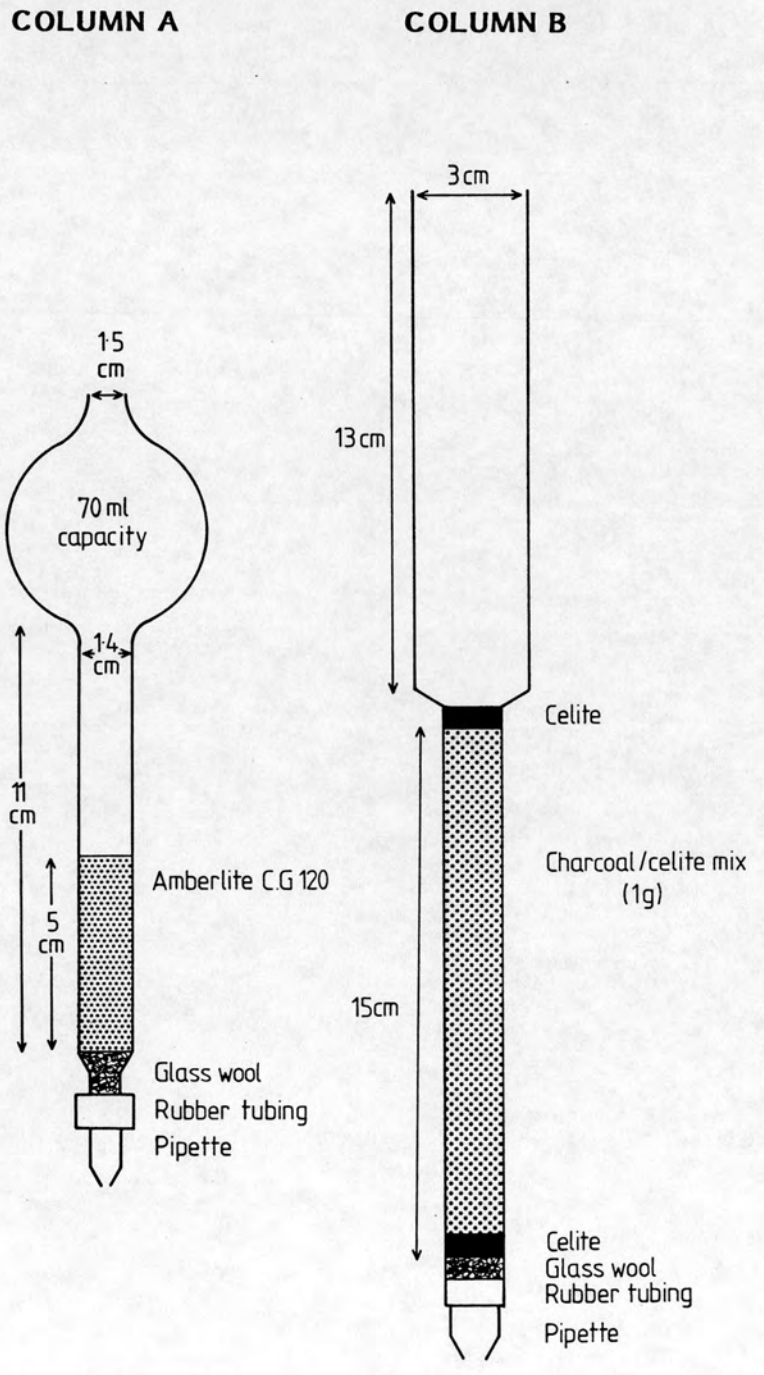
Amberlite (G120 (Na) (type II 200 mesh BDH) was suspended on 0.2 N HCl several times decanting off the fine supernatant, and repeated using O.2 N NaOH, in which the amberlite could be stored. This suspension was then poured into column B (figure 2c), the tip having been plugged with a little glass wool, to a final height of 5 cm. The excess NaOH was allowed to drain off. This column was then eluted with 60 mls pH2 buffer until pH reached

The amberlite columns could be regenerated by eluting with 60 mls of O.2 N NaOH and then 60 mls pH2 buffer. Buffers used are detailed in Appendix 1c.

The hydrolsate: A 150-200 mg sample of freeze dried sample was hydrolysed in 6 mls 6N HCl overnight (at least 16 hours) at 105°C in Quickfit tubes (MF 24/1/5), with stoppers taped with autoclave tape to prevent leakage. The hydrolysate was then transferred onto the prepared charcoal/celite column, using 2-3 mls H_2O to wash out the tube, and allowed to run on collecting elution in a round bottom flask (R.B.F.). Elution proceeded with 10 mls 20% v/v ethanol/H_2O followed by 5 mls H_2O. Total eluate collected was then dried down on a rotary evaporator.

Separation of proline and DAPA: The dried sample was redissolved in a little pH2 buffer (Appendix 1c) and then transferred to a prepared Amberlite column. Elution continued with 5 x 50 mls of pH 3.4 citric acid buffer, under slight pressure, to remove proline. This was discarded. This

FIGURE 2c The columns used in the analysis of 2-6 DAPA.



was followed by elution with 50 mls of pH4.2 buffer which was subsequently collected in R.B.F. and taken to dryness on the rotary evaporator.

The Ninhydrin test: the dry samples were redissolved in 5 mls of H_2O. These could be diluted if necessary. To 2 mls of this was added 4 mls of the ninhydrin reagent in Quickfit conical tubes (BC 24/C 14T). A 2 ml blank of water was treated in the same manner, as was a standard of 20µg/ml DAPA in H_2O. A stock solution of the latter keeps well in the fridge. Blanks, standards and samples were then heated for 5 minutes in a boiling water bath (DAPA gives a yellow colour), removed and allowed to cool. Proline could also be estimated using the same method (if the pH3.4 eluate was retained). This gives a pink colour with ninhydrin. Once cooled 250µl out of each tube was read against the blank using a Microelisa auto-reader MR580 (Dynatech) using wavelengths 405 nm and 630 nm instead of reading at 425 nm on the Unicam SP600 series 2 spectrophotometer. Whilst the method of Czerkawski, 1974 suggested a wavelength of 425 nm, for convenience that of 405 nm was used. This was just as suitable a wavelength at which to read absorption, as 425 nm. This was tested by checking wavelength pattern on the Unicam SP800 ultraviolet scanning spectrophotometer (figure 2d). The relationship between increasing concentration of DAPA and increasing absorption was linear (figure 2e).

Quality control: With each run of DAPA analysis 2 faecal (or caecal) control samples were included. Coefficient of variation for repeat analysis of faecal control (n = 36) was 11% and for caecal samples (n = 40) 13%. Concomitant with each analysis, an Amberlite column was prepared to which only 500µl/ml DAPA was added. Recovery was good, averaging 95%. As was expected the DAPA content of the diet was negligible and recovery of added DAPA was good, Table 2j.

(ii) Short chain fatty acids

Short chain fatty acids (SCFA's) were analysed using the method of Spiller et al (1980). Using a SCFA stock solution, which contained 1 g each of acetic-, propionic-, isobutyric-, butyric, isovaleric- and valeric acids per 100 ml H_2O at pH7, an internal stock standard containing 1 g methylvaleric acid per 100 ml H_2O at pH7, a standard curve was constructed. Standard solutions contained from 10-20 µl of stock standard, 50µl of internal standard, 100µl

FIGURE 2d A scanning spectrograph for 2-6 Diaminopimelic acid showing the wavelengths for maximum absorption.

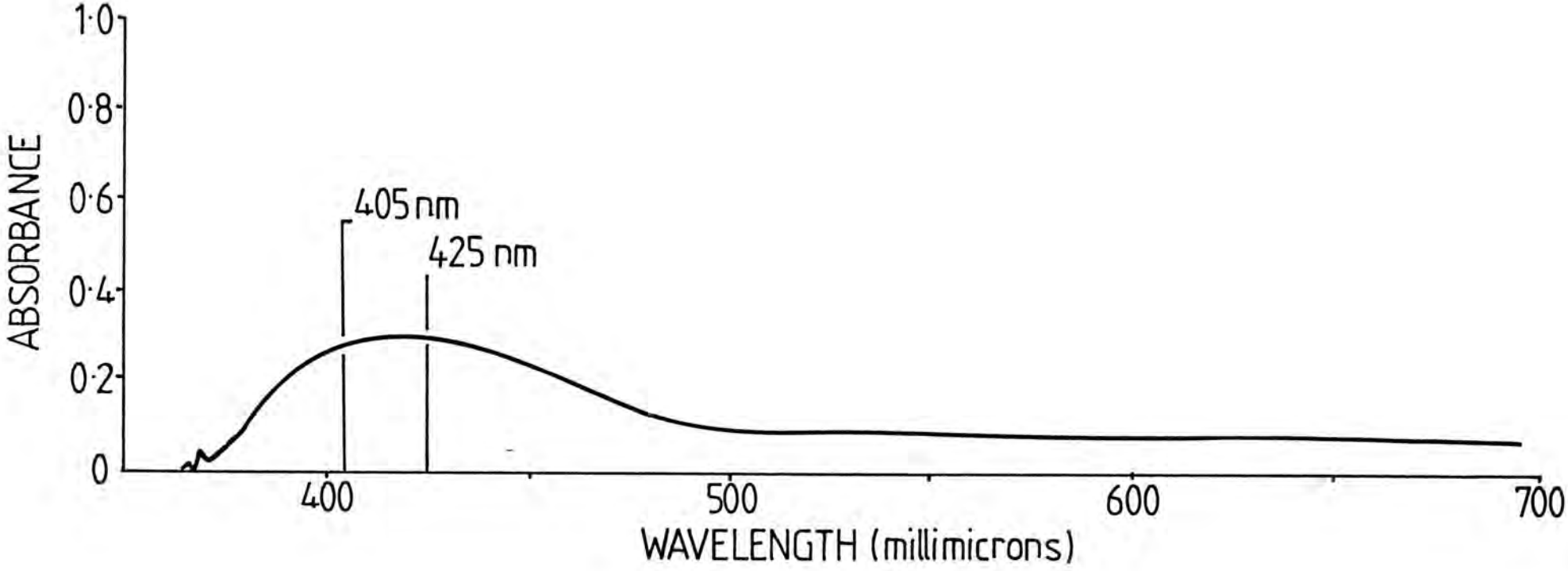


FIGURE 2e The linear relationship between the concentration of 2-6 Diaminopimelic acid and optical density.

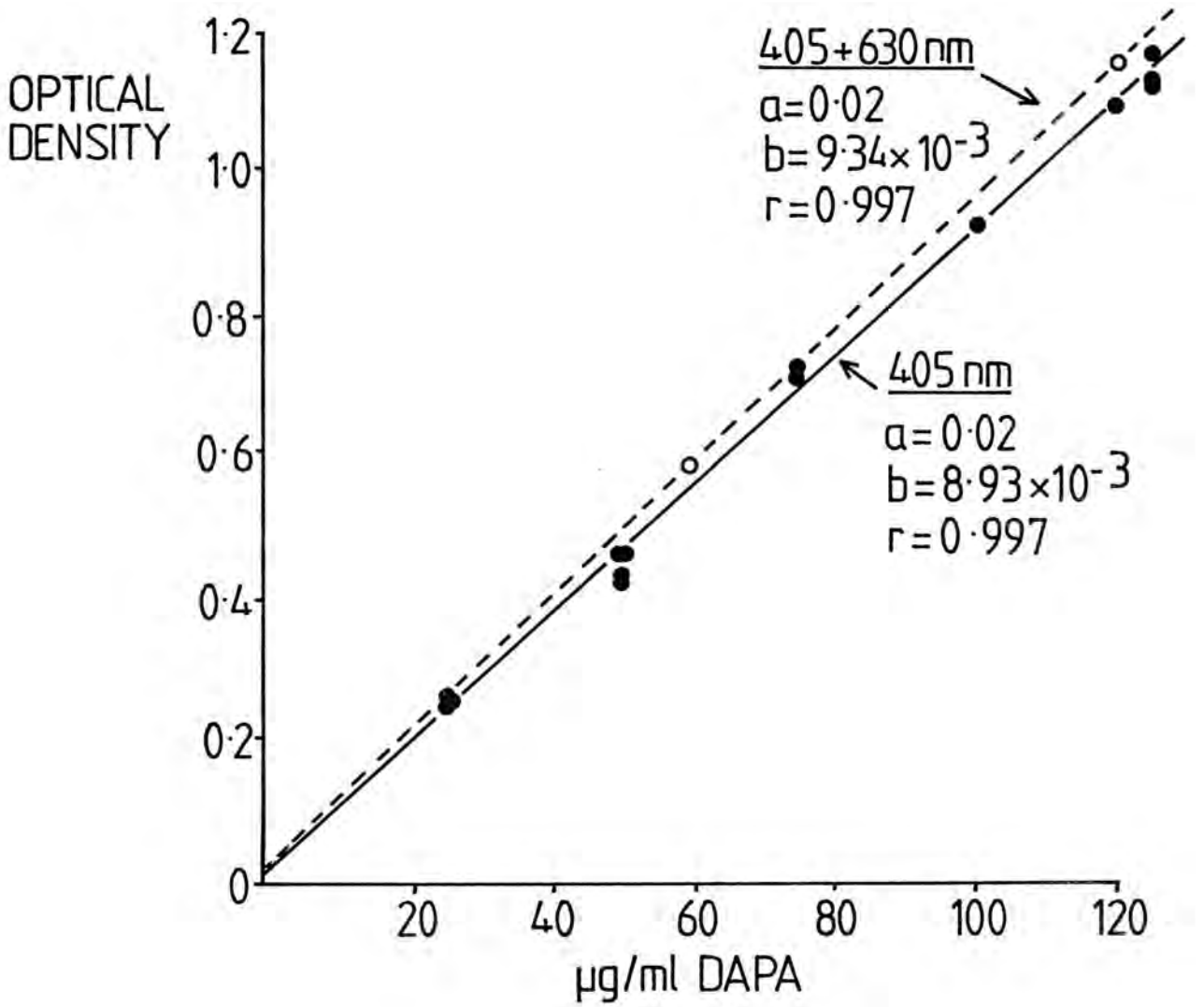


TABLE 2i The DAPA content of the Plant Origin diet, Gum arabic and Bran

SAMPLES*	µg DAPA	µg/g dry Wt
Plant origin (PF)	7.4	37
PF + 500 µg DAPA	497.0	2479
Gum arabic	1.2	6
Gum arabic + 500 µg DAPA	498.0	2484
Bran	8.1	41
Bran + 500 µg DAPA	539.0	2688
500µg DAPA	470.0	94% recovery

* With the exception of DAPA, a dry sample weight of 200 mg (0.2 g) was used for each dietary feedstuff analysed.

of orthosphoric acid and made upto 0.8 mls with water. Approximately 100 mg dried faeces or caeces were used for the test solution along with 0.8 mls H_2O, 50µl internal standard and 100 µl orthophosphoric acid. All solutions were mixed thoroughly, the samples requiring centrifugation at 3000 rpm for 10 mins to separate the ether layers. The solutions were extracted with 3 ml redistilled diethyl ether three times and the extracts combined and mixed. The SCFA's were separated using a flame ionization detector gas chromatograph, temperature programmed (80°C to 150°c at 16°c/minute) with column packing SP 2250. The flow rate of the oxygen free nitrogen carrier gas was 40 ml/min. Calibration curves were constructed for each SCFA by plotting the ratio of SCFA: internal peak heights against SCFA concentration of the standard. Standard solutions were made up and analysed and calibration curves drawn on each day of analysis. Samples were read from these curves.

(iii) Bile acids

Using the method of Brydon et al, (1979) bile acids were measured on pooled aliquots of faecal or caecal material. A 150-200 mg sample of dried material, together with 1 ml internal standard (0.5 mg/ml 23-nordeoxycholic acid) 4 ml glacial acetic acid and 10 ml toluene were mixed and extracted for 1 hour at 120°C stoppered in Quickfit boiling tubes (MF 24/3/8) after which they were taken to dryness. After hydrolysis in 20% w/v KOH in ethanediol at 220°C for 20 mins, and the subsequent addition of 20% w/v NaCl and methanol, neutral steroids were removed by extracting in 10 ml petroleum ether 3 times (Evrard and Janssen, 1968). Bile acids were then extracted with diethyl ether, 3 times, blown to dryness on a sandbath and then methylated using a methanol-dimethoxypropane-conc. HCl mixture (50 : 20 : 1) for 1 hour at room temperature before taking to dryness on a sandbath. It was necessary to further purify the bile acids by thin layer chromatography, having first redissolved them in 0.2 ml acetone. The spotted plates were first put into a tank containing 230 mls benzene and allowed to run. Once completed, the plate was removed and allowed to dry and then placed in a second tank containing 180 mls isooctane, 60 mls isopropanol and 1.5 mls acetic acid. Once examined under ultra violet light, each sample was then scraped into a Quickfit tube BC24/C24R washed in with 10 mls acetone, mixed and allowed to settle. This was then decanted into clean tubes making sure that all sediment was left

behind. To the sediment a further 5 mls of acetone was added, mixed and immediately filtered into the preceeding 10 mls. This was then take to dryness. The methylesters were then acetylated for 2 hours at +6°C on ice in 1 ml of an acetic acid: acetic anhydride: 70% perchloric acid mixture (14 mls : 10 mls : 1 drop). After this time the esters were removed from ice, 10 mls of 20% w/v NaCl was added and extraction proceeded with 5 ml diethyl ether three times. The pooled extracts were washed with water and then taken to dryness.

Separation of methyl ester acetate derivatives was carried out in a Pye Unicam Series 104 dual column chromatograph equipped with hydrogen flame ionization detectors. Five feet, silanized glass columns were packed with 3% SE-30 on 100-200 mesh supelcoport (Supelco, Bellefonte, Pa., USA). Carrier gas was oxygen free nitrogen at a flow rate of 40 mls/minute operating temperature of the column was 270°C.

Concomitant with the test samples two standards were run, containing 1 ml of internal standard, 1 ml of internal mixture (0.5 mg/ml of lithocholic -chenodeoxycholic-deoxycholic-and hyodeoxycholic acid and 1 mg/ml of cholic acid), 3 mls of glacial acetic acid and 10 ml of toluene. Bile acids were calculated by relating the standard ratios of the component acids to the internal standard both in standard solutions and tests. The ratio's within the samples were then related to the ratio's within the standards, converting to 1 mg where necessary.

STATISTICAL ANALYSIS AND THE PRESENTATION OF RESULTS

a(i) Statistical analysis

The statistical methods used were:

- a one-way analysis of variance (ANOVA) with replication (Chapter 7)
- a two factor ANOVA with replication (Chapters 3,4 and 5)
- a three factor ANOVA with replication (Chapter 6)
- analysis of covariance (Chapter 8-discussion)

The first two were calculated using a Casio FX180p calculator.
The three factor ANOVA and the analysis of covariance were calculated by Dr. R. Elton of the Medical Computing and Statistics Department, University

of Edinburgh, using an SPSS package on the I.C.L. computer 2988 at the Edinburgh Regional Computing Centre.

The design of an ANOVA allows the main effects of diet and time to be studied and at the same time the interaction between diet and time (DXT), that is, whether the effect of diet varies across time points. If such an interaction reaches significance (Chapter 3: dry caecal contents), the significance of the main effects (diet and time) become much less meaningful, if they too are significant. An interaction highlights the different trends occurring over time with different diets. That is every diet does not behave in the same manner over a given time course, and this is not reflected in the mean of the diet or time interval.

(ii) Significant differences

Levels of significance chosen were: $p<0.05$, $p<0.01$, $p<0.001$.

If diet, time or the interaction reached significance, as indicated by the F-statistics, a follow up test was then used. The value arrived at with this test, determines between which pairs of means of a group (diet, time or DXT) show a significant difference. The follow up test used was that of least Significant Difference (L.S.D.) given by:

L.S.D. = t x standard error of the difference (S.E.D.)

Where t is a given value for the degrees of freedom associated with the residual mean square (R.M.S.). It is obtained from the table of 't' distribution for a specified level of significance and

where $S.E.D. = \frac{1}{n} + \frac{1}{n}$ (RMS)

or $\frac{2}{n} \times$ RMS

(Clarke 1969; Snedecor and Cochran, 1967).

In this thesis L.S.D. has as its subscript the degrees of freedom associated with the R.M.S., thereby indicative of the 't' value. A worked example is

given in Appendix 1d.

If diet is significant, a comparison between the three diet groups, irrespective of time, is made, to determine which pairs of means significantly differ. The mean for each diet group is calculated by the summation of the 15 values spanning the three time periods.

For example:

the mean for the diet PG = $\frac{PG(4\text{ weeks}) + PG(8\text{ weeks}) + PG(12\text{ weeks})}{n}$

where n = 15

This can then be compared with the means for diets PF and PB arrived at in the same way. If the difference between two means exceeds the value of the L.S.D., then the difference is significant.

Where time is significant, the mean for each time interval is calculated in the same way.

For example:

the mean for 4 weeks = $\frac{(PF)4\text{ weeks} + (PG)4\text{ weeks} + (PB)4\text{ weeks}}{n}$

where n = 15.

Both diet and time can be simultaneously significant: whilst one diet, overall, has a significant influence on a chosen parameter, with time each diet is acting in the same way. That is at any given time point the difference in the response between any two diets will be the same. In effect when the values are plotted the lines will appear to be parallel.

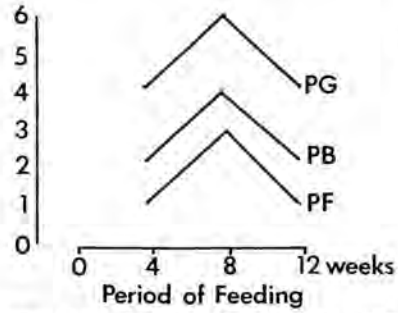


Diagram 2a

Diagram 2a shows that diet PG significantly increases the mean weight (g) of the chosen indicator, but with any diet, the mean weight (g) at 8 weeks exceeds that at either 4 or 12 weeks.

If an interaction and the individual effects of diet and time are all significant, then the significance of the latter two becomes less meaningful, as already

mentioned, and the results should be interpreted with care. An interaction is indicative of the dissimilar trends, over time, with diet.

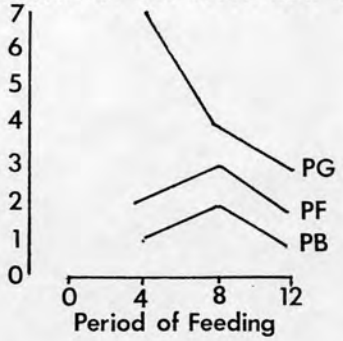


Diagram 2b

Diagram 2b illustrates that with time the mean weight (g) of the chosen indicator decreases with diet PG, being very high at 4 weeks. Diets PB and PF show that weight (g) shows a transient peak at 8 weeks. If the overall means for 4,-8 and 12 weeks were looked at alone then these trends would not be seen as a false impression, that of an overall decrease with time of weight, would be given. That is the means associated with the main effects of diet and time, obscure the magnitude of the changes occurring within each diet group over the prescribed time periods. The main advantage of an ANOVA is that it allows the interaction of diet and time to be assessed.

b(i) Presentation of results

All results have been expressed as mean ± standard error of the mean (S.E.M.). Coefficients of variation are used in Chapter 7.

(ii) Figures

In Chapters 3,4 and 5 all graphs drawn are line graphs, and show the nine mean ± S.E.M.'s. Solid lines have been used to join the plots as an aid for visual interpretation, even though rats within any dietary group were killed every four weeks. In Chapter 6, where a three factor ANOVA has been used, bar graphs have been used to allow easier interpretation of the results. The average means for the main effects of diet and time are quoted in the text and not given as a single value on a figure. They can of course be estimated from the figure. The relevant L.S.D.'s, and level of significance, are given on the figures. All the F-statistics are given in the appendices.

(iii) Tables (Chapters 3,4 and 5)

Where results are tabulated then the table is split into two parts:

a) the main table which lists the nine individual means $\pm$ S.E.M.'s.

b) The table of significant differences which details the significant differences shown to exist between diet, time or the 9 DXT groups, the L.S.D.'s and the level of significance. Any significant difference is denoted by a different superscript.

All F-statistics are given in Appendix 2 .

The results of Chapter 6, with the exception of the three-way-interaction, have been graphed. The results of Chapter 7 have been tabulated.

CHAPTER 3

EXPERIMENT I: TO INVESTIGATE THE EFFECTS OF THE DURATION OF FEEDING A LOW FIBRE DIET OF PLANT ORIGIN WITH AND WITHOUT A FIBRE SUPPLEMENT, UPON THE CAECAL METABOLISM AND STOOL WEIGHT OF THE ALBINO, ADULT MALE, WISTAR RAT

MATERIALS AND METHODS

The experimental design and animal housing conditions, materials (diets and supplements) and methods for this experiment have been described in Chapter 2. All significant changes due to a supplemented diet are expressed with respect to the unsupplemented diet unless otherwise stated. The F-statistics are given in Appendix 2a.

The results showed a normal distribution and are given as mean±S.E.M.

RESULTS

LIVE-WEIGHT

Time alone was significant upon final live-weight ($p<0.05$). Final live-weights after 12 weeks of feeding. (530± 16.1 g) were significantly different from those at 4 weeks (475 ±13.7 g). The live-weight after 8 weeks of feeding was intermediate and not significantly different from either of those two (Table 3a). Initial live-weights are given in Table 3a. When the average live-weight gain is considered, Table 3a, it can be seen that rats fed for 12 weeks gained approximately 50% ($p<0.01$) more weight than rats fed for 4- and 8 weeks, respective live-weight changes being 108± 6.09 g (12 weeks), 65±7.16 g (8 weeks) and 5[illegible]±3.16 g (4 weeks).

LIVER WET WEIGHT

Time alone was significant ($p<0.05$). Table 3b shows that liver wet weight decreased after 8 weeks (15.5± 0.62 g) but increased to near the original 4 week weight at 12 weeks (17.1± 0.60 g). There was no significant difference between 4- and 12 weeks. When expressed as a function of live-weight (g/kg) these significant differences were no longer apparent.

STOOL WEIGHT

Figure 3a shows the dry stool weight of the three diet groups over time. Diet alone was significant ($p<0.01$). Figure 3a shows that the addition of bran (PB) increased stool weight by approximately 65% from 1.20± 0.08 g (unsupplemented diet, PF) to 1.98± 0.08 g/day, and by 40%, with respect to the gum supplemented diet, (PG). There was no significant difference between the PG (1.43± 0.09 g/day) and PF diets. When expressed as a function of live-weight, the PB diet resulted in a significant increase ($p<0.01$) of stool weight, 4.14 ±0.14 g/kg. There was no significant difference between the PF diet (2.30±0.17 g/kg) and the PG diets (2.91±0.18 g/kg).

TABLE 3a The live-weights (g) and live-weight changes (g) of rats fed either a plant origin diet (PF) alone, or supplemented with 10% gum arabic (PG) or 10% wheat bran (PB), for three time periods.
Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	PF	PG	PB	PF	PG	PB	PF	PG	PB
Rat Initial Weight	457 ± 12.4	447 ± 16.1	418 ± 19.4	417 ± 16.9	375 ± 15.1	414 ± 18.1	448 ± 25.5	415 ± 12.0	396 ± 27.6
Rat Final Weight	511 ± 18.1	496 ± 13.5	468 ± 21.4	509 ± 21.5	451 ± 10.1	460 ± 27.2	556 ± 28.1	526 ± 22.2	509 ± 33.9
Live-weight change	54.2 ± 7.38	49.5 ± 6.07	50.0 ± 3.22	83.1 ± 8.79	76.1 ± 4.75	46.2 ± 15.3	108 ± 10.5	111 ± 12.5	113 ± 9.87

Table of significant differences in ascending order from left to right within each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
Final Live-weight	NS	8^a 4^{ab} 12^b	NS
LSD (36)		38.3	
		*	
Live-weight change	NS	4^a 8^a 12^b	
LSD (36)		21.6	NS
		**	

A different superscript denotes a significant difference.
* = $p<0.05$
** = $p<0.01$
NS = not significant.

TABLE 3b The liver wet weight of rats fed a plant origin diet (PF) alone or supplemented with either 10% gum arabic (PG) or wheat bran (PB), for three time periods.
Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	F	G	B	F	G	B	F	G	B
Liver Wet Weight (g)	19.0 ± 0.64	18.1 ± 1.00	15.2 ± 0.66	16.7 ± 1.02	14.4 ± 0.97	15.3 ± 1.15	17.7 ± 1.19	16.8 ± 1.05	16.8 ± 1.04
Liver Wet Weight (g/kg)	37.2 ± 0.94	36.5 ± 1.51	33.9 ± 0.63	32.7 ± 1.02	33.1 ± 1.32	33.2 ± 0.64	31.9 ± 0.46	31.9 ± 1.02	33.0 ± 0.59

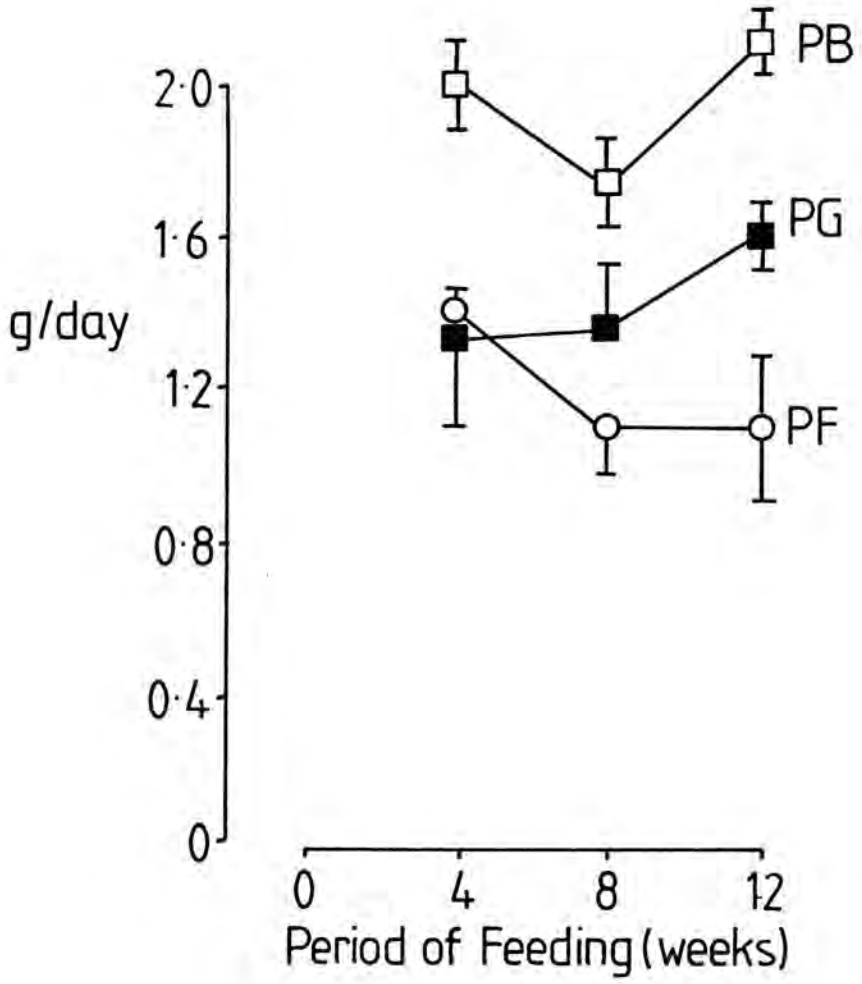
Table of significant differences, in ascending, order from left to right, in each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
Liver wet weight (g) LSD (36)	NS	8^a 12^b 4^b 1.66 *	NS
Liver wet weight g/kg LSD (36)	NS	12^a 8^a 4^b 2.20 **	NS

different superscript denotes a significant difference
* = $p<0.05$
** = $p<0.01$
NS = not significant.

FIGURE 3a Daily dry stool weight of rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods.

Results are mean ± S.E.M.
n = 5 except PG (8 weeks), where n = 4.



Diet alone was significant ($p<0.01$)

	DIET
L.S.D. (35)	0.302
n =	15 except diet PG where n = 14

THE CAECUM AND ITS CONTENTS

(i) The caecal sac wet weight (C.S.W.W.)

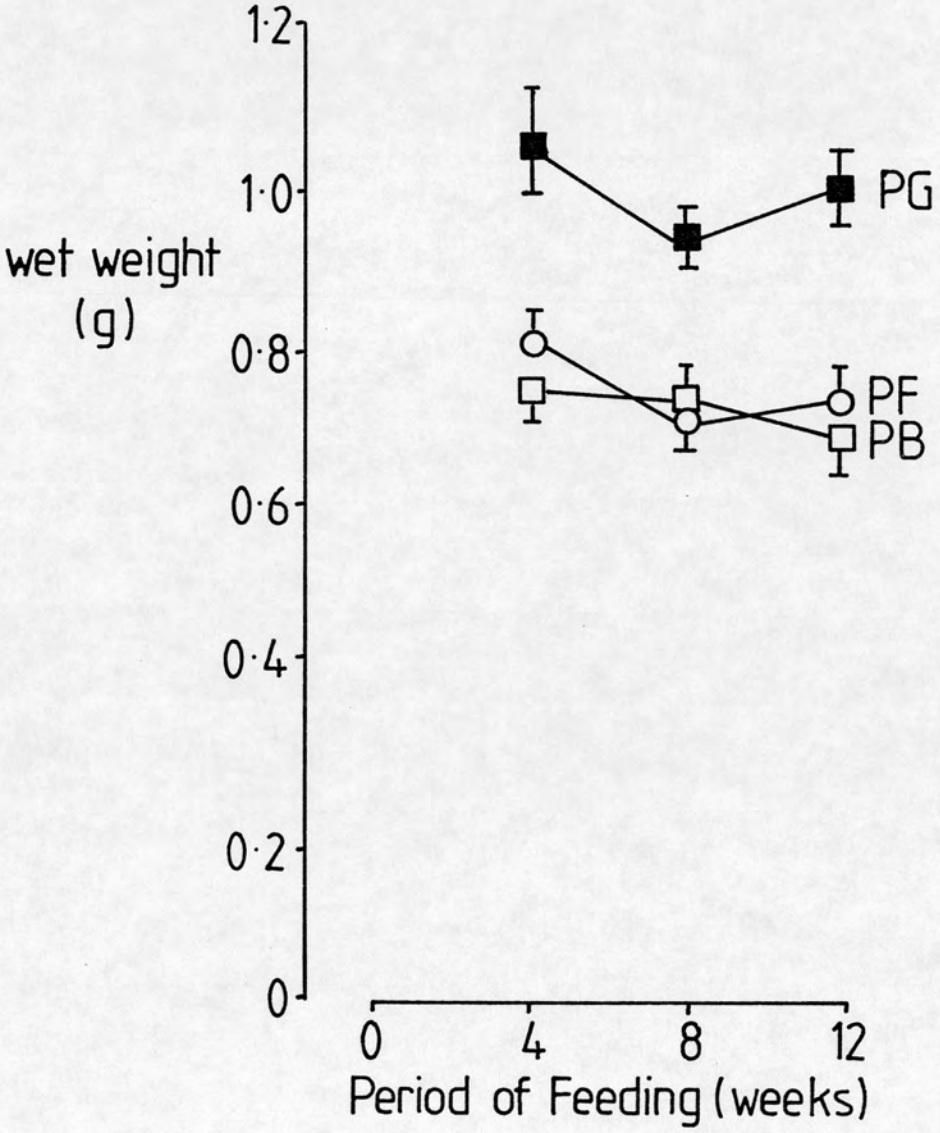
Diet alone was significant ($p<0.01$). The addition of gum arabic significantly increased the C.S.W.W. (1.02 ± 0.03 g) with respect to both the PF and PB diets (0.76 ± 0.02 g and 0.73 ± 0.03 g respectively). Figure 3b. These latter two were not significantly different from each other. When expressed as a function of body weight (g/kg), both diet and time were significant at ($p<0.01$). There was no significant difference between PF (1.45 ± 0.05 g) and PB diets (1.52 ± 0.05 g). C.S.W.W. with diet PG (2.10 ± 0.06 g) was significantly greater ($p<0.01$). The proportion of C.S.W.W. : live-weight decreased with time. The C.S.W.W. at 12 weeks (1.55 ± 0.08 g) was significantly lower than at either 8- (1.74 ± 0.10) or 4 weeks (1.79 ± 0.08 g).

(ii) Dry caecal content weight (C.C.)

Diet, time and interaction of DXT were all significant ($p<0.01$). An interaction of DXT looks at the nine individual groups. It describes the similarities that exist between different diets fed for different times. It highlights the fact that different diets do not behave in the same way with time. A difference between any two of the nine diet x time groups, or between the three dietary means, or the three time period means, is significant if it exceeds the tabulated L.S.D. A detailed explanation is given in Chapter 2. The addition of gum arabic caused a significant increase in dry C.C. to 1.43 ± 0.13 g. There was no significant difference between the overall means of the PF (0.40 ± 0.03 g) and PB diets (0.60 ± 0.03 g) (Figure 3c). Only the difference between 4-(1.03 ± 0.26 g) and 12 weeks (0.71 ± 0.08 g) was significant. Dry C.C. at 8 weeks was intermediate and not significantly different from either of these two. Figure 3c shows the similarity between the PF and PB diets, and the dietary interaction with time. There was no significant difference between any of the six PF and PB groups whose weights ranged from PF (12 weeks) 0.46 ± 0.03 g to PB (8 weeks), 0.64 ± 0.04 g. Whilst there was no significant difference between diet PG, 8 weeks and diet PG 12 weeks, the weight with diet PG fed for 4 weeks (2.00 ± 0.20 g) was significantly greater than all the other 8 groups. Figure 3c shows how the significance of an interaction makes the significance of main effects less meaningful: whilst C.C. decreased over time on diet PG, with the diets PF and PB values remained fairly constant. Thus the magnitude of the change between time periods is not the same for each diet, and is/....

FIGURE 3b Caecal sac wet weights of rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods.

Results are mean ± S.E.M.
n = 5



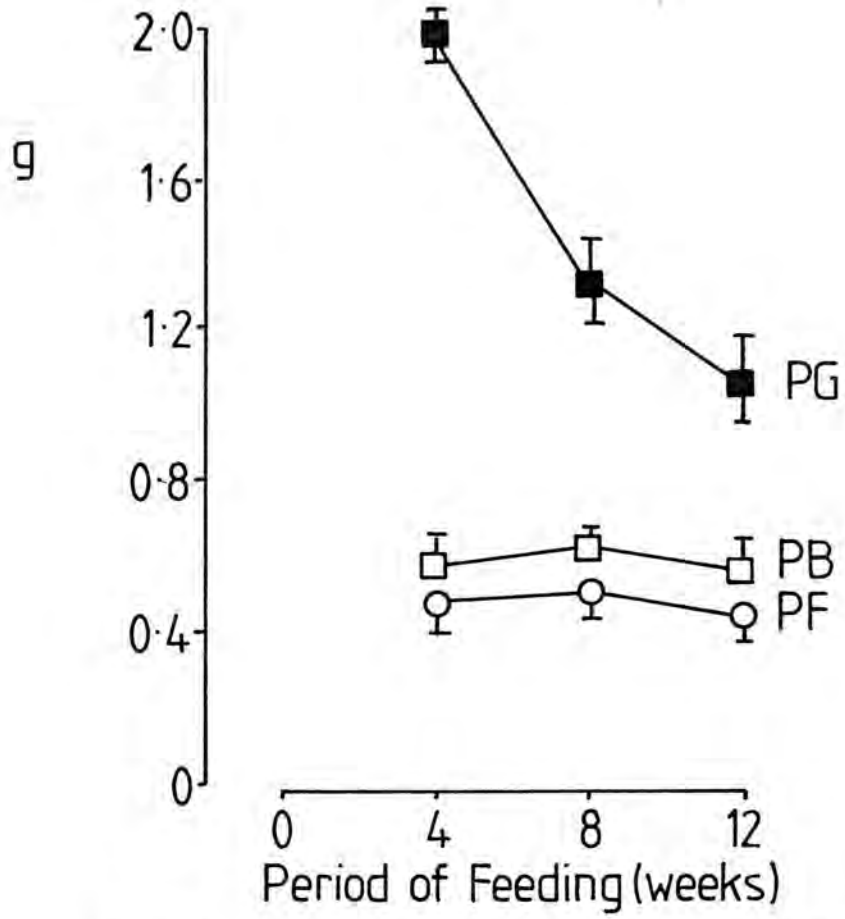
Diet alone was significant ($p<0.01$)

	DIET
$L.S.D_{(36)}$	0.011
n =	15

FIGURE 3c Dry caecal content weight of rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M

n = 5



Diet, time and the interaction of diet and time (DXT) were significant ($p<0.01$)

	DIET	TIME	DXT
L.S.D$_{(36)}$	0.22	0.22	0.08
n =	15	15	5

distorted by looking only at the means of the main effects. When expressed as a function of live-weight (g/kg), the same pattern of events with C.C. occurred. Diet, time and the interaction of DXT were significant ($p<0.01$).

2-6-DIAMINOPIMELIC ACID (DAPA)

a) Total DAPA

(i) Caecal (μmols)

Diet alone was significant ($p<0.01$). Figure 3d (i) shows how the addition of gum arabic significantly increased total caecal DAPA, (10.9 ± 0.70 μmols). There was no significant difference between total DAPA from the PF (3.3 ± 0.3 μmols) and PB diets (2.9 ± 0.22 μmols).

(ii) Faecal (μmols/day)

Both diet ($p<0.01$) and time ($p<0.05$) were significant (Figure 3d (ii)). The addition of gum significantly increased total faecal DAPA, (12.5 ± 0.9 μmols/day). There was no significant difference between the PF (7.75 ± 0.51 μmols/day) and PB diets (7.58 ± 0.49 μmols/day). Only the difference between 4- (11.0 ± 0.98 μmols/day) and 8 weeks (8.30 ± 1.03 μmols/day) was significant. Total DAPA after 12 weeks was intermediate and not significantly different from either value.

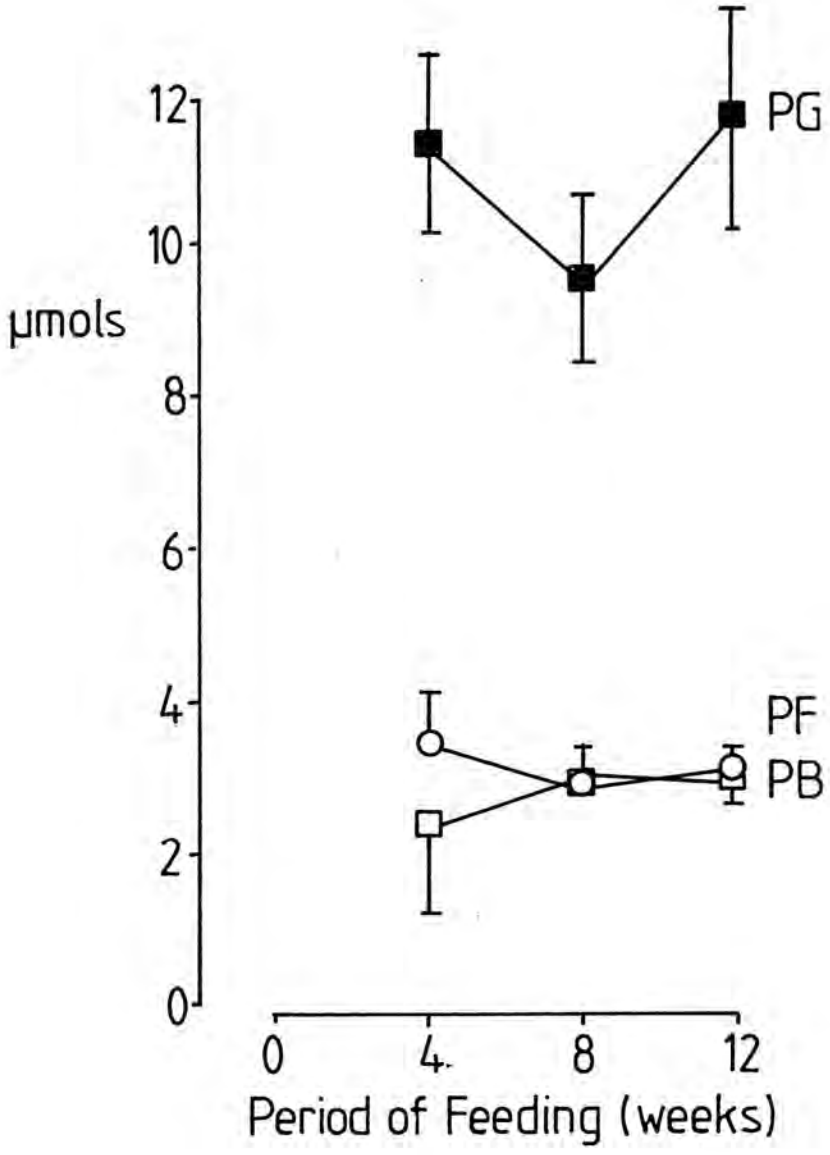
b) Concentration of DAPA

(i) Caecal (μmols/g)

Diet, time and the interaction of DXT were all significant ($p<0.01$) and are shown in Figure 3 e (i). Only the difference between the PG (8.03 ± 0.76 μmols/g), and the PB diet (4.71 ± 0.24 μmols/g) was significant ($p<0.01$). Concentration with the PF diet was intermediate and was not significantly different from either of these two. Considering the main effect of time, concentrations increased with time: after 12 weeks of feeding, concentration was significantly (7.08 ± 0.80 μmols/g) larger than either of the concentrations at 4-(5.70 ± 0.42 μmols/g) and 8 weeks (5.90 ± 0.38 μmols/g). However Figure 3e (i) shows that the interaction was significant ($p<0.01$). The changes with time were not the same for each diet, thus making any conclusions drawn from the main effects less meaningful. The value for the PG diet (12 weeks) was significantly greater than all the others, whilst there was no significant difference between the concentrations with diets PF (4 weeks), the PB (12 weeks) and the 4 intermediate dietary diets demonstrating that similarities between different diets can exist between/....

FIGURDE 3d(i) Total caecal 2-6 DAPA from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M
n = 5

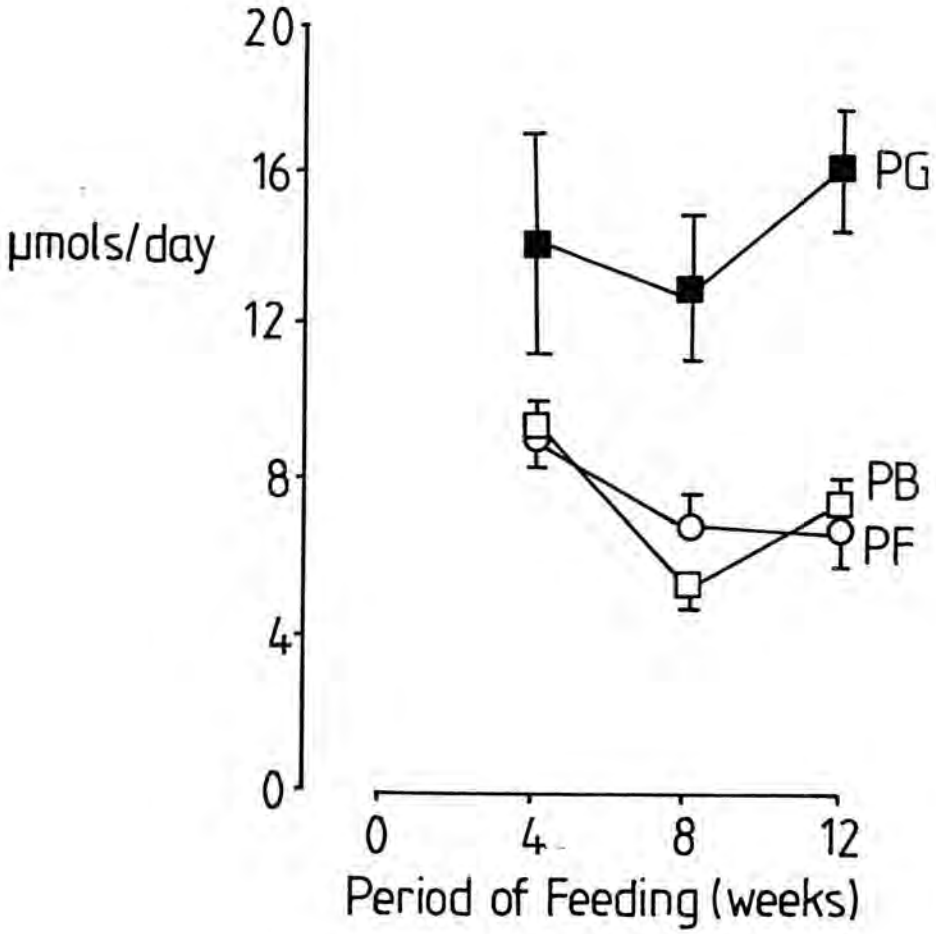


Diet alone was significant ($p<0.01$)

	DIET
$L.S.D_{(36)}$	1.77
n =	15

FIGURE 3d(ii) Total faecal 2-6 DAPA from rats fed a plant origin diet (PF) alone and supplement with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M.
n = 5 except PG (8 weeks), where n = 4



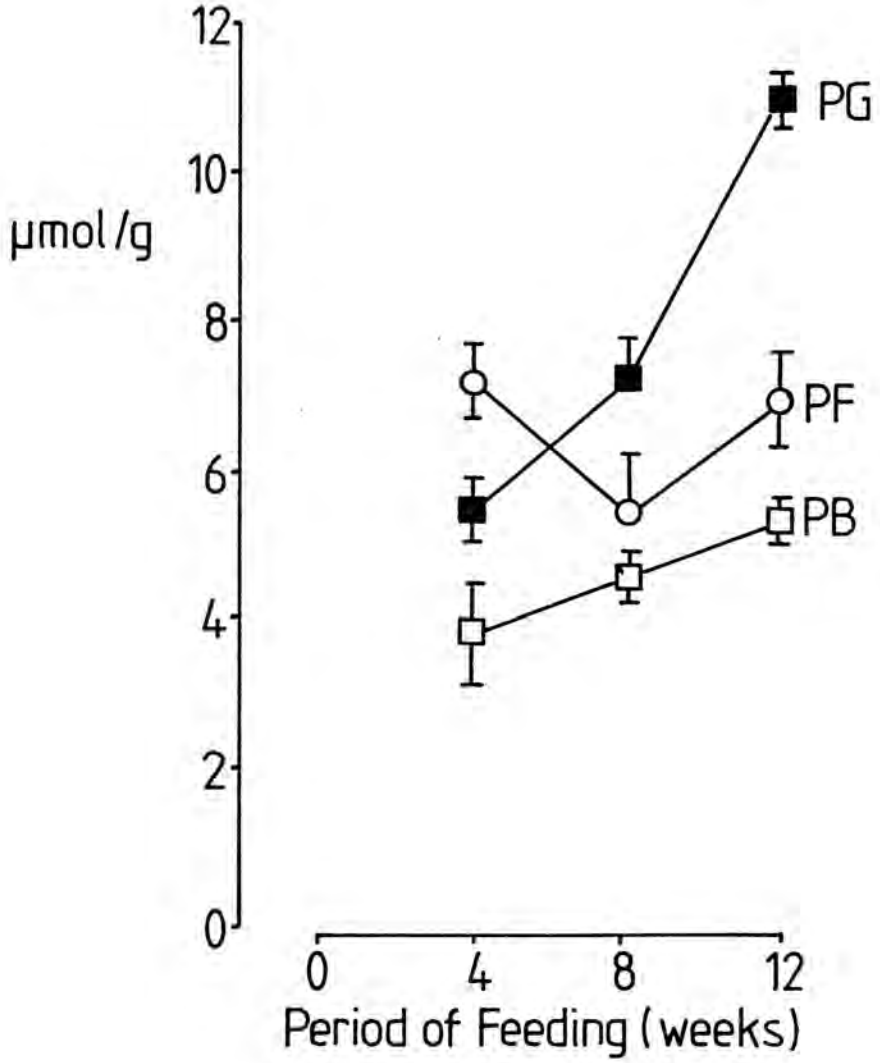
Diet ($p<0.01$) and time ($p<0.05$) were significant.

	DIET	TIME
$L.S.D_{(35)}$	2.78	2.03
n =	15	15

except diet PG and time period 8 weeks where n = 14

FIGURE 3e(i) Caecal concentration of 2-6 DAPA from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M
n = 5



Diet, time and the interaction of diet and time (DXT) were significant ($p<0.01$).

	DIET	TIME	DXT
L.S.D$_{(36)}$	1.41	1.41	2.45
n =	15	15	5

different diets fed for different time periods.

(i) Faecal (μmols/g)

Diet alone was significant ($p<0.01$), as shown in (Figure 3e (ii)). The addition of gum arabic caused a significant increase in the concentration of faecal DAPA (10.2 ± 0.28 μmols/g). The three overall dietary concentrations were significantly different from each other (PG = 6.49 ± 0.18 μmols/g; PB = 3.84 ± 0.20 μmols/g). It appears that the presence of bran reduced the concentration of DAPA.

SHORT CHAIN FATTY ACIDS (SCFA's)

a) Total SCFA's

(i) Caecal (μmols)

Diet alone was significant ($p<0.01$). The presence of gum arabic caused a significant increase in total SCFA's (745 ± 57 μmols). There was no significant difference between the overall means of the PF (238 ± 38 μmols) and PB diets (234 ± 16 μmols) Figure 3f(i).

(ii) Faecal (μmols/day)

Both diet and time were significant ($p<0.01$). Total faecal SCFA's were greatest on the PG diet (261 ± 48 μmols/day). There was no significant difference between the PF (100 ± 20 μmols/day) and PB diets (154 ± 15 μmols/day) Figure 3f (ii), shows that the total faecal SCFA's decreased with time ($p<0.01$). Total SCFA's after 4 weeks (251 ± 42 μmols/day) were significantly greater than at either 8-or 12 weeks (101 ± 13 μmols/day and 153 ± 28 μmols/day respectively), between which there was no significant difference. Total caecal SCFA's exceeded total faecal SCFA's.

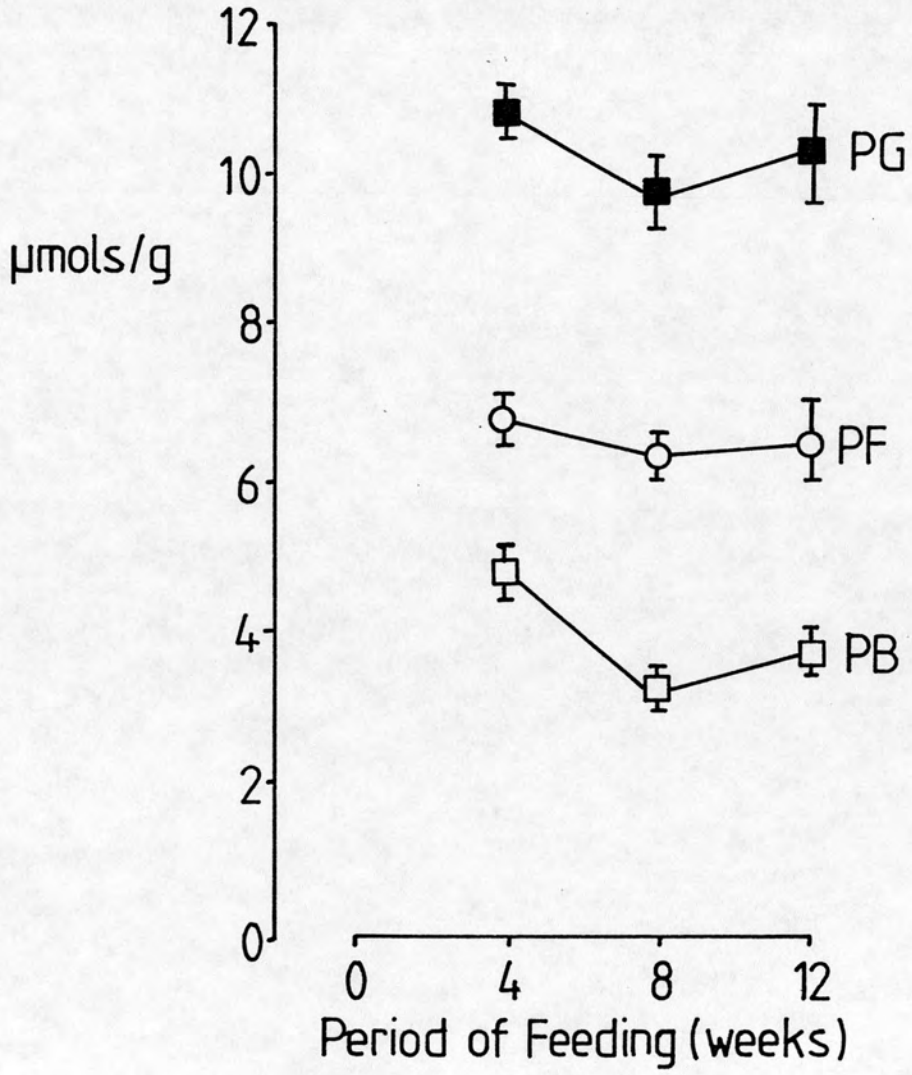
b) Concentration of SCFA's

(i) Caecal (μmols/g)

Both diet and the interaction of DXT were significant ($p<0.01$), Figure 3g (i). Considering the main effect of diet, only the difference between the PB (390 ± 101 μmols/g) and PG diets (528 ± 31 μmols/g) was significant ($p<0.01$). The overall mean for the PF diet was intermediate and not significantly different from either of these two. However from Figure 3g (i) the nature of the interaction of DXT can be seen, and the similarities that exist between

FIGURE 3e(ii) Faecal concentration of 2-6 DAPA from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M
n = 5 except PG (8 weeks), where n = 4

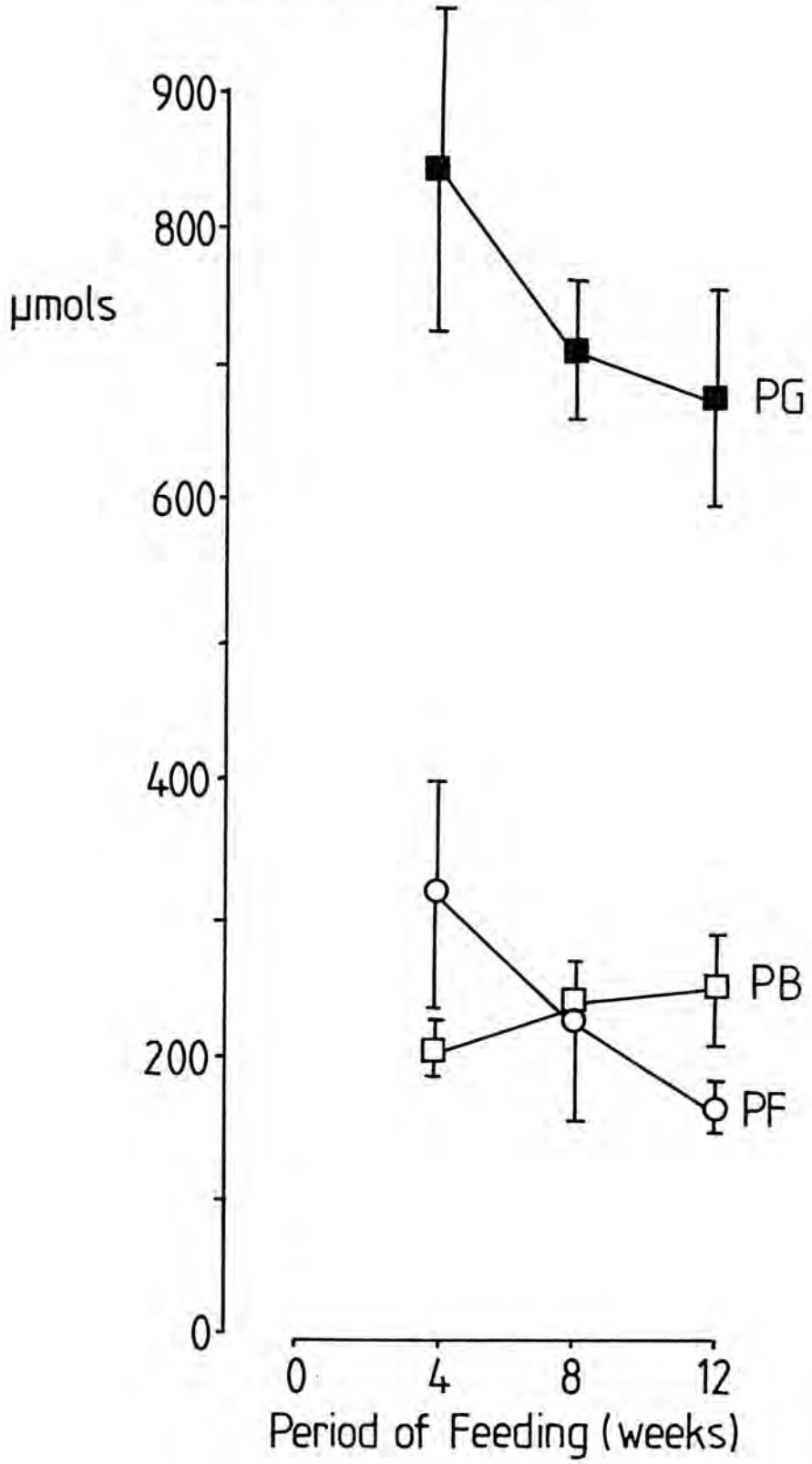


Diet alone was significant ($p<0.01$)

	DIET
L.S.D$_{(35)}$	0.811
n =	15 except diet PG where n = 14

FIGURE 3f(i) Total caecal SCFA's from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M; n = 5

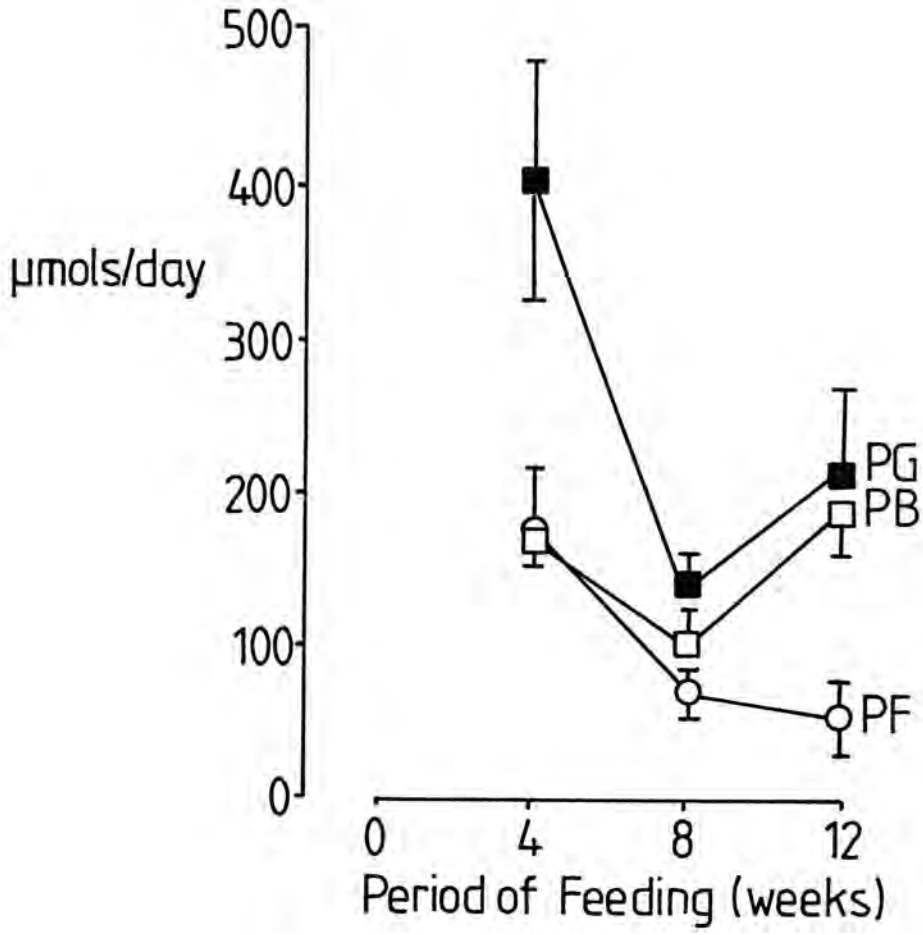


Diet alone was significant ($p<0.01$)

	DIET
L.S.D $_{(36)}$	0.144
n =	15

FIGURE 3f(ii) Total faecal SCFA's from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are mean ± S.E.M
n = 5 except PG (8 weeks), where n = 4



Diet and time were significant ($p<0.01$)

	DIET	TIME
L.S.D$_{(35)}$	0.095	0.095
n =	15	15

except diet PG and time period 8 weeks where n = 14

different diets fed for different time intervals. For example, the concentration with the PB diet after 4 weeks of feeding (351 ± 15 μmols/g), was not significantly different from that with the PF diet fed for 4 weeks, and the four intermediate groups. Figure 3g (i) shows that the trends with time and the changes between time periods were not the same for the three diets and are obscured by considering only the means of the main effects of diet and time.

(ii) Faecal (μmols/g)

Diet, time and the interaction of DXT, were significant ($p<0.01$), and are shown in Figure 3g (ii). Considering the main effect of diet, the addition of gum arabic increased the concentration of SCFA's (176 ± 27 μmols/g.) There was no significant difference between the PF (79 ± 13 μmols/g) and PB diets (84 ± 8 μmols/g). With time, concentrations decreased the concentration at 4 weeks (170 ± 27 μmols/g) being significantly greater than at either 8or 12 weeks. Figure 3g (ii) shows that the changes with time were not the same for each diet, thus making any conclusions drawn from the main effects alone, less meaningful, and that there are similarities between different diets at different time intervals. For example the concentration with the PB (8 weeks: 57 ± 7 μmols/g) diet is not significantly different from that of the PF diet fed for 4 weeks (131 ± 25 μmols/g) and the five intermediate groups.

c) The composition of the SCFA's (mmols/mol)

a) Acetate

(i) Caecal

Diet alone was significant ($p<0.01$). The addition of gum arabic resulted in an increase of caecal acetate (704 ± 21 mmols/mol) of 24% and 6% with respect to the PB (570 ± 10.2 mmols/mol) and PF diets (663 ± 11.9 mmols/mol) between which there was no significant difference, Table 3c.

ii) Faecal

Diet alone was significant ($p<0.01$). The addition of gum resulted in a decrease of faecal acetate (831 ± 19 mmols/mol) 12% with respect to the PB (927 ± 23 mmols/mol) and PF diets (948 ± 19 mmols/mol), between which there was no sigificant difference, Table 3d.

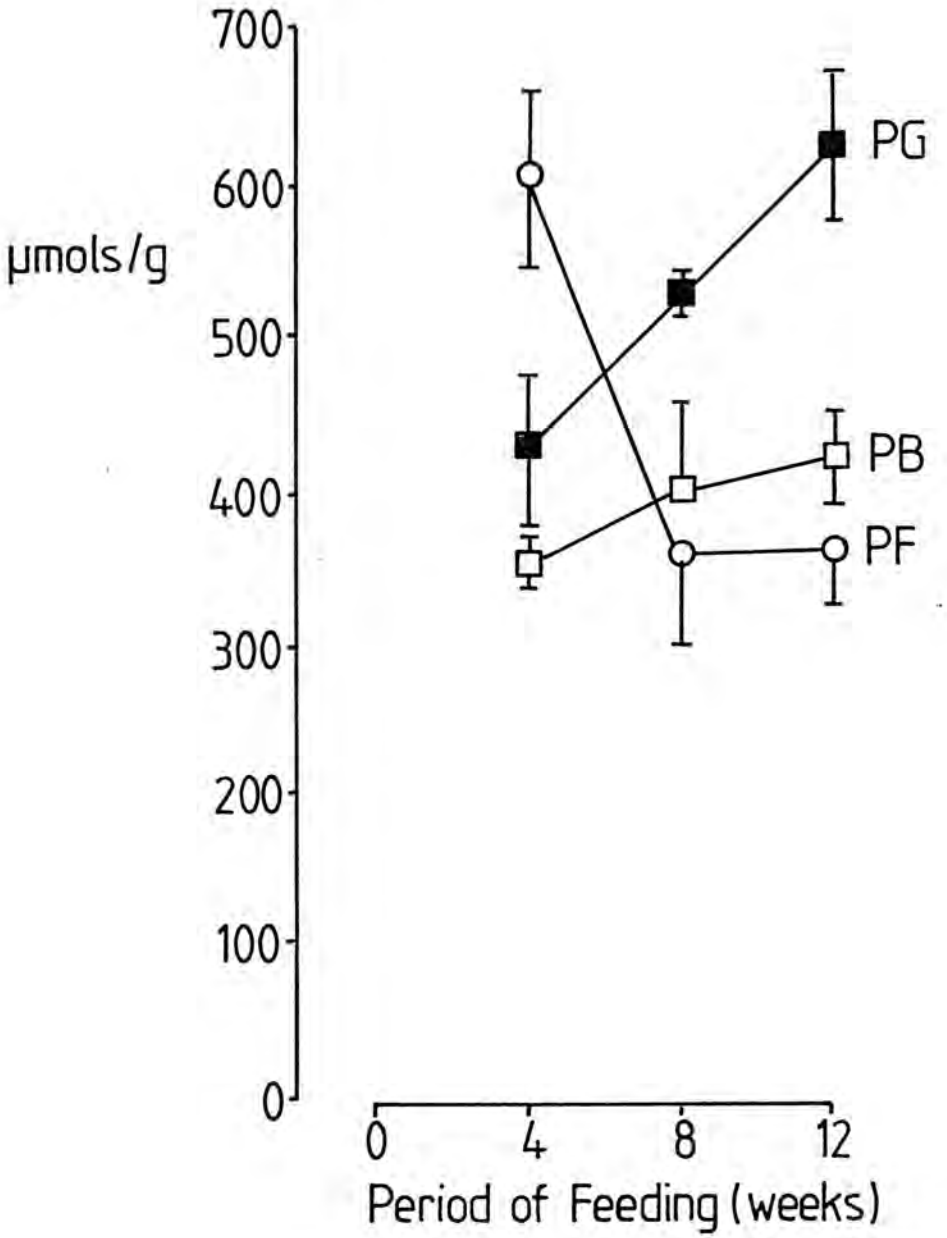
b) Propionate

i) Caecal

Time alone was significant ($p<0.01$). The proportion of caecal propionate

FIGURE 3g(i) Caecal concentration of SCFA's from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10 %wheat bran (PB) for three time periods

Results are mean ± S.E.M
n = 5

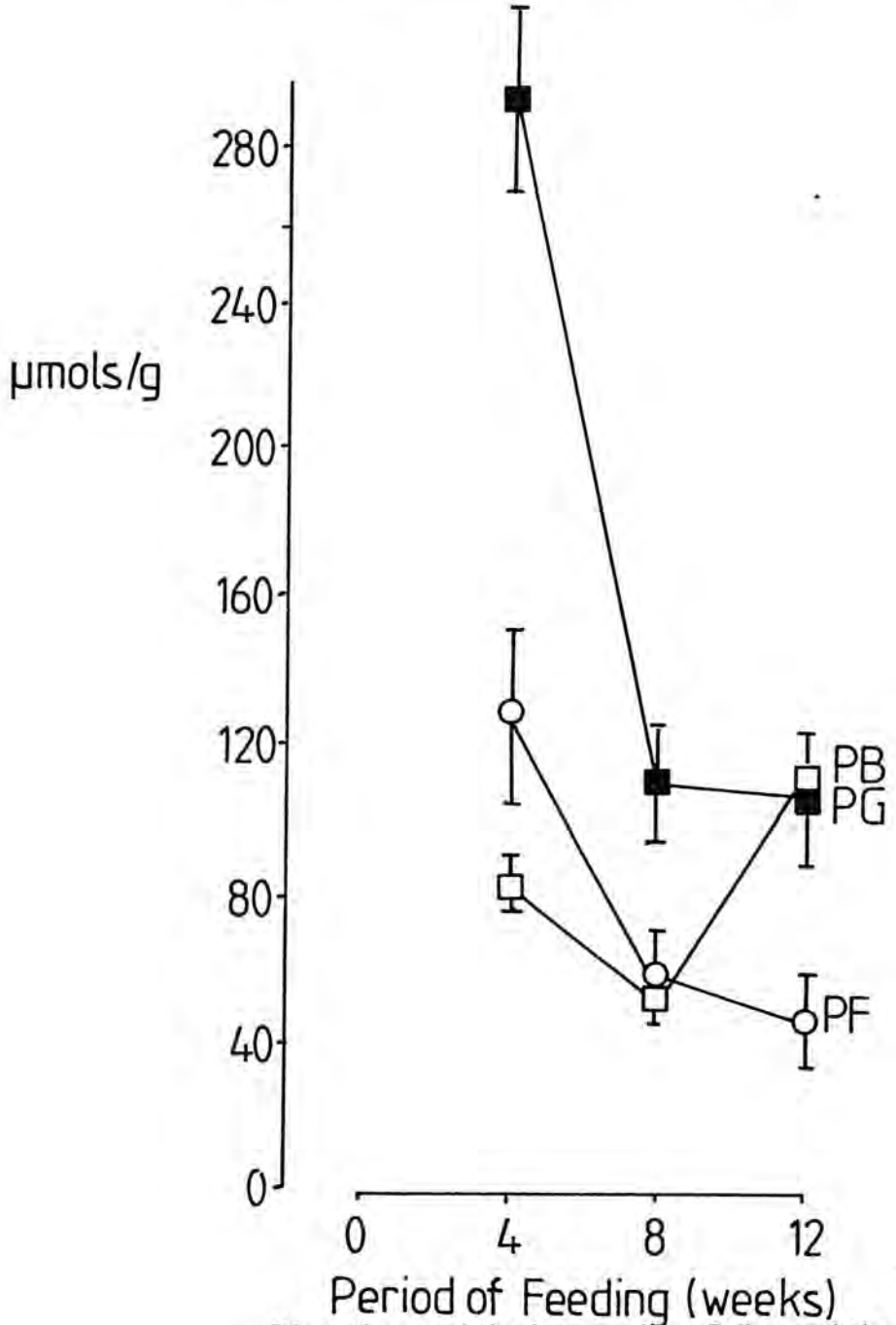


Diet and the interaction of diet and time (DXT) were significant ($p<0.01$)

	DIET	DXT
L.S.D$_{(36)}$	97.7	169
n =	15	5

FIGURE 3g(ii) Faecal concentration on SCFA's from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are ± S.E.M
n = 5 except PG (8 weeks) where n = 4



Diet, time and the interaction of diet and time (DXT) were significant ($p<0.01$)

	DIET	TIME	DXT
L.S.D$_{(35)}$	41.3	41.3	69.7
n =	15	15	5

except diet PB, time period 8 weeks, where n = 14 and diet PB (8 weeks) where n =4.

decreased with time (Table 3c). The proportion at 4 weeks (206± 6 mmols/mol) was significantly greater than at either 8- (179±9 mmols/mol) or 12 weeks (165±4 mmols/mol), between which there was no significant difference.

ii) Faecal

Diet was significant ($p<0.01$) as was the interaction of D X T ($p<0.05$). Concomitant with a reduction in faecal acetate, the presence of gum arabic stimulated a 6 fold increase in faecal propionate (123 ±11 mmols/mol). There was no significant difference between the PF (26 ±12 mmols/mol) and PB diets (21±11 mols/mol) (Table 3d). The significance of the interaction could be partially explained by the high individual within group variation, as indicated by the high S.E.M. values with respect to the means.

c) Butyrate

i) Caecal

Diet alone was significant ($p<0.01$). Bran significantly increased the proportion of caecal butyrate (236 ±14.7 mmols/mol). The overall proportions with the different diets were significantly different from each other, (PG : 100±12.5 mmols/mol)., PF : 142±23.3 mmols/mol (Table 3c)).

ii) Faecal

There were no significant changes associated with faecal butyrate.

Isobutyrate, Isovalerate and valerate were not detected.

HYDROGEN AND METHANE

a) Hydrogen (H_2)

Diet was not significant. Time alone was significant ($p<0.01$) and the results are shown in Table 3e. Only the difference between 4-(0.62±0.10 mls/hr/kg) and 12 weeks (0.25± 0.05 mls/hr/kg) was significant. The level of H_2 after 8 weeks was intermediate and not significantly different from either of these two.

b) Methane (CH_4)

Diet alone was significant ($p<0.05$). No CH_4 was detected from rats which had been fed the PB diet for 8- or 12 weeks. After 12 weeks on the PG diet

TABLE 3c The composition of SCFA's (mmols/mol) in dry caecal contents from rats fed a plant origin diet (PF) alone, or supplemented with either 10% gum arabic (PG) or wheat bran (PB) for three time periods. Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	F	G	B	F	G	B	F	G	B
ACETATE	642 ± 24	687 ± 26	530 ± 15	658 ± 20	699 ± 48	591 ± 9	689 ± 15	725 ± 88	588 ± 13
PROPIONATE	193 ± 6	217 ± 14	208 ± 15	156 ± 13	190 ± 23	160 ± 6	165 ± 1.2	165 ± 11	164 ± 7
BUTYRATE	130 ± 9	88 ± 13	254 ± 11	151 ± 13	107 ± 65	219 ± 21	145 ± 15	106 ± 29	237 ± 8

Table of least significant differences in ascending order from left to right in each group, (Chapter 2).

	DIET	TIME	INTERACTION(DXT)
ACETATE	PG^a PB^b PF^b	NS	NS
$LSD_{(36)}$	58.6		
	**		
PROPIONATE	NS	12^a 8^a 4^b	NS
$LSD_{(36)}$		25.0	
		**	
BUTYRATE	PG^a PF^b PB^c	NS	NS
$LSD_{(36)}$	40.2		
	**		

A different superscript denotes a significant difference.
** = $p<0.01$
NS = not significant.

TABLE 3d The composition of SCFA's (mmols/mol) in dry faecal contents from rats fed a plant origin diet (PF) alone or supplemented with either 10% arabic (PG) or 10% wheat bran (PB) for three time periods. Results given are mean ± SEM where n = 5 per group except PG (8 weeks) where n = 4.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	F	G	B	F	G	B	F	G	B
ACETATE	902 ± 35	848 ± 17	936 ± 27	942 ± 36	793 ± 28	974 ± 26	1000	842 ± 44	872 ± 55
PROPIONATE	54 ± 24	114 ± 9	0	24 ± 24	137 ± 6.	0	0	120 ± 31	63 ± 27
BUTYRATE	43 ± 18	37 ± 11	64 ± 60	0	71 ± 25	26 ± 26	0	37 ± 23	64 ± 29

Table of least significant differences in ascending order from left to right in each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
ACETATE	PG^a PB^b PF^b		
LSD (35)	75	NS	NS
	**		
PROPIONATE	PB^a PF^a PG^b		PF^a PB^a PB^a PF^a PF^{ab} PB^{bc} PG^{bc} PG^{bc} PG^c
LSD (35)	41.2	NS	12 4 8 8 4 12 4 12 8
	**		74.5
			*
BUTYRATE			
LSD (35)	NS	NS	NS

A different superscript denotes a significant difference.
* = $p<0.05$
** = $p<0.01$
NS = not significant

there was a 60% decrease in CH_4 production, with respect to CH_4 production on the same diet after 8 weeks of feeding (Table 3e). The results suggest that the addition of 10% bran significantly reduced CH_4 production by 85%, relative to CH_4 detected from rats fed the PF (0.66 ± 0.11 mls/hr/kg) and PG diets (0.17 ± 0.12 mmols/mol(Table 3e).

BILE ACIDS

A pooled sample of faecal and caecal material was used for analysis. As a result, no statistical analysis was made.

a) Total bile acids

(i) Caecal (µmols)

Total caecal bile acids are given in Figure 3h (i). The presence of gum arabic increased total caecal bile acids. (23.8 ± 3.69 µmols). There was little difference between the overall means of the PB and PF diets (10.1 ± 0.64 µmols and 9.67 ± 0.17 µmols respectively). With the exception of the PG diet fed for 12 weeks, within any dietary group there was little change in the total bile acids between 4- 8 and 12 weeks (Figure 3h(i)). The overall means are 15.8 ± 6.00 µmols . 16.3 ± 5.36 µmols., and 11.8 ± 2.34 µmols respectively., for 4- 8 and 12 weeks.

(ii) Faecal (µmols/day)

Total faecal bile acids were greatest on the PB diet (35.0 ± 2.86 µmols/day) and least on the PF diet (17.8 ± 0.27 µmols/day). Total faecal bile acids with the PG diet was intermediate (Figure 3h (ii)). With the exception of the PG diet, total faecal bile acids exceeded total caecal. Total faecal bile acids, within each group, increased with time from an overall mean of 22.6 ± 3.72 µmols/day (4 weeks) to 27 ± 6.50 µmols/day (12 weeks). The increase with time was most noticeable with the PB diet.

b) Concentration of total bile acids

(i) Caecal (µmols/g)

From Figure 3j (i) it can be seen that the changes, over time, are different for each diet, even though there is little difference between the three overall dietary means : PF = 20.5 ± 1.22 µmols/g., PG = 16.3 ± 1.87 µmols, and PB = 17.4 ± 0.92 µmols. There appears to be no significant overall difference between the PF and PB diets: concentrations increased after 8 weeks and thereafter

TABLE 3e H_2 and CH_4 (mls/hr/kg) from rats fed a plant origin (PF) diet alone or supplemented with 10% arabic (PG) or 10% wheat bran (PB) for three time periods.
Results given are mean±SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	PF	PG	PB	PF	PG	PB	PF	PG	PB
H_2	0.62 ± 0.27	0.58 ± 0.07	0.65 ± 0.11	0.54 ± 0.10	0.71 ± 0.21	0.32 ± 0.06	0.27 ± 0.08	0.08 ± 0.04	0.40 ± 0.04
CH_4	0.56 ± 0.26	0.90 ± 0.26	0.25 ± 0.12	0.94 ± 0.17	0.69 ± 0.24	0	0.48 ± 0.11	0.29 ± 0.13	0

Table of significant differences, in ascending order from left to right, in each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
H_2	NS	12^a 8^{ab} 4^b	NS
LSD (36)		0.30	
		**	
CH_4	PB^a PF^b PG^b	NS	NS
LSD (36)	0.38		
	**		

A different superscript deontes a significant difference.
** = $p<0.01$
NS = not significant

FIGURE 3h(i) Total caecal bile acids from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are means where n = a pooled sample from five rats

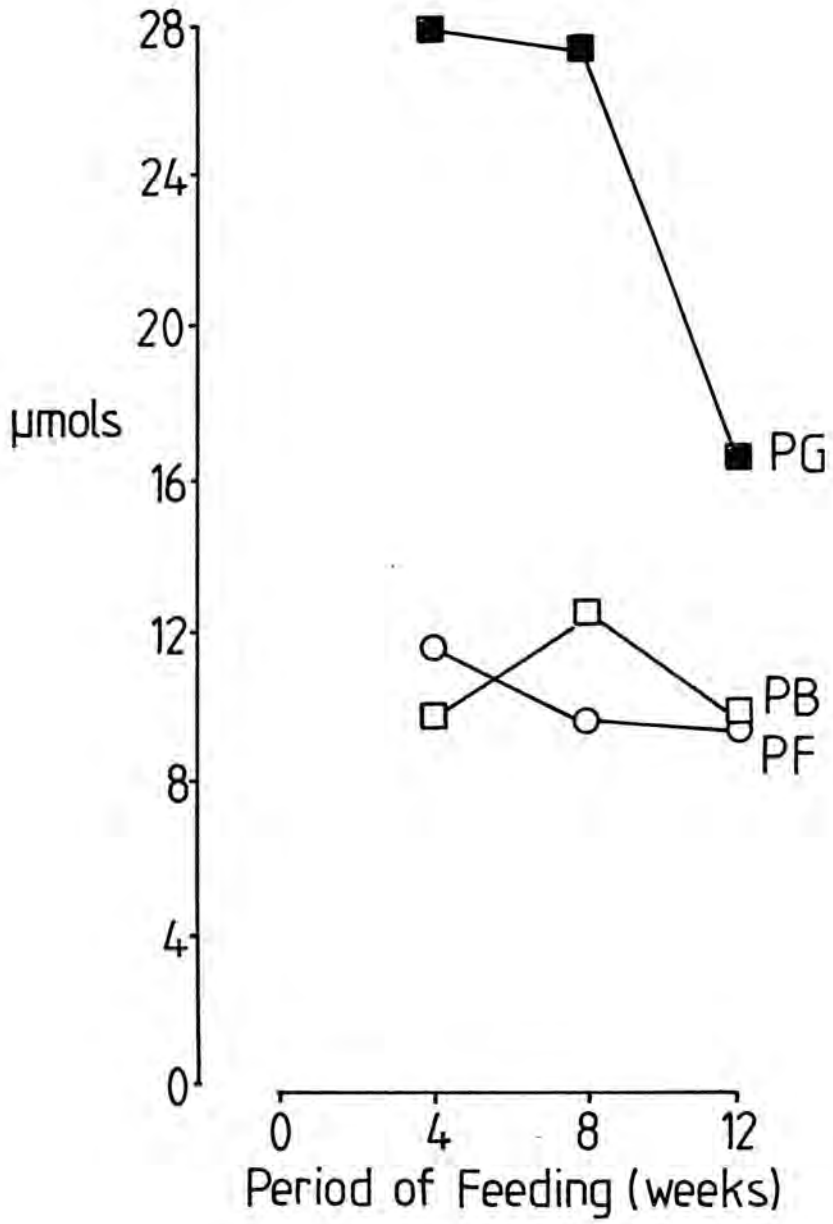
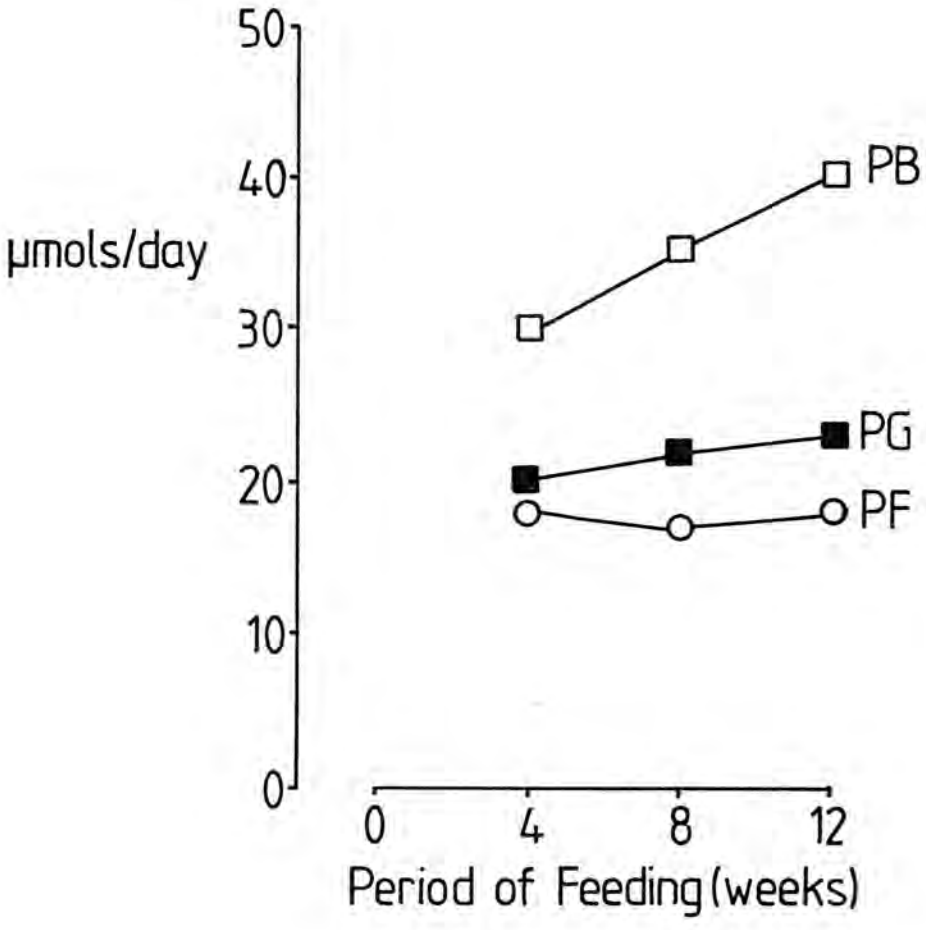


FIGURE 3h(ii) Total faecal bile acids from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are means, where n = a pooled sample from five rats except PG (8 weeks) where n = four rats.



declined. With the PG diet, concentrations decreased after 8 weeks and rose again. Considering the main effect of time alone the concentration at 8 weeks (19.2 $\pm$0.44 µmols/g) was greater than at 4- (17.7$\pm$ 2.6 µmols/g) and 12 weeks (17.3$\pm$1.60 µmols/g). Figure 3j (i) shows that the means of the main effects give a misleading picture of the changes with time, for each diet.

