FEED INTAKE AND MILK PRODUCTION IN DAIRY COWS WITH SPECIAL

REFERENCE TO DIETS CONTAINING GRASS AND LUCERNE

SILAGES WITH BARLEY SUPPLEMENTS

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Summary

1. The thesis provides a review of the literature concerned with food intake regulation and digestion in the ruminant, and with the utilization of nutrients for milk production in the dairy cow. The survey identified that benefits on food intake and milk production might accrue through the treatment of cereal grains with acidified formaldehyde solution prior to feeding. The main part of the thesis reports a series of 8 experiments.

2. In Experiment 1 treatment of rolled barley with 8 l/t with a reagent containing (g/kg) 410 formalin, 220 isobutyric acid, 210 acetic acid, 75 lignone sulphonate and 75 urea/utropine stabiliser led to a reduction in the rate of degradation of the cereal starch and protein in the rumen as determined using the Dacron bag technique.

3. In Experiment 2, 12 cows were used in a cyclic changeover experiment to study the effect of formaldehyde-treatment of barley on milk production. The animals were given a low protein (107 g/kg DM) silage ad libitum together with either untreated or formaldehyde-treated barley at rates of 4.0, 6.5, or 9.0 kg/d. Increasing the rate of barley supplementation reduced silage intake and increased milk production but there were no treatment effects attributable to the type of barley given.

4. In Experiment 3, 8 cows were used in a duplicated 4 x 4 Latin square experiment. The treatments were grass silage ad libitum with concentrates of barley (7 kg/d; B) or barley and fishmeal (6 kg/d and 1 kg/d; BF), with the barley in untreated or in formaldehyde-treated (T) form. The formaldehyde reagent was applied at a rate of 15 l/t. The results showed that both

inclusion of fishmeal in the diet and treatment of barley led to improvements in food intake and milk production. Milk yields for the B, BF, TB and TBF treatments were 16.91, 18.25, 18.46 and 19.60 kg/d (SED 0.30; P < 0.001). Differences between treatments in milk composition were not statistically significant but fat content tended to be slightly reduced by fishmeal supplements and by the formaldehyde-treated barley. Trends in protein content were opposite to those in fat content.

5. Experiment 4 was a duplicated Youden square experiment with 6 cows and 4 dietary treatments. These consisted of a higher protein (149 g/kg DM) grass silage ad libitum given without supplement or with supplements (9 kg/d) of barley, formaldehyde-treated barley or barley and $NaHCO_3$ (250 g/d). All supplements reduced silage intake but the effect was more pronounced with barley than with treated-barley or barley-$NaHCO_3$. Milk yields for the unsupplemented diet, for the barley diet, for the treated-barley diet and for the barley-$NaHCO_3$ diet were 16.36, 18.10, 19.50 and 18.72 kg/d. There were no significant effects on milk composition but the formaldehyde-heated barley led to similar trends in composition to those in Experiment 3.

6. In Experiment 5, 12 first-lactation cows were used in a cyclic changeover experiment to study the effect of type of silage given with or without barley or formaldehyde-treated barley on milk production. The animals were given grass (G) or lucerne (L) silage ad libitum either with no supplement or with untreated (B) or formaldehyde-treated (TB) barley (15 l/t) at 7.0 kg/d. The barley supplement reduced silage intake more than the treated barley but the effect was only evident with the lucerne silage.

Milk yields for G, GB, GTB, L, LB, and LTB treatments were 14.37, 16.23, 16.12, 13.79, 14.96 and 15.91 kg/d (SED 0.40; P < 0.001). Differences between treatments in milk composition were non-significant but were similar to those observed in Experiment 3.

7. In Experiment 6, 12 cows were used in a cyclical changeover experiment in which the treatments consisted of grass silage (G) or lucerne silage (L) or the mixture of both silages (GL) ad libitum together with barley (B) or formaldehyde-treated barley (T) (15 l/t) at 7 kg/d. Treatment of barley with formaldehyde reagent had no effect on the intake of grass silage or the mixture of grass and lucerne silage but there were slight increases in the intake of lucerne silage. Milk yields for GB, GTB, GLB, GLTB, LB and LTB treatments were 22.88, 23.19, 21.86, 22.53, 20.49 and 22.29 kg/d (SED 0.646; P < 0.01). Differences between treatments in milk composition were not statistically significant but were similar to those observed in Experiment 3.

8. In Experiment 7, 8 cows were used in a duplicated 4 x 4 Latin Square experiment. The treatments consisted of lucerne silage ad libitum together with barley (B) or barley treated with 8, 11.5 or 15 l/t of acid-formaldehyde reagent. The results showed that 8 l/t had no effect on silage intake but the application rate of 11.5 and 15 l/t increased silage intake slightly. Milk yields for the 0, 8 l, 11.5 l and 15 l treatments were 21.36, 22.42, 22.21 and 22.74 kg/d (SED 0.57). Differences between treatments in milk composition were not statistically significant but fat content tended to be reduced by acid-formaldehyde reagent especially with the 15 l/t treatment.

9. Experiment 8 investigated the possibility that differences in intake between grass and legume silage noted in earlier experiments were oropharyngeal in origin. Five rumen cannulated sheep were used in an experiment of Youden Square design. The treatments consisted of three diets: grass silage alone; lucerne silage alone; and a mixture of grass and lucerne silage in equal parts. For the diets containing grass silage, lucerne silage and grass and lucerne silage (2 diets) half the silage was consumed by mouth and half the silage was given via the rumen cannula.

   The digestibility of lucerne silage was 24% less than the grass silage and the sheep consumed more lucerne silage DM than grass silage DM. For the mixed grass silage/lucerne silage diets the administration either of the grass or lucerne silage through the rumen cannula had no effect on intake.

10. The Discussion of the thesis considers the influence of the type of silage given to cows (grass or lucerne) and the type of supplement (barley or formaldehyde-treated barley) on food intake and milk production. The main conclusion is that formaldehyde treatment of barley supplements can lead to significant improvements in silage intake and/or milk production. However the quantitative importance of the effect depends on the nature and composition of the silage given the level of supplementation and the rate of application of the formaldehyde reagent.

SECTION I

INTRODUCTION

Ruminant animals are characterised by a multicompartmental stomach having four parts: rumen, reticulum, omasum and abomasum, which is most like the gastric stomach of simple-stomached animals. At birth the rumen and reticulum are undeveloped but they increase in size as the animal matures and is weaned, and in the adult the organs and their contents may account for as much as 20% of the total body weight.

Like other mammals, ruminants do not secrete cellulase and hemicellulase - the enzymes responsible for the breakdown of the cellulose and hemicellulose which comprise the main structural carbohydrates in plants. However, as a result of a symbiotic relationship between ruminants and anaerobic microorganisms which colonize and proliferate in the rumen and reticulum, the animals can utilize coarse, bulky forage foods. The essential cellulase and hemicellulase are provided through the activities of the rumen microbes.

The main nutrients absorbed by simple-stomached animals are sugars, long-chain fatty acids and amino-acids which are taken up in the small intestine. However, in the ruminant intestinal digestion is preceded by rumen fermentation. This leads to the production of volatile fatty acids - mainly acetic, propionic and butyric acids with small amounts of isobutyric, valeric and isovaleric acids - which are absorbed and form a major portion of the animal's nutrient supply (Dobson and Phillipson, 1968).

The nature of the ruminant digestive processes and the anatomical

and physiological adaptations which have taken place during their evolution confer major advantages in the animal's ability to consume and utilize forage foods, and the absorption of fermentation end-products has led to adaptive changes in the animal's intermediary metabolism (Ballard et al., 1968). The synthesis of microbial protein in the rumen enables ruminants to use dietary sources of non-protein-nitrogen, such as ammonia and urea, and makes the animals less dependent on a balanced supply of dietary protein (Virtanen, 1969). The rumen microbes also act as a source of supply of vitamins and trace nutrients for the animal (Hungate, 1966).

Ruminants are not without disadvantages, however, and some of these are accentuated under modern conditions of animal production. In the high-yielding dairy cow, in particular, the high demand for energy exceeds the animal's ability to derive that energy supply solely from forage foods, because of limitations on forage intake. Similarly, the cow's specific requirements for glucose for lactose synthesis and for amino acids for milk protein synthesis may be difficult to meet because of constraints on their supply imposed by the composition of forage foods and the fermentation of dietary carbohydrate and protein constituents in the rumen. Limitations on the supply of nutrients may be overcome through supplementation of the diet with concentrate foods, and starch-rich cereal grains are widely used for this purpose. However, such supplements have drawbacks because their inclusion in the diet leads to a reduction in the ad libitum intake of forage. Where high levels of starchy concentrates are used there may be additional problems of metabolic imbalances in rumen fermentation, disorders in intermediary metabolism and depressions in milk fat content (Kronfeld, 1969; Rook, 1983).

The following review of the literature considers aspects of food intake regulation and milk production in the dairy cow, with particular reference to the prospect of manipulating food intake and milk production through the use of grain supplements chemically treated to modify their effects on rumen fermentation and forage intake.

FOOD INTAKE IN THE RUMINANT

In their review of the literature on food intake in the ruminant, Baile and Forbes (1974) pointed out that the characteristic features of ruminants, indicated above, must be borne in mind in any discussion of intake regulation. They also drew attention to the fact that for generations domesticated ruminants have been selected for various desirable characteristics and that this probably has had an influence on their anatomy and physiology. For example, in the high-yielding dairy cow, selection for enhanced mammary size and activity has almost certainly had consequences in terms of the basic regulation of food intake and energy balance. Similarly, genetic selection for beef production and for rapid growth and early fattening may have been linked with changes in the hypothalamic control of appetite and feeding behaviour. Baile and Forbes (1974) emphasized that the regulation of food intake in domesticated ruminants may differ substantially from that in non-domesticated ruminants found in the natural state.

Regulation of energy balance

Under many, even extreme, climatic conditions, losses of energy to the environment are met with accuracy by the amount of energy consumed in food. Energy requirements increase during exposure to low environmental temperatures (Baile and Forbes, 1974) and in animals fed

ad libitum the increased energy need is generally met by an increased
energy intake. Thus the body weight of mature adult animals is
normally maintained relatively stable, despite variations in energy
loss. However, this may not be the case in animals given diets of low
caloric density (digestible or metabolizable energy per unit mass or
volume). Rats (Peterson and Baumgardt, 1971), chicks (Hill and Dansky,
1954) and pigs (Owen and Ridgman, 1968) have not been able to eat
enough to maintain their energy balance when given diets of low energy
content, and it is evident that ruminants can be similarly affected
(Nelson et al., 1968). Whereas simple-stomached animals are rarely
given food with low-energy content, ruminants commonly consume
substantial amounts of bulky low-energy forage foods.

Effects of environment

At high environmental temperatures food intake is reduced
(Ragsdale et al., 1950; Reik et al., 1950) whilst under cold conditions
food intake is increased (Ragsdale et al., 1950; Moose et al., 1969).
Under hot conditions depressions in food intake may cause the animal to
be in negative energy balance, and at temperatures above 40°C ruminants
become anorexic (Ragsdale et al., 1950; Appleman and Delouche, 1958).
These effects may be due to the general influences of exposure to high
temperatures on the animal's behaviour or metabolism or to more
specific influences operating at a ruminal level. Gengler et al.
(1970) showed that increasing the temperature of rumen contents in
cattle from 38.0^{o}C to 41.3^{o}C using heating coils, depressed food intake
by 15%. Also Bhattacharya et al. (1968) found that in animals held at
a temperature of 30°C reducing the temperature of the rumen by adding
cold water (5°C) resulted in a decrease in body temperature and a 24%

increase in food intake. Surprisingly, in the same experiment, addition of warm water (49°C) did not depress intake significantly. Heat-stressed cattle increased their food intake when given cold water to drink (Thompson et al., 1949; Ittner et al., 1951).

Grazing sheep or cattle require 20-70% more energy for maintenance than stall-fed animals. Part of this increase in maintenance requirement is due to increased heat loss to meet the environmental demands of pasture conditions and part is due to the energy required for exercise (Blaxter, 1962). Lactating ewes grazing good pastures have been able to compensate for much of the energy expenditure required for grazing by eating more to maintain energy balance (Coop and Drew, 1963; Hadjipieris and Holmes, 1966), and this appears to be true for cattle also (Huffman, 1959; Stiles et al., 1968).

Effect of lactation

The increased energy requirement of lactating animals is generally associated with increased food intake (Cook et al., 1961; Daves, 1962; Arnold, 1969). Lactating cows were shown to eat more than their non-lactating counterparts in comparisons of monozygotic twins given fresh grass (Hutton et al., 1964) or the same amount of concentrates, with hay ad libitum (Campling, 1966). Forbes (1970) summarised data showing that food intake and milk yield in lactating cows are generally positively correlated, and higher food intakes were reported for ewes rearing twin lambs than for those with singles (Coop and Drew, 1963; Hadjipieris and Holmes, 1966). Forbes (1970) also pointed out, however, that the increase in food intake that occurs after parturition lags behind the corresponding increase in milk yield. Peak levels of food intake are not usually achieved until milk yield is declining in

the period after peak milk production.

There is a broad positive correlation between food intake and milk yield during the mid-lactation period, and a similar correlation between the food intake and milk production for the complete lactation period (Curran and Holmes, 1970; Curran et al., 1970). However, early in lactation many cows, especially high-yielding animals, mobilise body tissue due to the failure of the animals to regulate energy balance to maintain body weight (see Forbes, 1977). Thus during lactation the animal does not always adjust its energy intake to meet demand in the short-term.

Various physiological adaptations occur in the lactating animal during the early phase of lactation including an increase in the capacity of the ruminoreticulum and intestines (Tulloh, 1966). This adaptation may take place over a period of several weeks to allow the animal to adjust its energy intake to meet its requirement. In mid- and late-lactation the weight lost in the early lactation period is normally recovered as milk yield declines and food intake remains at a high level.

Effect of metabolic rate and growth

Several studies have shown that body weight decreases for a period after thyroxine implantation (Blaxter et al., 1949; Ferguson, 1958; Kirton and Barton, 1958). This is probably due in part to a reduction in gastrointestinal contents resulting from an increasing gastric motility (Balch, 1952) and intestinal peristalsis (Kirton and Barton, 1958). Food intake has also been increased in most experiments but not always enough to prevent the mobilization of energy from body tissue to meet the animal's needs (Ferguson, 1958; Lambourne, 1964).

Implantation with diethylstilbestrol has been shown to stimulate growth in ruminants (Dinusson et al., 1950; Davey and Wellington, 1959). In a study with steers given an all-concentrate ration, body weight increased about 20% faster with a 6% increase in food intake in animals implanted with the anabolic agent (Oltjen et al., 1965). Such data indicate that the animals may adjust food intake to meet energy needs arising from increasing metabolic requirements for growth.

Effect of dietary energy concentration

Studies on the effects of dilution of the energy content of the diet have been conducted using various diluent materials such as water, which is easily absorbed and adds no digestible calories, or straw, which contains some digestible energy but lowers the overall digestibility and energy content of the diet. The experiments have shown that there is an ability to compensate for dilution of the diet with water in the calf (Pettyjohn et al., 1963), sheep (Davis, 1962) and cow (Thomas et al., 1961) so long as the degree of dilution is not extreme. However, silage intake in steers was not depressed by addition of water to the diet even when the water content was raised to over 80% (Thomas et al., 1961). Since superficial water on a food can be removed rapidly by absorption from the rumen, extracellular water is unlikely to have an important effect on rumen distension. Wilson (1978) found that even with wet immature herbages there was no correlation between DM content of the crop and voluntary intake in sheep, although there was an indication that intake was impaired at DM levels below 125g-145g DM per kg. This critical level is in practice likely to occur only with very immature herbage in the spring.

When ground straw or hay was used as a diluent for a high energy

food to prepare diets with digestibilities of energy varying from 54 to 80%, growing lambs (Montgomery and Baumgardt, 1965; Owen et al., 1967; Owen et al., 1969; Moose et al., 1969) and mature ewes (Donefer et al., 1963) increased their food intake to completely accommodate the reduction in dietary energy concentration. Similarly in studies with other diluents - sawdust, kaolin and verxite (Baile and Pfander, 1967; Baumgardt, 1969; Baumgardt and Peterson, 1971) - sheep compensated for dietary additions so long as the digestible energy concentrate of the complete pelleted food was approximately 10.5 MJ/kg or higher in adult sheep or 16.7 MJ/kg in young growing sheep.