(ii) Faecal (µmols/g)

Figure 3j (ii) shows the concentration of total bile acids in stool. The addition of bran increased total bile acids (17.8 $\pm$1.63 µmols/g), with respect to the PF (15.0 $\pm$ 1.16 µmols/g) and PG diets (15.9 $\pm$0.42 µmols/g). With all three diets concentrations increased with time, particularly after 8-weeks feeding. Concentrations ranged from 14.2$\pm$ 0.71 µmols/g (4 weeks) to 17.2$\pm$ 0.66 µmols/g (12 weeks). Figure 3 j (ii) shows that the difference between 4- and 8 weeks on the PB diet was greater than the difference between these two time periods on the PF and PG diets.

c) The composition of the total bile acids (mmols/mol).

(i) Caecal

Table 3f (i) gives the individual proportions of each bile acid in dry caecal contents. On all diets, with the exception of the PG diet, deoxycholic, cholic and hyodeoxycholic acid, formed the largest proportion of the individual bile acids. The PG diet reduced the proportion of hyodeoxycholic acid. Of the three muricholic acids, ω- and β-muricholic acids formed the largest proportion. The addition of gum arabic increased the proportion of the three muricholic acids, whilst the addition of bran increased the proportion of lithocholic- and hyodeoxycholic acid.

Both the PG and PB diets reduced the proportion of deoxycholic acid present, with respect to the PF diet. Only with lithocholic acid does there appear to be no interaction of D X T.

Lithocholic acid: on the PG diet the proportion remained constant with time (36 mmols/mol). With the PF diet, the proportion decreased between 4- and 12 weeks. Whilst with the PB diet the proportion decreased between 4- and 8 weeks thereafter remaining constant.

Deoxycholic acid: the proportion decreased between 4 (194 mmols/mol) and 8 weeks (177 mmols/mol) on the PB diet, thereafter remaining

FIGURE 3j(i) Caecal concentration of bile acids from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods.

Results are means where n = à pooled sample from five rats.

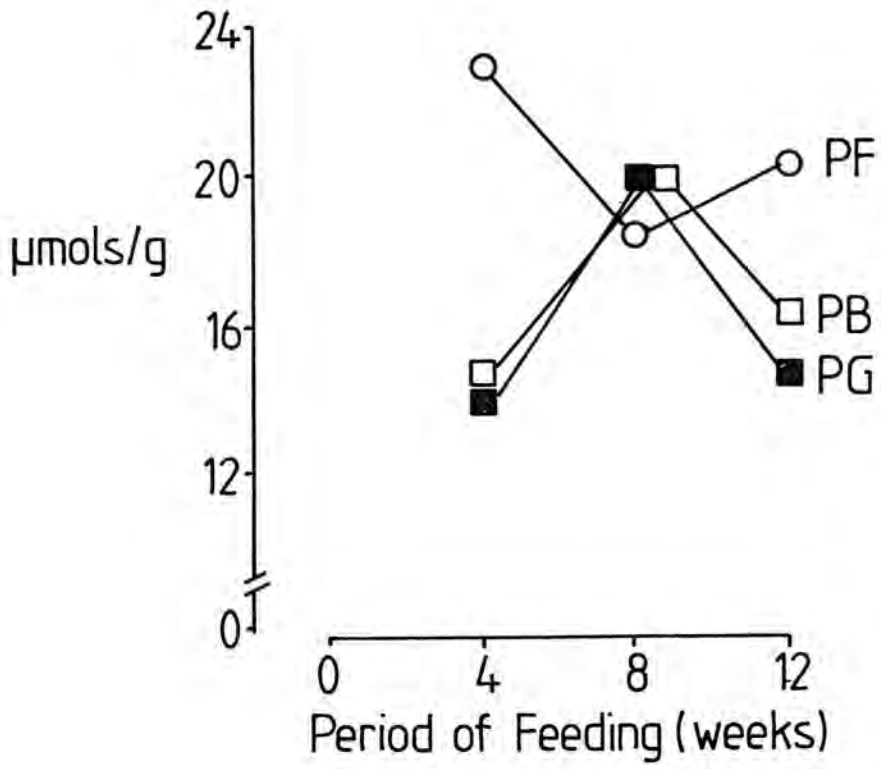
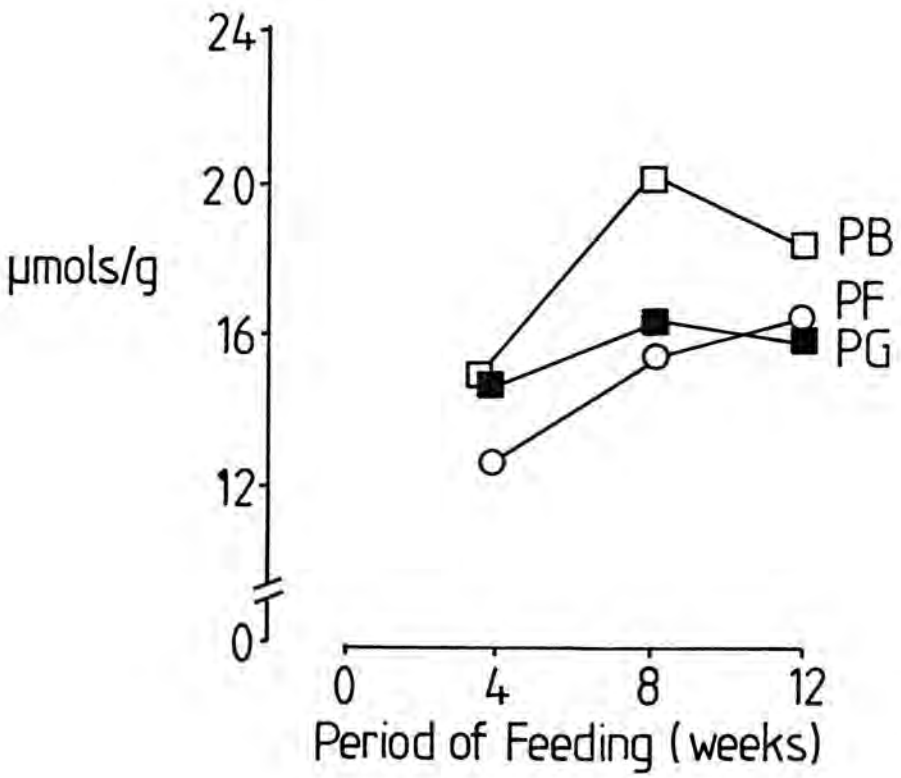


FIGURE 3j(ii) Faecal concentration of bile acids from rats fed a plant origin diet (PF) alone and supplemented with 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods

Results are means where n = a pooled sample from five rats except PG (8 weeks) where n = four rats.



constant;increased between 4- and 8 weeks with the PG diet decreasing again at 12 weeks, and with the PF diet the proportion increased between 4- (234 mols/mol) and 8 weeks (265 mmols/mol) with a decrease at 12 weeks (200 mmols/mol). The PF diet gave the largest proportion of the acid.

Chenodeoxycholic acid: at 4 weeks there was little difference between the proportions associated with each diet (25 mmols/mol). With time, the PF and PB diets showed similar changes, the proportion at 12 weeks being greater than at 4 weeks. By 12 weeks, the proportion on the EG diet had decreased to 13 mmols/mol.

Cholic acid: with time, the proportion decreased on the PB diet from 152 mmols/mol to 97.6 mmols/mol. On the PF diet, the proportion decreased from 178 mmols/mol (4 weeks) to 152 mmols/mol (12 weeks). On the PG diet the proportion increased between 4- and 8 weeks (128 mmols/mol) and thereafter decreased. At 8 weeks there was little difference between the three diets. At 12 weeks there was no difference between the lower proportions of the PG and PB diets.

Hyodeoxycholic acid: the proportion of hyodeoxycholic acid remained constant with time on the PG diet (65 mmols/mol), and increased over time from 262 mmols/mol to 436 mmols/mol with the PF diet. The proportion was highest after 8 weeks on the PB diet (576 mmols/mol).

α-muricholic acid: with the PG diet, the proportion decreased in a linear fashion with time from 71.9 mmols/mol to 33.1 mmols/mol. With the PB diet the overall proportion was lower and decreased between 4- (48.5 mmols/mol) and 8 weeks (10.4 mmols/mol), with a subsequent increase after 12 weeks, a trend that was not reflected with the PF diet. At 12 weeks there was little difference between the proportions exhibited by the PG and PB (415 mmols/mol) diets.

ω-muricholic acid: the overall proportion was largest on the PG diet and increased with time from 281 mmols/mol to 371 mmols/mol. The proportions were lower on the PB and PF diets, with little differences between the two. On the PB diet, the proportion increased with time, and on the PF diet decreased.

β-muricholic acid: the proportion decreased in a linear fashion, with time,

TABLE 3f(i) The composition of bile acids (mmols/mol) in pooled caecal material from rats fed a plant origin diet (PF) alone or supplemented with either 10% gum arabic (PG) or 10% wheat bran (PB) for three time periods. Results are means.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	PF	PG	PB	PF	PG	PB	PF	PG	PB
Lithocholic acid	30.0	36.0	57.0	27.0	36.0	41.7	20.6	36.4	42.7
Deoxycholic acid	234	151	194	265	212	177	200	181	176
Chenodeoxycholic acid	26.4	21.6	24.2	16.2	27.6	20.8	19.6	13.3	24.4
Cholic acid	178	79.1	152	135	128	135	152	106	97.6
Hyodeoxycholic acid	262	64.8	309	286	103	516	436	99.34	415
α-muricholic acid	26.4	71.9	48.5	37.8	62.1	10.4	14.7	33.1	30.5
ω-muricholic acid	106	281	103	124	222	62.5	73.5	371	122
β-muricholic acid	132	295	115	108	212	36.5	83.3	166	91.6

with the PG diet, (295 mmols/mol to 166 mols/mol) and on the PF diet (132 mmols/mol to 83.3 mmols/mol). The magnitude of the difference between PG (4 weeks) and PG (12 weeks) was greater than that for PF (4 weeks) and PF (12 weeks). The proportion was greatest on the PG diet and lowest on the PB diet, where proportions ranged from 62.5 mmols/mol (8 weeks) to 103 mmols/mol (4 weeks).

(ii) Faecal

Table 3f (ii) gives the individual proportions of each bile acid in dry stool material. With all diets, deoxycholic hyodeoxycholic and muri cholic-acid, formed the largest proportions with the exception of hyodeoxycholic acid on the PG diet. The addition of gum arabic reduced the proportion of hyodeoxycholic- and cholic acid. The addition of bran decreased the proportion of lithocholic- deoxycholic and chenodeoxycholic acid, with respect to the PF and PG diets. Without exception, the addition of gum arabic increased the proportion of the three muricholic acids. Of the three muricholic acids, β-muricholic acid formed the largest proportion. With all the bile acids, there appeared to be some degree of an interaction of DXT: the trends with time were not the same for each diet for each individual bile acid.

Lithocholic acid: the proportion with the PF diet increased after 12 weeks (60 mmols/mol) and then decreased. There was very little difference between 4- and 12 weeks. With the PB diet, the proportions decreased with time from 56.3 mmols/mol (4 weeks) to 42.2 mmols/mol (12 weeks). With the PG diet, the proportion decreased only after 8 weeks of feeding. After 12 weeks there was no discernible difference between the proportions associated with the three diets.

Deoxycholic acid: the proportion at 4 weeks was largest with the PF diet (186 mmols/mol) and smallest with the PG diet (161 mmols/mol). With the PF diets the proportion increased after 8 weeks (213 mmols/mol). At 12 weeks, the proportion had decreased with the PF diet (173 mmols/mol) and increased with the PG diet (166 mmols/mol). There was no discernible difference between the three diets at 12 weeks. With the PB diet the proportion had decreased after 8 weeks and then increased.

Chenodeoxycholic acid: there was no difference between the PF and PG diets at either 4- (62 mmols/mol) or 8 weeks (46 mmols/mol), proportions

were greater than with the PB diet. However, the magnitude of the change between 4- and 8 weeks was the same for all three diets. At 12 weeks, the proportion was largest with PG (73.5 mmols/mol) and smallest with PB (37.3 mmols/mol).

Cholic acid: both the PF and PG diets showed similar trends of increasing proportions in a linear fashion with time. With the PF diet proportions increased from 117 mmols/mol (4 weeks) to 140 mmols/mol (12 weeks). With the PG diet proportions increased from 76.2 mmols/mol (4 weeks) to 110 mmols/mol, and on the PB diet the proportion increased from 92.9 mmols/mol (4 weeks) to 130 mmols/mol (12 weeks).

Hyodeoxycholic acid: With the PF and PG diets the proportions increased with time from 373 mmols/mol and 213 mmols/mol respectively (4 weeks) to 452 mmols/mol and 324 mmols/mol respectively 12 weeks. With the PB diet, the proportion increased with time, that at 8 weeks (499 mmols/mol) being the largest.

α-muricholic acid: the proportion decreased slightly with time on the PF diet (19.5 mmols/mol, (4 weeks), 17.3 mmols/mol (12 weeks). With the PG diet, the proportion of α-muricholic acid decreased to zero after 12 weeks. With the PB diet no α-muricholic was detected after 8 weeks but levels were detected after 12 weeks (18.4 mmols/mol). Proportions were in the same range as those of cholic acid.

ω-muricholic acid: there appeared to be no interaction of DXT. The proportion decreased between 4- and 8 weeks, followed by a rise after 12 weeks with all diets. The magnitude of the differences between the time periods were similar for each diet.

β-muricholic acid: at 4 weeks there was little difference between the PF (92.4 mmols/mol) and PB diets (76.7 mmols/mol), nor at 12 weeks (58.6 mmols/mol and 56.8 mmols/mol respectively. With the PG diet, the proportion increased from 187 mmols/mol (4 weeks) to 216 mmols/mol (8 weeks) and then decreased to 88 mmols/mol (12 weeks).

TABLE 3f(ii) The composition of bile acids (mmols/mol) in pooled faecal material from rats fed a plant origin diet (PF) alone or supplemented with either 10% gum arabic (PG) or wheat bran (PB), for three time periods. Results are means.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	PF	PG	PB	PF	PG	PB	PF	PG	PB
Lithocholic acid	49.9	57.0	56.3	60.3	57.5	35.5	43.6	42.9	42.2
Deoxycholic acid	186	176	161	213	174	144	173	166	167
Chenodeoxycholic acid	61.6	62.9	50.2	47.5	45.3	31.6	44.2	73.5	37.3
Cholic acid	117	76.2	92.9	113	88.7	144	140	110	130
Hyodeoxycholic acid	373	213	404	397	278	499	452	324	449
α-muricholic acid	19.5	49.0	25.1	18.6	37.3	0	17.3	0	18.4
ω-muricholic acid	101	179	133	53.2	103	83.4	73.5	195	99.5
β-muricholic acid	92.4	187	76.7	97.5	216	63.2	58.6	88.8	56.8

SUMMARY

This chapter has given the results of the experimental feeding trial using a plant origin diet (PF). The PF diet was fed unsupplemented and with 10% (by dry weight) gum arabic (PG) or 10% wheat bran (PB). The effect of these diets upon chosen measurements was investigated, concomitant with the effect of the feeding duration. A two-factor ANOVA was used to analyse the results. A summary of the results is given below and discussed in detail in chapter 8.

(1) The diet was acceptable to the rats as indicated by the significant increase in live-weight (g) over time ($p<0.01$).

(2) Liver wet weight (g) was influenced by time alone ($p<0.05$). There was no significant difference between weights at 4- and 12 weeks. Weights at 8 weeks were lower.

(3) Stool weight (g/day) was increased with wheat bran ($p<0.01$) but not with gum arabic. There was no significant change in stool weight with time. The same result was observed when expressed as a function of live-weight (g/kg).

(4) The C.S.W.W. (g) was increased with gum arabic ($p<0.01$) but not with wheat bran. When expressed as a function of live-weight (g/kg) the same dietary significances were observed ($p<0.01$). Time was also significant ($p<0.01$). The C.S.W.W.: live-weight increased with time.

(5) The weight of the dry C.C. (g) was increased with gum arabic ($p<0.01$) but not with the PB diet. With time the weight of C.C. decreased on the PG diet, and remained relatively constant on the PF and PB diets. The interaction of D X T was significant ($p<0.01$). When expressed as a function of live-weight the same significances were observed ($p<0.01$).

(6) Caecal DAPA (µmols), was increased with gum arabic ($p<0.01$), but not with bran. There was no effect of time. Faecal DAPA (µmols/day) was increased with gum arabic ($p<0.01$). Faecal DAPA significantly decreased after 8 weeks, on each diet ($p<0.05$).

(7) Caecal DAPA (µmols/g) increased with gum arabic ($p<0.01$). Concentration

increased with time ($p<0.01$). The trends of the change over time within each diet were not the same: concentration increased with the PG and PF diets and remained constant on diet PB. Faecal DAPA (μmols/g) increased with gum arabic and decreased with bran ($p<0.01$).

(8) Caecal SCFA's (μmols) increased with gum arabic ($p<0.01$) and not with bran. Similarly for faecal SCFA's (μmols/day). Total faecal SCFA's decreased with time on each diet ($p<0.01$).

(9) Caecal and faecal SCFA's (μmols/g) increased by gum arabic and not by wheat bran. The interaction of D X T was significant indicating the difference in changes over time associated with each diet.

(10) Of the SCFA's, acetate formed the largest proportion (mmols/mol), in caecal and faecal contents. Acetate in the stool exceeded that in the caecum. Gum arabic reduced the proportion of acetate whilst bran increased it. Time alone influenced caecal propionate, values decreasing with time. Gum arabic increased faecal propionate. Bran increased caecal butyrate ($p<0.01$) whilst gum arabic decreased caecal butyrate.

(11) H_2(mls/hr/kg) decreased with time ($p<0.01$) but not CH_4(mls/hr/kg). Bran abolished CH_4. The addition of gum arabic increased CH_4 with respect to the PF and PB diets, which decreased with time.

(12) Caecal bile acids (μmols) were increased with gum arabic. There was little change with time. Faecal bile acids (μmols/day) were increased with bran, and values increased with time, for each diet, the increase being most noticeable with the PB diet. After 8 weeks the concentration (μmols/g) of the total caecal bile acids decreased on the PG diet, but increased on the PF and PB diets. The addition of bran increased the concentration of the total faecal bile acids, and concentrations were increased with time on all three diets.

(13) Of the individual bile acids in the caecal contents deoxycholic- cholic- and hyodeoxycholic acids, formed the largest proportion (mmols/mol) of the bile acids on all three diets, with the exception of the PG diet. The PG diet reduced the proportion of the hyodeoxycholic acid. Of the muric holic acids,

ω-and β formed the largest proportions. Bran increased the proportion of lithocholic- and hyodeoxycholic acid. Gum increased the proportion of all three of the muricholic acids. The pattern of the changes in the proportion of the individual bile acids over time were not similar for each diet and were indicative of the interaction of D X T. In the faecal bile acids deoxycholic-,hyodeoxycholic- and β-muricholic acid formed the largest proportion on each with the exception of the PG diet. The PG diet reduced the proportion of hyodeoxycholic- and cholic acid . The addition of bran decreased the proportion of lithocholic- deoxycholic- and chenodeoxycholic acid. Gum increased the proportion of the three muricholic acids, of which β-muricholic acid formed the largest proportion. The pattern of the changes in the proportion of the individual bile acids over time were not similar for each diet and were indicative of the interaction of D X T.

CHAPTER 4

EXPERIMENT II: TO INVESTIGATE THE EFFECTS OF THE DURATION OF FEEDING A LOW FIBRE DIET OF ANIMAL ORIGIN WITH AND WITHOUT A FIBRE SUPPLEMENT UPON THE CAECAL METABOLISM AND STOOL WEIGHT OF THE ALBINO, ADULT MALE, WISTAR RAT

MATERIALS AND METHODS

The experimental design, animal housing conditions, materials (diets and supplements) and methods for this experiment have been described in Chapter 2. All significant changes due to a supplemented diet are expressed with respect to the unsupplemented diet unless otherwise stated. The F-statistics are given in Appendix 2b.

The results showed a normal distribution and are given as mean ± S.E.M.

Unlike the rats on the plant origin diet (Chapter 3), or the elemental diet (Chapter 5) these rats did not like the animal origin diet, either supplemented or unsupplemented. Flavouring the mashes with vanilla, lemon or strawberry essence did not help.

RESULTS

LIVE-WEIGHT

Diet and the interaction of D X T were significant ($p<0.01$) upon final live-weight. Bran significantly increased live-weight (373 ± 10 g). There was no significant difference between the final live-weights of the rats fed the unsupplemented (AF) (290 ± 6 g) and gum supplemented (AG) diets (315 ± 7 g). The nature of the interaction of D X T is given in Table 4a. Rats on the bran supplemented (AB) diet showed an increase of weight over time. There was no significant difference between the three live-weights of the three AB groups. Live-weight on the AF diet decreased with time, again not significant. When considered as live-weight gain, diet alone was significant ($p<0.01$). Rats on diet AF lost the most weight (-62 ± 7 g). Those rats fed the AG diet lost -30 ±7 g, and rats being fed the (AB) diet gained an average 9 ± 7 g. (Table 4a). Initial live-weights are given in Table 4a.

LIVER WET WEIGHT

Both diet and the interaction of D X T were significant ($p<0.01$) Table 4b. Those livers from rats fed diet AB (12.3 ± 0.38 g) were significantly heavier ($p<0.01$) than those from rats fed diets AG (13.9 ± 0.49 g) and AF (10.6 ± 0.21 g). All three overall means were significantly different from each other ($p<0.01$). Table 4b shows the interaction of D X T. This shows that the changes with time were not the same for each diet, and this is obscured by considering only the means of the main effect, diet. Similarities exist between different diets fed for different time periods. For example the weight of livers from the AF diet after 8 weeks is not significantly different from that of the AB diet for 4 weeks and the 4 intermediate diet groups. When expressed as a function of body/...

TABLE 4a The live-weights (g) and live-weight changes (g) of rats fed either an animal origin diet (AF) alone, or supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.
Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	AF	AG	AB	AF	AG	AB	AF	AG	AB
Rat Inital Weight	374 ± 11	374 ± 13	335 ± 10	355 ± 24	317 ± 11	390 ± 17	328 ± 6	362 ± 13	367 ± 5
Rat Final Weight	306 ± 5.0	320 ± 11	346 ± 25	288 ± 12	290 ± 5	383 ± 13	278 ± 8	336 ± 9	340 ± 7
Live-weight Change	-67 ± 9	-53 ± 5	+11 ± 18	-71 ± 18	-12 ± 15	-7 ± 5	-50 ± 4	-26 ± 8	+23 ± 7

Table of significant differences in ascending order from left to right in each group, (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Final Live-weight	AF^a AG^a AB^b		AF^a AF^a AG^a AF^{ab} AG^{ab} AB^b AB^{bc} AB^c AB^c
LSD (36)	26.6	NS	12 8 8 4 4 12 4 8 12
	**		46 **
Live-weight Change	AF^a AG^b AB^c	NS	NS
LSD (36)	25		
	**		

A different superscript denotes a significant difference.
** = $p<0.01$
NS = not significant

TABLE 4b The liver-wet weights of rats fed an animal origin diet (AF) alone or supplemented with either 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.
Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	F	G	B	F	G	B	F	G	B
Liver wet weight (g)	11.3 ± 0.21	10.2 ± 0.28	10.4 ± 0.41	12.8 ± 0.66	12.0 ± 0.93	12.1 ± 0.37	12.5 ± 0.84	13.8 ± 0.67	15.4 ± 0.46
Liver wet weight (g/kg)	36.9 ± 0.44	39.7 ± 0.79	36.1 ± 0.56	35.9 ± 1.39	41.3 ± 3.04	37.1 ± 1.53	37.3 ± 0.71	36.0 ± 1.06	39.7 ± 1.03

Table of significant differences in ascending order, from left to right, in each group, (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Liver wet weight (g)	AF^{a} AG^{a} AB^{c}		AF^{d} AF^{de} AF^{def} AG^{defg} AG^{defg} AB^{efg} AG^{fg} AB^{gh} AB^{h}
			8 12 4 8 12 4 4 8 12
LSD (36)	1.28	NS	2.20
	**		**
Liver wet weight	NS	NS	AF^{a} AG^{a} AB^{a} AF^{ab} AB^{ab} AF^{ab} AB^{ab} AG^{ab} AG^{b}
			8 12 4 4 8 12 12 4 8
LSD (36)			3.98
			*

A different superscript denotes a significant difference
* = $p<0.05$;
** = $p<0.01$;
NS = not significant.

weight (g/kg) diet was no longer significant. The significance of the interaction between D X T was ($p<0.05$). This suggests that liver weight was explained by live-weight.

STOOL WEIGHT

Figure 4a shows the dry stool weight of the three diet groups over time. Diet alone was significant ($p<0.01$). Figure 4a shows the addition of bran resulted in an increase of 79% in stool weight from 1.29 ± 0.07 g/day (AF) to 2.31 ± 0.14 g/day; and by 37% with respect to the AG diet (1.61 ± 0.11 g/day). There was no significant difference between the AF and AG diets. When expressed as a function of live-weight (g/kg) only the difference between the AF (4.48 ± 0.27 g/kg) and AB diets (6.25 ± 0.37 g/kg) was significant ($p<0.01$). The mean for AG was intermediate and not significantly different from either of these two.

THE CAECUM AND ITS CONTENTS

(i) The caecal sac wet weight (C.S.W.W.)

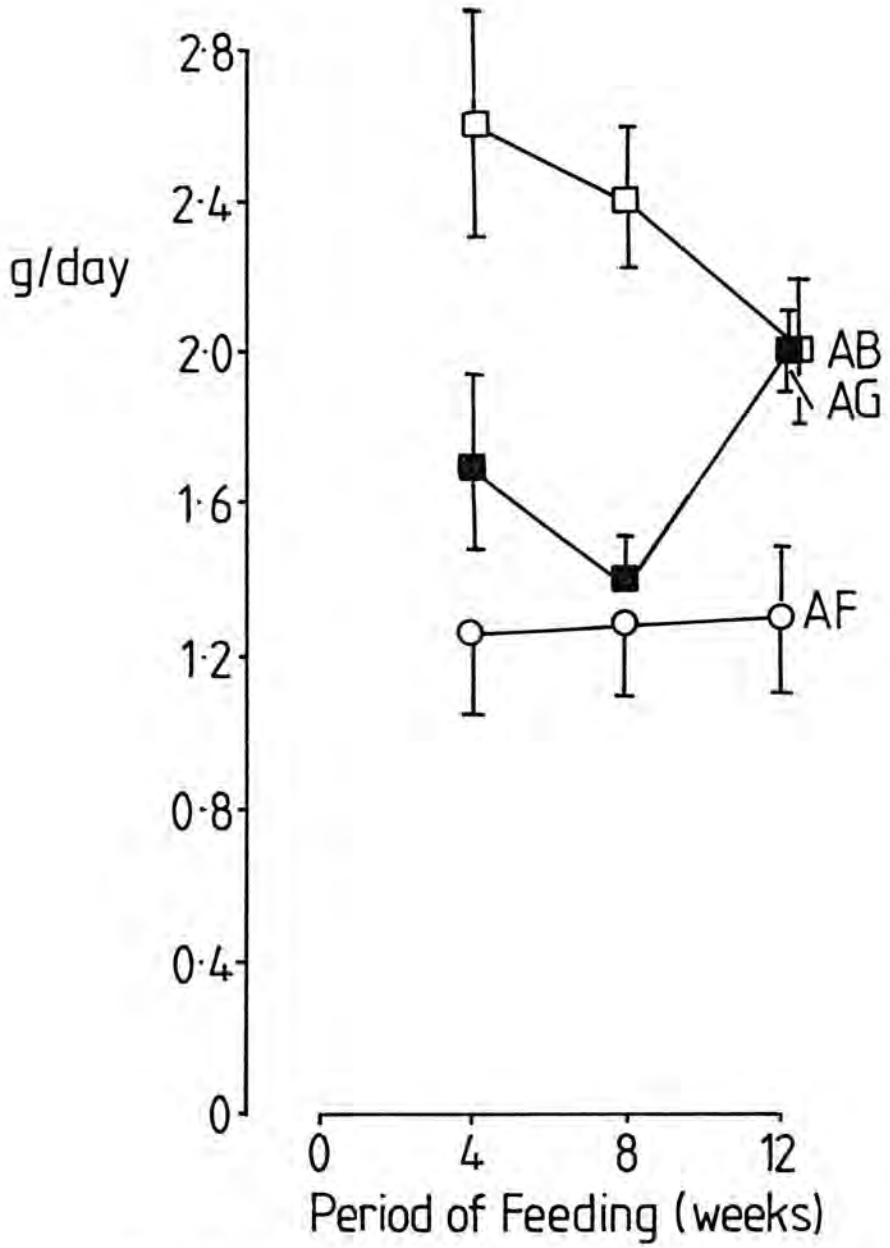
Diet, time and the interaction of D X T were significant ($p<0.01$). Considering the main effect of diet, the addition of gum arabic significantly increased C.S.W.W. (1.07 ± 0.13 g). There was no significant difference between the AB (0.60 ± 0.02 g) and AF diets (0.62 ± 0.03 g). With time, weights increased ($p<0.01$) after 8 weeks of feeding (0.97 ± 0.15 g), and as can be seen from Figure 4b, this is due to the heavier C.S.W.W. at week 8 on the AG diet. There was no significant difference between the overall means for 4- (0.62 ± 0.02 g) and 12 weeks (0.70 ± 0.03 g). Figure 4b shows how the significance of an interaction makes the significance of the main effects, diet and time, less meaningful. Similarities exist between the different diets fed for different time intervals. For example the weight after feeding the AB diet for 4 weeks (0.54 ± 0.01 g) was not significantly different from that of AG fed for 4 weeks (0.70 ± 0.04 g) and the five intermediate groups. The weight from the AG diet fed for 8 weeks (1.74 ± 0.09 g) was significantly greater than all the other 8 groups. When expressed as a function of live-weight (g/kg), the same pattern of events occurred. Diet, time and the interaction of D X T were significant ($p<0.01$).

(ii) Dry caecal content weight (C.C)

Diet alone was significant ($p<0.01$) as shown in Figure 4c. The addition of

FIGURE 4a Daily dry stool weight of rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5

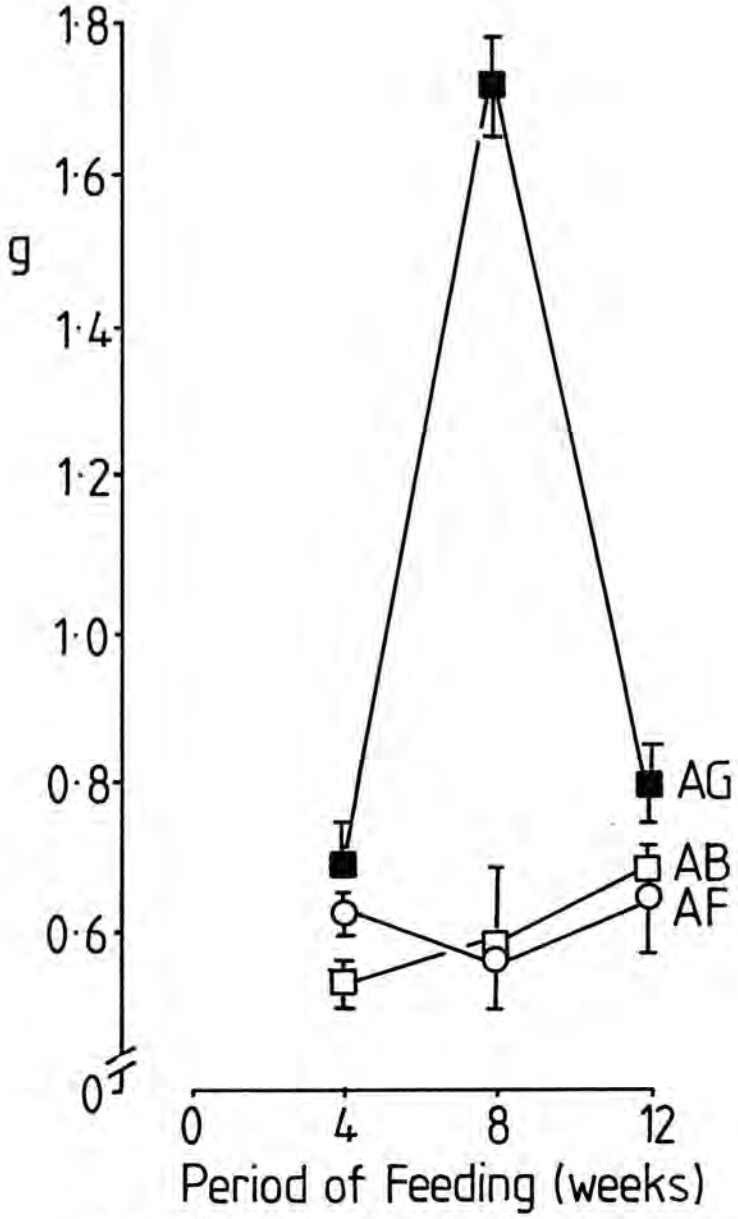


Diet alone was significant ($p<0.01$)

	DIET
L.S.D. $_{(36)}$	0.40
n =	15

FIGURE 4b Caecal sac wet weight of rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5



Diet, time and the interaction of diet and time (DXT) were significant ($p<0.01$).

	DIET	TIME	D X T
L.S.D.$_{(36)}$	0.11	0.11	0.20
n =	15	15	5

gum arabic resulted in an increase in weight (0.93 ±0.04 g) of 37% with respect to both the AF (0.65 ±0.04 g) and AB diets (0.71 ±0.04 g), between which there was no significant difference. When expressed as a function of liveweight (g/kg), the same pattern of events was apparent. Diet alone was significant ($p<0.01$).

2-6 DIAMINOPIMELIC ACID (DAPA)

a) Total DAPA

(i) Caecal (μmols)

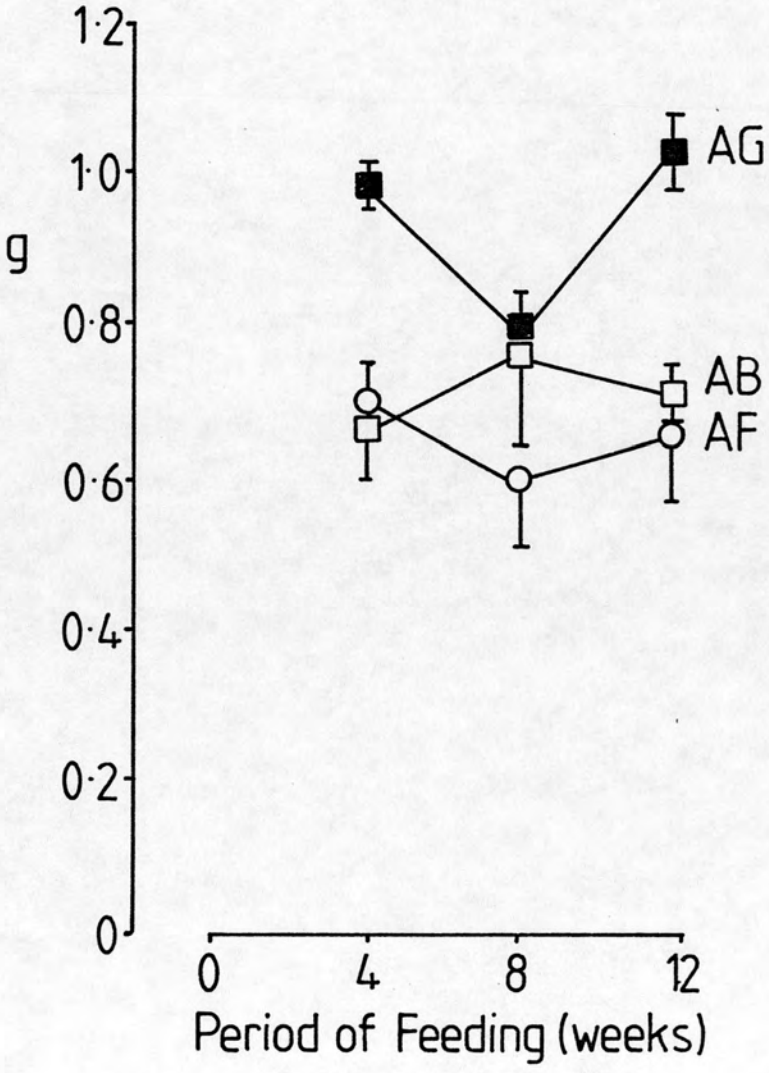
Diet was significant ($p<0.01$) as were time and the interaction of D X T ($p<0.05$). Figure 4d (i) shows that the addition of gum arabic significantly increased total caecal DAPA (6.79 ± 0.43 μmols) ($p<0.01$). There was no significant difference between the AF (2.69 ± 0.21 μmols) and AB diets (2.34 ±0.19 μmols). Only the difference between 8- (3.43 ±0.41 μmols) and 12 weeks (4.49 ± 0.73 μmols) was significant. Total caecal DAPA after 4 weeks was intermediate and not significantly different from either of these two. Figure 4d (i) shows that the concentration from the AG diet fed for 8 weeks was significantly different from the other 8 groups. Within all three diet groups, there was no significant difference between 4- and 8 weeks. Figure 4d (i) shows that the difference between time periods was not the same for each diet, thus making the significance of the main effects less meaningful. Similarities exist between different diets fed for different time periods. For example, total DAPA from the AB diet fed for 4 weeks (1.70 ±0.26 μmols) was not significantly different from that of the AB fed for 12 weeks (2.75 ±0.27 μmols) and the three intermediate groups (Figure 4d (i) . It can be seen that the changes with time were similar for the AF and AB diets.

(ii) Faecal (μmols/day)

Both diet ($p<0.01$) and time ($p<0.05$) were significant. (Figure 4d (ii)). The addition of gum arabic significantly increased total faecal DAPA (10.0 ±0.71 μmols/day). There was no significant difference between the AF (5.07 ±0.41 μmols/day) and AB diets (5.73 ±0.40 μmols/day). Only the difference between 8- (6.16 ±0.54 μmols/day) and 12 weeks (8.00 ±0.90 μmols/day) was significant. Total DAPA after 4 weeks was intermediate and was not significantly different from either of these two. Despite the different pattern of changes occurring within each dietary group, (Figure 4d (ii)), the interaction between diet and time was not significant.

FIGURE 4c Dry caecal content weight of rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5

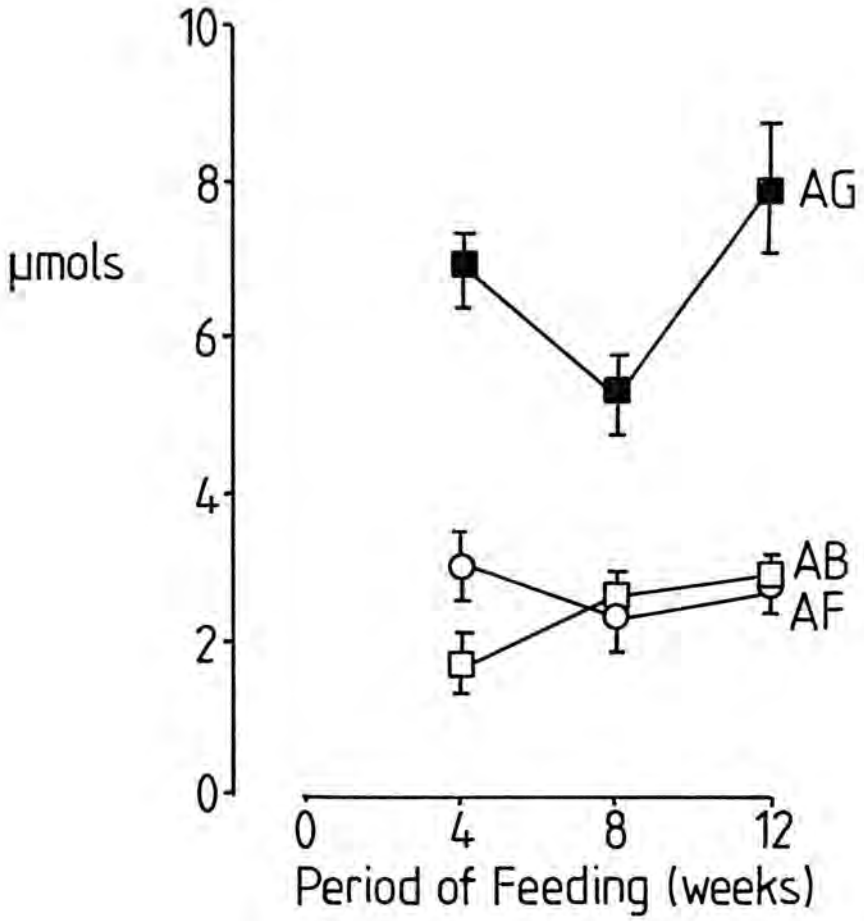


Diet alone was significant ($p<0.01$)

	DIET
L.S.D.$_{(36)}$	0.14
n =	15

FIGURE 4d(i) Total caecal 2-6 DAPA from rats fed an animal origin diet(AF) alone and supplemented with 10 % gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5

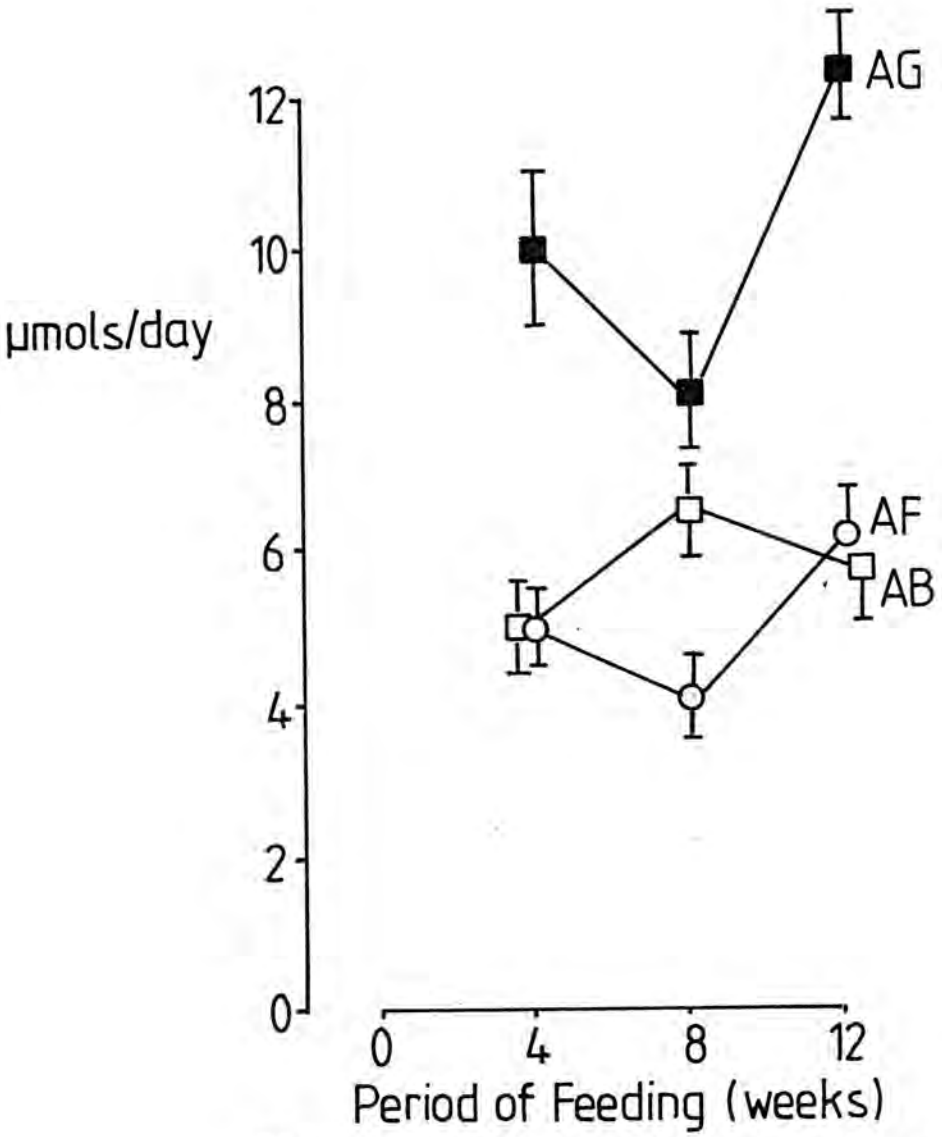


Diet ($p<0.01$), time and the interaction of diet and time(DXT) ($p<0.05$) were significant.

	DIET	TIME	D X T
L.S.D.$_{(36)}$	0.92	0.69	1.20
n =	15	15	5

FIGURE 4d(ii) Total faecal 2-6 DAPA from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5



Diet ($p<0.01$) and time ($p<0.05$) were significant.

	DIET	TIME
L.S.D. (36)	1.77	1.32
n =	15	15

b) Concentration of DAPA

(i) Caecal (μmols/g)

Both diet ($p<0.01$) and time ($p<0.05$) were significant. (Figure 4e (i)). The addition of gum arabic caused a significant increase in the concentration of caecal DAPA (7.23 ± 0.25 μmols/g). All three dietary means were significantly different from each other. Bran decreased the concentration (3.31 ± 0.22 μmols/g) of DAPA. The mean for AF was 4.14 ± 0.17 μmols/g. Concentration increased with time on all diets being greatest after 12 weeks (5.33 ± 0.54 μmols/g). The difference between 4- (4.46 ± 0.53 μmols/g) and 8 weeks (4.72 ± 0.40 μmols/g) was not significant.

(ii) Faecal (μmols/g)

Diet, time ($p<0.01$) and the interaction of D X T ($p<0.05$) were significant, and are shown in Figure 4e (ii). The addition of gum arabic significantly increased faecal DAPA concentration (5.92 ± 0.17 μmols/g). All three dietary means were significantly different from each other. The addition of bran decreased DAPA concentration (2.52 ± 0.12 μmols/g), the concentration with the AF diet was 3.92 ± 0.17 μmols/g. Only the difference between 8(3.84 ± 0.37 μmols/g) and 12 weeks (4.50 ± 0.39 μmols/g) was significant. The concentration of faecal DAPA at 4 weeks was intermediate and not significantly different from either of the two. Figure 4e (ii) shows the nature of the interaction, and that the changes over time was not the same for each diet. Similarities existed between different diets fed for different times. For example all three AG diets were not significantly different from each other, but were significantly greater than the other six groups.

Overall faecal excretion of DAPA (μmols/day) exceeded amounts present in the caecal contents. Gum arabic resulted in the most excretion of faecal DAPA. There appeared to be little difference between faecal concentration of DAPA and caecal concentration of DAPA.

SHORT CHAIN FATTY ACIDS (SCFA's)

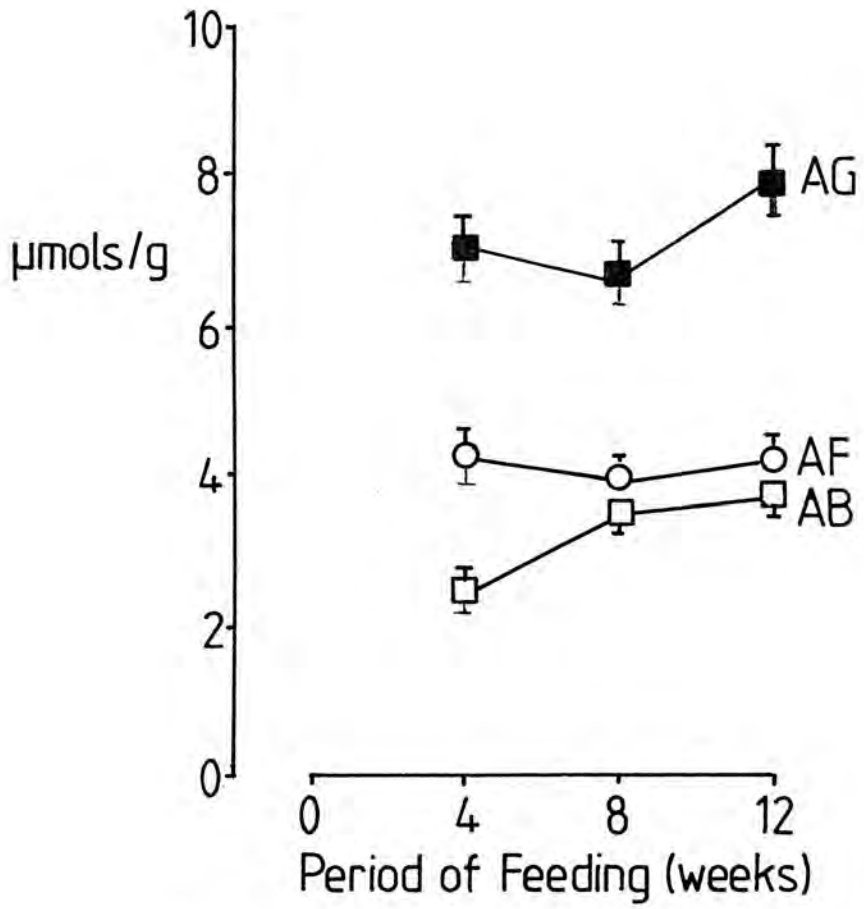
a) Total SCFA's

(i) Caecal (μmols)

Diet, time and the interaction of D X T were significant ($p<0.01$) and are shown in Figure 4f (i). Considering the main effect of diet the addition of gum arabic increased total SCFA's (429 ± 57 μmols) from 129 ± 14.3 μmols (AF).

FIGURE 4e(i) Caecal concentration of 2-6 DAPA from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5

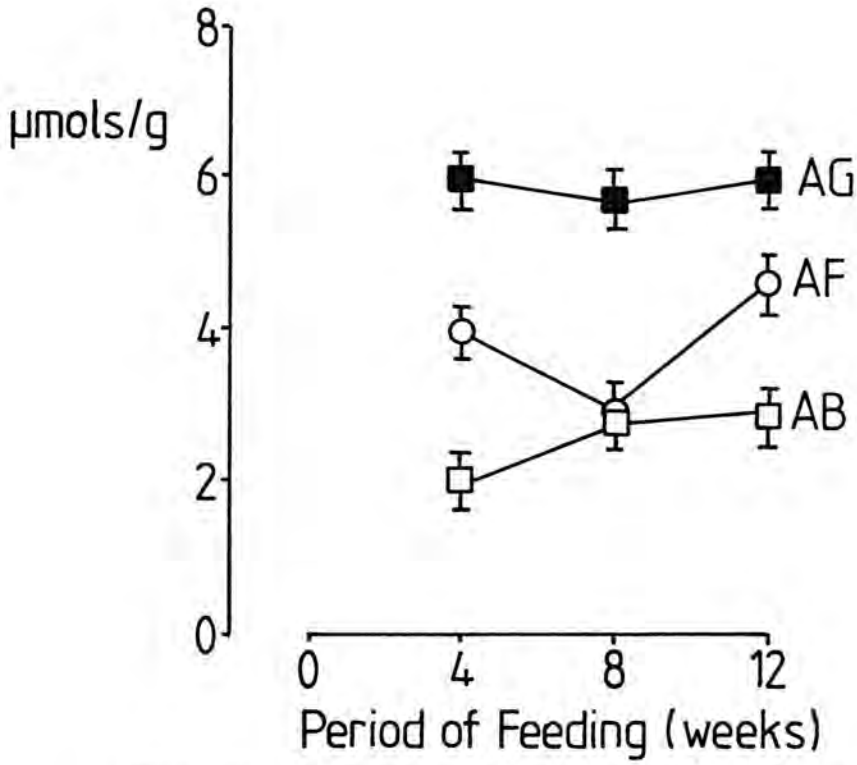


Diet ($p<0.01$) and time ($p<0.05$) were significant.

	DIET	TIME
L.S.D.$_{(36)}$	0.73	0.27
n =	15	15

FIGURE 4 e(ii) Faecal concentration of 2-6 DAPA from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5



Diet , time ($p<0.01$) and the interaction of diet and time (DXT) ($p<0.05$) were significant.

	DIET	TIME	D X T
L.S.D.$_{(36)}$	0.56	0.56	0.73
n =	15	15	5

Total SCFA's with the AB diet was intermediate and significantly different from either of these two. Total SCFA's at all three time intervals, irrespective of diet were significantly different from each other ($p<0.01$): 409 ± 59.5 µmols, 12 weeks; 247 ± 41 µmols, 4 weeks; 153 ± 20.8 µmols, 8 weeks.

Whilst the changes over time were similar for each diet (Figure 4f (i) the interaction of D X T was significant ($p<0.01$), thus making the significance of the main effects less meaningful. Similarities exist between different diets fed for different time periods (Figure 4f(i) For example there was no significant difference between the AF diet fed for 8 weeks and AG fed for 8 weeks and the 4 intermediate values. The AB diet fed for 12 weeks and AG fed for 4 weeks were not significantly different from each other. Total SCFA's from the AG diet fed for 12 weeks was significantly different from the other eight groups ($p<0.01$).

(ii) Faecal (µmols/day)

Diet, time ($p<0.01$) and the interaction of D X T ($p<0.05$) were significant and are shown in Figure 4f (ii). Total faecal SCFA's were less than total overall SCFA's.

Unlike total caecal SCFA's, the addition of bran increased total faecal SCFA's (138 ± 17 µmols/day). The three overall means for the dietary group were significantly different from each other ($p<0.01$): 29.9 ± 4.14 µmols/day, AF and 76 ± 12 µmols/day, AG. Total SCFA's increased with time ($p<0.01$), being greatest after 12 weeks (120 ± 22 µmols/day). The difference between 4- (73.6 ± 44.7 µmols/day) and 8 weeks (50.8 ± 9.11 µmols/day) was not significant. Figure 4f(ii) shows the nature of the interaction ($p<0.05$) and the similarities that exist between different diets fed for different times. For example there was no significant difference between total SCFA's from the AF diet fed for 8 weeks (15.1 ± 3.65 µmols/day) the AG diet fed for 4 weeks (58.7 ± 9.72 µmols/day) and the three intermediate values. Total SCFA's with the AB diet fed for 12 weeks (209 ± 22.6 µmols/day) was significantly from the other eight.

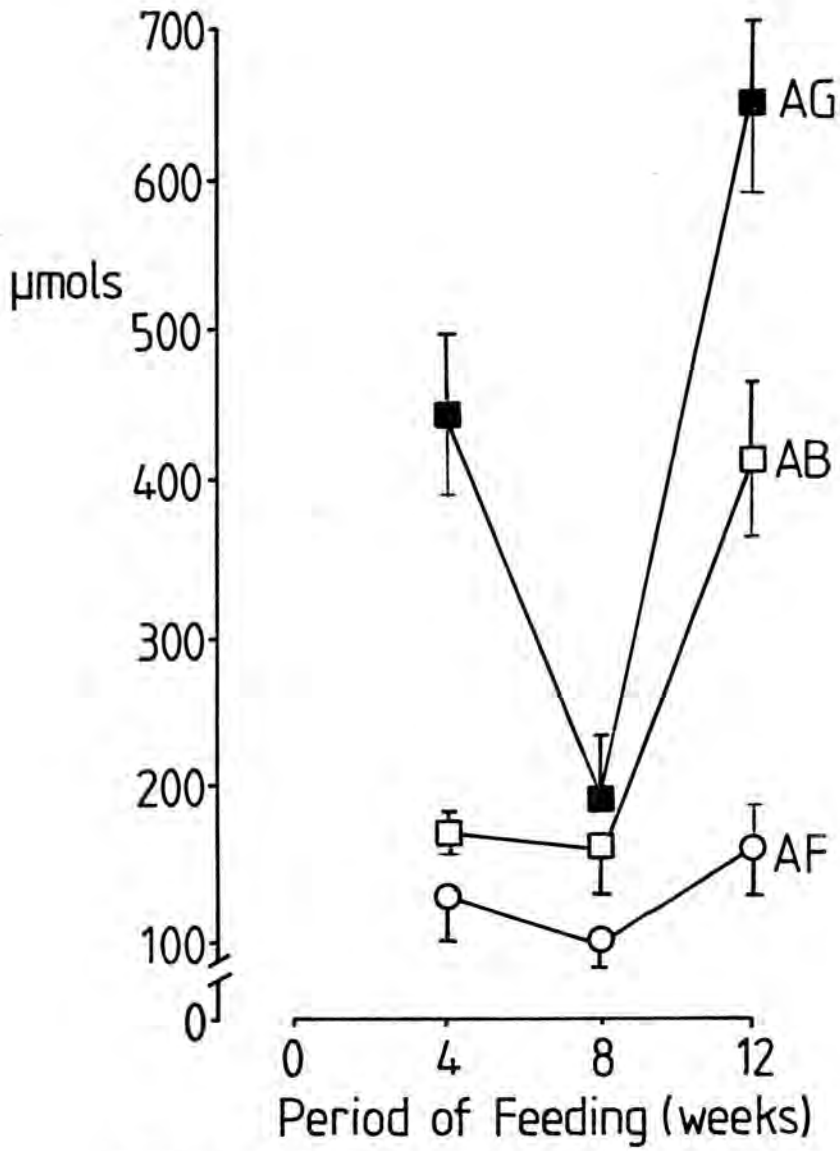
b) Concentration of SCFA's

(i) Caecal (µmols/g)

Diet, time and the interaction of diet with time were all significant ($p<0.01$)

FIGURE 4f(i) Total caecal SCFA's from rats fed an animal origin diet (AF) alone and supplemented with 10 % gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5

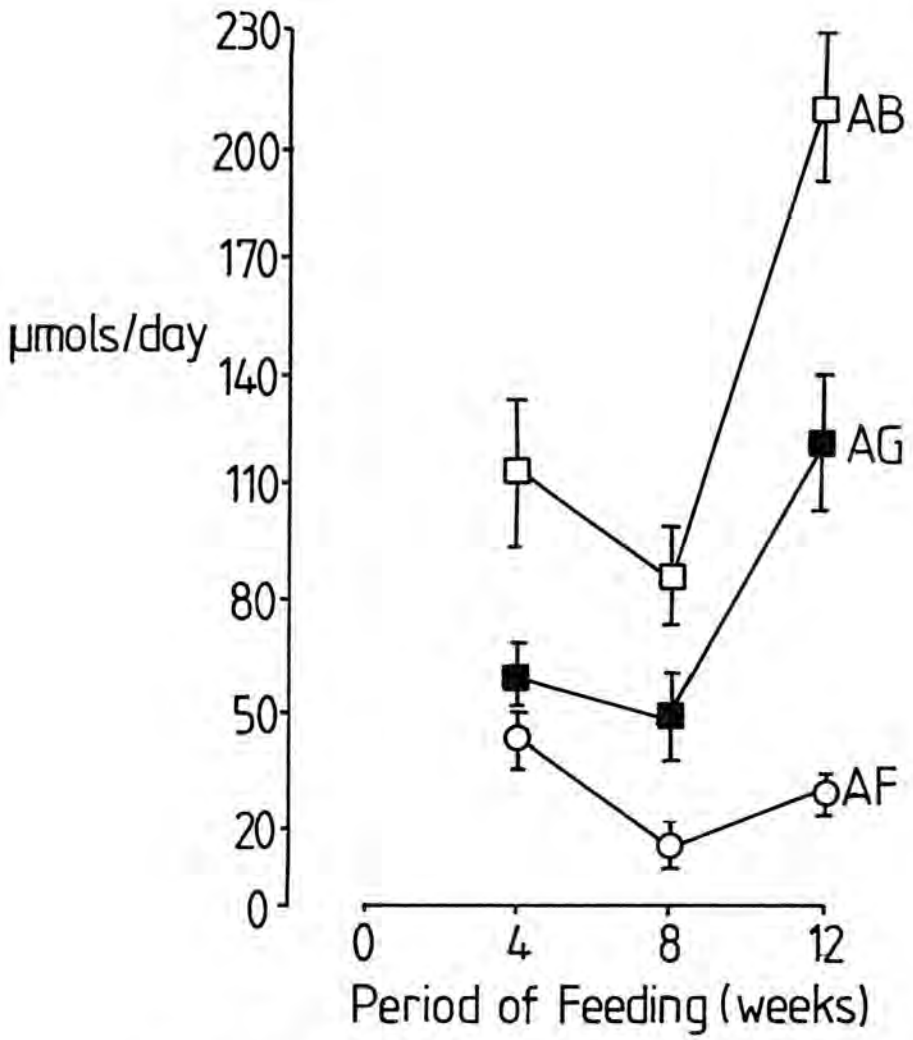


Diet , time and the interaction of diet and time (DXT) were significant ($p<.0.01$).

	DIET	TIME	D X T
L.S.D.$_{(36)}$	85.8	85.8	149
n =	15	15	5

FIGURE 4f(ii) Total faecal SCFA's from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10 % wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5



Diet , time ($p<0.01$) and the interaction of diet and time (DXT) ($p<0.05$) were significant.

	DIET	TIME	D X T
L.S.D.$_{(36)}$	34.1	34.1	44.0
n =	15	15	5

and are shown in Figure 4g(i). Considering the main effect of diet, the overall means for the three dietary groups were significantly different from each other. The addition of gum increased the concentration to (439±49 μmols/g) whilst bran increased the concentration to 354±49 μmols/g from that of the AF diet, 195±17 μmols/g. Within each dietary group, concentrations decreased after 8 weeks of feeding. Considering the main effect of time, the concentration of SCFA's at the three time periods were significantly different from each other ($p<0.01$). Concentration was greatest after 12 weeks (487± 52.8 μmols/g). Concentration for 8 weeks was 204±19 μmols/g and for 4 weeks (297±35 μmols/g). From figure 4 g (i) the nature of the interaction can be seen, and the similarities that exist between different diets fed for different time intervals. For example the concentration with the AF diet fed for 8± weeks (166±22 μmols/g), AB diet fed for 4 weeks (265±12 μmols/g) and the four intermediate values.

(ii) Faecal (μmols/g)

Diet, time and the interaction of D X T were significant ($p<0.01$) and are shown in Figure 4g (ii). The addition of bran, increased the faecal concentration of SCFA's (65±11 μmols/g). The difference between the AF (24.6±4.7 μmols/g) and AG diets (42.9±4.4 μmols/g) was not significant. With time, concentration increased, the concentration at 12 weeks (63.6±11.4 μmols/g) being significantly greater than at either 8- (28.7±3.95 μmols/g) or 4 weeks (39.9±4.3 μmols/g). The decrease with time was most noticeable with the AF diet. From Figure 4g (ii) it is evident that the changes with time were not the same for different diets, making the significance of the main effects less meaningful. Similarities exist between different diets fed for different time intervals. For example, diet AB fed for 12 weeks (110±18 μmols/g) gave significantly greater concentrations than all the other groups. Diet AF fed for 8 weeks (11.3±1.73 μmols/g), diet AF, 4 weeks (39.8±11.4 μmols) and the four intermediate groups were not significantly different from each other. Faecal concentration was less than caecal concentration, but the trends observed were common to both (Figures 4g (i) and (ii)).

c) The composition of the SCFA's (mmols/mol)

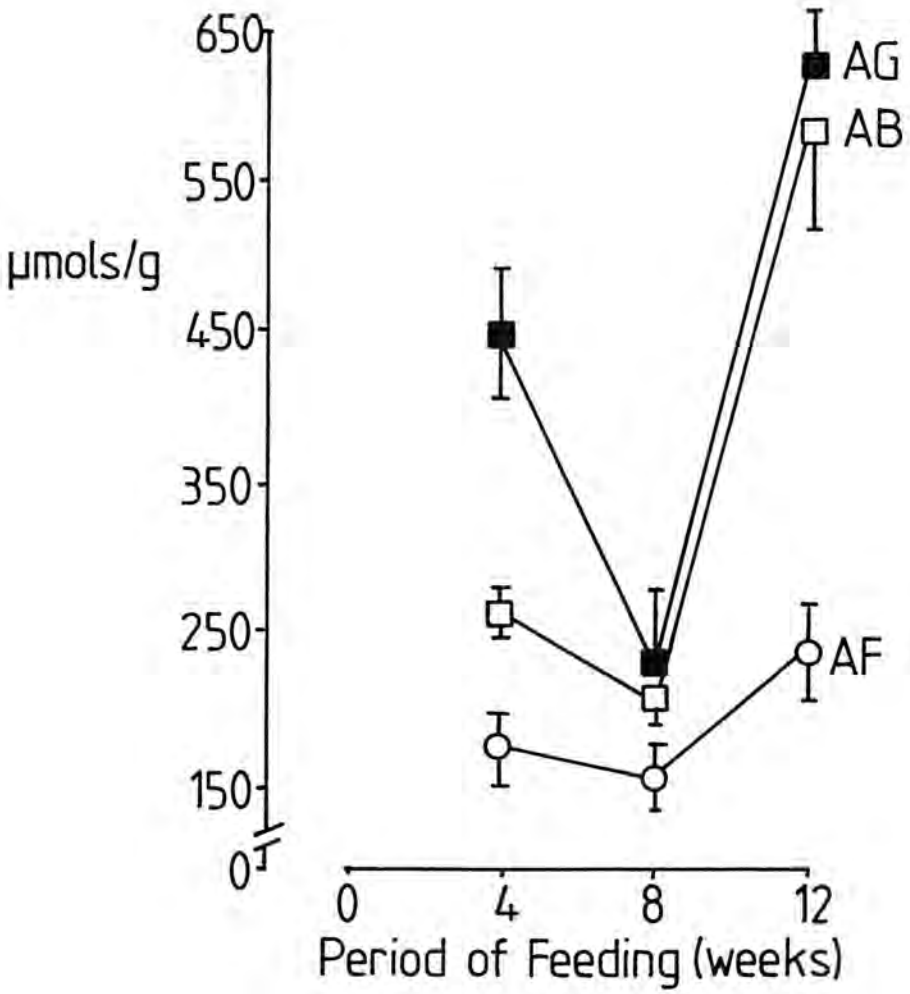
a) Acetate

(i) Caecal

Both diet and the interaction of D X T were significant ($p<0.01$). Considering the main effect of diet, the addition of gum arabic caused a significant increase

FIGURE 4g(i) Caecal concentration of SCFA's from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5

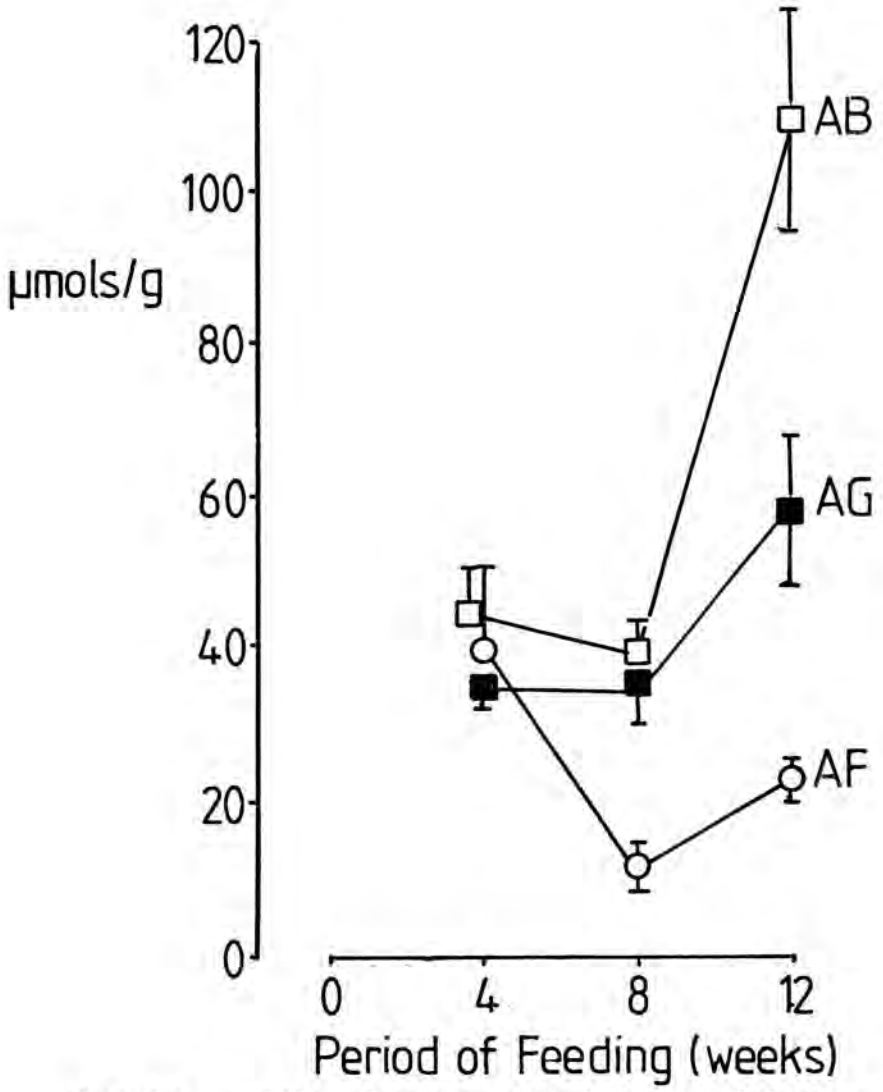


Diet, time and the interaction of diet and time (DXT) were significant.

	DIET	TIME	D X T
L.S.D.$_{(36)}$	82.7	82.7	143
n =	15	15	5

FIGURE 4g(ii) Faecal concentration of SCFA's from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are mean ± S.E.M.
n = 5



Diet, time and the interaction of diet and time (DXT) were significant ($p<0.01$).

	DIET	TIME	D X T
L.S.D.$_{(36)}$	18.5	18.5	32.08
n =	15	15	5

in caecal acetate (712±10 mmols/mol). There was no significant difference between the AB (641±11 mmols/mol) and AF diets (656±12 mmols/mol). From Table 4c the nature of the interaction can be seen, and the different pattern of events that occur, over time with the different diets, and the similarities that exist. For example, the proportion of acetate from diet AB fed for 12 weeks (609±9 mmols/mol), diet AF (647±33 mmols/mol) and the intermediate groups were not significantly different from each other.

(ii) Faecal

Both diet ($p<0.01$) and time ($p<0.05$) were significant. Both gum (882±15 mmols/mol) and bran (914±14 mmols/mol) reduced the proportion of acetate, between which there was no significant difference, with respect to the AF diet (1000 mmols/mol), Table 4d. With time, the proportion significantly increased after 8 weeks (958±14 mmols/mol). There was no significant difference between the overall means of 4- (918±19 mmols/mol) and 12 weeks (921±18 mmols/mol).

b) Propionate

(i) Caecal

Diet alone was significant ($p<0.01$). The addition of bran significantly reduced the proportion of caecal propionate (149±4 mmols/mol). There was no significant difference between the overall mean of the AG (190±5 mmols/mol) and AF diets (192±11 mmols/mol), Table 4c.

ii) Faecal

Both diet ($p<0.01$) and time ($p<0.05$) were significant. Results are given in Table 4d. No propionate was detected with the AF diet. The difference between the AB (86±14 mmols/mol) and AG diets (105±12 mmols/mol) was not significant. Faecal results were considerably less than caecal. Only the difference between 8- (42±14 mmols/mol) and 4 weeks (78±18 mmols/mol) was significant. The proportion after 12 weeks was intermediate and not significantly different from either of these two.

c) Butyrate

(i) Caecal

Diet, and the interaction of D X T were significant ($p<0.01$). Time was significant ($p<0.05$). Considering the main effect of diet, all three dietary means were significantly different from each other. The addition of bran significantly increased the proportion of butyrate (180±9 mmols/mol), whilst gum reduced the proportion of caecal butyrate (83±7 mmols/mol). The mean

TABLE 4c The composition of SCFA's (mmols/mol) in dry caecal contents from rats fed an animal origin deit (AF) alone or supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods. Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	AF	AG	AB	AF	AG	AB	AF	AG	AB
ACETATE	636 ± 12	749 ± 6	639 ± 14	647 ± 33	705 ± 20	675 ± 21	686 ± 6	680 ± 10	609 ± 9
PROPIONATE	208 ± 15	181 ± 5	152 ± 3	198 ± 27	191 ± 4	145 ± 9	169 ± 8	197 ± 15	152 ± 9
BUTYRATE	116 ± 9	60 ± 5	160 ± 13	113 ± 14	85 ± 13	171 ± 7	94 ± 5	104 ± 7	211 ± 13

Table of significant differences, in ascending order from left to right, for each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
ACETATE	AB^a AF^a AG^b		AB^c AF^{cd} AB^{cd} AF^{cde} AB^{de} AG^{de} AF^{def} AG^{ef} AG^f
LSD (36)	38	NS	12 4 4 8 8 12 12 8 4
	**		65 **
PROPIONATE	AB^a AG^b AF^b		
LSD (36)	28.3	NS	NS
	**		
BUTYRATE	AG^a AF^b AB^c	4^a 8^{ab} 12^b	AG^c AG^{cd} AF^{cd} AG^d AF^d AF^d AB^e AB^e AB^f
			4 8 12 12 8 4 4 8 12
LSD (36)	23	17	39
	**	*	**

A different superscript denotes a significant difference.
* = $p<0.05$
** = $0<0.01$
NS = not significant.

TABLE 4d The composition of SCFA's (mmols/mol) in dry faecal material from rats fed an animal origin diet (AF) alone or supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods. Results are given as mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	AF	AG	AB	AF	AG	AB	AF	AG	AB
ACETATE	1000	872 ± 17	881 ± 33	1000	929 ± 29	944 ± 24	1000	844 ± 20	918 ± 4
PROPIONATE	0	116 ± 5	119 ± 33	0	70 ± 29	56 ± 24	0	130 ± 5	82 ± 4
BUTYRATE	0	0	0	0	0	0	0	0	0

Table of significant differences in ascending order from left to right for each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
ACETATE LSD (36)	AG^a AB^a AF^b 42 **	4^c 12^c 8^d 31 *	NS
PROPIONATE LSD (36)	AF^a AB^b AG^b 38 **	8^a $12^{bc}4^c$ 28 *	NS

A different superscript denotes a significant difference.
* = $p<0.05$
** = $p<0.01$
NS = not significant.

for the AF diet was 108±6 mmols/mol. Only the difference between 4- (112± 12 mmols/mol) and 12 weeks (136± 15 mmols/mol) was significant ($p<0.05$): proportions increased with time. From Table 4c, the nature of the interaction of D X T can be seen, and the similarities that can exist between different diets fed for different time intervals. The diet AB fed for 12 weeks (211± 13 mmols/mol) was significantly different from the other eight groups.

(ii) Faecal

No butyrate was detected in any of the nine diet/time groups.

The molar proportions of isobutyrate, isovalerate and valerate are given in Appendix 3a. These were detected in small and fluctuating amounts. None of these were detected in the stool. Diet alone influenced isobutyrate ($p<0.01$). No isobutyrate was detected on the AG diet. There was no significant difference between the AB (12±3 mmols/mol) and AF diets (18±4 mmols).

Both diet and time were significant (12 ±3 mmols/mol) and AF diets (18±4 mmols/mols).

Both diet and time were significant ($p<0.05$) upon isovalerate. No isovalerate was detected with the AG diet. Only the difference between the AG and AF diets (5±2 mmols/mol) was significant. The value for the AB diet was intermediate and not significantly different from either of these two. No isovalerate was detected at 8 weeks on any diet. Only the difference between 8- and 12 weeks (5±2 mmols/mol) was significant.

Only the interaction of DXT was significant upon valerate ($p<0.01$).

HYDROGEN AND METHANE

Hydrogen and methane were not measured.

BILE ACIDS

A pooled sample of faecal and caecal material was used for analysis. As a result, no statistical analysis was made.

a) Total bile acids

(i) Caecal (μmols)

Total caecal and faecal bile acids are given in Figure 4h (i) and (ii). The addition of/....

bran reduced the amount of total bile acids (12.9±2.3 μmols). The mean for the AF diet was 31.2±6.21 μmols, and for the AG diet 23.4±4.90 μmols. Figure 4h (i) shows that the changes over time, were not the same for each diet, which is obscured when considering only the main effects of diet and time. Values, overall, decreased slightly after 8 weeks (20 ± 1.39 μmols) of feeding. The overall mean for 4 weeks was 26± 8.5 μmols and 23 ± 9.36 μmols (12 weeks). Figure 4h(i) shows that there was a similarity between the AF and AB diets fed for 4- and 8 weeks. With time, total caecal bile acids decreased on the AG diet, remain fairly constant on the AB diet and decrease on the AF diet at 8 weeks thereafter increasing to near their 4 week value at 12 weeks.

(ii) Faecal (μmols/day)

Figure 4h (ii) shows that bran resulted in an increase of total faecal bile acids (58.6 ±2.96 μmols/day). There was little difference between the overall means of the AF (50 ± 10.8 μmols/day) and AG diets (41 ± 6.13 μmols). The means for the three time intervals were 52 ± 10.2 (4 weeks), 53 ± 6.9 μmols/day (8 weeks) and 45 ± 7.8 μmols/day (12 weeks). From Figure 4h(ii,) it is apparent that there was an interaction with time, obscured by the means of the main effects. With time, the concentration of bile acids decrease in a linear fashion, on the AF diet, increased on the AG diet, in a linear fashion, and remained fairly constant with the AB diet.

b) Concentration of total bile acids

(i) Caecal (μmols/g)

The concentration was greatest with the AF diet (50± 8.5 μmols/g). The addition of a fibre supplement reduced the concentration of bile acids (Figure 4j (i) The mean for the AG diet was 27.8 ±6.5 μmols/g and for the AB diet, 18.5± 2.5 μmols/g). The overall means of concentration for the three time periods were 33 ±11 μmols/g (4 weeks), 28± 3.70 μmols/g (8 weeks) and 32±16 μmols/g (12 weeks). Figure 4j (i) shows that the changes over the time periods were not the same for each diet.

(ii) Faecal (μmols/g)

There was a close similarity between the diet groups AG (24.9 ±2.8 μmols/g) and AB (25.7± 1.21 μmols/g). Concentration was greatest with the AF diet (39±9.0 μmols/g). (Figure 4j (ii)). Considering time, there was little difference between the three time periods(4 weeks = 31±10 μmols/g; 8 weeks = 32± 6 μmols/g 12 weeks 25 ± 1.9 μmols/g). Figure 4j (ii) shows that the changes with time periods/....

FIGURE 4h(i) Total caecal bile acids from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are means, where n = a pooled sample from five rats.

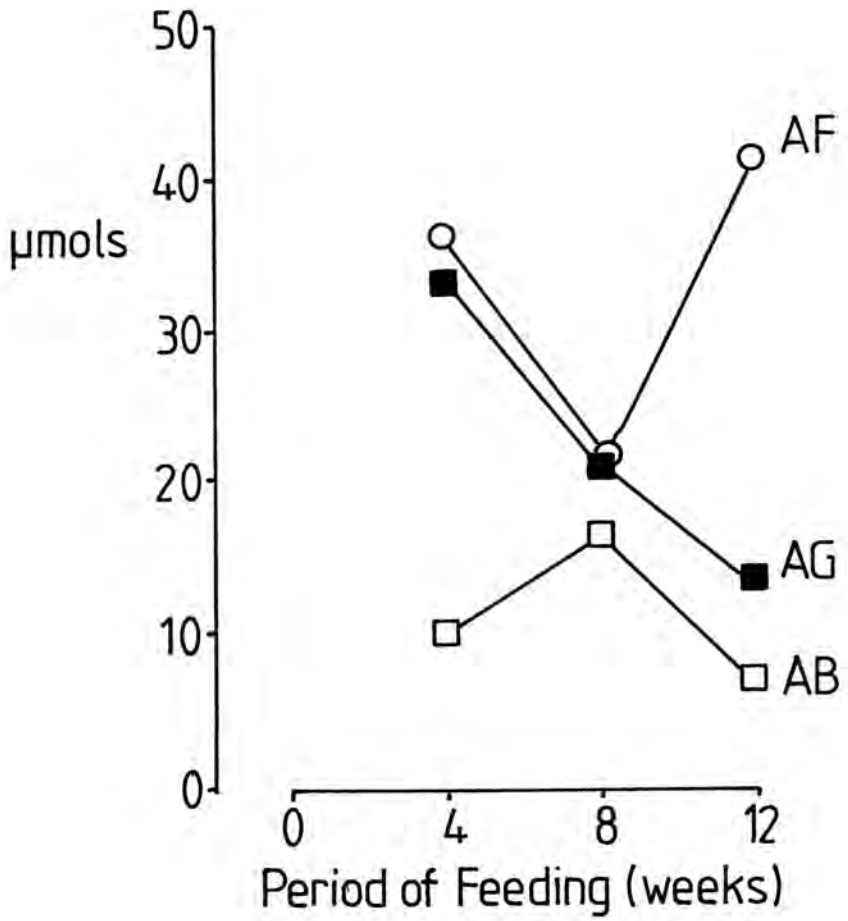


FIGURE 4h(ii) Total faecal bile acids from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are means where n = a pooled sample from five rats.

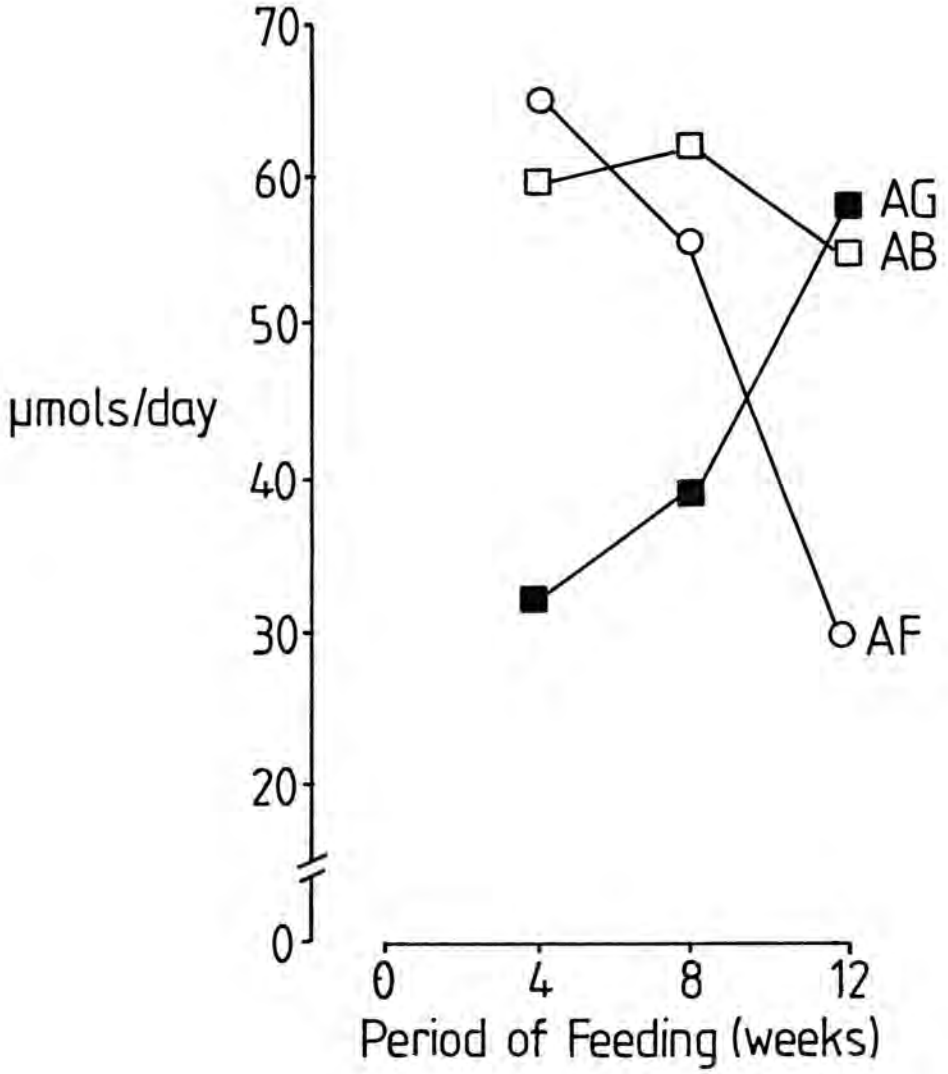


FIGURE 4j(i) Caecal concentration of bile acids from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are means, where n = a pooled sample from five rats

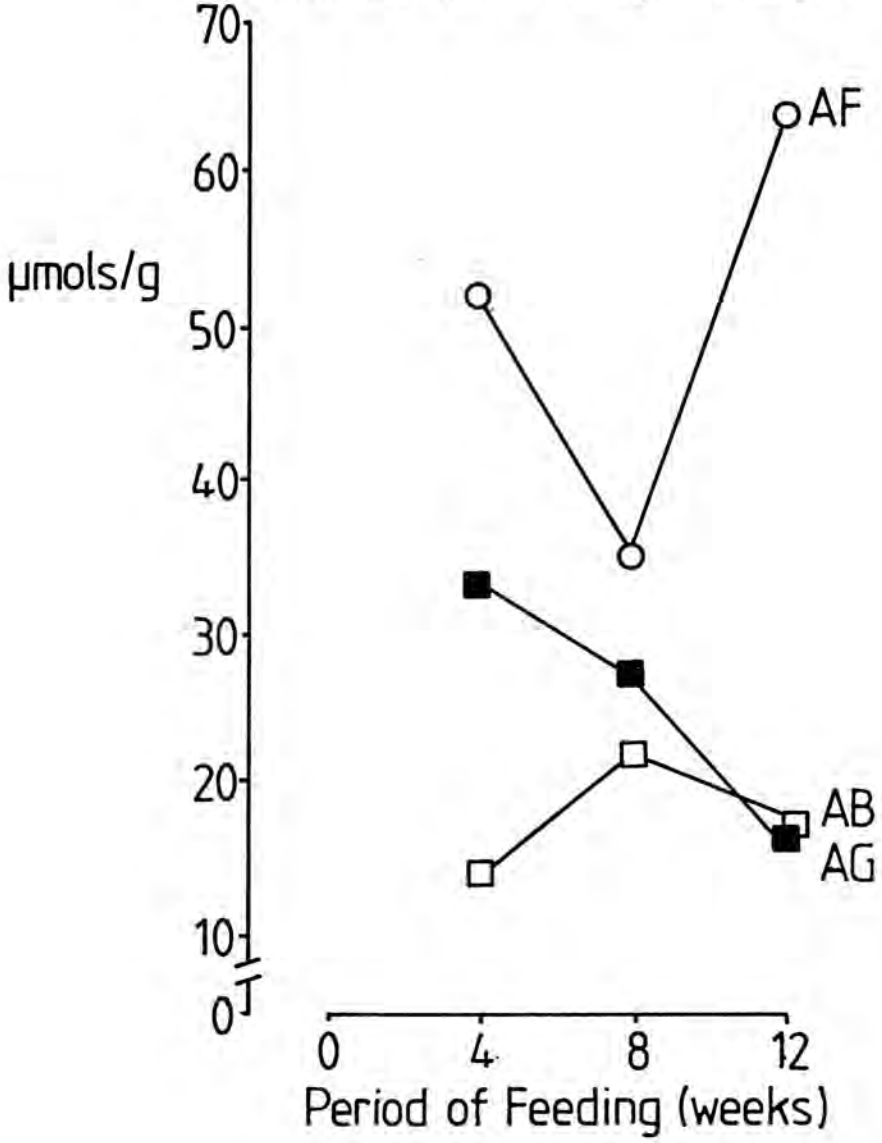
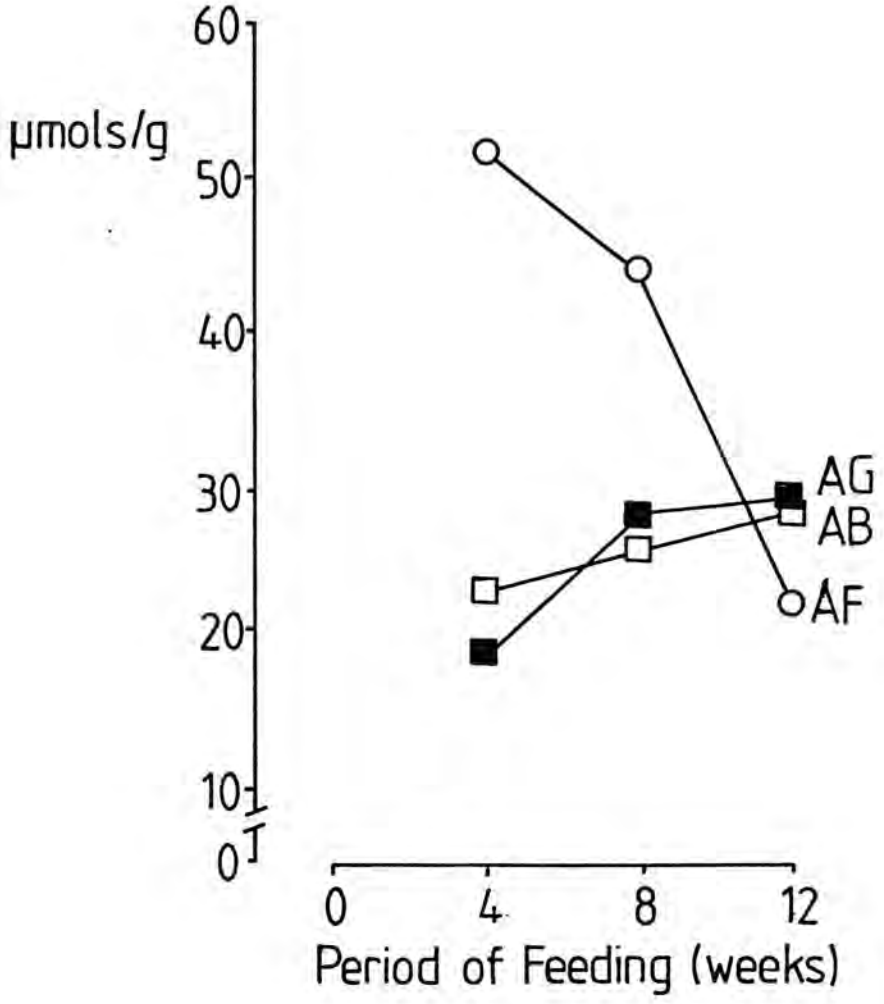


FIGURE 4j(ii) Faecal concentration of bile acids from rats fed an animal origin diet (AF) alone and supplemented with 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Results are means, where n = a pooled sample from five rats.



were not the same for each diet.

Both the AG and AB diets show increasing trends with time, with little difference between the points. The AF diet, however, shows a decrease in the concentration of bile acids over time, the difference between 8- and 12 weeks being greater than that on either the AG or AB diets.

The composition of the total bile acids (mmols/mol)

(i) Caecal

Table 4e (i) gives the individual proportions of each bile acid in dry caecal contents. Deoxycholic- and hyodeoxycholic formed the largest proportion of the individual bile acids. With respect to the other diets, the AG diet substantially reduced the proportion of hyodeoxycholic acid, and to a lesser extent, deoxycholic acid.

The addition of gum arabic did increase the overall proportion of lithocholic- and chenodeoxycholic acid. The appearance of the three muricholic acids was varied and showed no pattern with diet or time. With all the bile acids there appeared to be some degree of an interaction with D X T.

Lithocholic acid: With the AG diet, the proportion of lithocholic acid increased with time from 150 mmols/mol to 194 mmols/mol. With the AF diet the proportion was greatest at 12 weeks (174 mmols/mol) proportions having decreased after 8 weeks (142 mmols/mol) of feeding, thereafter rising. With the AB diet, the proportion peaked after 8 weeks (203 mmols/mol) thereafter decreasing to give the lower proportion at 12 weeks (148 mmols/mol) of the three diet groups AG, AF and AB.

Deoxycholic acid: The AF diet exhibited the greatest proportion at all three time periods. The proportion of deoxycholic acid decreased, in a linear fashion, with time on the AF and AG diets. With time, the proportion increased on the AB diet, the peak being observed at 8-weeks, being slightly greater than at 12 weeks. There was very little difference between the AF (250 mmols/mol) and AB (238 mmols/mol) proportions observed at 12 weeks.

Chenodeoxycholic acid: Both the AF and AB diets exhibited similar proportions. There was little difference between the two diets. Values remained constant with time, increasing slightly after 12 weeks of feeding from an average of 23 mmols/mol to 35 mmols/mol. The AG diet increased the proportion

of chenodeoxycholic acid, by approximately 3 fold at 4 weeks (80 mmols/mol) and 10 fold at (8- and 12 weeks with respect to these diets. The increase between AG (4 weeks) and AG (8 weeks) was greater than the difference between AG (8 weeks) and AG (12 weeks).

Cholic acid: the proportion on the AF diet decreased at 8 weeks (14 mmols/mol) thereafter increasing (127 mmols/mol). On the AB diet the proportion increased between 4-, (43 mmols/mol) and 8 weeks (93 mmols/mol), there being little difference between the proportion at 8- and 12 weeks. On the AG diet the proportion differed little at 4- and 12 weeks.

Hyodeoxycholic acid: Both the AG and AB diets showed similar trends with time: the proportion, decreased after 8 weeks (256 mmols/mol and 378 mmols/mol respectively) and rose again after 12 weeks (338 mmols/mol and 418 mmols/mol respectively) to near to their 4 week values. The proportion on the AF diet in creased between 4- (372 mmols/mol) and 8 weeks (524 mmols/mol) and increased at 12 weeks (379 mmols/mol).

α- muri cholic acid: None was detected with the AG diet. With the AB diet the proportion at 4 weeks was the highest of all the nine groups (75 mmols/mol) with none present at 8 weeks, and the proportion at 12 weeks being near that observed at 4 weeks. None was detected on the AF diet at 4- and 8 weeks. A small amount was detected at 12 weeks (24 mmols/mol).

ω- muri cholic acid: Only at 8 weeks was ω-muri cholic acid detected with the AB diet (49 mmols/mol). Only at 12 weeks was a small amount detected with the AF (40 mmols/mol). With the AG diet (67 mmols/mol) the proportion at 4 weeks was greater than all the other 8 groups. After 12 weeks none was detected. The decrease was of a linear fashion.

β- muricholic acid: none was detected with the AB diet. Only at 8 weeks (47 mmols/mol) was a small amount detected on the AG diet. Only at 12 weeks was a small amount detected on the AF diet (36 mmols/mol).

(ii) Faecal

Table 4e (ii) gives the individual proportions of each bile acid in dry stool material. Lithocholic-deoxycholic- and hyodeoxycholic acid formed the largest

TABLE 4e (i) The composition of bile acids (mmols/mol) in pooled caecal material from rats fed an animal origin diet (AF) alone or supplemented with either 10% gum arabic (AG) or 10% wheat bran (PB), for three different time periods. Results are means

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	AF	AG	AB	AF	AG	AB	AF	AG	AB
Lithocholic acid	154	150	151	142	167	203	179	194	148
Deoxycholic acid	331	273	194	293	191	256	250	163	238
Chenodeoxycholic acid	24.8	79.9	21.5	28.3	200	23.3	38.1	194	32.8
Cholic acid	119	92.0	43.0	14.2	83.7	93.0	127	113	98.4
Hyodeoxycholic acid	372	337	516	524	256	378	379	338	418
α-muricholic acid	0	0	75.3	0	0	0	23.8	0	65.6
ω-muricholic acid	0	67.5	0	0	55.8	48.8	40.5	0	0
β-muricholic acid	0	0	0	0	46.5	0	35.7	0	0

proportions with each diet. Of the three muri cholic acids, ω-mur cholic acid formed the largest proportion. With respect to the other diets the addition of gum reduced the proportion of deoxycholic acid whilst the addition of bran reduced the proportion lithocholic- and cholic acid, and increased the overall proportion of β-muri cholic acid. With all the bile acids there appeared to be some degree of an interaction of D X T; the trends with time were not the same for each diet.

Lithocholic acid: both the AF and AB diets exhibited similar trends, the proportion increased between 8- and 12 weeks from 195 mmols/mol to 259 mmols/mol (AF) and from 176 mmols/mol to 203 mmols/mol (AB). The proportion decreased from 4- to 12 weeks on the AG diet, from 250 mmols/mol to 181 mmols/mol. There was little difference between the proportions with AG and AB diets at 12 weeks (181 mmols/mol and 203 mmols/mol respectively).

Deoxycholic acid: with the AG diet the proportion increased from 125 mmols/mol (4 weeks) to 153 mmols/mol (8 weeks) and then decreased to 132 mmols/mol (12 weeks). With the AB diet the proportion increased with time from 184 mmols/mol (4 weeks) to 196 mmols/mol (12 weeks). With the AF diet, the proportion decreased from 4- (269 mmols/mol) to 12 weeks (171 mmols/mol) the difference between 4- and 8 weeks being greatest. At 12 weeks the AB diet gave the greatest proportion of deoxycholic acid.

Chenodeoxycholic acid: with the AG diet values decreased at 8 weeks (20.4 mmols/mol) of feeding, increasing again after 12 weeks (45.4 mmols/mol) to near the proportion observed at 4 weeks. With the AB diet values increased with time, the proportion at 8 weeks (38.6 mmols/mol) being slightly greater than at 12 weeks. With the AF diet, the proportion decreased from 43.1 mmols/mol (4 weeks) to 28.4 mmols/mol at 8 weeks of feeding and remained constant. At 12 weeks, the AG diet gave the greatest proportion. There was no difference between the AF and AB diets.

Cholic acid: the AB diet gave the lowest proportions which changed little with time (65.8 mmols/mol, 12 weeks to 72.0 mmols/mol, 4 weeks). With the AF diet the proportion increased linearly with time from 106 mmols/mol (4 weeks) to 130 mmols/mol (12 weeks). With the AG diet the proportion was high (147 mmols/mol) at 4 weeeks and increased slightly at 8 weeks (158 mmols/mol) but decreased after 12 weeks. There was no difference between the AG and AB diets at 12 weeks.

TABLE 4e (ii) The composition of bile acids (mmols/mol) in pooled faecal material from rats fed an animal origin diet (AF) alone or supplemented with either 10% gum arabic (AG) or 10% wheat bran (AB) for three different time periods. Results given are means.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	AF	AG	AB	AF	AG	AB	AF	AG	AB
Lithocholic acid	198	250	184	195	214	176	259	181	203
Deoxycholic acid	269	125	184	182	153.0	195	171	132	196
Chenodeoxycholic acid	43.1	37.5	13.4	28.4	20.4	38.6	30.8	45.4	29.3
Cholic acid	106	147	72.0	123	158	80.5	130.	71.8	65.8
Hyodeoxycholic acid	332	403	358	433	418	385	373	470	397
α-muricholic acid	6.15	0	0	0	0	0	0	0	14.6
ω-muricholic acid	29.2	37.5	137	23.0	20.4	80.5	37.7	56.7	43.9
β-muricholic acid	15.4	0	51.8	10.7	15.3	45.1	0	41.6	51.2

Hyodeoxycholic acid: there was little difference between the proportions associated with each of the nine groups. Both the AG and AB diets showed increasing trends with time. With the AF diet, the proportion peaked at 8 weeks thereafter decreasing to near its 4 week value, at 12 weeks.

α-muricholic acid: Only 6.15 mmols/mol was detected with diet AF (4 weeks) and 15 mmols/mol with diet AB (12 weeks). None was detected with diet AG.

ω-muricholic acid: the proportion decreased from 137 mmols/mol (4 weeks) to 44 mmols/mol (12 weeks) on the AB diet. The AG and AF showed similar trends with time, there was very little difference between the six values. At 4 weeks the proportion with the AB diet was 3 fold greater than that of either the AF or AG diets. At 12 weeks there was little difference between the three diet groups.

β-muricholic acid: the AB diet produced the greatest proportion, and this remained constant with time (52 mmols/mol to 52 mmols/mol). The proportion on the AG diet increased from zero to 42 mmols/mol whilst that on the AF diet decreased from 15 mmols/mol to zero.

SUMMARY

This Chapter has given the results of the experimental feeding trial using an animal origin diet (AF). The AF diet was fed unsupplemented and with 10% (by dry weight) gum arabic (AG) or 10% wheat bran (AB). The effect of these diets upon chosen measurements was investigated, concomitant with the effect of the feeding duration. A two factor ANOVA was used to analyse the results. A summary of the results is given below and discussed in detail in Chapter 8.

(1) The rats did not like the diet, as indicated by the decrease in live-weight (g). Those on the AF diet lost most weight. The addition of bran increased live-weight ($p<0.01$). An interaction between D X T was significant ($p<0.01$).

(2) Liver wet weight (g), reflected the decrease in live-weights. The addition of bran increased weights ($p<0.01$).

(3) Stool weight (g/day) was increased with wheat bran, but not gum arabic. When expressed as function of live-weight (g/kg), the difference between the AG and AB diet, stool weight was not significant.

(4) The C.S.W.W. (g) was increased with gum arabic ($p<0.01$) but not with wheat bran. Weights were significantly greater at 8 weeks. The interaction of D X T was significant ($p<0.01$): the trends of changes over time within each diet were not the same. When expressed as a function of live-weight (g/kg) the same pattern of events was observed ($p<0.01$).

(5) The weight of the dry C.C. was increased with gum arabic but not wheat bran ($p<0.01$). When expressed as a function of live-weight (g/kg) the same dietary significance was observed ($p<0.01$).

(6) Caecal DAPA (µmols) was increased with gum arabic but not with wheat bran. DAPA decreased after significantly increasing. The interaction of D X T was significant ($p<0.05$). Faecal DAPA (µmols/day) was increased with gum arabic but not with wheat bran ($p<0.01$). DAPA mass decreased after 8 weeks thereafter significantly increasing ($p<0.05$).

(7) Caecal DAPA (µmols) was increased with gum arabic and decreased with wheat bran ($p<0.01$). Concentration increased with time on all diets, the concentration at 12 weeks being significantly greater ($p<0.01$). Faecal DAPA (µmols) increased with gum arabic and decreased with wheat bran ($p<0.01$). Concentration decreased at 8 weeks and thereafter significantly increased ($p<0.01$). The interaction of D X T was significant ($p<0.01$).

(8) Caecal SCFA's (µmols) increased with gum arabic and wheat bran ($p<0.01$) with respect to AF. Total SCFA's increased with time ($p<0.01$). The interaction of D X T was significant ($p<0.01$) indicating the different trends over time associated with each diet. Faecal SCFA's (µmols/day) were increased with bran ($p<0.01$), the means from the three diets being significantly different from each other. SCFA's increased significantly between 8- and 12 weeks ($p<0.01$). The interaction of D X T was significant ($p<0.05$).

(9) Caecal SCFA's (µmols/g) were increased with gum arabic and wheat bran the difference being significant ($p<0.01$). All three diet means were significantly different from each other. Concentration increased with time and was greatest after 12 weeks ($p<0.01$). Concentrations at all three time periods were significantly different from each other. The interaction of D X T was significant ($p<0.01$). Faecal SCFA's (µmols/g) were increased with bran and not gum arabic. Concentrations increased with time the concentration at 12 weeks being significantly greater than the other two. The interaction of D X T was significant ($p<0.05$).

(10) Of the SCFA's acetate formed the largest proportion (mmols/mol) in caecal and faecal contents. Caecal acetate was increased with gum arabic. The interaction of D X T was significant ($p<0.01$). Faecal acetate was decreased with gum arabic and wheat bran ($p<0.01$) with respect to the AF diet. The proportion was significantly greater at 8 weeks ($p<0.05$). Caecal propionate was reduced with wheat bran ($p<0.01$). No faecal propionate was detected with the AF diet ($p<0.01$). There was no significant difference between the AG and AB diets. Caecal butyrate was increased with wheat bran ($p<0.01$) and decreased with gum arabic. The proportion increased with time ($p<0.05$). The interaction of D X T was significant ($p<0.01$).

(11) H_2 and CH_4 were not measured.

(12) Caecal bile acids (µmols) were reduced by the addition of wheat bran. There appeared to be little difference between the overall means for the time periods, but the trends over time for each diet were different. Faecal bile acids (µmols) were increased with bran. There was little difference between the three overall means for the three time periods, but the trends over time for each diet were different. The concentration (µmols/g) of bile acids was reduced with bran and gum in both caecal and faecal material. The trends over time were not the same for the different diets.

(13) Of the individual bile acids in the caecal contents deoxycholic- and hyodeoxycholic acid formed the largest proportion (mmols/mol) with all three diets. The AG diet reduced the proportion of hyodeoxycholic acid and deoxycholic acid. The addition of gum did increase the proportion of lithocholic- and chenodeoxycholic acids, - and muricholic acids, which decreased to zero between 8- and 12 weeks of feeding. The pattern of the changes in the proportion of the individual bile acids over time were not similar for each diet and were indicative of the interaction of D X T. Of the faecal bile acids, lithocholic- deoxycholic- and hyodeoxycholic acid formed the largest proportions for each diet. Overall the addition of gum reduced the proportion of deoxycholic acid and the addition of bran reduced the proportion of lithocholic and cholic acid. Of the three muricholic acids, ω-muricholic acid formed the largest proportion. Bran increased the proportion of β-muricholic acid. The pattern of the changes in the proportion of the individual bile acids over time were not similar for each diet and were indicative of the interaction of D X T.

CHAPTER 5

EXPERIMENT III: TO INVESTIGATE THE EFFECTS OF THE DURATION OF FEEDING AN ELEMENTAL DIET (FLEXICAL) WITH AND WITHOUT A FIBRE SUPPLEMENT UPON THE CAECAL METABOLISM AND STOOL WEIGHT OF THE ALBINO, ADULT MALE WISTAR RAT

MATERIALS AND METHODS

The experimental design, animal housing conditions materials (diets and supplements) and methods for this experiment have been described in Chapter 2. The only difference in the final composition of the elemental diets was the inclusion of a known quantity of gelatine to enable the diets to set. The dry weights of diet and supplement, w/w, were otherwise the same as used for the PF and AF experiments of Chapters 3 and 4.

All changes due to a supplemented diet are expressed with respect to the unsupplemented diet unless otherwise stated. The F-statistics are given in Appendix 2c.

The results showed a normal distribution and are given as mean $\pm$ S.E.M.

RESULTS

LIVE-WEIGHT

Diet and time were significant ($p<0.01$) upon final live-weight (Table 5a). Only the difference between the gum supplemented diet (EG) (476$\pm$ 11 g) and the bran supplemented diet (EB) (515$\pm$14 g) was significant. The final liveweight for the unsupplemented diet (EF) was intermediate and not significantly different from either of these two. Live-weight was greatest after 12 weeks (545$\pm$ 9 g). On any one diet, final live-weights were greatest at 12 weeks. There was no significant difference in weight after 4- (462$\pm$ 10 g) and 8 weeks (477$\pm$11 g). Initial live-weights are given in Table 5a. When the average live-weight changes are considered (Table 5a) it can be seen that rats fed the EG diet had gained less weight (92$\pm$8 g) than rats fed the EF and EB diets, between which there was no significant difference. Live-weight gain increased with time ($p<0.01$) the three means for the time periods being significantly different from each other (4 weeks = 71$\pm$5 g; 8 weeks = 110$\pm$4 g;12 weeks = 148$\pm$7 g).

LIVER WET WEIGHT

Both diet ($p<0.01$) and time ($p<0.05$) were significant. Table 5b shows that liver wet weights were lightest on the EG diet (16.2$\pm$ 1.82 g). There was no significant difference between the weights from the EF (18.3$\pm$ 0.61 g) and EB diets (19.3$\pm$2.40 g). Only the difference between 8- (16.8 $\pm$0.69 g) and 12 weeks (19.1$\pm$ 0.59 g) was significant, the weight after 4 weeks being intermediate and not significantly different from either of these two. When

TABLE 5a The live-weight (g) and live-weight changes (g) of rats fed an elemental diet (EF) alone or supplemented with 10% gum arabic (EG) or 10% wheat bran (EB), for three time periods.
Results given are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
Rat Initial Weight	402 ± 9	386 ± 20	387 ± 21	337 ± 12	373 ± 13	409 ± 17	390 ± 12	394 ± 5	389 ± 16
Rat Final Weight	482 ± 9	441 ± 16	464 ± 24	436 ± 24	469 ± 13	524 ± 19	560 ± 18	518 ± 17	557 ± 10
Live-weight change	80 ± 1	55 ± 6	76 ± 6	120 ± 9	117 ± 19	115 ± 5	170 ± 11	124 ± 8	149 ± 7

Table of significant differences in ascending order, from left to right, within each group (Chapter 2).

	DIET			TIME			INTERACTION (DXT)
Final live-weight	EG^a	EF^{ab}	EB^b	4^a	8^a	12^b	NS
LSD (36)		36.7 **			36.7 **		
Live-weight change	EG^a	EB^b	EF^b	4^b	8^d	12^e	NS
LSD (36)		15.4 **			15.4 **		

A different superscript denotes a significant difference.
** = $p<0.01$
NS = not significant

TABLE 5b The liver-wet weight of rats fed an elemental diet (EF) alone or supplemented with either 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.
Results given are mean±SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
Liver wet weight (g)	18.7 ± 0.6	16.1 ± 0.8	18.9 ± 1.2	16.1 ± 1.1	15.5 ± 1.1	18.9 ± 1.1	20.2 ± 0.5	17.0 ± 0.5	20.1 ± 1.2
Liver wet weight (g/kg)	38.8 ± 0.97	36.4 ± 0.65	40.7 ± 0.80	36.8 ± 0.97	33.0 ± 1.60	35.9 ± 0.76	36.1 ± 0.73	32.7 ± 0.70	36.0 ± 1.89

Table of significant differences, in ascending order, from left to right, for each group, (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Liver wet weight (g)	EG^a EF^b EB^b	8^c 4^{cd} 12^d	
$LSD_{(36)}$	2.07	1.57	NS
	**	*	
Liver wet weight g/kg	EG^a EF^b EB^b	12^a 8^a 4^b	
$LSD_{(36)}$	2.15	2.15	NS
	**	**	

A different superscript denotes a significant difference
* = $p<0.05$
** = $p<0.01$
NS = not significant.

expressed as a function of live-weight, the same dietary significances were observed ($p<0.01$). With time, the liver wet weight: live-weight was greatest at 4 weeks (3.87 ± 0.01 g). There was no significant difference between the weights at 12- (3.50 ± 0.08 g) and 8 weeks (3.52 ± 0.29 g).

STOOL WEIGHT

Figure 5a shows the dry stool weight of the three diet groups over time. Both diet ($p<0.01$) and time ($p<0.05$) were significant. The addition of bran increased stool weight by approximately 56% to 1.09 ± 0.08 g/day with respect to the EF and EG diets. There was no significant difference of stool weight from the EF (0.70 ± 0.05 g/day) and EG diets (0.67 ± 0.08 g/day). Only the difference between 4- (0.68 ± 0.08 g/day) and 12 weeks (0.91 ± 0.33 g/day) was significant, the value for 8 weeks being intermediate and not significantly different from either of these two. When expressed as a function of live-weight only diet was significant. Bran increased the stool weight: live-weight ratio (2.12 ± 0.14 g/kg). There was no significant difference between the EF (1.44 ± 0.09 g/kg) and the EG diets (1.40 ± 0.16 g/kg).

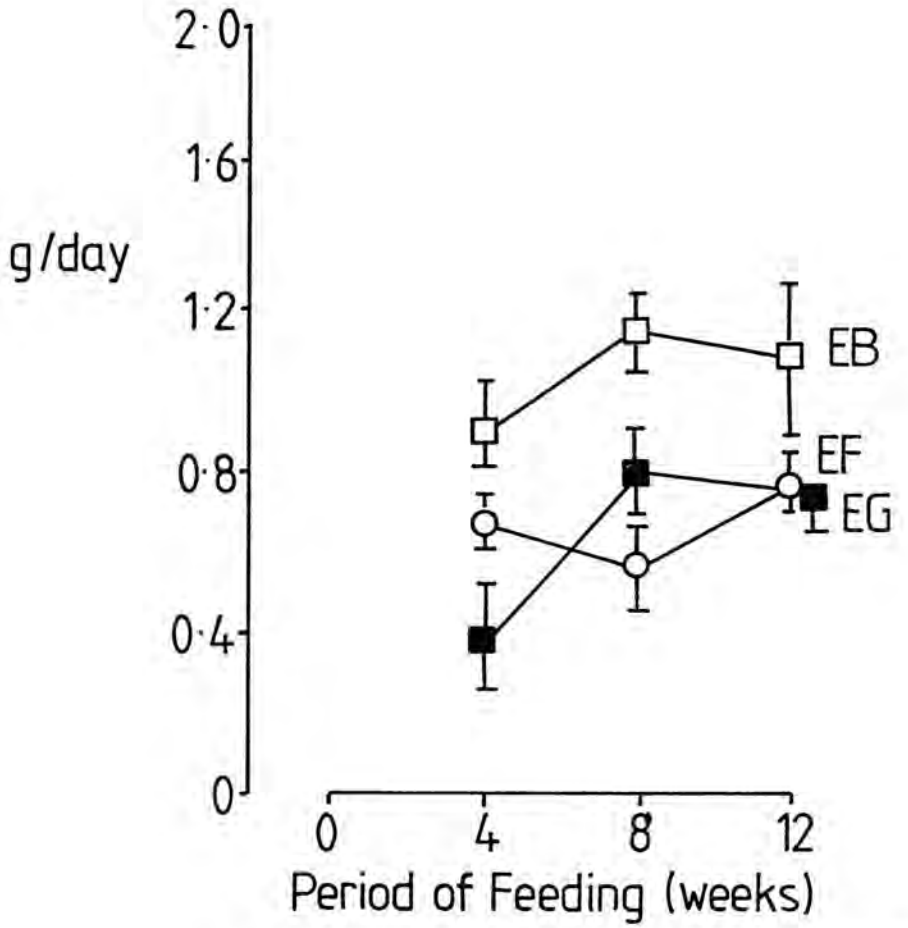
THE CAECUM AND ITS CONTENTS

(i) Caecal sac wet weight (C.S.W.W.)

Diet and the interaction of D X T were significant ($p<0.01$). An interaction of D X T looks at the nine individual groups and describes the similarities that exist between diets fed for different times. It highlights the fact that different diets do not behave in the same way with time. A difference between any two of the nine diet x time groups or between the three dietary means or the three time periods means is significant if it exceeds the tabulated L.S.D. A detailed explanation is given in Chapter 2. Considering the main effect of diet, the addition of gum arabic significantly increased C.S.W.W. (1.20 ± 0.03 g) by 74% with respect to the EF diet (0.69 ± 0.03 g). The C.S.W.W. from the EB diet (0.82 ± 0.04 g) was significantly different from these two. Figure 5b shows that the significance of an interaction makes the significance of the main effects less meaningful. Similarities exist between different diets fed for different times. The C.S.W.W. decreased with time with the EG and EF diets. C.S.W.W. remained constant between 4- and 12 weeks on the EB diet. There was no significant difference between any of the three EG groups all of which were significantly greater than all the others. When expressed as a function of live-weight (g/kg) diet, time and the interaction of D X T were all significant ($p<0.01$). The same changes with time were

FIGURE 5a Daily dry stool weight of rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5

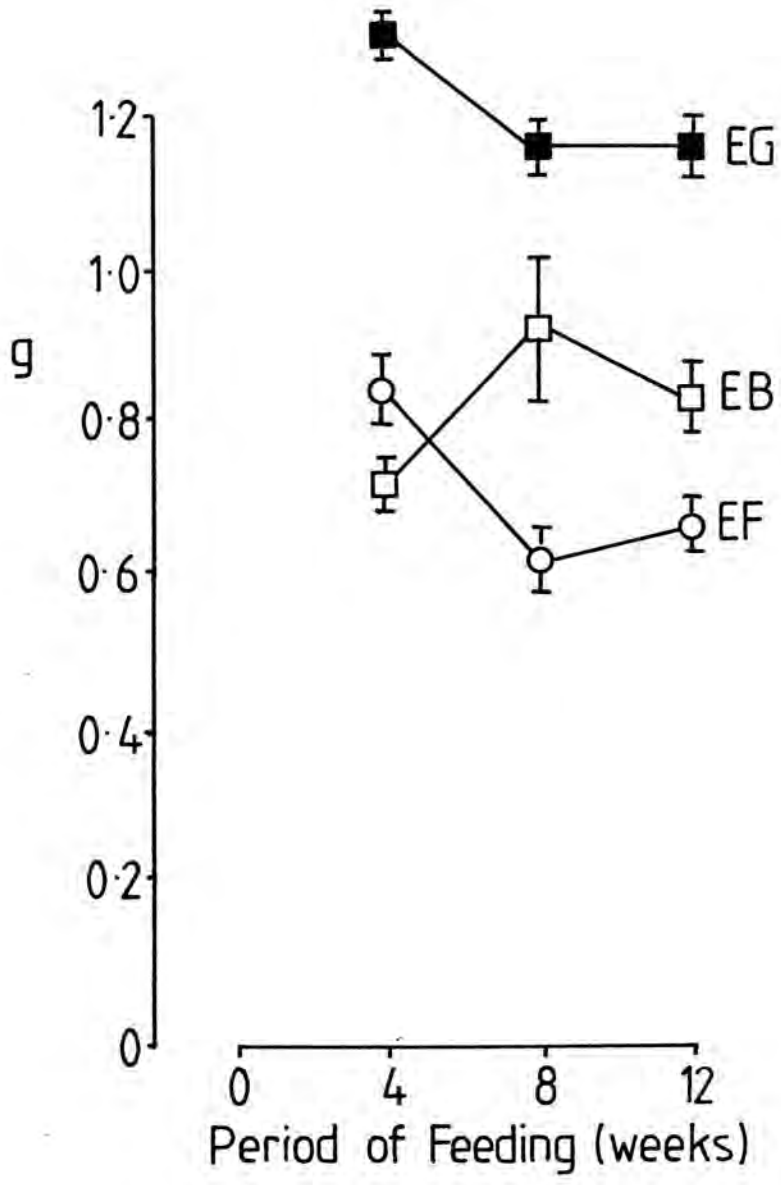


Diet ($p<0.01$) and time ($p<0.05$) were significant.

	DIET	TIME
L.S.D.$_{(36)}$	0.26	0.19
n =	15	15

FIGURE 5b Caecal sac wet weights of rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5



Diet and the interaction of diet and time (DXT) were significant ($p<0.01$).

	DIET	D X T
L.S.D $_{(36)}$	0.10	0.17
n =	15	5

evident. The C.S.W.W. : live-weight ratio was lowest after 12 weeks (1.62 ± 0.13 g/kg) when considering the main effect of time. There was no significant difference between 8- (1.87 ± 0.13 g) and 4 weeks (2.68 ± 0.18 g/kg).

(ii) Dry caecal contents (C.C.)

Diet alone was significant, $p<0.01$, as shown in Figure 5c. The addition of gum arabic significantly increased C.C. (1.06 ± 0.05 g). There was no significant difference between the EF (0.46 ± 0.04 g) and EB (0.54 ± 0.03 g) diets (Figure 5c). When expressed as a function of live-weight (g/kg), diet alone was significant ($p<0.01$). The dry weight of C.C.: live-weight ratio was significantly greater with the EG diet (2.22 ± 0.09 g/kg) ($p<0.01$). There was no significant difference between the EF (0.46 ± 0.04 g/kg) and EB diets (0.54 ± 0.03 g/kg).

2-6 DIAMINOPIMELIC ACID (DAPA)

a) Total DAPA

(i) Caecal (μmols)

Diet alone was significant ($p<0.01$). The addition of gum arabic significantly increased total caecal DAPA by 3 fold, with respect to the other diets, to 10.7 ± 0.61 μmols (EG). There was no significant difference between the EB (2.99 ± 0.25 μmols) and EF diets (3.74 ± 0.33 μmols). Figure 5d (i) shows that the trends observed with EF and EB diets closely paralleled each other.

(ii) Faecal DAPA (μmols/day)

Diet alone was significant ($p<0.01$). Figure 5d (ii) shows that gum arabic significantly increased (7.78 ± 0.73 μmols/day) total faecal DAPA by 68% and 62% with respect to the faecal DAPA from rats fed the EF (4.63 ± 0.36 μmols/day) and EB (4.79 ± 0.38 μmols/day), between which there was no significant difference. The trends associated with the EF and EB diets closely paralleled each other.

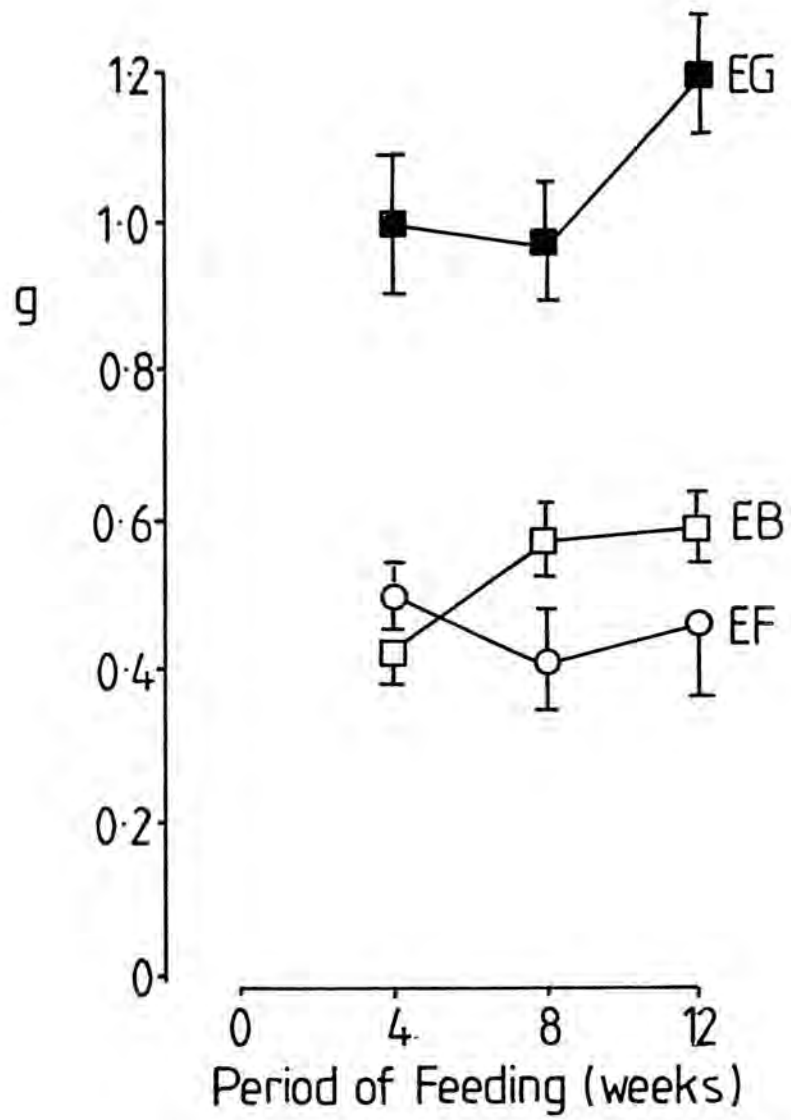
b) Concentration of DAPA

(i) Caecal (μmols/g)

Diet ($p<0.01$) and time ($p<0.05$) were significant (Figure 5e (i)). The addition of gum arabic significantly increased the concentration of caecal DAPA (10.4 ± 0.65 μmols/g) by 26% with respect to the EF diet. Bran significantly reduced

FIGURE 5c Dry caecal content weight of rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5

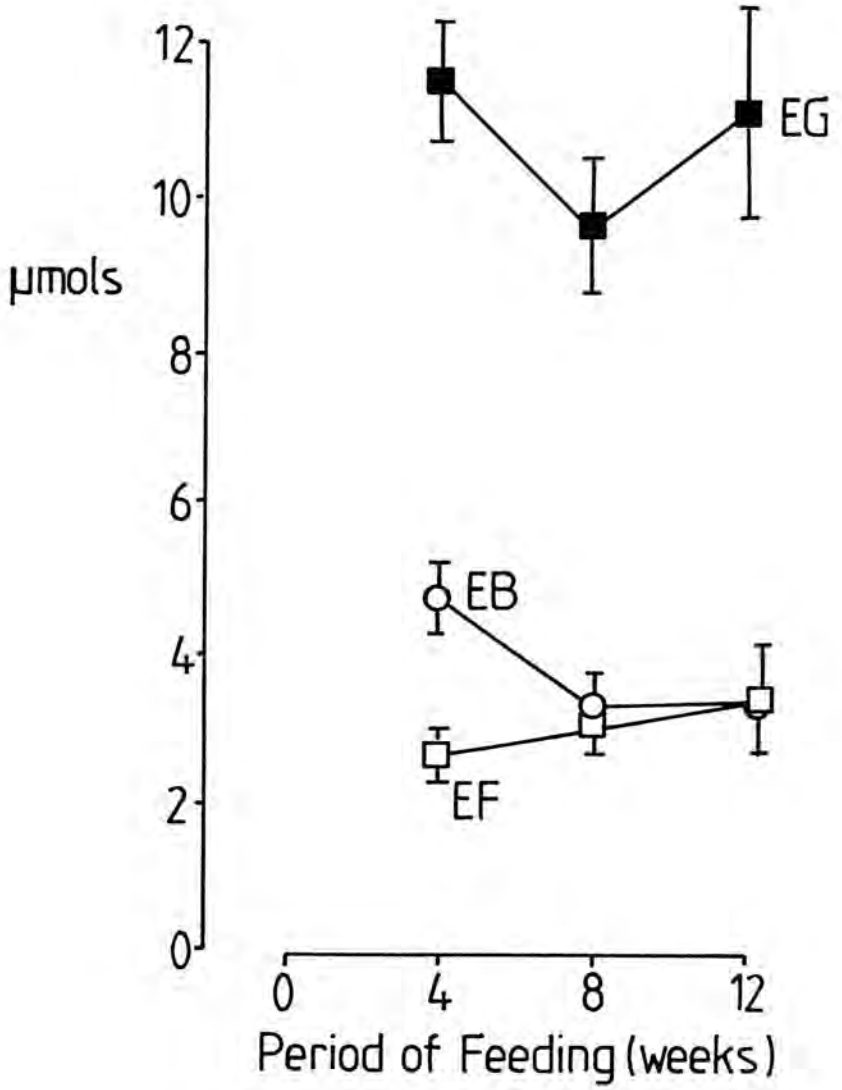


Diet alone was significant ($p<0.01$).

	DIET
L.S.D$_{(36)}$	0.14
n =	15

FIGURE 5d(i) Total caecal 2-6 DAPA from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10 % wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5

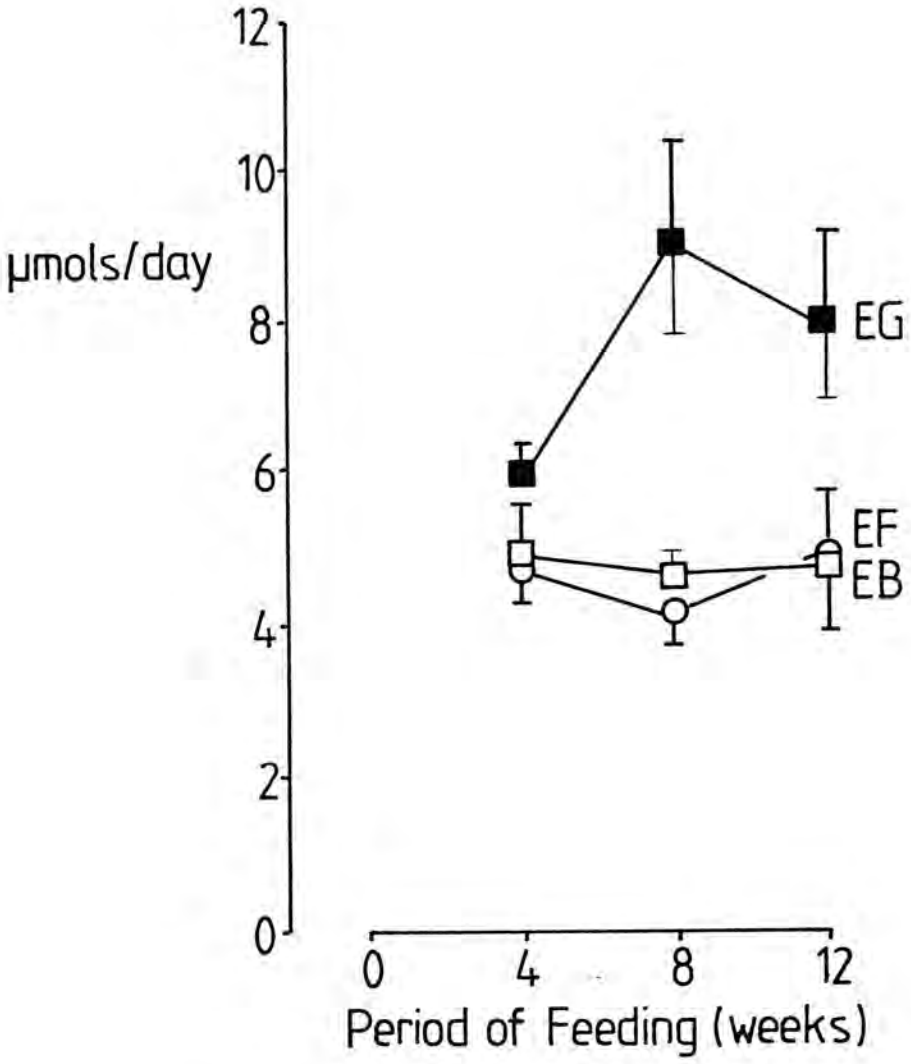


Diet alone was significant ($p<0.01$)

	DIET
$L.S.D._{(36)}$	1.63
n =	15

FIGURE 5d(ii) Total faecal 2-6 DAPA from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5 except EG (4 weeks) where n = 4



Diet alone was significant ($p<0.01$).

	DIET
L.S.D.$_{(35)}$	1.96
n =	15 except diet EG where n= 14

the concentration of DAPA (5.66± 0.42 μmols). The three dietary means were significantly different from each other, the concentration with the EF diet being intermediate (8.26 ± 0.45 μmols/g). Concentration decreased with time by 20% from 9.21± 0.74 μmols/g at 4 weeks to 7.41 ± 0.64 μmols/g ($p<0.05$). There was no significant difference between the concentration at 8- and 12 weeks.

(ii) Faecal (μmols/g)

Diet ($p<0.01$), time and the interaction of D X T ($p<0.05$) were significant. Figure 5e (ii) shows that gum arabic increased the concentration of faecal DAPA (10.9 ± 0.40 μmols/g). Bran decreased the concentration (4.43 ± 0.19 μmols/g). All three dietary means, (EF: 6.66± 0.28 μmols/g), were significantly different from each other. Considering the main effect of time, only the difference between 4- (7.77 ± 0.27 μmols/g) and 12 weeks (6.7 ± 1.20 μmols/g) was significant. The concentration at 8 weeks was intermediate and not significantly different from either of these two. Figure 5e (ii) shows that the changes with time were different from each diet and that similarities exist between different diets fed for different times. For example the concentration with the EG diet (4 weeks) was significantly greater than all the other groups, but that there was no significant difference between the EB diet fed for 4 weeks and the EF diet fed for 12 weeks.

SHORT CHAIN FATTY ACIDS (SCFA's)

a) Total SCFA's

(i) Caecal(μmols)

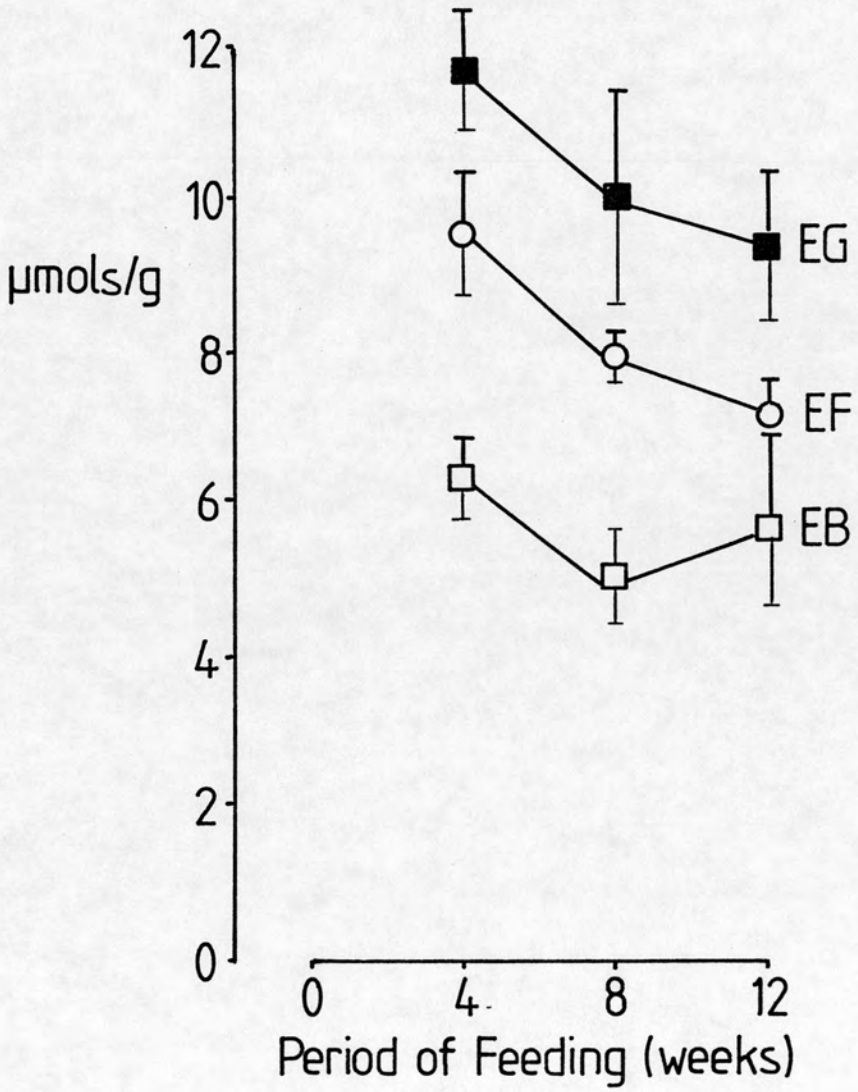
Diet alone was significant ($p<0.01$). The addition of gum arabic caused an increase of 95% in total caecal SCFA's (517 ± 49 μmols) with respect to the overall means of EF (263 ± 27 μmols) and EB (259± 16 μmols) between which there was no significant difference. Figure 5f (i) shows that, the pattern of caecal SCFA's with time, was similar for both EG and EF diets whilst with the EB diet, SCFA's remained virtually constant over time.

(ii) Faecal (μmols/day)

Diet ($p<0.01$) and time ($p<0.05$) were significant, and are shown in Figure 5f (ii). Both the gum arabic and bran supplemented diets increased the total amount of SCFA's in the stool, with respect to the EF diet (57.5 ± 11.5 μmols/day) ($p<0.01$). There was no significant difference between the overall means of the EG diets (116 ± 14.5 μmols/day) and EB (134 ± 16 μmols/day). With time, total SCFA's increased by 2 fold, from 71.3 ± 11.5 μmols/day at 4 weeks. There

FIGURE 5e(i) Caecal concentration of 2-6 DAPA from rats fed an elemental diet alone (EF) and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.EM.
n = 5

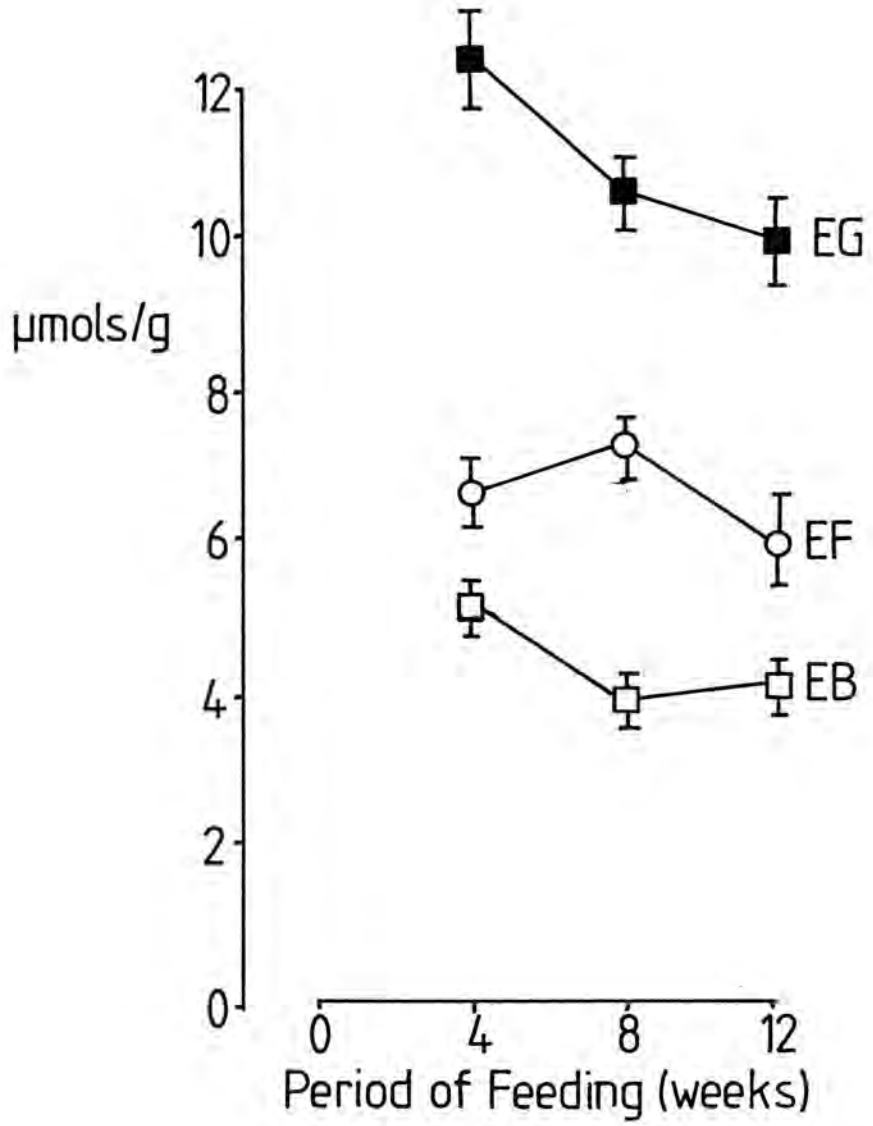


Diet ($p<0.01$) and time ($p<0.05$) were significant.

	DIET	TIME
L.S.D.$_{(36)}$	1.96	1.42
n =	15	15

FIGURE 5e(ii) Faecal concentration of 2-6 DAPA from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic(EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5 except EG (4weeks) where n = 4.



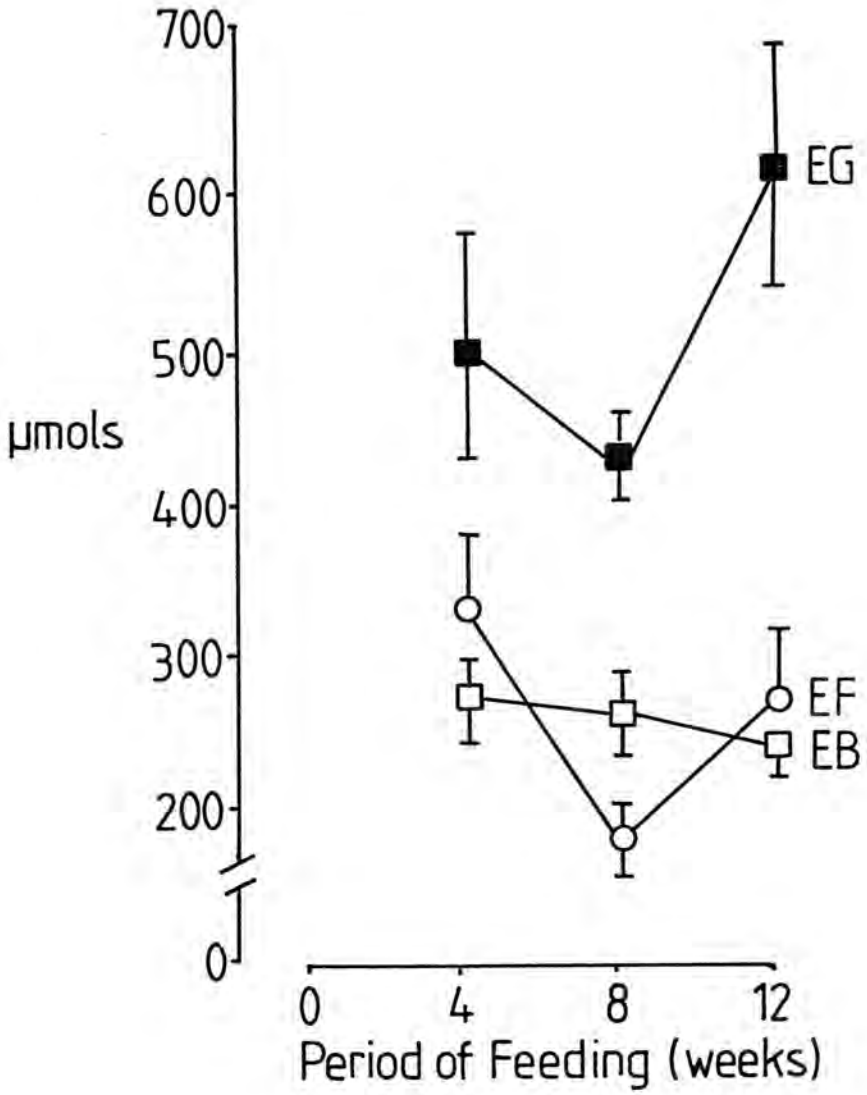
Diet ($p<0.01$), time and the interaction of diet and time (DXT) ($p<0.05$) were significant

	DIET	TIME	D X T
L.S.D.$_{(35)}$	0.93	0.69	1.18
n =	15	15	5

except diet EG, time period 4 weeks where n = 14 and diet EG (4 weeks) where n =4

FIGURE 5f(i) Total caecal SCFA's from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n =5

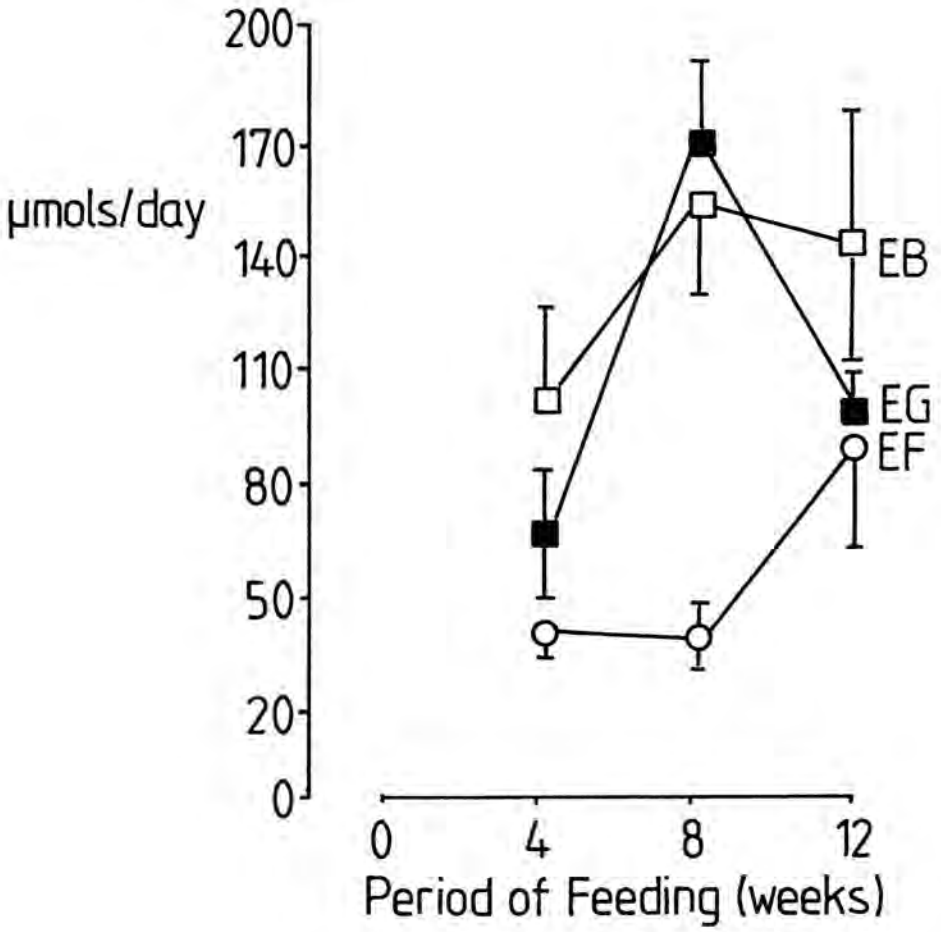


Diet alone was significant ($p<0.01$).

	DIET
L.S.D.$_{(36)}$	123
n =	15

FIGURE 5f(ii) Total faecal SCFA's from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5 except EG (4weeks) where n = 4.



Diet ($p<0.01$) and time ($p<0.05$) were significant.

	DIET	TIME
L.S.D.$_{(35)}$	48.7	36.3
n =	15	15

except diet EG and time period 4 weeks where n = 14

was no significant difference between 8- (120 ± 19 µmols/day) and 12 weeks (112±15.2 µmols/day).

b) Concentration of SCFA's

(i) Caecal (µmols/g)

Time alone was significant ($p<0.01$), Figure 5g (i). With all diets, concentration decreased at 8 weeks (444±21 µmols/g). Concentration was highest at 4 weeks (596 +29 µmols/g). The concentration at 12 weeks was 520± 41 µmols/g . The means for each time interval were significantly different from each other ($p<0.01$).

(ii) Faecal (µmols/g)

Diet, time ($p<0.01$) and the interaction of D X T ($p<0.05$) were significant, (Figure 5g (ii)). Considering the main effect of diet, the addition of gum arabic increased the concentration of SCFA's (158 ± 11 µmols/g). All three dietary means were significantly different from each other. The mean for the EF diet was (78.3 ±10.3 µmols/g) and for the EB diet (120± 8µmols/g). Only the difference between 4- (99± 12 µmols/g) and 12 weeks (133± 16 µmols/g) was significant. The concentration at 8 weeks was intermediate and not significantly different from either of these two. Figure 5g (ii) shows the nature of the interaction. Concentration on the EG diet (8 weeks) was significantly greater than with the other 8 groups. The changes between time periods were not the same for the three diets, thus making the significance of the main effects less meaningful. Figure 5g (ii) shows that similarities exist between different diets fed for different times. For example the concentrations with the EF diet (12 weeks), EG diet (12 weeks) and the four intermediate diet groups EB (4-, 8 and 12 weeks) and diet EG (4 weeks) were not significantly different.

c) The composition of the SCFA's (mmols/mol)

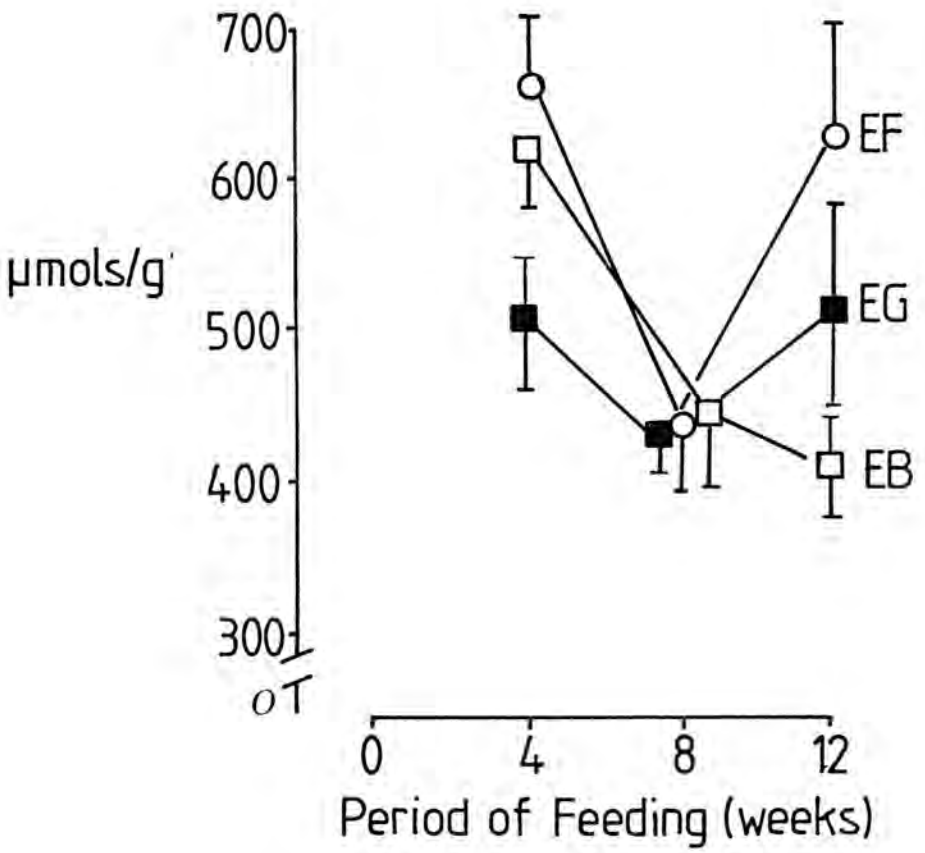
a) Acetate

(i) Caecal

Diet ($p<0.05$) and the interaction of D X T ($p<0.01$) were significant. Results are given in Table 5c. Considering the main effect of diet, only the difference between the EG (614 ± 19 mmols/mol) and EF diets (669 ± 15 mmols/mol) was significant. The proportion with the EB diet was intermediate and not significantly different from either of these two. The/....

FIGURE 5g(i) Caecal concentration of SCFA's from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5

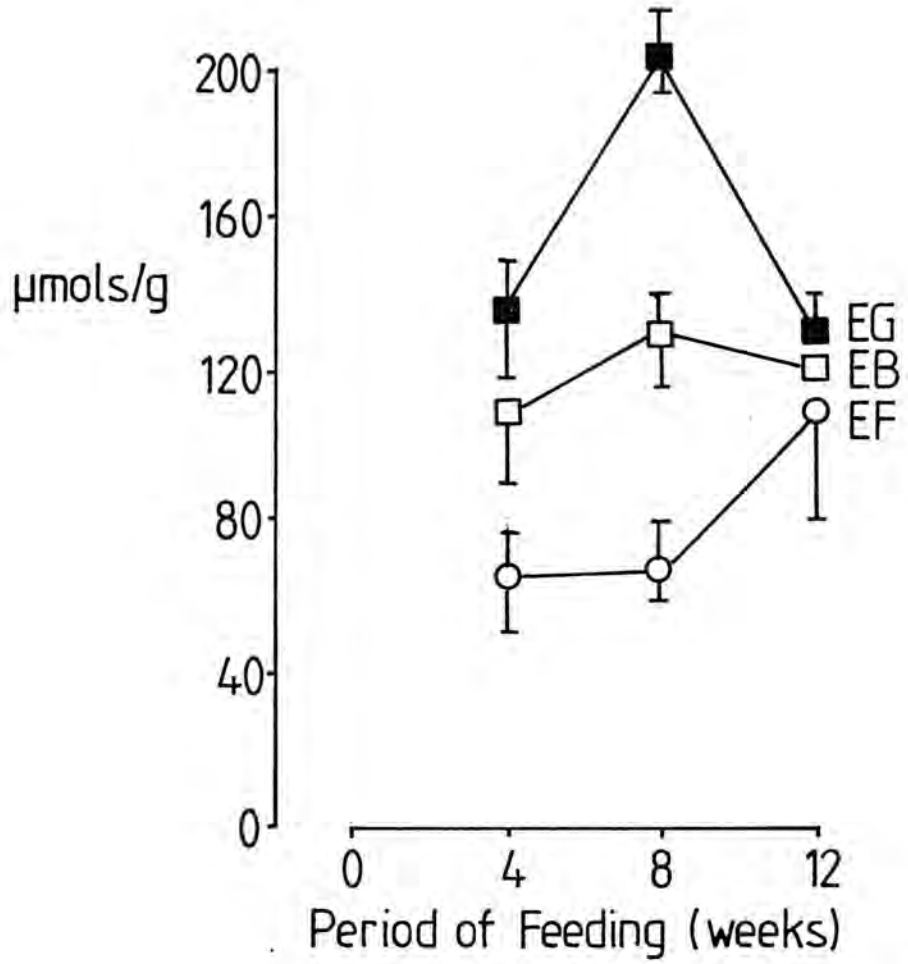


Time alone was significant($p<0.01$).

	TIME
L.S.D.$_{(36)}$	28.2
n =	15

FIGURE 5g(ii) Faecal concentration of SCFA's from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are mean ± S.E.M.
n = 5 except EG (4weeks) where n =4.



Diet($p<0.01$), time and the interaction of diet and time (DXT)($p<0.05$) were significant

	DIET	TIME	D X T
L.S.D.$_{(35)}$	32.2	24.0	43.1
n =	15	15	5

except diet EG, the time period 4 weeks, where n =14 and diet EG (4 weeks) where n = 4.

interaction shows that similarities exist between different diets fed for different times. For example only the proportions with the EG (12 weeks) and EF (12 weeks) diets were outrightly significantly different from each other ($p<0.01$); whilst there was no significant difference between the EB diet (12 weeks) EF diet (12 weeks) and the intermediate six groups; Within the three EF diet groups, there was no significant difference between the three values. Similarly for the EB diet groups. With the EG and EF diets, the proportion increased with time and remained stable on the EB diet.

(ii) Faecal

Diet, time ($p<0.01$) and the interaction of D X T ($p<0.05$) were significant (Table 5d). The addition of gum arabic reduced the proportion of acetate (626 ± 12 mmols/mol) with respect to the EF diet (755 ± 19 mmols/mol). There was no significant difference between the proportions from the EF and EB (745 ± 33 mmols/mol). Only the difference between 4- (643 ± 45 mmols/mol) and 8 weeks (753 ± 26 mmols/mol). The proportion at 12 weeks was intermediate and not significantly different from either of these two. Table 5d shows that the changes between time periods were not the same for each diet. Similarities do exist between diets fed for different time intervals. For example the proportion of acetate with the EG diet (4 weeks), EF diet (4 weeks) and the three intermediate groups was not significantly different. There was no significant difference between the proportions from any of the diets. With the exception of 4 weeks, the proportion of faecal acetate exceeded caecal.

b) Propionate

(i) Caecal

Diet alone was significant ($p<0.01$). The addition of gum arabic significantly increased caecal propionate (231 ± 10 mmols/mol) by 40%, concomitant with a decrease in acetate, with respect to the EF diet. There was no significant difference between the overall means of the EB (160 ± 6 mmols/mol) and EF diets (165 ± 5 mmols/mol), Table 5c.

(ii) Faecal

Only the interaction of D X T was significant ($p<0.01$) Table 5d. Whilst the proportion of propionate decreased with time on the EB and EG diets, the

proportion increased with the EF diet. There was no significant difference between any of the diets fed for 12 weeks. The results suggest that without a fibre as a substrate, propionate accumulates. With a fibre the initial proportion is high, after which the proportion decreases, perhaps indicative of production. The caecal proportion of propionate exceeded the faecal.

c) Butyrate

(i) Caecal

Diet alone was significant ($p<0.01$). The addition of bran, significantly increased the caecal proportion of butyrate (133 ± 7 mmols/mol), by 43% with respect to the EF diet (93 ± 3 mmols/mol) $p<0.01$. There was no significant difference between the EG (104 ± 7 mmols/mol) and EF diets. (Table 5c).

(ii) Faecal

Diet, time and the interaction of D X T were significant ($p<0.01$). Considering the main effect of diet, the addition of gum increased the proportion of butyrate (104 ± 5 mmols/mol) by 46% with respect to the EF diet (71 ± 16 mmols/mol). Whilst the proportion of butyrate was lower with the EB diet (43 ± 8 mmols/mol), this was not significantly different from the EF diet. With time, the proportion of butyrate decreased ($p<0.01$). The proportion at 4 weeks (97 ± 13 mmols/mol) was significantly greater than at 8- (57 ± 12 mmols/mol) and 12 weeks (63 ± 10 mmols/mol) between which there was no significant difference (Table 5d). The significance of an interaction makes the significance of the main effects less meaningful. From Table 5d it is evident that the trends with time were different for each diet, and that similarities exist between diets for different times. For example there was no significant difference between the three EG groups or the three EB groups; and there was no significant difference between the EB diet fed for 8 weeks, EB (12 weeks) and the EF diet fed for 8- and 4 weeks. The proportion of caecal butyrate exceeded faecal.

The molar proportion of isobutyrate, isovalerate and valerate are given in Appendices 3b (i) and (ii). These were detected in small and fluctuating amounts. The interaction of D X T was observed with caecal isobutyrate ($p<0.05$) the different trends associated with each of the diets. Time alone influenced faecal isobutyrate ($p<0.01$). Only the difference between 4- (23 ± 4.1 mmols/mol) and 8 weeks (9.3 ± 3.0 mmols/mol) was significant. The proportion at 12 weeks

TABLE 5c The composition of SCFA's (mmols/mol) in dry caecal contents from rats fed an elemental diet (EF) alone or supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods. Results given are mean±SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
ACETATE	644 ±10	664 ± 26	659 ± 9	655 ± 10	621 ± 27	667 ± 18	708±40	557±30	621 ± 13
PROPIONATE	179 ± 10	228 ± 29	161 ± 4	162 ± 5	234 ± 13	146 ± 15	155 ± 9	230 ± 6	171 ± 4
BUTYRATE	96 ± 3	90 ± 11	116 ± 10	96 ± 11	138 ± 14	279 ± 30	84 ± 5	125 ± 11	144 ± 9

Table of significant differences, in ascending order from left to right, for each group, (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
ACETATE LSD (36)	EG^{a} EB^{ab} EF^{b} 37.8 *	NS	EG^{c} EB^{cd} EG^{cd} EF^{cd} EF^{d} EB^{d} EG^{d} EB^{d} EF^{d} 12 12 8 4 8 4 4 8 12 87.9 **
PROPIONATE LSD (36)	EB^{a} EF^{a} EG^{b} 28.5 **	NS	NS
BUTYRATE LSD (36)	EF^{a} EG^{a} EB^{b} 20.6 **	NS	NS

A different superscript denotes a significant difference.
* = $p<0.05$
** = $p<0.01$
NS = not significant.

TABLE 5d The composition of SCFA's (mmols/mol) in dry faecal material from rats fed an elemental diet (EF) alone or supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.
Results given are mean SEM where n = 5 per group except EG (4 weeks) where n = 4.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
ACETATE	675 ± 41	601 ± 27	646 ± 61	758 ± 24	639 ± 12	865 ± 10	774 ± 20	634 ± 24	730 ± 34
PROPIONATE	121 ± 31	213 ± 7	249 ± 60	160 ± 3	179 ± 8	118 ± 6	190 ± 7	179 ± 5	148 ± 8
BUTYRATE	141 ± 15	105 ± 12	47 ± 14	41 ± 14	16 ± 2	17 ± 8	31 ± 19	94 ± 8	64 ± 10

Table of significant differences, in ascending order from left to right, for each group (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
ACETATE	EG^{a} EF^{b} EB^{b}	4^{c} 12^{cd} 8^{d}	EG^{e} EG^{ef} EG^{ef} EB^{ef} EF^{efg} EB^{fgh} EF^{gh} EF^{hj} EB^{j}
			4 12 8 4 4 12 8 12 8
LSD (35)	72.2	72.2	96.5
	**	**	*
PROPIONATE			EB^{a} EF^{a} EB^{ab} EF^{abc} EG^{bc} EG^{bc} EF^{bc} EG^{cd} EB^{d}
			8 4 12 8 12 8 12 4 4
LSD (35)	NS	NS	53.3
			**
BUTYRATE	EB^{a} EF^{a} EG^{b}	8^{c} 12^{c} 4^{d}	EB^{e} EF^{e} EF^{e} EB^{ef} EB^{efg} EG^{fgh} EG^{gh} EG^{gh} EF^{h}
			8 12 8 4 12 12 4 8 4
LSD (35)	28.1	28.1	50.3
	**	**	**

A different superscript denotes a significant difference.
* = $p<0.05$
** = $p<0.01$
NS = not significant.

was not significantly different from either of these two. An interaction of D X T was observed with caecal and faecal isovalerate ($p<0.01$). Diet, time and the interaction of D X T were significant ($p<0.01$) upon caecal valerate. The EG (32 ± 7 mmols/mol) and the EB diets (38 ± 2 mmols) reduced caecal valerate. The proportion with the EF diet (59 ± 5 mmols/mol) was significantly greater than either. Only the difference between 4- (34 ± 6 mmols/mol) and 12 weeks (56 ± 7 mmols/mol) was significant, the proportion at 8 weeks not being significantly different from either. Diet ($p<0.01$), time ($p<0.05$) and the interaction of D X T ($p<0.01$) were all significant upon faecal valerate. Considering the main effects, the EB diet (36 ± 4.7 mmols/mol) significantly increased faecal valerate. There was no significant difference between the EF (65 ± 4.4 mmols/mol) and EB diets (12.9 ± 5.4 mmols/mol). Only the difference between 8- (11 ± 4 mmols/mol) and 12 weeks (25 ± 7 mmols/mol) was significantly different. The proportion at 4 weeks was not significantly different from either. A dietary interaction with time was also significant ($p<0.01$). This was also significant for faecal isovalerate and valerate ($p<0.01$). The caecal proportion of valerate was greatest with gum, and increased with time. With the EF feeding, the faecal proportion of isovalerate was abolished after 8 weeks of feeding, as was that on the EB diet.

HYDROGEN AND METHANE

(i) Hydrogen (H_2)

Diet, time ($p<0.01$) and the interaction of D X T ($p<0.05$) were significant. Table 5e it shows that all H_2 levels were low. Considering the main effect of diet the addition of gum arabic significantly increased the level of H_2 (0.8 ± 0.21 mls/hr/kg). The difference between the EF (0.19 ± 0.07 mls/hr/kg) and EB diets (0.38 ± 0.07 mls/hr/kg) was not significant. H_2 decreased with time. All three means for the time periods were significantly different from each other (4 weeks = 0.83 ± 0.19 mls/hr/kg; 8 weeks = 0.15 ± 0.08 mls/hr/kg; 12 weeks = 0.06 ± 0.03 mls/hr/kg). Table 5e shows that the changes between time periods were not the same for each diet. The level of H_2 with the EG diet (4 weeks) was significantly greater than the other 8 groups. Between 4- and 8 weeks with the EG diet a decrease of 53% was observed. For the EF diet this was 33% and for the EB diet 20%. Similarities exist between diets fed for different times. For example there was no significant difference between any of the diets fed for 12 weeks or for 4 weeks. There was no significant difference between the EF diet (12 weeks), EB diet (4 weeks) and five intermediate groups.

TABLE 5e H_2 and CH_4 (mls/hr/kg) from rats fed an elemental diet (EF) alone or supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.
Results given are mean $\pm$ SEM, where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
H_2	0.30 $\pm$0.10	1.70 $\pm$ 0.30	0.50 $\pm$ 0.10	0.20 $\pm$ 0.10	0.80 $\pm$ 0.10	0.40 $\pm$ 0.03	0	0	0.20 $\pm$ 0.04
CH_4	0	1.00 $\pm$ 0.60	0	0	0.35 $\pm$ 0.16	0	0	0	0

Table of significant differences, in ascending order from left to right, each each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
H_2	EF^a EB^a EG^b	12^c 8^d 4^e	EF^f EG^f EB^f EF^{fg} EF^{fg} EB^{fg} EB^g EG^g EG^h
LSD (36)	0.33 **	0.33 **	0.54 **
CH_4	EF^a EB^a EG_b		
LSD (36)	0.42 **	NS	NS

A different superscript denotes a significant difference
** = $p<0.01$;
NS = not significant.

(ii) Methane (CH_4)

Diet alone was significant ($p<0.01$). No CH_4 was detected with either the EF or EB diets. The addition of gum arabic gave a mean CH_4 of 0.47 ± 0.21 mls/hr/kg over the three time periods. Although time was not significant, CH_4 production did diminish over the three time periods, (Table 5e), from 1.0 ± 0.6 mls/hr/kg to zero.

BILE ACIDS

A pooled sample of faecal and caecal material was used for analysis. As a result, no statistical analysis was made.

a) Total bile acids

(i) Caecal (μmols)

Total caecal and faecal bile acids are given in Figures 5h(i) and (ii). The addition of gum arabic caused an overall 2 fold increase of total caecal bile acids (36.4 ± 8.07 μmols). The addition of bran reduced the overall amount of total bile acids 14.7 ± 1.5 μmols). Considering time alone, total bile acids were greatest at 8 weeks (27.2 ± 10.9 μmols). However Figure 5h (i) shows that the trends with time for each diet were different, and are not evident from the overall means for diet and time. With the EF and EB diets, total bile acids decreased with time in a linear fashion. With the EG diet, total bile acids, increased greatly between 4- and 8 weeks and remain at a high level at 12 weeks.

(ii) Faecal (μmols/day)

Figure 5h (ii) shows that, over time trends were different for each diet. Considering diet alone the addition of gum arabic and bran decreased total bile acids (12.1 ± 2.7 μmols/day; 14.6 ± 1.12 μmols/day, respectively) with to the EF diet (18.3 ± 3.31 μmols/day). Considering time alone faecal bile acids increased between 4 weeks (12.9 ± 3.11 μmols/day) and 12 weeks (17.5 ± 3.77 μmols/day). It is, however evident from Figure 5h (ii) that these means obscure the different trends associated with diet. On the EG diet were lowest at 4 weeks: there was little difference between the higher total values of the EF and EB diets. Whilst there was a substantial increase between 4- and 8 weeks, and further still at 12 weeks, with the EG diet, total faecal bile acids decreased with the EB diet over all three time periods and decrease between 4- and 8 weeks on the EF diet with a substantial increase at 12 weeks. At 8 weeks there was little different between the three diet toals; at 12 weeks totals are greatest with the EF diet and lowest on the EB diet.

b) Concentration of total bile acids

(i) Caecal (μmols/g)

The addition of bran decreased the concentration of caecal bile acids ($28.4 \pm$

FIGURE 5h(i) Total caecal bile acids from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) and 10% wheat bran (EB) for three time periods.