Various studies with growing cattle have investigated the effect of dilution of concentrate diets with forage. When the forage was long or chopped, the cattle consumed a constant amount of digestible energy with forage additions up to 30% of the total diet, while above that level the animals ate a reduced weight of DM, and showed reduced growth rate as a result of the reduced energy intake (Parrott et al., 1968; McCullough, 1969; Theurer, 1970). Studies with lactating cows given four milled diets having hay to concentrate ratios of 90:10, 60:40, 30:70 and 0:100 showed that except for the 90:10 diet similar amounts of digestible energy were consumed. There were no differences in milk yield between treatments during a 24 week experiment (Ronning and Laben, 1966) except that those cows given the 90:10 diet were unable to consume sufficient food to meet their requirement, and produced less milk. Similarly in a comparison involving three complete diets containing different sources of forage (alfalfa, cotton seed hulls or native grass hay) and concentrates in a 30:70 ratio and a conventional system of feeding forage and concentrates separately, the digestibility of DM of the four diets was between 63 and 73%. The results showed

that the cows consumed an approximately constant digestible energy intake irrespective of the composition of the diet (Villavicencio et al., 1968). In another study by Nelson et al. (1968), twenty Holstein cows were given five completely pelleted diets containing 100, 75, 50, 25, or 0% hay, giving energy digestibilities of 41, 49, 58, 66, and 78% respectively. The cows ate approximately the same quantity of digestible energy with the 25 and 0% hay diets, and although digestible energy intakes with the 75 and 50% hay diets were reduced, milk yield was unaffected. With the 100% hay diet digestible energy intake was sufficiently reduced to cause a significant fall in milk yield.

In a summary of the results for voluntary food intake and dry matter digestibility in 114 experiments with lactating dairy cows (283-660 kg liveweight) given rations with DM digestibilities between 52% (all forage) and 80% (all concentrate) Conrad et al. (1964) showed that cows compensated for dilution of the digestible energy content of diet if the digestibility of DM in the food was above approximately 67%. Below that level, digestible energy intake declined with digestibility value. However, Conrad et al. (1964) pointed out that the value of 67% would be applicable only for the conditions under which their experiments were conducted. Baile and Forbes (1974) also emphasised that the dry matter digestibility value of 67% has sometimes been regarded with unjustified significance as an exact point above which energy balance can be maintained by ruminants. However, it appears that lactating cows, like sheep and growing cattle, can regulate food intake to maintain a constant digestible energy supply provided that the diet has a digestible energy concentration above the 'critical point' in the intake-digestibility relationship described by Conrad et al. (1964) (Figure 1.1).
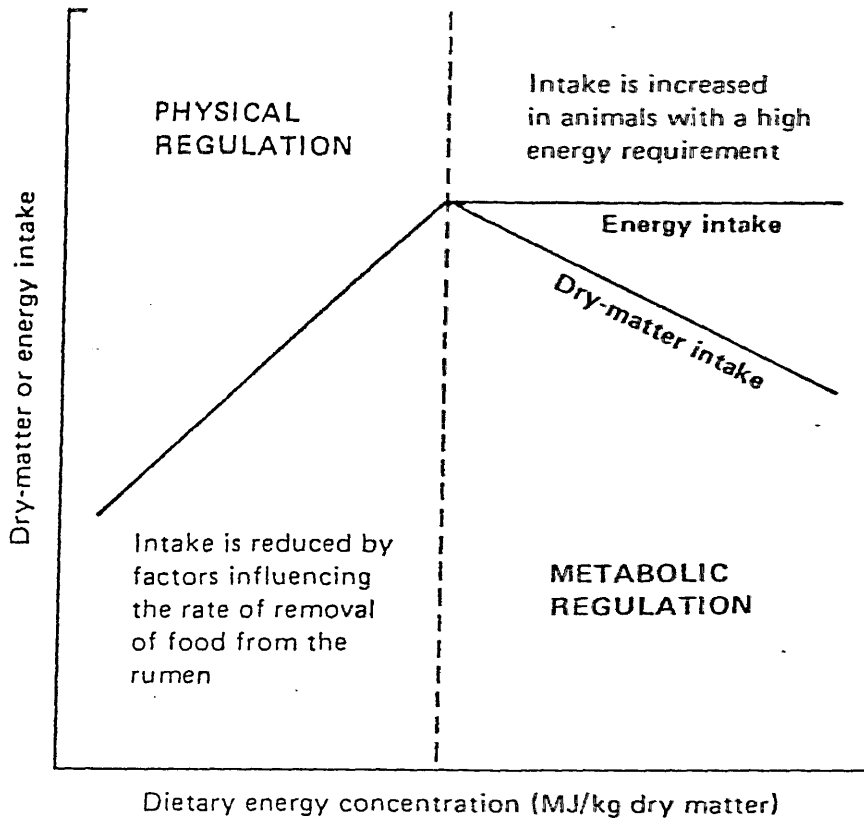
Figure 1.1. A schematic representation of the relationship between
voluntary food intake in the cow and the concentration of
energy in the diet. The diagram shows the change that occurs
as the dominant mechanism for intake regulation changes from
a physical to a metabolic type. Where intake regulation is
physical, energy intake and dry-matter intake both increase
with the energy content of the diet. Under these conditions
also, intake is reduced by physical characteristics of the
diet (e.g. increase in chop-length), and by nutritional and
physiological factors (e.g. low dietary protein contents, high
dietary starch contents and low efficiency of rumination) that
reduce the rate of removal of plant fibre constituents from the
rumen. Where intake regulation is metabolic, energy intake
remains constant as dietary energy concentration is increased,
and dry-matter intake is reduced. At all stages of the relation-
ship intake tends to be increased in animals with a high energy
requirement.
(After Thomas and Chamberlain, 1982)

Regulation of energy balance: conclusions

The results of experiments involving variation in energy requirements of the animals through alterations in loss of heat, deposition of body tissue, or milk yield, and studies of the effects of varying the concentration of digestible energy in the diet, have shown that ruminants tend to maintain a constant energy balance by changing their food intake to accommodate the effects of change in physiological or environmental conditions. But their ability to compensate for changes in energy demand or diet composition is insufficient to cope with diets of high bulk and low energy concentration. Under these circumstances intake is limited apparently by physical constraints on the amount that can be consumed.

Physical regulation of food intake

Physical constraints on intake have been envisaged to occur in at least three different ways: oropharyngeal regulation; intestinal-fill; and rumen-fill. It is now considered that oropharyngeal factors are probably not very important in the regulation of food intake in ruminants. As evidence of this, cattle in which food boluses were continuously removed from the rumen continued to eat for much longer periods than normal and consumed larger quantities of food than during normal feeding (Campling and Balch, 1961). Similarly intestinal fill is regarded not to be of great importance except under specific circumstances (Blaxter et al., 1961; Purser and Moir, 1966; Campling and Freer, 1966; Ulyatt et al., 1967). In contrast there is strong evidence that the intake of forage diets may be regulated through a rumen-fill mechanism and this regulation can be illustrated by the effects observed with intraruminal addition or removal of food,

intraruminal addition of water-filled balloons, and by a variety of results from experiments involving changes in the chemical composition or physical form of the diet.

Intraruminal addition of food or removal of food from the rumen

With cannulated cows given hay ad libitum removal of hay from the reticulorumen led to changes in food intake. The cows, which were fed in one meal per day, normally ate 8.47-10.70 kg hay DM/d but when hay boluses were removed from the rumen, eating was prolonged and total intake was increased by 70-85% to 14.4-17.75 kg/d (Campling and Balch, 1961). The authors reasoned that at any particular meal the intake of forage diets was probably governed by the amount of digesta in the reticulo-rumen and that this amount was unlikely to be affected by habituation of the cows to the amount of diet consumed on previous days. However, in other experiments designed to investigate the effect of altering the amount of digesta in the reticulo-rumen immediately before feeding or during feeding or after a meal, transfer of ruminal digesta from a donor animal to the reticulo-rumen of an experimental animal resulted in a decrease in the voluntary intake of hay but the reduction was not as great as the amount of dry matter added in the transferred digesta (Campling and Balch, 1961). The greatest reduction in food intake was 2.6 kg/d when the digesta transfer was made immediately before a meal, the corresponding value was 1.76 kg/d when the addition of digesta was made 11.0 hours after feeding. These results show the ability of the animal to compensate for a change in the weight of ruminal digesta by decreasing or increasing voluntary intake of hay (Campling and Balch, 1961).

Carr and Jacobson (1967) also conducted experiments to study the

effect of additional inert material placed in the rumen on voluntary food intake. Containerised polyethylene cubes or water were added to the rumen at levels of 1.6, 4.9 and 8.2% of metabolic body weight. Voluntary intake of chopped alfalfa hay was reduced by 0.5, 0.4 and 0.1 kg/d respectively. These differences were not significant, however. In three additional experiments, digesta was removed from the rumen at levels of 1.6 and 4.9% of metabolic body weight prior to feeding and 8.2% metabolic body weight after feeding. A significant increase in DM intake of 0.4 kg/d occurred when 8.2% of metabolic body weight was removed three hours after feeding, but other treatments were without effect. These results were interpreted to mean that bulk added to the rumen in physiological amounts was not an important factor determining the DM consumed.

Addition of water to the rumen

In studies in which the volume of rumen contents of cows was increased by injection of water into rubber bladders placed in the ventral rumen, the introduction of 45 kg of water reduced hay intake by 2.4 kg/d (Campling and Balch, 1961); and similar results were obtained with a grass silage diet (Farhan and Thomas, 1978).

Injection of water either directly into the rumen or into a balloon in the rumen of goats eating a concentrate diet did not reduce intake until the ratio of water injected to food eaten approached 10:1 (Baile et al., 1969). It was concluded that in goats fed a concentrate ration, ruminoreticular distension was not a factor determining meal size.

Rumen-fill

Many studies have indicated that meals of various forages are terminated when the weight or volume of contents in the rumen or the distension of the rumen approaches a certain critical level, and 'rumen-fill' has been proposed to be a major factor in intake regulation in animals given long- or chopped-forage foods (Blaxter et al., 1961; Campling and Balch, 1961; Purser and Moir, 1966). In studies by Ulyatt et al. (1967) sheep given two hay diets and a dried grass diet consumed different amounts of dry matter ad libitum but had similar volumes of rumen digesta, as determined by dilution estimates made using polyethylene glycol as marker. Similarly in studies with dairy cows Freer and Campling (1963) found that animals given hay and dried grass diets consumed different amounts of food but had a similar weight of dry matter in the rumen determined 5 hours after feeding.

Constant amounts of digesta in the rumen have not been found in all dietary comparisons, however. Campling et al. (1961) found that there was 35% more dry matter in the rumen in cows fed hay than in those fed oat straw, and Campling (1966a) and Waldo et al. (1965) both reported differences in rumen dry matter weight between cows given hay and silage diets. In their review of the literature Baile and Forbes (1974) calculated that in cattle given a range of diets for approximaely 6 hours per day there was an average increase in the weight of total digesta of 48% during the feeding period and an average increase in the weight of digesta dry matter of 96%. However, for both total and dry weights of digesta, the response on feeding was highly variable between diets, and there was a substantial spread in the values for post-feeding digesta weights. On the basis of this information and taking account of the fact that the weight of rumen

contents increases in response to physiological stimuli, eg lactation, Baile and Forbes (1974) concluded that rumen-fill mechanisms based on some perception of distension or stretch were operative with forage diets but that the 'critical' rumen-fill was a variable rather than a constant term.

Ruminal digestion and outflow

Under circumstances where intake is regulated by rumen-fill mechanisms the rate of removal of food residues from the rumen is of crucial importance. Food residues are effectively removed by digestion and by onward passage through the alimentary tract and these processes are influenced by the chemical composition and physical form of the food and by the digestive activities of the rumen microbes. In cows given diets of hay or oat straw, Campling et al. (1961) found that the weights of dry matter in the rumen after feeding were 11.9 kg and 10.4 kg respectively. However the rate of digestion of the straw was low compared with the hay and as a consequence the weights of dry matter in the rumen prior to the next meal were similar for the two diets. Where the rate of digestion of straw in the rumen was increased through supplementation of the diet with an intraruminal infusion of urea, the intake of straw and the weight of dry matter in the rumen after feeding were both substantially raised (Campling et al., 1962).

Reflecting the influence of rate of digestion on rumen-fill and voluntary intake, various studies have demonstrated significant correlations between intake and the digestibility of forage dry matter or organic matter (Campling et al., 1961; Blaxter et al., 1961; Freer and Campling, 1963; Castle et al., 1983). Also, as discussed later, starchy concentrate supplements, which depress ruminal cellulolytic

activity, reduce the rate of forage intake (Blaxter and Wilson, 1963; Campling and Murdoch, 1966; Tayler and Wilkins, 1976).

The outflow of food residues from the rumen can be increased through processing of forage by fine grinding and this also increases the intake of low digestibility forages. For example, Campling and Freer (1966) found that in cows given straw diets fine grinding of the diet increased voluntary intake by 26% but similar effects were not obtained with diets of hay (Campling et al., 1963) or dried grass (Campling and Freer, 1966), which were of higher digestibility. In a review of these experiments Campling and Freer (1966) suggested that the beneficial effect of fine grinding of forages may be lost with hay and dried grass diets because of an accumulation of food residues in the lower gut, leading to effects on ad libitum intake attributable to 'intestinal-fill' mechanisms.

Nitrogen supplementation

The effects of dietary nitrogen supply on ruminal digestion and food intake have already been referred to and have been demonstrated in various studies (Morris, 1958; Campling et al., 1962; Hemsley and Moir, 1963; Crabtree and Williams, 1971). However, nitrogen supply can also affect intake through influences on the supply of amino acids to the small intestine. This was first demonstrated by Egan (1965) who showed that in sheep given a poor-quality straw diet intake was substantially increased by an infusion of casein into the abomasum, with almost no effect on ruminal cellulolytic activity and the rate of ruminal digestion of the forage. Corresponding experiments with silage supplemented with intra-abomasal infusion of casein did not change the silage intake (Hutchinson et al., 1971). Whilst marked intake responses

to postruminal or parenteral supplements of methionine have been noted
in some experiments, these effects are not always obtained (Barry et
al., 1973; Kelly and Thomas, 1975; Gill and Ulyatt, 1977; Barry et al.,
1978; Shamoon, 1984).

Factors affecting the intake of specific diets

Silage

Cows generally consume less dry matter when given the same forage
conserved as silage than as hay (Moore, Thomas and Sykes, 1960; Thomas
et al., 1961; Brown et al., 1963). For example, with lactating dairy
cows given silage and hay prepared from the same sward, Murdoch and
Rook (1963) recorded intakes of 14.4 kg DM/d with hay and 9.06 kg DM/d
with silage. Similarly in a corresponding study with non-lactating
fistulated cows, Campling (1966a) found that although silage and hay
had similar digestibilities, silage residues had a longer retention
time in the digestive tract. This was associated with an intake
reduction of 28% and with a reduced weight of digesta in the
reticulo-rumen immediately after feeding. Results corresponding to
those in the cow have also been reported with beef cattle (Culpin,
1962) and with sheep (Harris and Raymond, 1963; Murdoch, 1964).

Numerous studies have been undertaken to establish relationships
between the presence of fermentation end-products in silage and dry
matter intake, and to identify individual products having a role in
intake regulation. Several studies have found statistically
significant relationships between intake and the fermentation quality
of silages, expressed in terms of silage pH, lactic acid content,
volatile fatty acid content, ammonia content etc. (Wilkins et al.,
1971; Wilkins et al., 1978; Thomas and Thomas, 1985) but unequivocal

demonstrations that these constituents themselves influence intake have been less easy to obtain.

In several experiments effects of silage pH and free acid content on intake have been investigated through the partial neutralization of silage with sodium bicarbonate. In some experiments statistically significant improvements in intake have been observed but the responses are inconsistent and not always obtained (McLeod et al., 1970; Thomas and Wilkinson, 1975; Lancaster and Wilson, 1975; Farhan and Thomas, 1978). In other studies free acid content has been altered through supplementation of the diet with lactic acid or through infusion of lactic acid into the rumen. In an experiment in which silage pH was reduced from 5.4 to 3.8 by lactic acid addition, McLeod et al. (1970) found that the intake of sheep was reduced by 22%; there were associated disorders in acid-base balance and a reduction in blood and urine pH but not in rumen pH. Similarly Morgan and L'Estrange (1977) observed adverse effects of lactic acid on the intake of dried grass by sheep when the acid was given at high levels as an intraruminal infusion. In an experiment with calves, Thomas et al. (1980) found that dietary supplements of lactic acid reduced DM intake by 11.5% but the effect was lost when the animals were also given a supplement of 50g of fishmeal per day. The authors suggested that the responses to fishmeal were related more to correction of the energy to protein balance in the diet than to an effect of fishmeal on digestion or acid-base balance. Similarly the supplementation of silage with fishmeal significantly increased silage DM intake and liveweight gain compared with non protein nitrogen supplements given to young calves (Cottrill et al., 1976).