Results are means, where n = a pooled sample from five rats

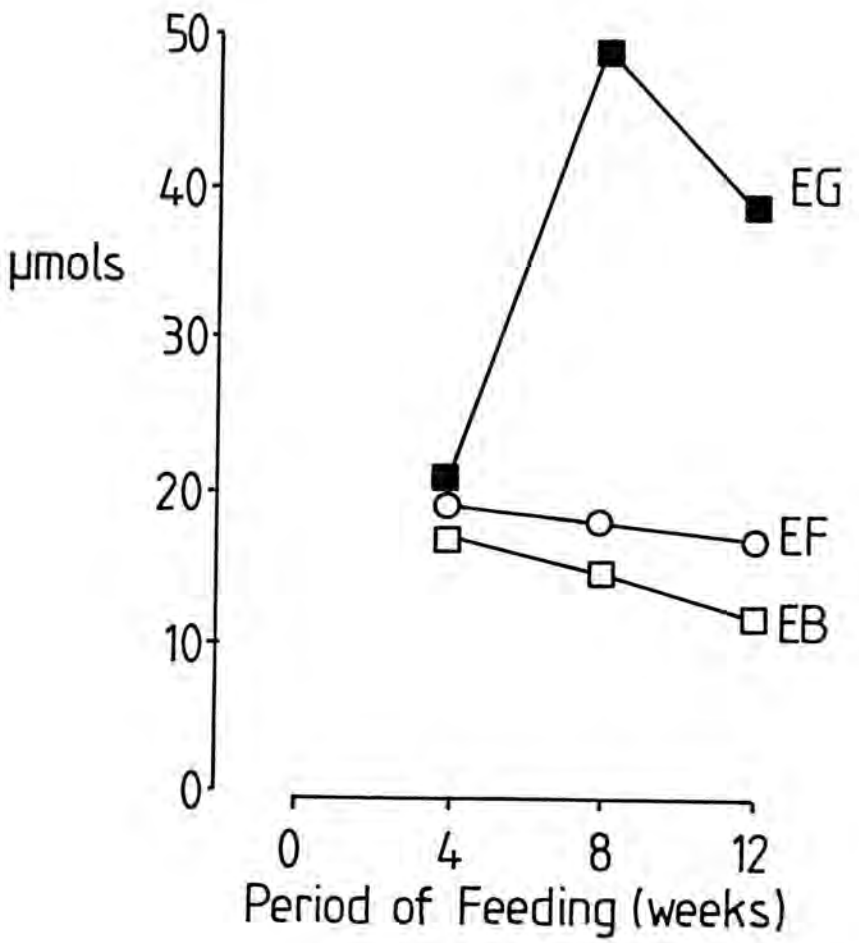
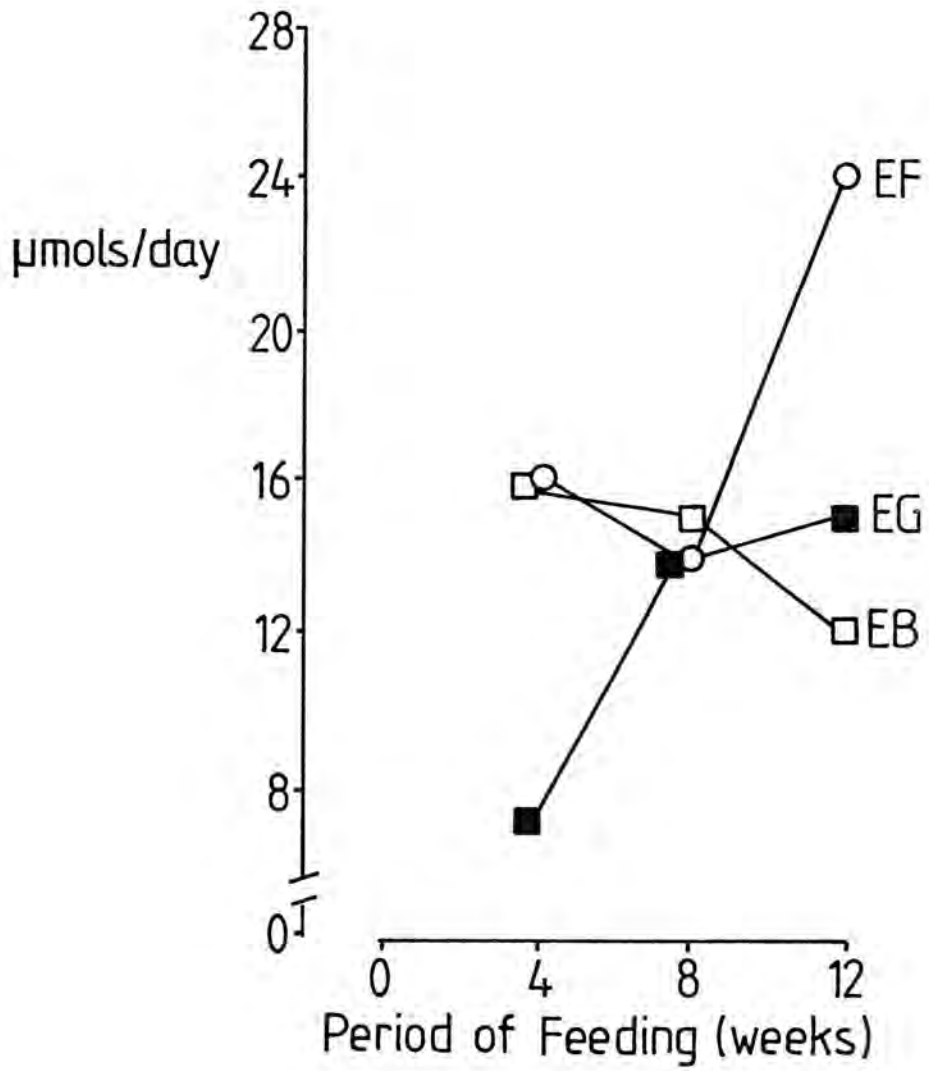


FIGURE 5h(ii) Total faecal bile acids from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) and 10% wheat bran (EB) for three time periods.

Results are means, where n = a pooled sample from five rats except EG (4 weeks) where n = four rats.



5.68 μmols/g). Concentration was greatest on the EF diet (39.5±1.70 and intermediate with the EG diet (34.8±8.47 μmols/g). Overall, concentration appeared to be greatest at 8 weeks (39.5± 7.46 μmols/g), with little difference between 4- (33.1±5.91 μmols/g) and 12 weeks (30.1±4.96 μmols/g). However Figure 5j (i) shows that each diet behaved in a different way with time. At 4 weeks there was little difference between the EF and EB diets. Concentration on the EG diet was lower. Concentration decreased, in a linear fashion, with the EB diet. With the EF diet, concentrations remained fairly steady over time. With the EG diet, concentration increased 2 fold between 4- and 8 weeks decreasing slightly at 12 weeks. At 12 weeks concentration was lowest on the EB diet, and highest on the EF diet.

(ii) Faecal (μmols/g)

The addition of bran decreased the concentration of faecal bile acids (13.6 ± 1.76 μmols/g; Figure 5j (ii). The addition of gum arabic also reduced the concentration of bile acids (17.8 ± 0.79 μmols/g) with respect to the concentration on the EF diet (25.9 ±2.39 μmols/g). Overall concentration increased from 18.9 ± 1.98 (4 weeks) to 20.3 ± 5.72 μmols/g (12 weeks). Over time the EF and EG exhibited very similar trends, increasing concentration after 8 weeks. Concentration decreased in a linear fashion on the EB diet.

The composition of the total bile acids (mmols/mol)

(i) Caecal

Table 5f (i) gives the individual proportions of each bile acid on dry caecal contents. Deoxycholic- hyodeoxycholic and ω-muricholic acid formed the largest proportion of the bile acids with all diets. The EG diet reduced the overall amount of hyodeoxycholic acid and cholic acid, whilst the addition of gum arabic increased the overall proportion of chenodeoxycholic acid and the three muricholic acids. There was little difference in the overall effects of the EF and EB diets. With all the bile acids, there appeared to be some degree of an interaction of D X T: the changes with time were not the same for each diet.

Lithocholic acid: both the EF and EG diets showed similar changes between time periods. The proportion decreased between 4- and 8 weeks and rose again at 12 weeks. With the EB diet, the proportion increased between 4- and 8 weeks (the increase being greater than the decrease for EF and EB

FIGURE 5j(i) Caecal concentration of bile acids from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are means where n = a pooled sample from five rats.

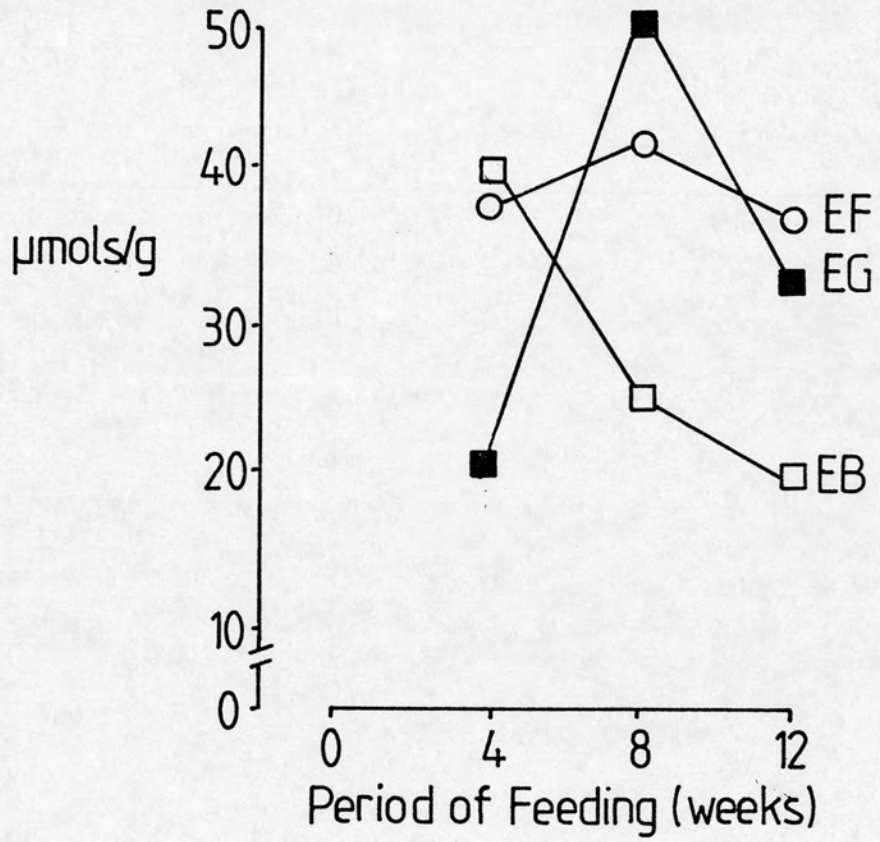
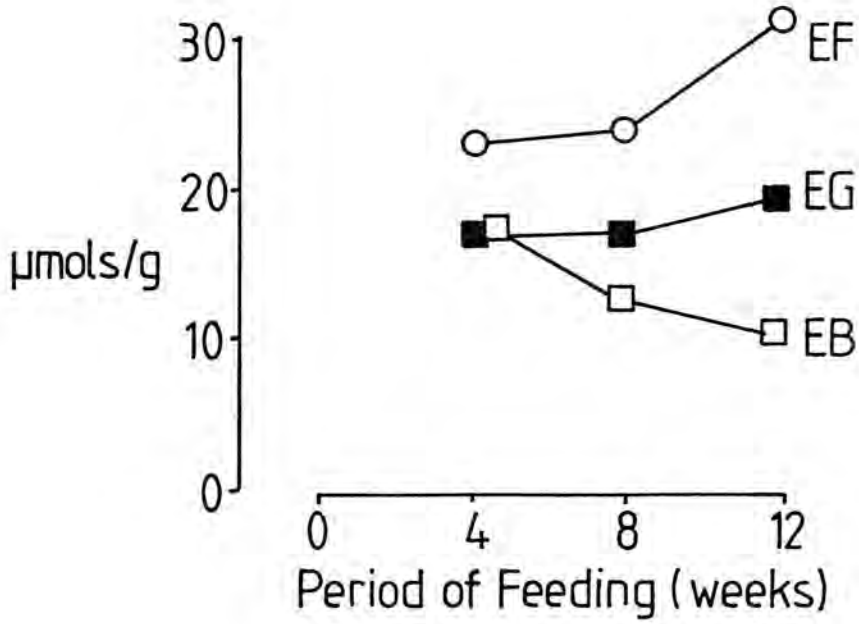


FIGURE 5j(ii) Faecal concentration of bile acids from rats fed an elemental diet (EF) alone and supplemented with 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Results are means where n = a pooled sample from five rats except EG (4 weeks) where n = four rats.



diets) decreasing slightly at 12 weeks. At 12 weeks there was little difference between the EB and EG diets.

Deoxycholic acid: on the EF diet, proportion decreased from 358 mmols/mol (4 weeks) to 238 mmols/mols (8 weeks) thereafter increasing back to near the 4 week proportion. With the EG diet, proportion increased with time from 192 mmols/mol (4 weeks) to 347 mmols/mol (12 weeks) the increase between 8- and 12 weeks being greatest. On the EB diet the proportion increased between 4- (160 mmols/mol) and 8 weeks (278 mmols/mol) and thereafter remained constant. At 12 weeks there was little difference between the three proportions.

Chenodeoxycholic acid: the proportion remained constant with time on diet EF. With diet EG the proportion increased from 32.3 mmols/mol (4 weeks) to 158 mmols/mol (8 weeks) and decreased to near the 4 week value after 12 weeks. With diet EB, values decreased from 147 mmols/mol (4 weeks) to 41.8 mmols/mol (8 weeks). There was no difference between the values of diets EB and EG after 12 weeks.

Cholic acid: all the diets showed similar changes with time for the proportion of bile acids. At 8 weeks, on any diet, the proportion was greatest than that at 4- and 12 weeks. The proportion of cholic acid was largest on the EF diet and lowest on the EG diet. At 12 weeks the proportion on the EB diet (123 mmols/mol) exceeded that on the EF (107 mmols/mol) and EG diets (103 mmols/mol).

Hyodeoxycholic acid: the proportion increased linearly on the EG diet from 22 mmols/mol (4 weeks) to 277 mmols/mol 12 weeks. Similarly on the EB diet. On the EF diet, the proportion peaked at 8 weeks (438 mmols/mol). There was little difference between the 4- (267 mmols/mol) and 12 week proportions. Similarly at 4- and 12 weeks there was little difference between the EF and EB diets. At 12 weeks there was little difference between all three diets.

α-muricholic acid: gum resulted in the largest proportion of the acid. The proportion decreased with time from 28 mmols/mol (4 weeks) to 20 mmols/mol

TABLE 5f (i) The composition of bile acids (mmols/mol) in pooled caecal material from rats fed an elemental diet (EF) alone or supplemented with either 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.
Results are means.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
Lithocholic acid	52	45	18.3	28.9	33.5	50.5	31.0	44.0	44.5
Deoxycholic acid	358	192	160	38	208	274	303	347	278
Chenodeoxycholic acid	40.3	32.3	147	49.1	158	41.8	41.3	25.0	42.4
Cholic acid	124	36	115	181	124	153	107	103	123
Hyodeoxycholic acid	267	22	265	438	162	306	313	277	329
α-muricholic acid	0	28.6	15.7	18.9	26.7	0	9.17	20.1	12.2
ω-muricholic acid	114	501	208	169	187	140	134	137	122
β-muricholic acid	44.8	145	70.3	76.7	101	55.4	62.0	46.4	53.6

(12 weeks). No α-muricholic was detected at 4 weeks on the EF diet, only at 8- and 12 weeks. With the EB diet no α-muricholic acid was detected at 8 weeks, only 4- and 12 weeks, there being little difference between the two values.

ω-muricholic acid: at 4 weeks the EG diet gave the largest proportion of the acid (501 mmols/mol). This decreased with time. At 8- and 12 weeks there was very little difference between the proportions associated with each diet. Of the three the EB gave the least proportion. On the EF diet, the proportion increased with time from 114 mmols/mol (4 weeks) to 134 mmols/mol (12 weeks). On the EB diet the proportion decreased slightly over time from 169 mmols/mol (4 weeks) to 122 mmols/mol (12 weeks).

β-muricholic acid: at 4 weeks, the EG diet gave the largest proportion (145 mmols/mol), which decreased with time. At 12 weeks there was little difference between the proportions associated with each diet. With the EF diet, the proportion was greatest at 8 weeks. With the EB diet, the proportion decreased after 4 weeks (70 mmols/mol) to 55 mmols/mol (8 weeks) and thereafter remained constant.

(ii) Faecal

Table 5f (ii) gives the individual proportions of each bile acid in dry stool material. Deoxycholic- hyodeoxycholic and ω-muricholic formed the largest proportions for each diet. Overall the addition of gum reduced the proportion of chenodeoxycholic-, cholic- and hyodeoxycholic acid. No β-muricholic acid was detected with the E B diet. With all the bile acids there appeared to be some degree of an interaction of D X T: the changes with time were not the same for each diet.

Lithocholic acid: there was very little difference between the EG and EB diets at any of the three time periods. The proportion increased with time on both diets from 60 mmols/mol (4 weeks) to 90 mmols/mol (12 weeks). On the EF diet, the proportion was lowest at 4 weeks, increased 5 fold at 8 weeks (64 mmols/mol) and was no different from the proportion observed with the EG and EB diets, and decreased at 12 weeks.

Deoxycholic acid: the proportion decreased with time on the EF diet from 287 mmols/mol (4 weeks) to 210 mmols/mol (12 weeks). It increased on the

EG diet between 4- (219 mmols/mol) and 8 weeks (301 mmols/mol) and thereafter remained constant. On the EB diet, the proportion also increased between 4- (191 mmols/mol) and 8 weeks (231 mmols/mols) and decreased slightly at 12 weeks. At 12 weeks the proportion on the EG diet exceeded that on the EF and EB diets, between which there was little difference.

Chenodeoxycholic acid: the change in proportion between time periods for the EF diet were similar for the EB diets. At 4 weeks, the proportion was largest on the EG diet (57 mmols/mol) with no difference between the EF and EB diets at 4 weeks. By 8 weeks, the proportion increased on both the EF and EB diets, and thereafter remained constant. With the EG diet, the proportion decreased at 8 weeks and remained constant. At 12 weeks there was no difference between the EF and EB diets; on the EG diet had substantially reduced the proportion of the acid.

Cholic acid: the EF and EG diets showed similar trends with time. At 8 weeks the proportion was greater than at either 4- or 12 weeks, the change between 8- and 12 weeks being the same for each diet. The proportion increased in a linear fashion with the EB diet from 109 mmols/mol (weeks) to 138 mmols/mol 12 weeks. At 12 weeks the EG results in the lowest proportion (96 mmols/mol) and the EB diet, the largest. Overall, gum decreased the proportion of cholic acid.

Hyodeoxycholic acid: overall, the proportion decreased with gum. The proportion was lowest at 4 weeks with the EG diet (159 mmols/mol), with little difference between the EF (365 mmols/mol) and the EB diets (341 mmols/mol). The proportion increased with time on the EB diet. With the EF diet after 8 weeks the proportion decreased. With the EG diet, the proportion peaked at 8 weeks and decreased at 12 weeks (274 mmols/mol). At 8 weeks there was little difference between the proportions on the three diets. At 12 weeks the EG produced the lowest proportion and the EB diet the largest.

α-muri cholic acid: the proportion decreased in a linear fashion on the EB diet from 62 mmols/mol to zero. The decrease between 4- and 8 weeks was the same for the EB and EG diets. After 8 weeks the proportion of

TABLE 5f (ii) The composition of bile acids (mmols/mol) in pooled faecal material from rats fed an elemental diet (EF) alone or supplemented with either 10% gum arabic (EG) or 10% wheat bran (EB), for three time periods.
Results are means.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
Lithocholic acid	11.8	59.6	60.4	64.3	64.5	68.8	44.5	93.8	88.5
Deoxycholic acid	287	219	191	221	301	231	210	296	221
Chenoxycholic acid	48.1	57.0	46.8	67.8	37.5	72.7	62.8	37.1	66.4
Cholic acid	137	76.4	109	144	124	128	115	95.9	138
Hyodeoxycholic acid	365	159	341	377	336	388	321	274	486
α-muricholic acid	22.4	57.6	62.2	16.2	31.7	37.5	124	60.1	0
ω-muricholic acid	78.3	327	190	87.5	105	75.0	75.2	142	0
β-muricholic acid	50.2	43.5	0	24.5	0	0	46.5	0	0

α-muricholic acid on the EG diet increased to 60 mmols/mol (12 weeks). With the EF diet, the proportion remained constant between 4- (22 mmols/mol) and 8 weeks (16 mmols/mol). By 12 weeks the proportion had increased to 124 mmols/mol.

ω-muricholic acid: overall gum increased the proportion of the acid. Concentration was highest at 4 weeks on the EG diet (327 mmols/mol). By 8 weeks the proportion had decreased by one third, increasing slightly at 12 weeks. On the EF the proportion remained constant with time. (80 mmols/mol). On the EB diet, the proportion decreased from 190 mmols/mol to zero.

β-muricholic acid: no β-muricholic acid was observed with the EB diet. At 4 weeks, on the EG diet, the proportion was 44 mmols/mol. At 8- and 12 weeks no β-muricholic acid was detected. On the EF diet, there was little difference between the proportion observed at 4- and 12 weeks (50 mmols/mol and 47 mmols/mol). At 8 weeks, the proportion had decreased by 50%.

SUMMARY

This Chapter has given the results of the experimental feeding trial using an elemental diet (EF). The EF diet was fed unsupplemented and with 10% gum arabic (EG) or 10% wheat bran (EB). The effect of these diets upon chosen measurements was investigated, concomitant with the effect of feeding duration. A two factor ANOVA was used to analyse the results. A summary of the results is given below and discussed in detail in Chapter 8.

(1) The diet was acceptable to the rats as indicated by the significant increase in liveweight ($p<0.01$). Rats fed the EG diet gained significantly less weight(g) The rats fed for 12 weeks on any diet, gained the most weight ($p<0.01$).

(2) The liver wet weights (g) reflected live-weight. The liver from rats fed the EG diet were lightest ($p<0.05$). The weight at 8 weeks was significantly lower than at 12 weeks ($p<0.05$).

(3) Stool weight (g/day) was increased with wheat bran ($p<0.01$) but not with gum arabic. Stool weight increased between 4- and 12 weeks on any diet ($p<0.05$). When expressed as a function of live-weight (g/kg), only diet was significant ($p<0.01$). Stool: live-weight ratio was greatest on the EB diet.

(4) The C.S.W.W. (g) was heaviest on the EG diet ($p<0.01$) and lightest on the EF diet ($p<0.01$). The interaction of D X T was significant ($p<0.01$). time: the tends of the changes with time for each diet were not the same. When expressed as a function of live-weight (g/kg) diet, time and the interaction of D X T were significant.

(5) The weight of C.C. (g) was increased with the addition of gum arabic ($p<0.01$) but not with wheat bran. The same result was apparent when the results were expressed as a function of live-weight (g/kg) ($p<0.01$).

(6) Caecal DAPA (µmols) was increased by the addition of gum arabic ($p<0.01$) but not by wheat bran. Faecal DAPA (µmols/day) was increased by the addition of gum arabic, and not by wheat bran ($p<0.01$).

(7) Caecal DAPA (µmols/g) was significantly increased by the addition of gum arabic and significantly reduced by the addition of wheat bran ($p<0.01$). Concentrations significantly decreased between 4- and 12 weeks ($p<0.05$). Faecal DAPA (µmols/g) was increased with the addition of gum arabic and decreased with the addition of wheat bran ($p<0.01$). The concentration significantly decreased between 4- and 12 weeks. The interaction of D X T was significant ($p<0.05$).

(8) Caecal SCFA's (µmols) were increased with the addition of gum arabic ($p<0.01$) and not with wheat bran. Faecal SCFA's (µmols/day) were increased with both gum arabic and wheat bran ($p<0.01$), between which there was no significant difference. Total SCFA's were significantly increased ($p<0.05$) between 4- and 8 weeks thereafter remaining constant.

(9) Caecal SCFA's (µmols/g) were significantly influenced by time alone ($p<0.01$). Concentrations were highest at 4 weeks and lowest at 8 weeks. All three means were significantly different from each other. Faecal SCFA's (µmols/g) were increased with gum arabic and wheat bran. All three means were significantly different from each other. Concentration increased significantly between 4- and 12 weeks ($p<0.01$). The interaction of D X T was significant ($p<0.01$).

(10) Of the SCFA's, acetate formed the largest proportion in caecal and faecal material. The addition of gum arabic decreased the proportion of acetate ($p<0.05$). The interaction of D X T was significant ($p<0.01$). Faecal acetate

was decreased with gum arabic but not with wheat bran ($p<0.01$). The proportion increased with time ($p<0.01$). The interaction of D X T was significant ($p<0.05$). Caecal propionate was significantly increased with the addition of gum arabic ($p<0.01$) and not with wheat bran. Only the interaction of D X T was significant ($p<0.01$) with faecal propionate. There was no significant difference between any of the diets fed for 12 weeks. Caecal butyrate increased with the addition of bran ($p<0.01$) and not with gum arabic. Faecal butyrate was increased with the addition of gum arabic ($p<0.01$) and decreased with the EB diet. The proportion decreased between 4- and 8 weeks and thereafter remained stable. The interaction of D X T was significant ($p<0.01$).

(11) H_2 (mls/hr/kg) increased with the addition of gum arabic ($p<0.01$) and not with wheat bran. H_2 decreased with time, the three means being significantly different from each other ($p<0.01$). The interaction of D X T was significant ($p<0.05$). CH_4 (mls/hr/kg) was detected only on the EG diet ($p<0.01$).

(12) Caecal bile acids (μmols) were increased with gum arabic and decreased with wheat bran, with respect to the EF diet. The changes with time were not the same for each diet. Total caecal bile acids decreased over time with the EF and EB diet and increased with the EG diet. Total faecal bile acids (μmols/day) were decreased with gum arabic and wheat bran. Total increased between 4- and 12 weeks. The changes with time were not the same for each diet. The concentration (μmols/g) of caecal bile acids was decreased with wheat bran and increased with gum arabic. Wheat bran and gum arabic both decreased the concentration of faecal bile acids.

(13) Of the individual bile acids in the caecal contents, and faecal material, deoxycholic-, hyodeoxycholic and ω-muricholic acid formed the largest proportion on all three diets. Overall, the addition of gum arabic reduced hyodeoxycholic and cholic acid and increased the proportion of chenodeoxycholic and the three muricholic acids in the caecal contents. There was little difference in the effects of the EF and EB diets. The pattern of the changes in the proportion of the individual bile acids over time were not similar for each diet and were indicative of the interaction of D X T. In faecal material, the addition of gum arabic decreased the proportion of chenodeoxycholic-cholic and hyodeoxycholic acids. No β-muricholic acid was detected with the EG diet. Wheat bran decreased the proportion of ω-muricholic acid. The pattern of the changes in the proportion of the individual bile acids over time were not similar for each diet, and were indicative of the interaction of D X T.

CHAPTER 6

THE ANALYSIS OF THE THREE DIETARY TRIALS USING A THREE-WAY ANALYSIS OF VARIANCE

The results of the three individual dietary trials using the three different basal diets, plant origin (PF), animal origin (AF) and elemental (EF), fed with and without gum arabic and coarse wheat bran, have been recorded in the preceeding three chapters. The results of a comparison of these three dietary trials, using a three way ANOVA with replication, are given in this chapter. The nature of this statistical analysis allows the following overall effects to be assessed:

MAIN EFFECTS

i) the significant effect, if any, upon caecal metabolism and faecal excretion, of the three dietary trials, irrespective of supplement and time.

ii) the significant effect, if any, upon caecal metabolism and faecal excretion, of the three time periods, irrespective of basal diet and supplement.

iii) the significant effect, if any, upon caecal metabolism and faecal excretion, of the three supplements (no supplement, gum arabic and wheat bran), irrespective of basal diet and time.

TWO-WAY INTERACTIONS

i) the significant effect, if any, of the "two-way interactions"upon caecal metabolism and faecal excretion. These are diet with time (D X T) diet with supplement (DXS) and time with supplement (SXT).

THREE-WAY INTERACTION

i) The significant effect, if any, upon caecal metabolism and faecal excretion of the interaction between the different basal diets, time periods and supplements (DXTXS).

The relevant L.S.D's (Chapter 2) are given on the figures and appendices. The F-statistics are given in appendix 2d as are the tables of the three-way interaction, where they reach significance.

The significance of the main effects, diet, time and supplement, indicate the overall, most effective basal diet, supplement and time period. Where the two-way- and three-way interactions reach significance, the significance of any main effect becomes less meaningful (Chapter 2). The overall mean for each of the main effects, dietary trial, time period and supplement, obscure the different trends associated with each combination of D X T, D X S and S X T and D X T X S. These interactions are important and must not be

discounted. In the gastrointestinal tract, a diet and supplement may form interacting complexes, whose combined effects are different from those of the individual components.

RESULTS

LIVE-WEIGHT

Figure 6a (i) shows rats from the AF dietary trial were significantly lighter ($p<0.001$). There was no significant difference in effect between dietary trials PF and EF. Overall, rats gained weight with time ($p<0.001$). All rats, were heaviest after 12 weeks of feeding (figure 6a (ii)). Overall, bran significantly increased live-weight ($p<0.001$). Only the difference between the gum and bran supplements was significant (figure 6a (iii)).

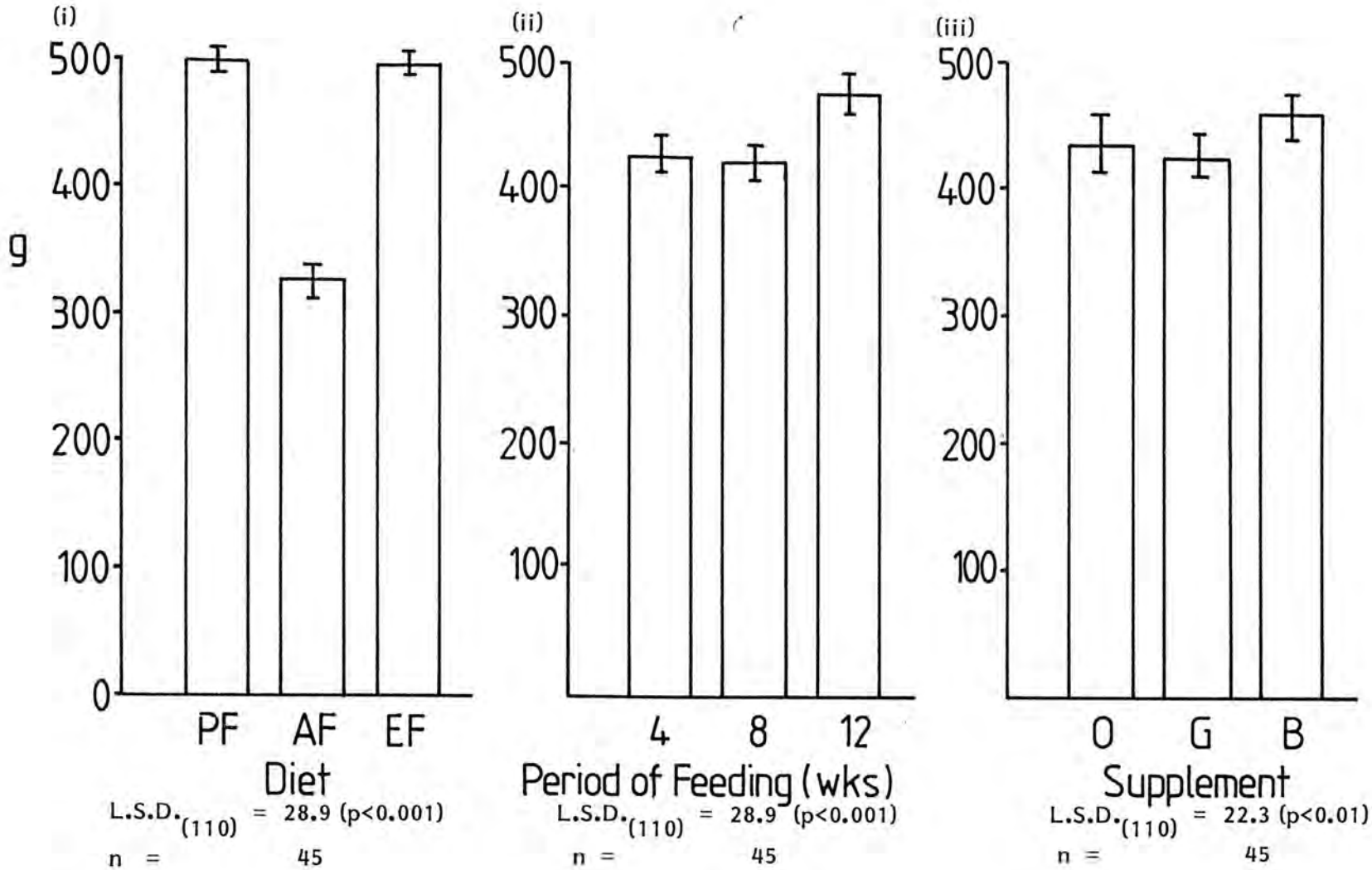
The two-way- interaction of D X T as was that of D X S ($p<0.001$) was significant ($p<0.01$). Figure 6a (iv) shows that within the dietary trials EF and PF, weights after 12 weeks were significantly greater than at the other two time periods. Both these two results were significantly different from each other. Rats given diet AF showed loss of live-weight, the weights at each of the three time periods not being significantly different from each other. Figures 6a (v) shows that with the PF dietary trial a supplement of bran significantly decreased live-weight with respect to diet PF and significantly increased live-weight when given with diet AF. There was no significant difference between the three EF diets. All rats fed the three AF diets had final live-weights significantly lower than the other six diet groups.

Figure 6b (i) shows that overall live-weight gain was greatest on the EF trial and lowest with the AF dietary trial ($p<0.001$). Live-weight change increased with time ($p<0.001$) on any trial. The live-weight changes after 4-, 8 and 12 weeks were significantly different from each other (figure 6b (ii). Overall bran gave the largest live-weight gain ($p<0.001$). There was significant difference between the effect of the absence of a supplement (O) and gum supplementation (figure 6b (iii)).

The two-way interactions of D X T and D X S were significant ($p<0.001$). Irrespective of diet, the live-weight change increased in a linear fashion with time (figure 6b (iv)). The magnitude of the increase was influenced by the basal diet. There was no significant difference between any of the three

FIGURE 6a Final live-weights of rats as influenced by (i) basal diet, Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF), (ii) time and (iii) supplement- no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.



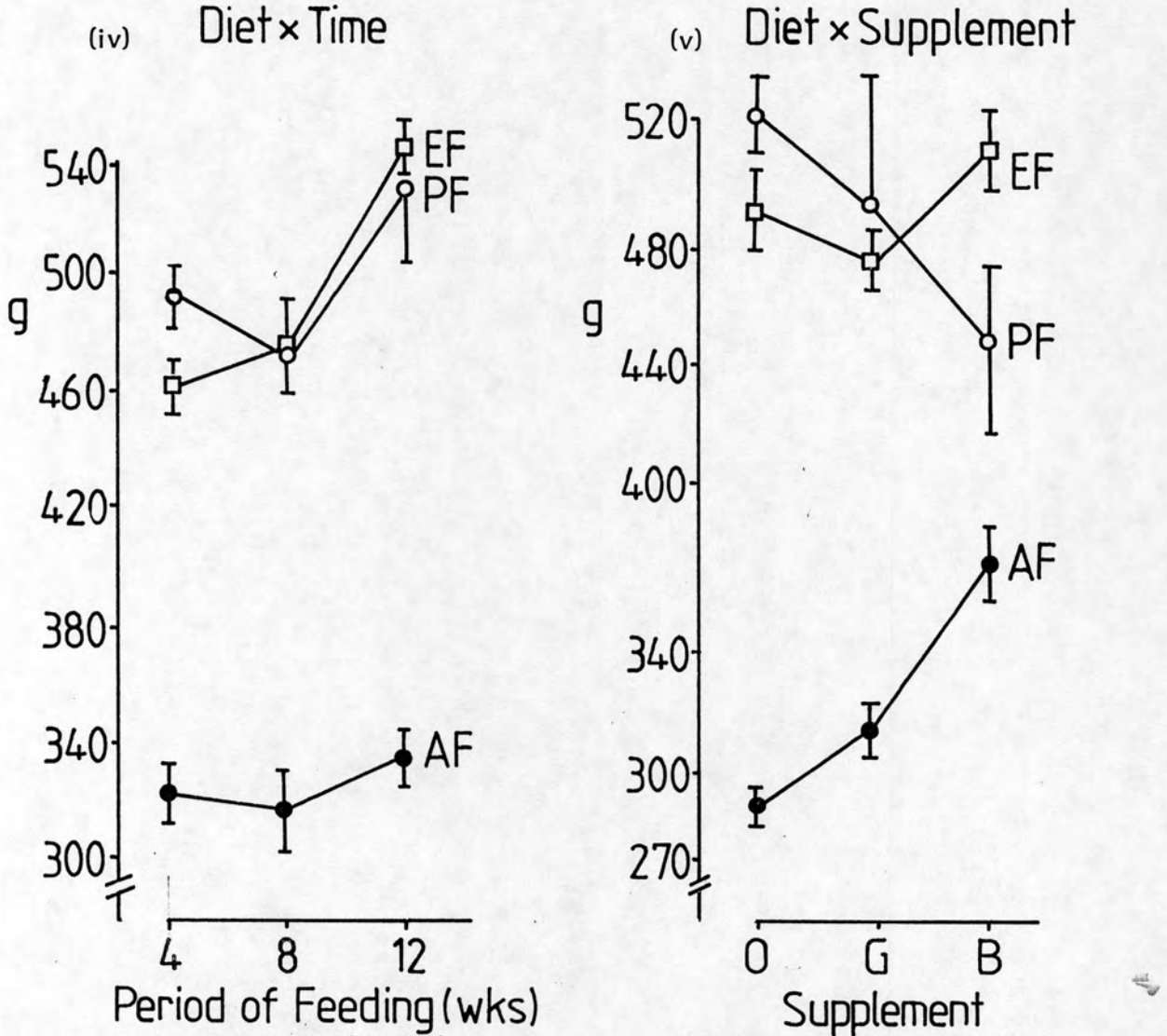
L.S.D.$_{(110)}$ = 28.9 ($p<0.001$) L.S.D.$_{(110)}$ = 28.9 ($p<0.001$) L.S.D.$_{(110)}$ = 22.3 ($p<0.01$)

n = 45 n = 45 n = 45

FIGURE 6a

Final live-weights of rats as influenced by the two-way interactions of (iv) basal diet- Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time and (v) basal diet and supplement- no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.



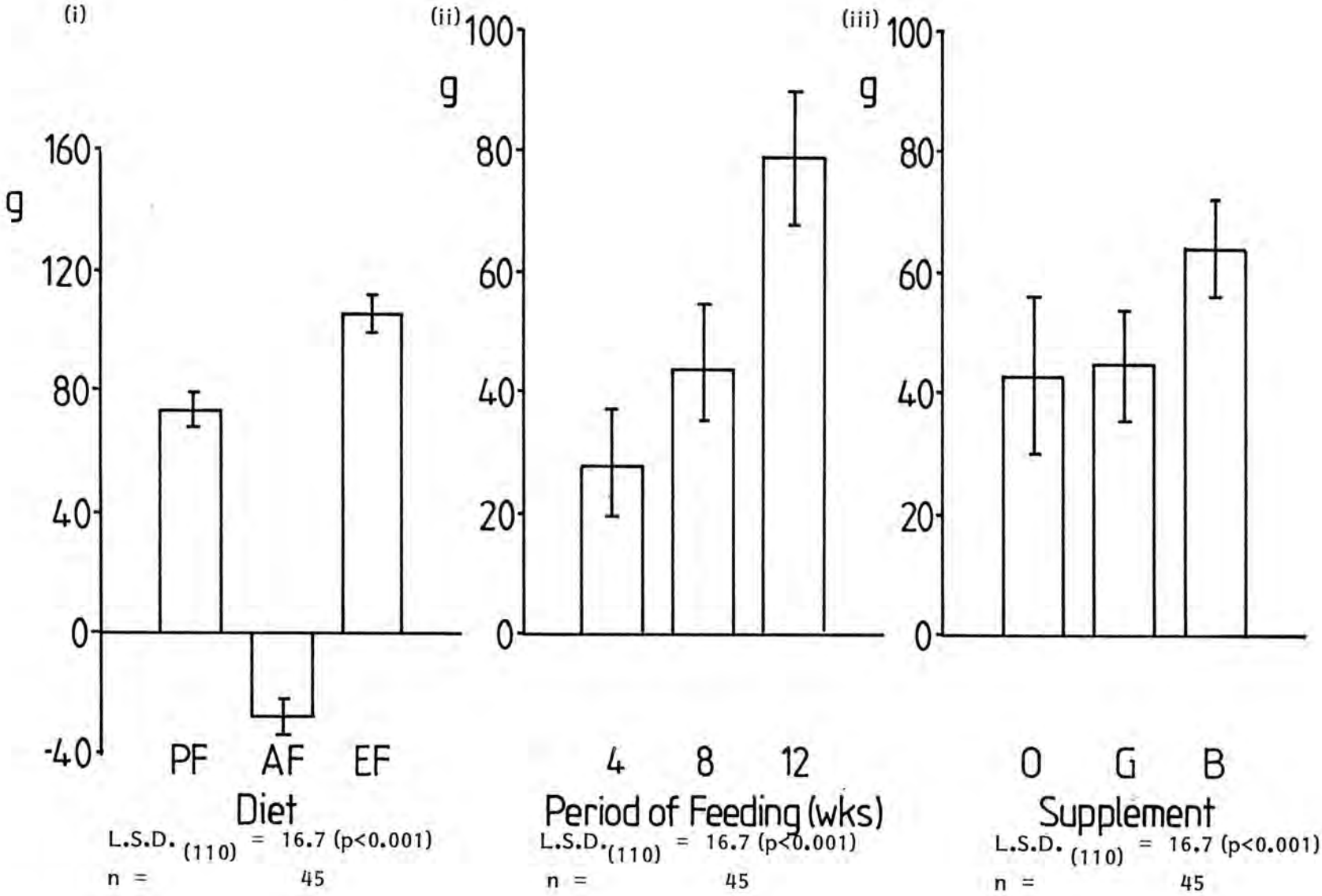
L.S.D.$_{(110)}$ = 33.2 ($p<0.01$)

n = 15

L.S.D.$_{(110)}$ = 49.7 ($p<0.001$)

n = 15

FIGURE 6b Live weight changes of rats as influenced by (i) basal diet – Plant Origin (PF), Animal Origin(AF) and an elemental diet(EF), (ii) time and (iii) supplement – no supplement(O), 10% gum arabic (G) and 10% wheat bran (B).
Results are mean ± S.E.M.



AF X time groups. All three EF X time period values were significantly different from each other. The live-weight with the PF diet fed for 12 weeks was significantly greater than the other two PF groups (Figure 6b (iv)). Figure 6b (v) shows the interaction of D X S. The most noticeable effect of supplement is evident with the AF trial, where live-weight gains ranged from -62.5 ± 6.80 g to 8.9 7.06 g. Only with the basal diet EF did gum supplementation significantly reduce liveweight ($p<0.001$), whilst overall bran may increase live-weight. When fed with PF or EF, there was a slight decrease in live-weight (7%-11%) respectively. This could be due to nutritional dilution of the diet by the bran. The interaction of D X T X S was significant ($p<0.05$). Appendix 4a gives the individual group means S.E.M. and shows the variation that exists between the component means, that constitute the overall means of the main effects of each of the two-way interactions, for each dietary trial. For example the difference between the unsupplemented EF diet and the gum supplemented diet EF, (EG) is not the same as that for the unsupplemented PF diet and the gum supplemented PF diet (PG), at 4 weeks.

Where further D X T X S interactions are significant, this explanation will be inferred.

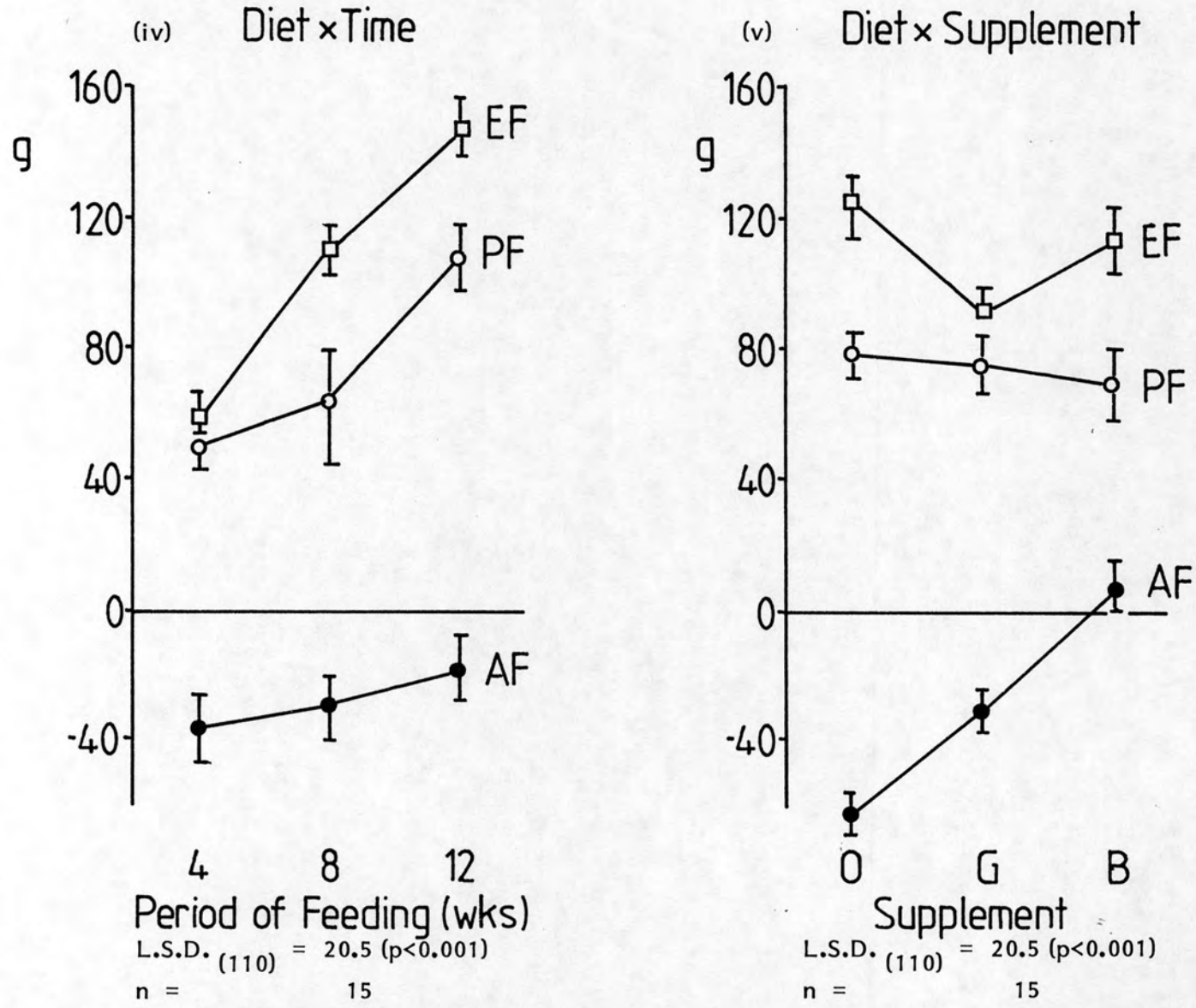
Where the three-way interactions are statistically significant only at the $p<0.05$ level, and the main effects and two interactions reach significant levels of $p<0.01$ and $p<0.001$, then the three way interaction may be regarded as far less important. The fact that this has attained statistical significance may possibly be due to chance, and poor replication ($n = 5$) and that there is no real influence of D X T X S. In this situation the significances of the main effects and two-way interactions are of more interest.

STOOL WEIGHT

Figure 6c (i) shows that stool weight was halved as a result of the dietary trial EF ($p<0.001$) with respect to dietary trials PF and AF, between which there was no significant difference. Of the supplements, bran increased stool weight by 78% and 40% with respect to the absence of supplementation and gum arabic supplementation (figure 6c (iii)). The interaction of D X S alone was significant ($p<0.05$). Figure 6c (iii) shows that, irrespective of the basal diet, the addition of bran increased stool weight. The absolute value was determined by the basal diet. With the PF trial the increase between PF

FIGURE 6b Live-weight changes of rats as influenced by the two-way interactions of (iv) basal diet- Plant Origin (PF), Animal origin (AF) and an elemental diet (EF) and time, and (v) basal diet and supplement- no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ±S.E.M.



and bran supplemented diet PF (PB) was 65%; between EF and bran supplemented EF, (EB), 56% and between AF and bran supplemented diet AF (AB), 78%. A bran supplement will increase stool weight, and that the percentage difference between an unsupplemented and bran supplemented diet is similar irrespective of basal diet. The three-way interaction was significant ($p<0.05$) and results are given in appendix 4b.

When expressed as a function of live-weight (g/kg) the stool: live-weight ratio was greatest with the AF trial (1.76 0.09 g/kg) and lowest on the EF dietary trial (0.84 0.5 g/kg). Overall the stool: live-weight ratio was greatest with bran ($p<0.001$). There was no significant difference between the unsupplemented and gum supplemented groups. Stool weight does not appear to be related to live-weight, but to diet and supplement.

The two-way interactions were not significant. The three-way interaction was significant ($p<0.05$) and the results are given in appendix 4c.

THE CAECUM AND ITS CONTENTS

(i) The caecal sac wet weight (C.S.W.W.)

Figure 6d (i) shows that only the difference between the AF and EF dietary trials was significant ($p<0.001$). Of the three time periods, only the difference between 8- and 12 weeks was significant ($p<0.001$, figure 6d (ii)). The L.S.D was 0.07 g. The difference between 8- and 12 weeks was 0.09 g. It may therefore be reasonable to conclude that there is no real effect of time. Of the supplements, gum arabic significantly increased ($p<0.001$) C.S.W.W. (figure 6d (iii)).

The two-way interactions of D X T, D X S and S X T were significant ($p<0.001$). There was no significant difference between the six EF and PF diet groups fed over time even though C.S.W.W. was heaviest after 4 weeks (figure 6d (iv)). With the AF diet, C.S.W.W. was heaviest after 8 weeks. Figure 6 d (v) shows, that, irrespective of basal diet gum arabic increased C.S.W.W. The difference between diets PG and EG was significant. The increase between the unsupplemented and gum supplemented groups was 34%, 73% and 74% for the PF, AF and EF trials respectively. Basal diet thus influence the increase. Figure 6d (v) shows the similarity between the unsupplemented

FIGURE 6c Daily dry stool weight of rats as influenced by (i) basal diet, Plant Origin (PF), Animal Origin(AF) and an elemental diet (EF), (ii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran(B) and (iii) the two-way interaction of basal diet and supplement.

Results are mean ± S.E.M.

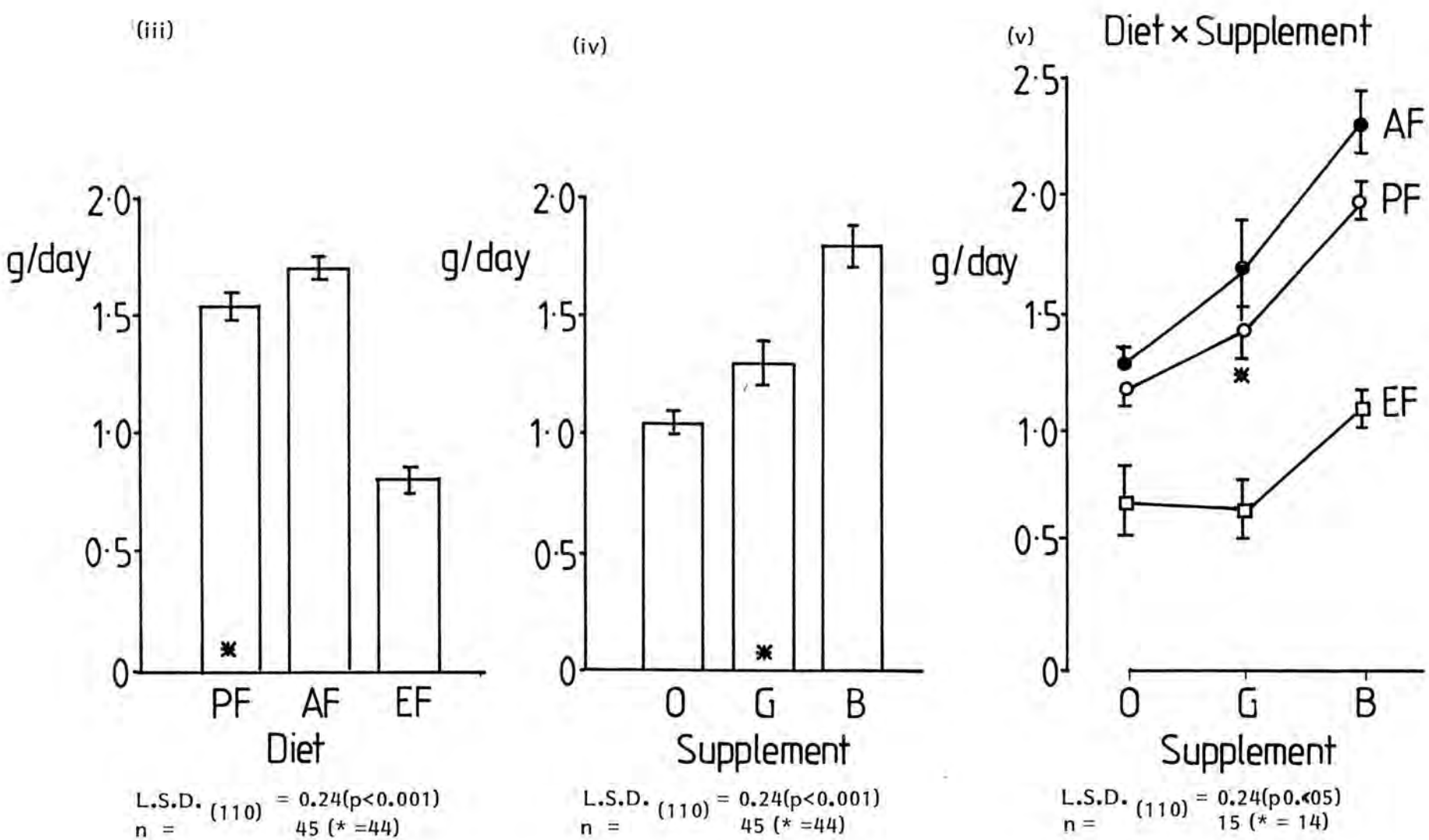
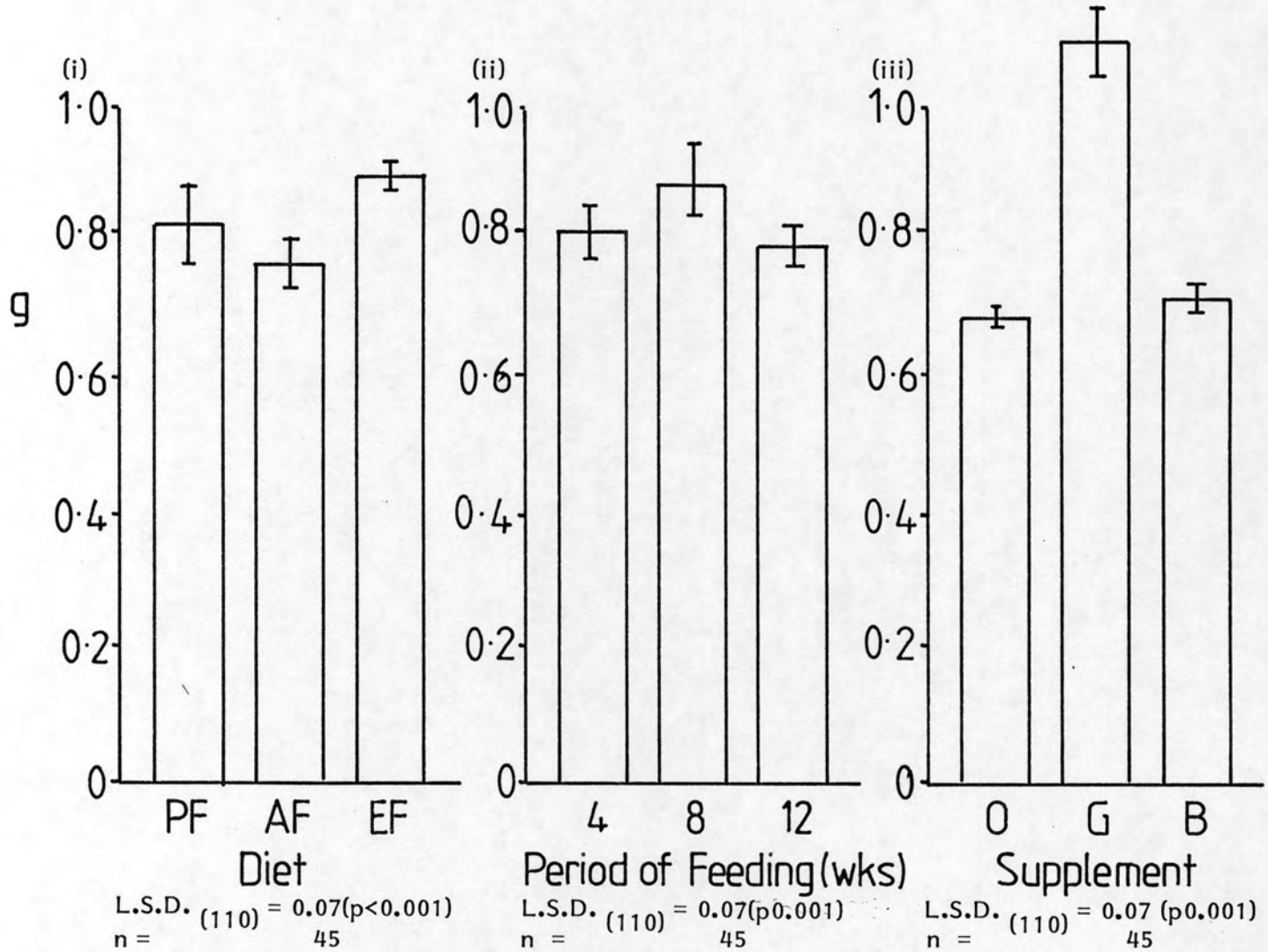


FIGURE 6d Caecal sac wet weight of rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin(AF) and an elemental diet(EF) (ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.



and bran supplemented groups within and between dietary trials. For example, with diet EB, C.S.W.W. was not significantly different from that of PF or PB. Figure 6d (vi) shows that gum arabic fed for 8 weeks (S X T) gave significantly greater C.S.W.W. than all the other groups ($p<0.001$). There was no significant difference between gum given for 4- or 12 weeks, or the other six groups.

The interaction of D X S X T was significant ($p<0.001$) and the results are given in appendix 4d. When expressed as a function of live-weight, the same trends were observed. The interaction of D X S X T was significant (Appendix 4e).

ii) Dry caecal contents (C.C.)

Figure 6e (i) shows that only the difference between the effects of the PF and EF dietary trials was significant ($p<0.001$). Overall, gum arabic significantly increased the dry C.C. weight ($p<0.001$). There was no significant difference between the effects of the absence of a supplement or bran (figure 6e (ii)).

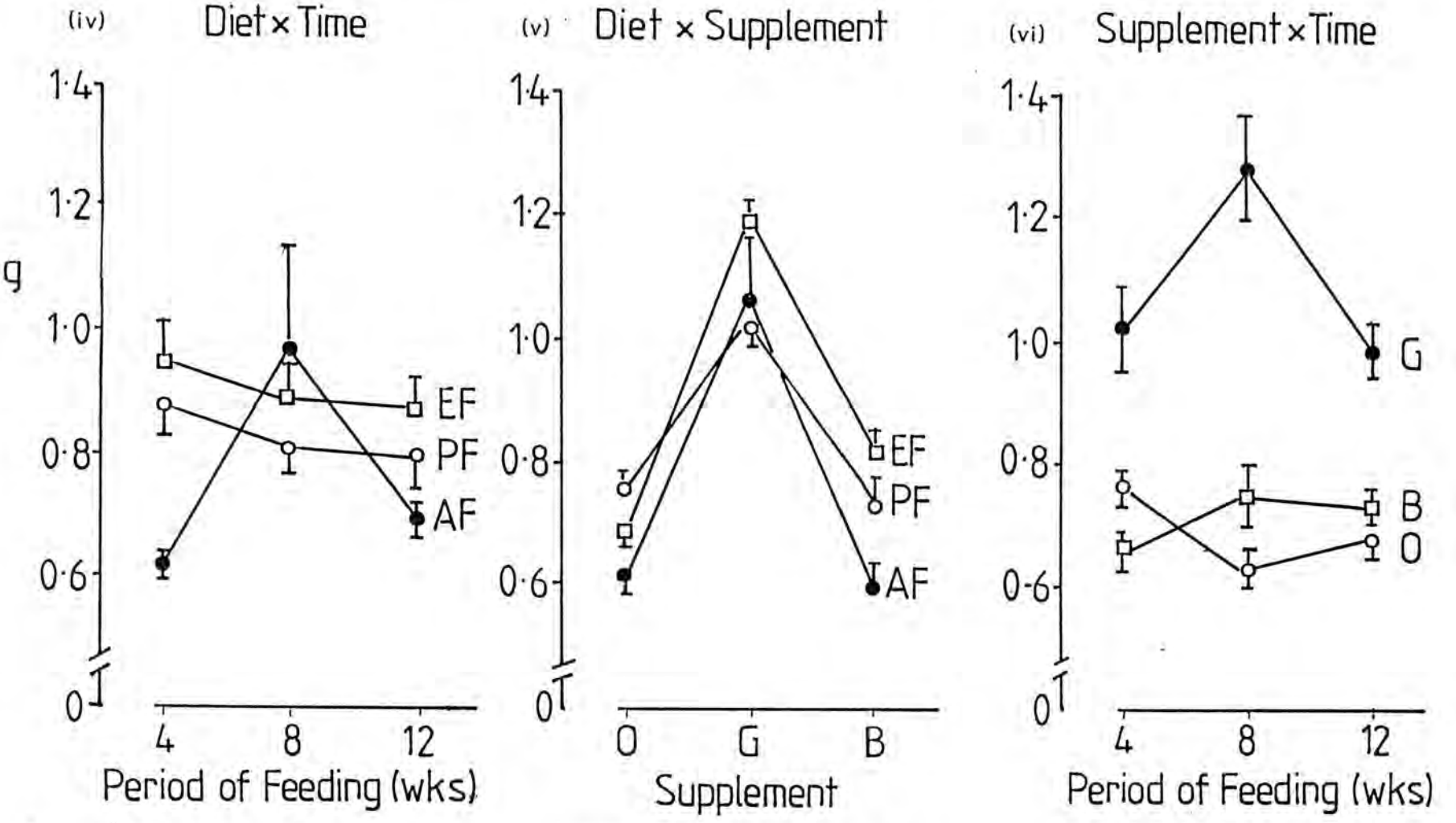
The two-way interactions of D X T, D X S and S X T were significant ($p<0.001$). Figure 6e (iii) shows that with time, the basal diets AF and EF exhibited the same trends with no significant difference between these six results. With diet PF, dry C.C. weight decreased with time ($p<0.001$). There was no significant difference between any of the results after 12 weeks of feeding (figure 6e (iii)). Figure 6e (iv) shows that, irrespective of basal diet, the addition of gum arabic significantly increased ($p<0.001$) dry C.C. with respect to the unsupplemented and bran supplemented diets. of the three gum groups, diet PG gave a significantly higher dry C.C. weight. There was close similarity of results between the remaining unsupplemented and bran supplemented diets. Figure 6e (v) shows that with time, there was no significant difference in effect between the unsupplemented and bran supplemented groups. With time gum arabic significantly altered C.C. weight the weight at 4 weeks being significantly greater than at 8- or 12 weeks. The three gum arabic results, with time, were significantly grater than the other six groups.

The three-way interaction was significant ($p<0.001$) and the results are given in appendix 4f. Despite this, it is reasonable to conclude that gum arabic increased C.C. weight and was greatest at 4 weeks with the PF diet.

When expressed as a function of live-weight, the C.C.: live-weight ratio was

Animal origin (AF) and an elemental diet(EF) and time, (v) basal diet and supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B) and (vi) supplement and time.

Results are mean ± S.E.M.



L.S.D. $_{(110)}$ = 0.14 ($p<0.001$)
n = 15

L.S.D. $_{(110)}$ = 0.14 ($p<0.001$)
n = 15

L.S.D. $_{(110)}$ = 0.14 ($p<0.001$)
n = 15

FIGURE 6e Dry caecal content weight from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and (ii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.

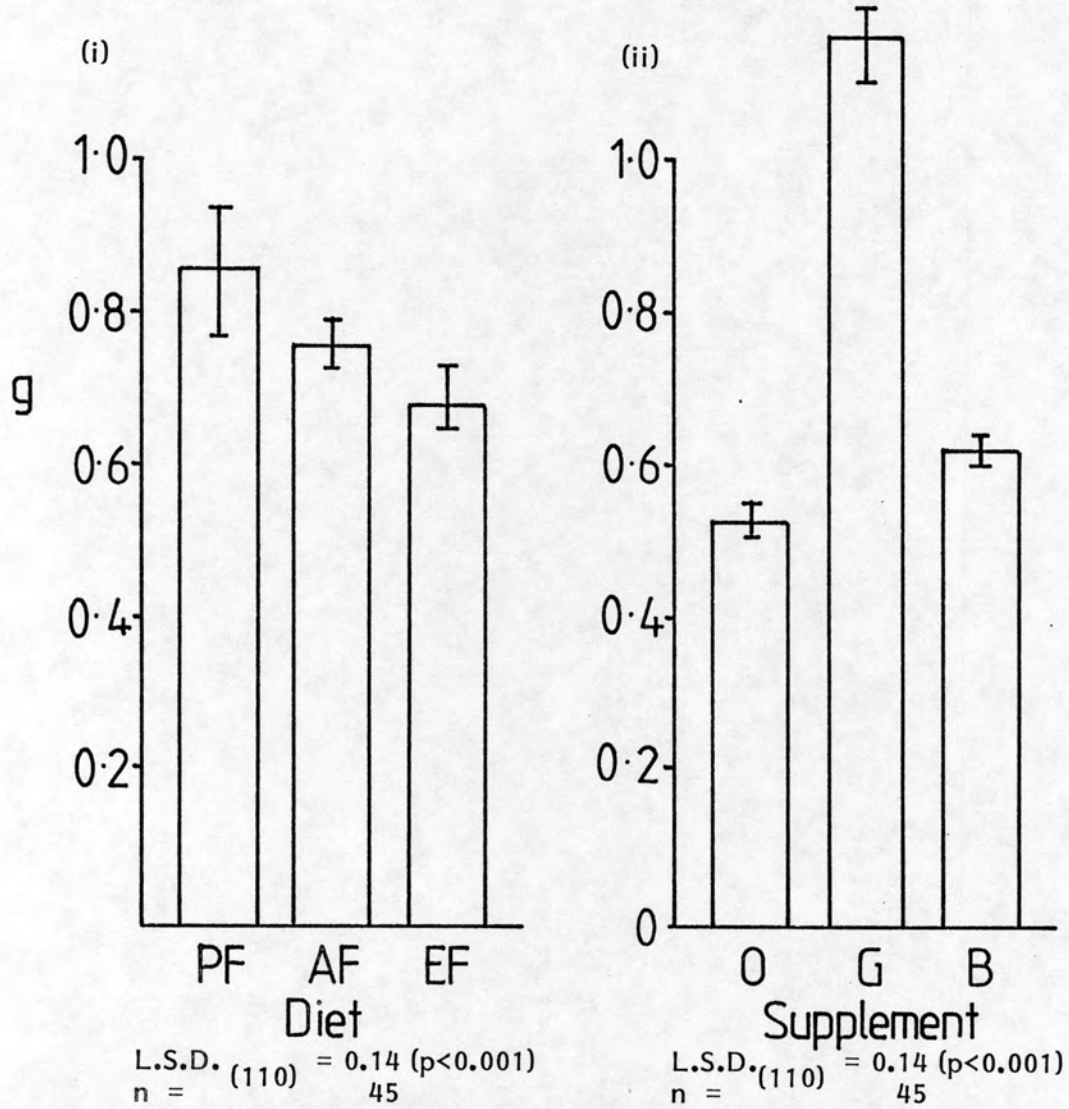
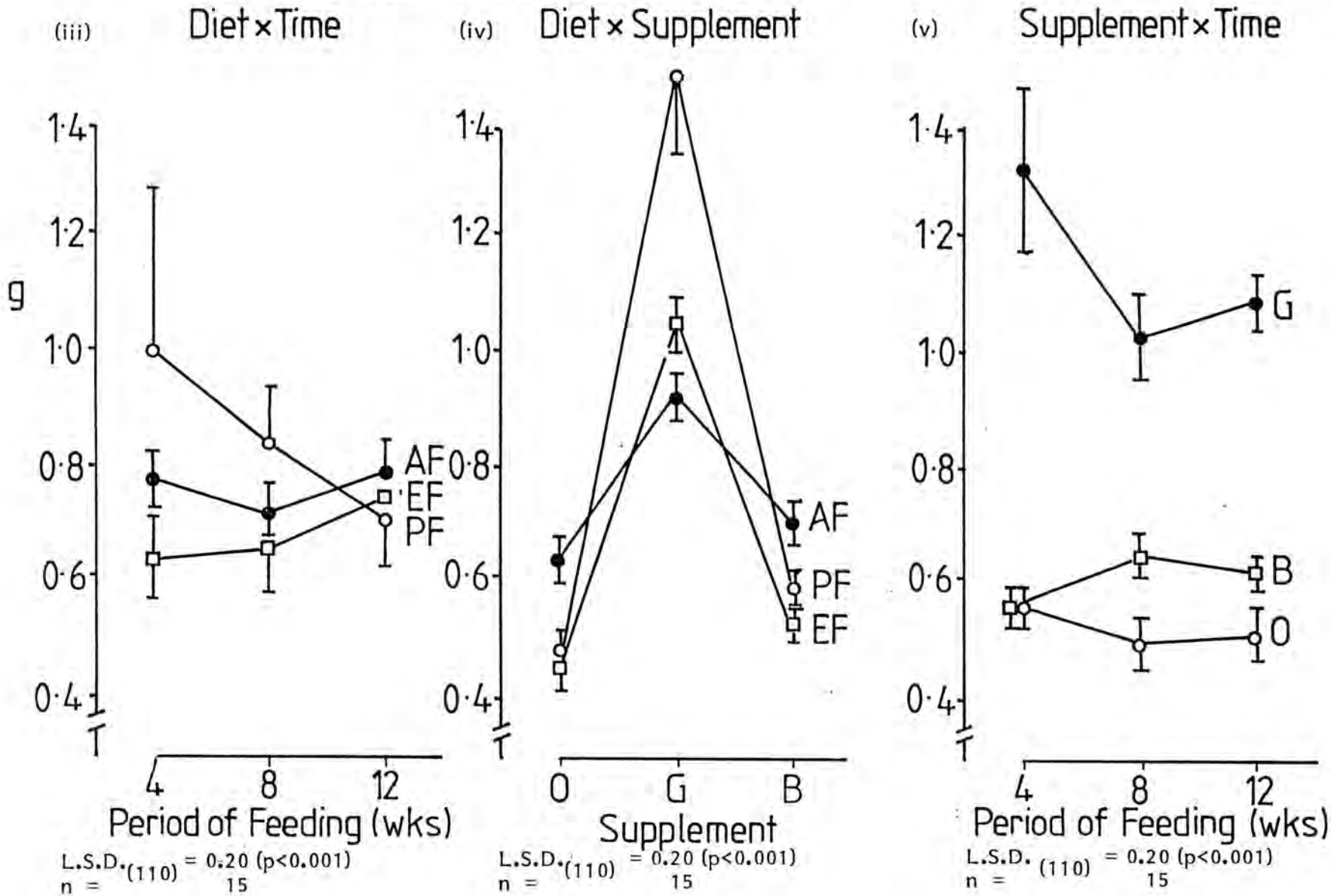


FIGURE 6e Dry caecal content weight from rats as influenced by the two-way interactions of (iii) basal diet – Plant Origin(PF), Animal Origin (AF) and an elemental diet (EF) and time, (iv) basal diet and supplement – no supplement(O), 10% gum arabic (G) and 10% wheat bran (B) and (vi) supplement and time.

Results are mean ± S.E.M.



lowest with diet EF (1.41± 0.17 g/kg) and greatest with diet AF (2.40±0.10 g/kg). All three ratios were significantly different from each other ($p<0.001$). Only the difference between 4- (2.00 ± 0.16 g/kg) and 12 weeks (1.70± g/kg) was significant ($p<0.01$). Gum arabic significantly increased ($p<0.001$) the C.C.: live-weight ratio (2.73 ± 0.12 g/kg). There was no significant difference in effect between the absence of a supplement and bran supplementation (1.40 g/kg for both groups). The two-way interactions of D X T and D X S were significant ($p<0.001$) as was that of S X T ($p<0.05$). With time there was no significant difference between diet EF given for 4,- 8- and 12 weeks and diet PF given for 8- and 12 weeks. After 12 weeks of diet PF the C.C: live-weight ratio was significantly lower than PF (4 weeks).

Irrespective of basal diet, gum arabic increased theC.C.: live-weight ratio. There was no significant difference between diets PG and gum supplemented diet AF (AG). Within each dietary trial there was no significant difference between the unsupplemented and bran supplemented diets. There was no significant difference between the unsupplemented and bran supplemented EF and PF diets.

The supplement gum arabic significantly increased the C.C.: live-weight ratio, which decreased with time. The C.C.: live-weight ratio after 4 weeks of gum arabic was significantly greater than the other 8 S X T groups. There was no significant difference between any of the six unsupplemented and bran supplemented X time groups.

The three way interaction was significant ($p<0.001$) and the results are given in appendix 4g.

2-6 DIAMINOPIMELIC ACID (DAPA)

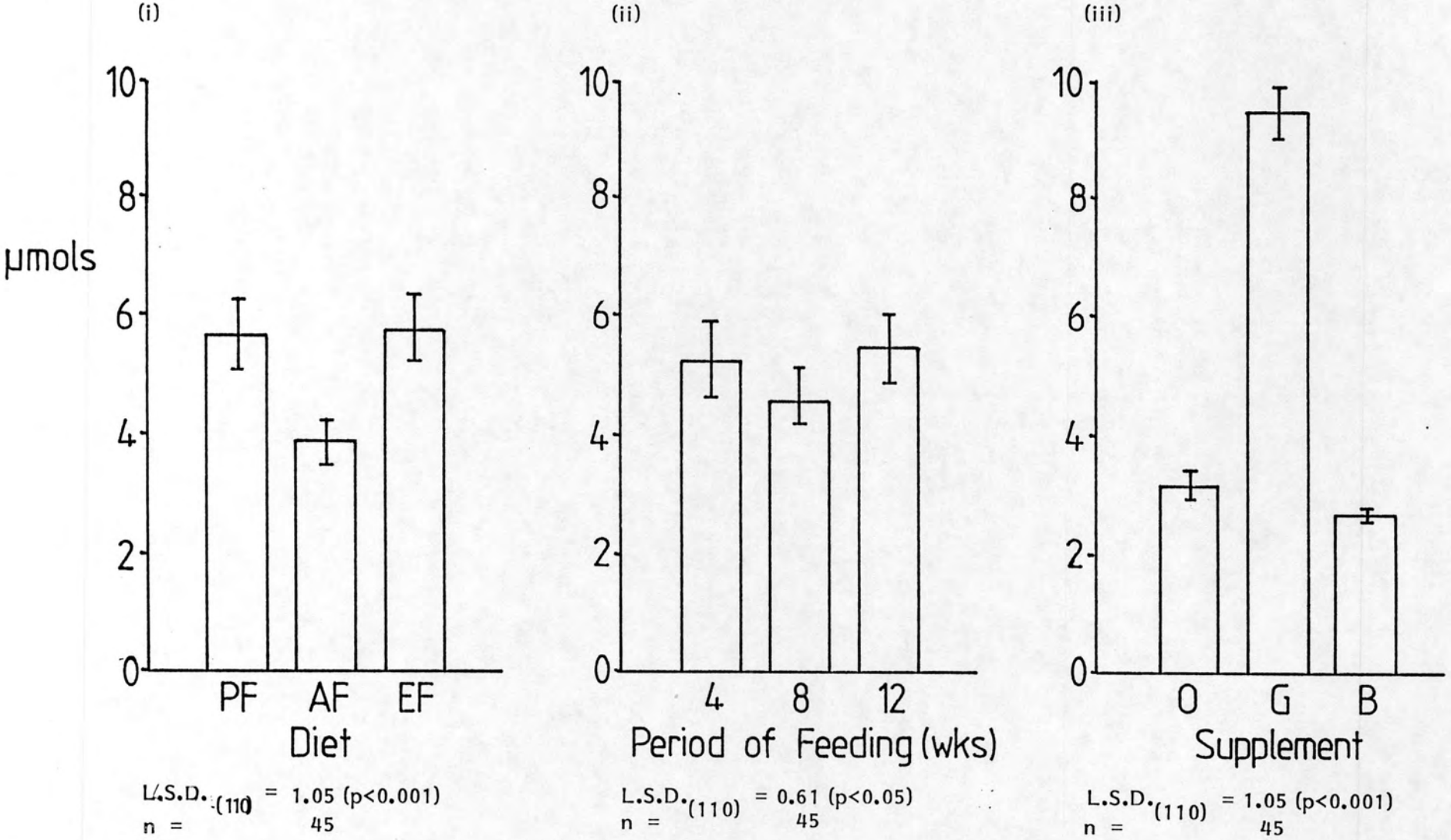
a) Total DAPA

(i) Caecal (µmols)

Figure 6f (i) shows that total caecal DAPA was significantly lower with dietary trial AF ($p<0.001$). There was no significant difference between dietary trials PF and EF. Total caecal DAPA decreased significantly after 8 weeks ($p<0.05$). There was no significant difference between 4- and 12 weeks (figure 6f (ii)). Gum arabic caused a significant 3 fold increased in total caecal DAPA ($p<0.001$). There was no significant difference in effect from the absence of supplement and a bran supplement (figure 6f (iii)).

FIGURE 6f . Total caecal 2-6 DAPA from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) (ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.



L.S.D.$_{(110)}$ = 1.05 ($p<0.001$)
n = 45

L.S.D.$_{(110)}$ = 0.61 ($p<0.05$)
n = 45

L.S.D.$_{(110)}$ = 1.05 ($p<0.001$)
n = 45

The interaction of D X S was significant ($p<0.001$) as was that of S X T ($p<0.01$). Irrespective of basal diet, the addition of gum arabic significantly increased total caecal DAPA (figure 6f (iv)). The basal diet influenced the magnitude of the increase. The AG diet group gave significantly less DAPA than either diet groups PG or EG, between which there was no significant difference. However the effect of diet AG was not significantly different from diet groups PF, EF, AF and AB (figure 6f (iv)). There was no significant difference between any of the unsupplemented or bran supplemented groups. Figure 6f (v) shows that with time the trends of the unsupplemented and bran supplemented groups closely paralleled each other, with no significant difference between any of the six values. These six values were lower than the results of the effects gum with time. After 8 weeks of gum arabic, total caecal DAPA had decreased by 20% ($p<0.01$). There was, however, no significant difference between the effect of feeding gum arabic for 4- or 12 weeks.

The three way interaction was not significant.

ii) Faecal (μmols/day)

Total faecal DAPA was significantly greater with the dietary trial PF ($p<0.001$). There was no significant difference between dietary trials AF and EF (figure 6g (i)). With the exception of dietary trial EF, total faecal DAPA exceeded total caecal DAPA. Overall, time was significant ($p<0.05$). Only the difference between 8- and 12 weeks was significant (figure 6g (ii)). Gum arabic significantly increased total faecal DAPA by 2 fold ($p<0.001$). There was no significant difference in effect with the absence of a supplement and a bran supplement (figure 6g (iii)).

Only the interaction of D X S was significant ($p<0.01$). Irrespective of basal diet gum arabic increased total faecal DAPA (figure 6 g (iv)). The choice of basal diet influenced the increase i.e. the difference between PF and PG was 61%, between EF and EG, 67% and between AF and AG 97%, all with respect to the unsupplemented diets. Within each dietary trial there was no significant difference between the unsupplemented and bran supplemented diets (figure 6g (iv)). There was no significant differences between diets PB, PF, EB and EG.

The three way interaction was not significant.

FIGURE 6f Total caecal 2-6DAPA as influenced by the two-way interactions of (iv) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and supplement - no supplement (O), 10% gum arabic (G) and (v) supplement and time.

Results are mean ± S.E.M

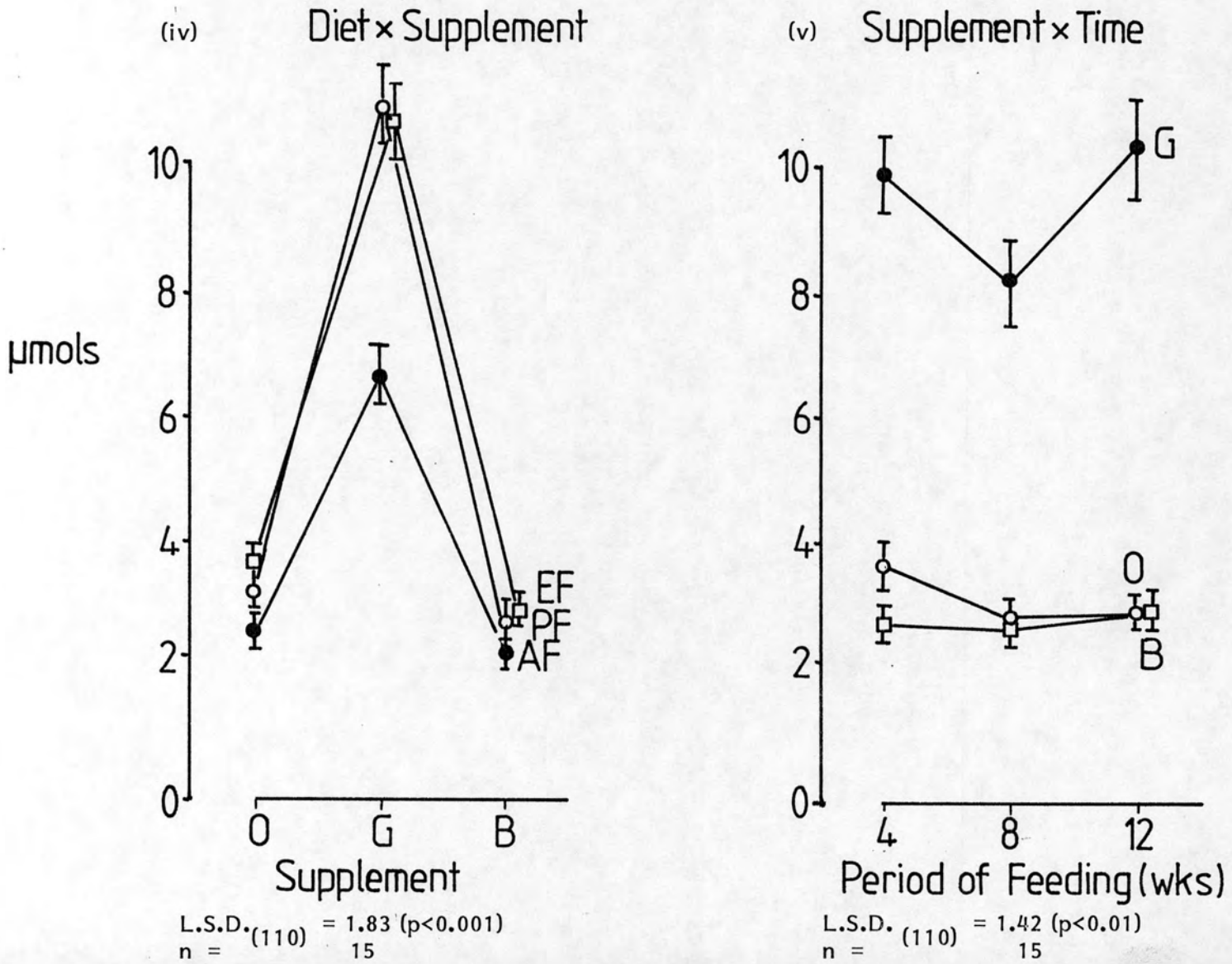
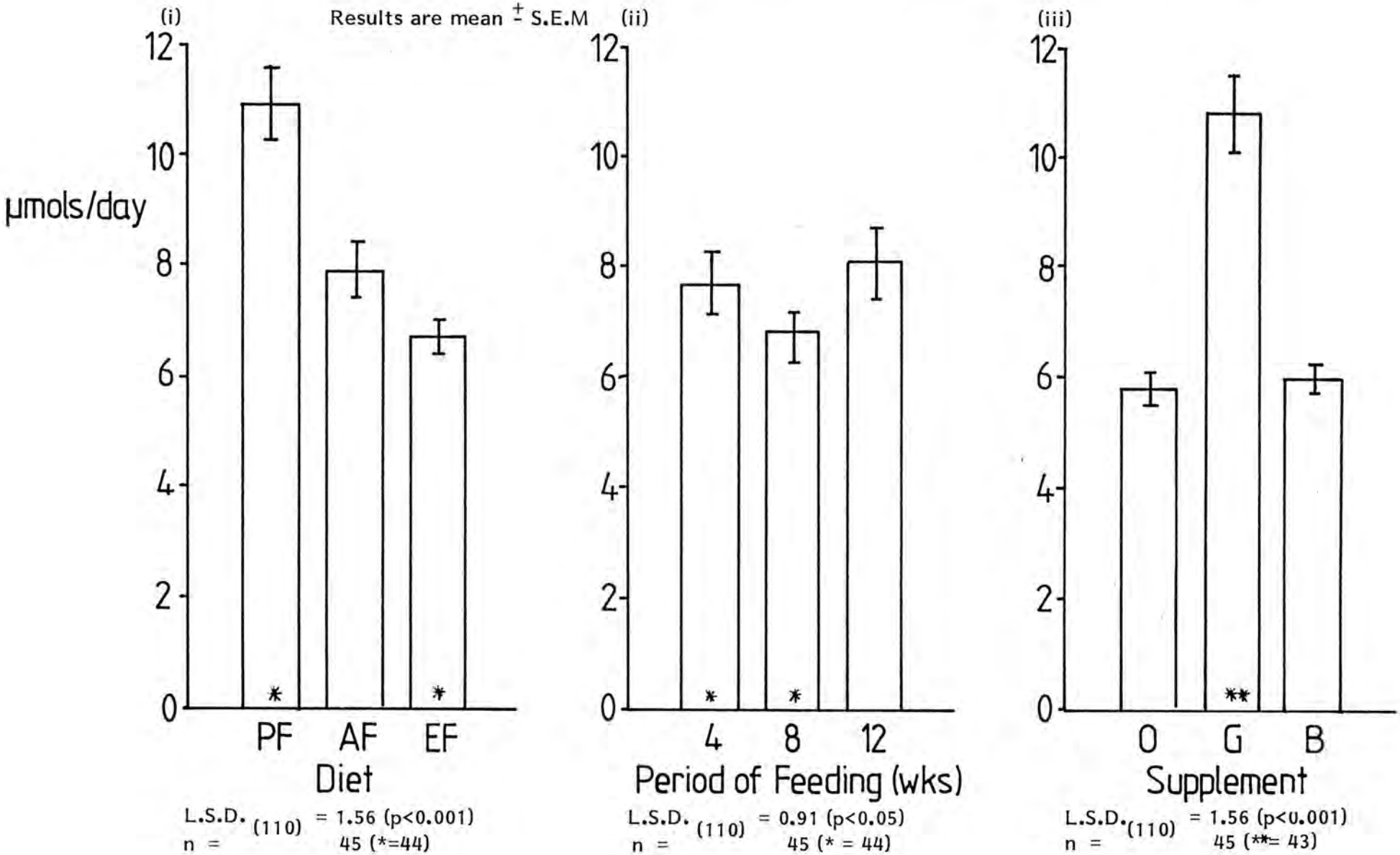


FIGURE 6g Total faecal 2-6 DAPA from rats as influenced by (i) basal diet (PF) , Animal Origin (AF) and an elemental diet (EF) ,(ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).