In a study of silage treated to give a range of acetic acid

contents (20-88 g/kg DM) and to maintain the pH at 4.1 and moisture

content at 75% by adding solutions of the acid and potassium hydroxide,

Hutchinson and Wilkins (1971) found that the treatment had no effect on

DM intake by the sheep. They concluded that high acetate levels per se

were unlikely to reduce silage intake when the pH of the silage was

constant. Ulyatt (1965) found that herbage intake was reduced by

intraruminal infusion of acetic acid but the sigificance of this

observation in relation to the regulation of silage intake is difficult

to assess.

Intraruminal infusion of nitrogenous silage-fermentation products

such as tyramine, tryptamine (Thomas et al., 1961; Neumark et al.,

1964), histamine (McDonald et al., 1963) or γ-amino butyric acid

(Clapperton and Smith, 1983) has not generally reduced silage intake.

On the other hand reductions in intake have been obtained in response

to infusions of ammonia salts (Thomas et al., 1961). Also in goats

given high-concentrate diets (mostly rolled grains and 17g N/kg) the

injection of ammonium chloride intraruminally shortened meal length by

20 to 30% and reduced the rate of eating and meal frequency (Conrad et

al., 1977).

Farhan and Thomas (1978) found that the placement of water-filled

balloons in the rumen of cows reduced silage intake by 16.5%, an

observation suggesting that intake is regulated through a rumen-fill

mechanism. Consistent with this, sheep given minced silage as compared

with unprocessed chopped silage had a significantly higher DM intake,

though the digestibility of minced silage was reduced (Thomas et al.,

1976). Corresponding results have also been obtained by French workers

(Demarquilly, 1973; Dulphy et al., 1975) who found a reduction in chop

length to the range 1-2 cm consistently to increase silage intake in

sheep. Also in a study with sheep, Deswysen et al. (1978) found dry matter intake was higher with short than with long silage of similar digestibility; the sheep on long silage had a lower rate of eating and shorter ruminating time than those on short silage, and the incidence of pseudo-rumination (i.e. rumination reflex not accompanied by the regurgitation of a digesta bolus) was significantly greater. In an experiment with cows, dry matter intake was increased as silage chop-length was reduced but the retention time of silage residues in the entire digestive tract was not significantly affected by chop-length (Castle et al., 1979).

In their review of the literature, Thomas and Chamberlain (1982) concluded that the "feeding behaviour of animals given silage diets is influenced by the presence of some as yet unidentified compounds". They postulated that chemical end-products of silage fermentation may affect silage intake through influences on "rumen fill".

Rapid infusion of 2.25 l of lucerne silage juice into the rumen of sheep given hay reduced rumen motility and rate of eating and intake was depressed for the first 1.5 hours after feeding; smaller effects were obtained when the juice of formaldehyde treated silage was used (Clancy et al., 1977). Various chemical solutions designed to simulate the composition of silage juice were also tested and these at best were found to be approximately 40% as effective as the juice itself. Infusion of silage juice was associated with irregular rumen motility patterns which did not return to normal until 4 hours after feeding (Clancy et al., 1977). In another study the intraruminal infusion of silage juice in sheep given hay or frozen grass ad libitum reduced intake during the fist 4 hours after feeding, especially in sheep given hay, but after that intake was relatively unaffected and total daily

intake was reduced by 19% and 14% of control levels of the hay and frozen grass respectively (Smith and Clapperton, 1981).

Grass and legume crops

The liveweight gains for sheep grazing pastures of white clover or mixtures of white clover and ryegrass are greater than those when pastures of ryegrass alone are grazed (Butler et al., 1968). Consistent with this, controlled indoor experiments have shown that the feeding value of fresh or dried legumes is superior to that of fresh or dried grass of corresponding digestibility (Moseley and Jones, 1979; Beever et al., 1980; Greenhalgh, 1981; Thomson et al., 1985). In a recent study Thomas et al. (1982) found that cows given red clover silage ate 0.21 kg more silage dry matter per day and yielded 2.4 kg/d more milk than those given perennial ryegrass silage of similar digestibility. Corresponding results have been obtained with white clover silage by Castle et al. (1983) and Castle et al. (1984), and higher DM intakes with lucerne silage than with grass silage have been reported by Chamberlain et al. (1984).

The superior feeding value of legumes is thought to be related to the behaviour of the crops during digestion. In a review of the literature Greenhalgh (1981) pointed out that about 70% of the digestible organic matter (OM) of grass is digested in the rumen, about 20% in the abomasum and small intestine and about 10% in the large intestine. However for legumes, organic matter digestion in the rumen is typically 50-55% of digestible OM intake and there is a shift in digestion to the small intestine and large intestine (Beever et al., 1972; Moseley and Jones, 1979; Beever et al., 1980), though the overall digestibility of the crops is generally lower than that of grass. In

calves, Beever et al. (1980) observed that the digestibility of OM of grass was 81.7% and of white clover was 75.0%; for the grass 71% of the digested organic matter disappeared in the rumen whilst for the clover the corresponding figure was 52%. Associated with this, the duodenal flow of non-ammonia nitrogen was similar for both diets, the rate of protein production in the rumen being 32 and 44.5g NAN/kg OM digested for the grass and clover respectively. Moseley and Jones (1979) reported that in sheep given three diets consisting of ryegrass or red clover and a mixture of two, the digestibility of organic matter (OM) was similar for the three diets and there were no differences between diets in the digestibility of N over the whole digestive tract. However the amount of N absorbed post-ruminally was 60% increased by the diet of ryegrass and clover and 115% increased by the diet of clover alone.

Differences in rate of digestion in the rumen have also been reported in comparisons of grass and legumes. Moseley and Jones (1984) measured the differences by the time taken to reach a point at which there were corresponding percentages of large (> 1 mm) and small (< 1 mm) particles in the rumen. They found that with clover the defined point was reached in 3.5 hours whereas with grass 12 hours was required. The passage of forage out of the rumen is thought to be affected by the rate of breakdown of forage material to sufficiently small particles (Balch and Campling, 1962) and very little material greater than 1 mm length is found in the abomasum or omasum (Troelson and Campbell, 1968; Grenet, 1970; Reid et al., 1977). Troelson and Bigsby (1964) and Troelson (1967) reported a direct relationship between the voluntary intake of hay by sheep and the rate of breakdown to fine particles and Troelson and Campbell (1968) showed that as

voluntary intake increased, the degree of fineness of particles appearing in the omasum became greater. However, they also showed that the size of omasal particles was greater in sheep fed alfalfa (Medicago sativa) than in sheep fed grass hay. They concluded that this difference was associated with variation in the size of alfalfa and grass particles passing from the rumen and suggested that the effects were attributable to between-crop differences in the structure and composition of the cell wall. As compared with grass, legumes have relatively less of their digestible organic matter in the form of cell wall (Osbourn et al., 1975). Additionally, however, microscopic examination of particulate material collected from the rumen has shown differences in the shape of particles between grass and legumes (Troelson and Campbell, 1968; Moseley and Jones, 1984). These may be important in influencing the rate of particle flow through the rumino-omasal orifice.

Forage and concentrate mixtures

In animals given forage foods ad libitum, supplementation of the diet with concentrate foods leads to a change in forage intake (Morris, 1958; Blaxter et al., 1961; Campling et al., 1962; Coombe and Tribe, 1963; Hemsley and Moir, 1963; Campling and Murdoch, 1966). The change in intake depends on the type of forage and its digestibility (Campling and Murdoch, 1966) and on the stage of lactation, since forage intake is increased in the mid and late compared to the early stages of lactation (Ostergaard, 1979). The effects also vary with the nature and the level of supplementary food (see Campling and Lean, 1983). Generally, pronounced negative effects occur when supplementary foods consist of readily digestible starchy materials (Blaxter and

Wilson, 1963; Campling and Murdoch, 1966; Castle and Watson, 1976;

Tayler and Wilkins, 1976; Bines, 1979; Castle and Watson, 1979; Harb

and Campling, 1983). However, with poor-quality forages containing

small amounts of nitrogen, such as cereal straws, there may be

pronounced increases in voluntary intake when high protein foods or

urea are given as supplements (Morris, 1958; Campling et al., 1962;

Coombe and Tribe, 1963; Hemsley and Moir, 1963; Crabtree and Williams,

1971). For example, the intake of barley straw by cattle was increased

by 25% when the diet was supplemented with a concentrate rich in

soyabean meal (Lyons et al., 1970).

Protein supplements. Increases in the voluntary intake of low protein

foods in response to non-protein nitrogen supplements (Campling et al.,

1962; Coombe and Tribe, 1963) are thought to be partly related to an

improvement in cellulolytic activity in the rumen and partly to

"improvement in the protein status of the animal" (Campling, 1966).

Infusions of casein or urea at a rate of 4.5g nitrogen/d into the

duodenum of sheep resulted in an increase in the intake of a chaffed

oat hay (35g crude protein/kg) of 42% with casein and 12% with urea

(Egan, 1965). This increase in intake was associated with a

substantial improvement in nitrogen balance. Egan also reported that

the rate of digestion of cotton threads in the rumen, digestibility of

DM, mean retention time of feed residues and number of rumination chews

were all unaltered by casein infusion. In contrast, infusion of urea

into the duodenum increased the rate of cotton thread digestion in the

rumen and led to a non-significant reduction in the retention time of

food residues in the alimentary tract. Egan suggested that the amount

of protein absorbed from the small intestine has a direct effect on

voluntary intake not associated with recycling of nitrogen to the rumen.

In research concerned with the development of high forage diets for the winter feeding of cows there has been a particular emphasis on selection of the supplementary food as a means of maximizing silage intake. The effect of various supplements on silage intake has been summarized by Castle (1982; Table 1.1). Highest substitution rates were observed with hay and with barley while protein supplements gave much reduced substitution values. The crude protein in grass silage is rapidly degraded in the rumen, especially when the NPN content of the silage is high (Chamberlain et al., 1986), and this may partly explain the pronounced effects of the protein foods. However, other workers have also shown in the dairy cow that when the protein content of forage and concentrate diets is increased, food intake and OM digestibility are increased also (see Oldham and Alderman, 1982), and there are associated increases in milk yield (Polan et al., 1976; Castle et al., 1979; Gordon, 1979; Majdoub et al., 1978; Van Horn et al., 1979; Gordon, 1980). The responses tend to vary with the source of dietary protein - increments of nitrogen given as soyabean meal had greater effects on food intake than equal amounts of urea (Jones et al., 1975; Polan et al., 1976; Wohlt and Clark, 1978; Poos et al., 1979). In lactating cows given diets containing N sources of high or low solubility (22 and 42%) at two different levels (130 and 150g crude protein/kg DM), intake and milk yield were increased at the high protein level when solubility was low but not when solubility was high (Majdoub et al., 1978). However, when the protein contents of basal diets (corn silage containing 116-136g crude protein/kg DM) were increased to 152-170 g/kg by adding urea or soya bean meal, food intake

Table 1.1. A summary of the effect of supplements on silage intake.
(Values are changes in the intake of silage dry matter with
different supplementary foods)

Supplementary food	Change in silage intake (kg DM per kg supplement DM)[†]
Hay	−0.84
Barley	−0.51
Dried grass cubes	−0.36
Barley + groundnut	−0.32
Sugar beet pulp	−0.40
Soya	+0.06
Groundnut	+0.13

[†]− Denotes a decrease and + an increase in intake

(After Castle, 1982)

and digestibility were improved by either supplement. However, a milk production response was seen only in cows in their second or third lactations; cows in their first lactation did not show an increase in milk production. In another experiment in which the crude protein content at a basal diet was increased from 86 g/kg to 165-170 g/kg by the addition of urea or soyabean meal, milk production was significantly increased by both supplements. The supplements also had equal effects on digestibility of DM, but the soyabean meal supplement led to a larger response in food intake and milk production (Poos et al., 1979). Milk production was not affected by the supplements in first-lactation cows but older cows responded to both supplements equally (Poos et al., 1979).

In several experiments supplementation of grass silage with fishmeal (Garstang et al., 1979; Kirby and Chalmers. 1982; Kirby et al., 1983a; Kay and Scott, 1984) or with soyabean meal (Kirby et al., 1983b; Waterhouse et al., 1983) has increased the liveweight gain of growing cattle. The responses in gain are due partly to increased silage DM intake and possibly to an increased supply of dietary amino acids passing from the rumen to the small intestines. However, in experiments with silage diets intra-abomasal infusion of casein did not change the silage intake (Hutchinson et al., 1971) whilst intake responses to postruminal or parenteral supplements of methionine have been noted in some experiments but not others (see Thomas and Thomas, 1985).

Starch supplements. As already indicated, supplementary starchy foods have a pronounced depressive effect on forage intake. For example, with steers given ad libitum a hay with a digestible organic matter in

dry matter (DOMD) value of 600 g/kg, a reduction of 0.92 kg in day DM intake per kg of rolled barley supplement was reported (Sriskandarajah et al., 1980). Campling and Murdoch (1966) pointed out that the reduction in intake tended to be greatest with hay of high digestibility but in their experiments replacement rates ranged from 0.20 to 0.40 kg hay DM per kg supplement given. In experiments with silage diets an average reduction of 0.50 kg silage DM intake per kg of barley supplement has been found (Castle and Watson, 1975, 1976; Harb and Campling, 1983) but higher values have also been reported. For example, Ettala and Lampila (1978) found a reduction of 0.64 kg silage DM per kg of barley supplement.

The depressive effects of starch supplements on forage intake have been attributed to the influence of starch on the digestion of fibrous carbohydrates in the rumen and the associated reduction in the rate of removal of food residues. Adverse effects of starch supplementation on the digestibility of crude fibre and cellulose have been reported in animal experiments and in incubation studies with rumen fluid (El Shazly et al., 1961; Stewart, 1977; Thomas et al., 1980; Mould et al., 1983).

Salivation is normally reduced in animals receiving concentrate supplements (Emery and Brown, 1961; Balch, 1971), and the effects of starch on fibre digestion in the rumen have been associated with a reduction in salivation coupled with an increased ruminal production of VFA, leading to a low rumen pH. There is evidence that the numbers and activity of cellulolytic bacteria in the rumen are pH-dependent (Terry et al., 1969; Stewart, 1977), but El Shazly et al. (1961) showed that with starch supplementation, cellulolysis in vitro was still inhibited even when pH was controlled.

In recent experiments with sheep given hay diets with or without

concentrate supplements, rumen pH was manipulated using continuous infusions of acids (H_2SO_4:HCl:H_3PO_4; 1:1:1, W/W) and bicarbonates ($NaHCO_3$, 66% : $KHCO_3$, 34%, W/W). It was found that reduction of rumen pH to 6.0-6.1 in sheep given hay alone caused inhibition of cellulolysis and associated changes in rumen microbial population. Dry matter degradation in the rumen and the dry matter intake were also depressed. On the other hand, increasing rumen pH in sheep given hay plus concentrate diets did not greatly alter the rumen microflora nor increase cellulolysis; dry matter degradation and dry matter intake were also unaffected (Mould and Ørskov, 1984). It was concluded that both type of substrate present in the rumen and rumen pH have important effects on ruminal cellulolytic activity.

In an attempt to reduce the adverse effect of grain on forage intake Ørskov and Fraser (1975) have examined the way in which grain is processed before being fed. In initial studies they demonstrated that in sheep given dried grass diets whole barley led to a much smaller reduction in rumen pH than ground-pelleted barley and there was an associative benefit in the intake of grass. Later, an alternative approach was investigated in which the whole grain was treated with sodium hydroxide to delignify the grain coat. In comparison with rolled, ground, or ground and pelleted barley, alkali-treated grain was found to give a marked improvement in forage intake (Ørskov et al., 1978). Similar results have also been found by Sriskandarajah et al. (1980).

LACTATIONAL RESPONSES TO FEEDING

It is well established that milk yield and composition in dairy cows is influenced by the dietary supply of materials providing energy

and protein. An increase in the amount of food given typically results
in an increase in the yield of milk and milk constituents and an
increase in liveweight gain, or in early lactation a decrease in
liveweight loss. The classic studies of Blaxter (1966) and Broster
(1976) have shown that the relationship between milk yield or
liveweight and food intake are curvilinear, the response in milk yield
diminishing and that in liveweight gain increasing as the level of
feeding raised (Figure 1.2). Corresponding separate response curves in
relation to the dietary supply of energy and protein can also be
constructed and these responses are considered more fully later. In
practice, dietary protein and energy supplies are most commonly
influenced by the level of allocation of supplementary concentrate
foods to the cow and by the level of ad libitum forage intake that the
cow can achieve.

Dietary energy supply

Conventionally the level of energy supplied in the diet is
described using the metabolizable energy (ME) system, first proposed by
Blaxter (1962) and now adopted as a standard basis for rationing dairy
cows in the UK. Calorimetric studies have shown that the partial
efficiency of utilization of energy for milk production (k_{lo}) varies
with diet over a narrow range of around 60-67%, and an average value of
62% is used for rationing purposes (Ministry of Agriculture, Fisheries
and Food, 1975). The partial efficiency of utilization of ME for body
tissue gain during lactation is also rather constant and similar to
that for lactation so that there is a linear relationship between ME
intake and the total energy secreted in milk and retained in body
tissue. However, this linearity conceals the fact that a number of
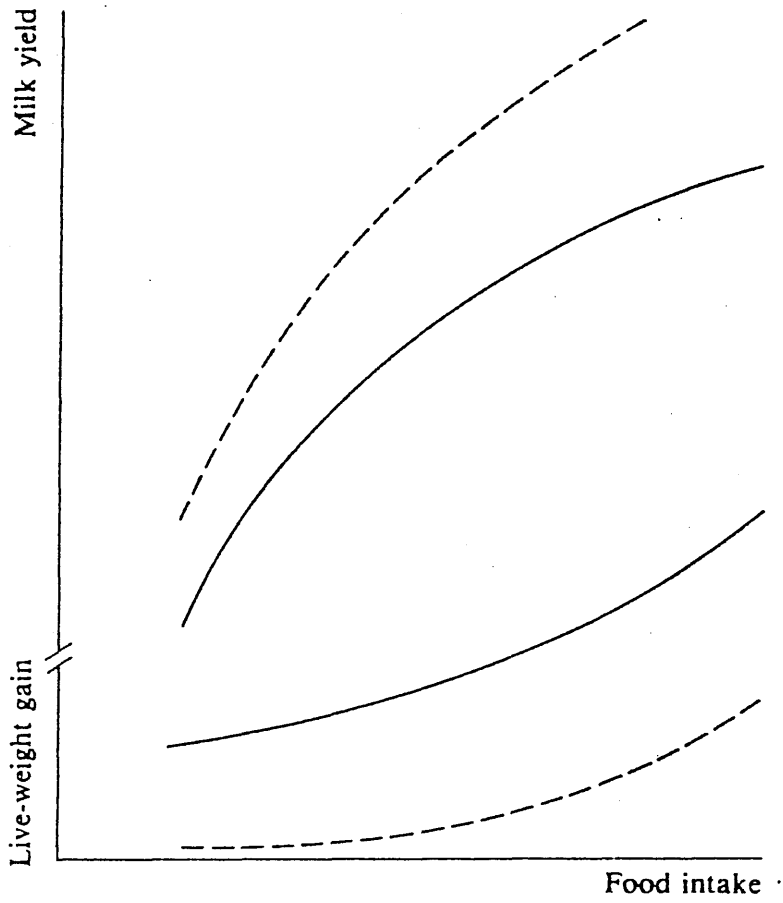
Figure 1.2.   A schematic representation of the relationships between
milk yield, live-weight gain and food intake in the cow
(-----, cows of high milk yield potential; ———, cows
of low milk yield potential).
(After Thomas and Rook, 1983)

factors influence the partition of ME use between milk secretion and body tissue deposition. In this regard the most important influences are related to the level of feeding, the cow's stage of lactation, and the chemical composition of the diet. As indicated in Figure 1.2, as level of feeding is raised a greater proportion of energy intake is partitioned towards body weight gain, though the characteristics of the response are influenced by the stage of lactation. A variety of studies have shown that the responsiveness of milk yield is related to the cow's current yield and thus is higher in early than in late lactation. These effects can, however, be overridden by the influences of diet, and some types of diet lead to a marked reduction in energy secretion in milk and a corresponding increase in energy deposition in body fat. The most fully described of these situations is that occurring with diets containing an excessively high proportion of concentrates leading to a high-propionate fermentation in the rumen and a depression in milk fat content and fat yield. Such effects can also occur, however, when there is a high uptake of starch from the small intestine (Sutton et al., 1980).

Dietary protein supply

A number of workers have reported on the lactational responses to dietary protein supply and have pointed out the importance of the interaction between dietary protein and dietary energy (Castle et al., 1979; Laird et al., 1979; Gordon, 1979; Murdoch and Hodgson, 1979; Claypool et al., 1980; Cowan et al., 1981; Oldham and Alderman, 1982; Oldham, 1984). From an analysis of all the data available Oldham (1984) concluded that under conventional conditions protein responses derive from a number of associated effects including effects on food

intake, effects on the digestibility of dietary OM and effects arising through an increased amino acid supply to the animal's tissues.

It is now recognized that amino acid supply is influenced by microbial protein synthesis in the rumen and by the passage of undegraded dietary protein to the small intestine. Thus lactational responses to dietary inclusion of proteins depend on the rumen degradability of the proteins, on the basal diet, on the effects of the protein source on microbial protein synthesis in the rumen and on the changes in the pattern of amino acid supply to small intestine relative to the requirement for milk and tissue synthesis. Twigge and Van Gils (1984) demonstrated that rumen degradable protein (RDP) deficiency occurred in animals fed on straw, and that there was an improvement in animal performance when supplementary urea was given. Also, they pointed out that RDP deficiencies can occur in less extreme circumstances, as in the work of Baraton and Pflimlin (1978) who reported a negative effect of formaldehyde-protected soyabean meal when compared with untreated soya on the performance of dairy cows receiving a basal diet of maize silage.

Feeding formaldehyde-protected soya to cattle given a basal diet of hay plus barley produced only a small non-significant increase in protein supply to the small intestine (Rooke et al., 1982) whilst a larger significant positive effect was found when the basal diet consisted of grass silage (Rooke et al., 1983). There were differences between the two experiments in the degradabilities of the untreated soya (0.90 and 0.74 for the silage and hay diets respectively) and this may in part explain the differences in response to HCHO treatment. In addition, the authors drew attention to the low rates of rumen microbial synthesis found with silage diets as a possible explanation of the

differences in response. Ørskov (1980) has pointed out that the
production of the acids by fermentation in silage effectively reduces
the supply of fermentable, carbohydrate substrates to the rumen
microflora. However, Twigge and Van Gils (1984) have commented that with
a maize silage/maize grain/soyabean meal diet given to cows in early
lactation milk yield responses to dietary protein content were absent
at levels above 140 g/kg DM. In contrast with diets consisting of
grass silage/barley and soyabean meal positive responses have been
obtained up to 220 g/kg DM. They explained these results by suggesting
that the protein degradability may be lower in maize silage than in
grass silage (see Baraton and Pflimlin, 1978).

The requirement for total dietary protein to satisfy the tissue
protein needs of the high yielding cow clearly will depend upon the
combined effects of the degradability of dietary protein and synthesis
of rumen microbial protein (ARC, 1980). Theoretical relationships
between milk yield and dietary nitrogen requirement with different
dietary and rumen conditions are presented in Figure 1.3.

Diet and milk composition

Whilst it is conventional to describe responses in lactation in
terms of dietary supply of energy and protein it is now recognised that
the composition of the diet, or more accurately the mixture of products
of digestion absorbed from the gut, has a marked influence on the
secretion of individual milk constituents.

The digestion of dietary carbohydrate, protein and lipid
components leads, as already described, to the production of volatile
fatty acids in the rumen and in the caecum and colon, and glucose,
amino acids and medium and long-chain fatty acids in the small

intestine. Changes in diet leading to changes in the composition of the mixture of products of digestion, are reflected in the effects of diet on milk composition.

Carbohydrate supply

Thomas and Chamberlain (1984) in a review of the literature concluded that the infusion of dilute solutions of acetic acid, propionic acid or butyric acids into the rumen of cows receiving basal diets of hay and concentrates brought characteristic responses in milk yield and composition. With acetic acid there was an increase in milk yield, a specific increase in milk fat content and a tendency for milk protein content to be depressed, whereas with propionic acid, fat content was depressed and protein content was increased. In experiments with cows given silage and concentrate diets, Chalmers et al. (1980) confirmed that intraruminal infusion of propionate depressed milk fat content but no positive effect on milk protein was obtained.

Experiments with cows infused intraabomasally or intravenously with glucose have generally shown an increase in milk yield and a reduction in milk fat and protein contents. Associated with these changes there is a reduction in the yield of fat and an increase in the yield of protein (see Rogers et al., 1979; Thomas and Chamberlain, 1984).

Under farm conditions, increased allowances of starchy concentrates, especially with animals given forage ad libitum, result in a reduction in forage intake. Thus the increase in dietary ME supply is associated with a change in the composition of the diet, with a reduction in forage to concentrate ratio and an increase in the dietary
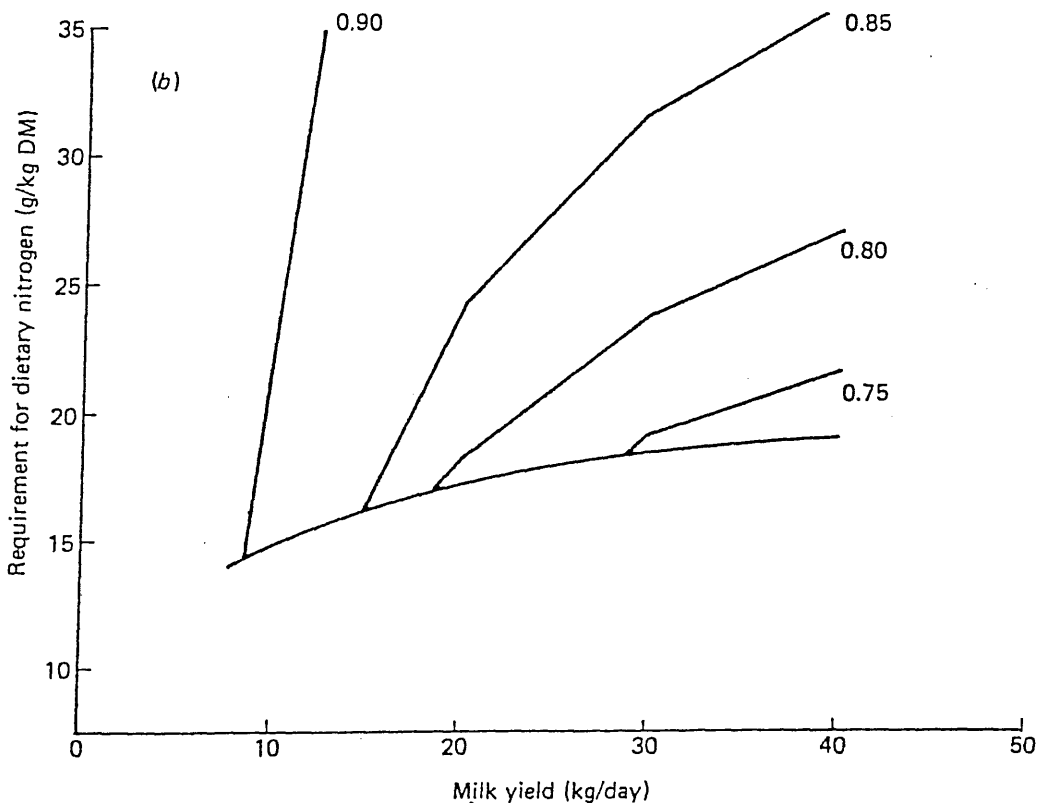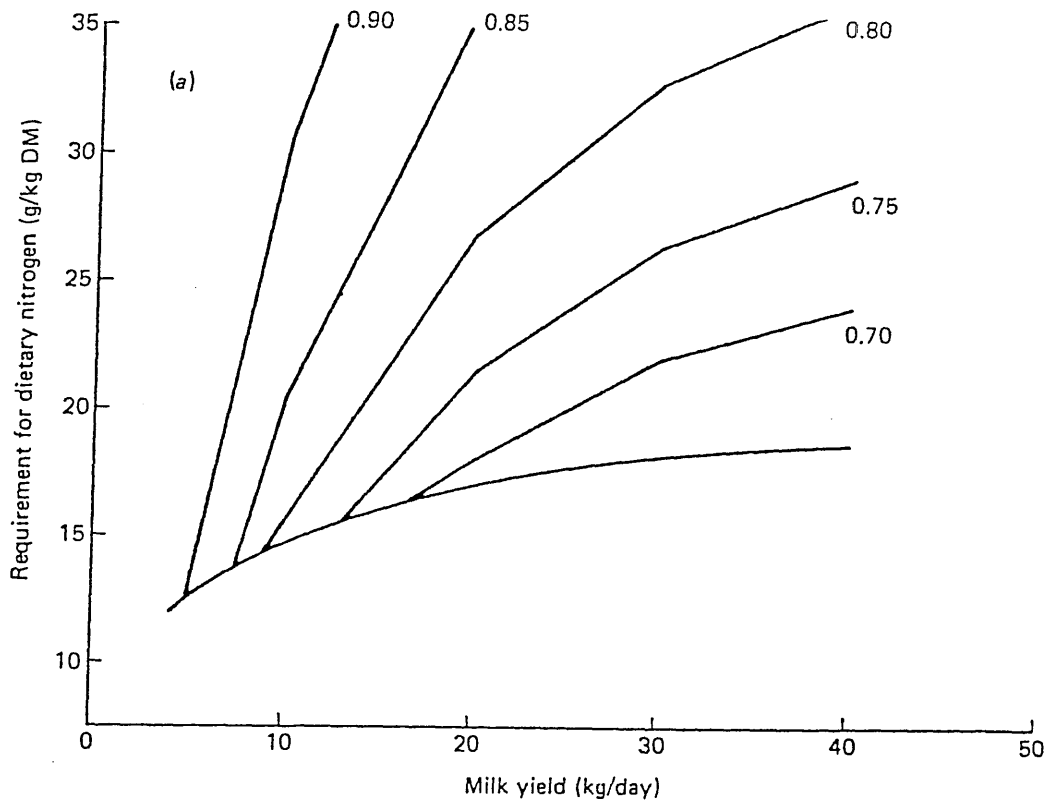
Figure 1.3. Relationship between milk yield and required dietary nitrogen concentration at protein degradabilities ranging from 0.70 to 0.90 assuming (a) microbial N yield of 25 g/kg DOM fermented, (b) microbial N yield of 30 g/kg DOM fermented.

(After Twigge and Van Gils, 1984)

content of readily fermentable carbohydrate. This modification generally leads to a reduction in milk fat content and less consistently to an increase in protein content (Thomas, 1983; Thomas and Chamberlain, 1984). The changes in milk composition have been attributed to the effect of diet on proportions of acetate, propionate and butyrate produced in the rumen and in specific cases to the amount of glucose absorbed in the small intestine (Armstrong and Prescott, 1971; Rook, 1976). Thomas and Chamberlain (1984) concluded that the quantitative changes in milk composition vary widely, depending on the types and amount of forage and concentrate in the diet and their effects on the digestion processes. This is illustrated by the results of Sutton et al. (1980) shown in Table 1.2.

Amino acid supply

Thomas and Chamberlain (1984) summarised data on the effects of amino acid supply on milk secretion derived from experiments involving the intra-abomasal infusion of casein. The results were from experiments with cows receiving basal diets containing between approximately 110 and 240g crude protein/kg, and they demonstrated that the infusions increased milk yield and milk protein content but often reduced milk fat content. There was generally an increase in the yield of protein, fat and lactose as a result of the change in milk yield. This is illustrated in Figure 1.4. Responses similar to those found with casein have been repoted by Trigg et al. (1982) using intra-abomasal infusions of lactalbumin and by Schwab et al. (1976) using a mixture of ten essential amino acids. These latter workers also observed responses to amino acid mixtures containing lysine plus methionine; they showed that individual amino acids were not so

Table 1.2. The digestion of some dietary constituents, and milk yield
and composition, in cannulated and intact cows given diets
containing hay and concentrates of rolled barley or
ground maize in various proportions

| | Proportion of dietary hay:concentrate | | | |
| | 40:60 | | 10:90 | |
	Barley	Maize	Barley	Maize
Cannulated cows				
Gross energy intake (MJ/day)	232^a	227^{ab}	220^b	217
Proportion digested				
In whole tract	0.696^b	0.693^b	0.730^a	0.686^b
In rumen	0.515	0.482	0.522^b	0.429
Starch intake (kg/day)	4.10^a	4.33^a	5.75^b	6.37^c
Proportion digested				
In whole tract	0.99^a	0.92^b	0.99^a	0.89^c
In rumen	0.89^a	0.72^b	0.90^a	0.67^c
Starch absorbed from the small intestine (kg/day)*	0.41	0.87	0.52	1.40
Rumen volatile fatty acids (mmol/mol)				
Acetic acid	612^a	629^a	434^c	530^b
Propionic acid	219^a	210^a	410^b	280^a
Butyric acid	119	116	92	112
Intact cows				
Gross energy intake (MJ/day)	221	229	219	218
Milk yield (kg/day)	16.1^b	18.9^{ab}	20.6^a	15.6^b
Fat content (g/kg)	44.9^a	40.4^a	20.3^c	29.7^b
Fat yield (g/day)	725^a	761^a	419^b	461^b
Protein content (g/kg)	31.5^b	30.0^b	30.3^b	34.3^b
Protein yield (g/day)	506^b	562^{ab}	617^a	535^b
Lactose content (g/kg)	45.2	45.2	46.2	45.5
Lactose yield (g/day)	729^b	852^{ab}	954^a	714^b

Means in a line that do not share the same letter differ significantly
(P < 0.05).