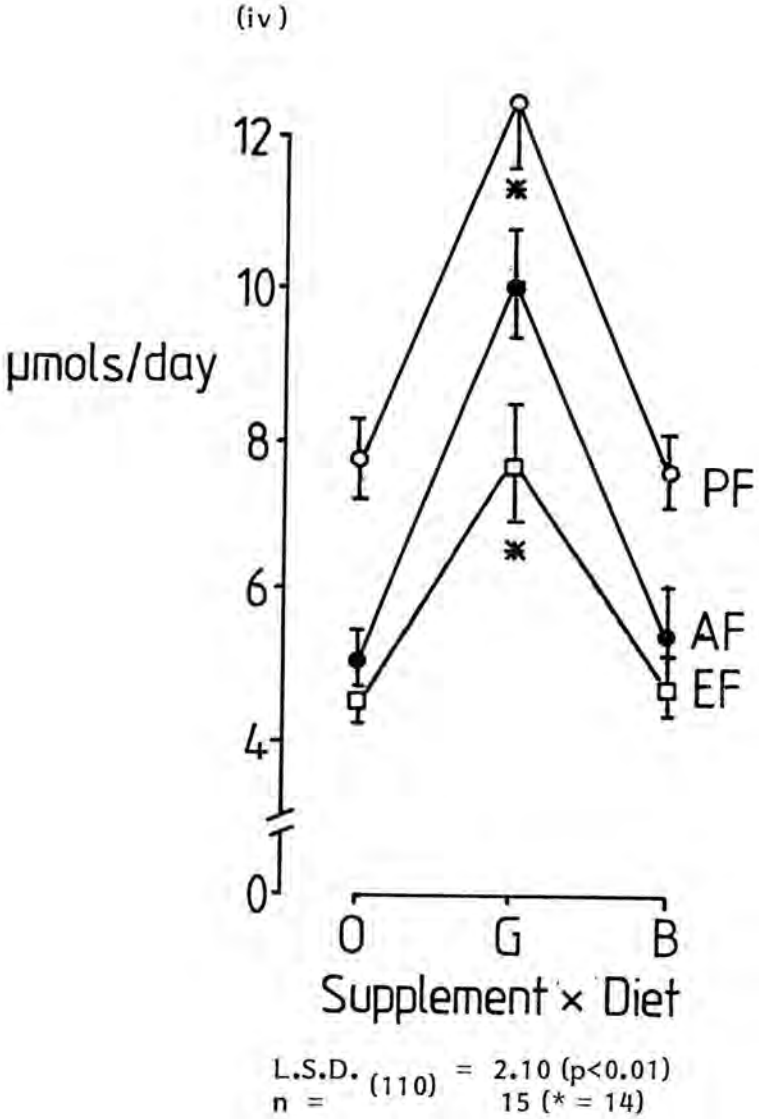
L.S.D. $_{(110)}$ = 1.56 ($p<0.001$)
n = 45 (*=44)

L.S.D. $_{(110)}$ = 0.91 ($p<0.05$)
n = 45 (* = 44)

L.S.D. $_{(110)}$ = 1.56 ($p<0.001$)
n = 45 (**= 43)

FIGURE 6g(iv) Total faecal 2-6 DAPA from rats as influenced by the two-way interaction of basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and supplement - no supplement (O), 10% gum arabic (G) 10% wheat bran (B).

Results are mean ± S.E.M



L.S.D. $_{(110)}$ = 2.10 ($p<0.01$)
n = 15 (* = 14)

b) Concentration of DAPA

i) Caecal (μmols/g)

Of the three basal diets, the dietary trial EF resulted in a significantly increased concentration of caecal DAPA ($p<0.001$). All three basal diets gave significantly different concentrations of DAPA (figure 6h (i)).

A supplement of bran significantly reduced the concentration of caecal DAPA ($p<0.001$), whilst gum arabic significantly increased the concentration of caecal DAPA ($p<0.001$) as shown in figure 6h (ii).

The two way interactions of D X T ($p<0.001$) and S X T were significant ($p<0.05$). With diets PF and AF, concentration increased with time. Only within dietary trial PF was this increase significant. Within the EF dietary trial the concentration decreased with time, but was not significant (figure 6h (iii)). Figure 6h (iii) shows the similarities that exist between different diets fed for different time periods. Figure 6h (iv) shows that with gum arabic and bran, the concentration of caecal DAPA increased with time. Concentration was lowest with all bran groups fed for each of the three time periods. Of the three bran groups, only a bran supplement fed for 12 weeks was not significantly different from the effect of no supplement fed for 8- and 12 weeks. Figure 6h (iv) shows other such similarities.

The three way interaction was significant ($p<0.05$), and the results are given in appendix 4h.

ii) Faecal (μmols/g)

Of the three basal diets, diet AF gave the lowest concentration of faecal DAPA ($p<0.001$). There was no significant difference between the overall effects of dietary trials PF and EF (figure 6j (i)). Of the three time periods, the concentration of faecal DAPA decreased after 8 weeks ($p<0.001$). There was no significant difference between the values at 4- and 12 weeks (figure 6j (ii)). Of the supplements, gum arabic significantly increased the concentration of faecal DAPA ($p<0.001$). A supplement of bran significantly decreased the concentration of faecal DAPA (figure 6j (iii)), with respect to effect in the absence of a supplement.

The interactions of D X T and D X S were significant ($p<0.001$). All the AF

FIGURE 6h Caecal concentration of 2-6 DAPA from rats as influenced by (i) basal diet- Plant Origin (PF), Animal Origin(AF) and an elemental diet (EF) and (ii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M

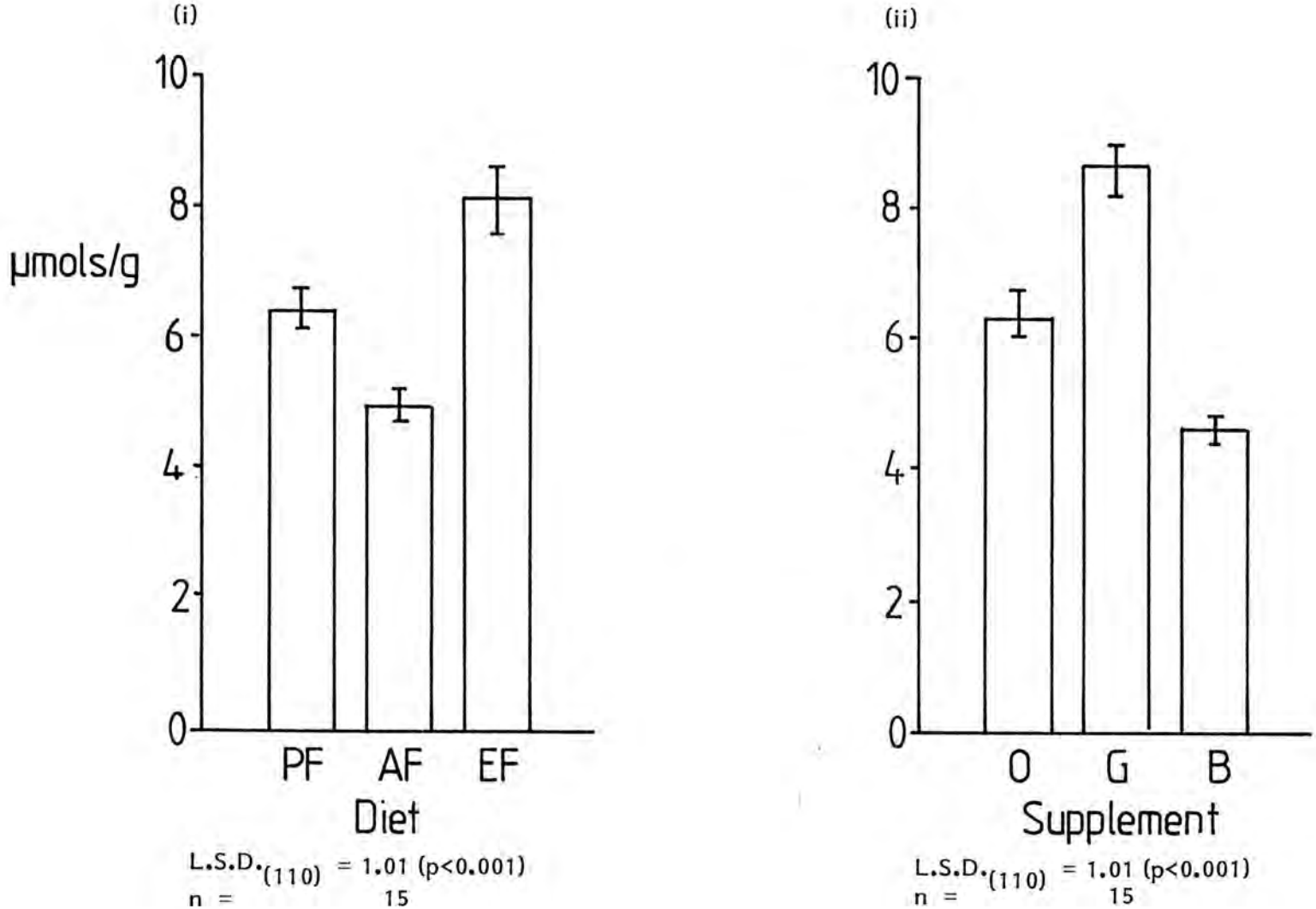


FIGURE 6h Caecal concentration of 2-6 DAPA as influenced by the two-way interactions of (iii) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time and (iv) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B) and time.

Results are mean ± S.E.M.

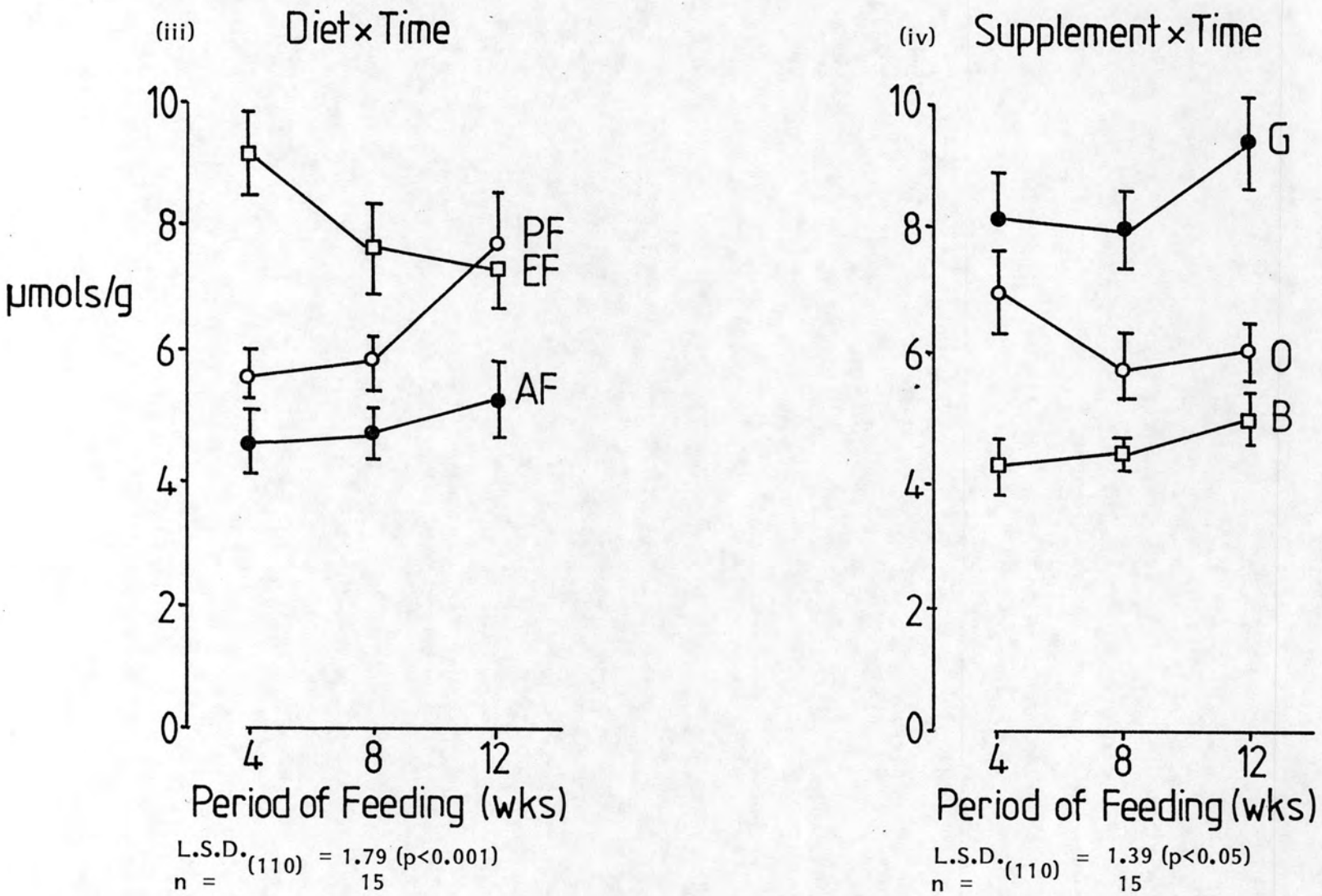
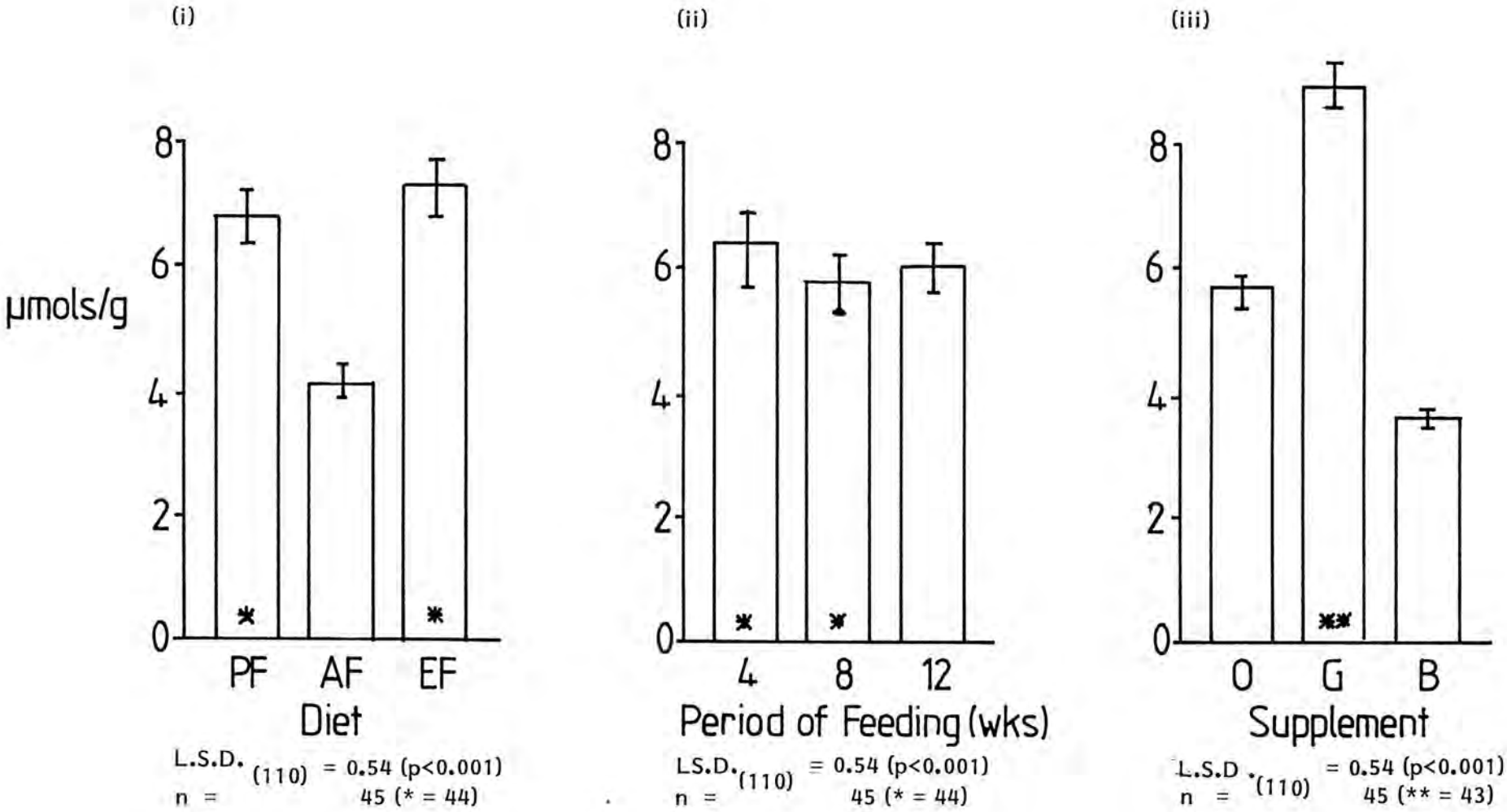


FIGURE 6j Faecal concentration of 2-6 DAPA from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) ,(ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M



diet groups gave significantly lower concentrations than any of the other six diet X time groups (figure 6j (iv)). There was no significant difference between diet PF fed for 4- and 12 weeks and diet EF fed for 8- and 12 weeks. Figure 6j (iv) shows other such similarities between these two basal diets. Figure 6j (v) shows that irrespective of basal diet, gum arabic significantly increased ($p<0.001$) the concentration of faecal DAPA within all dietary trials. The addition of bran reduced the concentration of DAPA. There was no significant difference between diets AF, PB and EB or diets PG and EG. The increase in faecal DAPA with gum arabic was related to basal diet: the increase between diet AF and AG was 39%, between diet PF and PG 57% and between EF and EG 64%, all with respect to the unsupplemented diets.

The three way interaction was significant ($p<0.01$), and the results are given in appendix 4i.

SHORT CHAIN FATTY ACIDS (S C F A's)

a) Total S C F A's

i) Caecal (µmols)

Basal diet was influential upon the total caecal S C F A's (figure 6k (i)). Only the difference between the dietary trials PF and AF was significant ($p<0.001$). Of the three time periods, only the difference between 8- and 12 weeks was significant ($p<0.001$) as shown in figure 6k (ii). Of the three supplements, gum arabic significantly increased ($p<0.001$) the total caecal S C F A's by 3 fold (figure 6k (iii)).

The interactions of D X T and D X S were significant ($p<0.001$) as was the interaction of S X T ($p<0.01$). With time, the dietary trials of AF and EF exhibited similar trends (figure 6k (iv)). With the dietary trial PF, total caecal S C F A's decreased with time. Figure 6k (iv) shows that there was no significant difference between any of the diets after 12 weeks of feeding. Figure 6k (iv) also shows the other similarities that existed between the different diets fed for different time periods. For example there was no significant difference between the diet PF fed for 4- and 12 weeks and the intermediary values. Figure 6k (v) shows that irrespective of diet, gum arabic increased total caecal S C F A's ($p<0.001$), that the magnitude of the increase was related to basal diet. There was no significant difference between diets AG and EG, whilst total caecal S C F A's as a result of diet PG were significantly increased. There was no significant difference between any

FIGURE 6j Faecal concentration of 2-6 DAPA as influenced by the two -way interactions of (iv) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time and (v) supplement - no supplement (O), 10% gum arabic (G), and 10% wheat bran (B) and basal diet.
Results are mean ± S.E.M

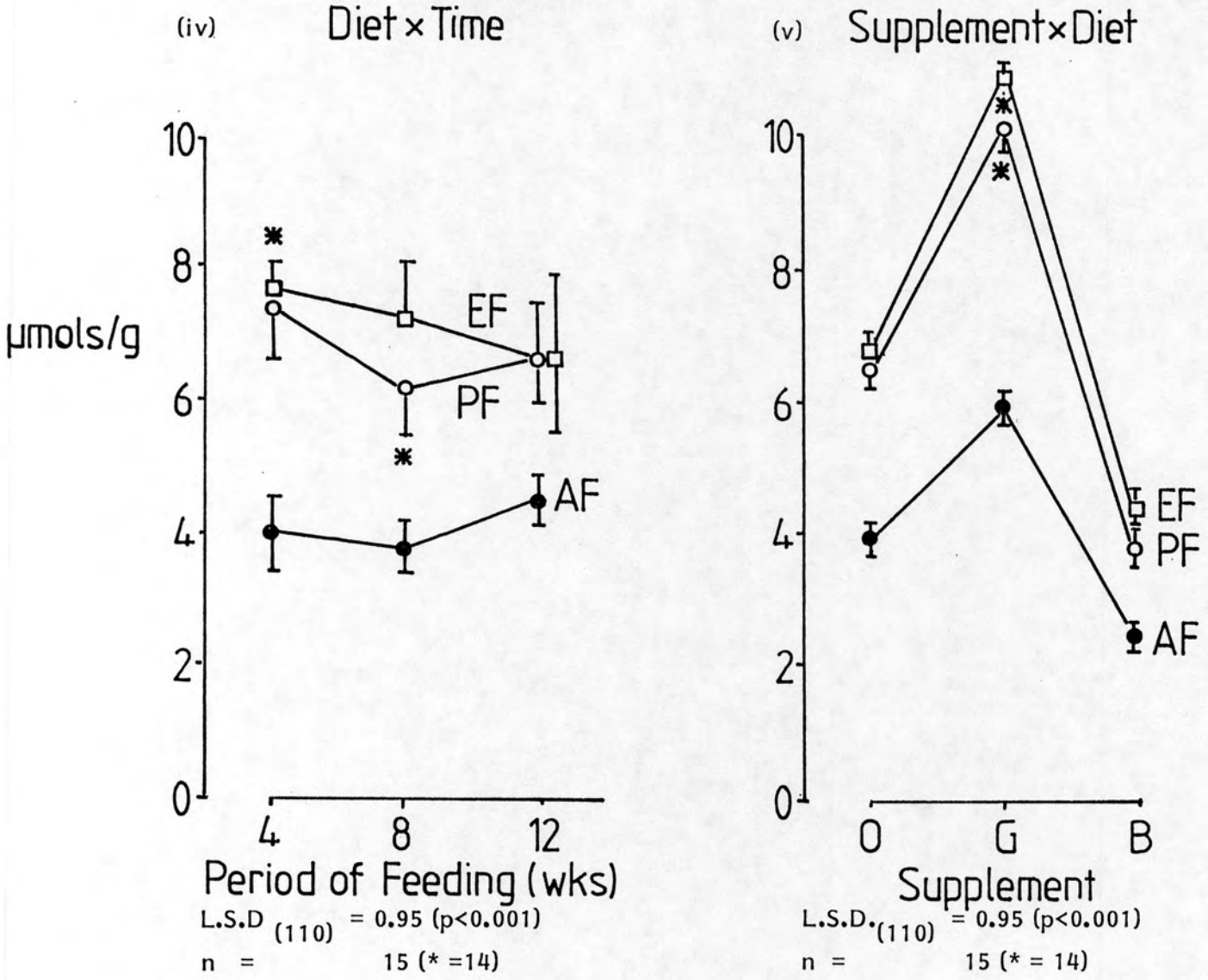


FIGURE 6k Total caecal SCFA's from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF), (ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.

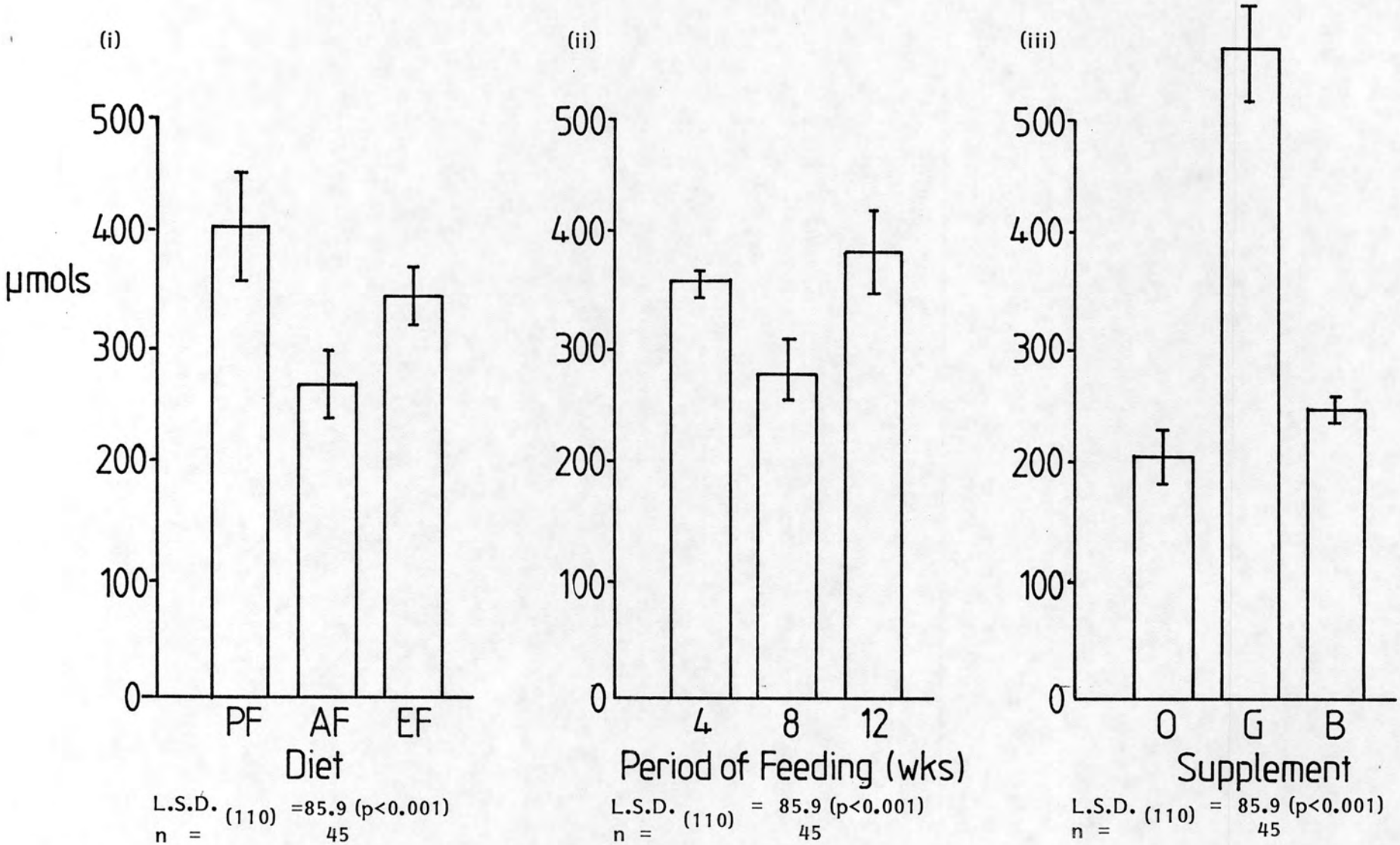
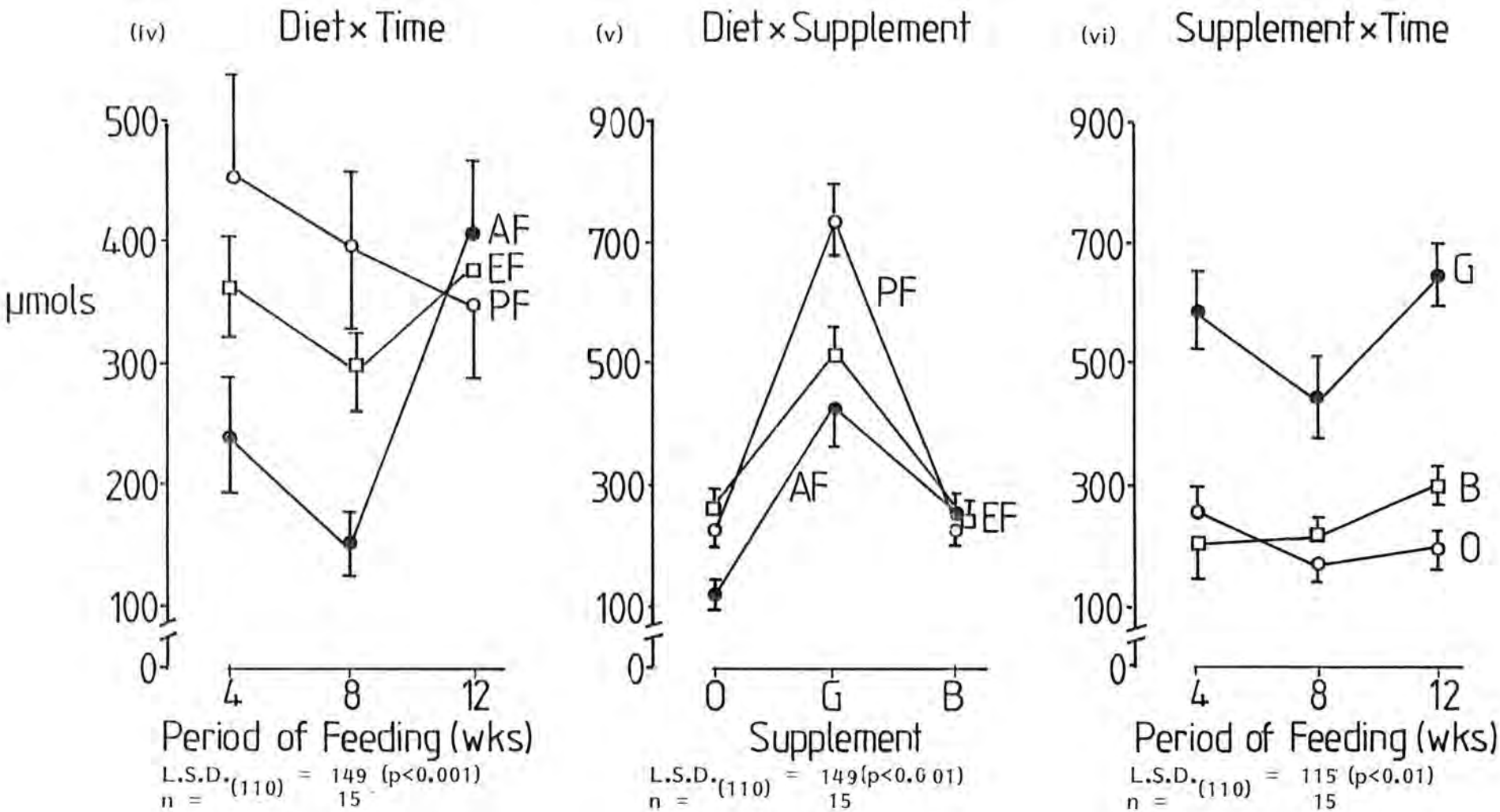


FIGURE 6k Total caecal SCFA's from rats as influenced by the two-way interactions of (iv) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time, (v) basal diet and supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B), and (vi) supplement and time.

Results are mean ± S.E.M



of the unsupplemented and bran supplemented diets. Figure 6k (vi) shows that gum arabic fed for 8- and 12 weeks gave significantly higher total caecal S C F A's ($p<0.01$). There was no significant difference between the three bran supplemented X time period groups and the unsupplemented groups fed for 4- and 12 weeks.

The three way interaction was not significant.

ii) Faecal (µmols/day)

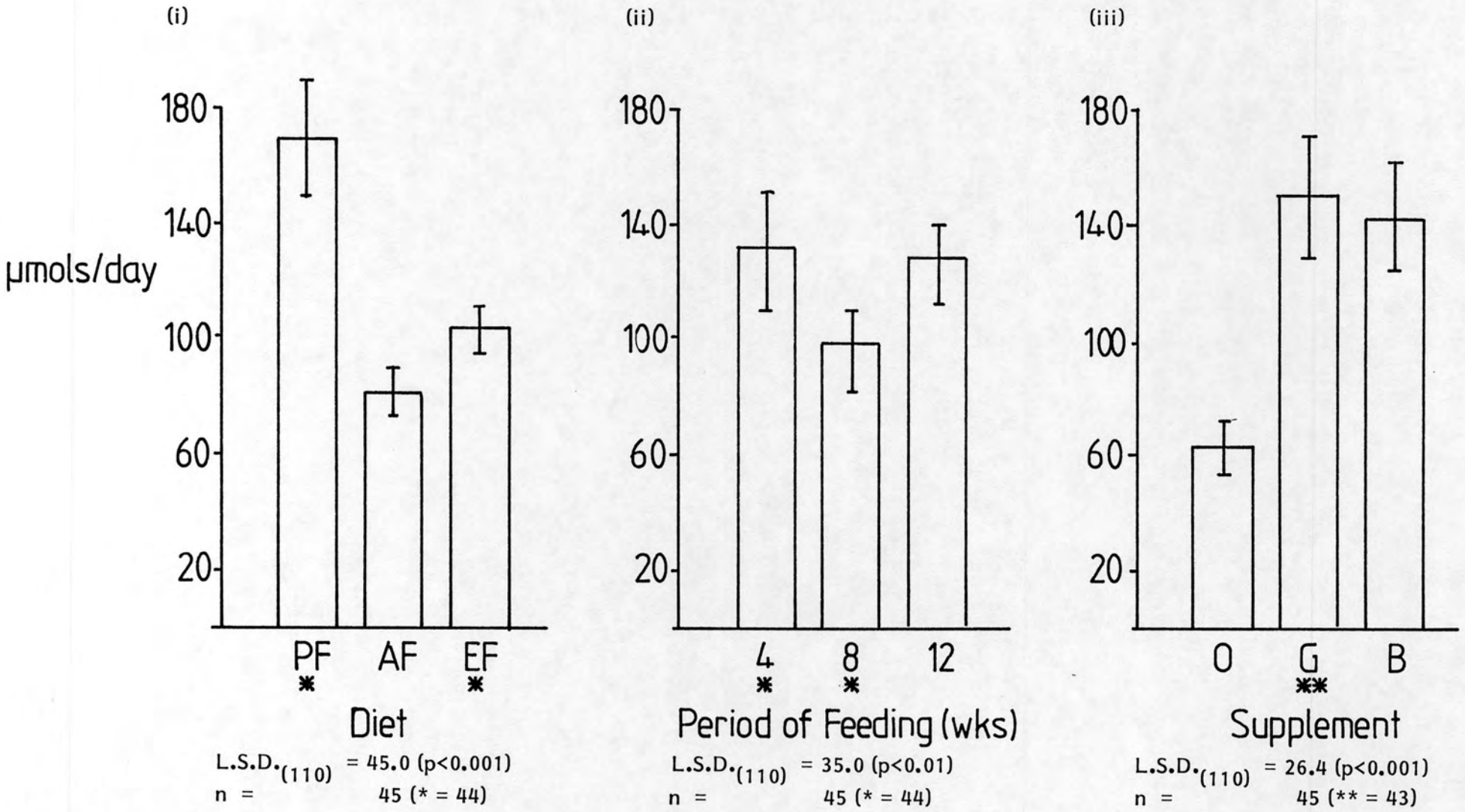
Figure 6 m (i) shows that total faecal S C F A's were significantly increased with basal diet PF ($p<0.001$). There was no significant difference between the dietary trials AF and EF. Overall, all faecal S C F A's were one-quarter those of their respective caecal values. Time was significant ($p<0.01$), and figure 6 m (ii) shows that after 8 weeks there was an overall decrease in total faecal S C F A's. Both gum arabic and bran significantly increased total faecal S C F A's ($p<0.001$) with respect to the effect of no supplement (figure 6m (iii)). The overall values observed with the unsupplemented group represented one-quarter that of the respective caecal values; that of the bran supplemented groups represented approximately one half.

The two way interactions of D X T and D X S were significant ($p<0.001$). With time, the dietary trials PF and AF showed similar trends, total faecal S C F A's decreasing after 8 weeks of feeding. There was no significant difference between diets AF and EF both fed for 8 weeks, and the five intermediate groups (figure 6m (iv)). The value observed with diet PF fed for 4 weeks was significantly greater than the other 8 groups. Figure 6m (iv) shows that, whilst there was no significance between the three AF groups or between the three EF groups, this was not so for the three PF groups. Figure 6m (v) shows that whilst gum arabic did increase total faecal S C F A's, only with the dietary trial PF was the effect of gum arabic, with respect to the unsupplemented diet PF, significant. With all dietary trials, the addition of bran also increased total faecal S C F A's. The increase was only significant between diets AF and AB. There was no significant difference between any of the three EF diet groups (figure 6m (v)). There was no significant difference between any of the unsupplemented forms. Figure 6m (v) shows the similarity of the effects of the different diet groups. For example diet EF is not significantly different in effect from diets AG, PF, EG and EB.

The three-way interaction was significant ($p<0.001$), and the results are given

FIGURE 6m Total faecal SCFA's from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF), (ii) time and (ii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.

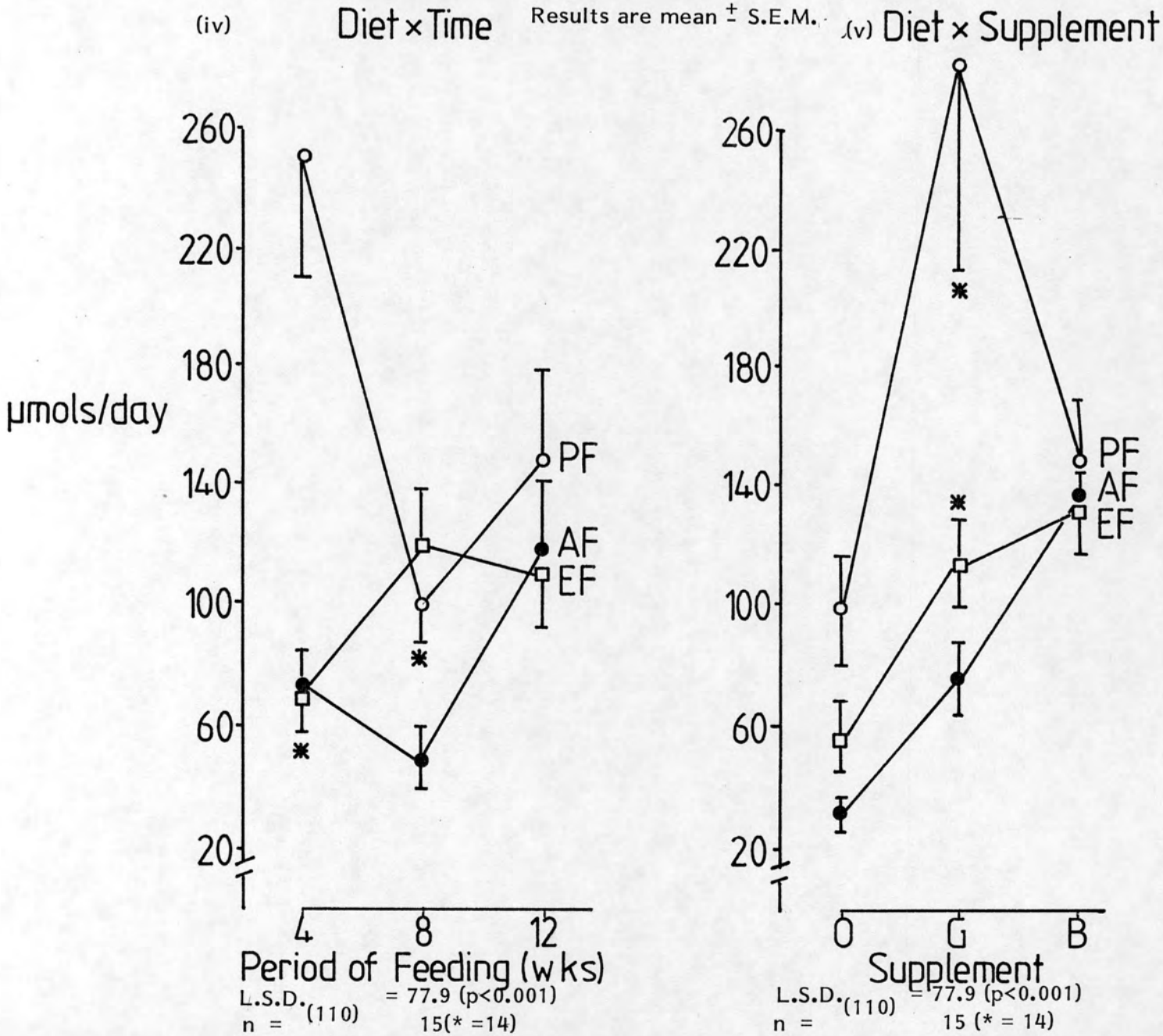


L.S.D.$_{(110)}$ = 45.0 ($p<0.001$)
n = 45 (* = 44)

L.S.D.$_{(110)}$ = 35.0 ($p<0.01$)
n = 45 (* = 44)

L.S.D.$_{(110)}$ = 26.4 ($p<0.001$)
n = 45 (** = 43)

FIGURE 6m Total faecal SCFA's from rats as influenced by the two-way interactions of (iv) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet(EF) and time and (v) basal diet and supplememt - no supplement(O), 10% gum arabic (G) and 10% wheat bran (B)



in appendix 4j .

b) Concentration of S.C.F.A's (μmols/g)

i) Caecal

Figure 6n (i) shows that the concentration of S C F A's was significantly lower with the AF dietary trial ($p<0.001$). There was no significant difference between the overall effects dietary trials PF and EF. Time was significant ($p<0.001$), the concentration of S C F A's significantly decreasing after 8 weeks (figure 6n (ii)). Supplementation was significant ($p<0.001$) but only the difference between the effects of the absence of a supplement, and supplementation with gum arabic was significant (figure 6n (iii)).

The two way interactions of D X T and D X S were significant ($p<0.001$) as was that of S X T ($p<0.01$). Irrespective of basal diet, caecal concentration of SCFA's was decreased after 8 weeks of feeding, although this did not always reach significance within each dietary trial (figure 6n (iv)). There was no significant difference between the three PF diet x time period groups. Within the AF dietary trial, the concentration was significantly greater at 12 weeks, whilst within the EF dietary trial only the difference between 4- and 8 weeks was significant. There was no significant difference between any of the three basal diets fed for 12 weeks. Figure 6n (iv) shows the similarities that do exist between the diets fed for different time periods. For example there was no significant difference between diets PF (8 weeks), EF (12 weeks) and the four intermediate values. Figure 6n (v) shows that, with the exception of diet EG, gum arabic increased the concentration of S C F A's, although this did not reach significance within the PF dietary trial with respect to diet PF. Only with the dietary trial AF, did the addition of bran significantly increase the concentration of S C F A's with respect to an unsupplemented diet. Despite the different trends associated with each D X S combination (figure 6n (v)), there do exist similarities between the different diet groups. For example there was no significant difference between diets AB, PB, AG and PF, or diets EG, EB, PG and EF. Figure 6n (vi) shows that, irrespective of supplementation, the concentration of S C F A's decreased after 8 weeks, whilst concentrations increased on all diets after 12 weeks. Within the unsupplemented- and bran supplemented x time groups, there was a significant difference between 8- and 12 weeks. With the gum supplementation x time group, the difference occurred between 4- and 12 weeks. Figure 6n (vi) shows there to be no significant difference between bran fed for 4- and 8 weeks

FIGURE 6n Caecal concentration of SCFA's from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet EF, (ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M

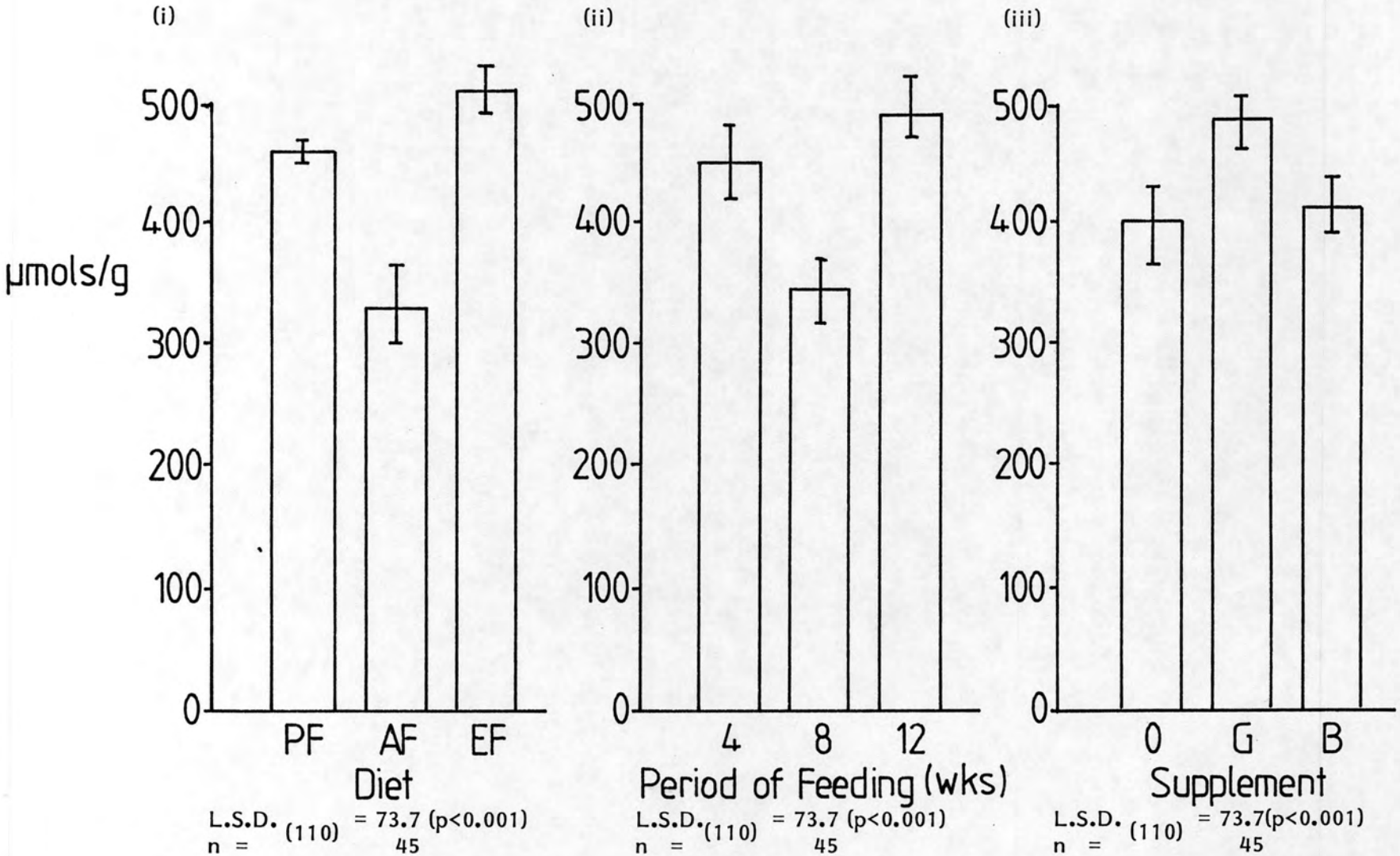
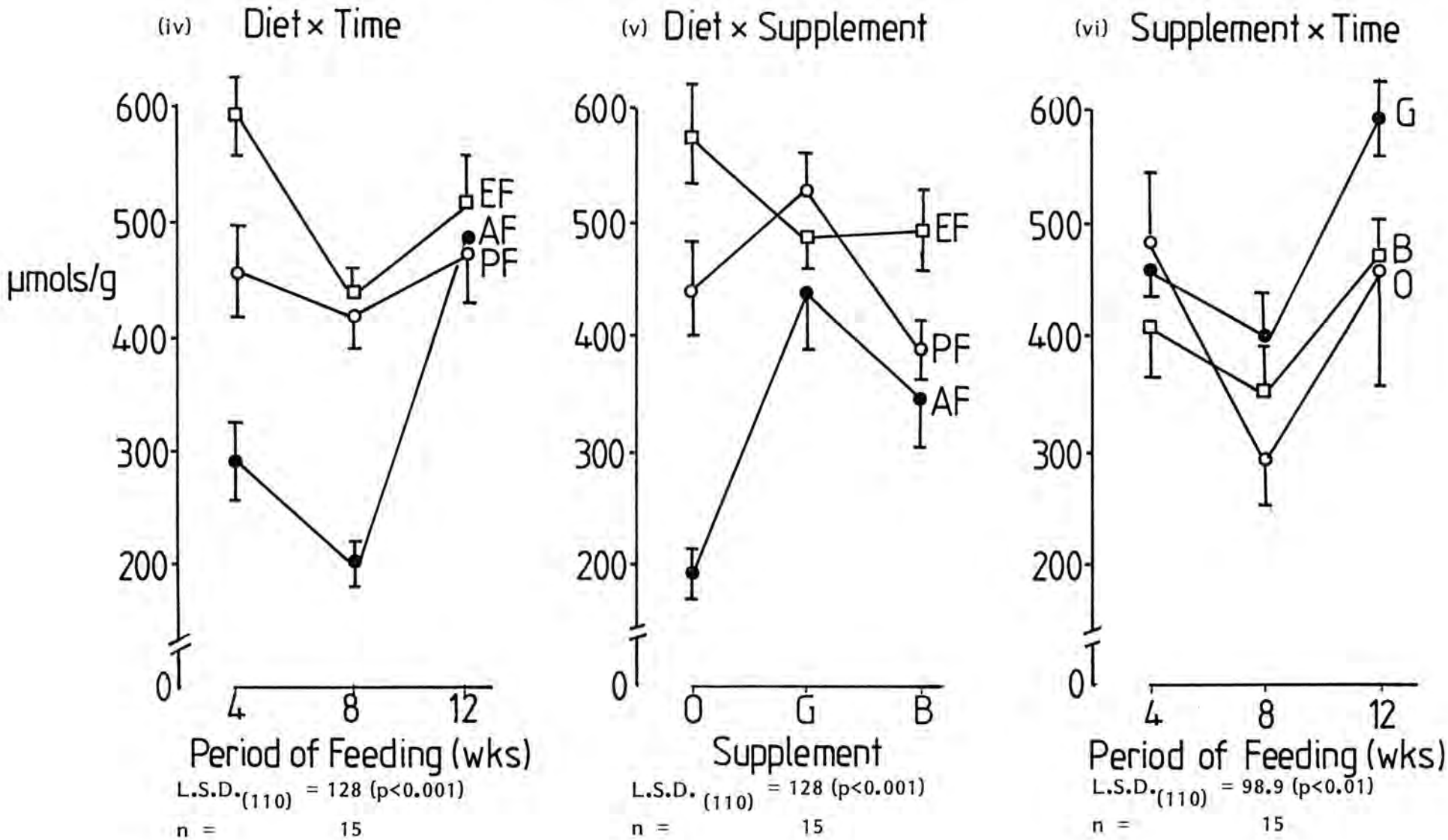


FIGURE 6n Caecal concentration of SCFA's from rats as influenced by the two-way interactions of (iv) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time, (v) basal diet and supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B) and (vi) supplement and time.

Results are mean ± S.E.M.



and the two intermediary groups, and no significant difference between gum arabic fed for 8 weeks and the absence of a supplement over a period of 4 weeks, and the four intermediary groups.

The three-way interaction was significant ($p<0.001$) and the results are given in appendix 4k.

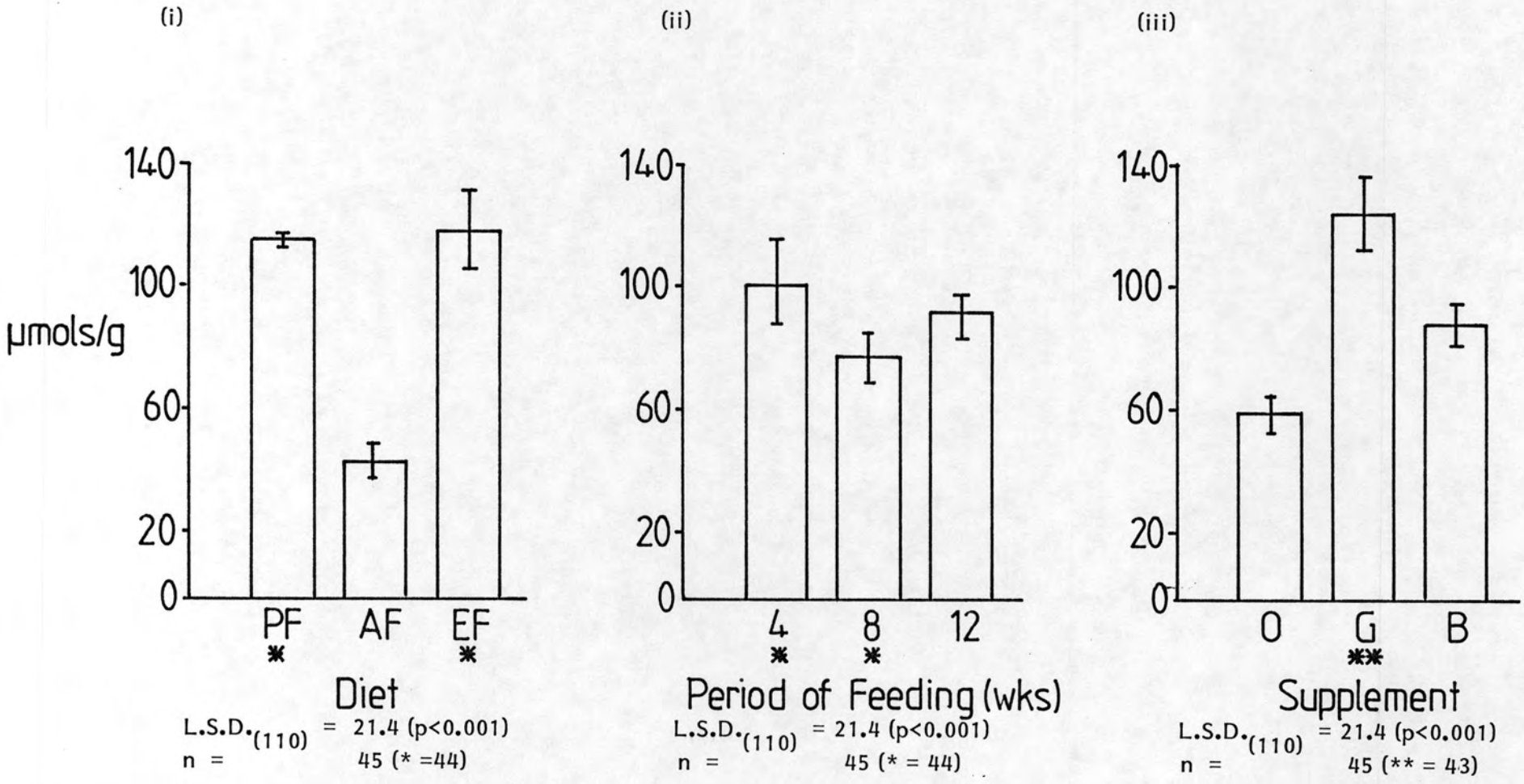
ii) Faecal

Figure 6p (i) shows that the concentration of faecal S C F A's was significantly less with dietary trial AF ($p<0.001$). There was no significant difference between the other two trials. Of the three time periods (figure 6p (ii)) only the difference between 4- and 8 weeks was significant ($p<0.001$). Figure 6p (iii) shows that a supplement significantly increased the concentration of faecal S C F A's, the increase being greatest with gum arabic.

The two way interactions of D X T, D X S and S X T were significant ($p<0.001$). Figure 6p (iv) shows that the trends with time, associated with each dietary trial, were dissimilar. Concentrations increased between 4- and 12 weeks with the AF and EF dietary trials. Only with the PF dietary trial did the concentration significantly decrease after 4 weeks of feeding. Within the AF and EF dietary there was no significant change in the concentration over 12 weeks. Of the six diet x time period groups, the results from the three AF groups gave concentrations that were significantly lower than the other diet groups. Figure 6p (v) shows that the supplementation of basal diets PF and EF gave similar results. With these two trials gum arabic increased the faecal concentration of S C F A's. ($p<0.001$) There was no significant difference between diets EG and PG. With dietary trials AF and EF the addition of bran significantly increased ($p<0.001$) the concentration of faecal S C F A's with respect to the unsupplemented diets. There was, however no significant difference between diets AG and AB. With all the dietary trials lack of a supplement resulted in the lowest faecal concentration of S C F A's, although this was not always significantly different from the supplemented groups (figure 6p (v)). There was no significant difference between diets PB or EB, or diets AB, EF, PF and PB, demonstrating the similarities that exist between different diet and supplement combinations. Figure 6p (vi) shows that a gum arabic supplement did increase the concentration of faecal S C F A's ($p<0.001$), and that the concentration decreased with time. Concentration of faecal S C F A's was significantly greater at 4 weeks with gum arabic ($p<0.001$)

FIGURE 6p Faecal concentration of SCFA's from rats as influenced by (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemetal diet (EF), (ii) time and (iii) supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B).

Results are mean ± S.E.M.



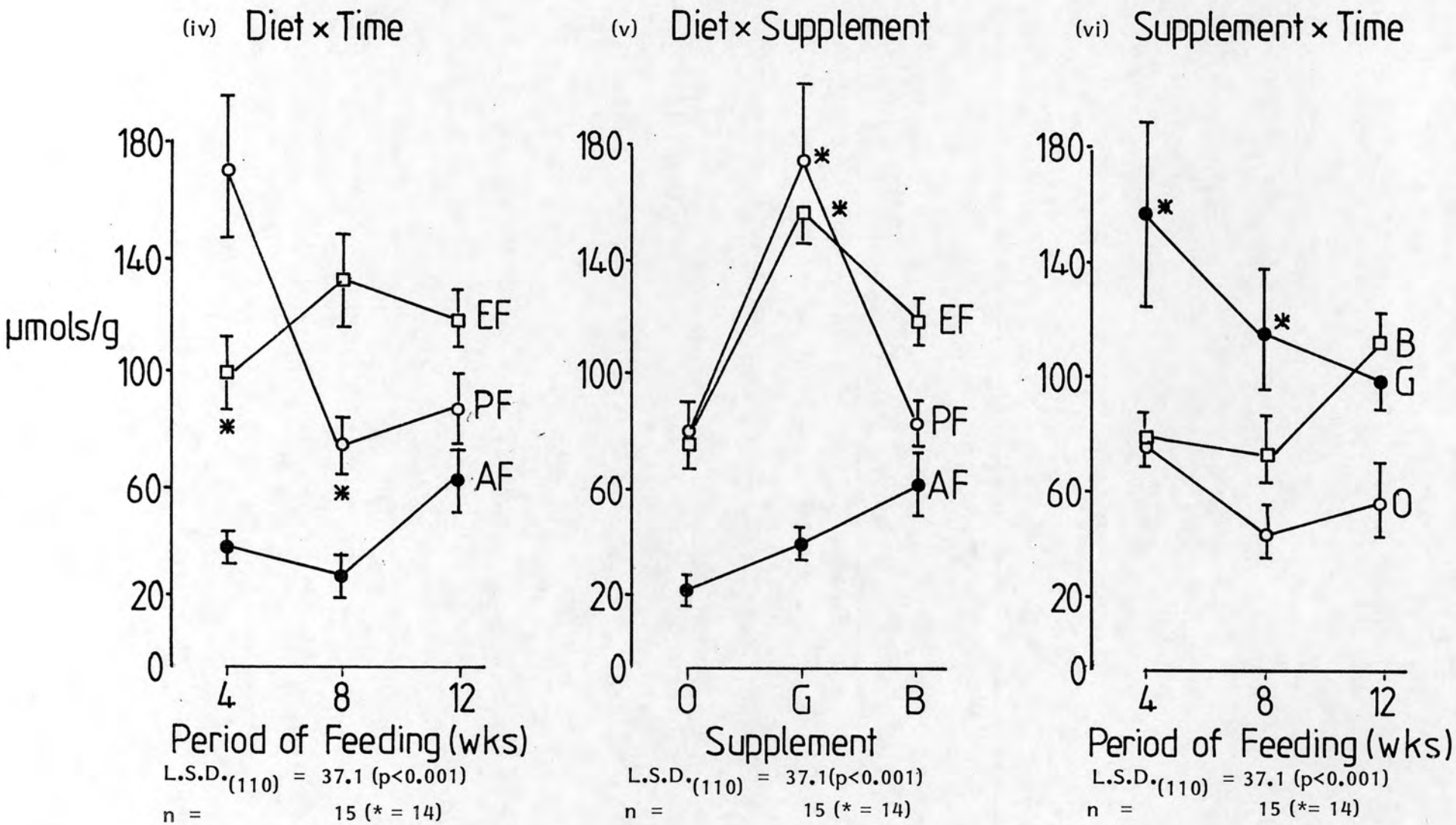
L.S.D.$_{(110)}$ = 21.4 ($p<0.001$)
n = 45 (* = 44)

L.S.D.$_{(110)}$ = 21.4 ($p<0.001$)
n = 45 (* = 44)

L.S.D.$_{(110)}$ = 21.4 ($p<0.001$)
n = 45 (** = 43)

FIGURE 6p Faecal concentration of SCFA'S as influenced by the two-way interactions of (iv) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time, (v) basal diet and supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B) and (vi) supplement and time.

Results are mean ± S.E.M



than all the other S X T groups. There was no significant change in the concentration, over time, in the absence of a supplement. With bran the concentration of faecal S C F A's increased with time, in contrast to gum arabic (figure 6p (vi)). After 12 weeks there was no significant difference in effect between a bran or gum arabic supplement. Figure 6p (vi) also shows the similarities between different S X T groups. For example there was no significant difference between the three unsupplemented x time period groups, and bran fed for 8- and 4 weeks.

The three-way interaction was significant ($p<0.001$), and the results are given in appendix 4l .

The composition of the S C F A's (mmols/mol)

a) Acetate

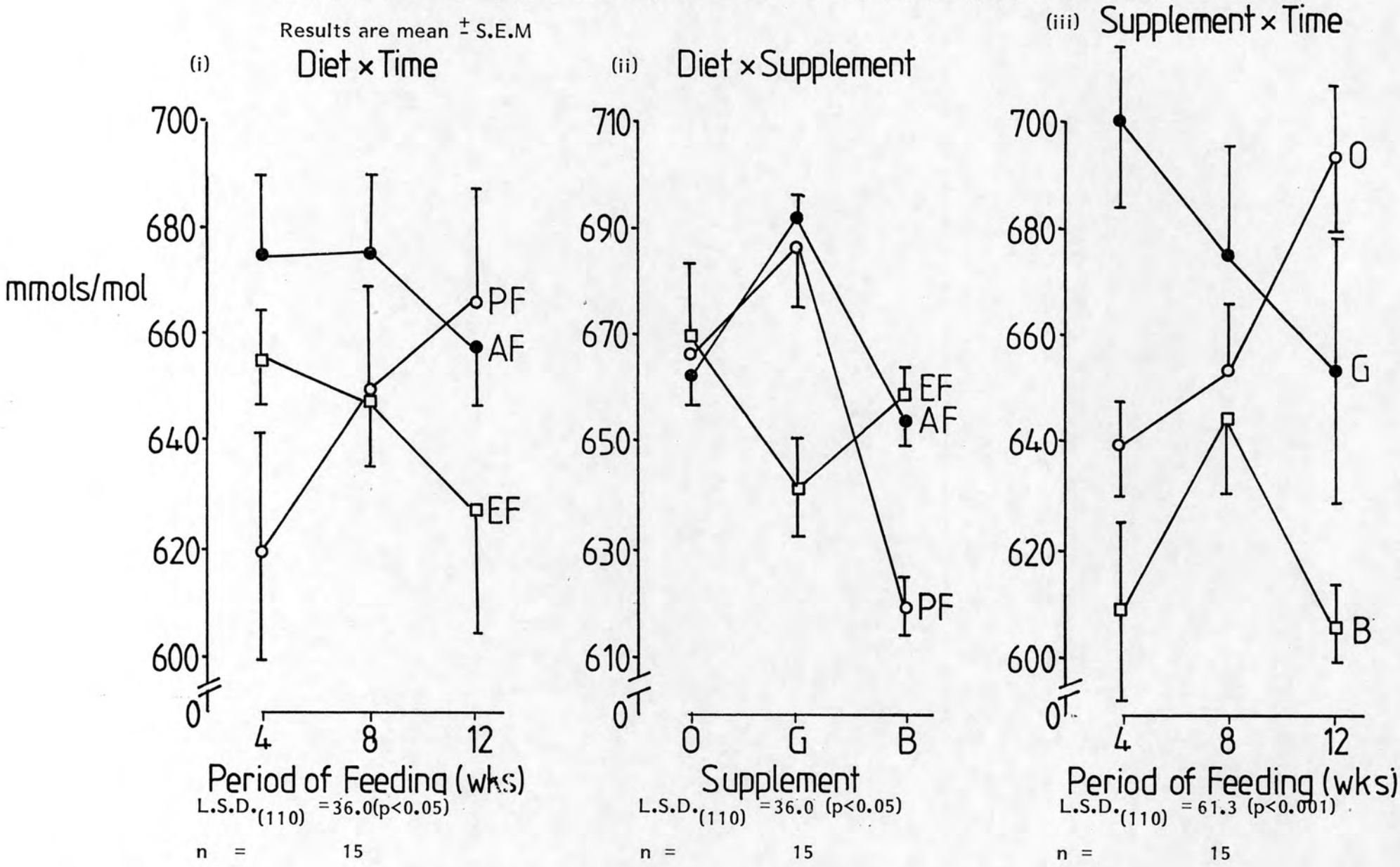
i) Caecal

The proportion of caecal acetate was greatest with the AF dietary trial (670 ± 7.8 mmols/mol, $p<0.05$). There was no significant difference / in effect between dietary trials PF (646 ± 12 mmols/mol) and EF (644 ± 9.1 mmols/mol). Overall, caecal acetate was significantly reduced ($p<0.001$) with a bran supplement (620 ± 7.9 mmols/mol). There was no significant difference in effect between the absence of a supplement (633 ± 7.5 mmols/mol) and gum arabic (676 ± 11.9 mmols/mol).

The two way interaction of D X T was significant ($p<0.05$) as were those of D X S and S X T ($p<0.001$). Figure 6q (i) shows that there were similar trends over time associated with the dietary trials AF and EF: the molar proportion of caecal acetate decreased after 8 weeks. With the dietary trial PF, the molar proportion increased, in a linear fashion, with time. Irrespective of diet there was no significant difference between the three 8 week values. Within each of the dietary trials AF and EF there was no significant change in the molar proportion of acetate over time, unlike the PF dietary trial (figure 6q (i)). Figure 6q (i) shows that there was no significant difference in the effect of diets AF and EF fed for 8 weeks and the five intermediary values from the other groups. Figure 6q (ii) shows that the supplementation of dietary trials PF and AF gave similar results. Gum arabic did increase the molar proportion of caecal acetate in both these trials, but this was not significant with respect to the respective unsupplemented diets. With the EF dietary trial, there was no significant difference between diets EF, EG and EB. In

FIGURE 6q. The proportion of caecal acetate from rats as influenced by the two-way interactions of (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet(EF) and time (ii) basal diet and supplement - no supplement(O), 10% gum arabic (G) and 10% wheat bran (B), and supplement and time.

Results are mean ± S.E.M



the dietary trials PF and AF, bran reduced the molar proportion of caecal acetate, but this was only significant in the former trial ($p<0.001$). Figure 6q (ii) shows that similarities exist between different D X S groups. For example there was no significant difference between diet groups EB, AF, PF, EF and PG. Figure 6q (iii) shows that there were no similar trends between the different S X T groups. Irrespective of the absence, or presence of a supplement, there was no significant change in the molar proportions of acetate over time. With gum arabic, the proportion decreased with time, but increased in the absence of a supplement. Figure 6q (iii) shows there to be no significant difference in effect of gum arabic fed for 4 weeks, the absence of a supplement for 4 weeks and the results from the five intermediate groups.

The three-way interaction was not significant.

ii) Faecal

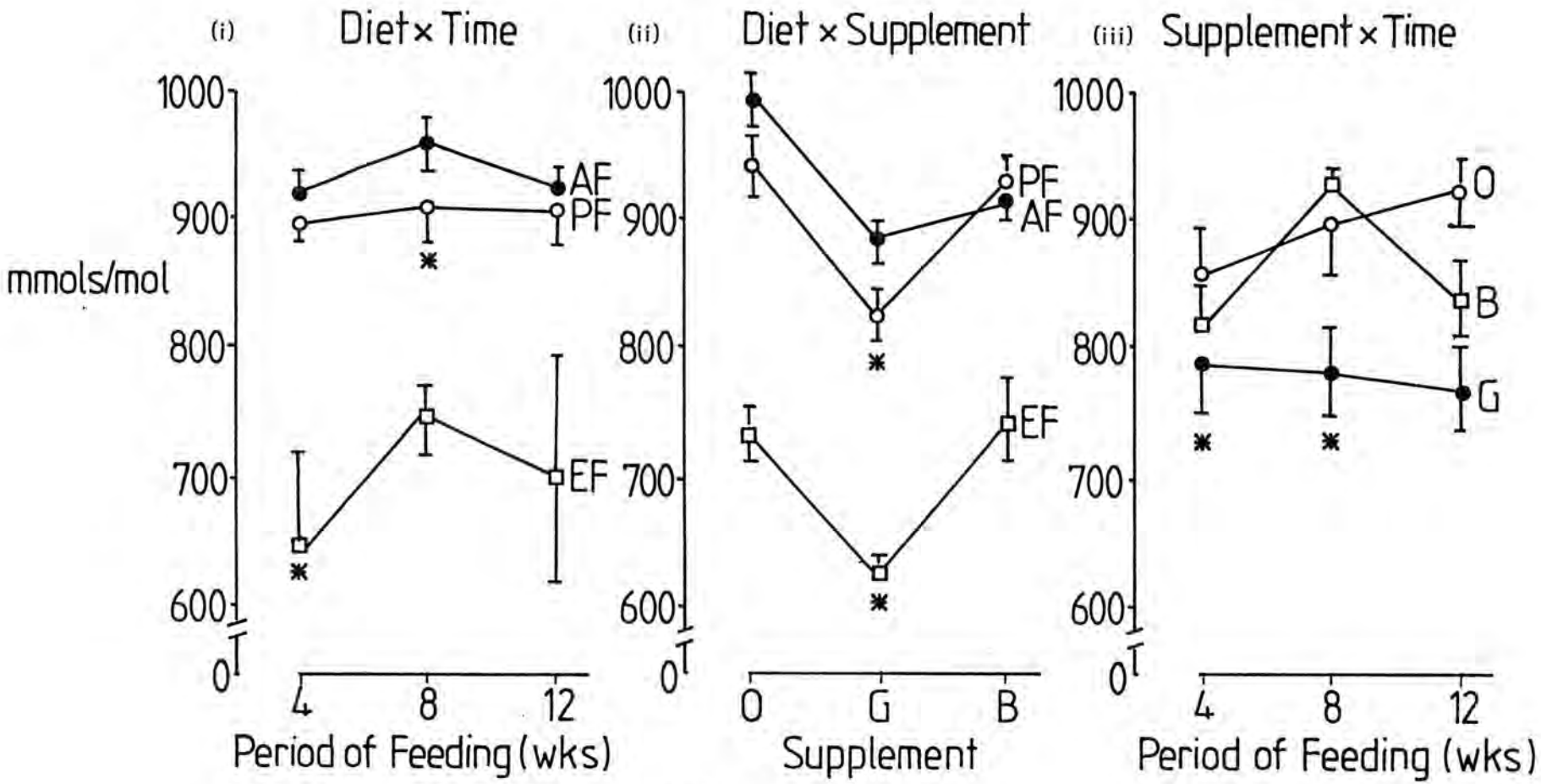
The overall faecal proportion of acetate was significantly lower with the EF dietary trial (705 ± 15.6 mmols/mol, $p<0.001$). There was no significant difference in effect between the PF (904± 13.9 mmols/mol) and AF (933± 9.85 mmols/mol) dietary trials. Overall, only the difference between 4- (824±22.3 mmols/mol) and 8 weeks (873± 19 mmols/mol) was significant ($p<0.001$).

Overall, gum arabic reduced the molar proportion of faecal acetate (783 ± 19.2 mmols/mol, $p<0.001$). There was no significant difference between the effects of lack of supplement (895 ± 19.4 mmols/mol) and bran (863 ± 19.0 mmols/mol).

The two way interactions of D X T and D X S were significant ($p<0.05$) as was that of S X T ($p<0.01$). Figure 6r (i) shows that, over time, there were similar trends between the dietary trials AF and PF. There was no significant difference between the three results, within each of the AF, EF and PF dietary trials. The three EF results were significantly lower than the other 6 values, whilst there was no significant difference between the results from the PF D X T groups and diet AF fed for 4- and 12 weeks. Figure 6r (ii) shows that supplementation of the three dietary trials gave similar results: gum arabic significantly reduced the faecal proportion of acetate ($p<0.05$) with respect to the unsupplemented diets. Within the PF and AF dietary trials there was no significant difference in effect between gum arabic and bran supplementation. Figure 6r (ii) also shows that there was no significant

FIGURE 6r The proportion of faecal acetate from rats as influenced by the two-way interactions of (i) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and time, (ii) basal diet and supplement - no supplement (O), 10% gum arabic (G) and 10% wheat bran (B) and (iii) supplement and time.

Results are mean ± S.E.M



difference between diets PG, AG, AB, and PF. Figure 6r (iii) shows that duration of supplementation was significant ($p<0.01$). With gum arabic, there was no significant change of concentration over time. In the absence of a supplement, concentration significantly increased with time. With bran, the concentration was greatest after 8 weeks. Despite the different trends, there were similarities between different S X T groups. For example, there was no significant difference between the results of bran fed for 4 weeks and the lack of supplement for 8 weeks and the two intermediate results.

The three-way interaction was not significant.

b) Propionate

i) Caecal

Overall the caecal proportion of propionate significantly decreased after 4 weeks, (192 ± 5.29 mmols/mol, $p<0.01$). There was no significant overall change between 8- and 12 weeks. (174±4.38 mmols/mol). Overall, gum arabic significantly increased ($p<0.001$) the proportion of caecal propionate (204± 5.8 mmols/mol). There was no significant difference in effect between the absence of a supplement (179 ± 4.55 mmols/mol) and bran (162 3.62± mmols/mol).

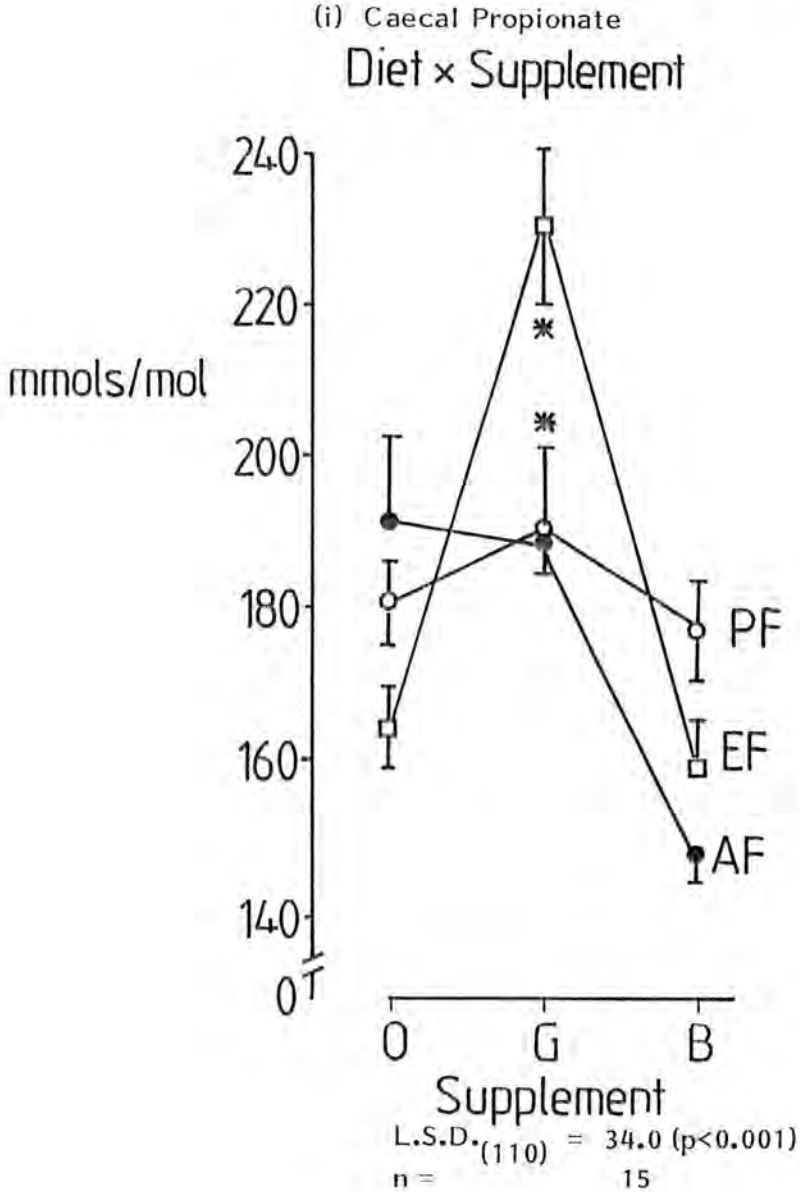
The interaction of D X S was significant ($p<0.001$). With the exception of diet AG, gum arabic increased the proportion of caecal propionate, with respect to the unsupplemented diets. Within all the dietary trials, bran reduced the proportion of caecal propionate, with respect to the unsupplemented diets, but this was only significant in the AF dietary trial (figure 6s (i)). The results do indicate that similarities do exist between different D X S combinations. For example, there was no significant difference between the results from diets EB, AF, and the five intermediate results.

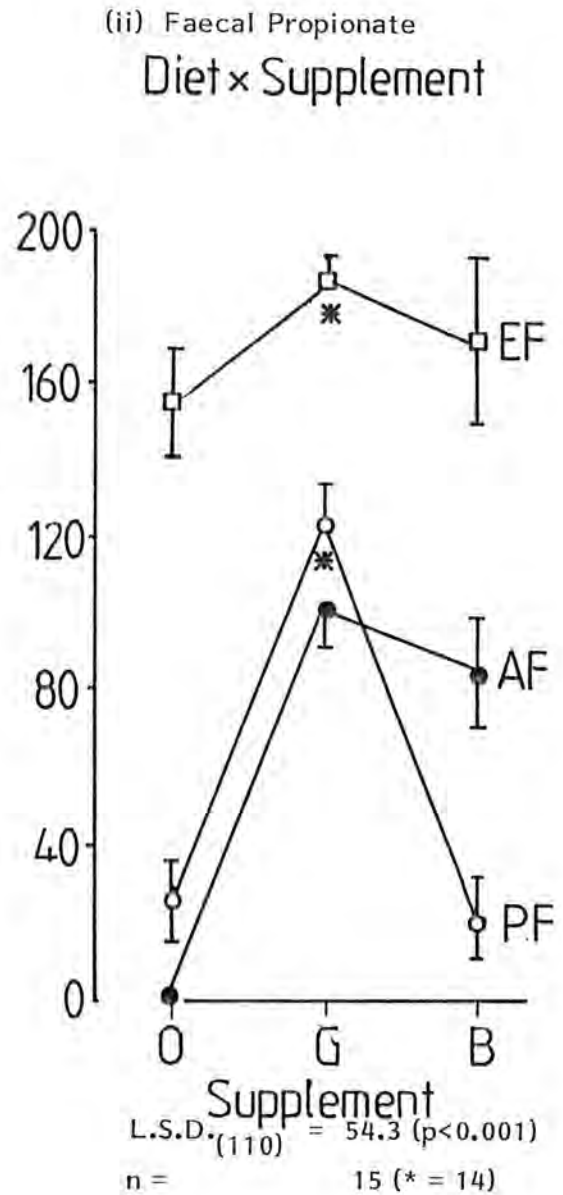
The three way interaction was not significant.

ii) Faecal

Overall, faecal propionate was significantly greater with the EF dietary trial (172±9.34 mmols/mol, $p<0.001$). Time was significant ($p<0.001$), but overall, only the decrease in concentration between 4- (107 14.6±mmols/mol) and 8 weeks was significant (82 ± 11 mmols/mol). The addition of a supplement was a significant influence upon faecal proportion ($p<0.001$). The molar proportion of propionate was greatest with gum arabic (138±7.9 mmols/mol)

FIGURE 6s The proportion of (i) caecal- and (ii) faecal propionate from rats as influenced by the two-way interaction of basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and supplement - no supplement (0), 10% gum arabic (G) and 10% wheat bran (B). Results are mean ± S.E.M.





and was also significantly increased with bran (93 ± 13.5 mmols/mol), with respect to the absence of a supplement (61 ±11.8 mmols/mol).

The two way interaction of D X S was significant ($p<0.001$). All three dietary trials, showed similar trends with supplementation (figure 6s (ii)). Irrespective of diet, gum arabic increased the molar proportion of faecal propionate, but only within the dietary trial EF was there no significant difference between the three diet groups. Only within the dietary trials AF and PF did gum arabic significantly increase the molar proportion of propionate, with respect to the unsupplemented diets. Only within the AF dietary trial, did bran significantly increase the proportion of propionate with respect to AF,(figure 6s (ii)). The diet groups EF, EB and EG gave results that were significantly greater than the other 6 groups with the exception of diet PG. Figure 6t illustrates the similarities that exist between different D X S combinations.

The three-way interaction was not significant.($p<0.001$), and the results are given in appendix 4m.

c) Butyrate

i) Caecal

The proportion of caecal butyrate was significantly greater with the PF dietary trial (160 ± 7.42 mmols/mol, $p<0.001$). There was no significant difference between the dietary trials AF (124 ± 4.16 mmols/mol) and EF (110 ± 10.3 mmols/mol). Overall, time was significant ($p<0.05$), but only the increase between 4- (123 ±8.55 mmols/mol) and 12 weeks (139± 8.51 mmols/mol) was significant. The addition of bran significantly increased the molar proportion of caecal butyrate (183± 7.8 mmols/mol). There was no significant difference between the effects of an absence of supplement (114± 4.5 mmols/mol) and gum arabic (96± 5.6 mmols/mol).

The two-way interaction of D X S was significant ($p<0.001$). Figure 6t (i) shows that supplementation caused similar trends, irrespective of basal diet. With all three dietary trials, bran significantly increased the molar proportion of butyrate with respect to the other diet groups. With the exception of diet EG, butyrate appeared to decrease with gum supplements. There was no significant difference between diet groups PG, AG and EG. Within the dietary trials AF and EF, there was no difference between the unsupplemented and gum arabic supplemented groups.

The three-way interaction was not significant.

ii) Faecal

No butyrate was detected with the AF dietary trial. The difference between the overall results from the EF (72 ± 7.2 mmols/mol) and PF dietary trials (40 ± 7.6 mmols/mol) was significant ($p<0.001$). Overall, with time, the proportion decreased after 4 weeks (47.3± 8.2, $p<0.05$). There was no significant difference between 8- (32 ±7.4 mmols/mol) and 12 weeks (32.3± 6.67 mmols/mol). Overall, a supplementation of gum arabic significantly increased ($p<0.05$) the molar proportion of faecal butyrate (48.1 ±7.71 mmols/mol). There was no significant difference between the effect of the absence of a supplement (32.2 ±7.96 mmols/mol) and bran (31.4±6.53 mmols/mol).