*Mean values calculated from the reported data.

(After Sutton, Oldham and Hart, 1980)
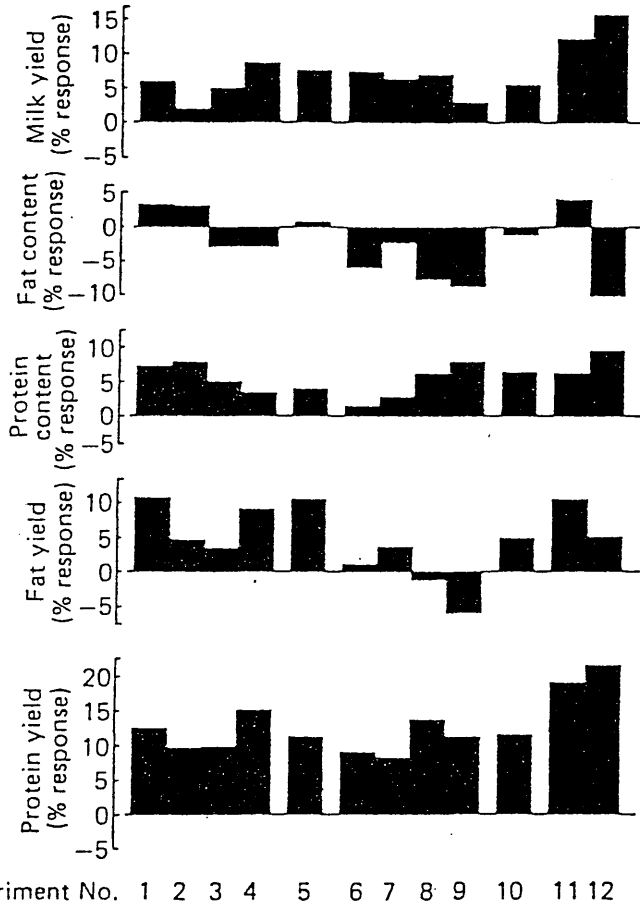
Figure 1.4.   Responses in milk yield, milk fat and protein content,
              and milk fat and protein yield in cows given intra-
              abomasal infusions of casein.   Data are from: Schwab,
              Satter and Clay (1876) (Experiments 1-4); Clark et al.
              (1977) (Experiment 5); Vik-Mo, Emery and Huber (1974)
              (Experiments 6-9); Broderick, Kowalczyk and Satter (1971)
              (Experiment 10); Rogers, Bryant and McLeay (1979)
              (Experiments 11 and 12).   (Results are calculated as the
              difference between the control and the casein infusion
              treatment, expressed as a percentage of the control.
              Infusion rates were 300-880 g/day)
              (After Thomas and Chamberlain, 1984)

effective when given alone.

No response in milk yield was recorded in experiments in which lysine or methionine were given intravenously though methionine induced significant increases in protein yield (Fisher, 1972) or fat yield (Chamberlain and Thomas, 1982) through effects on milk composition. In recent studies, Wong et al. (1983) suggested that the response in fat secretion may be due to a specific action of methionine on triglyceride synthesis in the mammary gland.

Lipid supply

Dairy cows have an absolute requirement for fatty acids and, if their diet is deficient in this respect, lipid supplements produce a massive increase in the yield of milk and milk constituents (Banks et al., 1976). Under more usual circumstances the main influences of lipids arise through an increase in long-chain fatty acid supply to the mammary gland and a corresponding response in the secretion of 'preformed' fatty acids in milk fat. This does not invariably increase milk fat content and yield, however.

Lipid supplements may depress de novo synthesis of short and medium chain fatty acids in the mammary gland either indirectly, through a suppression of acetate production in the rumen, or directly, through the inhibition of mammary acetyl-CoA carboxylase by preformed long-chain fatty acid (Fogerty and Johnson, 1980; Palmquist and Jenkins, 1980; Storry, 1980; Storry and Brumby, 1980; Banks et al., 1982, 1983; Clapperton and Steele, 1983). Under some circumstances lipid supplements reduce milk protein content (Palmquist and Moser, 1981) though the reason for this is unknown.

Responses in milk yield and composition to dietary lipid

supplements are thus variable and rather inconsistent depending on the type of basal diet, the type of lipid supplement, its level of inclusion in the diet, and other unknown factors.

FORMALDEHYDE TREATMENT OF FOODS

Recognition of the extensive and often wasteful proteolysis of dietary protein in the rumen has led to a search for methods whereby protein can be protected from ruminal attack. Such protection has been achieved through the use of a number of chemical agents but the most fully researched technique has been formaldehyde (HCHO) treatment of the protein (Ferguson, 1975).

The chemistry of the reaction of HCHO with protein has been discussed by Van Dooren (1972) in his survey of the literature. He reported that in most instances the initial step appears to be the rapid formation of a methylol compound

$$R - N_tH_2 + HCHO \rightleftharpoons R - N_tH - CH_2OH \qquad (1)$$
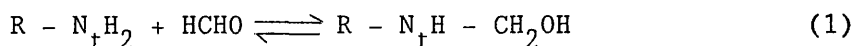
where N_tH_2 represents the terminal amino groups of the protein. After that condensation reaction, further reactions (2, 3, 4) take place slowly over time, with the formation of stable methylene cross-linkages between protein chains.

$$R - N_tH_2 - CH_2OH + R - NH_2 \longrightarrow RN_t CH_2 - NH - R + H_2O \qquad (2)$$

$$R - NH_2 + HCHO \longrightarrow R - NH - CH_2OH \qquad (3)$$

$$R - N_tH_2 + R - NH - CH_2OH \longrightarrow R - N_t - CH_2 - NH - R + H_2O \qquad (4)$$
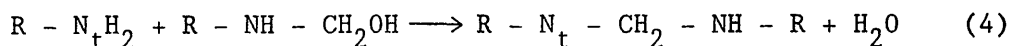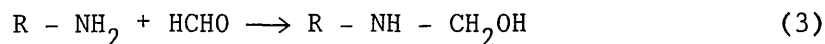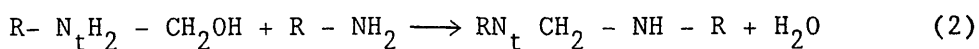
These reactions of HCHO with the amino group are influenced by conditions of pH and temperature. At neutral pH and room temperature Ferguson (1975) considered the principle reactions to be those involving terminal amino groups.

In practice formaldehyde application rates of 30-52 g/kg degradable true protein have been found necessary to achieve protein protection in the rumen (see Barry, 1976). Using that level of application a number of workers have found formaldehyde treatment of the diet to increase the passage of dietary protein to the duodenum. For example Beever et al. (1976) found increases in duodenal protein flow in response to formaldehyde treatment of perennial ryegrass, and corresponding results have been reported where the formaldehyde has been applied during ensilage (see Table 1.3). Protein concentrate foods have similarly been successfully treated with formaldehyde with beneficial effects on duodenal protein flow (Table 1.4). Similarly treatment of barley with formaldehyde (10g HCHO per kg CP) decreased the nitrogen disappearance in sacco after 8 hours of incubation in the rumen from 64% to 16% (Armstrong, 1982).

In addition to its effects on protein, formaldehyde will cross-link starch, particularly under acid conditions and this cross-linkage or bridging of the molecular chains, leads to a more rigid macromolecular network within the starch granules. This affects the physical properties of the starch such that the starch granule is toughened. For example, resistance to gelatinization is determined by the degree of cross-linking (see Greenwood, 1970). Modifications to the physical properties of the starch brought about by cross-linking would be expected to result in a reduced susceptibility to enzymic attack and evidence of this was obtained in studies conducted at the University of Newcastle. The studies involved a formaldehyde reagent originally designed to reduce moulding of grain during storage. The reagent contained (g/kg) of 410 formalin, 220 isobutyric acid, 210

Table 1.3. The effect of formaldehyde application at ensilage on silage composition and on the digestion of silage nitrogen in the rumen

Experiment no.[a]	1		2		3		4		5	
Rate of formaldehyde[b] application (g/kg crude protein)	0[c]	15	0	30	0[c]	35	0	50	0[c]	60
Silage total N (g/kg DM)	19.9	19.6	32.0	29.9	32.0	30.7	26.4	26.7	31.3	31.3
Silage NPN (g/kg total N)	-	-	578	431	769	388	-	-	-	-
Silage water-soluble N (g/kg total N)	615	512	600	451	737	404	462	494	820	776
Silage ammonia-N (g/kg total N)	95	61	53	47	134	36	98	Tr.	-	-
Total N intake (g/day)	91.3	90.7	27.9	24.7	32.3	30.1	66.3	70.6	32.6	32.3
Duodenal N flow (g/day)	92.4	94.1	19.3	22.9	21.6	35.5	61.9	84.0	27.8	35.5
Duodenal N flow (kg/kg N intake)	1.01	1.04	0.69	0.93	0.67	1.18	0.93	1.19	0.85	1.10
Response to formaldehyde[d](% of control duodenal flow)	+3.0		+34.7		+76.1		+27.9		+29.4	

[a]Expt. 1 with cows from Rooke, Greife and Armstrong (1983). Expt. 2 with sheep from Siddons et al. (1984). Expt. 3 with sheep from Siddons, Evans and Beever (1979). Expt. 4 with cattle from Thomson et al. (1981). Expt. 5 with sheep from Beever et al. (1977).

[b]Additives also contained formic acid except in Expt. 5.

[c]Control silage made without additive, other control silages made with application of formic acid.

[d]Values have been adjusted to equalized N intakes.

(After Thomas and Thomas, 1985)

Table 1.4. Effect of formaldehyde treatment of protein concentrate on the flow of non-ammonia nitrogen (NAN) to the small intestine

Diet	N intake g/d	NAN at duodenum g/d		Reference
		Untreated	Treated	
Dried grass plus treated or untreated casein	29.6	26.3	31.1	MacRae et al. (1972)
Grass silage plus treated or untreated soyabean meal	125	83.9	109	Rooke et al. (1983)
Starch concentrate plus treated or untreated peanut meal at two levels of dietary crude protein	20.3	26.4 [†]	29.3 [†]	Faichney (1974)
	36.1	31.0 [†]	37.8 [†]	

[†] Abomasal flows

acetic acid, 75 lignone sulphonate and 75 urea and utropine stabiliser.

Treatment of barley grain with this material substantially reduced the rate of disappearance of starch and protein from samples of grain incubated in Dacron bags in the rumen over 8-hour periods; the loss of barley starch was reduced from 97% for the non formaldehyde control to 64% for a 20g HCHO/kg CP treatment. Corresponding values for barley protein were 68% and 5% (Armstrong, 1982). Treatment of grain in this way might be expected to lead to increases in the passage of grain starch to the small intestine and to a reduction in the adverse effects of grain supplements on forage intake. However, the effects of the treatment on food intake or animal performance have not been reported.

AIMS AND OBJECTIVES OF PRESENT RESEARCH

The series of experiments reported in the following sections of this thesis was designed to investigate aspects of the treatment of barley supplements with acidified formaldehyde reagent. The experiments were to provide results on the influence of the formaldehyde treatment on the rate of the degradation of barley starch and protein in the rumen and on the rate of degradation of silage dry matter and crude protein. More especially they were to provide data on the effects of formaldehyde-treated grain supplements on the ad libitum intake of silage, on milk yield and composition and on the yield of milk constituents in cows given a range of diets based on perennial ryegrass and lucerne silage. One experiment was also conducted to investigate whether palatability effects were important in explaining the differences in dry matter between grass and lucerne silages which were observed during the course of work.

SECTION II

MATERIALS AND METHODS

PREPARATION AND MANAGEMENT OF SURGICALLY PREPARED ANIMALS

Rumen cannulation

Solid nylon cannulas of a design similar to that described by Jarret (1948) were used. The cannulas were mushroom shaped with a hollow stem which was threaded on its external surface. The head of the cannula was inserted into the rumen and the stem which protruded through the body wall was held in place by a nylon ring which was screwed into position. The cannula was closed with a rubber bung or a screw cap.

The field of operation was prepared by clipping and shaving the animal's left side over the desired site and when the animal was on the operating table, this area was thoroughly cleansed with an antiseptic solution. Anaesthesia was achieved by an initial intravenous injection of sodium barbitone (in sheep) or Rompun (in cows) and was maintained throughout the operation by a mixture of Fluothane and air administered through a cuffed endotracheal tube. For the insertion of the cannula an incision about 10 cm long was made 5 to 8 cm below the transverse processes of the lumbar vertebrae and 10 cm posterior to the last rib. The muscles were retracted, the peritoneum opened and the rumen exposed. A cone-shaped pouch of rumen was brought to the exterior and punctured with an incision about 10 cm long. The base of the cannula was inserted through the incision and the wound closed to the stem of the cannula with a continuous suture. A second pursestring suture was

made around the stem of the cannula and closed to prevent seepage of digesta. The stem of the cannula was 'stoppered' with swabs and then exteriorised through a knife-stab made anterior to the initial incision. The peritoneum and muscle layers of the incision were closed using continuous sutures and the skin with single stay sutures. To assist the adhesion of the rumen to the body wall the perspex retaining wing was screwed into position on the stem of the cannula.

Management of prepared animals

Prior to the insertion of rumen cannulas the animals were fasted for 24 hours and held without water. At time of surgery all animals were given an intramuscular injection of a broad spectrum antibiotic and the external wounds around the stem of the cannula and the skin incision were dressed with an antibacterial powder.

Animals normally recovered from the effects of anaesthesia within 1-2 hours and showed interest in food and water at this time; they were given half their daily ration of food and a little water. Within a further 2 to 3 hours they were given *ad libitum* access to water. The day following the operation they were offered full rations of food. Usually the full rations were consumed but occasionally appetite did not recover completely for a further 2 to 3 days. The skin stitches were removed 7 to 10 days after surgical preparation and following this the cannula was washed at 3 to 5 day intervals and where necessary the area was clipped.

EXPERIMENTAL TECHNIQUES AND COLLECTION OF SAMPLES

Sampling of rumen digesta

Samples of rumen digesta were obtained by suction from the ventral region of the rumen immediately below the rumen cannula, using a 1.25 cm diameter stainless steel tube, about 40 cm long. Holes (0.5 cm diameter) were bored in the side of the tube to increase the rate of flow and to allow sampling from a larger volume of rumen. The sample was strained immediately through a single layer of cheesecloth, and the pH was taken as quickly as possible. The procedure for the use of rumen liquor in in vitro studies of cellulolytic activity is described later.

Sampling of milk

Cows were milked twice daily at 0600 hours and 1600 hours; the milk yields were recorded at each milking. Milk was sampled and held in 300 ml bottles containing 280 mg potassium dichromate (Thompson and Capper Ltd., Runcorn, Cheshire) as a preservative. The bottles were stored in a refrigerator at $4^{o}C$. At the end of each sampling period, milk samples were gradually warmed to $40^{o}C$ in a water bath to disperse the fat globules. Successive samples from individual cows were then bulked according to milk yield and the weighted bulk sample was taken for analysis.

METHODS OF CHEMICAL ANALYSIS

Chemical analysis of food

Dry matter and ash

These were determined according to standard procedures. A known

weight of sample of food was oven-dried at 100°C to constant weight and the dry matter expressed as a percentage of fresh weight. For the silage the dry matter was determined by distillation of a minced silage sample with toluene following the procedure of Dewar and McDonald (1961). Ash was determined by ignition of a known weight of dry sample in a muffle furnace at 550°C.

Total nitrogen

The nitrogen content of samples of food were determined using a macro-Kjeldahl technique. The analysis was carried out using a Kjeltec apparatus, which included a digestion unit, a distillation unit, and a titration unit (Tecator Ltd., Thornbury, Bristol). Samples containing 1-2 mg nitrogen were first digested with nitrogen-free, concentrated sulphuric acid, using tablets containing 3.5 g potassium sulphate and 0.10 g copper as a catalyst. The digested samples were distilled and the ammonia collected in a 250 ml conical flask containing 25 ml boric acid solution (40 g/l). The total nitrogen was determined by titration with 0.01 M hydrochloric acid.