The two-way interaction of D X S was significant ($p<0.001$) as was that of S XT ($p<0.01$). Figure 6t (ii) shows the different trends associated with the supplementation of diets EF and PF. Supplementation did increase the molar proportion of faecal butyrate within the PF dietary trial. Despite the difference between the diet groups PF, and PG, this was not statistically significant. Only the difference between diets EB and EG was significant in the EF dietary trial. Figure 6t (ii) does show that similarities existed between the effects of different D X S combinations. For example, there was no significant difference between diet groups EB, PG, PB and EF. Figure 6t (iii) shows the dissimilar trends associated with the duration of supplementation. Whilst with time, the proportion of faecal butyrate significantly decreased with lack of a substrate ($p<0.01$), the proportion did not significantly alter with time with a gum arabic or bran supplement, despite the decrease at 8 weeks with the latter. After 4 weeks of feeding, there was no significant difference in the proportion of faecal butyrate with any of the three forms of supplementation. After 12 weeks, the proportion of faecal butyrate was significantly lower due to the absence of a supplement, only with respect to gum supplementation for a period of 12 weeks. Figure 6t (iii) shows that, in general, there was very little significant difference between the nine groups.

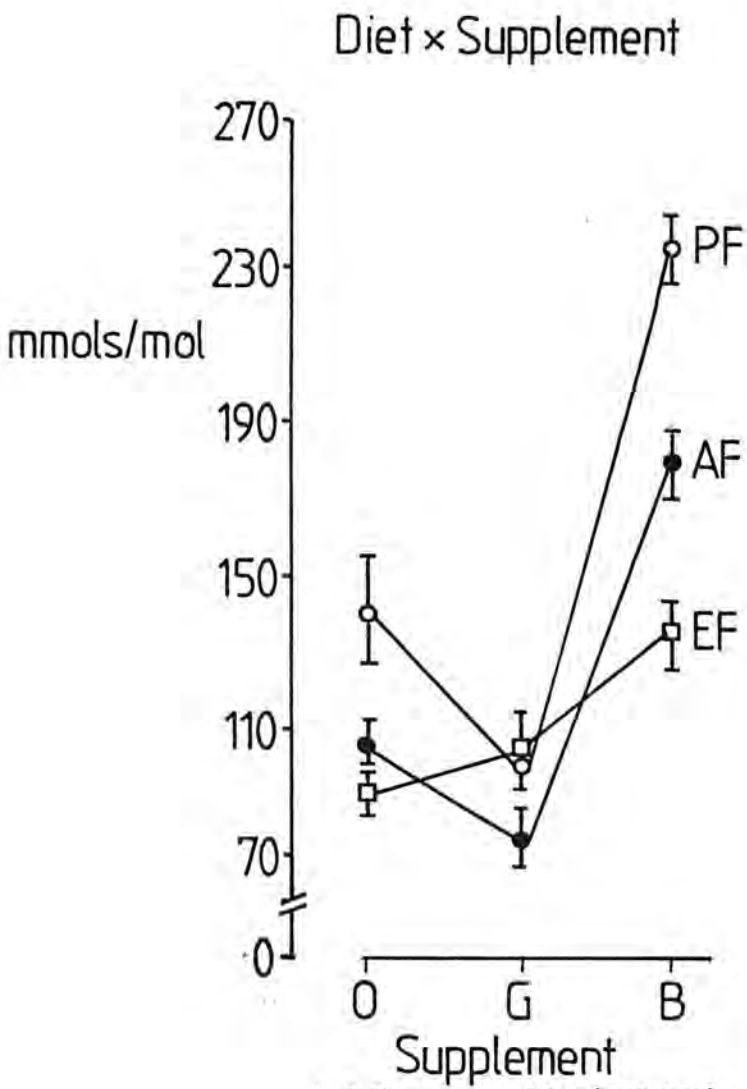
The three-way interaction was not significant.

HYDROGEN AND METHANE H_2 AND CH_4

The determination of both H_2 and CH_4 was not possible for the AF dietary

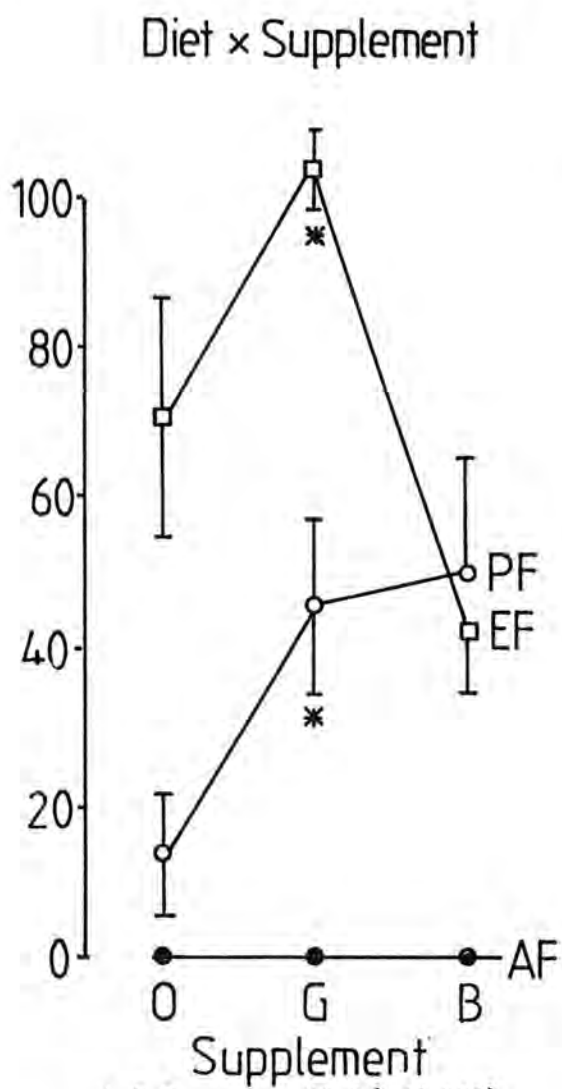
FIGURE 6t The proportion of (i) caecal- and (ii) and (iii) faecal butyrate from rats as influenced by two-way interactions of (i) and (ii) basal diet - Plant Origin (PF), Animal Origin (AF) and an elemental diet (EF) and supplement- no supplement (0), 10% gum arabic (G) and 10% wheat bran (B), and (iii) supplement and time. Results are mean ± S.E.M.

(i) Caecal Butyrate



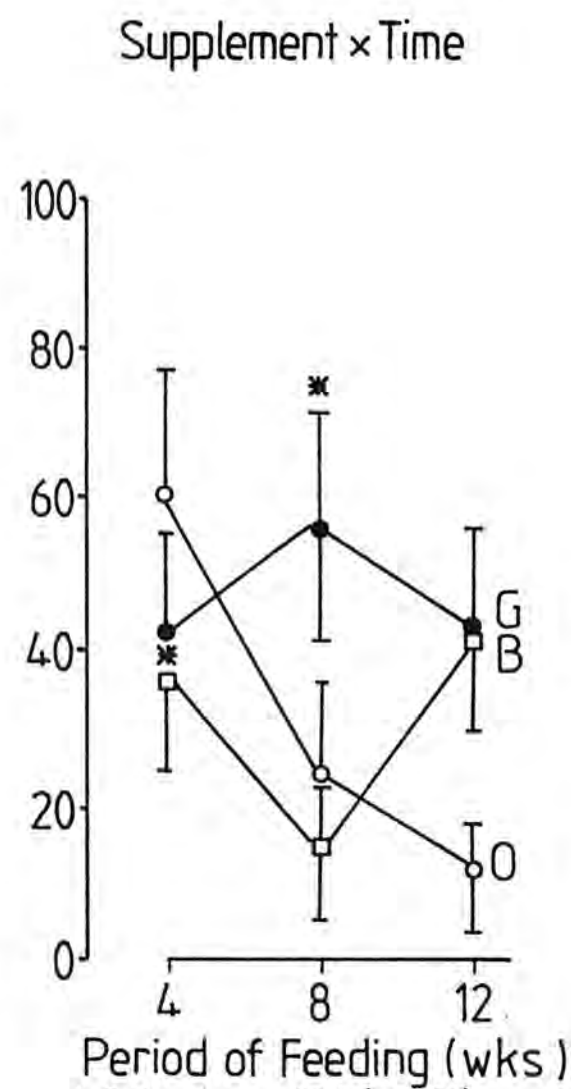
L.S.D.$_{(110)}$ = 62.9 ($p<0.001$)
n = 15

(ii) Faecal Butyrate



L.S.D.$_{(110)}$ = 41.9 ($p<0.001$)
n = 15 (* = 14)

(iii) Faecal Butyrate



L.S.D.$_{(110)}$ = 32.5 ($p<0.01$)
n = 15 (* = 14)

trial.

(i) Hydrogen (mls/hr/kg)

The results indicate that overall, time was a significant influence ($p<0.001$) as was supplementation ($p<0.01$). The levels of H_2 significantly decreased after 12 weeks (0.16 ± 0.03 mls/hr/kg). There was no significant difference between 4- (0.73 ± 0.11 mls/hr/kg) and 8 weeks (0.47 ± 0.06 mls/hr/kg). Supplementation did influence the level of H_2, but only the difference between the absence of supplement (0.34 ± 0.06 mls/hr/kg) and gum arabic supplementation (0.61 ± 0.12 mls/hr/kg) was significant.

The two-way interaction of D X T was significant ($p<0.05$) as were those of D X S and S X T ($p<0.001$). Figure 6v (i) shows how with both the PF and EF dietary trials show decreased levels of H_2 over time. Figure 6v (ii) shows that H_2 levels remained unaltered with the PF dietary trial, irrespective of supplement. Supplementation of the EF diet does significantly influence H_2 levels ($p<0.001$). When gum arabic is added to the EF diet levels of H_2 were significantly increased with respect to diets EF and EB. Diet EG gave levels of H_2 twice that of diets PG, PB and PF, but there was no significant difference between these four diets. Figure 6v (iii) shows that irrespective of supplement, the levels of H_2 decreased with time, the change with time being related to the supplement. Despite the 70% decrease of H_2 excretion between 4- and 12 weeks in the absence of a supplement, there was no significant difference between the three groups. With gum arabic H_2 excretion was virtually abolished after 12 weeks, with no significant difference between 4- and 8 weeks. With bran, there was no significant difference in excretion between the three time periods. Figure 6v (iii) shows that, whilst H_2 excretion was greatest with gum arabic, after 12 weeks there was no significant difference between H_2 excretion from groups given no supplement, and those given gum arabic or bran, for 12 weeks.

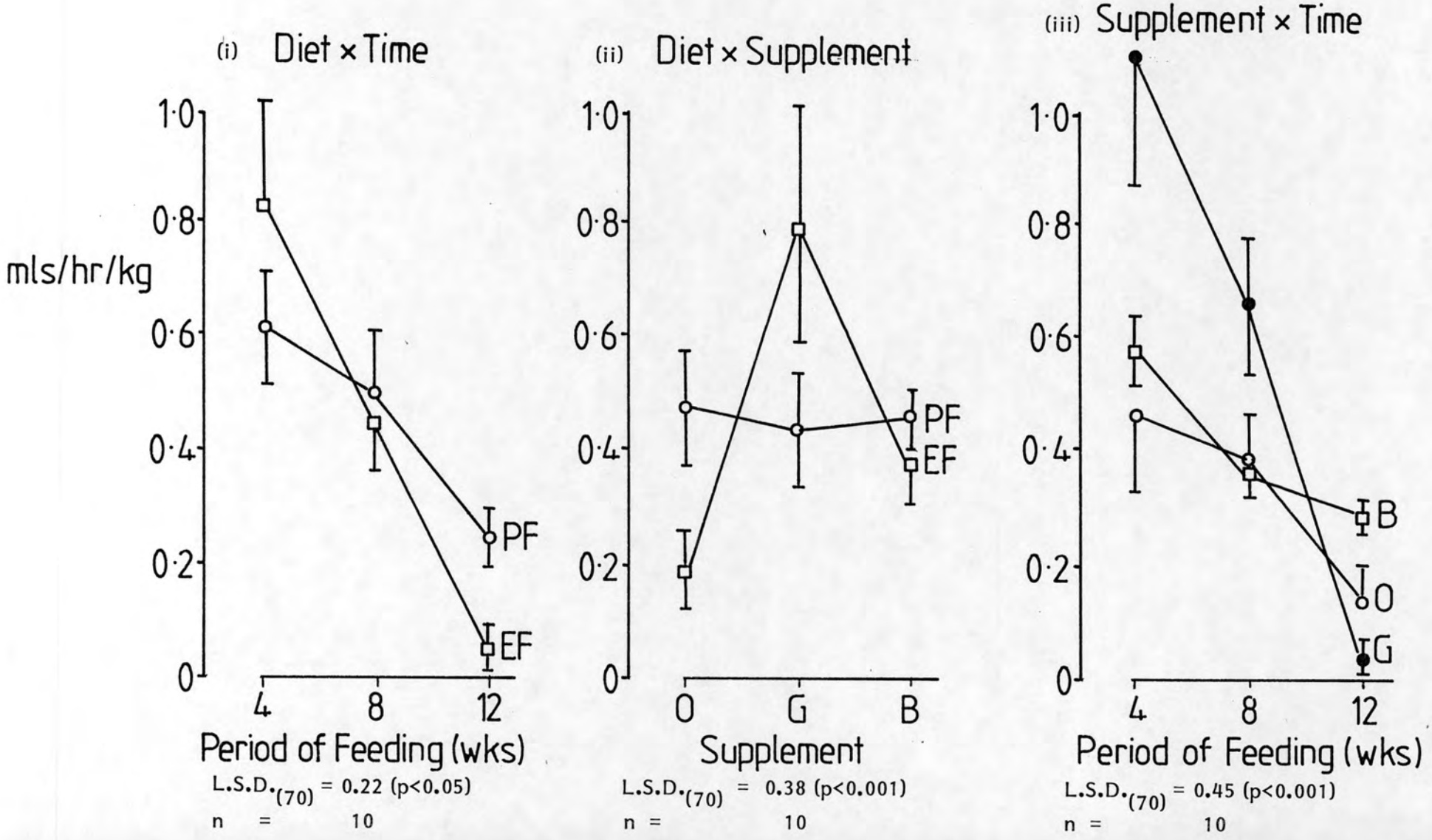
The three way interaction was significant ($p<0.01$) and the results are given in appendix 4n. There was immense individual variation within and between the 27 groups, and as a consequence, these values ought to be treated with care. H_2 is a product of fermentation (Chapter 1) and can be influenced by diet. Variable though these results may be there is a common pattern and shows that fibre supplement is influential upon H_2 excretion.

(ii) Methane (mls/hr/kg)

Of the two dietary trials, overall the dietary trial EF produced significantly

FIGURE 6v Hydrogen production from rats as influenced by the two-way interactions of (i) basal diet - Plant Origin (PF), and an elemental diet (EF) and time, (ii) basal diet and supplement - no supplement (0), 10% gum arabic (G) and 10% wheat bran (B) and (iii) supplement and time.

Results are mean ± S.E.M.



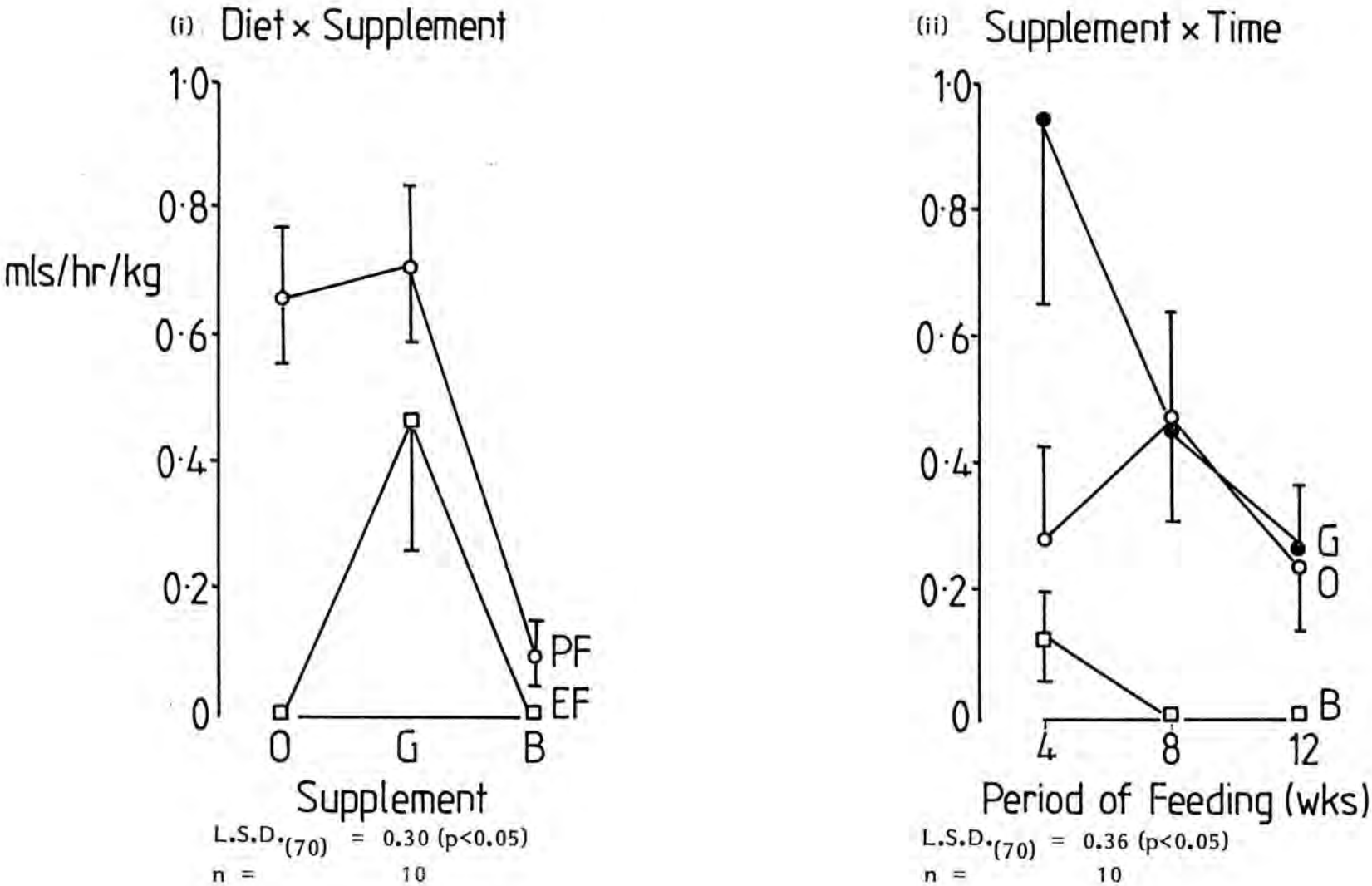
less H_2 (0.15 ± 0.07 mls/hr/kg) than dietary trial PF (0.47 ± 0.07 mls/hr/kg, $p<0.001$). Overall, with time, the excretion of H_2 decreased ($p<0.05$), the value after 12 weeks (0.19 ± 0.05 mls/hr/kg), being less than that detected at 4- (0.45 ± 0.13 mls/hr/kg) and 8 weeks (0.31 ± 0.08 mls/hr/kg), between which there was no significant difference. Overall, bran abolished CH_4 excretion (0.04 ± 0.03 mls/hr/kg, $p<0.001$). Gum arabic increased CH_4 excretion (0.55 ± 0.12 mls/hr/kg) but this was not significantly different from the excretion observed with no supplement (0.33 ± 0.08 mls/hr/kg).

The two-way interactions of D X T and S X T were significant ($p<0.05$). Figure 6w (i) shows that irrespective of basal diet gum arabic significantly increased CH_4 excretion, although there was no significant difference between diets PF and PG. There was no significant difference between diets EG, PF and PG, nor between diets EF, EB and PB. Figure 6w (ii) shows that CH_4 excretion was greatest after 4 weeks with gum arabic. By 8 weeks there was no significant difference in effect between lack of supplement and gum arabic, and after 12 weeks there was no significant difference in effect between lack of supplement, gum arabic and bran. Figure 6w (ii) shows that bran abolishes CH_4 excretion.

The three way interaction was not significant.

FIGURE 6w Methane production from rats as influenced by the two-way interactions of (i) basal diet - Plant Origin (PF), and an elemental diet (EF) and supplement - no supplement (0), 10% gum arabic (G) and 10% wheat bran (B) and (ii) supplement and time.

Results are mean ± S.E.M.



SUMMARY

The three way analysis presented in this chapter, brings together the results of the three individual dietary trials. Chapter 6 identifies common trends and differences associated with the three basal diets, time periods, supplements and the interactions that occur between those three main effects.

The results show that overall, significant differences did occur between the three dietary trials. Time was important. The addition of gum arabic or wheat bran did have a significant influence upon physical (live-weight, stool weight) and metabolic measurements (bacterial mass, S.C.F.A's). The results have shown that time and/or supplement may have the same effect upon a measurement irrespective of the basal diet used, only the absolute values are influenced. For example, bran increased stool weight (g/day) irrespective of basal diet employed (figure 6c (iii)). Similarly, irrespective of basal diet, gum arabic increased C.S.W.W (g). Apparent, also, is that different D X T combinations may give the same, or similar result. Similarly for D X S and S X T.

The difference between an unsupplemented and supplemented basal diet of one dietary trial fed for one time period, may not be the same as that in another and this is reflected in the three-way interaction. This illustrates that the effect of a supplement or time period may be more, or less, noticeable with one basal diet than with another. From the results of this chapter it is apparent that the basal diets have inherent characteristics which will influence the change in the measurement under study, depending upon the duration of feeding unsupplemented, supplemented diets and the nature of the supplement.

Overall, the results suggest that the characteristics of basal diet PF causes elevated results before supplementation and that the effect of the supplement may be masked. The dietary trial EF gives similar absolute values.

A period of greater than 4 weeks is necessary to measure the changes in metabolic measurements and to ensure that these have stabilised. With stool weight, 4 weeks seems to be an adequate time period.

A supplement of bran will increase stool weight, but has little influence upon caecal metabolism, whilst gum arabic is a significant influence upon the

products of caecal metabolism, and not on stool weight.

These results confirm the findings of the individual dietary trials, chapters 3, 4 and 5.

CHAPTER 7

A STUDY OF THE SMALL ANIMAL DIETS GIVEN CONCOMITANT WITH THE THREE DIETARY TRIALS

Concomitant with each of the three dietary trials, described in the Chapters 3,4 and 5, additional rats were fed a standard small animal diet (S.A.D) used in the animal unit at the time of each dietary trial. The S.A.D. used at the time of the elemental trial (S.A.D.3) was of a different pelleted form, and originated from a different company, than that used with the plant origin - and animal origin dietary trials (S.A.D. 1and S.A.D. 2).
Formulations are given in Tables 7a (i) (ii) and (iii).

MATERIALS AND METHODS

Five rats were killed three days after the start of each trial (0 weeks) to facilitate a three day stool collection. The remaining 5 rats were fed 150 g S.A.D./day for 12 weeks and then killed. All analytical techniques used were the same as described in Chapter 2.

The following points have been investigated: (i) the differences that may occur between nutritionally complete, fibre containing diets suitable for the maintenance of small laboratory animals. (ii) the inherent biological variation that may exist between rats.

The results of the effect of these three S.A.D's fed for two time periods are given in part A.

A comparison of the results from the 30 rats fed for 12 weeks on S.A.D.1, S.A.D.2, and S.A.D.3, which contain fibre, and those results from rats fed for 12 weeks on the nine other diets with/without a 10% fibre supplement i.e. PF, PG, PB..... EF, EG, EB, is given in part B of this chapter.

STATISTICAL ANALYSIS AND PRESENTATION OF RESULTS

The effect of diet (S.A.D1, S.A.D2, S.A.D3), time (0- and 12 weeks) and any interaction between diet and time (D X T) was analysed using a oneway ANOVA. All results are presented in a tabular form. The upper table shows the group mean $\pm$ S.E.M. and the coefficient of variation (C.V. (%)). The lower table gives the results of the statistical analysis, detailing, where appropriate, the significance of the main effects, the interaction of diet and time, and the accompanying least significant difference, L.S.D. (Chapter 2).

i) Significant effect: this allows the main effects of diet and time to be regarded independently of each other, as well as any interaction (D X T) effect.

TABLE 7a(i) Spratts (Spillers) small animal diet (autoclaved).

CRUDE COMPOSITION

8.6%	Moisture
21.3%	Crude Protein (N x 6.25)
3.4%	Crude Fat (by SOXHLET)
5.6%	Mineral matter (Ash)
2.2%	Crude Fibre - 2.7% = cellulose 0.8% = lignin
48.0%	Calculated Digestible Carbohydrates
4.154	Gross Energy Kcal /g Diet
3.425	Digestible Energy Kcal /g Diet (clac)
41.8%	Starch
2.8%	Sugars (SUCROSE)

VITAMINS

9.0	Vitamin A i.u/g
2.4	Vitamin D_3 i.u/g
39.0	Vitamin E (as TOCOPHEROL) μg/g
10.0	Vitamin K_3 μg/g
5.3	Thiamine (Vit. B1) μg/g
5.9	Riboflavine (Vit. B_2) μg/g
0.015	Vitamin B_{12} μg/g
0.84	Choline μg/g
1.5	Folic Acid μg/g
59.0	Nicotinic Acid μg/g
12.0	Pantothenic Acid μg/g
8.5	Pyridoxine μg/g
0.22	Biotin μg/g

MINERALS (%)

Calcium	1.1
Phosphorus	0.77
Sodium	0.27
Potassium	1.30
Chloride as CL'	0.40
Iron p.p.m.	170.00

TRACE ELEMENTS (ppm)

Copper p.p.m.	1500
Manganese p.p.m.	45
Zinc p.p.m.	125
Iodine p.p.m. added before manufacture	0.25
Molybdenum (not greater p.p.m.	1
Fluorine p.p.m.	100.00
Magnesium	0.152

AMINO ACIDS (%)

Total Lysine	1.13
Methionine	0.38
Cystine (contains sulphur)	0.26
Tryptophan	0.27
Leucine	1.67
Isoleucine	0.85
Valine	0.99%

TABLE 7a(i) (contd)

Threonine	1.00%
Phenylalanine	0.96%
Argenine	1.34%
Histidine	0.58%
Tyrosine	0.75%
Glycine	1.23%
Alanine	1.13%
Proline	1.69%
Serine	1.14%
Glutamic Acid	3.90%
Aspartic Acid	1.69%
Taurine	Trace

Information provided by Spratts.

TABLE 7a (ii) Composition of small animal diet 3 (S.A.D 3)

Proximate Analysis

Crude oil	2.4%
Crude protein	18.1%
Crude fibre	3.6%
Metabolisable energy	2855 Kcal/kg
Carbohydrate (as %)	56.97

Vitamins added (per kg)

Vitamin A	8,000 iu
Vitamin D_3	1,000 iu
Vitamin B_2	8 mg
Nicotinic acid	50 mg
Pantothenic acid	12 mg
Vitamin B_{12}	12 µg
Vitamin E	60 iu
Vitamin K	10 mg
Folic acid	10 mg
Choline chloride	200 mg
Vitamin B_1	4 mg
Vitamin B_6	6 mg

Minerals added (%)

Calcium (as Ca)	0.8
Phosphorus (as P)	0.7
Salt	0.7

Trace Elements (ppm)

Manganese	25
Copper	7
Cobalt	0.4
Iron	30
Iodine	1.3
Magnesium	102

Amino Acids (% age of feed)

Threonine	0.6	Tyrosine	0.6
Glycine	0.9	Phenylalanine	0.8
Valine	0.8	Lysine	1.0
Cystine	0.2	Histidine	0.4
Methionine	0.3	Arginine	1.2
Isoleucine	0.7	Tryptophan	0.2
Leucine	1.4		

TABLE 7a (iii) Dietary composition of the small animal diets (S.A.D 1, S.A.D 2 and S.A.D 3)

	*SAD1 and SAD2 (SPRATTS, SPILLERS)	** SAD3 (LABSURE CRM)
CRUDE FIBRE:	2.2%	3.6%
CELLULOSE	2.7%	2.28%
LIGNIN	0.8%	NA
SOLUBLE NCP	NA	1.99%
INSOLUBLE NCP	NA	6.42%
TOTAL NCP	10.8%	8.41%
DIETARY FIBRE	14.4%	10.69
TOTAL NSP		9.69%
CRUDE PROTEIN	21.7%	18.1%
CRUDE OIL	NA	2.4%
CRUDE FAT	3.4% (BY SOXHLET)	-
CARBOHYDRATES	NA	56.97%
METABOLISABLE ENERGY	NA	2.855 k Cal/g
DIGESTIBLE ENERGY	3.425 K Cal/g	NA
STARCH	41.8%	NA
SUGARS	2.8%	NA

NCP = Non-cellusic polysaccharides
NSP = Non-starch polysaccharides

NA = Not available.

* By Van Soest method of fibre determination
** By Englyst " " " "

Their significance is derived from the main body of the ANOVA (Chapter 2, and appendix 1d.

ii) Level of significance: this is either $p<0.05$ or $p<0.01$. No significant difference is given by N S.

iii) Least significant difference: Where diet, time or D X T, weresignificant (variance ratio) then a follow up test was used and the L.S.D. arrived at, as described in Chapter 2. If the difference between any pair of means (main effects, or interaction) is greater than the calculated L.S.D., it is considered to be significant. For example, Table 7c gives liver weights. Only the main effect of diet was significant. Thus the relevant diet means, irrespective of time, are (mean $\pm$ SEM)

S.A.D1^{b}	S.A.D2^{ab}	S.A.D3^{a}
19.1 $\pm$ 0.84 g	18.1 $\pm$ 0.50 g	16.4 $\pm$ 0.43 g

Where S.A.D1 = $\frac{\text{S.A.D1 (0 weeks) + S.A.D1 (12 weeks)}}{10}$

and similarly for S.A.D2 and S.A.D3

For time period 0 weeks:-

$$\frac{\text{S.A.D1 (0 weeks) + S.A.D2 (0 weeks) + S.A.D3 (0 weeks)}}{15}$$

and similarly for 12 weeks.

L.S.D. = 1.77, thus the liver weights of rats fed S.A.D1 were significantly greater than those from S.A.D3, but not S.A.D2. This is indicated by superscript notation.

The overall means for the main effects of diet and time are not given as a value on the tables but are quoted in the text. They can of course be estimated from the main body of the table.

The F - statistics are given in appendix 2e (i) and 2e (ii).

A A STUDY OF THE EFFECTS OF THE THREE SMALL ANIMALS DIETS FED FOR TWO TIME PERIODS

RESULTS

LIVE-WEIGHT

Table 7b shows a significant difference between the initial live-weights of

TABLE 7b The live-weight (g) and live-weight changes (g) of rats fed three small animal diets (S.A.D.) for two time periods. Results given are mean± SEM, where n = 5 per group.

Period of Feeding	0 WEEKS			12 WEEKS		
DIET	S.A.D1	S.A.D2	S.A.D3	S.A.D1	S.A.D2	S.A.D3
Initial Live-weight	457 ± 19 (9%)	400 ± 10 (6%)	351 ± 9 (6%)			
Initial Live-weight				414 ± 16 (9%)	406 ± 12 (7%)	351 ± 9 (6%)
Final Live-weight				513 ± 25 (11%)	502 ± 18 (8%)	452 ± 7 (4%)
Live-weight Change				98.4 ± 11 (26%)	96.8 ± 7 (16%)	106 ± 4.7 (10%)

Figures in paranthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right, in each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Initial Live-weight (0 weeks) L.S.D (12)	S.A.D3[a] S.A.D2[ab] S.A.D1[b] 57.7**	NS	NS
Initial Live-weight (12 weeks) L.S.D (12)	S.A.D3[a] S.A.D2[ab] S.A.D1[b] 55.7**	NS	NS

A different superscript denotes a significant difference
** = $p<0.01$;
NS = not significant.

the rats in the 0 weeks and 12 weeks groups ($p<0.01$). There was no significant difference between the average weight gain over 12 weeks (Table 7b). There was no significant difference between the final live-weights of those rats fed for 12 weeks. There was no significant difference between the live-weight changes of the three groups.

LIVER WET WEIGHT

Diet alone was significant ($p<0.05$). Livers were heaviest on the S.A.D1 (19.1 ± 0.84 g) and lightest on the S.A.D3 (16.4 ± 0.43 g) Table 7c. When expressed as a function of live-weight, both time ($p<0.01$) and D X T ($p<0.05$) were significant. The liver: live-weight ratio was less after 12 weeks of feeding the S.A.D3 (35.1 ± 0.78 g). Table 7c shows that the liver: live-weight ratio was greatest with S.A.D3 at 0 weeks. There was no significant difference between any of the values after 12 weeks of feeding.

STOOL WEIGHT

Diet, time and the interaction of D X T were all significant ($p<0.01$). Stool weight was greatest with S.A.D3 (3.93 ± 0.26 g/day) and least with S.A.D1 (3.15 ± 0.15 g/day). All three stool weights were significantly different from each other ($p< 0.01$). Stool weight decreased with time from 3.80 ± 0.19 g/day to 3.12 ± 0.09 g/day after 12 weeks ($p<0.01$). Table 7d shows that only S.A.D3 (0 weeks) gave a significantly greater stool weight than all the other diets ($p<0.05$). All the means are of the order of magnitude 3-4 g/day, and so it may be reasonable to conclude that there is no significant difference between the stool weights with the three diets. When expressed as a function of live-weight (g/kg), the same differences are apparent. Diet, time and the interaction of D X T were all significant ($p<0.01$). Stool: live-weight ratio was greatest with the S.A.D3 (10.2 ± 1.07 g/kg) and least with S.A.D1 (6.45 ± 0.40 g/kg). The ratio decreased with time from 9.7 ± 0.77 g/kg (0 weeks) to 6.7 ± 0.29 g/kg (12 weeks). Stool weight does not seem to be directly related to live-weight, as stool output does not proportionally increase with live-weight (Table 7d) over a period of time.

THE CAECUM AND ITS CONTENTS

(i) The caecal sac wet weight (C.S.W.W.)

Time alone was significant ($p<0.01$). Weight decreased by 21% from 0.95 ± 0.05 g to 0.72 ± 0.06 g after 12 weeks of feeding (Table 7e). When expressed

TABLE 7c The wet liver weights of rats fed three small animal diets (S.A.D.) for two time periods. Results given are mean $\pm$ S.E.M. where n = 5 per group.

Period of Feeding	0 WEEKS			12 WEEKS		
DIET	S.A.D1	S.A.D2	S.A.D3	S.A.D1	S.A.D2	S.A.D3
Liver (g)	19.7 $\pm$0.5 (6%)	18.5 $\pm$ 0.5 (6%)	17.4 $\pm$ 0.5 (7%)	18.6 $\pm$ 1.38 (20%)	17.7 $\pm$ 0.91 (11%)	15.4 $\pm$ 0.25 (4%)
Liver (g/kg)	42.8 $\pm$ 0.9 (5%)	46 $\pm$ 0.45 (2%)	49.8 $\pm$ 2.7 (12%)	36.1 $\pm$ 0.08 (0.5%)	35 $\pm$ 0.86 (5%)	34.1 $\pm$ 0.61 (4%)

Figures in paranthesis are the coefficients of variation.

Table of significant differences in ascending order from left to right, in each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Liver (g)	$S.A.D3^{a}$ $S.A.D2^{ab}$ $S.A.D1^{b}$	NS	NS
L.S.D (24)	1.77 *		
Liver (g/kg)		12^{a} 0^{b}	$S.A.D3^{c}$ $S.A.D2^{c}$ $S.A.D1^{c}$ $S.A.D1^{d}$ $S.A.D2^{dc}$ $S.A.D3^{c}$
L.S.D (24)	NS	3.5 **	12 12 12 0 0 0 4.5 *

A different superscript denotes a significant difference.
* = $p<0.05$;
** = $p<0.01$;
NS = not significant.

TABLE 7d The dry stool weights of rats fed three small animal diets (S.A.D) for two time periods. Results given are mean ± S.E.M. where n = 5 per group.

Period of Feeding	0 WEEKS			12 WEEKS		
DIET	S.A.D1	S.A.D2	S.A.D3	S.A.D1	S.A.D2	S.A.D3
Stool (g/day)	3.21 ± 0.30 (21%)	3.66 ± 0.14 (9%)	4.5 ± 0.23 (12%)	3.09 ± 0.10 (7%)	3.30 ± 0.11 (8%)	3.33 ± 0.23 (15%)
Stool (g/kg)	7.00 ± 0.72 (23%)	9.2 ± 0.41 (10%)	12.3 ± 0.96 (17%)	6.07 ± 0.28 (10%)	6.61 ± 0.32 (11%)	7.39 ± 0.60 (18%)

Figures in paranthesis are the coefficients of variation.

Table of significant differences in ascending order from left to right in each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Stool (g/day)	$S.A.D1^{a}$ $S.AD2^{b}$ $S.A.D3^{c}$	0^{d} 12^{e}	$S.A.D1^{f}$ $S.A.D1^{f}$ $S.A.D2^{f}$ $S.A.D3^{fg}$ $S.A.D2^{g}$ $S.A.D3^{h}$
			12 0 12 12 0 0
L.S.D (24)	0.20 **	0.17 **	0.29 **
Stool (g/kg)	$S.A.D1^{a}$ $S.A.D2^{a}$ $S.A.D3^{b}$	12^{c} 0^{d}	$S.A.D1^{e}$ $S.A.D2^{e}$ $S.A.D1^{ef}$ $S.A.D3^{ef}$ $S.A.D2^{f}$ $S.A.D3^{g}$
			12 12 0 12 0 0
L.S.D (24)	1.60 **	1.31 **	2.27 **

A different superscript denotes a significant difference.
** = $p<0.01$;
NS = not significant.

as a function of live-weight, time alone was significant. The C.S.W.W.: live-weight ratio decreased by 36% from 2.37±0.10 g to 1.57±0.13 g after 12 weeks of feeding.

(ii) Dry caecal content weight (C.C.)

The results are given in Table 7e. There was no significant effect of diet, time or the interaction D X T upon C.C. Values ranged from 0.96±0.08 g - 1.01±0.16 g. When expressed as a function of live-weight no significant differences were observed. Weights ranged from 1.93±0.20 - 2.47±0.18 g/kg, Table 7e.

Due to limitations of time, only the S.A.D1 and S.A.D3 were analysed for 2-6,DAPA and S C F A's.

2-6, DIAMINOPIMELIC ACID

a) Total DAPA

(i) Caecal (µmols)

Diet alone was significant ($p<0.01$). The S.A.D3 resulted in a decrease of 59% from 6.77±0.59 µmols (S.A.D1) to 2.77±0.20 µmols (Table 7fi).

(ii) Faecal (µmols/day)

Time alone was significant ($p<0.05$). After 12 weeks of feeding, total faecal DAPA (µmols/day) had fallen by 59% from 12.4±1.05 µmols/day to 8.29±1.10 µmols/day (Table 7fi).

b) Concentration of DAPA

(i) Caecal (µmols/g)

Diet alone was significant ($p<0.01$). The S.A.D3 diet reduced the concentration of DAPA by 55% from 6.81±0.45 µmols/g to 3.00±0.24 µmols/g (Table 7fii).

(ii) Faecal (µmols/g)

Diet, time and the interaction of D X T were not significant upon the concentration of faecal DAPA. (Table 7f ii). Faecal concentration, as a result of S.A.D1 was less than the equivalent caecal concentrations at both time periods.

SHORT CHAIN FATTY ACIDS (SCFA's)

a) Total SCFA's

TABLE 7e The wet caecal sac weight and the dry caecal content weight of rats fed three small animal diets (S.A.D) for two time periods.
Results given are mean ± S.E.M. where n = 5 per group.

Period of Feeding	0 WEEKS			12 WEEKS		
DIET	S.A.D1	S.A.D2	S.A.D3	S.A.D1	S.A.D2	S.A.D3
Caecal sac (g)	1.06 ± 0.09 (19%)	0.92 ± 0.10 (25%)	0.88 ± 0.03 (7%)	0.81 ± 0.06 (16%)	0.51 ± 0.13 (51%)	0.78 ± 0.05 (15%)
Caecal sac (g/kg)	2.32 ± 0.19 (19%)	0.92 ± 0.10 (25%)	0.88 ± 0.03 (7%)	0.81 ± 0.06 (16%)	0.57 ± 0.13 (51%)	1.72 ± 0.05 (15%)
Caecal contents (g)	1.01 ± 0.16 (13%)	1.01 ± 0.09 (15%)	0.85 0.05 (13%)	0.96 ± 0.06 (15%)	0.96 ± 0.08 (18%)	1.01 ± 0.16 (14%)
Caecal contents (g/kg)	1.98 ± 0.08 (9%)	2.47 ± 0.18 (17%)	2.42 ± 0.11 (10%)	2.13 ± 0.23 (24%)	1.93 ± 0.20 (23%)	2.23 ± 0.11 (11%)

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right for each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Caecal sac (g) L.S.D (24)	NS	12^a 0^a 0.19 **	NS
Caecal sac (g/kg) L.S.D (24)	NS	12^a 0^b 0.42 **	NS

A different superscript denotes a significant difference.
** = $p<0.01$;
NS = not significant.

TABLE 7f (i) Total 2-6,DAPA in dry caecal (μmols) and faecal (μmols/day) material of rats fed two small animal diets (S.A.D) fed for two time periods.
Results given are mean ± S.E.M. where n = 5 per group.

(i) TOTAL

Period of Feeding	0 WEEKS		12 WEEKS	
DIET	S.A.D1	S.A.D3	S.A.D1	S.A.D3
Caecal	7.04 ± 0.60 (19%)	2.52 ± 0.21 (18%)	6.49 ± 1.09 (17%)	3.00 ± 0.34 (26%)
Faecal	1.5 ± 2.01 (39%)	13.3 ± 0.73 (12%)	6.49 ± 1.09 (37%)	10.1 ± 1.64 (36%)

Figures in paranthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right, for each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Caecal L.S.D (16)	S.A.D3[a] S.A.D1[b] 1.02 **	NS	NS
Faecal L.S.D (16)	NS	12[a] 0[b] 3.08 *	NS

A different superscript denotes a significant difference
* = $p<0.05$;
** = $p<0.01$;
NS = not significant.

TABLE 7f (ii) The concentration (μmols/g) of 2-6,DAPA in dry caecal and faecal material from rats fed two small animal diets (S.A.D) for two time periods.
Results given are mean±S.E.M. where n = 5 per group.

(ii) CONCENTRATION

Period of Feeding	0 WEEKS		12 WEEKS	
DIET	S.A.D1	S.A.D3	S.A.D1	S.A.D3
Caecal	7.03 ±0.56 (18%)	2.99 ±0.30 (22%)	6.59 ±0.76 (12%)	3.05 ±0.39 (29%)
Faecal	3.39 ±0.37 (24%)	2.99 ±0.30 (22%)	4.04 ±0.30 (7%)	3.01 ±0.41 (30%)

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right, for each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Caecal	S.A.D3[a] S.A.D1[b]		
L.S.D (16)	1.55 **	NS	NS

A different superscript denotes a significant difference.
** = p< 0.01;
NS = not significant.

(i) Caecal (µmols)

Diet and time were significant ($p<0.05$). The S.A.D3 caused an increase of 17% from 399 ±19 µmols to 466± 25 µmols. Total caecal S C F A's increased with time on both diets. An increase of 16% was observed (Table 7g i) from 401±20.3 µmols (0 weeks) to 465±24 µmols (12 weeks).

(ii) Faecal (µmols/day)

Diet alone ($p<0.01$) was significant.

Totals were greatest with the S.A.D3 (696±58 µmols/day) compared to 353 ± 32 µmols/day of S.A.D1, a difference of 70%. This can be partially explained by the 25% increase in stool weight exhibited by S.A.D3. With the exception of S.A.D1 (12 weeks) total faecal SCFA's exceeded total caecal. (Table 7g (i)).

b) Concentration of S C F A's

(i) Caecal (µmols/g)

Diet alone was significant ($p<0.01$).

Table 7g(ii) shows that concentration was greatest on the S.A.D3 (503± 20 µmols/g). The concentration with S.A.D1 was 407±55 µmols/g.

(ii) Faecal (µmols/g)

Diet and the interaction of D X T were significant ($p<0.01$). Concentration was significantly lower with the S.A.D1 (114 ±9 µmols/g) compared to the S.A.D3 (182±14 µmols) ($p<0.01$). Table 7 g(ii) shows that the trends with time within each diet group were different. Overall concentration decreased with time on S.A.D1 and increased on the S.A.D3. Only the S.A.D3 (12 weeks) and S.A.D1 (0 weeks) were significantly from each other (Table 7g (ii)

c) The composition of the SCFA's (mmols/mol)

a) Acetate

(i) Caecal

Diet, time and the interaction of D X T were not significant upon the molar proportion of acetate Table 7h (i).

(ii) Faecal

Time alone was significant ($p<0.01$). Between 0 weeks (765± 10 mmols/mol) and 12 weeks (855 ± 11 mmols/mol) there was a 12% increase (Table 7h (ii)). The proportion of faecal acetate exceeded that in the caecal contents, on

TABLE 7g (i) Total S C F A's in dry caecal (μmols) and faecal (μmols/day) material of rats fed two small animal diets (S.A.D.) for two time periods.
Results given are mean ±S.E.M. where n = 5 per group.

(i) TOTAL

Period of Feeding	0 WEEKS		12 WEEKS	
DIET	S.A.D1	S.A.D3	S.A.D1	S.A.D3
Caecal	378 ±32 (19%)	424 ±24 (13%)	419 ±21 (5%)	509 ±36 (16%)
Faecal	399 ±41 (23%)	664 ±99 (34%)	306 ±42 (14%)	729 ±68 (21%)

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right, in each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Caecal	S.A.D1[a] S.A.D3[b]	0[c] 12[d]	
L.S.D (16)	61 *	61 *	NS
Faecal	S.A.D1[a] S.A.D3[b]		
L.S.D (16)	195 **	NS	NS

A different superscript denotes a significant difference
* = $p<0.05$;
NS = not significant.

TABLE 7g (ii) The concentration of S C F A's (μmols/g) in dry caecal and faecal material from rats fed two small animal diets (S.A.D) for two time periods.
Results given are mean ± S.E.M. where n = 5 per group.

(ii) CONCENTRATION

Period of Feeding	0 WEEKS		12 WEEKS	
DIET	S.A.D1	S.A.D3	S.A.D1	S.A.D3
Caecal	378 ± 26 (16%)	503 ± 35 (7%)	437 ± 15 (3%)	504 ± 24 (11%)
Faecal	139 ± 9 (14%)	144 ± 14 (22%)	96 ± 12 (13%)	219 ± 14 (15%)

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right for each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
Caecal	$S.A.D1^a$ $S.A.D3^b$	NS	NS
L.S.D (16)	76.5 **		
Faecal	$S.A.D1^a$ $S.A.D3^b$	NS	$S.A.D1^c$ $S.A.D1^{cd}$ $S.A.D3^d$ $S.A.D3^e$
L.S.D (16)	31.5 **		12 0 0 12
			44.7 **

A different superscript denotes a significant difference
** = $p<0.01$;
NS = not significant.

average, by 48%

b) Propionate

(i) Caecal

Diet ($p<0.01$) and the interaction of D X T ($p<0.05$) were significant. (Table 7h (i)) S.A.D3 gave significantly less caecal propionate, (123 ±6 mmols/mol) than S.A.D1 (157±11 mmols/mol). The interaction of D X T, only just reached significance ($p<0.05$). The tabulated F-ratio is 4.49, the observed F-ratio was 4.59.

After 12 weeks, the S.A.D1 gave significantly less caecal propionate than S.A.D1 (0 weeks), the reverse of the trend associated with S.A.D3. Table 7h (i) shows that there was no significant difference between S.A.D3 (0 weeks), S.A.D3 (12 weeks) and S.A.D1 (12 weeks). Similarly S.A.D1 (0 weeks) and S.A.D1 (12 weeks).

(ii) Faecal

Time alone was significant ($p<0.05$). Faecal propionate decreased by 9% from 0 weeks (106±4 mmols/mol) to 12 weeks (69±13 mmols/mol). Individual variation was high (92%) for group S.A.D1 (12 weeks). (Table 7 h (ii)). The concentration of faecal propionate was lower than the corresponding caecal values.

c) Butyrate

(i) Caecal

Diet, time and the interaction of D X T were not significant upon the molar proportion of butyrate.

(ii) Faecal

Both time and the interaction of D X T were significant ($p<0.01$). Between 0 weeks (121±13 mmols/mol) and 12 weeks (69.8 ±13 mmols/mol) there was an overall decrease of 42%, Table 7h (ii). Table 7h (ii) shows that only the S.A.D3 (0 weeks) was significantly different from the other three diets. High individual variation (Table 7h (ii)) could possibly explain the highly significant interaction. Values for S.A.D1 (12 weeks) ranged from 0–121 mmols/mol and for S.A.D3 (12 weeks) 37–120 mmols/mol.

HYDROGEN AND METHANE

Hydrogen (H_2) and methane (CH_4) were only measured in groups S.A.D1 and

TABLE 7h(i) The molar proportions (mmols/mol) of the individual SCFA's in dry caecal material of rats fed two small animal diets (S.A.D) for two time periods.
Results given are mean ±S.E.M. where n = 5 per group.

(i) CAECAL

Period of Feeding	0 WEEKS		12 WEEKS	
	S.A.D1	S.A.D3	S.A.D1	S.A.D3
ACETATE	552 ± 22 (9%)	542 ± 20 (8%)	516 ± 7 (3%)	573 ± 30 (12%)
PROPIONATE	174 ± 11 (14%)	116 ± 5.5 (11%)	140 ± 15 (24%)	130 ± 11 (8%)
BUTYRATE	251 16 (14%)	331 16 (11%)	335 21 (14%)	279 30 (24%)

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order, from left to right for each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
PROPIONATE	S.A.D3[a] S.A.D1[b]		S.A.D3[c] S.A.D3[c] S.A.D1[cd] S.A.D1[d]
			0 12 12 0
L.S.D. (16)	32.7	NS	33.7
	**		*

A different superscript denotes a significant difference
* = $p<0.05$;
** = $p<0.01$;
NS = not significant.

TABLE 7h (ii) The molar proportions (mmols/mol) of the individual SCFA's in the dry faecal material of rats fed two small animal diets (S.A.D) for two time periods.
Results given are mean ±S.E.M. where n = 5 per group.

(ii) FAECAL

Period of Feeding	0 WEEKS		12 WEEKS	
DIET	S.A.D1	S.A.D3	S.A.D1	S.A.D3
ACETATE	789 ± 7 (2%)	740 ± 8.4 (3%)	855 ± 17 (4%)	854 ± 15 (4%)
PROPIONATE	109 ± 8 (16%)	103 ± 3 (6%)	66 ± 27 (92%)	72 ± 8 (25%)
BUTYRATE	85 ± 6 (15%)	157 ± 16 (15%)	78 ± 22 (64%)	61 ± 16 (57%)

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right for each group (Chapter 2).

	DIET	TIME	INTERACTION (DXT)
ACETATE	NS	0^a 12^b	
L.S.D. (16)		36.5 **	NS
PROPIONATE	NS	12^a 0^b	
L.S.D. (16)		31.4 **	NS
BUTYRATE	NS	12^a 0^b	$S.A.D3^c$ $S.A.D1^c$ $S.A.D1^c$ $S.A.D3^d$
L.S.D. (16)		14.3 **	12 12 0 0
			61.3 **

A different superscript denotes a significant difference
** = $p<0.01$;
NS = not significant.

S.A.D3. There was no significant effect of diet or time upon H_2 (Table 7j). Levels of H_2 production were very low, and individual variation was high, particularly S.A.D3 (0 weeks). Only one group did not produce H_2 (S.A.D2). Similarly, only one group (S.A.D1 0 weeks) produced CH_4. Diet, time and D X T were all significant upon CH_4 production ($p<0.01$). Table 7j indicates that, overall, no CH_4 was produced, particularly as only a very little amount was produced with S.A.D1 (0 weeks) and C.V. was 52%.

BILE ACIDS

a) Total bile acids*

(i) Caecal (μmols)

Total caecal bile acids are given in Table 7k (i). There was little difference in the overall mean of S.A.D1 (18.1±3.41 μmols) and S.A.D3 (16.2±8.24 μmols). The overall mean for S.A.D2 was 39.6± 15.6 μmols. Table 7k (i) shows that individual variation was high within the diet groups. The overall mean for 0 weeks was 17.8 ±4.98 μmols and for 12 weeks 31.4±12.2 μmols. With the S.A.D2 and S.A.D3 total bile acids increased with time.

(ii) Faecal (μmols/day)

Total faecal bile acids are given in Table 7k (ii). The overall mean for S.A.D2 was 58.5± 15.9 μmols/day. The overall means for S.A.D1 and S.A.D3 were 50.9± 7.25 μmols/day and 31.9± 4.20 μmols/day respectively. With time, the overall means for the bile acids remained constant (48.6± 16.6 μmols/day, 0 weeks; 45.6 ±6.55 μmols/day, 12 weeks). Total faecal bile acids did not increase with time with each diet as shown in Table 7k (ii).

b) Concentration of total bile acids

(i) Caecal (μmols/g)

Table 7k (i) gives the total concentration of bile acids. Concentration was highest on S.A.D2 (44.7 ±19.7 μmols/g). There was little difference between S.A.D3 and S.A.D1 whose values were approximately 60% lower. With time the concentration of caecal bile acids increased from 18.3± 4.51 μmols/g to 32.2 ±12.7 μmols/g. Table 7k (i) shows the variation within each diet group and between the six individual groups.

(ii) Faecal (μmols/g)

Concentrations were lowest on the S.A.D3 diet (8.48±2.36 μmols/g) and highest

* = a pooled sample of faecal and caecal material was used for analysis. As a result no statistical analysis was made.

TABLE 7j Hydrogen (H_2) and methane (CH_4) (mls/hr/kg) from rats fed two small animal diets (S.A.D.) for two time periods.
Results given are mean $\pm$ S.E.M. where n = 5 per group.

Period of Feeding	0 WEEKS		12 WEEKS	
DIET	S.A.D1	S.A.D3	S.A.D1	S.A.D3
H_2	0.29 ± 0.08 (62%)	0.12 ± 0.05 (92%)	0.61 ± 0.68 (25%)	0
CH_4	0.56 ± 0.13 (52%)	0	0	0

Figures in parenthesis are the coefficients of variation.

Table of significant differences, in ascending order from left to right, for each group (Chapter 2).

	DIET	TIME	INTERACTION (D X T)
CH_4	S.A.D3[a] S.A.D1[b]	0[c] 12[d]	S.A.D[e] S.A.D1[e] S.A.D3[e] S.A.D1[f]
			12 12 0 0
L.S.D. (16)	0.18	0.18	0.26
	**	**	**

A different superscript denotes a significant difference
** = $p<0.01$;
NS = not significant.

on the S.A.D2 (25.5 ± 6.20 μmols/g). Concentrations with S.A.D1 were 94% greater than S.A.D3 (Table 7k(i)). The overall concentrations increased with time from 13.2 ± 3.83 μmols/g to 20.4 ± 6.08 μmols/g. Table 7k(ii) shows that the trends with time are not the same for each diet.

c) The composition of the total bile acids (mmols/mol).

(i) Caecal

Table 7k (i) gives the individual proportions of each bile acid in dry caecal contents. Deoxycholic and hyodeoxycholic acids (with the exception of S.A.D2 and S.A.D3) formed the largest proportions of the bile acids. The S.A.D2 (0 weeks) produced more chenodeoxycholic acid than the other diets. Of the three muricholic acids, ω-muri cholic formed the largest proportion.

Lithocholic acid: On the S.A.D1 and S.A.D2 the proportion decreased by approximately 20% from 65 mmols/mol and 57 mmols/mol respectively. There was a 2 fold decrease from 118 mmols with S.A.D3. At 12 weeks there was no difference between the S.A.D1 and S.A.D3. The S.A.D2 diet gave slightly lower proportions.

Deoxycholic acid: the proportion decreased on the S.A.D1 diet with time by 28% from 391 mmols/mol. The decrease with the S.A.D3 diet with time, from 378 mmols/mol was only 10%. On the S.A.D2 the proportion increased with time by 3 fold from 181 mmols/mol. After 12 weeks the S.A.D2 diet gave the greatest proportion and S.A.D1 the least.

Chenodeoxycholic acid: There was no change in the proportion, over time with S.A.D3 (31.4 mmols/mol). With the S.A.D1 the proportion decreased with time by 45% and with the S.A.D2 diet by 90%. At 0 weeks the proportion was greatest on the S.A.D2. After 12 weeks there was very little difference between the three diets.

Cholic acid: the proportion decreased slightly over time with S.A.D1 (15%). On the S.A.D2 and S.A.D3 the proportion increased with time by 18% and 48% respectively. At 12 weeks there was no difference between the S.A.D1 and S.A.D2. The proportion with S.A.D3 was 26% lower at 12 weeks than the latter two.

Hyodeoxycholic acid the proportions increased with time on the S.A.D1 by

62% from 214 mmols/mol and increased by 68% from 133 mmols/mol, with the S.A.D2. There was no change over time with the S.A.D3. After 12 weeks, the proportion associated with S.A.D3 was 10 times lower than that of the S.A.D1 and S.A.D2.

α-muricholic acid: the proportion on the S.A.D1 and S.A.D2 did not alter with time. At 0 weeks, the S.A.D1 gave the lowest proportion (14 mmols/mol) and the S.A.D3 the highest (62.8 mmols/mol). After 12 weeks the latter value had decreased to 12 mmols/mol. At 12 weeks there was little difference between S.A.D1 and S.A.D3, the S.A.D2 diet gave the highest proportion (36 mmols/mol).

ω-muricholic acid: At 0 weeks the S.A.D1 diet produced the lowest proportion (60 mmols/mol) which increased by 2 fold after 12 weeks. The S.A.D3 produced the most ω-muricholic acid (334 mmols/mol) which had decreased by 2 fold after 12 weeks. With the S.A.D2 the proportion decreased by 2 fold after 12 weeks from 171 mmols/mol, to give the lowest proportion, at 12 weeks, of the three diets (87 mmols/mol).

β-muricholic acid: at 0 weeks the proportion was greatest on the S.A.D1 (88 mmols/mol). This had decreased by 40% after 12 weeks. The proportion with S.A.D2 (67 mmols/mols) remained stable over time. There was no β-muricholic acid detected with S.A.D3 (0 weeks). At 12 weeks, this diet had produced 89.2 mmols/mol of this acid.

(ii) Faecal

Table 7k (ii) gives the individual proportions of each bile acid in dry faecal material. Deoxycholic- and hyodeoxycholic acid formed, overall, the largest proportion of the bile acids. Of the three muricholic acids, ω-muricholic acid formed the largest proportion with the exception of β-muricholic, S.A.D2 (12 weeks).

Lithocholic acid: with the exception of S.A.D1, the proportion increased

with time. At 0 and 12 weeks the proportion was lowest with S.A.D1 (59 mmols/mol and 43 mmols/mol respectively) and highest with S.A.D3 (118 mols/mol). At 12 weeks the proportion was highest with S.A.D3 (128 mmols/mol).

Deoxycholic acid: there was little difference between the proportions at 0 weeks which ranged from 228 mmols/mol (S.A.D2) to 266 mmols/mol (S.A.D1). After 12 weeks the proportions decreased with S.A.D1 and S.A.D2 to 217 mmols/mol. The proportion on S.A.D3 increased by 36% to 340 mmols/mol.

Chenodeoxycholic acid: there was no difference between the proportion at 0 and 12 weeks with S.A.D3 (61.5 mmols/mol). The proportion increased by 14% from 76 mmols, on the S.A.D1 over time, and by 3 fold from 20.7 mmols/mol, with S.A.D2. The difference between the three diets at 12 weeks was less than at 0 weeks.

Cholic acid: at 0 weeks the proportion was lowest with S.A.D3, with no difference between S.A.D1 (107 mmols/mol) and S.A.D2 (103 mmols/mol). After 12 weeks, the proportion remained unchanged with the S.A.D1 and decreased by only 17% to 85 mmols/mol S.A.D2. There was a 20% increase with S.A.D3 after 12 weeks. At 12 weeks there was no difference between the S.A.D2 and S.A.D3.

Hyodeoxycholic acid: there was little difference between the proportions of S.A.D1 and S.A.D3 at 0 weeks. The proportion with S.A.D2 was approximately 56% lower (166 mmols/mol). After 12 weeks, there was virtually no change in the proportion with S.A.D1 (341 mmols/mol). With S.A.D2 the proportion had increased by 58% (263 mmols/mol) and by 20% with S.A.D3 (385 mmols/mol).

α-muri cholic acid: none of this acid was detected with S.A.D3. At 0 weeks, none was detected with S.A.D1, and at 12 weeks 18 mmols/mol was detected. The decrease between S.A.D2, 0 weeks (52 mmols) and S.A.D2 12 weeks, was 23%.

ω-muri cholic acid: at 0 weeks the proportion was greatest with S.A.D2

TABLE 7k (i) The composition of bile acids in pooled caecal material from rats fed three small animal diets (S.A.D) for two time periods.
Results are means

Period of Feeding	0 WEEKS			12 WEEKS		
DIET	S.A.D1	S.A.D2	S.A.D3	S.A.D1	S.A.D2	S.A.D3
Caecal bile acids (μmols)	21.5	24	7.98	14.7	55.1	24.2
Caecal bile acids (μmols/g)	21.5	24	9.39	15.3	57.2	24.5
BILE ACIDS (mmols/mol)						
Lithocholic acid	65	57	118	52	44	56.2
Deoxycholic acid	391	181	378	282	434	344
Chenodeoxycholic acid	47	257	30	26	25	31.8
Cholic acid	121	83	50	103	98	74
Hyodeoxycholic acid	214	134	26	347	203	24
α-muricholic acid	14	40	62.8	18	36	12
ω-muricholic acid	60	171	334	118	87	154
β-muricholic acid	88	67	0	52	73	89.2

TABLE 7k (ii) The composition of bile acids in pooled faecal material from rats fed three small animal diets (S.A.D) for two time periods.
Results are means

Period of Feeding	0 WEEKS			12 WEEKS		
DIET	S.A.D1	S.A.D2	S.A.D3	S.A.D1	S.A.D2	S.A.D3
Faecal bile acids (μmols/day)	43.6	74.4	27.7	58.1	42.6	36.1
Faecal bile acids (μmols/g)	14.2	20.3	6.12	18.8	12.9	10.8
BILE ACIDS (mmols/mol)						
Lithocholic acid	59	78	118	43	94	128
Deoxycholic acid	266	228	250	217	217	340
Chenodeoxycholic acid	76	20.7	62	87	70	60.9
Cholic acid	107	103	71.8	103	85	85.9
Hyodeoxycholic acid	351	166	322	341	263	385
α-muricholic acid	0	52	0	18	40	0
ω-muricholic acid	100	300	176	103	108	0
β-muricholic acid	38	98	0	87	124	0

(300 mmols/mol), which decreased by 64% after 12 weeks. There was no change between 0- and 12 weeks with S.A.D1 (100 mmols/mol). At 0 weeks the S.A.D3 diet gave 176 mmols/mol. There was none produced after 12 weeks.

β - muricholic acid: none was detected with S.A.D3. The proportion increased from 98 mmols/mol to 124 mmols/mol after 12 weeks with the S.A.D2. The proportion increased by 2 fold with time, from 38 mmols/mol to 87 mmols/mol with the S.A.D1.

SUMMARY

Concomitant with the three main dietary trials, 10 additional rats of comparable weights to the rats studied in Chapters 3,4 and 5, were fed for 12 weeks with three small animal diets, used to maintain small animals in the animal unit. These diets were nutritionally balanced for maintenance, and contained some fibre.

The results demonstrate the inherent biological variation associated with individual rats and standard small animal diets. Diet was the cause of most differences. Out of 26 measurements (stool weight etc.) only 11 were associated with the effect of time. Where time was significant, the majority of results had decreased by 12 weeks, indicating that two time periods are insufficient to determine where changes occur. Dry C.C. appeared to remain unchanged with time. Whilst stool weights do decrease between 0- and 12 weeks the difference is one gram. This suggests that stool weight may be assumed to be unaffected by time. Stool weight, DAPA and S C F A's reflected the change of diet. Individual S C F A's fluctuated with time, indicating that less than 12 weeks (4 weeks as indicated by Chapters 3, 4 and 5), may be suitable for monitoring stool weight, but for metabolic activities a longer time period is required for changes to manifest themselves, as indicated by Chapters 3, 4 and 5).

B A COMPARISON BETWEEN THE THREE SMALL ANIMAL DIETS AND THE NINE OTHER DIETS (GROUPS PF-EB) FED FOR 12 WEEKS

A one-way ANOVA was used to examine any differences between the three S.A.D's and the nine other dietary groups fed for 12 weeks. The results are given in Tables 7m - 7t, as the mean and coefficient of variation (C.V.). The main table gives the mean and C.V. whilst the lower table gives the level of significance, where significant differences occur, and the least significant difference (L.S.D). A difference between two means is significant if it exceeds the L.S.D (Chapter 2). This section serves to indicate how different three S.A.D's are compared to three fibre free and fibre supplemented diets.

RESULTS

LIVE-WEIGHT

Table 7 m shows that the final live-weights of the 12 groups were variable. Live-weights of all the rats fed the S.A.D's were only significantly heavier than the rats fed the AF diets. With the exception of the 3 AF groups, and the EF diet, there was no significant difference in live-weight change as a result of different diets, provided that diet was acceptable and enjoyed by the rats. The AF diet was not and this was reflected in live-weight change.

LIVER WET WEIGHTS

The wet liver weights (Table 7n), from rats given the three S.A.D's were not outrighly greater or lower than those from the other rats. Significant differences were observed among individual groups ($p<0.01$).

STOOL WEIGHT

All three S.A.D's gave stool weights (g) that were significantly greater than all of the other nine groups, ($p<0.01$), Table 7p . When expressed as a function of live-weight (g/kg), the S.A.D's also gave the greatest stool : live-weight ratio. The difference was less apparent than that with absolute stool weights, ($p<0.01$). Stool output appears to be related to diet and not directly proportional, or related to, live-weight.

THE CAECUM AND ITS CONTENTS

i) Caecal sac wet weight (C.S.W.W.)

Overall, the weights from rats given the S.A.D.'s were not outrightly greater than the weights from rats given the other 9 diets (Table 7q). The gum supplemented diets gave the heaviest C.S.W.W. ($p<0.01$), those from rats given PG and EG being the heaviest. The C.S.W.W. of S.A.D2 was significantly

TABLE 7m The live-weights (g) and live-weight changes (g) of rats fed the small animal diets (S.A.D) and the other nine diets (PF - EB) for 12 weeks.
Results given are the mean and coefficient of variation, where n = 5 per group.

DIET	S.A.D1	S.A.D2	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Final live-weights	513 (11%)	502 (8%)	452 (4%)	556 (11%)	526 (9%)	509 (15%)	274 (7%)	336 (7%)	390 (4%)	560 (7%)	518 (3%)	557 (4%)
Live-weight changes	98 (26%)	97 (16%)	106 (10%)	100 (23%)	111 (25%)	114 (19%)	-50 (18%)	-26 (65%)	+23 (74%)	170 (15%)	124 (15%)	149 (11%)

The least significant difference (L.S.D.) between any 2 means is:

	$L.S.D._{(48)}$	Significance
Final live-weight	175	$p<0.01$
Live-weight change	33.7	$p<0.01$

TABLE 7n The wet liver weights of rats fed the three small animal diets (S.A.D) and the other nine diets (PF - EB) for 12 weeks
Results given are the mean and coefficient of variation, where n = 5 per group.

DIET	S.A.D1	S.A.D2	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Liver (g)	18.6 (20%)	17.7 (11%)	15.4 (4%)	17.7 (15%)	16.8 (14%)	16.8 (14%)	10.4 (9%)	12.1 (7%)	15.4 (6%)	20.2 (5%)	17.0 (7%)	20.0 (14%)
Liver (g/kg)	36.1 (13%)	35 (5%)	34.1 (4%)	32.0 (6%)	32.0 (3%)	33.0 (2%)	37.0 (4%)	36.0 (6%)	40.0 (5%)	36.0 (4%)	33.0 (5%)	36.0 (12%)

The least significant difference (L.S.D) between 2 means is:

	L.S.D. (48)	Significance
Liver (g)	3.35	$p<0.01$
Liver (g/kg)	4.07	$p<0.01$

TABLE 7p The stool weights of rats fed the three small animal diets (S.A.D) and the nine other diets (PF - EB) for 12 weeks
Results given are the mean and coefficient of variation, where n = 5 per group.

DIET	S.A.D1	S.A.D2	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Stool g/day	3.09 (7%)	3.30 (8%)	3.33 (15%)	1.09 (35%)	1.59 (10%)	2.15 (10%)	1.34 (21%)	2.01 (11%)	1.99 (28%)	0.81 (20%)	0.78 (29%)	1.14 (40%)
Stool g/kg	6.07 (10%)	6.61 (11%)	7.39 (18%)	1.95 (29%)	3.03 (2%)	4.26 (10%)	4.86 (25%)	6.03 (16%)	5.14 (29%)	1.53 (20%)	1.51 (28%)	2.06 (40%)

The least significant difference (L.S.D.) between any 2 means is:

	L.S.D. (48)	Significance
Stool (g)	0.56	$p<0.01$
Stool (g/kg)	2.20	$p<0.01$

lighter than those from EB, PG and EG. The C.S.W.W. of S.A.D1 and S.A.D3 were not significantly different from the latter three groups. Table 7q shows C.S.W.W. expressed as a function of live-weight. The C.S.W.W. appears to alter with diet, not live-weight.

(ii) Dry caecal contents (C.C.)

The S.A.D's gave dry C.C. that were not significantly different from the gum supplemented diets. (Table 7q). These six diets gave significantly greater dry C.C. than the unsupplemented and bran supplemented diets ($p<0.01$). With the exception of AF, the same trend was apparent when dry C.C. was expressed as a function of liveweight ($p<0.01$).

2-6 DIAMINOPIMELIC ACID

a) Total

(i) Caecal (μmols)

Diet significantly influenced total (μmols) and concentration of (μmols/g) caecal DAPA. S.A.D3 reduced total caecal DAPA ($p<0.01$). There was no significant difference between the S.A.D3, AF, EB and the intermediary groups (Table 7r (i)). S.A.D1 did not differ in effect from EB or AG. Gum arabic increased total caecal DAPA ($p<0.01$).

(ii) Faecal (μmols/day)

Total faecal DAPA was greatest on diet PG ($p<0.01$). This was not significantly different from diet AG (Table 7r (i)). The S.A.D1 and S.A.D3 were not significantly from diets EB, EG and the intermediate diet groups. The S.A.D3 was similar in effect to the AG diet.

b) Concentration of DAPA

(i) Caecal (μmols/g)

Concentrations are given in Table 7r (ii). Gum arabic supplementation increased caecal DAPA, as did the S.A.D1. The S.A.D3 significantly decreased the caecal DAPA concentration with respect to the gum arabic supplemented diets ($p<0.01$).

(ii) Faecal (μmols/g)

Concentration of faecal DAPA was significantly lowered with the S.A.D1 and S.A.D3. ($p<0.01$). The latter two were not significantly different from the bran supplemented diet groups; AF and EF diet groups (Table 7r ii).

TABLE 7q The wet caecal sac weights and dry caecal contents of rats fed the three small animal diets (S.A.D) and the nine other diets (PF - EB) for 12 weeks.
Results given are the mean and coefficient of variation, where n = 5 per group.

DIET	S.A.D1	S.A.D2	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Caecal Sac (g)	0.81 (16%)	0.57 (23%)	0.78 (15%)	0.74 (13%)	1.02 (11%)	0.69 (16%)	0.65 (25%)	0.80 (11%)	0.69 (10%)	0.65 (9%)	1.15 (10%)	0.82 (13%)
Caecal Sac (g/kg)	1.69 (27%)	1.74 (79%)	1.72 (16%)	1.35 (16%)	1.93 (2%)	1.36 (11%)	2.32 (25%)	2.32 (12%)	1.80 (7%)	1.16 (10%)	2.23 (12%)	1.48 (14%)
Caecal Contents (g)	0.96 (15%)	0.96 (18%)	1.01 (14%)	0.46 (13%)	1.09 (22%)	0.58 (26%)	0.65 (28%)	1.02 (14%)	0.71 (13%)	0.47 (45%)	1.19 (13%)	0.59 (12%)
Caecal Contents (g/kg)	2.13 (11%)	1.93 (23%)	2.23 (5%)	0.83 (20%)	2.09 (10%)	1.13 (22%)	2.37 (30%)	3.05 (15%)	1.83 (16%)	0.85 (52%)	2.30 (13%)	1.07 (15%)

The least significant difference (L.S.D) between any two means is:

	L.S.D. (48)	Significance
Caecal Sac (g)	0.24	$p<0.01$
Caecal Sac (g/kg)	0.54	$p<0.01$
Caecal Contents (g)	0.24	$p<0.01$
Caecal Contents (g/kg)	0.35	$p<0.01$

TABLE 7r Total and concentration of 2-6,DAPA in dry caecal and faecal material of rats fed two small animal diets (S.A.D) and the nine other diets (PF - EB) for 12 weeks.
Results given are the mean and coefficient of variation, where n = 5 per group.

(i) TOTALS

DIET	S.A.D1	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Caecal (μmols)	6.49 (17%)	3.00 (26%)	3.23 (29%)	11.8 (30%)	3.09 (25%)	2.65 (23%)	8.09 (22%)	2.75 (22%)	3.26 (42%)	11.1 (28%)	3.41 (21%)
Faecal (μmols/day)	6.49 (37%)	10.09 (36%)	6.93 (31%)	16.4 (21%)	7.46 (13%)	6.06 (31%)	12.2 (17%)	5.73 (25%)	4.96 (34%)	7.93 (37%)	4.88 (45%)

(ii) CONCENTRATIONS

Caecal (μmols/g)	6.59 (12%)	3.05 (29%)	7.01 (18%)	11.1 (29%)	5.36 (9%)	4.15 (13%)	7.91 (15%)	3.92 (23%)	7.15 (14%)	9.35 (24%)	5.72 (46%)
Faecal (umols/g)	4.04 (7%)	3.01 (30%)	6.43 (30%)	10.21 (14%)	3.56 (6%)	4.55 (11%)	6.08 (10%)	2.87 (13%)	5.98 (17%)	9.95 (13%)	4.2 (7%)

The least significance difference (L.S.D.) between any 2 means is:

		L.S.D. (44)	Significance
(i) TOTALS	Caecal	3.21	p<0.01
	Faecal	5.30	p<0.01
(ii) CONCENTRATIONS:	Caecal	2.89	p<0.01
	Faecal	1.86	p<0.01

Concentration of faecal and caecal DAPA were of similar magnitude.

SHORT CHAIN FATTY ACIDS (SCFA's)

a) Total SCFA's

(i) Caecal (µmols)

There was no significant difference between S.A.D3 and EG, AG and PG (Table 7s (i)). The S.A.D1 was not significantly different from S.A.D3 or the three bran supplemented groups. The unsupplemented groups gave the lowest total S C F A's. The bran supplemented-and unsupplemented diets gave significantly lower caecal S C F A's than the three gum supplemented groups ($p<0.01$).

(ii) Faecal (µmols/day)

Both the S.A.D1 and S.A.D3 gave significantly higher total S C F A's ($p<0.01$) than any of the other diet groups (Table 7s (i)).

b) Concentration of total SCFA's

(i) Caecal (µmols/g)

There was no significant difference between S.A.D3 the bran supplemented groups and the gum supplemented groups. The S.A.D1 did not significantly differ from the S.A.D3, EG, PB, EB, PF and AF. diets (Table 7s (ii)).

(ii) Faecal (µmols/g)

Faecal concentration of S C F A's was significantly greater with S.A.D3 ($p<0.01$). Concentration with S.A.D1 did not significantly differ from any of the remaining 9 groups (Table 7s (ii). The S.A.D3 increased S C F A's and stool weights. With the S.A.D1, S C F A's appeared to be diluted by the increase in stool weight.

c) The composition of the SCFA's (mmols/mol)

a) Acetate

(i) Caecal

The S.A.D1 and S.A.D3 gave the least caecal acetate (Table 7t (i). The S.A.D3 was not significantly different in effect from the EB and the intermediate diet groups. S.A.D1 was not significantly different from the PB and intermediate groups. Table 7t (i) shows that caecal acetate was significantly greater with the PF, AF, EF diets ($p<0.01$) than the S.A.D1 and S.A.D3.

ii) Faecal

There was no significant difference between any of the groups (Table 7t (ii)).

TABLE 7s Totals and concentrations of S C F A's from dry caecal and faecal material of rats fed two small animal diets (S.A.D) and the nine other diets (PF-EB) for 12 weeks.
Results given are the mean and coefficient of variation where, n = 5 per group.

(i) TOTAL

DIET	S.A.D1	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Caecal (μmols)	419 (5%)	509 (16%)	162 (11%)	678 (27%)	250 (36%)	160 (40%)	653 (19%)	415 (29%)	276 (34%)	622 (37%)	243 (19%)
Faecal (μmols/day)	306 (14%)	729 (21%)	54 (61%)	211 (60%)	194 (35%)	30 (21%)	120 (44%)	209 (10%)	92 (70%)	102 (30%)	142 (51%)

(ii) CONCENTRATIONS

Caecal (μmols/g)	437 (3%)	504 (11%)	365 (10%)	626 (8%)	426 (15%)	243 (28%)	632 (13%)	585 (26%)	632 (27%)	517 (29%)	411 (18%)
Faecal (μmols/g)	96 (13%)	219 (15%)	47.5 (52%)	109 (43%)	111 (30%)	22.6 (10%)	58.3 (34%)	110 (36%)	106 (55%)	133 (17%)	121 (17%)

The least significant difference (L.S.D) between any 2 means is:

	L.S.D. (44)	Significance
(i) TOTALS		
Caecal	196	p<0.01
Faecal	31.3	p<0.01
(ii) CONCENTRATIONS		
Caecal	142	p<0.01
Faecal	55.4	p<0.01

TABLE 7t The molar proportions (mmols/mol) of individual S C F A's in the dry caecal and faecal material from rats fed two small animal diets (S.A.D) and the nine other diets (PF-EB) for 12 weeks.
Results given are the mean and coefficient of variation, where n = 5 per group.

(i) CAECAL

DIET	S.A.D1	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
ACETATE	514 (3%)	573 (12%)	689 (5%)	725 (12%)	588 (5%)	686 (2%)	680 (3%)	609 (3%)	708 (13%)	557 (12%)	621 (5%)
PROPIONATE	140 (24%)	130 (8%)	165 (2%)	162 (16%)	164 (9%)	169 (10%)	197 (17%)	152 (13%)	155 (13%)	230 (6%)	171 (5%)
BUTYRATE	335 (14%)	279 (24%)	145 (23%)	106 (61%)	237 (8%)	94 (12%)	104 (15%)	211 (14%)	84 (13%)	125 (20%)	144 (14%)

(ii) FAECAL

ACETATE	855 (4%)	854 (4%)	1000	842 (12%)	872 (14%)	1000	844 (5%)	918 (1%)	773 (6%)	634 (8%)	730 (11%)
PROPIONATE	66 (92%)	72 (25%)	0	120 (58%)	63 (95%)	0	130 (9%)	82 (11%)	190 (8%)	179 (10%)	148 (12%)
BUTYRATE	78 (64%)	61 (57%)	0	37 (138%)	64 (100%)	0	0	0	31 (137%)	94 (19%)	64 (35%)

The least significant difference (L.S.D) between any 2 means is:

	L.S.D (44)	Significance		L.S.D (44)	Significance
(i) Caecal ACETATE	87	p<0.01	(ii) Faecal ACETATE	NS	NS
PROPIONATE	NS	NS	PROPIONATE	59.7	p<0.01
BUTYRATE	62	p<0.01	BUTYRATE	59.1	p<0.01

Propionate

(i) Caecal

There was no significant difference between any of the groups despite the lower proportions associated with the S.A.D1 and S.A.D3 (Table 7t (i)).

(ii) Faecal

There was high individual variation within the S.A.D1 group (92%), PG group (58%) and PB group (95%). The results for S.A.D1 and S.A.D3 were significantly lower than those of the EB, EG and EF groups ($p<0.01$), Table 7t (ii).

Butyrate

(i) Caecal

The S.A.D1 and S.A.D3 gave significantly greater proportions of caecal butyrate ($p<0.01$). They were not significantly different from each other. The S.A.D3 was not significantly different from the PB diet (Table 7t (i)).

(ii) Faecal

With the exception of the non-butyrate producing groups, the two S.A.D diet groups did not significantly differ from the remaining 5 groups.

Acetate formed the major SCFA. Faecal acetate exceeded caecal in all groups. On average caecal propionate would be considered the next major acid, although caecal butyrate values for AB, PB, S.A.D1 and S.A.D3 were greater than the corresponding caecal propionate values. Very little butyrate was detected in the stool.

HYDROGEN AND METHANE H_2 AND CH_4 (mls/hr/kg)

Individual variation was high within groups for both H_2 and CH_4 (Table 7v). The S.A.D1 and S.A.D3 formed the extremes of the range of H_2 values (0 mls/hr/kg to 0.61 ml/hr/kg). The S.A.D1 gave significantly greater H_2 than all other groups ($p<0.01$). No CH_4 was produced with the S.A.D1 and S.A.D3. This was only significantly different from the PF and PG diet groups ($p<0.01$).

TABLE 7v Hydrogen (H_2) and methane (CH_4) (mls/hr/kg), from rats fed two small animal diets (S.A.D) and the six other diets (PF - EB) for 12 weeks.
Results given are the mean and coefficient of variation, where n = 5 per group.

DIET	S.A.D1	S.A.D3	PF	PG	PB	EF	EG	EB
H_2	0.61 (28%)	0	0.27 (70%)	0.08 (100%)	0.40 (20%)	0	0	0.22 (40%)
CH_4	0	0	0.48 (52%)	0.29 (103%)	0	0	0	0

The least significant difference (L.S.D) between any 2 means is:

	L.S.D (32)	Significance
H_2	0.16	$p<0.01$
CH_4	0.24	$p<0.01$

BILE ACIDS

a) Total bile acids *

(i) Caecal (μmols)

Table 7w (i) shows that total caecal bile acids were greatest with the three EG diets, S.A.D3 and S.A.D2, with the exception of diets AF and EF. Of the twelve groups the three bran supplemented groups, PF gave the lowest total caecal bile acids. Total caecal bile acids from the rats fed the three S.A.D's were varied and ranged from 14.1 μmols to 55.1 μmols.

(ii) Faecal (μmols/day)

Table 7w (ii) shows that total faecal bile acids were greatest with the bran supplemented diets and the three S.A.D's groups, with the exception of diets AG and EB. Of the twelve groups, S.A.D1 gave the greatest total faecal bile acids and diet EB, the least. Total faecal bile acids from the three S.A.D's were varied and ranged from 36.1 μmols/day to 58.1 μmols/day. The results from the three S.A.D groups formed the upper range of total faecal bile acids from the twelve groups.

b) Concentration of total bile acids

(i) Caecal (μmols/g)

Table 7w (i) shows that the concentration of caecal bile acids, of the twelve groups, was greatest on the AF diet and lowest on diet AG. In general the concentration of bile acids was lowest in caecal material from rats in the gum-, and bran supplemented groups, with the exception of diet group EG, and the S.A.D1 groups. The S.A.D2 and S.A.D3 gave concentrations of bile acids similar to those of the unsupplemented diet groups. Concentrations associated with the three S.A.D groups were varied and ranged from 15.3 μmols to 57.2 μmols.

(ii) Faecal (μmols/g)

Table 7w (ii) shows that concentrations of faecal bile acids were lowest in the S.A.D1 and S.A.D2 groups and greatest in the diet groups AF, AG, AB and EF. The value for the S.A.D group fell within the middle of the twelve values. There was no overall uniform effect associated with either the gum arabic or bran supplements.

c) The composition of the total bile acids (mmols/mol)

(i) Caecal

Table 7 w(i) shows the individual proportions of each bile acid in dry C.C.

* = A pooled sample of faecal and caecal material was used for analysis. As a result no statistical analysis was made.

Deoxycholic- and hyodeoxycholic acids, with the exception of the S.A.D3 group, formed the largest proportion of the bile acids with all the diets. Of the three muricholic acids, ω-muricholic acid formed the largest proportion with all the diets, with the exception of the PF, AF and AB diets.

Lithocholic acid: the three S.A.D groups gave results that were similar to each other (44 mmols/mol, S.A.D2 to 56 mmols/mol, S.A.D3), the EG and EB groups. The result of the S.A.D2 group was not dissimilar to that found with diets PG and PB. The molar proportion was lowest with the PF group. The diets AB, AF and AG gave proportions that were at least 4 fold greater than the proportions from any of the other diet groups.

Deoxycholic acid: the diets EB, EF and EG gave the largest proportion of this acid, and these results were similar to the range exhibited by the three S.A.D groups (281 mmols/mol, S.A.D1 to 434 mmols/mol, S.A.D2). With the exception of diet EG, the unsupplemented diets gave larger proportions within each of their respective dietary trials.