True protein in silage

This was determined using a macro-Kjeldahl technique after precipitation of protein with tannic acid solution

Reagent

Tannic acid solution was prepared by dissolving 4.45g tannic acid in 100 ml distilled water containing 0.1 ml concentrated sulphuric acid. The solution was allowed to stand for 24 hours and was filtered through a Whatman No. 42 filter paper before use.

Procedure

Wet silage (1g) was weighed into a centrifuge tube. Boiling tannic acid (20 ml) was added and well mixed with the silage. The tube and its contents were heated in a boiling water bath for 15 minutes. After cooling for 10 minutes, the tube was centrifuged at 1700g for 10 minutes, and the supernatant was removed using a pipette, which had its end covered with fine, washed muslin to exclude any of the silage particles. The residue was washed with 25 ml of cold water, centrifuged, and the supernatant again removed though muslin. The washing was repeated twice as before, and the residue was taken for nitrogen determination using the procedure described above.

Ammonia-nitrogen in silage

This was determined on a sample of silage extract.

Preparation of silage extract

Wet minced silage (20g) was weighed into a 600 ml beaker and 200 ml of distilled water was added. After incubation of the mixture in a water bath at $40^{o}C$ for 30 minutes, the mixture was filtered through a double layer of muslin by squeezing the silage juice from the muslin. The filtrate was then centrifuged at 1700g for 20 minutes, transferred to plastic bottles and stored at $-20^{o}C$ until analysed.

Procedure

Sodium hydroxide (10M, 10 ml) was added to 10 ml of silage extract in a steam distillation apparatus (Tecator Ltd., Thornbury, Bristol). Ammonia was distilled from the sample and collected in 25 ml of boric acid solution (40 g/l). Ammonia was determined by titration with 0.01M hydrochloric acid.

Total and individual short-chain fatty acids in silage

The method used was that of Cottyn and Boucque (1968).

Reagents

(i) Preservative. This contained 30 ml metaphosphoric acid (25% W/V), 10 ml formic acid (90%, Analar) and 10 ml distilled water.

(ii) Internal standard. Hexanoic acid (2 g/l).

Procedure

Two ml of silage extract, prepared as described on page 43, was transferred to a 10 ml test tube; 1 ml of preservative was added plus 2 ml of hexanoic acid and the contents mixed well. After standing for 20 minutes, the tube and its contents were centrifuged at 1700g for 20 minutes. Supernatant (1-3 µl) was injected onto the column of a gas chromatograph (Model 8310; Perkin Elmer Ltd., Beaconsfield, Bucks.) fitted with a flame ionisation detector. The chromatograph was fitted with a glass-lined stainless steel column (1 m long, 3.125 mm diameter) uniformly packed with 5% carbowax 20 M/TPA on chromasorb G 80-100 mesh. The column temperature was programmed to hold at $90^{o}C$ for 5 minutes, to increase at a rate of $5^{o}C$ per minute up to a temperature of $130^{o}C$, and then to hold constant for a further 3 minutes. The flow rate of nitrogen carrier gas was 40 ml/min and the detector flame was maintained with hydrogen and air at pressures of 1.3 bar. The separation of the acids from acetic to hexanoic was completed in approximately 16 minutes. The molar concentration of each acid was calculated from the peak area of the acid on the chromatogram relative to area of hexanoic acid after allowances had been made for differences in the detector responses for each acid. These responses were

determined from the analysis of a standard mixture containing known concentrations of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids.

Ethanol in silage

This was determined by gas chromatography using the method of Huida (1982) using methanol as an internal standard. Thirty µl of dry methanol was added to 5 ml of silage extract and 1 µl injected on to the column of the gas chromatograph (Jeol 20K; Jeol Instruments, Stanmore, Middlesex) which was fitted with a flame ionization detector. The columns were 2 m long and 2 mm internal diameter and were packed with chromasorb 101. The oven setting was $100^{o}C$ and a carrier gas (N_2) flow of 60 ml/min.

Lactic acid in silage

Lactic acid was determined by the method of Barker and Summerson as outlined by Pryce (1969).

Reagents

(i) Protein precipitating reagent. This was prepared by dissolving 10g of sodium tungstate in 800 ml of distilled water and 22 ml of 90% (W/V) orthophosphoric acid. Five grams hydrated copper sulphate was added and the total volume made up to 1 l with distilled water.

(ii) Concentrated sulphuric acid (Analar)

(iii) Colour reagent. This was prepared by dissolving 1.5g of parahydroxybiphenyl in 100 ml dimethyl formamide.

(iv) Lactic acid stock solution. Pure lithium lactate (1.065g)

and 1 ml concentrated sulphuric acid was made up to 1 l with distilled water. Dilutions containing 10, 20, 30, 40, 50 mg lactic acid per 100 ml were prepared.

Procedure

Silage extracts (0.1 ml) prepared as described on page 43 were added to 3.9 ml of protein precipitating reagent in 15 ml centrifuge tubes. The tubes were shaken and centrifuged at 1500g for 5 minutes. One ml of the supernatant was transferred to a boiling tube and then 5 ml of the concentrated sulphuric was quickly added and the tubes left for 2 minutes and then cooled in a water bath. Parahydroxybiphenyl (0.1 ml) was added. The tubes were shaken and were allowed to stand at room temperature for 10 minutes to allow colour development. The tubes were placed in a boiling water bath for 90 seconds and then cooled. The optical density of the solution was read at 565 nm against a distilled water blank. The concentration of lactic acid in the silage was obtained by reference to a standard calibration curve.

Total soluble sugars

These were determined by a method similar to that of Somogyi (1945).

Reagents

(i) Somogyi reagent. This was prepared by dissolving 28g anhydrous di-sodium hydrogen orthophosphate and 40g potassium sodium tartrate in 700 ml distilled water; then 100 ml 1M sodium hydroxide and 80 ml of 10% (W/V) anhydrous sodium sulphate was added, and the volume made up to 1 l with distilled water.

(ii) Arsenomolybdate reagent. This was prepared by dissolving 25g
ammonium molybdate in 450 ml distilled water. To this solution, 21 ml
Analar concentrated sulphuric acid and 25 ml of 12% (W/V) di-sodium
hydrogen arsenate was added. The mixture was transferred to a brown
bottle, incubated for 24 hours at 37°C, cooled and held at 4°C until
used.

Procedure

A sample (5 ml) of silage extract (see page 43) was pipetted into
a glass stoppered test tube for hydrolysis. Sulphuric acid (0.1 ml;
1M) was added and the tube and its contents were boiled in a boiling
water bath for 30 minutes. After cooling in a water bath, 0.1 ml of 1M
sodium-hydroxide was added and 2 ml of hydrolysate was transferred to a
15 ml centrifuge tube. Four ml of 5% zinc sulphate and 4 ml of 0.15M
barium hydroxide was added for deproteinization. After mixing, the
tube and its contents were centrifuged at 1500g for 10 minutes.
Supernatant (2 ml) was transferred to a glass stoppered test tube
containing 2 ml of Somogyi reagent. The tube was heated in a boiling
water bath for 10 minutes. After cooling, 2 ml of arsenomolybdate
reagent was added, the solution transferred to a 50 ml volumetric flask
and made up to volume with distilled water. The absorbance was read on
a spectrophotometer at 500 nm against a blank of distilled water. The
total soluble sugars in the samples were calculated by reference to a
calibration graph derived with standard solutions of D-glucose
containing 50 to 250 mg/l.

<u>α-Linked glucose polymers</u>

α-Linked glucose polymers were deterined by the method of MacRae

and Armstrong (1968). α-Linked glucose polymers can be completely

degraded to glucose by an amyloglucosidase available commercially as

Agidex. The enzyme does not attack raw starch and this necessitates

a gelatinisation stage in the analysis procedure. By estimating the

glucose released by Agidex the concentration of α-linked glucose

polymers in the sample can be determined.

Reagents

(i) Acetate buffer pH 4.5. Three parts of 0.2M sodium acetate

(16.4 g/l) were added to two parts of 0.2M acetic acid (12.01 g/l) and

the mixture adjusted to pH 4.5

(ii) Sodium hydroxide solution (12.0 g/l).

(iii) Zinc sulphate (5%).

(iv) Amyloglucosidase (Agidex, Sigma Chemicals, Poole, Dorset).

(v) Glucose test combination kit (Boehringer Mannheim, W.

Germany) containing glucose oxidase and ABTS (di-ammonium 2,2'-azino-

bis(3-ethylbenzothiazaline-6-sulfonate)).

Procedure

A sample of 0.1-0.15g of food or partially digested food was

placed in a 150 ml flat bottomed Soxhlet flask and the flask and

contents weighed. Fifty ml distilled water was added and the mixture

was refluxed for 4 hours to gelatinise the starch. The flask was

cooled and 50 ml acetate buffer and 0.4g Agidex enzyme were added.

Assuming unit density the volume of liquid in the flask could then be

calculated. A thin layer of liquid paraffin was placed on the liquid's

surface, (about 10 ml poured down the side of the flask) to inhibit

microbial growth. The flask was stoppered and incubated at 60°C for 24

hours. Duplicate distilled water blanks were taken through this complete procedure to obtain the very small blank readings given with Agidex alone. After 24 hours incubation the flasks were cooled to room temperature and duplicate 0.5 ml samples withdrawn and placed in 15 ml centrifuge tubes. The samples were deproteinised by addition of 2 ml 5% zinc sulphate and 2 ml 0.3M sodium hydroxide solution. After standing for 5 minutes the mixture was centrifuged at 1500g for 10 minutes. The concentration of glucose in the supernatant was determined using the Boehringer glucose test-combination kit.

Neutral-detergent fibre

The neutral detergent fibre in food was determined by the method of Goering and Van Soest (1970).

Reagents

(i) Neutral-detergent solution. The following chemicals were dissolved in one litre of water.

(i) – Sodium lauryl sulphate 30g

(ii) – Ethylenediaminetetra-acetic acid (di-sodium salt-EDTA) 18.61g

(iii) – Di-sodium tetraborate (borax $Na_2B_4O_7$ $10H_2O$) 6.81g

(iv) – Di-sodium hydrogen orthophosphate (Na_2HPO_4) 4.56g

(v) – 2-Ethoxyethanol (Analar) 10 ml

(vi) – Dekalin

(vii) – Acetone

(viii)– Sodium sulphate (anhydrous)

The EDTA and $Na_2B_4O_7$ $10H_2O$ were placed together in a large beaker, distilled water added and mixture treated to dissolve the chemicals. The solution of sodium lauryl sulphate and 2-ethoxyethanol was added.

The Na_2HPO_4 was placed in a beaker and distilled water was added and heated until Na_2HPO_4 dissolved. Then the two solutions were mixed together and placed on a hot plate with a magnetic stirrer and left to dissolve. The mixture was allowed to cool and the volume was made up to 1 l with distilled water. The pH of the mixture was in the range of 6.9-7.1

Procedure

A sample of food weighing 1g was transferred to a 500 ml conical flask; 100 ml of neutral detergent solution, 2 ml Dekalin and 0.5g sodium sulphite were added. The mixture was heated under reflux. After the mixture began to boil, the heat was reduced to avoid foaming and refluxing was continued for 60 minutes from the onset of boiling. The contents of the flask were then transferred to a sintered glass crucible (porosity 1) which had been previously set on a filter manifold. The flask and the inside of the crucible were washed with boiling water. The mat of filtered solid was broken up with a small glass rod and washed twice with hot water; the washing was repeated with acetone. The crucible and its contents were then dried overnight in an oven at $100^\circ C$ and re-weighed after cooling in desiccator. The sample was then ashed at $580^\circ C$ for 3 hours.

The content of neutral detergent fibre in the sample was calculated using the following equation:

$$NDF \% = \frac{\text{loss in weight on ashing crucible}}{\text{weight of sample dry matter}} \times 100$$

Acid-detergent fibre

The acid-detergent fibre content of food was determined by the

method of Goering and Van Soest (1970).

Reagents

     (i) Decahydronaphathalene (Dekalin).

     (ii) Acetone, free from colour and leaving no residue upon evaporation.

     (iii) Hexane.

     (iv) Acid-detergent solution. This contains 20g cetyltrimethyl ammonium-bromide (CTAB) per litre 0.5M sulphuric acid.

Procedure

    A sample of food (1g) was transferred to a conical flask; 100 ml acid detergent solution and 2 ml Dekalin were added. The mixture was heated under reflux. After the mixture began to boil, the heat was reduced to avoid foaming and refluxing was continued for 60 minutes from the onset of boiling. The contents of the flasks were then transferred to a sintered glass crucible which had been previously set on a filter manifold. The flask and the inside of the crucible were washed with boiling water. The mat of filtered solid was broken up with a small glass rod and washed twice with hot water. The washing was repeated with acetone until all the colour and the lumps were removed. Hexane was added with the last acetone wash. The crucible and its contents were then dried overnight in a oven at $100^{o}C$ and re-weighed after cooling in a desiccator. The sample was then ashed at $580^{o}C$ for 3 hours.

    The content of acid detergent fibre (ADF) in the samples was calculated using the following equation:

$$\text{ADF \%} = \frac{\text{Loss in weight on ashing crucible}}{\text{Weight of sample dry matter}} \times 100$$

Digestible organic matter in the dry matter

The estimation of digestible organic matter in the dry matter (DOMD or "D" value) of dried forage samples was based on their lignin content which was analysed using the method of Morrison (1972). After removal of interfering phenolic materials, lipids, waxes and colouring matter, the lignin hydroxyl groups are acetylated and brought into solution with acetyl bromide in acetic acid. Hydroxylammonium chloride solution was added to remove excess reagent, bromine and polybromide. Following the separation of proteinaceous sediment, lignin was estimated as an "A" value by measurement of the optical density at 280 nm.

Reagents

(i) Ethanol: Absolute, 99.7-100%

(ii) Acetyl bromide-acetic acid reagent. A 25 ml quantity of acetyl bromide was mixed with Analar glacial acetic acid and made up to 100 ml with acetic acid.

(iii) Hydroxylammonium chloride solution (approximately 0.5 M). A 3.5g quantity of hydroxylammonium chloride was dissolved in water and made up to 100 ml.

Method

A 50 mg sample of food was weighed accurately into a 25 ml Quickfit test-tube and 20 ml of water added. The tube was stoppered, mixed and heated at 70°C in a water bath for 30 minutes. The tube was shaken at 10 minute intervals. The sample was then filtered through a GF/A filter paper on a filter crucible and washed in order with water, ethanol, acetone and diethyl ether. The filter paper and sample were transferred back to the test-tube and all traces of organic solvent

removed in the oven. To the residue in the tube was added 5.0 ml of
acetyl bromide/acetic acid reagent. The tube was shaken, stoppered and
heated in a water bath at 70°C for 30 minutes with shaking as before.
After allowing the tube to cool in a water bath at 20°C for 30 minutes,
20 ml of Analar glacial acetic acid was added and the contents mixed.
A 50 ml aliquot was transferred to a 50 ml volumetric flask to which
was also added 7.5 ml of glacial acetic acid and 1.0 ml of 2.0 M sodium
hydroxide. The volume was made up to approximately 45 ml with ethanol,
1.5 ml hydroxylammonium chloride was added, the flask shaken and
contents made up to the mark with ethanol. The flask was shaken again
and allowed to stand for 1 hour before the contents were filtered
through a 9 cm GF/A filter paper. A blank was prepared as above but
without the addition of sample.

A portion of supernatant was transferred to a 10 mm silica cell
and the optical density read at 280 nm against a cell containing
distilled water. An SP6-400 ultraviolet spectrophotometer was used
(Pye Unicam Ltd., Cambridge).

Calculation

The lignin content was reported as an "A" value from the following
formula:

$$\text{"A"} = \frac{OD_s - OD_b}{C} \text{ litre/g/cm}$$

Where: OD_s = optical density of sample

OD_b = optical density of blank

C = weight of sample dry matter x 4

The "D" value of the forages was calculated using the following
regression equation:

$$D \text{ (g/kg)} = 86.148 - 109.07A$$

Determination of digestibility in vivo

The nutritional value of the silages used was determined by evaluation of their digestibility in vivo. In each case the procedure was to offer the silage at an approximate maintenance level of feeding to three or four wether mature sheep held in metabolism cages designed for the separate collection of faeces and urine. When the sheep were established on the appropriate silage they were fed at a constant level of intake for a period of 21 days. Complete faecal collections were made over the last 7 days of this period.