Chenodeoxycholic acid: there was little difference between the three S.A.D groups (25 mmols/mol, S.A.D2 to 32 mmols/mol, S.A.D3). The AB, AF, AG and EB diet groups gave the largest proportion of this acid. The diets AB and AF were not dissimilar in effect from S.A.D3. The S.A.D1 and S.A.D2 were not dissimilar in effect from diets EG, and PB.

Cholic acid: the S.A.D3 group gave the lowest proportion of this acid (74 mmols/mol) of the twelve groups. There was very little difference between the higher values of S.A.D2 (98 mmols/mol), S.A.D1 (105 mmols/mol), diets PB, AB and EG. There was little difference between the result of the S.A.D1 group and diet groups PG, EF and AG. Table 7 w(i) shows that with the exception of the EB diet group, the proportion of cholic acid was lower with the bran supplemented groups, S.A.D3 and S.A.D2, and greater with the two unsupplemented groups AF and PF.

Hyodeoxycholic acid: the three S.A.D groups demonstrated a wide range of results from 24 mmols/mol (S.A.D3) to 346 mmols/mol (S.A.D1). There were no close similarities between any of the unsupplemented, gum-, and bran supplemented diets and the S.A.D groups. The proportion of this acid was increased when a diet was supplemented with bran. The S.A.D1 group gave

a result that was intermediate between that from diets EB, PB and AB.

α - muricholic: none of this acid was detected from diet AG. The proportion was highest with diet AB. The S.A.D2 gave a similar result to that from diets PB and PG. The proportion of cholic acid was lowest when diets were fed unsupplemented, with a similar result with the S.A.D1 and S.A.D2 groups. With the exception of diet AG, gum increased the proportion of this acid.

ω - muricholic acid: a wide spread of results were observed with the three S.A.D groups, 87 mmols/mol (S.A.D2) to 154 mmols/mol (S.A.D3). Bile acids were lowest with the AF, AG and AB diets. With the exception of the diet AG, the addition of gum arabic caused an increase of this, being greatest with the PG diet. The S.A.D1 and S.A.D3 groups results similar to those observed with the EF, EG, EB, and PB diets. The S.A.D2 group gave results comparable only to diet group PF.

β - muricholic acid: the three S.A.D groups gave a spread of results from 52 mmols/mol (S.A.D1) to 89 mmols/mol (S.A.D3). These results were similar to those results observed with the majority of the other diet groups. The PF, PB and PG diet groups gave the largest proportion of bile acids. The proportion associated with the S.A.D3 was similar to that of diets PF and PB. With the exception of diet PG, the addition of gum to a diet decreased the proportion of this acid.

(ii) Faecal

Table 7 w(ii) shows the individual proportions of each bile acid in dry stool material. Deoxycholic- and hyodeoxycholic acids formed the largest proportion of the bile acids with all the diets. Cholic acid was also increased on all diets except S.A.D2, S.A.D3, AG and AB. Not one of the three mur cholic acids was detected with S.A.D3 or diet EB. Of the three muri cholic acids, ω-muricholic acid formed the largest proportion. The proportion of ω-muri cholic acid were similar to those of cholic acid.

Lithocholic acid: the results of the S.A.D groups ranged from 43 mmols/mol (S.A.D1) to 128 mmols/mol (S.A.D3). The proportion of lithocholic acid was greatest on diets AG, AB and AF. The proportion given by S.A.D2 was similar to that from diets EB and EG. The proportion given by S.A.D1 was similar to that from diets PB, PG PF and EF.

Deoxycholic acid: the results of the S.A.D1 and S.A.D2 (216 mmols/mol) were the same as each other and similar to the results from diets AB and EB. The result from S.A.D3 (340 mmols/mol) gave the largest proportion of deoxycholic acid out of the twelve diets. The proportion was greater with diets EB, EF and EG than the remaining eight groups (excluding S.A.D3). With the exception of diet EG, the addition of gum arabic decreased the proportion of this acid.

Chenodeoxycholic acid: the three S.A.D groups gave similar results (61 mmols/mol, S.A.D3 to 87 mmols/mol, S.A.D1). These results were similar to those from diets EF, EB and PG, all of which were greater than the remaining six groups. With the exception of diet EG, the addition of gum to a diet increased the proportion of this acid.

Cholic acid: the results from S.A.D2 and S.A.D3 were the same (85 mmols/mol), and similar to the results from diets AG and EG which formed the lower end of the spread of results. The result from S.A.D1 was slightly higher (103 mmols/mol) and similar to the results from diets EG, PG and EF. With the exception of diets AG and EF, the addition of gum to a diet decreased the proportion of this acid, and an unsupplemented diet caused an increase.

Hydeoxycholic acid: the S.A.D2 group gave the overall lowest proportion of this acid (263 mmols/mol), similar only to that of diet EG. The S.A.D1 and S.A.D3 groups gave results of 341 mmols/mol and 385 mmols/mol respectively. These latter results and the results from diets EF, PG, AG and AB comprised the middle spread of results. With the exception of diet AB, the addition of bran to a diet increased the proportion of this acid.

α-muricholic acid: with the S.A.D group the proportion ranged from zero (S.A.D3) to 40 mmols/mol (S.A.D2). The former results was identical to that of diets PG, AF, AG and EB. The S.A.D1 group gave a result similar to that of diets AB, PF and PB. The diets EG and EF gave the highest proportion of this acid.

ω-muricholic acid: none of this acid was detected from diet groups S.A.D3 and EB. The results of S.A.D1 and S.A.D were almost identical and were similar to that of diet PB, at the top end of the range. The addition of gum arabic to a diet increased the proportion of this acid.

β-muricholic acid: none of this acid was detected from diet groups S.A.D3, AF, EG and EB. The S.A.D1 (87 mmols/mol), diet PG (88.8 mmols/mol) and S.A.D2, (124 mmols/mol), groups gave the three largest proportions of this acid.

The results show that the three small animal diets gave results which were compatible with the other nine diets. They gave values that were intermediate and did not fall at either the bottom or top end of the range of results. In nearly all of the results concerned with the proportions of the individual bile acids, the individual S.A.D groups gave results that were similar to the bran supplemented groups.

TABLE 7w (i) The composition of bile acids (mmols/mol) in pooled caecal material from rats fed the small animal diets (S.A.D) and the nine other diets (PF-EB) for 12 weeks.
Results given are means

DIET	S.A.D1	S.A.D2	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Caecal Bile Acids (μmols)	14.7	55.1	24.5	9.38	16.5	9.5	42	16	12.2	17.4	38.9	12.1
Caecal Bile Acids (mols/g)	15.3	57.2	24.2	20.4	15.1	16.4	64.4	14.8	17.3	37.1	32.8	20.5
BILE ACIDS (mmols/mol)												
Lithocholic acid	52	44	56	20.6	36.4	42.7	179	194	148	31	44	44.5
Deoxycholic acid	281	434	344	200	181	176	250	163	238	303	347	278
Chenodeoxycholic acid	26	25	32	19.6	13.3	24.4	38.1	194	32.8	41.3	25	42.4
Cholic acid	105	98	74	152	106	97.6	127	113	98.4	107	103	123
Hyodeoxycholic acid	346	203	24	436	99.3	415	379	338	418	313	277	329
α-muricholic acid	18	36	12	14.7	33.1	30.5	23.8	0	65.6	9.17	20.1	12.2
ω-muricholic acid	118	87	154	73.5	371	122	40.5	0	0	134	137	122
β-muricholic acid	52	73	89	83.3	166	91.6	35.7	0	0	62	46.4	53.6

TABLE 7 w(ii) The composition of bile acids (mmols/mol) in pooled faecal material from rats fed the small animal diets (S.A.D) and the nine other diets (PF-EB) for 12 weeks.
Results given are means.

DIET	S.A.D1	S.A.D2	S.A.D3	PF	PG	PB	AF	AG	AB	EF	EG	EB
Faecal bile acids (μmols/day)	58.1	42.6	36.1	18.2	23.4	39.8	29.2	52.9	54.7	24.8	15.2	12.4
Faecal bile acids (μmols/g)	18.8	12.9	10.8	16.7	16.3	18.5	21.7	28.9	27.5	30.6	19.4	10.9
BILE ACIDS (mmols/mol)												
Lithocholic acid	43	94	128	43.6	42.9	42.2	259	181	203	44.5	93.8	88.5
Deoxycholic acid	217	216	340	173	166	167	171	132	196	231	296	221
Chenodeoxycholic acid	87	70	61	44.2	73.5	37.3	30.8	45.4	29.3	62.8	37.1	66.4
Cholic acid	103	85	86	140	110	130	130	71.8	65.8	115	95.9	138
Hyodeoxycholic acid	341	263	385	452	324	449	373	470	397	321	274	486
α-muricholic acid	19	40	0	17.3	0	18.4	0	0	14.6	124	60.1	0
ω-muricholic acid	103	108	0	73.5	195	99.5	37.7	56.7	43.9	75.2	142	0
β-muricholic acid	87	124	0	58.6	88.8	56.8	0	41.6	51.2	46.5	0	0

SUMMARY

This section has given the results of the comparison between the S.A.D's and the nine other diets given to rats for 12 weeks.

The three S.A.D's gave significantly greater stool weights than the nine other diets, a possible reflection of the fibre content. The three S.A.D's gave dry C.C. equal to those observed with the gum arabic supplemented diets.

There was some discrepancy between diets S.A.D1 and S.A.D3. With total faecal DAPA, there was no difference between the S.A.D1, S.A.D3 and the three bran groups. The S.A.D3 group was not significantly different from the gum groups. With faecal DAPA concentration, the S.A.D1, S.A.D3 and bran groups gave lower concentrations than the gum supplemented groups.

Both the S.A.D1 and S.A.D3 gave significantly greater total faecal SCFA's. The S.A.D3 gave a significantly greater concentration of total faecal S.C.F.A's.

With total S C F A's (caecal), although there was no significant difference between the S.A.D1 and S.A.D3, S.A.D3 gave results of similar magnitude to those of the gum arabic diet groups, a reverse effect to that of caecal DAPA. The S.A.D1 resembled results from the bran supplemented groups. Similarly for the concentration of S C F A's. Such comparisons can be made for the remaining groups: there was no significant difference between trial live weights; S.A.D3 gave similar results to those from gum arabic supplemented groups for caecal contents; S.A.D3 and S.A.D1 emulated gum results for caecum sac, but S.A.D2 did not.

The differences in dietary composition of the three S.A.D's (Tables 7 (a) - (aiii) are slight and do not fully explain the differences observed between diets S.A.D1, S.A.D2 and S.A.D3. The pelleting technique may alter the effect of diet. With the exception of stool weight and dry C.C. results from S.A.D1, S.A.D2 and S.A.D3 tend to fall in the range of values observed from the other diets. Stool weight maybe explained by the greater crude fibre content (Tables 7a and 2a). The S.A.D's have 2.8% sugars as sucrose. The PF and AF diets are reported to have a total sugar composition of 0.8% and 0.1% respectively. This may explain why the S.A.D's gave dry C.C. weight similar to those on gum arabic diets. More sugar maybe reaching the caecum, and being degraded. Whilst in some measurements there are differences between S.A.D1 and S.A.D3

eg DAPA results, the S.A.D1, S.A.D2 and S.A.D3 do not outrightly resemble either an unsupplemented, gum supplemented or bran supplemented fibre free diets. From these few results there appears to be:

1) biological variation which maybe important and as well as the differences between standard small animal diets which are similar in composition.
2) Fibre in the diet is apparently important.
3) Type and amount of fibre is important.
4) What is fed in conjunction with the fibre is important, possibly more so than amount.
5) that for some parameters eg stool weight 4 weeks is adequate for any dietary effects to have stabilised. For more metabolic parameters such as bacterial mass, SCFA's and bile acids, a longer time period maybe required in which to assess the effect on bacterial metabolism and activity.
6) The composition of the non-cellulosic polysaccharide fraction of the diet, and amount of starch may be important.
7) the form of the diet, (pelleting, cooking etc) may be important.

CHAPTER 8

A DISCUSSION

GENERAL

Dietary fibre occupies an important place in human nutrition, and the aetiology and management of many diseases of Western Civilisation. Conditions such as appendicitis, haemorrhoids and diverticular disease have been related to dietary fibre intake (Burkitt et al, 1972; Burkitt, 1975). Indeed, diverticular disease has occurred only in developed countries whose diet has changed to that of a low residue, more refined diet (Painter and Burkitt, 1971; Painter et al, 1972). The incidence of Diabetes mellitus, ischaemic heart disease, obesity and cancer of the colon have also been related to dietary fibre intake (Rose et al 1974; Trowell 1973 and 1976).

Review articles and books have been written about dietary fibre and its effects, and various hypothesis proposed (Cummings, 1973; Eastwood, 1975; Cummings, 1978; Eastwood and Kay, 1979; Eastwood and Passmore, 1983; Cummings, 1984). Books on the subject include F-Plan diet (Eyton, 1982), Medical Aspects of Dietary Fibre (1980) and Medical Aspects of Dietary Fibre - a report of the Royal College of Physicians (1980). Whilst a wealth of knowledge exists concerning dietary fibre, its chemical and physical properties and subsequent effects, there is still a degree of uncertainty as to how the effects of dietary fibre are mediated. Human studies are used to indicate the potential of a dietary fibre, concomitant with the continuous development and improvement of in-vitro methods, whereby the effect of a fibre source in man can be predicted in the laboratory environment. Human trials are time consuming and demanding on laboratory and experimental personnel (Eastwood et al, 1983) and have the added complication, that unless a long time interval elapses after the previous assay, then there is a carry over effect (Eastwood et al, 1973).

Dietary fibre consists of a variety of complex polysaccharides which include the non-starch polysaccharides (the non -α-glucan polysaccharides), cellulose, hemicellulose, and lignin. Over the years it has become apparent that the original methods for the determination and quantification of fibre have been lacking as the knowledge of dietary fibre increases. More precise and sensitive methods are sought after, which are both convenient and short and will provide a low nitrogen and starch containing residue.

The original method for fibre determination is that of crude fibre, commonly referred to as the Weende method (Mangold, 1934; Van Soest and McQueen,

1973). However, there are major problems associated with the nature of the crude fibre analysis as reviewed by Van Soest and McQueen (1973). A major problem is the loss of cellulose, hemicellulose and lignin during extraction and filtration thus giving a false indication of the amount and quality of dietary fibre present. Other problems that have presented themselves are, the concepts of what constitutes fibre, the definition of the constituent components, the separation of these components, the non-uniformity of cellulose, hemicellulose and lignin between plant materials and the effective removal of starch.

Van Soest, amongst others, recognised the defects of the crude fibre method and developed improved methods. These methods use acid- and neutral detergents to measure the constituent components of fibre (Van Soest, 1963 (a) and (b); Van Soest and Wine, 1967, and 1968; Goering and Van Soest, 1970), resolving many of the problems associated with the older procedure of crude fibre . However, these more refined methods were developed with respect to ruminant, nutritional studies. Man lacks a rumen and is unable to make use of the hydrolytic activities of the intestinal microflora in the same way as the ruminant and non-ruminant herbivore (Southgate, 1973). The methods of Van Soest and colleagues are somewhat subject limited (Van Soest, 1967). The detergent system for the measurement of plant cell wall material fails to measure water soluble polysaccharides and incomplete removal of starch from starch rich foods. A major problem in all methods for measurement of dietary fibre is the removal of starch (Englyst and Cummings, 1985). The Southgate (1969) procedure for the measurement of unavailable carbohydrate in man, removed starch by enzymatic hydrolysis, with the colorimetric measurement of the sugars. The method was a step in the right direction to resolving the ubiquitous problem of starch and to the determination of cell wall material in human food. However it also fails to completely remove starch. Englyst and colleagues have recently further developed a method for the isolation and measurement of non-starch polysaccharides, which takes into account any starch that may have become resistant to α-amylase digestion through food processing, which may therefore be measured as non-starch polysaccharide (Englyst et al 1982; Englyst and Cummings, 1984). Plant polysaccharides can be divided into starch and non-starch polysaccharides. The latter group may be further divided into cellulose and non-cellulosic polysaccharides and the constituent sugars measured. The aim of the procedure is to completely recover all the non-starch polysaccharides with the complete

removal of starch (Englyst, et al 1982). However, the complete removal of starch from fibre preparations does not include what happens in the gut e.g. the interactions between retrograde starch and the non-starch polysaccharides in the caecum.

Chemical methods for the isolation and evaluation of the individual components of dietary fibre are essential to further our knowledge of the complex nature of different fibres. These analyses only give a result for the individual component. However, such in-vitro analysis are not easily related to the in-situ interactions with other dietary components, such as starch and intestinal bile acids and the metabolic production of short chain fatty acids (S C F A's). The extrapolation of such measurements to colonic function is not currently possible. The results of in-vitro work should only be treated as the substantiation of in-vivo work.

Whilst the measurement and qualification of dietary fibre components is the nucleus of the dietary fibre story, other in-vitro methods have been developed to evaluate the physical effects of dietary fibre. Such methods include water holding capacity (W.H.C.), (Robertson and Eastwood, 1981 (a), and (b)). In-vitro methods do have their limitations in as much as they do not portray the environment of the colon. An example is W.H.C. A high W.H.C. was thought to reflect the ability to increase stool bulk (Eastwood, 1975), but this depended on the method chosen and fibre source. Stephen and Cummings, (1979) demonstrated an inverse relationship between W.H.C. and stool bulk. Recently, McBurney, et al (1985) have shown that there could be a direct relationship between stool weight and the potential W.H.C. (pW.H.C.) of a fibre residue, where the pW.H.C. is given as a function of the extent of fermentability. This warrants further investigation.

In essence, there is an established effect of dietary fibre on the colon and faecal output. Both human and animal studies have attempted to show the site of degradation of dietary fibre sources, the individual components of dietary fibre and the resultant effect on certain metabolic functions of the gastrointestinal (G I) tract and the effect of the growth of organs (Elsden et al, 1946; Yang et al, 1969 and 1970; Walters et al, 1975; McLean Baird et al, 1977; Cummings et al, 1978; Younoszai et al, 1978; Elsenhans et al, 1980 and 1981; McKay and Eastwood, 1983; McLean Ross et al, 1983 and 1984). Studies have also included the toxicology of dietary fibres (Moinuddin

and Lee, 1959; Leegwater et al, 1974; Walker, 1978; Anderson et al, 1983; Mallett et al, 1983 (a) and (b); Mallett et al, 1984 (b). Dietary complexes and associated effects can only be assessed in a mammalian model, the choice of model being dependent upon the species to which the evaluation of feedstuffs is applicable. In this case, the human is the best choice. However, there are inherent problems, and consequently the most practical choice is the laboratory rat- easy to use, relatively cheap and easy to monitor.

Dietary fibre undergoes bacterial fermentation in the colon with the release of a number of chemical by products. These include carbon dioxide, hydrogen (H_2), methane (CH_4) and SCFA's. The best known effect of dietary fibre is that of stool weight and bulk, a measurement that is non-invasive. The stool can then be analysed for metabolic by-products such as SCFA's, bacteria and bile acids, all of which serve as indicators of fibre metabolism in the colon. Other non-invasive indicators are the gases excreted in the breath, H_2 and CH_4. The SCFA's have also been suggested as a mediator of stool weight (Williams and Olmsted, 1936 (b); Hellendoorn, 1978) but subsequent studies have shown that SCFA's can be absorbed in the rectum (McNeil et al, 1978) and that the relationship between stool weight and SCFA's is coincidental (Cummings, 1981).

Both population and laboratory studies have shown that the more fibre an individual eats, the greater the stool weight (Royal College of Physicians, 1980). Studies have also shown that stool weight is dependent on the fibre source (Williams and Olmsted , 1936 (a); Cummings et al, 1978; Kelsay 1978), and its physical properties. A wheat bran will increase stool weight by virtue of its water-holding properties, (Eastwood et al 1973; Eastwood et al, 1983) whilst fruit and vegetables will affect stool weight, either by increased bacterial mass (Stephen and Cummings, 1980(a) and (b)) or by the W.H.C. of the residual fibre (Eastwood et al, 1983). The in-vitro bulking ability of a fibre has a limited ability in forecasting the biological potential of a fibre (Stephen and Cummings, 1980(a)).The W.H.C. of wheat bran is a good predictor of faecal bulking, but has little predictive value with fruit and vegetable fibre. The physical form of a fibre, particle size and the physical changes that occur during cooking and preparation may profoundly affect the faecal bulking properties.

What about the duration of feeding? Whilst the duration of studies has spanned

periods of 7-9 days (Schneeman and Gallaher, 1980; Ullrich et al, 1981; Hoverstad and Bjørnklett, 1984), 3-5 weeks (Robertson et al, 1979(b) Spiller et al, 1980; Ross and Leklem, 1981; Mallett et al, 1983 (a), and (b)), 9 weeks (Yang et al, 1969; Elsenhans et al, 1981; Fleming and Lee, 1983; Jacobs and White, 1983), 9 months to one year (Dowling, 1967; McLean Baird et al, 1977; Tarpilla et al 1978). Few people have actually investigated the possible important influence of the duration of dietary supplements. McLean Ross et al, (1984) did comment on the effect of gum arabic on the production of H_2 and CH_4 over a period of 14 and 28 days. Anderson et al (1982) studied the long term, sub-chronic effect of gum arabic fed for 13 weeks. Elsenhans et al (1981) came close to studying the effect of feeding duration in their studies with unavailable carbohydrates gelling agents. They observed that changes in the parameters under study remained constant between 4- and 9 weeks. Ullrich et al (1981) suggested that 4 days was adequate to cause a large increase in primary bile acids with a concomitant decrease in secondary bile acids. The implication is that short term studies could be useful in screening diets for longer studies. Spiller et al, (1980) observed the influence of cellulose and pectin SCFA's over 2-, 4- and 5 weeks, but did not fully discuss the potential importance of the three time periods.

This study has attempted to establish an animal model which could be used to evaluate the effect of novel fibres on colonic function and stool output. Whilst Williams and Olmsted (1936 (a)) believed that the results for the study of stool weight were more convincing and applicable if a human subject was used, as already mentioned, human trials are laborious, time consuming and can be unreliable, (Eastwood et al 1983). It is virtually impossible to directly study events occurring in the caecum. Such techniques involve intubation. Indirect methods include breath H_2 and CH_4 (McKay, 1981; Tadesse, et al, 1979), stool weight and composition (Eastwood et al, 1973; Cummings et al, 1978; Spiller et al 1980; Smith et al, 1981), blood analysis (Robertson et al, 1979(b) Stasse-Wolthius et al, 1980).

By the means of three individual, but related, dietary trials, the effect of two fibre supplements, given in conjunction with three basal fibre-free diets, was examined. This accommodated the possibility that the diet given with a fibre is important. In practice, people are unlikely to increase their intake of fibre from a single source but rather to take a moderate amount of several different foods. (Cummings et al, 1978). Whilst interactions between dietary

components exist (Yang et al, 1969) much remains to be learnt about the interaction between fibres from different sources and indeed the interaction between the fibre and basal diet. The three basal diets were chosen with respect to the different origins of fat and protein and the low level of inherent fibre.

Gum arabic was chosen because of its complete degradation in the caecum and upper tract of the rat and human (McLean Ross et al, 1983 and 1984). Gum arabic is a water soluble, acidic heteropolysaccharide of very high molecular weight, composed of a highly branched array of galactose, arabinose, rhamnose and glucuronic acids, and is in wide dietary use. Coarse wheat bran, by way of contrast, is incompletely degraded in the caecum. Wheat bran is composed of cellulose, lignin, hemicelluloses, protein and starch and non-starch polysaccharides. Gum arabic and bran represent the wide spectrum of the group headed 'dietary fibres'. Both fibres have been used in previous dietary trials within the Wolfson Laboratory.

THE DIETARY TRIALS

Crude analysis of the three diets containing gum arabic and wheat bran show them to be virtually identical in their fibre content, that is plant origin diet (PF) plus bran (PB) or gum arabic (PG), animal origin diet (AF) plus bran (AB) or gum arabic (AG) and elemental diet (EF) plus bran (EB) or gum arabic (EG), when the inherent fibre content of PF, AF and EF is accounted for (pages 42-46). The fibre content of the three bran supplemented diets is 1.12% (PB), 1.14% (AB), and 1.2% (EB). With gum arabic, no fibre is present. The inclusion of 10% of each fibre supplement may have diluted the vitamin and mineral premix content but in practice did not , cause any problems (Rickett, 1984 pers comm.). Wheat bran will have contributed some vitamins and minerals. The protein content of all the bran supplemented groups was higher than the gum supplemented groups, which maybe reflected in live-weight changes, particularly with the EG group. The protein content of this group was 32% lower than diets PB and AB and 19% lower than the PG and AG groups. Whilst it is suggested that rats require a minimum of 20% protein of high biological value in an adequate diet for optimal growth (Coates et al, 1969), it is believed that levels of 10-13% protein in the diet of these mature adult rats was sufficient. The rats were chosen because they were mature (300-400 g live-weight) in order to study the effect of the diets on changes of metabolic and physical measurement rather than the diet induced

changes of growth. A group of 5 rats were given a total of 150 g dry weight of diet, which was considered to be more than enough to feed 5 rats, thereby creating an ad-lib feeding situation. This would then allow the rats to compensate for any nutritional imbalance.

A comparison of diets PF and AF shows them to be identical, with the exception of the source of fat and protein, minerals, vitamin K, lysine content, magnesium (Mg) salt, calcium (Ca) phosphorus (P) and potassium (K). The source of protein in diet AF was meat, and meat and bone meal, whilst the protein of diet PF came from soya concentrate. This could explain the addition of 1000 mg Mg instead of 100 mg (PF). The lack of Ca is possibly explained by the inclusion of bones. Similarly for P. The soya of diet PF may explain the lack of K in diet PF as soya is rich in K. The diets were nutritionally complete. The protein of diet EF was fractionally lower (10%) and the total carbohydrate content was 67%, which equalled that on the diet AF. Energy content was slightly higher.

Soyabean meal, when cooked, will reduce the availability of lysine which possibly is why lysine was added to diet PF. Soyabean meal also has a good biological score (75g-rat), but a low chemical score (49), meaning that soyabean meal has a less balanced amino acid composition (McDonald et al, 1973). Meat and bone meal has a biological balue of 67 (adult man) and is useful as a lysine supplement, but a poor source of methionine.

Gum arabic has a protein content of 1.9%. This is approximately 0.285g/15g of gum arabic and thus 0.19% of the total diet fed. Coarse wheat bran has a protein content of 1.5% and a starch content of 17.5%, approximately equal to 0.15% of the diet fed (protein) and 1.75% (starch).

If there had been nutritional inadequacies with the diets, these could possibly have been reflected in organ weights, as demonstrated by Anderson et al (1982). In the latter study the liver weight was reduced in a close dependent manner when rats were fed gum arabic. Only liver weights during the AF dietary trial appeared to decrease. This was attributed to poor acceptability of the diet and therefore limited food intake. In the PF dietary trial liver weights were unaffected by diet. Although weights were significantly lighter at 8 weeks, it does appear that this is a false indication of events, as there was no significant difference between 4- and 12 week weights. This slight

drop in weight after 8 weeks of feeding may have been as a result of delayed reaction to adaptation of the diet. It is clear that liver-weight did not alter with increasing live-weight but remained fairly stable, as live-weight steadily increased.

Whilst food intakes were measured, the presence of 5 rats per cage, and the nature of the diets, reduced the accuracy of estimated feed intake. The estimated dry food intakes of the rats fed diets EF (28g/day), EG (25g/day) and EB (26g/day) were very similar to those from rats fed diets PF (27g/day), PG (25g/day) and PB (26g/day) over 12 weeks. The lower protein content of diet EG may have resulted in the significantly lower live-weight gain than the rats fed diets EF and EB. Food intakes were lower with rats fed diets AF (21g/day), AG (19g/day) and AB (25g/day) over 12 weeks.

Diets PF ± supplement and EF ± supplement were obviously acceptable to the rats. All rats increased their body weights over 12 weeks, as indicated by estimated dry food intake and modest live-weight loss. Diet AF was disliked by the rats. The diet was nutritionally adequate in its unsupplemented and supplemented forms. Only the addition of bran had a beneficial effect upon live-weight. This could be due to the beneficial effect of the bran in conjunction with diet AF. The results indicate that a supplement helped to stem live-weight loss, those rats fed diet AF losing the most weight. Overall rats were lightest with the AF dietary trial, and bran significantly increased live-weight (p108 and 185-187.)This suggests that weight increase is related to the nature of the fibre supplemented. Bran is a more bulky fibre than gum arabic and a nutritional diluent. To meet nutritional requirements, it is possible that rats ate more, and thereby put on more weight. The rats on the gum arabic gained their nutritional requirement from less food intake and consequently the weight gain was less. Bran does have some carbohydrate and protein, whereas gum arabic is just gum arabic. Alternatively if bran has nutrients present and gum arabic is fermented it may be better nutritionally to absorb from the jejunum than the colon.

The presentation of the elemental diets differed from that of the PF and AF dietary trials, only in the respect that a known quantity of gelatine was added to the diets in order to make them acceptable to the rats. It is believed that gelatine had no effect on the final results. Work by de Bethizy and Street (1984) has shown that a purified hydrated gelatin diet meets the nutritional

requirements of growing rats and provides a suitable matrix in which to feed dietary fibres froma variety of sources during short term experiments. Previous work by McLean and Ross et al (1984) using flexical and gum arabic also used gelatine as a setting agent and the results were not influenced by the presence of the latter.

In all, the rats survived and thrived on the three elemental based diets, and results were not influenced by the presence of gelatine. The animals fed during the PF and EF dietary trials grew at equivalent rates. That is, they had not lost weight after 12 weeks, suggesting no complicating nutritional factor.

Stool collections and their analysis have long been the subject of investigations, as an assessment of the action of a test dietary fibre. As was anticipated, the presence of bran in the diet significantly increased dry stool weight (Williams and Olmsted, 1936 (a); Eastwood et al, 1973; Cummings et al, 1978), irrespective of the basal diet. From chapter 6 it is evident that, whilst, overall the elemental dietary trial reduced stool weight, the addition of bran to the diet had the same effect, (figure 6c (iii)p192)as the addition of bran to diets PF and AF, with respect to the respective unsupplemented diets. The percentage increase in stool weight, with respect to an unsupplemented diet,was 66%, 79% and 56% for the PB, AB and EB diets. Whilst the absolute values are altered by basal diet, the relative values are all very similar, particularly between diets PB and EB. Of the three bran supplemented diets, the stool weight associated with diet AB was significantly greater than that of diets PB and EB (figure 6c (iii), p 192) despite the unacceptability of diet AF by the rats. The results suggest that metabolic functions and body organ weights were unaffected by this decrease of nutritional intake. No deficiency symptoms were observed in the appearance of the rat. The rats were obviously not eating to maintenance level. Stool does not appear to be a function of live-weight, nor of basal diet, but of the supplement given. In this case, bran. The increase of stool weight with bran is possibly due to W.H.C. The slight, but non-significant increased observed with gum arabic is probably due to bacterial activity and proliferation. Time was not significant with either the PF or AF dietary trials.

In both the PF and AF dietary trials, the addition of bran to the diet was

seen to increase stool weight by approximately 60%. The dry stool weight with diets EF, EG and EBwassmall compared to the absolute values observed with the PF and AF dietary trials. Gum arabic when fed with any diet was not seen to significantly increase stool weight in any of the individual trials. When the nine diets are considered together it can be seen that stool weight with diet AG is again significantly greater than diets PG and EG, and even diet AF. The change in stool weight between diets PF and PG, and diets EF and EG, was not significant, although stool weight on the latter diets was half that observed with diets PF and PG. In the case of the elemental diets, the large intestine would receive the supplements free of nutrients as these would have been absorbed more proximally (McLean Ross et al, 1984). Therefore the exaggerated increase in stool weight observed with the PF and AF dietary trials may be partly due to undigested and unabsorbed components reaching the large intestine concomitant with the dietary fibre residues. The percentage increase between the stool weights from rats fed diets EG and EB was 63% and between diets PG and PB 40% which supports the hypothesis. The stool weight of 0.7 ± 0.05 g/day with the unsupplemented diet EF, exactly matched that observed in the study of McLean Ross, et al (1984) where rats of similar weights given an elemental diet gave a stool weight of 0.7 ± 0.07g/day (mean ± SEM), showing the reproducibility of the study and the use of the rat as an animal model. In that study a 43% increase in stool weight was observed when rats were fed the elemental diet with 130g gum arabic/kg (1.0 ± 0.1g/day, mean ± SEM). With this present trial there was no significant difference between stool weights from rats fed either diets EF or EG. These results thus suggest that what is fed along with the supplements is important, and that wheat bran, being of low caecal degradability will predictably increase stool weight. Only the magnitude of the increase in stool weight will be influenced by the basal diet. Thus the small, but insignificant, increase of stool weight observed with diets PG and AG is probably attributed to small amounts of retrograde materials such as starch, reaching the caecum being digested and fermented by caecal bacteria, or remaining untouched, and thus contributing to stool weight. With an elemental diet this cannot happen. This highlights the importance of methods such as those of Englyst et al (1982) which accurately analyse food materials, in vitro, for starch, retrograde starch and dietary fibre. Equally it highlights the discrepancy between the in-vitro and in-vivo digestion of individual foods.

The reduced daily faecal weight of those rats fed the elemental diets, is in

agreement with other studies on elemental diet in humans (Winitz et al, 1970) and in the rat (Greenstein et al, 1957). Elsenhans et al (1981) reported significant increased faecal weights of 0.1g/g ingested food when a basal diet was supplemented with 100g/kg gum arabic. When the feed intakes of this present study are considered, then the dry faecal weight on the diet EG is approximately 0.03g/day and for the diet EF, 0.03g/day. Cummings et al (1978) demonstrated the different effects on stool weight of different dietary fibres. The addition of 20g/day of guar gum, apple, carrot, cabbage or bran to a diet increased wet stool weight by 20%, 40%, 59% and 127% respectively. The increase associated with bran in that study is not dissimilar from the 65% increase in dry stool weight from the rats of this dietary study whose estimated intake of bran or gum arabic was 3g/day. If gum arabic is degraded extensively in the caecum (McLean Ross et al, 1984) with a resultant increase in bacterial mass, as indicated by the increased Diaminopimelic acid (DAPA) content of the dry caecal contents, then there is no reflection of this in the stool weight. Cabbage, which was reported to be extensively degraded in the large bowel during transit (Stephen and Cummings, 1980(a))resulted in marked increases in stool weight, (54.3g/day). Of this 8.5g/day were solids and of this, 4.8g was attributed to bacterial solids. With wheat bran, the increase in stool weight was 102g/day, of which 19g/day were solids and of this 2.3g was attributed to bacterial mass. The conclusion was that wheat bran altered stool weight by virtue of its W.H.C. and that vegetable fibre altered stool weight by virtue of increased microbial growth. However, both wheat bran and cabbage were not entirely digested in the G I tract thus allowing the possibility of some bacteria to adhere to remnents of fibre (cellulose fibres, lignin) and be excreted in the stool, thus showing evidence of increased microbial activity. This in turn suggested that undigested residue plays some part in the increase of stool weight, but not when gum arabic was given to humans. The chronic increase in bacterial metabolism did suggest altered bacterial metabolism. With gum arabic, degradation is complete. A reasonable suggestion is that stool weight comprises mainly of bacteria, and by-products of metabolic pathways Certainly total faecal DAPA was increased with the gum arabic but not with bran as were total SCFA's. Thus, bran increases stool weight partially by undigested residue and water holding properties. Gel forming, water holding polysaccharides may or may not increase stool weight by virtue of bacterial proliferation. McLean Ross et al (1984) reported increases in stool weight when rats were fed a pelleted Oxoid breeders diet (O.B.D.) + 10% gum arabic but not when gum arabic was given to humans. The chronic increase in bacterial metabolism did suggest altered bacterial

metabolism. This difference may have been due to increased food consumption on this diet by the rats, or an indirect result of the basal diet. The O.B.D. was reported to contain 15.2% dietary fibre. Significant stool weight increases were observed when an elemental diet gum arabic was also fed to rats. As the elemental diet was residue free, it would be expected that whatever supplement was added would solely increase stool weight, even if it was only through "excess to body requirements".

Bran has little additional effect on caecal metabolism as suggested by the CH_4, DAPA and SCFA results. Gum arabic did give a small but insignificant increase of stool weight which may be as a result of increased caecal activity. There does seem to be difficulty in relating the W.H.C. of all fibre sources in-vitro to their stool bulking abilities in-vivo. The W.H.C. of wheat cereal bran clearly relates to faecal bulking ability. McBurney et al (1985) have attempted to resolve the differences between in-vitro and in-vivo W.H.C. Their in-vitro results for W.H.C. using dialysis tubing showed pectin to have the greatest W.H.C. and cellulose the least. When the four test fibres were anaerobically fermented, the W.H.C. were in order of legumes > cellulose > cabbage > pectin. Thus it is not the W.H.C. we want to measure but the p W.H.C.* a function of the extent of fermentability and W.H.C. of the fermentation residues. A result of their study is that four factors might alter wet stool weight: the change in W.H.C./of the fibre remaining after fermentation; loss of organic matter; addition of microbial mass; particle size. The significantly lower W.H.C. of lucerne neutral detergent residue compared to neutral detergent fibre substantiated the hypothesis that fermentation directly reduced the W.H.C. of fibre. The result of cellulose (ethanol insoluble residue) W.H.C. was greater than the original cellulose, suggesting that the microbial organic matter generated from the fermentation of cellulose had a higher W.H.C. than the cellulose that was fermented. The pW.H.C. results ranked fibre sources in the same order as have been found in in vivo studies, giving better predictions of stool weight.

The amount of fibre fermented and its ability to hold water must be therefore considered as well as the amount of microbial organic matter produced, if one is to predict the effect of fibre on colonic contents.

The effect of time, of feeding the supplement, was seen only to be significant with the elemental dietary trial ($p<0.05$) where stool weight increased between

* = potential Water Holding Capacity

4- and 12 weeks with each diet. However there was no significant difference between 4- and 8 weeks or 8- and 12 weeks. With the PF and AF dietary trials stool weight had appeared to have stabilised after 4 weeks, and time was not significant. Thus if stool weight is to be used as an indicator of the effects of dietary fibre, then a period of between 4- and 8 weeks will be necessary depending on the basal diet chosen. It may well be, that with the elemental diet, 5 weeks may have been the feeding period beyond which no further significant differences would be observed. This requires further studies, with other dietary fibres. Elsenhans et al (1981) reported that a period of 7 to 8 weeks would appear to be reasonable to study adaptive responses as effects of long-term feeding of carbohydrate gelling agents added to a fibre free diet.

These three trials indicate that the extent of the degradation within the G I tract of a particular bulking agent, is the primary determinant for the resultant effect upon faecal excretion (Elsenhans et al, 1981). Bran increased stool weight, probably as a result of W.H.C. and undigested residue. Within each dietary trial gum arabic had an insignificant effect upon stool weight. These results compare favourably with those from human dietary trials and suggest that, as far as stool weight is concerned, the rat is a suitable model.

Stool weight increased with wheat bran. The weight of the wet caecal sac (C.S.W.W.) and the weight of the dry caecal contents (C.C.) were greatest on the gum supplemented diets. Only with the PF and AF dietary trials was there no significant difference in effect between the unsupplemented and bran supplemented diets. The C.S.W.W. from diet EB was significantly greater than that from rats fed diet EF.

Figure 6d (vi), (p195). shows that, irrespective of basal diet, gum arabic increased C.S.W.W. with an increase in weight after 8 weeks. This may reflect a delayed reaction to the supplement, which had steadied by 12 weeks. Considered alone, figure 5b (p150) shows that no significant effect of time was seen with diet EG between the three time periods, which suggests that adaptation to the diet has occurred before 4 weeks, whilst lack of fermentable substrate and nutrients with diets EB and EF caused an increased adaptation period. The increase in C.S.W.W. with diet EB with respect to diet EF may be due to increased retention time, and subsequent caecal distension. This does seem unlikely as raw bran is associated with increased transit times (Cummings, et al, 1978; Harvey et al, 1973; Wyman et al, 1976). Caecal degradation

of bran is unlikely: there was no increase of DAPA with bran. If bran increases stool weight by virtue of its W.H.C. (Eastwood et al, 1973) there may also be increased swelling of the bran in the caecum causing distension of the walls. From figures 3band 5b(p76 and p150) it is obvious that the diets EF, EB, PF and PB do not behave in the same manner over time. Whilst there is no difference between the diet groups PF and PB, this is not true for diet groups EF and EB. If, as is suggested, no nutrients are reaching the caecum in the diet EF, then the C.S.W.W. would be lower than would be expected thus exaggerating the difference between the diet groups EF and EB. With diet PF some material may be reaching the caecum and being degraded thus putting pressure on the caecum walls. By comparing the results between the 'PF and EF' dietary trials, this latter supposition can only partially explain the differences in the associated dietary trends, because there is close similarity between the various means: diet EG gave C.S.W.W. which were only 18% greater than those on diet PG; diet EB gave C.S.W.W. which were only 12% greater than those with diet PB; diet PF gave C.S.W.W. that were 10% greater than those with the EF diet. There must be a further explanation to the increased C.S.W.W. with the EB diet such as the possible bacterial adaptation to the diet and the breakdown of any starch, which is known to cause caecal enlargement, (Leegwater et al ,1974), or the increased work to propel bulky contents (Brown et al, 1979). Certainly, what is immediately apparent is that both diets, PG and EG caused significant increases in C.S.W.W. which did not alter between 4- and 12 weeks, and that these were coincident with the previous studies reported. The percentage increase between the unsupplemented and gum arabic supplemented diets is related to the basal diet fed. The percentage increase between diets EF and EG was 74% and between diets PF and PG, 34%. The results are similar to those of Elsenhans et al (1981) who found the weight of the caecum to be significantly greater in growing rats fed 10% gum arabic, compared to the unsupplemented diet. Inert polysaccharides had no effect on the weight of the caecum, a reflection of the accessibility of the different polysaccharides to degradation. Thus gum arabic is retained for longer in the caecum and is degraded, the result of which is for caecal volume to increase causing distention of the caecal walls.

With the AF dietary trial, the trend with time of C.S.W.W. was not uniform for the three diets. This may be a reflection of decreased live-weight and subsequent adaptation to the diet under stressed circumstances.

Caecal enlargement is a common finding when large quantities of an

undigestible bulk agent are fed to rats, and is now "fully attributable to normal physiological adjustment" (Zbinden, 1969). Such an increase could be related to prolonged caecal residence, increased bacterial products with the increased weight of C.C. causing caecal distension. Dowling, et al, (1967) found no macroscopic or histological evidence of rat caecal wall hypertrophy. Only a thickening of the large bowel muscle coat when the diets contained 66% and 80% by weight of powdered Kaolin. Younoszai et al, (1978) found that of a variety of diets, those containing fibre increased small intestinal and colonic- but not stomach- or caecal growth. Moinuddin and Lee, (1959), attributed increased caecal - and caecal content-weights, to the alteration in residues during transit and the ability of bacteria in the caecum to hydrolyse starch, with a subsequent stretching of the walls to accommodate increased bulk. Starches which are resistant to hydrolysis in the small intestine may be degraded by the caecal flora to compounds of lower molecular weight with higher osmotic activity, resulting in caecal enlargement. Similar studies have shown that starch will increase caecal enlargement (Leegwater et al, 1974; Walker et al, 1978). In the former study, caeca returned to normal sizes within four weeks when the animals reverted to the initial diet. Walker et al, (1978) observed that raw potato starch alone resulted in impaired nutritional performance, respiratory problems and mortality. Where caeal enlargement was noted, the tissues appeared to be histologically normal. The tuber starches increased filled caecal weight (9.55 g/kg body weight). The filled caecal weight with diet PG was 8.15 $\pm$ 0.42 g/kg; Visually the walls of the caecum appeared to be thicker (no histological examination was made), with no apparent detrimental effect on the well being of the rat. Cellulose has been shown to increase wet colonic weight in rats (Oku et al, 1982) with respect to a fibre free diet. Tissue increase was due to hypertrophic hyperplasia at 8 weeks. This was rapidly brought back to control level when the rats were switched back to a control diet. In rats, a 20% wheat bran supplement increased the caecum and distal colonic weights. Mucosal cell hyperplasia, in addition to muscle thickness, was most noticeable in the distal colon. The proximal colon (caecum) showed no significant increase in mucosal cell mass (Jacobs and Schneeman, 1981). These concur with the results of this present study. In a subsequent study (Jacobs and White, 1983) of longer duration (4-9 weeks), a 59% increase of intestinal mucosal weight of the caecum found after 4 weeks, with a decreased exfoliation in the caecum and 45% decrease in cell migration. The lack of effect of wheat bran in this present study may well be due to only 10% wheat bran (w/w) in the diet, compared to 20% of

the previous two studies.

Bran also increases transit time,(Burkitt et al, 1972) and thereby caecal residence and fermentation is decreased. Gum arabic, however, is known to be fully degraded in the caecum (Elsenhans et al, 1981; McLean Ross et al, 1984), thus metabolism will be altered, caecal residence will be increased, resulting with increased C.S.W.W. and C.C. Gum arabic did increase the C.C. by approximately 2 fold compared to no supplement or the addition of bran. The effects of diets PF and PB upon dry C.C. remained constant over time. When gum arabic was added to the diet, dry C.C. weight was significantly greater at 4 weeks than after 8- or 12 weeks. This suggests that adaptation to the diet takes at least 8 weeks, and for bacterial activity to reach a steady state situation after an initial response to the diet culminating with increased metabolic end products, and subsequent increased dry C.C. weight.

No such dietary interactions with time were observed with the AF and EF dietary trials. The difference in increase in weight between diets AF and AG was less than that between diets PF and PG. This was probably due to the weight of dry C.C. with diet PF being 25% less than that with diet AF. This increase of dry C.C. with diet AF is probably due to the dietary constituents being more liable to bacterial degradation than diet PF. The increase of C.C. could thus be a result of increased bacterial mass compared to the diet PF. The weight of C.C. with diet AG was 54% lower than with diet PG. Gum arabic is obviously being degraded in the caecum and increasing the weight of dry C.C. by bacterial mass. Dry C.C. also increased with diet EG and again there was no significant effect of time. By 4 weeks it is apparent that the change in dry C.C. had occurred. The overall mean weight of dry C.C. is equivalent to that observed after 12 weeks on diet PG. The different trend with time exhibited by diet PG may be as a result of extra substrate reaching the caecum, in the form of the accompanying diet PF, whilst with diet EG and diet EF is totally absorbed in the small intestine therefore any increase in dry C.C. weight because of the degradability of the gum is entirely a result of the gum and not of a complex between the EF and gum arabic. Elsenhans et al (1981) reported increased caecal weight with gum arabic, guar and tragacanth. Gum arabic (10%) resulted in a caecal weight of 1.4g after 52 days of feeding, a value comparable with the results of this present study. Colonic weight was more affected by feeding inert rather than degradable gelling agents. (carrageenan, gum karaya, methylcellulose). Therefore the nature of the fibre will have different effects upon anatomical

structure. Bran may not have increased C.S.W.W. or dry C.C., but could have influenced distal colonic growth (Jacobs and Schneeman, 1981) which might account for the increase of faecal DAPA and SCFA's. The increase in dry caecal content weight is most probably due to fermentation and bacterial degradation of the gum arabic in the caecum. The difference between the results of diet PG, diets AG and EG could be because diet PG was more easily degraded than either of the latter two diets. There was no significant difference between the effects of diets PF, AF, and EF. The difference in weight of dry C.C. may then be due to the interactions formed between the basal diet and gum arabic. With diet PG, the two components may be symbiotic; with diet AG, there may be a slight inhibitory reaction, but also the rats ate less on this diet, so less substrate may actually be reaching the caecum. With a completely absorbable diet (EG) only gum arabic is reaching the caecum to be degraded with no associated nutrients for bacterial growth. With both the AF and EF dietary trials, the results suggest an adaptation period of 4 weeks. With the PF dietary trial, a period of at least 8 weeks is required if the effects of a highly degradable substrate such as gum arabic be studied. Four weeks is adequate to observe the changes associated with the unsupplemented and bran supplemented diets.

The increase in stool weight associated with a bran supplement was not reflected by the dry C.C. which suggests that the activities in the rat caecum may not necessarily be directly associated with stool weight (McLean Ross et al, 1984). Despite the differences between the different basal diets, gum arabic increased dry C.C. by approximately 2.5 times compared to an unsupplemented diet. Only the adaptation time to a gum supplemented diet is affected by the basal diet.

The results of H_2, CH_4 and SCFA's confirm that a change of bacterial metabolism did occur with the gum supplemented diets and that the effect of each basal diet did not alter with time. The unsupplemented and bran supplemented diets were inert there being little or no H_2, CH_4 or SCFA's with the diets PF, AF and EF. The view point that carbohydrate gelling agents may increase caecal enlargement by way of altered and increased bacterial activity and mass is supported. In this present study, DAPA was used as an indicator of bacterial mass, despite its inherent problems (Work and Dewey, 1953; Czerkawski, 1974; Ling and Buttery, 1978). The presence of DAPA in the bacterial cell wall, allows it to be used as a marker of bacterial matter (Czerkawski, 1974) and microbial nitrogen (Ling and Buttery, 1978).

Dry C.C. was seen to be increased with diet PF after 4 weeks. Caecal DAPA was not. This implies that the weight of dry C.C.was not entirely due to increased bacterial mass. With all three diets total caecal and faecal DAPA was increased with a gum supplement and, with the exception of diet EG, total faecal DAPA exceeded total caecal DAPA. This suggests:

(i) increased bacterial matter in the caecum in the presence of a degradable polysaccharide.
(ii) an increase of bacteria with DAPA rich cell walls.
(iii) an alteration of bacterial activity. The results of this study do not allow the distinction to be made.

The diets EG, EF and EB gave similar trends with caecal DAPA to the respective diets PG, PF and PB as well as identical absolute values for total caecal DAPA. Similarly total faecal DAPA was increased by the EG diet as by diet PG. However the trends exhibited by diets PG and EG are dissimilar, whilst there is no difference between diets EF, EB, PF and PB. Total faecal DAPA in the EF dietary trial was exceeded by the value for the PF dietary trial althoughthe percentage increase between EF and EG (68%) was the same as that between PF and PG (62%). This decrease in total faecal DAPA would be expected because of the concomitant decrease in stool weight with the EF dietary trial. With the AF dietary trial it can be seen that, within each diet the total caecal DAPA fluctuates with time. This fluctuation with diet, maybe a reflection of decreased, and fluctuating, intakes and the possible utilisation of bacteria by bacteria to maintain vital metabolic processes, causing a decrease in total bacterial mass with respect to the diet PF. The nature of the accompanying diet and/or decreased food intake and energy may have restricted bacterial activity and consequently the magnitude of total bacterial matter, the difference between diets AG and PG being a decrease of 38% with respect to diet PG. There was a significant difference between the diets AF and AB after 4 weeks but not thereafter, suggesting lack of available substrate with diet AB. After 8 weeks the bacterial flora had adapted to give a total increase of bacterial flora. Total faecal DAPA with diet PG exceeded that with diet AG similarly for diets AF and AB. The difference is possibly related to increased retention of bacteria in the caecum, possibly to compensate for the lack of nutritional intake. With both diets EG and PG, total caecal DAPA and faecal DAPA are virtually identical within their respective dietary trials. The bacteria must be multiplying at a rate, such

as to be able to excrete as much as is being produced.

The mixture of complex carbohydrate which enters the human colon encompasses a host of different polysaccharides and lignin. All are degraded to different degrees. Reaction products can also influence digestibility (Salyers et al, 1983). In the colon, is a bacterial population which can digest these substrates, different bacteria for different substrates (Salyers et al, 1977 (a) and (b) 1978 and 1983).

The metabolic activity of the gut microflora is recognised as an important factor in the disposition, and potential for change to toxicity and carcinogeneity of many compounds (Goldman, 1981; Rowland, 1981). In the rat certain hydrocolloid materials have been shown to influence the total bacterial population (Mallett et al, 1984 (b) with resultant varying degrees of change in bacterial, enzymatic activity and resultant effects on the degradation of toxic compounds (Wise et al, 1982). Pectin has been shown to increase the nitrate reductase activity of bacteria (Wise et al, 1982; Mallett et al, 1983 (a); Rowland et al, 1983 (a)). Cellulose, by contrast has been reported to decrease the numbers of bacteria per total caecal contents, whilst increasing the total caecal contents (Mallett et al, 1983 (b)). The depression of microbial activities/gram C.C. and total activity, were related to the cellulose dilution of the C.C. Rowland et al (1983 (b)) demonstrated that the cell wall extracts of carrots and cabbage had a similar dilution on caecal bacteria. The metabolic activity of the remaining bacteria was increased.

In human studies counts of faecal flora have been used as a basis to estimate the whole colonic bacteria (Hill and Drasar, 1975). Aries et al (1969) found differences between the faecal flora of Ugandan and English people, and associated these with diet. However the effect of diet upon adult human faecal flora has been difficult to demonstrate reproducibly. Walters et al (1975) reported no change in faecal flora when either wheat bran or sugar cane bagasse was added to the diet. Similarly, Drasar et al (1976) found no change when wheat bran was added to the diet. The results presented in this thesis, indicate that diet is influential upon bacterial matter. No attempt was made to study bacterial activity, or to quantify species composition. Time was only a significant factor with faecal DAPA. On all diets faecal DAPA decreased with time.

A consideration of total bacterial mass is important. The environment within

the caecum is a culture, probably continuous, and thus it is important to know the total amount of bacteria at any one time within that unit.
Concentration is also important when considering the physiological importance of bacterial activities. With the PF dietary trial gum arabic increased the concentration of caecal DAPA. Wheat bran significantly reduced caecal DAPA with respect to diet PG, and faecal DAPA with respect to diet PF and PB.

There appears to be an inverse relationship between dry C.C. and DAPA concentration. In the stool, wheat bran appeared to have a dilution effect on DAPA: stool weight increased while concentration decreased.

By measuring DAPA content, an indication of the change in bacterial matter is possible. At 4 weeks on diet PG, DAPA does not contribute to C.C. as much as is thought, otherwise the concentration would have been greater. By 12 weeks, on diet PG, C.C. weight has decreased and the concentration of bacteria increased. A possible explanation is:

(i) the concentration of other constituents has decreased.
(ii) the amount of DAPA containing bacteria has increased.

The concentration of faecal DAPA was influenced only by diet. On all three unsupplemented diets, the concentration remained fairly stable with time, in contrast to the caecal DAPA. If the average concentration of caecal DAPA (PG diet) at 4 weeks is 5 μmols/g, it is difficult to find an explanation as to why the concentration in the stool is 10 μmols/g. The concentration in the caecum, (PB diet), exceeded that in the stool. This suggests that either bacteria from the PB diet were being utilised for metabolic purposes, increased stool bulk had a dilution effect on bacteria, or that the bran carried more dead bacteria out to the faeces.

With a fermentable polysaccharide such as gum arabic, 4 weeks is inadequate in which to qualify the adaptive responses to diet. With diets PF and PB 4 weeks would be suitable. Diet PG results in effects which are associated with an inverse relationship between the concentration of caecal DAPA and dry C.C., suggesting that the weight of C.C. at 4 weeks is not attributable to DAPA. This is not apparent with diets PF and PB.There may be a relationship between caecal and faecal DAPA

- gum arabic increases both, bran has no effect. Thus the stool may be used as an indicator of the effects of dietary changes on caecal flora. The increase of stool weight with bran is not attributable to bacteria, confirming the results of Stephen and Cummings (1980(a). Increased stool weight with gum arabic (N.S.) may be due to increased bacterial matter. Certainly, stool weight was greater at 12- than 4 weeks as was the concentration of caecal and faecal DAPA. The increase in total faecal DAPA, maybe attributed to the increase in total stool output as the concentration in the stool was less than that in the caecum. Caecal concentration increased on the gum diet with time, whilst bran decreased the overall concentation of DAPA. With all three diets the concentration was seen to increase with time, unlike the PF dietary trial. Concentration on diet AG was only 10% lower than that with diet PG, and 30% lower with diet AB with respect to diet PB. Dry C.C. with all these unsupplemented diets remained constant with time, and so are not having a dilution effect on the concentration of DAPA and subsequently bacterial matter. With diet PG dry C.C. were greatest after 4 weeks, and yet concentration of DAPA was at its lowest, suggesting dilution of the bacteria. Thereafter the concentration increased whilst the dry C.C. decreased, suggesting self perpetuation by the bacteria. In essense the same is occurring with the AF dietary trial, where the concentration is increasing with time on diets AG and AB and remaining fairly static on the AF diet. The bran must therefore be being fermented to a slight extent to create changes in the concentration. Such changes in concentration could have important physiological effects on the mucosal- and intestinal cell wall. Again with the PF dietary trial, concentrations increased with time being significantly greater after 12 weeks, and similar trends were associated with the PF and AF dietary trials. Obviously the diets and supplements are not having a dilutional effect upon caecal bacteria.

In the stool from rats on the AF dietary trial, the concentration of faecal DAPA was lower than that present in the caecum, and lower than that observed with the PF dietary trial. Thus the increased total DAPA coincided with increased stool weight. With respect to diet AF, bran had the effect of decreasing the concentration of DAPA, thus a dilution effect is seen, similarly with the PF dietary trial. These results suggest that the faecal flora may be a reasonable indicator of events occurring in the caecum. Again, the interaction of D X T was significant. The concentration of faecal DAPA did not significantly change with time on diet AG; the other diets did alter

DAPA with time. This shows the different ways the diets exert their effects over a varying period of time. Because the rats did not like diet AF, then food intake was reduced, and not constant. Consequently there might be fluctuating changes within the metabolic processes which would be altering accordingly with discontinuous, possibly irregular eating habits. However it is apparent that, despite the poor food intake of these rats, this has not been reflected in the results measured.

Whilst total caecal DAPA was identical with the diets PG and EG, the concentrations on these respective diets were not. Whilst both gum supplemented diets, overall, increased the concentration of caecal DAPA, with diet EG the effect was more constant with time. With diet PG, concentration significantly increased with time. This is a relfection of the change in dry C.C. weight, and unlike diet PG there is no inverse relationship between dry C.C. weight and the concentration of caecal DAPA. After 12 weeks feeding on diet PG, concentration was slightly greater than on diet EG.

With time the concentration on diets EG and EF decreased, a complete contrast to diets PG and PF. With diet EG there does seem to be an indirect relationship between dry C.C. and the concentration of DAPA. Whilst after 12 weeks there was an insignificant increase in dry C.C. weight, the concentration had decreased. With diet PG the reverse was true. The concentration was lowest with both diets PB and EB. The results suggested that, unlike diet PG, at 4 weeks DAPA containing bacteria are contributing more to dry C.C. weight with diet EG. This maybe because the bacteria are able to utilise the gum arabic more easily, than when it is fed with diet PF. Diet PF and gum arabic may form a complex that is not so easily degraded as gum arabic on its own. With the diet EF, only the supplement will reach the caecum, as diet EF is completely absorbed before reching the caecum. Hence the dry C.C. weight may increase after 4 weeks but, bacterial activity has not peaked until after 12 weeks of feeding diet PG, when the bacteria have adapted to the presence of gum arabic and are able to fully degrade it. The similar results of caecal DAPA concentration at 12 weeks diet PG and at all times diet EG support this supposition.

Salyers et al (1977(b)) reported that three strains of Bifidobacterium longum fermented gum arabic. Bacteroides spp, which comprise 20% of the faecal

flora did not ferment gum arabic. Polysaccharides such as gum arabic represent a wide range of carbohydrate linkages. The lack of effect on bacterial mass of the bran supplemented diets may be attributed to the inability of the colonic bacteria to breakdown the bran particles. This may be due to lack of penetration by bacterial enzymes or to shortened colonic transit time and caecal residence. Both the unsupplemented and bran supplemented diets must have some influence in the distal colon, as faecal DAPA exceeds caecal DAPA (by 160%), suggesting an effect on bacterial mass and faeces. Strains of Bacteroides are capable of recognising more than one substrate (Salyers et al, 1977(a)); Salyers et al 1983). Enzyme activities are inducible rather than constitutive. The same microbial flora could have very different overall metabolic activities depending on diet. This could explain the difference in the concentrations associated with diets EG and PG. With diet PB, caecal DAPA concentration insignificantly increased with time, but did not increase with time on diet EB. An explanation is that diet PF is of a more complex composition than diet EF, and consequently an unknown quantity of additional nutrients will be reaching the caecum and colon, concomitant with the bran or gum arabic supplement. The nature of diet EF allows it to be completely absorbed in the small intestine and thus no additional nutrients will be reaching the caecum. The only substrates available will be body excretions, and any additional dietary supplement in the case of diet PF. The DAPA containing bacteria are able to act upon those constituents of diet PF reaching the caecum instead of the bran, causing the concentration of DAPA to increase. With time the bacterial population may change, sufficiently in numbers or functionality, to enable degradation of the bran. As such bran is not fermented in the caecum.

The results show that diet EG is giving a more representative account of what is happening with gum arabic, and that 8 weeks is anecessary time period. Diet PG takes 12 weeks for adaptation to occur and gives similar results to diet EG at 8 weeks. And yet figure 3 e (i) (p 81) shows that 2 weeks may not be long enough.

In human studies the faecal flora are used to infer colonic bacteria (Hill and Drasar, 1975). Walters et al (1975) reported no change in faecal flora when bagasse was added to the diet of human volunteers. Winitz et al (1970) reported that chemically defined diets dramatically decreased stool excretion and concentration of types of faecal bacteria. A review of the

literature on the stability of human faecal flora, by Bornside (1978) concludes that faecal flora, in general, is not altered during the feeding of chemically defined diets. The results of both the PF and EF dietary trials suggest that diet does influence bacterial flora. However whether this is due to the different types of bacteria or an increase in one type of bacteria cannot be determined from this study. All that is apparent is that gum arabic causes an increase in DAPA. Certainly in both the PF and EF dietary trials, total faecal DAPA reflected the overall changes in total caecal DAPA and so too did the respective concentrations. Thus in future trials, as an initial indicator of bacterial alteration the stool may be used as an initial estimation of the bacterial events occurring in the caecum; changes in stool weight do not reflect changes in dry C.C. weight.

To summarise, irrespective of basal diet, wheat bran will increase stool weight and total faecal DAPA but not the concentration of faecal DAPA. Wheat bran has no effect on the C.S.W.W. or the weight of dry C.C. Gum arabic however, does not have any significance on dry stool weight, but increases the dry C.C. weight, C.S.W.W, and caecal DAPA. This increase in DAPA with gum arabic is related to the nature of the basal diet fed. With the more complex diets PF and AF, extra nutrients are available to the caecum, which are energy sources for bacteria, aiding the subsequent fermentation of gum arabic. With the EF diet, nutrients are absorbed in the small intestine and consequently only gum arabic reaches the caecum. The relative lack of substrate reduces the ability of the bacteria to ferment the gum arabic and as a consequence the increase in dry C.C. and caecal DAPA is less than that observed with the gum arabic supplemented diets PF and AF. Time cannot be disassociated from the results, the adaptation period required being a function of diet and supplement.

SCFA's are a major product of microbial fermentation of carbohydrates and are important to the nutrition of some mammals and to the normal secretory and absorptive functions of the large intestine of most mammals. Stevens (1978) showed the mean concentrations of SCFA's in the large intestine of dogs, pigs and ponies fed various diets equivalent to these proportions found in the ruminant forestomach, and that a greater proportion of plant fibre had no effect. Remesy and Demigne (1976) reported that SCFA's are absorbed in the rat, contributing 4-7% of the total energy requirement (Yang et al, 1970). The major SCFA's are acetate, propionate and butyrate with variable

quantities of the isomeric acids, and valerate. In the ruminant the proportions of the three major SCFA's have been shown to be influenced by changing from a diet high in roughage (hay) to a diet high in concentrates. Diets containing a high proportion of roughage favour bacteria which produce acetate as the major SCFA (Storry and Sutton, 1969). Diets high in concentrates favour the production of propionate, at the expense of acetate. Both situations are associated with a change in bacterial flora, from predominantly cellulolytic to amylolytic. (Topps et al, 1968; Kaufmann, 1976; Thomas and Rook, 1977). The nature of the diet was also influental upon changes in the make up of SCFA's (Orskov et al, 1974). Such clearchanges are not reported in the human. Cummings et al (1979 (b)) reported no significant changes in SCFA concentration when four subjects changed from low protein to a high protein diet, or when 30 g of dietary fibre were added to a high protein diet. Rubenstein et al (1969) reported that fasting can reduce the production and concentration of SCFA's.

In the rat, the ratio of the molar proportion of acetate: propionate: butyrate is reported to be 610:250:140. (Remesy and Demigne, 1976); in man, 600:240:160. (Cummings et al, 1981). Buckley and Williamson (1977) reported considerable concentrations of acetate in the portal blood, as a result of bacterial metabolism within the large bowel providing acetate as a source of nutrition.

In this study the gum supplemented diets increased total caecal SCFA's. No such increase was seen with the unsupplemented and bran supplemented diets. Only with the AF dietary trial was there no difference in effect between diets AG and AB. If diet AF was unpalateable to the rats and little was eaten, then very little nutrition would be obtained. Subsequently the addition of bran could become the predominate substrate for bacterial activity. Diet PF and gum arabic produce quantities of SCFA's, the latter giving rise to more than the former. Together the effect is maximised. With diet AF and gum arabic, when fed together, whilst SCFA's are significantly increased, the result is less than with diet PG, suggesting that there may be some other effect of diet PF and gum arabic. The most reasonable explanation is that the rats ate less of diet AG than diets PF or AB, and that production is related to overall intake. Alternatively the SCFA's produced by the caecal fermentation could be utilised and absorbed more quickly from diet AG than diet PG, because of the needs associated with live-weight loss. The effect

of the interaction, in the AF dietary between diet and time was significant. This illustrates that a diet fed for a specific time period can give the same result as another diet fed for a shorter or longer time period. By 12 weeks there was no significant difference between diet AB fed for 12 weeks or diet AG fed for 4 weeks. There was no significant interaction observed during the PF and EF dietary trials, but it is obvious from figures 3 f(i) and 5 f(i) (pages 84 and 158 respectively) that the changes occurring with time are different for all diets. With diet EF total caecal SCFA's decreased with time but not in a linear fashion as with diet PF. With diet EG, values are seen to increase with time, in contrast to the decrease in values with time with diet PG. This may be related to the increase in dry C.C. and the variation in bacterial activity associated with the diet PG. What is apparent is that when gum arabic is added to a diet, the total caecal SCFA's are increased two fold with a simple low residue diet, and by three fold with a more complex low residue diet eg diet PF. The discrepancies are related to variation in production, bacterial utilization and caecal absorption. This suggests that other dietary constituents and cojeners of a diet effect the manner in which gum arabic is metabolised (McLean Ross et al, 1984). The maximal and significantly elevated levels of SCFA's in the C.C. of rats fed gum arabic, and the concomitant reductions in stool weight indicate that the caecum represents a major site for gum arabic fermentation. The increased total SCFA's of the C.C. are a reflection of the increased caecal content weight. Whilst the latter altered with time, total SCFA's remained constant.

The absence of a significant effect of the three-way interaction upon caecal SCFA's (Chapter 6, pp 210-211) confirms how similar the effect of supplementation is irrespective of basal diet. Only the absolute values of the results change: there is no difference between the percentage change of SCFA's with a supplemented diet to an unsupplemented diet. There is also no difference between any of the unsupplemented and bran supplemented diets.

Gum arabic increased total faecal SCFA's with the exception of diet AG. This is probably a reflection of the increase in stool weight. Indeed total SCFA's with diet AB were 5 fold greater than with diet AF. Total faecal SCFA's were in conflict with those from the PF dietary trial, where diet PG increased total faecal SCFA's and all values were greater than observed with the dietary AF trial, In light of the increased dry C.C., DAPA and SCFA's

and C.S.W.W. it is apparent that the caecum is the site of great fermentive activity. However concomitant with this is a decrease in the amount of food eaten only 19 g dry weight/day over 12 weeks by those rats given diet AG and 21 g dry weight/day by those rats given diet AF for 12 weeks. Thus the amount of gum arabic eaten is less than that eaten by rats given diet PG (25 g/day for 12 weeks). This, firstly, will reduce the total amount of SCFA's being produced and secondly increase the amount retained and absorbed for metabolic functions and energy (Yang et al 1970; Remesy and Demigne 1976). Consequently the amount appearing in the stool will be reduced, in this case substantially. However it is difficult to explain why total faecal SCFA's increased with diet AB, except by virtue of the increase in stool weight when there was no increase in caecal SCFA's with bran. Between the caecum and stool there may have been increased bacterial activity. The significance of the interaction of D X T (Chapter 4, p.122), indirectly shows the instability of the internal environment. Lack of a constant nutritional source suggests a constantly adapting environment.

Within the PF and EF dietary trials entirely different patterns were observed. Despite the trend of faecal SCFA's increasing with time on diet EF, there was no significant interaction. With both the PF and EF dietary trials the gum supplemented diets gave the greatest total faecal SCFA's, but only with the EF dietary trial was there an absence of significant differences between diets EG and EB. No doubt the difference between the absolute values of total faecal SCFA's observed during the PF and EF dietary trials are due to differences in absolute stool weights. Neither are representative of the total caecal SCFA's.

Caecal concentrations of SCFA's increased with all of the gum arabic supplemented diets (PG, AG and EG), which reflects the increase of caecal DAPA concentration. Within the PF dietary trial, there was a significant increase of SCFA's over time, particularly with diet PG. Within the EF dietary trial there was an insignificant decrease of SCFA's with time. By 12 weeks the concentration of SCFA's with diet PG was only 15% greater than that with diet EG after 12 weeks of feeding. With diet EB, it appears that bran had a dilutional effect, as the concentrations are seen to decrease with time, in contrast to the effect with diet PB where no change in the concentration was observed. After 12 weeks on each diet the concentations are similar (400 μmols/g). Why there is such an increase in SCFA's after only 4 weeks on diet EB, is difficult to explain. In the absence of a fermentable substrate,

the likely substrates are then of endogenous origin, such as mucopolysaccharides and glycoprotein hexosamines from small intestinal mucus and desquamated mucosal cells (Vercellotti et al, 1978). After 4 weeks, adaptation to the diet has proceeded and the use of endogenous secretions declined. With diet PB there may be some change in bacterial activity possibly associated with the remnants of undigested diet PF and possibly associated with time. There may be a slow change in bacterial flora with bran feeding, due to the resistance to degradation. Diet PF may provide initial substrate and energy for the commercial bacteria, which encourages the presence of those bacteria which are able to degrade bran.

As with the PF and EF dietary trials, diet AG may have increased the concentration of caecal SCFA's, but so too did diet AB. Whilst the rats fed diet AB ate more than the rats fed diet AG (p294), these former rats may not have been producing more SCFA's, but that the rats given diet AG may be eating less, therefore having less substrate to ferment, resulting in a greater demand for what is produced. This may be a plausible explanation: the concentration of caecal SCFA's from rats given diet AB was 10% less than that with diet PB, where estimated dry food intakes were the same; caecal SCFA's from rats given diet AG was 17% less than that with diet PG, and estimated dry food intake was 23% less with diet AG. The difference in food intakes of diets AB and AG was 19%, with respect to diet AG, and 26% between diets PB and PG with respect to PG. Gum arabic and bran appear to have the same effect, irrespective of diet. Absolute values may, therefore, be influenced by diet and food intake by rats.

The faecal concentration of SCFA's was significantly increased with diets EG and PG, but not diet AG. Similarly there was little difference between the overall concentration of faecal SCFA's of diets EF, EB, PF and PB. From chapter 3, 4 and 5 (pp 88, 126 and 162) it is quite apparent that there were different trends associated with time for the three dietary trials, and that each diet within each trial behaved differently with time. Figure 3g/(ii) (page 88), shows that the concentration on diet PG at 4 weeks was double that after 8 weeks, whilst on diet EG, the concentrations remained fairly constant with time. This may be attributed to the nature of the basal diet, that some of diet PF is escaping, upper small intestinal absorption and passing into the colon where it is being fermented by bacteria, and thereby increasing the concentration of SCFA's. The results of the EF dietary trial better match those findings

of Spiller et al (1980), the effects of where cellulose and pectin and a natural low residue diet on SCFA's and stool weight in the adult, were studied. Faecal weight increased with cellulose and decreased with pectin over time (2,4 and 5 weeks). The SCFA's were shown to increase with cellulose. Differences in total SCFA's paralleled the changes in stool weight, thus concentrations did not change. Other reports of studies in the rat have shown that pectin and cellulose ingestion increase the concentration of SCFA (Yang et al, 1969; Hove and King, 1969). Yang et al (1969), Remesy and Demigne (1976) and McLean Ross et al (1984) showed there to be 59% decrease in the faecal concentrations of SCFA's after caecectomy of rats with detection of gum arabic in the stool, which is not found in the faeces of intact rats fed gum arabic. In the present PF dietary trial total faecal SCFA's did parallel stool weight, but not exactly. Concentrations did not remain the same with time (figure 3g/p 8 8(ii)). With pectin, in man, (Spiller et al, 1980), total SCFA's did not parallel stool weight. With diet AB, faecal concentrations of SCFA's were increased as was stool weight. Thus the increase in stool weight was not having a dilutional effect upon SCFA's. These changes may have significant physiological and clinical effects on the microecology of the colonic lumen, colonic pH mucosas of rectum, sigmoid colon and colonic function (Spiller et al, 1980). The fact that pectin increased the concentration of SCFA's, but not faecal bulk is attributed to the degradability of pectin in the caecum. In their study Kay and Truswell (1977(a)) recorded that pectin did not influence the concentration of faecal SCFA's.

The faecal concentration of SCFA's of the dietary trials was less than that observed by Remesy and Demigne (1976), in their study of the site of absorption of SCFA's although the results obtained from the rats of these present studies, fed for 12 weeks, were similar. With the PF dietary trial, the caecal concentration of SCFA's did not show a direct relationship with dry C.C. This was particularly noticeable with diet PG, and was similarly noted with caecal DAPA, where the increase in the caecal concentration of DAPA, like SCFA's, increased up until 12 weeks combining the hypothesis of increased bacterial matter, and supposed activity on diet PG, but not with diets PF and PB where substrate is limiting.

The caecal production of SCFA's and faecal excretion of SCFA's, and the concomitant concentrations appears to be a function of:

(i) absorption within the caecum, colon and rectum;
(ii) bacterial utilization for energy and subsequent growth, which will affect

bacterial activity;
(iii) time, where different trends are associated with each diet, as adaptation to the diet proceeds;
(iv) the nature of the low fibre basal diet, whether it is an elemental diet which is completely absorbed in the upper intestinal tract, or whether it is of a more complex composition here some residue may be carried to the caecum as in the case of the plant- and animal origin diets.
(v) the types of fibre supplement given, in this case bran or gum arabic.

The increases in total caecal SCFA's are to be expected as evidence of fermentation in association with increased caecal weight and bacterial mass. This did not occur with diets PF and PB, an indication perhaps of a lack of available, suitable substrate. Caecal SCFA's did not increase after 4 weeks; faecal SCFA's had stabilised by 8 weeks. As the results of DAPA analysis suggest, stabilization in the caecum occurs some time earlier than faecal output, in this case by more than 4 weeks but certainly by 8 weeks.

The major SCFA's produced from the breakdown of carbohydrate are acetate, propionate and butyrate, and these are rapidly absorbed in man (McNeil et al, 1978). Spiller et al (1980) reported acetate to be the major / anion in faecal matter, when three diets were given in human studies.

In this present study, acetate, propionate and butyrate were detected in caecal and faecal material. Acetate was found to be the major anion present in both caecal and faecal material, with the exception of faecal material from dietary trial AF. The proportion of acetate in the caecal contents of diets AG and PG were similar, as were the proportions of diets AB and AF. The proportion of caecal acetate falls within the values quoted by other authors. (Remesy and Demigne, 1976; Spiller et al, 1980; McKay and Eastwood, 1983). The values are however, in excess of those observed by McLean Ross et al (1984), who showed that the addition of gum arabic to two different diets increased the production of SCFA's. Only with diet EG, was gum arabic seen to decrease the molar proportion of caecal acetate by 10% with respect to diet EF, and from figure 6q(ii) (p222) it is quite apparent that this proportion was significantly lower than that observed with the other two diets. These results indicate that the diet to which gum arabic is added is important. Time was observed to be significant with the AF dietary trial, the proportion being largest after 8 weeks with no significant difference between 4- and 12 weeks. Obviously the change in diet is having an effect upon the metabolic activities

of the caecal flora. There is a subtle shift in bacterial activity stimulated by the presence of a degradable substrate. As a consequence, there ought to be a shift in the proportions of the other acids produced.

Diet did not influence caecal propionate during the PF dietary trial, but concomitant with the decrease of caecal acetate with the dietary trial EF, caecal propionate was seen to increase with gum arabic. Despite these differences, the overall means for caecal acetate compared favourably with those of the PF dietary trial. The two unsupplemented diets gave identical overall means, whilst the difference in caecal acetate was 15% greater with diet PG with respect to diet EG. This suggests that residue of diet PF was reaching the caecum and contributing to the increase in caecal acetate. With the AF dietary trial, diet was also important. Diet AB reduced the proportion of caecal acetate, whilst there was no significant differences between diets AG and AF. What you feed the dietary fibre supplement with is important. As can be seen from figure 6q(ii), (p 222), there was no significant difference between any of the unsupplemented and bran supplemented diets, with the exception of diet AB. The absolute values for all the diets confirm well with those previously reported (Remesy and Demigne, 1976; McKay and Eastwood, 1983; McLean Ross et al, 1984). Caecal propionate was 22% greater with diet EG, with respect to diet PG, and 10% lower with diets EF and EB, with respect to diets PF and PB. Obviously there has been a subtle change in bacterial flora, and activity, with a shift from acetate to propionate production. This is noticeable in the EF dietary trial where only the fibre supplement is reaching the caecum. With the PF dietary trial, there was no significant difference between the proportion of caecal propionate observed with diets PG and PF. This suggests that a 'complex' is being formed between the diet PF and gum arabic, which is somehow having a diminishing effect on propionate production from gum arabic. This 'complex' is removed when it is fed with a completely residue free diet. The results fit well with those observed by McLean Ross et al (1984), although the proportion of propionate with diet EG of the present study was 22% greater. Why diet AB should cause a decrease in propionate whilst bran has no such effect with diet PF is difficult to explain.

When all the diets are considered together it can be seen that bran reduced the proportion of caecal propionate. Propionate is produced in less quantities than acetate and dietary changes will alter the proportion produced due to changes of bacterial flora and activity and absorption. Because propionate is not in abundance there may be greater demands put upon it. For example,

conversion to
/liver glycogen and gluconeogenesis as reported by Schambye and Phillipson (1949), in their studies of volatile fatty acids in the portal blood of sheep or mucosal, bacterial utilization. From chapter 6 it is quite apparent that when the diets are considered on mass, basal diet alone is not influential upon caecal propionate, but in conjunction with a supplement the level of propionate will be changed. The difference between diets EF and EG was greater than that between diets PF and PG and was probably attributable to the fermentable residues of diet PF reach the caecum. Propionate was detected in the stool of all animals with the exception of the AF diet group. As can be seen from figure 6s(ii)(p226) most propionate was found in the three EF diets, with least in the other two dietary trials, a reflection of the nature of the diets and reliance upon the production of propionate. The quantities observed infer colonic absorption (McNeil et al, 1978; Cummings et al, 1979(b) The results reflect those found by other workers. McKay and Eastwood (1983), found that when wheat bran was fed to rats, a decrease in the proportion of propionate occurred. Similarly Farrell and Johnson (1972), reported that the proportion of propionate decreased with a concomitant increase in acetate.

All the bran supplemented diets significantly increased the proportion of caecal butyrate. Figure 6t(i), (p229), shows there to be no significant difference between any of the nine dietary groups with the exception of diets PB and AB where caecal values were significantly increased by the addition of bran. Whilst the different dietary trials gave different absolute values, the percentage differences between diets changed very little. The difference between diets AG and AB, and PG and PB, with respect to the gum supplemented diets was 2 fold. The difference between diets AF and AB and PF and PB with respect to the unsupplemented diets was 1.7 fold. In both of these trials, the gum arabic diets decreased caecal butyrate by 23% and 30% with respect to the AF and PF diets, although the decreases were not significant. The overall effect of time was significant, with the proportion of butyrate increased with time. However as detailed in Chapters 3 and 5 (pp 89 - 164)/ the trends with time were not the same for each diet. The proportion of caecal butyrate with diets EF and EB was half that of diets PF and PB, and lower than those values observed by McKay and Eastwood (1983). The molar proportion with diet EG was identical to that of diet PG but lower than that reported by McLean Ross et al 1984.

The AF dietary trial did not result in any faecal butyrate. Butyrate was

detected in the stool of rats in the EF and PF dietary trials in minor quantities. The AF and EF dietary trials did result in the appearance of isobutyrate isovalerate and valerate in the caecal and faecal contents of rats. These were not detected in the PF dietary trial. Thomsen et al (1982) reported the disappearance of isomeric forms and valerate with the addition of pectin to an elemental diet. In other studies the presence of these minor SCFA has not been reported (Yang et al, 1969; Remesy and Demigne, 1976 Hove and King, 1979) whilst other have reported their appearance (McKay and Eastwood 1983; Høverstad and Bjørneklett, 1985). Spiller et al (1980) reported small amounts of isovaleric and valeric acids (5% of each) and occasional traces of isobutyric acid. From appendix 5 it can be seen that these minor acids form only a small percentage of the total SCFA's (0.1% to 10%). Certain bacteria are known to be dependent upon such isomeric forms, eg Ruminococcus bromii (Bryant, 1974) and their appearance implies their altered production or consumption or modification of the bacterial flora and activity. The lowered caecal propionate and increased butyrate proportions must be linked to altered production rates in the face of an altered caecal environment. Further the alterations in SCFA proportions might be due to differential absorption. Whilst Dawson et al (1964) concluded that SCFA chain length was a determinant on absorption, McNeil et al (1978), however, suggested that absorption rates were similar for the three major SCFA's with similar rates in the proximal, mid and distal colon. (McNeil and Cummings, 1979). Roediger (1980) by means of in-vitro techniques showed that butyrate was a preferred energy source for isolated colonocytes; which would explain the increase in butyric acid.

Cummings et al (1979 (b)), found that neither the total faecal SCFA's concentration nor relative proportions of individual SCFA were altered with diet, which suggest colonic absorption. Spiller et al (1980) found little difference between the molar proportions of faecal propionic- and butyric acid, each one present at approximately 20%. There was, however a slight change in the ratio of acetic to propionic acid as total faecal SCFA's increased in the cellulose fed group.

Appendix 5 shows the average ratios of the individual SCFA's in the three dietary trials. It can be seen that the average caecal ratio of acetate: propionate changed very little with diet with the exception of diet EG and fluctuated greatly in faecal material. The caecal acetate: butyrate ratio was lowest in all the bran supplemented diets, being lowest on diet PB, and fluctuated in faecal material. The propionate : butyrate ratio was changed

very little with diet, the lowest being with diets PB and AB (0.8). and the highest with diet AG (2.3). The propionate : butyrate ratios match those of McLean Ross et al (1984) who observed decreases in the acetate : propionate ratio when gum arabic was fed with an elemental diet. When an oxoid Breeders diet (O.B.D.) was fed with 10% gum arabic the respective ratios were increased.

This was similar to the change in ratio observed with diets PF and PG. Yang et al, (1969) observed that rats fed diets of grain and cellulose gave increased SCFA's, the increase principally accounted for by butyrate.

These results confirm that dietary interactions have profound effects upon the metabolic fate of dietary polysaccharides in the rat and as in the ruminant are major factors in the alteration of SCFA metabolism (Stevens, 1978). Patterns of fermentation in the large bowel are not solely influenced by the quantity and nature of the polysaccharide consumed but the basal diet and time period as has been shown by the significance of the interactions.

Why there is an overall increase in faecal acetate with respect to caecal acetate, is hard to explain. McNeil et al (1978), suggested that absorption rates of the three major SCFA's are similar with absorption occurring at similar rates in the proximal-, mid-, and distal colon (McNeil and Cummings 1979). Differential absorption may be the full explanation (Yang et al, 1969), along with bacterial utilisation. Acetate which is being eliminated along the colon may be further acted upon in the distal colon. The high levels of these anions in the stool infer that they contribute to stool weight.

Diet can thus be seen to influence SCFA's and their composition. McKay and Eastwood (1983) reported a similar conclusion. Propionate was reduced when bran was fed to rats. Rats fed a meat diet had less acetate and butyrate. Similar dietary influences have been reported in the pig (Cranwell, 1968), rodent (McBee, 1970), dog, pig and pony (Stevens, 1978). Farrell and Johnson (1972) observed that propionate decreased with a concomitant increase in acetate when 26% cellulose was given to rats. Values compare favourably with those of the present studies.

In man, pectin, cabbage and cellulose have been reported as increasing the concentrations of faecal SCFA's (Spiller et al, 1980; Ehle, et al, 1982), but in general the concentration of SCFA's appear to be little affected by the

dietary fibre ingested.

The results from these present studies show that dietary interactions have profound effects on the total, concentrations and individual molar proportions of SCFA, and are dictated by the metabolic capabilities of the intestinal bacteria. As in the ruminant, diet is a major factor in the alteration of the production of SCFA's (Stevens, 1978). The ratios of the individual acids change very little with diet, showing that whilst absolute values maybe influenced by basal diet orsupplement, the changes between two diets or two acids is, ultimately, not altered.

Further effects of diet upon caecal metabolism are seen in the measurement of H_2 and CH_4. All H_2 produced in man is assumed to be of bacterial origin in the colon (Levitt, 1969) and indicates caecal carbohydrate metabolism (Calloway, 1966; Bond and Levitt, 1978). Wheat bran is thought to be a relatively poor substrate for gas production by colonic bacteria (Nyman and Asp, 1982). Dietary supplementation with a fermentable polysaccharide or carbohydrate has been shown to both increase the amount of H_2 production (Calloway, 1966; McKay and Eastwood, 1983) and to have no effect on the level of H_2 production (McLean Ross et al, 1984) in rats.

With the PF dietary trial, diet had no effect upon exhaled H_2, in contrast to findings in man. H_2 was detected in all rats, and for any given diet did significantly decrease with time, with minimal levels detected after 12 weeks of any dietary regime. The EF dietary trial produced H_2 levels that were lower than those of the PF dietary trial, particularly the unsupplemented diet, EF. Unsupplemented, it appears that diet PF is reaching the caecum and providing a substrate for H_2 production. Diet EF, is totally absorbed in the upper intestinal tract and therefore is not providing extra substrate for bacterial utilisation. Both diets PG and EG did produce more H_2 than the respective unsupplemented and bran supplemented diets, with the noticeable abolition of H_2 after 12 weeks of feeding of diets EG and PG. The results, although greater than those reported by McLean Ross et al, (1984) are coincident with his findings, that after 14 days on the unsupplemented elemental diet, no H_2 was produced, but when supplemented with gum arabic for 28 days, H_2 was detected.

Concomitant with the alterations in H_2 production, CH_4 was abolished with both diets PB and EB. In contrast to this CH_4 excretion was increased with

diet PG, although this was not significantly different from diet PF. With diet EG, after 4 weeks of feeding, CH_4 excretion was similar to that of diet PG. But by 8 weeks, CH_4 production with diet EG had decreased by 50% with respect to diet PG and by 12 weeks CH_4 had been abolished with diet EG. The abolition of CH_4 with diets PB and EB is similar to the findings of McKay and Eastwood (1983). The results of the EF dietary trial are similar to those of McLean Ross et al (1984).

McLean Ross et al (1984) found the level of H_2 to increase when gum arabic was added to the elemental diet. In this present study, H_2 rose with the gum supplemented elemental diet, to a level of CH_4 on the same EG diet. In the PF dietary trial more CH_4 than H_2, in general, was produced on diet PG suggesting that the consumption of gum arabic, concomitant with any residue of diet PF, disposes itself to increased CH_4 production. Diets EF and EB abolished CH_4 production, which with the reduced absolute levels of SCFA's reflects the reduction of available substrate, indicating that the little CH_4 produced with diets PF and PB was mainly due to residue diet PF.

The distinct lack of significant effect of diet upon H_2 production in the PF dietary trial, and the decrease with time, suggests that diet PF is sufficient substrate for fermentation, with diets PF and PB giving comparable levels to those exhibited by diet PG and that diet PF and gum arabic may form a complex to prevent excessive increased bacterial fermentation to H_2. With the EF dietary trial, gum arabic alone is entering the caecum and being fermented, the lack of effect with the EF and EB diets indicating that the low residue nature of the diets provides less substrate for bacterial activity. With CH_4 production, the abolition of CH_4 by diet PB suggests a lack of bacteria capable of fermenting the bran, and consequently a change in bacteria and metabolic activity. Bran could be acting as an inhibitor to methanogens. With diet PF, some substrate is reaching the caecum. The absence of CH_4 from diets EF and EB again confirms the lack of substrate provided by this low-residue diet. When gum arabic is added to a diet, its metabolic fate is in part dictated by other dietary constituents.

In man there may be an association between diet and CH_4, but not equally affecting H_2 and CH_4 production (Tadesse et al, 1980). The continuous and discontinuous feeding habits of rats and humans may make for quite different

relationships between CH_4 and H_2 production in the two species.

The duration of feeding did not significantly affect CH_4 production. During the PF dietary trial, here was an insignificant decrease of 60% of CH_4 production after 8 weeks of feeding. The excretion of H_2 was affected by time. This suggests that bacterial metabolic adaptation to dietary change occurred between 4- and 8 weeks. This agrees with the results of dry C.C. Diet PB appeared to be associated with a diminished total faecal and total caecal DAPA and the abolition of CH_4 excretion. This was not true for diet PG.

The lack of dietary effect upon H_2 coincides with the results of Tadesse and Eastwood (1978) where cellulose, lignin and pectin produced no effect on breath H_2 excretion in an acute feeding experiment. With carrots, H_2 excretion was not apparent until 10 days on the diet (Robertson et al, 1979 (b)) suggesting a period of bacterial adjustment to the diet. If a period of 4 weeks is assumed for bacterial adjustment, then this may have occurred unobserved in this present study. In-vitro studies (Bond and Levitt, 1978) have shown that fermentation of fibre by faecal suspensions taken from subjects accustomed to high bran intake showed no greater H_2 production than faeces from subjects of low fibre intake. No dietary changes were observed when 30 g bran was ingested, compared to 10 g lactulose.

CH_4 production in humans has been shown to be variable, with one-third being suggested as CH_4 producers (Calloway, 1968; Levitt and Bond, 1970). Whilst CH_4 production is an established feature of rumination (Czerawski, et al, 1966; Czerkawski and Breckenridge, 1969), its formation is more complex in the human, and cannot be related to one single factor such as diet or bacteria. All healthy human subjects may produce CH_4 but only when production reaches a threshhold does it appear in the breath (McKay et al, 1985). By lowering the oxygen tension, methanogenic bacteria are encouraged to grow and consequently CH_4 excretion is increased (McKay et al, 1983 and 1985). This may in part explain why no significant increase of CH_4 excretion was associated with gum arabic. Equally, Bjørneklett and Jenssen (1982) observed the pulmonary excretion of H_2 and CH_4 to be universely related, and concluded that CH_4 was produced by H_2 utilising flora. If, as in the PF dietary trial, diet has no influence on H_2, then there would be no expected change in CH_4 excretion. However there was, which suggests that

the explanation of Bjørneklett and Jenssen (1982) is not entirely applicable. H_2, CH_4 and SCFA's may be unrelated in the rat caecum, and thus reflect different aspects of bacterial metabolism.

Dietary fibre can interact with bile acids and intestinal flora, and hence alter the relative sizes of various bile salt pools, (Story and Kritchevsky, 1978) and may result in increased faecal excretion of bile salts. The extent to which fibre binds bile acids is characteristics for each fibre and substrate (Kritchevsky, 1978). More than 95% of the total bile acid pool in the mammalian body is found in the intestinal tract (Eastwood and Boyd, 1967). Distribution along the tract will depend upon the rate of absorption, transit time and diet. A high fibre diet may enhance excretion of bile acids, whilst a low fibre diet will retard faecal evacuation. If bile acids are retained in the colon for a longer period of time, then the opportunity for bacterial action and subsequent degradation increases. Studies of the faecal output of bile acids and plasma cholesterol concentation in relation to the amounts of dietary fibre eaten have given inconsistent and contradictory results (Royal College of Physicians, 1980; Eastwood and Passmore, 1983). The supplementation of a diet with large amounts of pectin (15g/day) was shown to increase faecal output of bile acids and reduce plasma cholesterol, whilst supplements of wheat bran (23 g/day) did not affect these paramaters (Kay and Truswell, 1977 (a), (b)). Wheat bran has been reported as a relatively poor binder of bile acids (Eastwood and Hamilton, 1968; Story and Kritchevsky, 1976).

In the PF, AF and EF dietary trials the presence of gum arabic increased the overall total caecal bile acids and was greater in rats fed diet EG than diet PG. Whilst bran increased total faecal bile acids in the diet groups AB and PB, the former diet increased total faecal bile acids by 67% with respect to diet AB. Similarly total faecal bile acids with diets AG and AF were three times that with diets PG and PF. However the effects of bran on faecal bile acids was most noticeable with diet PB where the difference between diets PB and PG was 94% (with respect to PG) and between diets AB and AG 43% (with respect to AG). With time, total caecal bile acids remained fairly constant whilst totals decreased and increased with diets AF and AG respectively.

The presence of a large bile acid mass in the caecum supports the concept of the caecum as an expanded fermenter. The increased bile acid mass

with diet EG suggests that this diet is associated with prolonged residence time in the caecum. The unsupplemented diets and bran supplemented diets had no effect on total caecal bile acids and as such altered very little with time. Both diets PG and EG exhibited decreases in total bile acids after 8 weeks. Unlike the PF and AF dietary trials, total faecal bile acids were not greatest on diet EB. At 8 weeks there was no difference between the three diets. After 12 weeks, total faecal bile acids were greatest on diet EF, and lowest on the unsupplemented diets. The results of the faecal bile acids, with diet EG agree with those of Eastwood et al (1973) and McLean Baird et al (1977) who found that bran did not alter total faecal bile acid excretion. Overall concentration was greatest with the unsupplemented PF and AF diets. Concentration with diet AF was 2 times greater than diet PF. The addition of bran reduced the concentration of total caecal bile acids but the difference between diets PBand PG was not as large as that between diets AB and AG. In the rats fed diet AB the dry C.C. weights did not alter drastically with time, neither did total bile acids. Consequently the concentration in the caecal contents remained virtually unchanged with time. Only with diet EB did concentrations decrease linearly with time. With diet AG concentration decreased over time as did total bile acids; dry C.C. weight did not alter statistically with time. Either gum arabic is having an inhibitory effect on the production of bile acids or they are being retained within the enterohepatic circulation for further metabolic purposes. With diet PG, there appears to be a relationship between dry C.C. and the caecal concentration of bile acids. With diet PB, the dry C.C. remain constant over time but not the concentration of bile acids. With diet PG, as dry C.C. decrease with time, concentration peaks after 8 weeks. With diet AB a direct relationship is evident between dry C.C. and the concentration of bile acids.

In the stool, it is apparent that diet PB is not having a dilution effect on the concentration of bile acids. As stool weight increases, the concentration also increases, for each time interval. With diet AF, concentration was greatest with diet AF, with no difference between diets AG and AB. Stool weight with diet AB was 37% greater than with diet AG, and yet the concentration of bile acids was unaltered. With diet EB the concentration of faecal bile acids was decreased. In both the AF and EF dietary trials, it is apparent that bran is having a dilution effect. These conflicting results indicate that

the diet that is fed with bran is important and will affect its properties with regard to the binding, production and excretion of bile acids. Walters et al (1975) reported that the concentrations of faecal bile acids were lower in humans given wheat bran, compared to those given a bagasse diet, with no effect on the resultant daily output. Eastwood et al (1973) showed bran to increase stool output and to have a concomitant diluting effect on total bile acids, i.e. the concentration decreased. There was an increase in faecal bile acid output, after the bran was stopped. Thiswas thought to be due to the nature of the experimental design and represented a carry over effect.

In the AF dietary trial daily faecal output was increased with diet AB, decreased by diet AF and increased with time on diet AG. With dietary trial PF, diet PB increased total faecal bile acids but not caecal bile acids. The unchanged faecal excretion from rats given diet PG, suggests extensive metabolism of the fibre source, with retention of bile acids within the enterohepatic circulation. With bran the conflict of results is hard to explain. If the caecal concentration is reduced, as with diet EB, then it is logical to expect that totalfaecal output and concentration will also decrease. The results of the AF dietary trial indicate that the different diets behave differently over the same time span. The bile acids produced from a fermentable fibre source maybe re-entering the enterohepatic circulation for further use before being excreted. Certainly faecal contents do not reflect the occurrences within the caecum.

The chemical nature of the bran and bile acids, suggest that hydrophobic bonding may be responsible. A dry grain will swell in water with the water penetrating the ligno-cellulosic material adding volume. This may partially explain why there was increased faecal excretion of bile acids with the bran diet. Increased transit time and decreased retention time must also play a part, explaining why faecal excretion exceeds caecal content. Eastwood and Boyd, (1967) reported that bran increased total bile acids in the small intestine, although Brydon et al (1980) observed that total intestinal pool was unaltered by diet. This was attributed to a difference in methodology. If bran did increase the small intestinal pool of bile acids, then this may explain why there was increased faecal excretion of bile acids with diet PF. The bran by virtue of its absorption capacities and transit time would bind the bile acids, to be excreted in the stool. Kritchevsky (1978) in his report of

fibre and bile acids, concludes that most, but not all, types of fibre increase stool weight and reduce the concentration of bile acids, whilst total excretion increases. This is not what was found in the PF dietary trial. With a fibre free diet, with or without a fermentable fibre source, the bile acids produced are not so readily excreted. They accumulate and consequently shut off further bile acid synthesis in the liver. Cholesterol then accumulates and degradation of the bile acids occurs. If the addition of fibre to diet alters the total and concentration of the caecal and faceal bile acids, then it is reasonable to suggest that the composition of the bile acids alters which may have significant effects in the colon. Pomare and Heaton (1973) reported that deoxycholic acid had pathogenic properties and that bran reduced the dehydroxylation of cholic acid to deoxycholic acid. Cholecystectomy was reported to have no effect on deoxycholic acid in patients given bran. Therefore the alteration in bacteria associated with bran does not explain the decrease in the acid. The decrease in deoxycholic acid must be due to the binding of the acid to bran thereby reducing the amount present for dehydroxylation. There was no change in bacterial matter in this present study which may substantiate the conclusions of Pomare and Heaton (1973).

The individual proportions of the bile acids are given in Pages 100,103,138,140,176, 179.The proportions did fluctuate with time. Deoxycholic acid was present in the greatest proportion with diet PF with the lowest amount be present with the addition of wheat bran. This coincides with the hypothesis of Pomare and Heaton (1973). The muricholic acids are seen to increase on the gum supplemented diet, and fit well with the results of Brydon et al (1980), who also found that the muricholic acids in colonic contents increased with low fibre diets. In the rat liver chenodeoxycholic acid is converted to lithocholic/ muricholic acids, with further degradation to hyodeoxycholic and β-muricholic acids (Brydon et al, 1930). The reduced level of hyodeoxycholic acid and concomitant increase in ω-muricholic with diet PG suggests that there may be inhibition of the conversion of β-muricholic acid to hyodeoxycholic acid, or interconversion between the two. Hyodeoxycholic acid is thought to be formed from lithocholic acid, which is in turn produced from chenodeoxycholic acid. All three acids were highest on the bran diet with resultant decreases in deoxycholic and the muricholic acids. The reduced level of hyodeoxycholic acid on the gum diet infers increased bacterial metabolism favouring the production of the three muricholic acids. Certainly the increased retention

of gum arabic has an influence upon the individual bile acids. Madsen et al (1976) showed that more than 50% of faecal bile acids in the rat on a regular chow diet consisted of hyodeoxycholic acid and ωmuri cholic acids.

In this present study the presence of bran did increase faecal hyodeoxycholic acid (500 mmols/mol) but not ω-muri cholic acid. Brydon et al (1980) reported that hyodeoxycholic acid was greater on a high fibre diet than a low fibre diet. With diet AF, the proportion of the secondary caecal bile acids increased substantially during the AF dietary trial, concomitant increase in the caecal primary bile acid, chenodeoxycholic. There are certain other subtle changes in the proportions of the other bile acids. For example, only 6% of the total bile acid pool comprises of each of ω-and β-muric holic acid, while this is a maximum 30% of each in the PF dietary trial. Diet thus influences the composition of the bile acid pool and their metabolic degradation, although it is uncertain as to how much of this is due to dietary effects. With the PF dietary trial, bran reduced the proportion of all the three muri cholic acids, as did diet PF, whilst gum gave the greatest proportion of these three acids. With the AF dietary trial, there were no such consistent trends within the three muri cholic acids in the caecal contents. Bran abolished βmuri cholic acid, and gum arabic α-muricholic acid, whilst only after 12 weeks did diet AF show any production of each of the three muri cholic acids. Certainly in both the PF and AF dietary trials, the bile acid content on the high fibre diets was not consistently lower than that of the low fibre diets (Brydon et al 1980). In both trials, the gum arabic supplemented diets reduced the proportion of hyodeoxycholic acid, although the proportion was greater with diet AG from lithocholic acid, which in turn is thought to be formed from chenodeoxycholic acid. Both in the PF and AF dietary trials, chenodeoxycholic acid showed a tendancy to decrease on the bran supplemented and unsupplemented diets. The difference was more noticeable in diet PF. In both trials the bran supplemented diets increased the proportion of lithocholic acid whilst the unsupplemented diet decreased the proportion. In both trials, gum arabic increased chenodeoxycholic acid, and decreased the proportion of hyodeoxycholic acid. No consistent trends were observed with the effect of gum arabic upon the proportion of lithocholic acid.

The results serve to show that the type and distribution of bile acids along the intestinal tract is a complex function of diet, colonic bacteria, enterohepatic circulation and intestinal motility.

Despite the differences associated with the bran supplemented diets, similar shifts in the proportions of the individual bile acids were noted in the caecal contents of rats of the PF and EF dietary trials. (Table 3 $f(i)$ and Table 5 $f(i)$ p 100 - 176). Less α-and β-muricholic acid were produced with the EF dietary trial whilst more ω-muricholic acid was produced. But, as with the PF dietary trial, the gum supplemented diet increased the proportion of the three muricholic acids with respect to the other two diets. The amount of hyodeoxycholic acid was lowest on diet EG but greater than that of diet PG. These changes are indicative of altered bacterial metabolism and the absence of a possible interacting effect with the main, basal diet. Faecal excretion of the bile acids does differ between the PF and EF dietary trials. The proportion of lithocholic acid excreted is higher in the latter trial as α-muricholic acid and ω-muricholic acid. The excretion of β-muricholic acid is reduced and completely abolished with diets EG and EB. Certainly, diet EG has a reduction effect on hyodeoxycholic acid, cholic acid as did diet PG.

The results of the three dietary trials show that diet does influence the caecal and faecal bile acids, and their individual composition. Period of feeding is also shown to have an effect, which again is not uniform for each diet. Irrespective of basal diet, it does seem that the presence of a fermentable fibre source increases caecal bile acids. The effect of bran needs to be further investigated.

From the results of these dietary trials, it is apparent that duration of feeding and diet have had noticeable effects on all measurements made and that they are not indiscriminate in their effects. The interactions observed between diet and time, show how, the adaptation time for one diet is not necessarily the same for another. If a long enough time period is allowed to elapse then two different diets may show outwardly no difference in overall trial effects indicative of an ever changing environment within the GI tract. With a highly fermentable diet such as gum arabic an initial reaction to diet change may be seen and thereafter a stabilising situation will occur. This is noticeable with the C.C.

The effects of bile acids have been suggested to be mediated through the physical properties of the fibre which alters their distribution along the GI tract and their excretion in the stool (Eastwood and Hamilton, 1968; Story and Kritchevsky, 1976; Kay, 1981). Gustaf son and Norman (1969), by means of a carmine faecal marker showed an increase in transit time in rats fed on low fibre synthetic diet as opposed to those fed on high fibre containing pellet. The high fibre diet by accelerating transit time, may enhance faecal excretion of bile acids while the fibre free diet retards evacuation and favours the accumulation of bile acids in the colon (Tadesse, 1979). This was not true for either the PF dietary trial or the AF dietary trials. Certainly, the gum arabic by virtue of its degradability, had a positive effect on the caecal bile acids in PF dietary trial particularly the muricholic acids, with a less consistent effect in the AF dietary trial. Gum arabic had the associated effect of increasing the primary bile acids, chenodeoxycholic acid, which may have stimulated increased metabolism to the muricholic acids. Concomitant with the increased retention time of gum arabic, more of the changed bile acids may have been absorbed into the enterohepatic circulation, resulting in a negative feedback effect on bile acid synthesis in the liver (Bergstrom and Danielsson, 1968; Danielsson 1973). However there was no evidence of increased faecal excretion associated with the less degradable, decreased transit time of wheat bran.

The results of the three dietary trials have been discussed as individual trials and, where applicable, with respect to each other. This seemed logical as the trials were separate entities. The results of the AF dietary trial must be treated with caution. The obvious dislike by the rats for this diet, as reflected by reduced intake and subsequent weight loss, gave sufficient cause to doubt the validity and the true influence, of dietary manipulation, upon the remaining results. As such any comparison between this dietary trial and the other two must take cogniscance of this. However it is of interest to note that the AF dietary trial did give similar trends to those of the other dietary trials. Examples of these similarities are the increase of stool weight when supplemented with bran, the increase of dry C.C. when supplemented with gum arabic and the major anion in the caecal and faecal contents being acetate.

Initial companions between these diets have shown that: (i) the choice of

basal diet will have an influence upon the trial results and can form a significant interaction with diet and for time.

(ii) The addition of bran to a diet increases stool weight irrespective of the basal diet.

(iii) the addition of gum arabic to a diet increases the dry C.C. weight, C.S.W.W. and caecal DAPA, irrespective of the basal diet. The unsupplemented and bran supplemented diets had very little effect.

(iv) The major anion in both caecal and faecal contents was acetate, the proportion of which was increased with gum arabic, except with the AF dietary trial. The proportion of caecal butyrate was increased when bran was added to the diet. Bran abolished the production of CH_4, whilst the addition of gum arabic to the diet increased the proportion of CH_4

(v) Time of feeding is a factor in dictating the pattern of results. The results of Chapters 3,4 and 5 have shown that the interactions of D X-T can be significant, indicating that a time period suitable for one diet may not be suitable for another diet. Similarly one diet fed for 12 weeks may give the same result as another diet fed for only 4 weeks. The adaptation time period is not the same for different diets.

For initial investigative studies into the effects of novel dietary fibre sources, stool weight would appear to most readily give clear results and a period of 4 weeks would seem appropriate. For metabolic studies, such as SCFA's H_2, CH_4 and C.C. then a period of greater than 4 weeks and less than 12 weeks is required.

The nature of the experimental design of each of the three dietary trials allowed the use of a three way analysis of variance, in due course, to compare all the results of the three dietary trials and enabled p-values to be assigned to the observations so far made. This has been detailed in Chapter 6. From the figures of Chapter 6 it is apparent that the choice of basal diet is important and will influence the absolute results of the parameters measured. That is the same effect may be recorded by two different diets, and the same percentage change may be observed between various unsupplemented and gum arabic supplemented diets, but the absolute values may be different. This has been best illustrated by the EF dietary trial with respect to the PF dietary trial. With both diets, the addition of bran increased stool weight by 65% (PF dietary trial) and 56% (EF dietary trial) with respect to the unsupplemented diets. The difference in increase is possible due to:

(i) slight difference in colonic metabolic activities of the individual rats.

(ii) Some components of diet PF reaching the caecum, being fermented, or passing straight through and thus contributing to stool weight. The elemental diet is completely residue free and absorbed before it reaches the caecum. Any increase in stool weight is entirely as a result of the supplement. This is substantiated by the significantly lower stool weight of the rats fed diet EF and the higher stool weight of rats fed diet PF. From Chapter 6 considering the main effect of diet alone, it is apparent that diet PF is having the most positive effect upon all the results.

Whilst bran increased stool weight, it was gum arabic that was shown to influence caecal metabolism and to a certain extent, the appearance of the products of caecal metabolism, in the stool. The faecal excretion is difficult to interpret because of the absorption along the large bowel. McNeil et al (1978) showed a marked absorption of acetate from the distal colon of man. Dawson et al (1964) indicated that the longer the chain length the faster the acids were absorbed, which might partially explain the lower proportions of propionate and butyrate appearing in the stool of this present study. Certainly a change in bacterial activity, and possibly the bacterial flora, is responsible for the change in fermentation patterns. With gum arabic total and concentrations of caecal and faecal DAPA were increased concomitant with increases in SCFA's CH_4, dry C.C., and C.S.W.W. The design of this trial did not allow a distinction to be made as to whether an increase in C.S.W.W. was due to a change in bacterial flow which might have encouraged hypertrophy or hyperplasia of the caecal cells.

The results reflect, the difference in fermentability of the two different dietary supplements. Gum arabic is a complex polysaccharide consumed in variable quantities by man and extensively degraded during intestinal transit in rat and man. Previous work has shown that degradation is primarily to the caecum (McLean Ross et al, 1984), thus caecal retention time is increased and is marked by the alterations in certain metabolic end products. Bran, by contrast, is a compressed lignified, cellulosic structure and as such is resistant to fermentation and bacterial degradation in the caecum. Caecal retention time is decreased and stool bulk increased.

It is thought that the increase of stool weight is related to W.H.C., but further work needs to be done on the W.H.C. and pW.H.C. of bran, if in vitro results are to reliably reflect the effects in vivo (Stephen and Cummings, 1979; Stephen and Cummings, 1980(a) McBurney et al, 1985).

Chapter 6 and the preceeding three chapters, showed how different dietary

combinations can have the same effect and that different dietary combinations fed for different time periods may result in the same effect. In the caecum and indeed the gastrointestinal tract, food particles are not likely to be found as discrete entities but will form complexes, bind water, and stimulate changes in bacterial activity. Thus it is reasonable to expect that such dietary interactions will have a bearing on the eventual result. Similarly the longer a minimally degradable fibre such as bran, is fed, the adaptation to the diet is increased and allows changes, if any, to occur. With a diet such as gum arabic, initial changes may occur, due to over stimulation of bacterial activity (figure 3c page 77), which after 8 weeks has reached a more stable level of activity. This is probably due to the combined effects of diet PF, which has escaped degradation in the upper gastrointestinal tract, and gum arabic by 12 weeks. The value is similar to that observed with diet EG. This latter diet showed no such changes with time. This further emphasises the importance of the choice of an adequate time period to allow adaptation to the diet to occur. This is of particular importance when a fermentable substrate such as gum arabic is being studied. With bran and unsupplemented diets there appears to be no changes occurring with time irrespective of basal diet.

The outcome of these dietary trials has shown that the physiological effect of a dietary fibre when eaten along with a normal low residue diet and an elemental diet can give similar resultant trends. The use of an elemental diet, discourages the interactions occurring between the basal diet and the dietary fibre under test whilst supporting growth and development of the rat. These results are perhaps a better indication of the action of a dietary fibre. However a more realistic representation of normal dietary circumstances is that of a normal low residue diet plus a dietary fibre supplement. To establish the effect of a fibre alone an elemental diet may have to be used for a period of 4-8 weeks. If knowledge of the effect of a dietary fibre when taken with a diet, and the resultant interactions is required, then a low residue diet ought to be used to represent a near normal environment.

Dietary trials in man are time consuming, laborious, tedious and may have carry over effects associated with them. Unlike ruminant experiments, cannulation of a human, to sample caecal contents and gastrointestinal fluids is not a plausible suggestion. This is only possible with patients who have had ileostomies or adostomies. The only indication of fibre fermentation is to collect stool samples and analyse them for the metabolic products of gastrointestinal fermentation. These include SCFA's bacterial mass and bile

acids. The gases H_2 and CH_4 may also be measured as further indicators of caecal fermentation. The measurement of transit time of a fibre will indicate its retention time in the GI tract and therefore the amount of time available for the fermentation of the substrate and the subsequent effects upon metabolic activities.

The stool is thought to represent and indicate occurrences in the caecum. Similarly relationships and correlations between metabolic products of the stool and stool weight have been reported. Williams and Olmsted (1936 (a)) attempted to correlate the increment in stool SCFA's with the increment in stool weight and the disappearnce of cellulose plus hemicellulose. They concluded that the quantity of SCFA's in the stool was greatest when a residue disappeared most during its passage through the GI. tract. In this present study, gum arabic was believed to be fermented in the caecum and increased SCFA's in the stool, thus supporting this theory. McLean Ross et al (1983), in a study of gum arabic in humans, showed there to be a significant relationship between wet stool weight andtotal faecal excretion per 24 hours and wet stool weight and faecal bile acid excretion. They also showed that faecal fat and bile acids correlated with dry stool weight. In their studies using rats, McLean Ross et al (1984) demonstrated a linear relationship ($r = 0.61$) between the ingestion of gum arabic and the concentration of total faecal SCFA's. Whilst this indicates that as gum arabic doseage increases the concentration of SCFA's increases, it does not demonstrate that the concentration of caecal SCFA's also increases or even decreases. Williams and Olmsted (1936 (a) and (b)) previously suggested that SCFA may be a mediator of increased stool weight, but it is generally felt that the association between SCFA and stool weight is conincidental (Cummings, 1981). Cummings et al (1978) showed that faecal weight was correlated with an increased intake of pentose containing polysaccharides from the fibre Southgate et al, (1976) showed that the excretion of faecal solids was highly correlated with the intake of unavailable carbohydrates. Contrary to the findings of Williams and Olmsted (1936 (a)), Kelsay et al (1981) found that linear regressions indicated that fibre intakes, rather than fibre disappearance, influenced faecal volume. Moinuddin and Lee (1959) attempted to determine possible relationships between cleaned caecum and colon, plus rectum and fresh weight of stool by using correlation and covariance methods. The caecal dry weight was possibly associated with length and maximum width of the caecum and weights of the caecal fill. The

dry weight and weight length ratio of the colon plus rectum were possibly asssociated with the fresh weight/faecal pellet. Moinuddin and Lee (1959) showed there to be little relationship between the weights of the caecal contents and faecal residues. This latter lack of association was attributed to the considerable alteration of residues during transit from the caecum through the colon before expulsion as faeces. Fischer, (1957) found that dry weights and numbers of faecal pellets, of rats, were not at all related to increases in small intestine or caecum weight.

In this present study, analysis of covariance was employed in an attempt to discover possible associations between the measurements used, and whether, in future studies, animal or human, it would be feasible to assume that the stool profile reflects events occurring in the caecum. The analysis of covariance was 'applied' to the three dietary trials. The relationships tested were stool weight with dry C.C. (N.S.); stool weight with the concentration of caecal DAPA ($p<0.001$); the concentration of faecal DAPA with the concentration of caecal DAPA ($p<0.001$); the correlations of total faecal SCFA's with dry C.C. ($p<0.001$); the concentration of faecal SCFA's with the concentration of caecal DAPA ($p<0.001$); the concentration of caecal SCFA's with dry C.C., (N.S.) and the concentration of caecal SCFA with the concentation of caecal DAPA ($p<0.001$). With the exception of stool weight with dry caecal content and the concentration of caecal SCFA's with dry C.C. all the covariates reached the significance level of $p<0.001$. Whilst the covariate was significant, indicating a relationship between two variables, the other P-values for the main effects, two way interactions and the three way interaction were the same as the original p-values of the three way analysis of variance. Thus there is a possible relationship between two variables within groups, i.e. within each of the nine dietary groups of each dietary trial, a total of 27 altogether, but this is not sufficient to explain the differences between each of the 27 groups.

These results show, to a certain degree, that the stool profile is indicative of events in the caecum. However an analysis of covariance does not show whether the correlations are positive or negative. This needs to be investigated further with work to determine the specific relationship between caecal and faecal material. An analysis of covariance only gives an average relationship, which could hide the nature of correlations within specific groups. Moinuddin and Lee (1959) found no relationship between caecal content and faecal

residues. This present study also showed there to be no general relationship between dry C.C. and stool weight. This present study indicated there to be a relationship between the concentration of faecal DAPA and the concentration of caecal DAPA. From the results of the preceeding chapters it is apparent that as caecal DAPA increases faecal DAPA increases, but the response may be different for different diets fed with different supplements and even fed for different time periods.

In the light of this analysis of covariance it is apparent that for certain chemical and biological entities, the stool is an indicator of metabolic activities in the caecum. The results from these studies also fit well with those previously alone in man and animals. In future dietary trials, human or animal, the analysis of the stool maybe a feasible way of predicting what is happening in the caecum, and allowing the design of long-term studies. In man this would be of particular use because of the lack of routine sampling techniques for sampling various parts of the gastrointestinal tract. Certainly the normal colonic flora is usually inferred from the composition of the normal faecal flora (Hill and Drasar, 1975).

SUMMARY

An animal model, for the evaluation of dietary fibres, and their action upon stool bulking and caecal metabolism, has been described. The experimental animal used was the adult male rat, 10-12 weeks of age, of the Albino Wistar strain. The rat, an omnivore, was considered to be a suitable and practical choice.

Three dietary trials were designed to study the effect of three different low fibre diets, when fed, supplemented and unsupplemented, to groups of rats for 4-, 8-, and 12 weeks. The plant origin and animal origin diets were chosen because of their different origins of protein and carbohydrate. An elemental diet, Flexical, was chosen because it is a nutritionally complete diet, completely absorbed in the small intestine and is frequently used in patients with gastrointestinal disorders. Gum arabic and Canadian Red Spring Wheat Bran were the selected fibre supplements. Gum arabic, a complex polysaccharide, is extensively degraded during intestinal transit in the rat and man and is used regularly as a food additive. Canadian Red Spring Wheat Bran, by contrast is not fermented in the caecum. These two supplements were chosen because of their different fermentabilities. The supplements were incorporated into the basal diets upto a concentration of 10% of the dry weight of any one of the three basal diets.

The effect of duration of feeding was investigated, as were the possible interactions between diet and time, diet and supplement, supplement and time and diet, time and supplement.

Chosen indices of fibre fermentation were: live-weight; liver wet weight; dry stool weight; wet caecum sac weight (voided by contents); dry caecal content weight (excluding the caecum sac); 2-6, Diaminopimelic acid; hydrogen and methane; short chain fatty acids and bile acids.

The results of the three dietary trials were analysed individually (Chapters 3, 4 and 5) by a two-way analysis of variance and together (Chapter 6) by a three-way analysis of variance.

An initial assessment of the action of a dietary fibre is that of daily stool output. Irrespective of basal diet, the presence of wheat bran in the diet significantly increased dry stool weight. Overall stool weight was lowest

with the elemental dietary trial. This may be due to the low residue nature of the diet. Some residue of the plant origin and animal origin diet may be escaping degradation and thus contributing to stool weight. With the elemental dietary trial, the fibre supplement alone will be contributing to stool weight. The slight but insignificant increase in stool weight observed with gum arabic was probably due to bacterial activity and proliferation. The increase in stool weight with wheat bran is thought to be associated with the water holding capacity of fibre. Only with the dietary trial was the duration of feeding significant ($p<0.005$). The results suggest that if stool weight is used as an indicator of fibre metabolism, then a minimum period of 4 weeks is necessary for a dietary trial. It is also apparent from the results that what the supplement is fed with is important. The results for stool weight are in accordance with similar findings about the effects of fibre on stool weight. Wheat bran will predictably increase stool weight, the magnitude of the increase being influenced by basal diet, while a readily fermentable dietary fibre will have little or no influence upon stool weight.

The major site of fermentation in the rat is the caecum, as indicated by a review of the literature and substantiated by the results of this thesis, and is marked by changes in wet caecal sac weight, dry caecal content weight, bacterial mass, hydrogen and methane and short chain fatty acids. The measurements of all these parameters were seen to be increased by the presence of gum arabic in the diet, irrespective of the basal diet, the magnitude of the changes between an unsupplemented and supplemented diets being influenced by basal diet and duration of feeding. Whilst gum arabic was seen to increase bacterial mass, caecal sac weight, dry caecal contents short chain fatty acids and methane, bran had little if any effect on these parameters, indicative of its resistance to caecal fermentation. Short chain fatty acid profile was altered with the addition of gum arabic and bran to the basal diets. The major short chain fatty acids were detected, gum arabic increasing total short chain fatty acids and the molar proportion of acetate. The appearance of minor short chain fatty acids (isobutyrate, isovalerate and valerate) was detected in the animal origin and elemental dietary trials. These are absent from the normal diet of the rat and the plant origin dietary trial but do form a normal part of the human short chain fatty acid spectrum. Wheat bran was found to increase the molar proportion of butyrate, and abolish the production of methane.

These changes indicate subtle changes in the bacterial flora, altered metabolic pathways, consequent upon different dietary fibres and the ability of the bacteria to ferment them. This is most noticeable with the elemental dietary trial, where only the dietary fibre supplement is reaching the caecum. Degradation of a complex molecule or diet may result in a delayed reaction, particularly noticeable with the appearance of hydrogen and methane. These results are in agreement with observations reported elsewhere.

The elemental dietary trial gave results that depicted similar trends to those of the plant origin and animal origin dietary trials, but often with lower absolute values, for example stool weight. This is possibly due to the nature of the basal diet. The elemental diet, being of a low residue nature, is completely absorbed in the small intestine, thus only the fibre supplement will reach the caecum. With the more complex low fibre diets, plant origin and animal origin, it is possible that some residue of the basal diet is reaching the caecum concomitant with the fibre supplement and thereby elevating results. To assess the dietary fibre alone, the elemental diet is an attractive method to use. However this is not representative of the normal nutritional environment, and so the plant origin and animal origin diets may be more useful to assess the interactions that may occur between different dietary constituents. Discrepancies related to variation in production, bacterial utilisation and caecal absorption of the end products of fibre fermentation, indicate that other dietary components and cogeners will affect the manner in which a dietary fibre is metabolised.

Bile acids are an additional way of assessing fibre metabolism. A fermentable polysaccharide such as gum arabic is thought to increase bile acids and serum cholesterol, whilst wheat bran is thought to bind bile acids and act as a diluent. The results from this thesis suggest that diet does influence the caecal and faecal bile acids and their individual composition. Irrespective of basal diet, gum arabic increased the caecal bile acids, in particular the muricholic acids. This agrees with other research work. The effect of bran, however, is less clear cut, appearing to be dependent on the basal diet, and requires further investigation.

The increase in stool weight with wheat bran was not reflected by the change in dry caecal content weight, with bran and vice versa for gum arabic. Gum arabic did increase both caecal and faecal 2-6 diaminopimelic acid. Increased

total faecal diaminopimelic was coincident with an increase in stool weight. A similar trend was observed with short chain fatty acids. This suggests, that whilst stool weight may not be directly related to the dry weight of caecal contents, the faecal flora may well be indicative of events occurring in the caecum. Faecal excretion is difficult to interpret, because of intestinal absorption, but it does appear that faecal composition is related to ingested polysaccharide. Further studies need to be done to identify the relationship between caecal and faecal components.

The changes affected with each fibre supplement, upon 'the chosen' measurements, did not always occur over the same time interval when different basal diets were used. This is indicative of interactions occurring between the basal diet and supplement, basal diet and time, supplement and time and basal diet, supplement and time. That is to say that different diets fed for different time intervals could give similar results, depending upon the adaptation period dictated by the diet and the resultant adaptive response. It is apparent that basal diet, duration of feeding and supplement have noticeable effects upon all measurements made and are not discriminate in their effects. Whilst stool weight may have stabilised by 4 weeks, it is evident that other metabolic measurements have not. For the initial assessment of a dietary fibre, a 4 week stool collection maybe adequate, but for the analysis of metabolic changes a longer period is required.

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APPENDIX 1a

B.P. NUTRITION (U.K.) LTD.

LABORATORY TEST METHOD Q.C./A/5

DETERMINATION OF FIBRE

Principle

The sample is defatted and treated successively with boiling solutions of sulphuric acid and sodium hydroxide of specified concentrations. The residue is separated by filtration, washed, dried, weighed and ashed. The loss of weight resulting from ashing corresponds to the fibre present in the test sample.

Reagents

1. Sulphuric acid, 0.255N solution.
2. Sodium hydroxide, 0.313N solution: the solution must be free or nearly free from sodium carbonate.
3. I.M.S.
4. Diethylether.
5. 1% V/V/ HC1

Apparatus

1. Conical flask, 1000 ml.
2. Buchner flask.
3. Buchner funnel.
4. Platinum or silica crucibles.
5. Electric muffle furnace.

Procedure

1. Weigh to the nearest 0.001 g 2.7 to 3.0 g of the prepared sample, and extract with light petroleum by stirring, settling and decanting three times. Air dry the extracted sample and transfer to a dry 11 conical flask.
   If the sample contains more than 3% calcium (chalk or limestone flour) wash by decantation with 100 ml of HCl and twice with 100 ml portions of water. Drain the residue thoroughly.

APPENDIX 1a (contd).

2. Bring 200 ml of 0.255N sulphuric acid to boiling point, add to the residue, and heat to boiling point within 1 minute.

3. Boil gently for exactly 30 minutes, maintaining a constant volume and rotating the flask every few minutes in order to mix the contents, and remove particles from the sides.

4. Allow the acid mixture to stand for 1 minute and then pour immediately into a shallow layer of hot water under gentle suction on Buchner funnel containing a Whatman 541 filter paper. The filtration time should not exceed 10 minutes.

5. Wash residue off the filter paper and back into the flask with 220 ml of 0.313N sodium hydroxide solution at its boiling point.

6. Boil for 30 minutes with the same precautions as those used in the earlier boiling and treatment.

7. Allow to stand for 1 minute and then filter immediately through a Whatman 541 filter paper. Wash the residue first with boiling water, then HC1 and again with water.

8. Finally, transfer the residue to a small, dried, weighed, conical Whatman 541 filter paper with water and wash twice with I.M.S. and once with ether.

9. Dry to constant weight at 100°C. Allow to cool in a desiccator and weigh.

10. Transfer the paper and insoluble residue to a crucible previously ignited to constant weight. Incinerate the paper and contents to an ash at a dull red heat, to a constant weight. Allow to cool in a desiccator and weigh.

Calculation

The fibre content, as a percentage of the sample, is given by the formula:

$$\frac{d-(p+a) \times 100}{w}$$

in which: d = weight of the paper + residue after drying (g);
p = weight of the paper (g);
a = weight of the ash (g); and
w = weight of the sample (g).

INFORMATION SUPPLIED BY S.D.S, WITHAM, ESSEX.

APPENDIX 11

BIOPSY REPORT

Department of Pathology
Western General Hospital
EDINBURGH EH4 2XU Telephone: 031-332-2525 ext 10

LABORATORY NO: WB 82 7183

surname of patient	forename(s)	hospital unit no.	date of birth	sex	ward	hospital	
RAT	%	%	%	F	GI	WG	Debbie Walker GI Unit

clinician	date received	date sent out	path.	associated laboratory no.	indicators - for laboratory use only
DWAL	19 11	22 11	B		0

part no.	description of specimen	left/right	diagnosis codes s.n.o.p. - 1	s.n.o.p. - 2	s.n.o.p. - 3	tumour information t.n. stage	grade			
1	RAT KIDNEY		0000 0000				.	0	.	2

Rat Kidney

Grossly this kidney shows evidence of hydronephrosis with numerous stones within its collecting systems. The largest stone measures about 0.3 cms in diameter.

Micro: 22.11.82

Histology confirms the presence of an acute on chronic pyelonephritis with some destruction of the renal parenchyma and ulceration of the pelvi-calyceal systems.

A. BUSUTTIL,
Consultant Pathologist.

APPENDIX 1c

Reagents for the 2-6 Diaminopimelic acid assay

ACID

concentrated 6N HCL: 540 mls concentrated HCL 1 litre with water (H_2O)

BUFFERS

Citric acid (A.R.)BDH 0.1 M = 21.0/g/litre

Sodium citre (A.R.)BDH 0.1 M = 29.4 g/litre

pH	Citric acid (vlms)	Sodium citrate (vlms)	H_2O (vlms
2	95	5	50
3.4	73	27	50
4.2	54	46	50

Using these proportions, the necessary total volumes required could be required could be prepared in advance.

Ninhydrin Reagent

- 6 M phosphoric acid 375 mls orthophosphoric acid 625 mls with water
- 12.5 M ninhydrin + 300 mls glacial acetic acid 200 mls 6M phosphoric acid.

APPENDIX 1d

A worked example of a 2 factor ANOVA with replication

Using daily stool output (g/day)
(Plant origin trial, PF).

WEEKS	4	8	12
DIET	1.58	1.58	1.14
	1.28	0.92	1.63
PF	1.28	1.09	0.78
	1.31	1.10	0.68
	1.50	0.84	1.23
mean	1.39	1.11	1.09
SD	0.14	0.29	0.38
SEM	0.06	0.13	0.17
	1.17	0.96	1.80
	0.66	1.60	1.39
PG	1.40	1.55	1.62
	1.41	-	1.69
	2.01	1.34	1.48
mean	1.33	1.36	1.59
SD	0.49	0.29	0.16
SEM	0.22	0.15	0.07
	1.62	1.55	2.40
	2.05	1.69	2.22
PB	1.88	1.92	2.26
	2.14	1.45	1.98
	2.44	2.14	1.88
mean	2.03	1.75	2.15
SD	0.30	0.28	0.21
SEM	0.14	0.12	0.10

	PF	PG	PB
DIET MEANS = (mean $\pm$SEM)	1.20 ± 0.08	1.43 ± 0.09	1.98 ± 0.08
	4	8	12
TIME MEANS = (mean $\pm$SEM)	1.58 ± 0.12	1.41 ± 0.10	1.62 ± 0.13

where SD = standard deviation
SEM = standard error of the mean.

APPENDIX 1d (contd)

(ii) Analysis of Variance for Stool Weight (g/day) for the Plant origin trial

	DF	SS	Mean Sum of Squares	(MS/EMS) F-ratio	Significance
Diet (D)	2	4.77	2.39	26.56	**
Time (T)	2	0.37	0.19	2.11	NS
DXT	4	0.53	0.13	1.44	NS
Residual	35	3.15	0.09		
Total	43				

Total sum of squares (SS) $= \Sigma x^2 - \frac{(\Sigma x)^2}{n}$

$= 112.78 - \frac{4574.07}{44}$

$= 8.82$

Treatment (diet) SS $= \frac{(\Sigma PF)^2}{15} + \frac{(\Sigma PA)^2}{14} + \frac{(\Sigma PB)^2}{15} - \frac{(\Sigma x)^2}{n}$

$= 21.49 + 28.71 + 58.53 - 103.96$

$= \underline{4.77}$

Treatment (time) SS $= \frac{(\Sigma 4 \text{ week})^2}{15} + \frac{(\Sigma 8 \text{ week})^2}{14} + \frac{(\Sigma 12 \text{ week})^2}{15} - \frac{(\Sigma x)^2}{n}$

$= 37.57 + 27.85 + 38.97 - 103.96$

$= \underline{0.37}$

DXT SS $= \frac{(\text{PF 4 weeks})^2}{5} + \ldots\ldots \frac{(\text{PB 12 weeks})^2}{5}$ $- \text{Diet SS} - \text{Time SS} - \frac{(\Sigma x)^2}{n}$

$= 9.69 + 6.12 + 5.96 + 8.80 + 7.41$
$+12.70 + 20.51 + 15.34 + 23.10$
$- 4.77 - 0.37 - 103.96$

$= \underline{0.53}$

where DF = degrees of freedom, and is n - 1.
SS = sum of squares
MS = mean sum of squares
EMS = error mean sum of squares = Residual mean square (RMS).
** = $p \leq 0.01$
NS = not significant
RMS = residual mean square

APPENDIX 1d (contd)

(iii) From the table, the F ratio for diet is 26.56. By looking at tables for the F-distribution, the value for diet with 2,35 degrees of freedom (DF) is 5.27.
Our value is greater than this and is significant ($p<0.01$).
Which diets are significantly different from each other:
The least significant difference LSD:

$$\text{is } t \times \sqrt{\frac{1}{15} + \frac{1}{14}\,(EMS)} = t \times \text{S.E.D.}$$

Where t is found by looking up the table of 't' distribution for $DF_{(35)}$ (the degrees of freedom for EMS). This is 2.724

$$\text{thus } 2.724 \times \sqrt{0.136 \times 0.09}$$

$$\text{LSD.} = \underline{0.30}$$

Therefore,
PF is not significantly different from PG. PB is significantly greater than both of them.

This onlyshows diet being significant. If time were significant at $p<0.01$ the same LSD would apply because n remains unchanged. If DXT were significant ($p<0.01$) then n would be equal to 5 and LSD would change. LSD would also change with the level of significance.

APPENDIX 2 a

A summary table of the F-statistics for the plant origin dietary trial (Chapter 3)

VARIABLE		DIET	TIME	INTERACTION
Live-weight				
Initial	g	1.36NS	3.87*	1.18NS
Final	g	3.05NS	4.35*	0.24NS
Live-weight Change	g	0.71NS	29.02**	1.84NS
Liver wet weight	g	2.65NS	3.95*	0.92NS
	g/kg	0.33NS	11.27**	1.73NS
Stool dry weight	g	26.6**	2.11NS	1.44NS
	g/kg	33.6**	0.38NS	1.67NS
Caecum sac wet weight (empty)				
	g	37.5**	2.50NS	1.50NS
	g/kg	64.0**	8.67**	1.00NS
Dry caecal contents				
	g	87.6**	8.2**	6.20**
	g/kg	99.3**	11.2**	6.75**
2-6 DAPA				
a) Total i) Caecal (µmols)		98.2**	0.82NS	0.08NS
ii) Faecal (µmols/day)		32.4**	3.89*	0.74NS
b) Concentration				
i) Caecal (µmols/g)		20.6**	10.1**	5.77**
ii) Faecal (µmols/g)		211**	0.40NS	NA
DEGREES OF FREEDOM		2,36	2,36	4,36
DEGREES OF FREEDOM FOR:				
final live-weights g, live-weight change g, liver weight g, stool g, g/kg, 2-6 DAPA (faecal) =		2,35	2,35	4,35

** = $p<0.01$
NS = not significant
NA = not available.

APPENDIX 2a

A summary of the F-statistics for the plant origin dietary trial (Chapter 3) Contd

VARIABLE			DIET	TIME	INTERACTION
SCFA's					
a) Total	i)	Caecal (μmols)	61.7**	1.66NS	0.67NS
	ii)	Faecal (μmols/day)	11.4**	9.55**	2.07NS
b) Concentration					
	i)	Caecal (μmols/g)	7.33**	0.86NS	7.93**
	ii)	Faecal (μmols/g)	17.4**	22.5**	12.7**
Individual SCFA's (mmols/mol)					
a) Caecal	i)	Acetate	20.3**	2.49NS	0.28NS
	ii)	Propionate	1.07NS	10.3**	1.09NS
	iii)	Butyrate	44.5**	0.05NS	0.78NS
b) Faecal	i)	Acetate	10.3**	0.15NS	2.59NS
	ii)	Propionate	28.2**	0.42NS	3.12NS
	iii)	Butyrate	2.82NS	0.63NS	0.84NS
HYDROGEN					
		mls/hr/kg	0.06NS	6.68**	1.88NS
METHANE					
		mls/hr/kg	12.7**	1.64NS	1.29NS
DEGREES OF FREEDOM			2,36	2,36	4,36
DEGREES OF FREEDOM FOR:					
Total and individual SCFA's (faecal)					
H_2					
CH_4			2,35	2,35	4,35

* = $p<0.05$
** = $p<0.01$
NS = not significant.

APPENDIX 2b

A summary table of the F-statistics for the animal origin dietary trial (Chapter 4)

VARIABLE		DIET	TIME	INTERACTION
Live-weight				
Initial	g	0.81NS	0.34NS	5.92**
Final	g	37.7**	1.34NS	4.02**
Live-weight change	g	30.9**	2.66NS	1.81NS
Liver wet weight	g	24.4**	0.91NS	4.19**
	g/kg	2.09NS	0.14NS	2.37*
Stool dry weight	g	24.7**	0.47NS	2.50NS
	g/kg	8.44**	0.35NS	2.64NS
Caecum sac wet weight (empty)				
	g	82.4**	37.6**	47.8**
	g/kg	54.7**	23.7**	30.8**
Dry caecal contents				
	g	17.0**	1.20NS	1.80NS
	g/kg	15.3**	0.65NS	0.33NS
2-6 DAPA				
a) Total i) Caecal (μmols)		104**	4.86*	4.28**
ii) Faecal (μmols/day)		33.8**	4.22*	2.57NS
b) Concentration				
i) Caecal (μmols/g)		110**	3.66*	2.12NS
ii) Faecal (μmols/g)		137**	5.44**	3.41*
DEGREES OF FREEDOM		2,36	2,36	4,36

* = $p<0.05$
** = $p<0.01$
NA = not significant.

APPENDIX 2 b

A summary table of the F-statistics for the animal origin dietary trial (Chapter 4) Contd

VARIABLE			DIET	TIME	INTERACTION
SCFA's					
a) Total	i)	Caecal (μmols)	45.7**	33.8**	8.08**
	ii)	Faecal (μmols/day)	37.3**	15.7**	4.30**
b) Concentration					
	i)	Caecal (μmols/g)	33.2**	44.8**	7.11**
	ii)	Faecal (μmols/g)	17.4**	13. 6**	6.96**
Individual SCFA's (mmols/mol)					
a) Caecal	i)	Acetate	14.7**	1.04NS	4.87**
	ii)	Propionate	10.5**	0.28NS	1.40NS
	iii)	Butyrate	73.5**	4.17*	4.23**
b) Faecal	i)	Acetate	31.7**	4.20*	2.00NS
	ii)	Propionate	33.1**	3.81*	1.55NS
	iii)	Butyrate	-	-	-
HYDROGEN					
	mls/hr/kg		-	-	-
METHANE					
	mls/hr/kg		-	-	-
DEGREES OF FREEDOM			2,36	2,36	4,36

* = $p<0.05$
** = $p<0.01$
NS = not significant.

APPENDIX 2c

A summary table of the F-statistics for the elemental dietary trial (Chapter 5)

VARIABLE		DIET	TIME	INTERACTION
Live-weight				
Initial	g	2.11NS	2.73NS	3.90*
Final	g	4.21**	21.5**	3.26NS
Live-weight Change	g	15.8**	91.4**	1.21NS
Liver wet weight	g	8.75**	4.43*	0.89NS
	g/kg	9.33**	10.8**	0.50NS
Stool dry weight	g	12.6**	3.48*	1.67NS
	g/kg	9.88**	1.72NS	1.56NS
Caecum sac wet weight (empty)				
	g	106**	2.00NS	4.50**
	g/kg	111**	15.6**	3.80*
Dry caecal contents				
	g	78.5**	2.50NS	2.00NS
	g/kg	177**	0.30NS	1.20NS
2-6 DAPA				
a) Total i) Caecal (μmols)		102**	1.50NS	0.97NS
ii) Faecal (μmols/day)		11.9**	0.81NS	1.05NS
b) Concentration				
i) Caecal (μmols/g)		22.8**	3.64*	0.42NS
ii) Faecal (μmols/g)		182**	4.85*	4.11*
DEGREES OF FREEDOM		2,36	2,36	4,36
DEGREES OF FREEDOM FOR				
2-6 DAPA (faecal) =		2,35	2,35	4,35

APPENDIX 2c

A summary table of the F-statistics for the elemental dietary trial (Chapter 5) contd.

VARIABLE		DIET	TIME	INTERACTION
SCFA's				
a) Total	i) Caecal (μmols)	21.3**	2.23NS	1.38NS
	ii) Faecal (μmols/day)	10.5**	4.43*	2.21NS
b) Concentration				
	i) Caecal (μmols/g)	2.92NS	7.23**	2.44NS
	ii) Faecal (μmols/g)	23.3**	4.60*	3.32*
Individual SCFA's (mmols/mol)				
a) Caecal	i) Acetate	4.43*	1.13NS	3.95**
	ii) Propionate	28.7**	0.38NS	0.81NS
	iii) Butyrate	14.5**	2.66NS	2.37NS
b) Faecal	i) Acetate	12.7**	8.91**	3.17*
	ii) Propionate	1.31NS	2.14NS	4.62**
	iii) Butyrate	1.80**	8.84**	10.1**
HYDROGEN				
	mls/hr/kg	14.5**	20.8**	6.60**
METHANE				
	mls/hr/kg	5.40**	2.50NS	2.20**
DEGREES OF FREEDOM		2,36	2,36	4,36
DEGREES OF FREEDOM FOR				
Total and individual SCFA's (faecal)		2,35	2,35	4,35

* = $p<0.05$
** = $p<0.01$
NS = not significant.

APPENDIX 2d

A summary table of the F-statistics for the three-way ANOVA (Chapter 6)

	MAIN EFFECTS			2 WAY INTERACTION			3 WAY INTERACTION
	DIET(D)	TIME(T)	SUPPLEMENT(S)	DXT	DXS	TXS	DXTXS
Live-weight							
Final g	65***	19.9***	6.44**	3.88**	9.66***	2.17NS	1.62NS
Live-weight change g	398***	55***	11.1***	6.31***	14.3***	1.31NS	2.23*
Liver wet weight							
g	108***	7.59***	6.11**	1.64NS	9.18***	1.97NS	0.85NS
g/kg	27.3***	11.5***	0.34NS	3.03**	5.74***		1.96NS
Stool dry weight							
g	9.31***	1.10NS	56.3***	1.82NS	3.35*	1.81NS	2.18*
g/kg	214***	0.08NS	32.8***	0.93NS	2.28NS	2.38NS	2.42*
Caecum sac Wet weight							
g	19.7***	8.92***	211***	19.9***	7.21***	17.7***	22.4***
g	53.0***	21***	156***	21.5***	11.5***	19.7***	19.3***
Dry caecal Contents							
g	11.6***	3.04NS	173***	6.60***	19.4***	5.34***	4.60***
g/kg	62.3***	5.87**	143***	5.20***	10.3***	3.16**	3.62***
2-6 DAPA							
a) Total							
(i) caecal (µmols)	23.2***	4.25*	295***	0.42NS	8.83***	3.84***	0.14NS
(ii) faecal (µmols/g)	44.0***	3.42*	74.0***	2.33NS	4.27**	1.67NS	1.44NS
b) Concentration							
(i) caecal (µmols/g)	54.8***	30.7NS	85.9***	7.06***	2.36NS	2.62*	2.15*
(ii) faecal (µmols/g)	228	7.65***	554***	6.19***	20.6***	1.61NS	2.82**

* = p<0.05; ** = p<0.01; *** = p<0.001; NS = not significant

APPENDIX 2d

A summary table of the F-statistics for the three-way ANOVA (Chapter 6) Contd

	MAIN EFFECTS			2-WAY INTERACTION			3 WAY INTERACTION
	DIET(D)	TIME(T)	SUPPLEMENT(S)	DXT	DXS	TXS	DXTXS
SCFA's							
a) Total							
(i) Caecal (µmols)	14.3***	8.99***	116***	6.50***	9.18***	3.58***	1.68NS
(ii) Faecal (µmols/day)	23.8***	5.42**	26.4***	10.8***	7.15***	0.07NS	2.76**
b) Concentration							
(i) Caecal (µmols/g)	36.3***	22.9***	9.29***	9.14***	10.2***	4.18**	5.96***
(ii) Faecal (umols/g)	84.5***	7.60**	50.4***	21.1***	14.3***	8.97***	7.91***
Individual SCFA's (mmols/mol)							
a) Caecal							
(i) Acetate	3.76*	0.31NS	15.8***	2.50***	12.9***	5.27***	1.04NS
(ii) Propionate	1.10NS	4.86**	26.1***	2.23NS	8.65***	1.54NS	0.87NS
(iii) Butyrate	34.6***	3.15*	111***	0.42NS	12.8***	2.30NS	1.46NS
b) Faecal							
(i) Acetate	173***	7.82***	37.1***	3.07*	3.00*	4.15***	1.85NS
(ii) Propionate	98.9***	4.78**	34.7***	0.87NS	7.74***	2.30NS	4.01**
(iii) Butyrate	50.9***	3.34*	3.85*	1.72NS	5.28***	4.34**	1.94NS
HYDROGEN (mls/hr/kg)	0.44NS	27.6***	6.60**	3.15***	10.0***	5.66***	3.66**
METHANE (mls/hr/kg)	14.1***	3.59*	12.0***	0.23NS	4.16*	2.53*	1.22NS
DEGREES OF FREEDOM	2,108	2,108	2,108	4,108	4,108	4,108	8,108
DEGREES OF FREEDOM FOR ALL STOOL RESULTS	2,107	2,107	2,107	4,107	4,107	4,107	8,107
H_2 AND CH_4	1,72	2,72	2,72	2,72	2,72	4,72	4,72

* = p<0.05; ** = p<0.01; *** = p<0.001; NS = not significant

APPENDIX 2e(i) **A summary table of the F-statistics for the small animal diets (Chapter 7, part A)**

VARIABLE		DIET	TIME	INTERACTION
Live-weight			-	-
Initial	(g)	0 weeks: 15.98**		
	(g)	12 weeks: 7.32**	-	-
Final	g	3.22NS	-	-
Live-weight Change	(g)	0.57NS	-	-
Liver wet weight	(g)	5.10*	3.19 NS	0.30 NS
	(g/kg)	1.25NS	78.55 **	4.35 *
Stool dry weight	g	7**	10.95 **	3.71 **
	g/kg	22.0**	42.0	8.28 **
Caecal sac wet weight (empty)				
	g	2.25NS	10.25 **	1.13 NS
	g/kg	2.61NS	30.73 **	0.98 NS
Dry caecal contents				
	g	0.95NS	0.5 NS	2.0 NS
	g/kg	0.63NS	4.25NS	2.25 NS
2-6 DAPA				
a) Total i) Caecal (μmols)		37.59**	69×10^{-3} NS	0.63 NS
ii) Faecal (μmols/day)		3.42NS	8.03 NS	0.39 NS
b) Concentration				
i) Caecal (μmols/g)		50.76**	0.15 NS	0.17 NS
ii) Faecal (μmols/g)		4.22NS	0.95 NS	0.82 NS
DEGREES OF FREEDOM :		2,24	1,24	2,24
DEGREE OF FREEDOM for total- and concentration of caecal and faecal 2,-6 DAPA:		1,16	1,16	1,16
DEGREES OF FREEDOM FOR live-weight results		2,12	-	-

* = $p<0.05$
** = $p<0.01$
NS = not significant.

APPENDIX 2e(i)

A summary table of the F-statistics for the small animal diets (Chapter 7, part A)

VARIABLE		DIET	TIME	INTERACTION
SCFA's				
a) Total	i) Caecal (μmols)	5.58 *	4.66 *	0.60 NS
	ii) Faecal (μmols/day)	26.4 **	0.05 NS	1.41 NS
b) Concentration				
	i) Caecal (μmols/g)	13.48 **	1.34 NS	1.20 NS
	ii) Faecal (μmols/g)	39.97 **	3.40 NS	31.18 **
Individual SCFA's (mmols/mol)				
a) Caecal	i) Acetate	1.18 NS	0.01 NS	2.45 NS
	ii) Propionate	9.42 **	0.81 NS	4.59 *
	iii) Butyrate	0.31 NS	0.56 NS	10.20 **
b) Faecal	i) Acetate	4.02 NS	51.84 **	3.65 NS
	ii) Propionate	9.18×10^{-4} NS	6.25 *	0.17 NS
	iii) Butyrate	3.45 NS	11.95 **	8.90 **
Hydrogen				
	mls/hr/kg	0.01 NS	0.56 NS	2.36 NS
Methane				
	mls/hr/kg	19.5 **	19.5 **	19.5 **
DEGREES OF FREEDOM		1,16	1,16	1,16

* = $p<0.05$
** = $p<0.01$
NS = not significant.

APPENDIX 2e(ii)

A summary table of the F-statistics for the small animal diets (Chapter 7, part B)

VARIABLE		DIET
Live-weight		
Initial	g	
Final	g	4.03 **
Live-weight Change	g	57.0 **
Liver wet weight	g	10.8 **
	g/kg	5.13 **
Stool dry weight	g	38.73 **
	g/kg	11.00 **
Caecal sac wet weight (empty)		
	g	7.00 **
	g/kg	9.20 **
Dry caecal contents		
	g	17.5 **
	g/kg	73.0 **
2-6 DAPA		
a) Total i) Caecal (μmols)		3.70 **
ii) Faecal (μmols/day)		4.90 **
b) Concentration		
i) Caecal (μmols/g)		10.0 **
ii) Faecal (μmols/g)		25.0 **
DEGREES OF FREEDOM :		11,48
DEGREES OF FREEDOM FOR total- and concentration of caecal and faecal 2-6 DAPA		10,44

* = $p<0.05$
** = $p<0.01$

APPENDIX 2e(ii)

A summary table of the F-statistics for the small animal diets (Chapter 7 Part B)

VARIABLE			DIET
SCFA's			
a) Total	i)	Caecal (μmols)	145 **
	ii)	Faecal (μmols/day)	598 **
b) Concentration			
	i)	Caecal (μmols/g)	16.6 **
	ii)	Faecal (μmols/g)	12.7 **
Individual SCFA's (mmols/mol)			
a) Caecal	i)	Acetate	9.29 **
	ii)	Propionate	1.79 NS
	iii)	Butyrate	26.3 **
b) Faecal	i)	Acetate	0.20 NS
	ii)	Propionate	16.99 **
	iii)	Butyrate	5.23 **
Hydrogen			
		mls/hr/kg	83.0 **
Methane			
		mls/hr/kg	13.5 **
DEGREES OF FREEDOM			10,44
DEGREES OF FREEDOM FOR hydrogen and methane			7,32

APPENDIX 3a The composition of SCFA's (mmols/mol) in dry caecal contents from rats fed an animal origin diet (AF) alone or supplemented with either 10% gum arabic (AG) or 10% wheat bran (AB) for three time periods.

Values are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	AF	AG	AB	AF	AG	AB	AF	AG	AB
ISOBUTYRATE	17 ± 7	0	23 ± 2	19 ± 8	0	0	18 ± 5	0	9 ± 0.8
ISOVALERATE	8 ± 5	0	0	0	0	0	9 ± 4	0	6 ± 1
VALERATE	15 ± 6	7 ± 3	26 ± 2	24 ± 10	12 ± 3	0	24 ± 7	18 ± 2	13 ± 1

Table of significant differences, in ascending order from left to right, for each group.

	DIET	TIME	INTERACTION (DXT)
ISOBUTYRATE	AG^a AB^b AF^b	NS	NS
LSD (36)	10		
	**		
ISOVALERATE	AG^a AB^{ac} AF^c	8^a 4^{ab} 12^b	NS
LSD (36)	3.4	3.4	
	*	*	
VALERATE	NS	NS	AB^a AG^{ab} AG^{abc} AB^{abc} AF^{abc} AG^{abc} AF^{bc} AF^{bc} AB^c
			8 4 8 12 4 12 8 12 4
LSD (36)			18.2
			**

* = $p<0.05$
** = $p<0.01$
NS = not significant.
A different superscript denotes a significant difference.

APPENDIX 3b(i) The composition of SCFA's (mmols/mol) in dry caecal contents from rats fed an elemental diet (EF) alone or supplemented with either 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Values are mean ± SEM where n = 5 per group.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
ISOBUTYRATE	16 ± 1	8 ± 2	14 ± 1	16 ± 2	12 ± 3	7 ± 2	13 ± 2	17 ± 5	17 ± 3
ISOVALERATE	11 ± 1	2 ± 1	11 ± 1	16 ± 2	9 ± 2	3 ± 2	6 ± 2	12 ± 5	12 ± 2
VALERATE	53 ± 5	8 ± 5	39 ± 2	52 ± 4	28 ± 7	38 ± 4	73 ± 14	59 ± 13	35 ± 4

Table of significant differences, in ascending order from left to right for each group.

	DIET	TIME	INTERACTION(DXT)
ISOBUTYRATE	NS	NS	EB^{a} EG^{a} EG^{ab} EF^{ab} EB^{ab} EF^{b} EF^{b} EG^{b} EB^{b} 8 4 8 12 4 8 4 12 12
LSD (36)			6.98 *
ISOVALERATE	NS	NS	EG^{a} EB^{ab} EF^{abc} EG^{abcd} EB^{abcd} EF^{bcd} EG^{cd} EB^{cd} EF^{d} 4 8 12 8 4 4 12 12 8
LSD (36)			
VALERATE	EG^{a} EB^{b} EF^{b}	4^{c} 8^{cd} 12^{d}	EG^{e} EG^{ef} EB^{fg} EB^{fgh} EB^{fgh} EF^{ghj} EF^{ghj} EG^{hi} EF^{j} 4 8 12 8 4 8 4 12 12
LSD (36)	16.8 **	16.8 **	2.17 *

* = $p<0.05$
** = $p<0.01$
NS = not significant
A different superscript denotes a significant difference.

APPENDIX 3b(ii) The composition of SCFA's (mmols/mol) in dry faecal contents from rats fed an elemental diet (EF) alone or unsupplemented with either 10% gum arabic (EG) or 10% wheat bran (EB) for three time periods.

Values are mean ± SEM where n = 5 per group except EG (4 weeks) where n = 4.

Period of Feeding	4 WEEKS			8 WEEKS			12 WEEKS		
DIET	EF	EG	EB	EF	EG	EB	EF	EG	EB
ISOBUTYRATE	17 ± 11	31 ± 2	24 ± 4	12 ± 8	16 ± 2	0	5 ± 5	17 ± 5	10 ± 5
ISOVALERATE	27 ± 11	20 ± 4	22 ± 6	29 ± 9	21 ± 1	0	0	23 ± 8	20 ± 2
VALERATE	20 ± 10	22 ± 8	12 ± 5	0	31 ± 3	0	0	53 ± 6	27 ± 14

Table of significant differences, in ascending order from left to right for each group.

	DIET	TIME	INTERACTION (DXT)
ISOBUTYRATE LSD (35)	NS	8^{a} 12^{ab} 4^{b} 12.5 **	NS
ISOVALERATE LSD (35)	NS	NS	EF^{a} EB^{a} EB^{ab} EG^{ab} EG^{ab} EB^{ab} EG^{ab} EF^{b} EF^{b} 12 8 12 4 8 4 12 4 8 25.3 **
VALERATE LSD (35)	EF^{a} EB^{a} EG^{b} 16.1 **	8^{c} 4^{cd} 12^{d} 12.1 *	EF^{e} EF^{e} EB^{e} EB^{ef} EF^{ef} EG^{f} EB^{f} EG^{f} EG^{g} 8 12 8 4 4 4 12 8 12 21.6 **

* = $p<0.05$
** = $p<0.01$
NS = not significant
A different superscript denotes a significant difference.

APPENDIX 4a

The live-weight changes (g) of rats, fed three different basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean S.E.M. where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING		
		4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	55 ± 7	83 ± 8	100 ± 10
	GUM ARABIC	50 ± 7	66 ± 5	111 ± 13
	BRAN	50 ± 3	46 ± 15	114 ± 10
ANIMAL ORIGIN	NONE	−67 ± 9	−71 ± 18	−50 ± 4
	GUM ARABIC	−53 ± 5	−12 ± 15	−26 ± 8
	BRAN	+11 ± 18	−7 ± 5	+23 ± 7
ELEMENTAL	NONE	80 ± 1	120 ± 9	170 ± 11
	GUM ARABIC	55 ± 6	117 ± 19	124 ± 8
	BRAN	77 ± 6	115 ± 5	149 ± 8

L.S.D. between any two means (110) 29.4 ($p<0.05$).

APPENDIX 4b

The dry stool weights (g/day) of rats fed three basal deits with and without gum arabic or wheat bran for three time periods.

Results given are mean ±S.E.M where n = 5 group except * where n = 4.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	1.40 ±0.06	1.11 ±0.13	1.10 ±0.17
	GUM ARABIC	1.33 ±0.22	*1.36 ±0.15	1.59 ±0.07
	BRAN	2.03 ±0.14	1.75 ±0.12	2.15 ±0.10
ANIMAL ORIGIN	NONE	1.25 ±0.14	1.28 ±0.14	1.34± 0.13
	GUM ARABIC	1.66 ±0.22	1.40 ±0.11	2.01± 0.10
	BRAN	2.55 ±0.26	2.37 ±0.15	1.99± 0.25
ELEMENTAL	NONE	0.71 ±0.06	0.58 ±0.11	0.82± 0.07
	GUM ARABIC	0.39 ±0.11	0.84 ±0.11	0.78± 0.10
	BRAN	0.94 ±0.10	1.19 ±0.10	1.14± 0.21

L.S.D. between any two means $_{(110)}$ 0.40 ($p<0.05$).

APPENDIX 4c

The dry stool weights (g/kg) of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean ± S.E.M. where n = 5 per group except * where n = 4.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	2.73 ± 0.14	2.23 ± 0.38	1.95 ± 0.25
	GUM ARABIC	2.68 ± 0.44	*3.03 ± 0.38	3.03 ± 0.03
	BRAN	4.33 ± 0.23	3.84 ± 0.29	4.26 ± 0.20
ANIMAL ORIGIN	NONE	4.08 ± 0.44	4.50 ± 0.42	4.86 ± 0.55
	GUM ARABIC	5.27 ± 0.84	4.85 ± 0.39	6.03 ± 0.43
	BRAN	7.37 ± 0.44	6.23 ± 0.46	5.14 ± 0.66
ELEMENTAL	NONE	1.47 ± 0.13	1.32 ± 0.21	1.53 ± 0.13
	GUM ARABIC	0.89 ± 0.24	1.79 ± 0.25	1.51 ± 0.19
	BRAN	2.04 ± 0.23	2.27 ± 0.16	2.06 ± 0.36

L.S.D. between any two means (110) 1.07 ($p<0.05$).

APPENDIX 4d

The caecal sac wet weight (g) of rats fed three basal diets, with and without gum arabic or wheat bran for three time periods.

Results given are mean±S.E.M where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING		
		4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	0.82 ± 0.04	0.71 ± 0.02	0.74 ± 0.04
	GUM ARABIC	1.08 ± 0.07	0.96 ± 0.03	1.02 ± 0.05
	BRAN	0.75 ± 0.03	0.74 ± 0.05	0.69 ± 0.05
ANIMAL ORIGIN	NONE	0.63 ± 0.02	0.57 ± 0.07	0.65 ± 0.07
	GUM ARABIC	0.70 ± 0.04	1.74 ± 0.09	0.78 ± 0.04
	BRAN	0.54 ± 0.01	0.59 ± 0.10	0.69 ± 0.03
ELEMENTAL	NONE	0.83 ± 0.04	0.61 ± 0.01	0.65 ± 0.03
	GUM ARABIC	1.30 ± 0.01	1.15 ± 0.03	1.15 ± 0.05
	BRAN	0.71 ± 0.03	0.91 ± 0.09	0.82 ± 0.05

L.S.D between any two means (110) 0.24 ($p<0.001$).

APPENDIX 4e

The caecal sac wet weights (g/kg) of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean$\pm$S.E.M where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING		
		4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	1.61 $\pm$ 0.05	1.40 $\pm$ 0.05	1.35 $\pm$ 0.09
	GUM ARABIC	2.16 $\pm$ 0.10	2.22 $\pm$ 0.10	1.93 $\pm$ 0.02
	BRAN	1.60 $\pm$ 0.09	1.61 $\pm$ 0.02	1.36 $\pm$ 0.07
ANIMAL ORIGIN	NONE	1.94 $\pm$ 0.18	2.00 $\pm$ 0.22	2.32 $\pm$ 0.25
	GUM ARABIC	2.19 $\pm$ 0.15	5.43 $\pm$ 0.42	2.32 $\pm$ 0.13
	BRAN	1.59 $\pm$ 0.15	1.58 $\pm$ 0.05	1.80 $\pm$ 0.05
ELEMENTAL	NONE	1.71 $\pm$ 0.06	1.42 $\pm$ 0.06	1.16 $\pm$ 0.05
	GUM ARABIC	2.98 $\pm$ 0.12	2.45 $\pm$ 0.04	2.23 $\pm$ 0.12
	BRAN	1.56 $\pm$ 0.12	1.74 $\pm$ 0.14	1.48 $\pm$ 0.09

L.S.D. between any two means (110) 0.68 ($p<0.001$).

APPENDIX 4f

The dry caecal content weight (g) of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean$\pm$S.E.M where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	0.50 $\pm$ 0.09	0.52 $\pm$ 0.04	0.46 $\pm$ 0.03
	GUM ARABIC	2.00 $\pm$ 0.20	1.34 $\pm$ 0.10	1.09 $\pm$ 0.11
	BRAN	0.59 $\pm$ 0.05	0.64 $\pm$ 0.04	0.58 $\pm$ 0.07
ANIMAL ORIGIN	NONE	0.70 $\pm$ 0.05	0.60 $\pm$ 0.09	0.65 $\pm$ 0.08
	GUM ARABIC	0.98 $\pm$ 0.03	0.80 $\pm$ 0.05	1.02 $\pm$ 0.06
	BRAN	0.66 $\pm$ 0.06	0.76 $\pm$ 0.12	0.71 $\pm$ 0.04
ELEMENTAL	NONE	0.50 $\pm$ 0.04	0.42 $\pm$ 0.05	0.47 $\pm$ 0.09
	GUM ARABIC	1.00 $\pm$ 0.09	0.97 $\pm$ 0.06	1.19 $\pm$ 0.07
	BRAN	0.44 $\pm$ 0.04	0.58 $\pm$ 0.04	0.59 $\pm$ 0.03

L.S.D. between any two means (110) 0.37 ($p<0.001$).

APPENDIX 4g

The dry caecal content weight (g/kg) of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean ±S.E.M where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	1.00 ±0.04	1.04 ±0.08	0.83 ±0.08
	GUM ARABIC	4.04 ±0.10	3.14 ±0.28	2.09 ±0.21
	BRAN	1.28 ±0.04	1.40 ±0.07	1.13 ±0.11
ANIMAL ORIGIN	NONE	2.45 ±0.19	2.10 ±0.29	2.37 ±0.32
	GUM ARABIC	3.07 ±0.09	2.76 ±0.21	3.05 ±0.20
	BRAN	1.96 ±0.26	1.98 ±0.28	1.83 ±0.13
ELEMENTAL	NONE	1.03 ±0.07	0.98 ±0.08	0.85 ±0.19
	GUM ARABIC	2.26 ±0.16	2.09 ±0.17	2.30 ±0.13
	BRAN	0.96 ±0.11	1.12 ±0.10	1.07 ±0.07

L.S.D. between any two means (110) 0.91 ($p<0.001$).

APPENDIX 4h

The concentration (μmols/g) of caecal DAPA of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean±S.E.M where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	7.33 ± 0.32	5.63 ± 0.64	7.01 ± 0.57
	GUM ARABIC	5.74 ± 0.29	7.29 ± 0.53	11.1 ± 1.43
	BRAN	4.02 ± 0.57	4.79 ± 0.15	5.36 ± 0.21
ANIMAL ORIGIN	NONE	4.30 ± 0.41	3.98 ± 0.25	4.15 ± 0.24
	GUM ARABIC	7.08 ± 0.34	6.70 ± 0.30	7.91 ± 0.53
	BRAN	2.53 ± 0.23	3.48 ± 0.24	3.92 ± 0.40
ELEMENTAL	NONE	9.49 ± 0.93	7.97 ± 0.34	7.15 ± 0.44
	GUM ARABIC	11.7 ± 0.84	10.1 ± 1.39	9.35 ± 1.03
	BRAN	6.27 ± 0.56	5.00 ± 0.10	5.72 ± 1.12

L.S.D. between any two means (110) 1.80 ($p<0.05$).

APPENDIX 4i

The mean concentration (μmols/g) of faecal DAPA of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean ±S.E.M where n = 5 per group except * where n = 4.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING		
		4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	6.74 ± 0.22	6.32 ± 0.34	6.48 ± 0.39
	GUM ARABIC	10.7 ± 0.22	* 9.66 ± 0.43	10.2 ± 0.66
	BRAN	4.73 ± 0.24	3.23 ± 0.19	3.56 ± 0.18
ANIMAL ORIGIN	NONE	4.08 ± 0.32	3.13 ± 0.12	4.55 ± 0.34
	GUM ARABIC	5.96 ± 0.31	5.71 ± 0.24	6.08 ± 0.37
	BRAN	1.98 ± 0.12	2.70 ± 0.14	2.87 ± 0.13
ELEMENTAL	NONE	6.69 ± 0.45	7.30 ± 0.30	5.98 ± 0.61
	GUM ARABIC	* 12.4 ± 0.56	10.6 ± 0.48	9.95 ± 0.57
	BRAN	5.16 ± 0.27	3.95 ± 0.25	4.20 ± 0.13

L.S.D. between any two means (110) 1.26 ($p<0.01$).

APPENDIX 4j

The total faecal S C F A's (μmols/day) of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean ± S.E.M where n = 5 per group except * where n = 4.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	181 ± 34.1	63.5 ± 12.2	54.0 ± 14.9
	GUM ARABIC	405 ± 93.6	*147 ± 18.8	211 ± 57.0
	BRAN	167 ± 9.06	103 ± 18.6	194 ± 30.0
ANIMAL ORIGIN	NONE	44.8 ± 7.10	15.1 ± 3.65	30.0 ± 2.87
	GUM ARABIC	58.7 ± 9.70	50.4 ± 8.63	119 ± 23.8
	BRAN	117 ± 23.0	86.9 ± 11.8	209 ± 22.6
ELEMENTAL	NONE	41.7 ± 5.90	39.4 ± 9.50	93.4 ± 28.7
	GUM ARABIC	*67.6 ± 15.7	169 ± 22.0	102 ± 13.7
	BRAN	104 ± 23.0	156 ± 25.0	142 ± 32.3

L.S.D between any two means (110) 105 ($p<0.01$).

APPENDIX 4k

The concentration (μmols/g) of total caecal S C F A's of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean ± S.E.M where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	611 ± 61.0	356 ± 51.0	365 ± 37.0
	GUM ARABIC	426 ± 51.0	530 ± 10.0	626 ± 48.0
	BRAN	351 ± 15.0	396 ± 16.0	426 ± 28.0
ANIMAL ORIGIN	NONE	177 ± 28.0	166 ± 22.0	243 ± 30.0
	GUM ARABIC	449 ± 44.0	236 ± 48.0	632 ± 35.0
	BRAN	265 ± 12.0	213 ± 12.0	585 ± 67.0
ELEMENTAL	NONE	660 ± 44.0	435 ± 34.0	632 ± 78.0
	GUM ARABIC	505 ± 40.0	442 ± 28.7	517 ± 68.0
	BRAN	623 ± 41.0	454 ± 49.0	411 ± 33.0

L.S.D between any two means (110) 221 ($p<0.001$).

APPENDIX 41

The concentration (μmols/g) of total faecal S C F A's of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean±S.E.M where n = 5 per group except * where n = 4.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING		
		4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	131 ± 24.5	58.3 ± 13.2	47.5 ± 11.0
	GUM ARABIC	295 ± 26.0	*112 ± 15.0	109 ± 20.6
	BRAN	82.9±3.38	57.0 ± 6.97	111 ± 14.5
ANIMAL ORIGIN	NONE	39.8±11.4	11.3 ± 1.73	22.6 ± 0.97
	GUM ARABIC	35.0±2.12	35.5 ± 5.39	58.3 ± 8.93
	BRAN	44.9±6.62	39.3 ± 4.01	109 ± 17.9
ELEMENTAL	NONE	62.1±10.9	66.5 ± 5.11	106 ± 26.0
	GUM ARABIC	*132 ± 15.4	203 ± 8.64	133 ± 10.3
	BRAN	109 ± 19.9	128 ± 12.4	121 ± 8.96

L.S.D between any two means (110) 64.2 ($p<0.001$).

APPENDIX 4m

The molar proportion (mmols/mol) of faecal propionate of rats fed three basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean ± S.E.M. where n = 5 per group except * where n = 4.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING		
		4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	54.0 ± 24.2	23.7 ± 23.7	0
	GUM ARABIC	114 ± 8.94	*137 ± 5.13	120 ± 30.7
	BRAN	0	0	65.3 ± 26.7
ANIMAL ORIGIN	NONE	0	0	0
	GUM ARABIC	116 ± 5.0	70.0 ± 29.0	130 ± 5.00
	BRAN	119 ± 33.0	56.0 ± 24.0	82 ± 4.00
ELEMENTAL	NONE	121 ± 31.0	160 ± 2.60	190 ± 6.80
	GUM ARABIC	*213 ± 7.0	179 ± 7.60	179 ± 7.90
	BRAN	249 ± 60.0	118 ± 6.40	148 ± 7.50

L.S.D between any two means (110) 72.9 ($p<0.001$).

APPENDIX 4n

The excretion of H_2 (mls/hr/kg) from rats fed two basal diets with and without gum arabic or wheat bran for three time periods.

Results given are mean±S.E.M. where n = 5 per group.

BASAL DIET	SUPPLEMENT	PERIOD OF FEEDING 4 WEEKS	8 WEEKS	12 WEEKS
PLANT ORIGIN	NONE	0.62 ± 0.27	0.54 ± 0.10	0.27 ± 0.08
	GUM ARABIC	0.58 ± 0.07	0.71 ± 0.21	0.08 ± 0.04
	BRAN	0.65 ± 0.11	0.32 ± 0.06	0.40 ± 0.04
ELEMENTAL	NONE	0.30 ± 0.14	0.24 ± 0.11	0
	GUM ARABIC	1.70 ± 0.30	0.76 ± 0.11	0
	BRAN	0.50 ± 0.06	0.40 ± 0.03	0.22 ± 0.04

L.S.D between any two means (110) 0.50 ($p<0.01$).

APPENDIX 5

The Average Ratio of the Individual Short Chain Fatty Acids

(i) Caecal

DIET	PF	PG	PB	AF	AG	AB	EF	EG	EB
RATIO									
Acetate : Propionate	3.6	4	3	3.4	3.7	4.3	4	4	2.6
Acetate : Butyrate	4.7	7	2.4	6.1	8.5	3.6	7	6	4.5
Propionate:Butyrate	1.3	1.8	0.8	1.7	2.3	0.8	1.8	1.5	1.7
(ii) Faecal									
Acetate : Propionate	36	7	44	0	8.4	11	6	3	4
Acetate : Butyrate	66	18	18	48	74	70	11	6	17
Propionate:Butyrate	2	3	0.4	0	8.8	6.6	2	2	4