Analysis of milk

Milk total solids

Total solids was determined gravimetrically according to British Standard 1741 (1963). A known weight of milk was dried initially by evaporating on a boiling water bath, and finally in an oven at 100°C.

Milk fat

Milk fat content was determined by the 'Gerber' method (British Standard 696 (1969)). The fat was separated from the milk by addition of concentrated sulphuric acid and determined by direct measurement using a Gerber butyrometer.

Milk protein

Total nitrogen in milk was determined by a macro-Kjeldahl method (Association of Official Agricultural Chemists, 1975). Protein was calculated as 6.38 x Total nitrogen.

Milk lactose

Lactose was determined polarimetrically using the method of Grimbleby (1956).

Determination of the microbial degradability of foods

At the start of the programme of experiments there was considered to be a need for a technique to provide an index of the cellulolytic activity of the rumen contents in sheep and cows given different diets. Two approaches seemed to be suitable. The first was to obtain rumen liquor from sheep or cows on a given diet and determine its cellulolytic activity by incubation in vitro with a cellulose substrate under controlled conditions (Halliwell, 1957). The second was to use the in vivo, Dacron bag procedure as described by Mehrez and Orskov (1977).

Cellulolytic activity of rumen liquor in vitro

The cellulolytic activity of rumen liquor was determined by a modification of the method of Halliwell (1957).

Reagents

    (i) Buffer solution as described in Table 2.1.

    (ii) Hydrochloric acid (3.8M).

    (iii) Ammonium hydroxide (0.7M).

    (iv) Teepol XL (1% (W/V)).

    (v) Distilled samples of ethanol, chloroform and methanol.

Procedure

The rumen liquor was prepared as described by Stewart (1977).

Table 2.1. Composition of buffer solution used in the _in vitro_ study

	Concentration g/l
Sodium bicarbonate ($NaHCO_3$)	10.00
Potassium chloride (KCl)	0.40
Potassium dihydrogen-orthophosphate (KH_2PO_4)	0.18
Magnesium sulphate ($MgSO_4$)	0.33
Ammonium hydrogen-orthophosphate ($(NH_4)_2HPO_4$)	0.89
Calcium chloride ($CaCl_2$)	0.43
Sodium sulphide (Na_2S)	3.07

Samples of rumen contents were taken 2 hours after morning feeding and strained through one layer of cheesecloth. Rumen liquor (2.5 ml) was added to a test tube (150 x 15 mm) fitted with a Bunsen valve and containing 17.5 ml buffer solution and a known weight (30, 60, 90, 150 and 200 mg) of dewaxed cotton fibre. The cotton fibre used in the incubations was dewaxed by successive soaking in chloroform and methanol (70g of yarn; 700 ml of solvent). After extraction it was washed in hot tap water and distilled water, dried at 37°C and stored in a desiccator. In vivo estimates of the degradation of cotton yarn were also carried out. Two g of cotton were incubated for 24 and 48 hours in Dacron bags in the rumen of sheep from which the rumen contents used in the in vitro studies were obtained. Residual cotton thread in the bags was washed and weighed using the same procedure as described for the in vitro incubations. The incubation mixture was thoroughly gassed with CO_2 to pH 6.8-6.9 and incubated for 48h at 39°C. After incubation, the cotton yarn was washed in the solvents as described by Halliwell (1957), as follows. Sintered glass filters (Grade 3) were used, on which the residual cotton fibre was washed successively with 5 ml volumes of 3.8M hydrochloric acid, 0.7M ammonium hydroxide, 1% (W/V) Teepol XL, and distilled ethanol (10 ml), with a 20 ml water-wash between each solvent. The residual cotton was subsequently dried at 100°C for 16 hours and weighed.

Dacron bag incubation procedure

A Dacron bag technique was used to estimate the rumen degradability of foods (Mehrez and Orskov, 1977).

Procedure

Dacron bags of approximately 20 x 8 cm with a mesh size of 45 μm with double stitched seams and curved corners, were used to hold a sample of food _in situ_ in the rumen of fistulated animals. A known fresh weight of barley (5g), silage (8g) and cotton fibre (2g) was placed in the bags. They were then tied at the neck and attached by a length of nylon string to a ring which in turn was tied to the cap of the rumen cannula. The total length of string was around 55 cm with cows and 30 cm with sheep. At the end of each incubation interval the bags were removed.

Comparative studies on the in vitro incubation and Dacron bag techniques

Before the main series of experiments were undertaken preliminary studies were carried out to investigate the suitability of the _in vitro_ and Dacron bag procedures as a means of assessing cellulolytic activity.

Materials and methods

Animals and their management

Two sheep (mean weight 60 kg) were used. The animals were fitted with permanent rumen cannulas. Food was given twice daily at 09.00 hr and 16.00 hr and water and mineralized salt licks were freely available.

Experimental treatments and plan

Three diets A, B and C were used. Diet A was a low-starch diet consisting of 0.5 kg/d of chopped hay and 0.5 kg/d of dried grass

cubes. Diet B was a medium-starch diet consisting of 0.5 kg/d of hay

and 0.5 kg/d of a concentrate mixture containing barley and soyabean

meal (80:20 on a fresh weight basis). Diet C was a high starch diet

consisting of 0.3 kg/d of hay and 0.7 kg/d of the concentrate mixture.

The composition of the dietary ingredients and the complete diets is

given in Table 2.2.

Initially diet A was given to one of the sheep and diet B was

given to the other. The animals were established on the diets for 14

days under controlled conditions prior to the experimental tests which

were conducted over a period of 10 days. Subsequently one of the sheep

was transferred to diet C and the establishment and sampling procedure

were repeated. In each sampling period the protocol was similar. On

two occasions a sample of rumen digesta was taken 2 hours after the

morning meal. The sample was strained through a single layer of

cheesecloth, as described by Stewart (1977) and then used for in vitro

incubation tests as described by Halliwell (1957). The procedure is

described in detail on page 55 . In brief, the pH of the rumen fluid

was measured initially and then adjusted to pH 6.8-6.9 by addition of

buffer. The buffered digesta was then used for incubations with graded

amounts of cotton thread (30, 60, 90, 150 and 200 mg per incubation).

Each incubation test was replicated three times giving a total of 6

observations over the two occasions on which the tests were undertaken.

All tests were conducted over a 48h period of incubation and

cellulolytic activity assessed by the loss in weight of the threads.

On the day following the sampling of rumen digesta for the

incubation studies cellulolytic activity in the rumen was determined in

vivo using the Dacron bag technique (see page 56) using a incubation

substrate of cotton threads. Incubations were carried out over a 48h

Table 2.2. The chemical composition of the dietary ingredients and the
complete diets given to the sheep.

	Hay	Dried grass	Concentrate	Diet A[†]	Diet B	Diet C
Dry matter (g/kg)	860	870	873	865	867	869
Organic matter (g/kg$_{DM}$)	948	883	905	906	927	918
Total nitrogen (g/kg$_{DM}$)	9.1	35.0	28.8	22.1	19.0	11.4

† Diet A consisted of hay and dried grass (50:50) on a fresh weight

   basis). Correspondingly diet B and diet C consisted of hay and

   concentrate (50:50 and 30:70 respectively).

period, bags being removed at 24h and 48h. Dacron bag measurements were carried out on two separate occasions and in each instance were replicated three times. Cellulolytic activity was assessed by the disappearance of cotton threads over the incubation period.

Statistical analysis

Results from in vitro and in vivo incubations were analysed by analysis of variance (Snedecor, 1956).

Results and Discussion

As is shown in Table 2.3 and Figure 2.1 the percentage of the cotton thread disappearing in the in vitro incubation tests varied with the weight of cotton thread included in the incubation. As illustrated in Figure 2.1 the change in percentage digestion was not simply a mathematical consequence of the difference in the weights of cotton threads used. When the results were expressed in terms of the total digestion of cotton over the incubation period (Figure 2.2) it was clear that the absolute quantity of cotton digested was increased as the amount of cotton incubated was raised. There were also significant differences in the percentage disappearance of cotton threads and in the absolute quantities digested which were related to the diet of the animal from which the rumen fluid was obtained. Highest rates of digestion were obtained with diet B. Values for diet A were lower and those for diet C were especially low.

The results obtained from the Dacron bag incubations (Table 2.4) also showed significant differences between diets but in this case the determined rate of disappearance of cotton threads decreased from diet A to diet B to C.

Table 2.3. Percentage loss of cotton thread dry matter from different
weights of cotton incubated in vitro with rumen liquor from
sheep given diets A, B and C (Each value is the mean
of 6 observations, SEM ± 2.4)

Weight of cotton (mg)	Diet		
	A	B	C
30	60.0	87.2	43.0
60	52.0	67.2	33.6
90	45.3	57.6	26.9
150	36.5	43.6	23.5
200	34.3	37.0	18.4
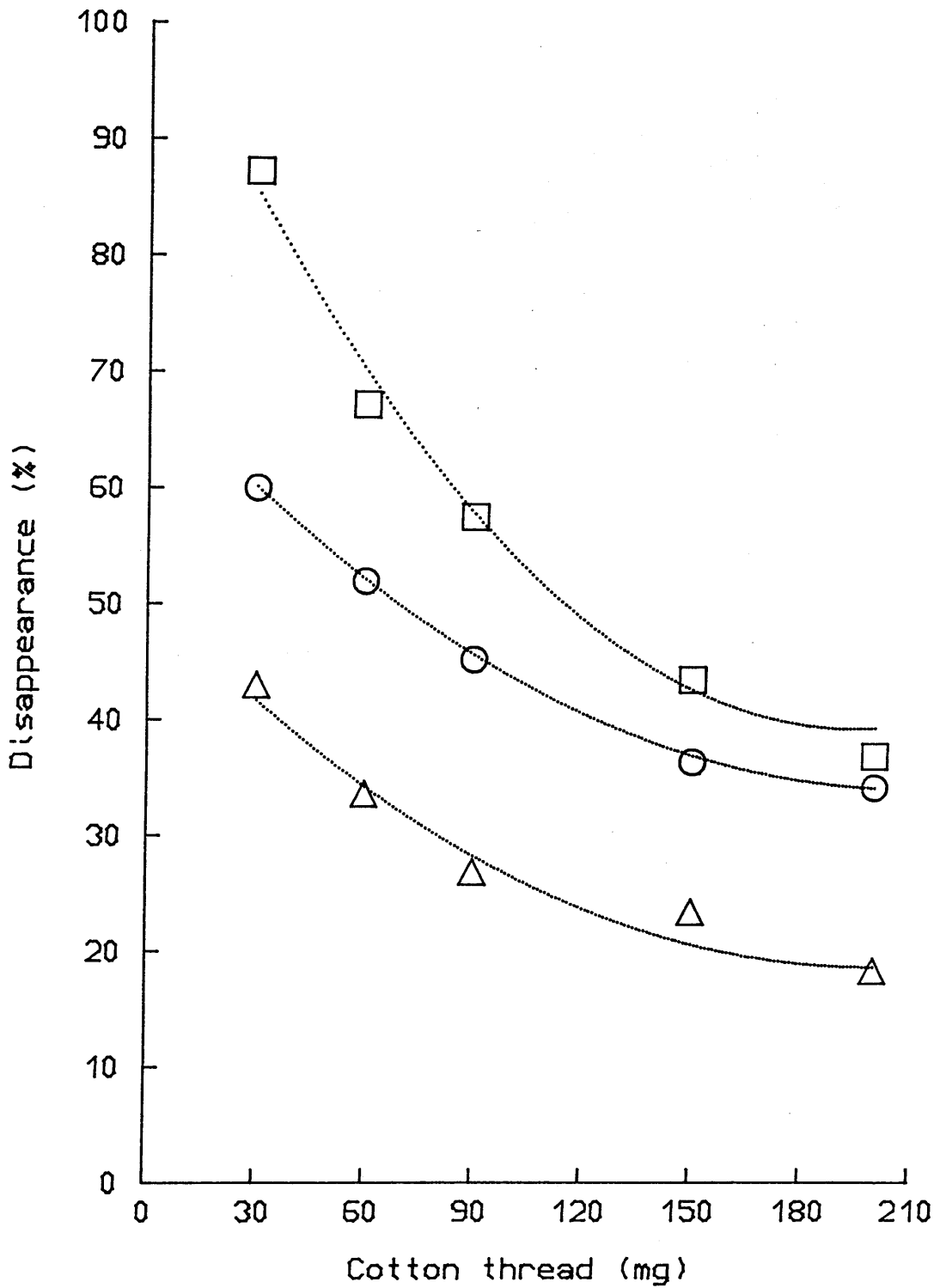
Figure 2.1. Percentage loss of cotton thread dry matter from different
weights of cotton incubated _in vitro_ with rumen liquor from
sheep given diet A (**O**), B (**◻**) and C (**▲**).
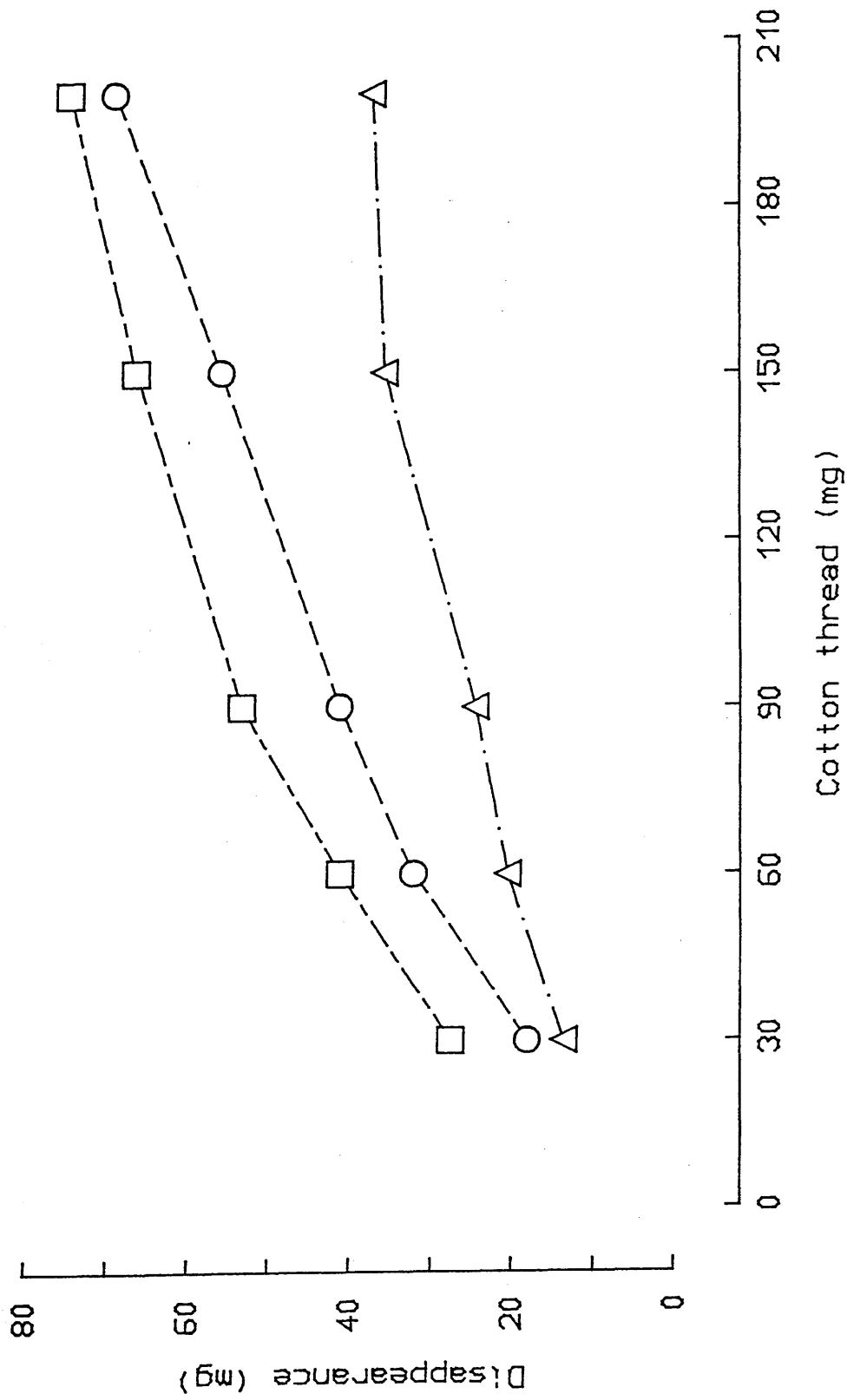
Figure 2.2. Weight of cotton thread (mg) disappeared from different weights of cotton incubated _in vitro_ with rumen liquor from sheep given diet A (**O**), B (**□**) and C (**△**).

Table 2.4. Percentage loss of dry matter from cotton thread incubated in Dacron bags in the rumen of sheep given diets A, B and C (Each value is a mean of 6 observations, SEM ± 1.5)

Incubation time (h)	Diet		
	A	B	C
24.0	25.3	13.9	5.7
48.0	64.9	29.7	14.6

In Table 2.5 the relative values for the three diets for cellulolytic activity are given together with mean rumen pH values recorded in the sheep at 2 hours after feeding.

The in vivo incubations showed a progressive decrease in the extent of breakdown of cotton with increasing levels of starch in the diet. However, the results for the in vitro incubation showed that the extent of cotton breakdown was disproportionately high with diet B. These results may be explained by the fact that in the in vitro study the pH of the rumen liquor samples were all adjusted to 6.8-6.9 and for diet B the pH may have been moved away from the critical point at which the cellulolytic activity would be affected (Mould, Orskov and Mann, 1983).

On the basis of these results and with the experience of using both in vivo and in vitro methods it was decided that the Dacron bag method should be preferred and this technique was therefore adopted. This also offered experimental convenience since the same procedure was used to determine the influence of treatment with formaldehyde-reagent on the ruminal degradability of barley dry matter, starch and protein.

Table 2.5. Percentage loss of cotton thread dry matter from the cotton
incubated in vitro and % loss of cotton dry matter incubated
(2g) in vivo in animals given diets A, B and C

Diet	Rumen liquor pH [†]	% DM loss in vitro [††]	% DM loss in vivo [§]
A	6.56	45.6	64.9
B	5.99	58.5	29.7
C	5.95	29.1	14.6

† Mean of two successive days.

†† Values are means for 30 mg – 200 mg of cotton thread.

§ Values are for 48 hours incubation.

SECTION III

EXPERIMENTAL

Experiment 1. The effects of treatment of barley with formaldehyde reagent on the rate of digestion of barley dry matter, starch and total nitrogen in the rumen.

Armstrong (1982) reported that treatment of barley grain with acidified-formaldehyde reagent led to a reduction in the rate of breakdown of the barley starch and protein in the rumen. The experiments described below were therefore conducted as preliminary observations to confirm the observations of Armstrong (1982) and to assess the effectiveness of formaldehyde reagent on the treatment of barley in rolled and ground forms.

Experimental

Animals and their management

One non-lactating cow fitted with a permanent rumen cannula was used. The cow was given ad libitum access to one of two hays, the composition of which is given in Table 3.1.

Experimental treatments and plan

The studies were conducted in two experiments (Expt 1a and 1b). The first was with hay 1 (Table 3.1) as the basal diet and the second was with hay 2 as the basal diet. The first experiment involved a comparison between untreated rolled barley and the same barley treated with formaldehyde reagent at a rate of 8 l/t. The second experiment

Table 3.1. Composition of the hay diets given to the cow used in Experiments 1a and 1b.

	Hay 1	Hay 2
DM (g/kg)	839	830
OM (g/kg DM)	960	939
Crude protein (g/kg DM)	47	62
ADF (g/kg DM)	357	391

involved a similar comparison but with barley in a ground form.

The formaldehyde reagent used was a mixed reagent containing (g/kg) 410 formalin, 220 isobutyric acid, 210 acetic acid, 75 lignone sulphonate and 75 utropine stabilizer (Farmos UK Ltd., West Bromwich, England). The reagent was applied dropwise directly to the grain (DM 820 g/kg) while it was continuously mixed in a small commercial cement mixer. Mixing was continued for approximately 10 minutes after the reagent was added. The grain was allowed to stand, open to air, for a period of at least 3 days prior to its use.

For each food tested incubations were made for five periods of 2.5, 5.0, 7.5, 16.0 and 24 hours respectively. Each incubation test was replicated 4 times on 3 days to give a total of 12 observations for each time period.

Chemical analysis

The barley samples and residues from each bag incubated were analysed for DM and the barley and 12 residues representing each time period for a given food were each bulked and analysed for total nitrogen and starch.

Statistical analysis

Rates of degradation of DM, starch and nitrogen were calculated from the estimated disappearance of the constituents over the time periods for which incubations had been conducted. Data for DM disappearance were analysed by analysis of variance.

Results

Expt 1a. The results for the disappearance of DM, starch and nitrogen

are shown in Table 3.2 and Figure 3.1. Treatment of the rolled grain
with the formaldehyde reagent at a rate of 8 l/t led to a marked
reduction in the rate and extent of degradation in the rumen of DM,
starch and nitrogen. For example, after 24 hours of incubation the
degradation of DM had been reduced by 26.1% by the formaldehyde
reagent. The corresponding reductions for starch and nitrogen
degradation were 21.0% and 62.6% respectively.

Expt 1b. As in Expt 1a, in Expt 1b treatment of barley with
formaldehyde reagent reduced the rate of degradation of DM, starch and
nitrogen in the rumen (Table 3.3, Figure 3.2). However, as indicated
by the effects measured after 24 hours of incubation the treatment with
formaldehyde reagent reduced DM disappearance by only 15.1%, starch
disappearance by 13.7% and nitrogen disappearance by 54.5%. These
values were lower than observed in Expt 1a. However it should also be
noted that as compared with Expt 1a the measured extents of degradation
of untreated barley samples were low.

Discussion

In both experiments, treatment of barley with the formaldehyde
reagent resulted in a marked reduction in the rate of disappearance of
DM, starch and nitrogen. However, as indicated by Expt 1b, the
formaldehyde treatment was less effective on ground than on rolled
grain. Whether this effect was real or was an artifact of the Dacron
bag procedure is not clear. There was evidence from the low rate of
digestion observed with the untreated barley that when present in
ground form the breakdown of the material in the bag was impaired.

As indicated by the effect observed after 24 hours of incubation,

Table 3.2. Expt 1a. The disappearance (%) of dry matter (DM), starch and total nitrogen from samples of untreated rolled barley (U) or rolled barley treated with formaldehyde reagent (T) at a rate of 8.0 l/t

Incubation time (hrs)	DM			Starch		Nitrogen	
	U	T	SEM	U	T	U	T
2.5	9.3	3.8	±0.85	27.9	11.7	4.0	0.0
5.0	25.1	10.4	±1.96	38.0	18.3	17.0	0.1
7.5	37.2	17.3	±1.94	51.9	30.4	24.3	3.9
16.0	62.3	41.1	±2.57	80.3	57.2	50.5	14.4
24.0	70.4	52.0	±1.33	88.6	70.0	63.6	23.8
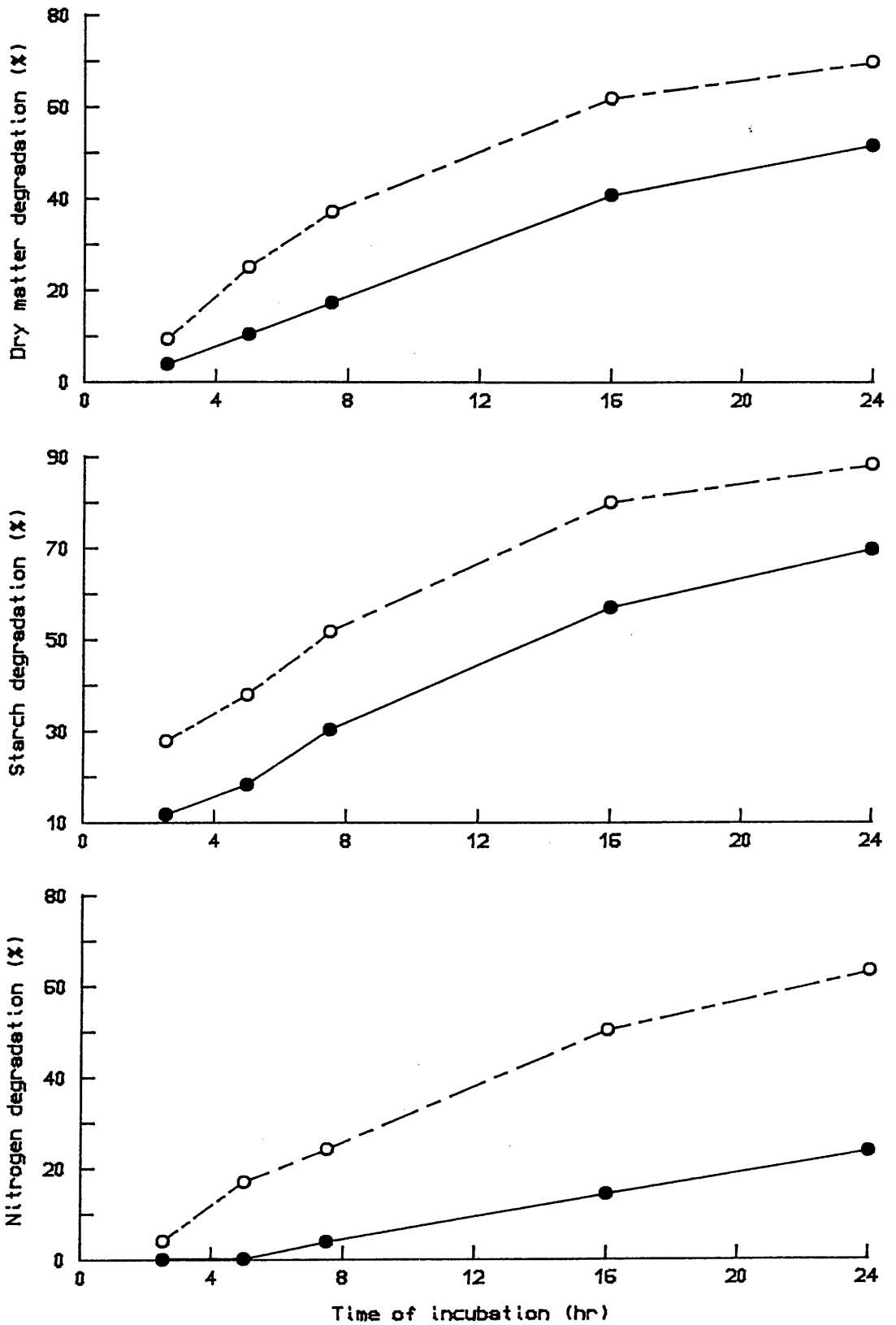
Figure 3.1.   The disappearance (%) of dry matter (DM), starch and
              nitrogen from samples of untreated rolled barley (O---O)
              or rolled barley treated with formaldehyde reagent (●——●).

Table 3.3. Expt 1b. The disappearance (%) of dry matter (DM), starch and total nitrogen from samples of untreated ground barley (U) or ground barley treated with formaldehyde reagent (T) at a rate of 8.0 l/t.

Incubation time (hrs)	DM			Starch		Nitrogen	
	U	T	SEM	U	T	U	T
2.5	16.3	12.3	±0.80	23.0	17.3	10.2	5.8
5.0	24.1	16.6	±1.35	31.3	22.6	13.6	3.8
7.5	30.4	24.3	±1.25	42.9	31.2	9.4	6.2
16.0	49.4	41.5	±1.20	69.1	58.0	21.8	13.6
24.0	54.8	46.5	±1.40	77.6	66.9	33.4	15.2
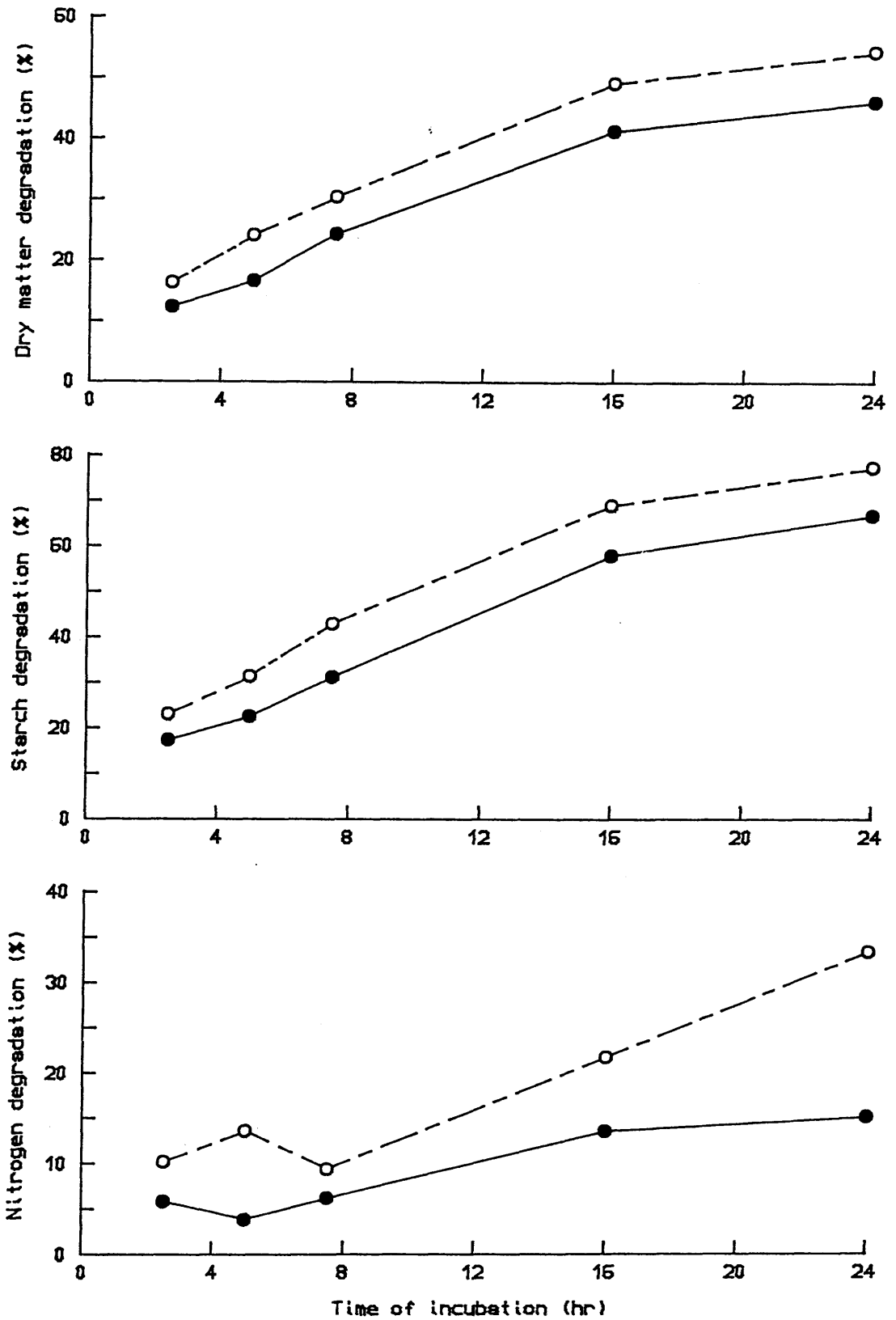
Figure 3.2. The disappearance (%) of dry matter (DM), starch and
nitrogen from samples of untreated ground barley (O---O)
or ground barley treated with formaldehyde reagent (●——●).

the formaldehyde treatment of grain reduced DM and starch disappearance but the effects on nitrogen disappearance were particularly marked. The formaldehyde reagent contained 152 g formaldehyde/l and thus the application at the 8 l/t rate was equivalent to a rate of 34.8 g formaldehyde/kg barley protein.

Experiment 2. Food intake and milk production in cows given silage <u>ad</u> <u>libitum</u> with increasing levels of barley or formaldehyde-treated barley supplements.

The results of Experiment 1 showed that when rolled barley grain was treated with the acidified-formaldehyde reagent the rates of starch and protein digestion in the rumen were reduced. To investigate the importance of these effects on silage intake and milk production in dairy cows, the experiment described below was conducted with animals given grass silage together with increasing amounts of supplements in the form of untreated barley or barley treated with formaldehyde at the rate of 8 l/t.

Experimental

Animals and their management

The experiment was conducted using six Friesian and six Ayrshire cows. The animals were in their 2nd-4th lactations and were in the 17-24th week of lactation at the start of the experiment. They were housed in individual stalls in a cattle byre and milked at 6.00 am and 16.00 pm in a herring-bone milking parlour. They were fed twice daily following milking and water was available <u>ad</u> <u>libitum</u>. The cows received a daily supplement of a commercial mineral mixture sufficient to meet their nutritional requirements (Scottish Agricultural Colleges, 1982).

Foods

The silage used in the experiment was made from a second crop of S23 perennial ryegrass (Lolium perenne) cut on the 27th July 1982. The

crop was cut with a disc mower and wilted for 5 hours before being

harvested with a precision-chop machine set to give a chop-length of 20

mm. During harvesting the grass was treated with an additive mixture

supplying 2.2 l of formic acid (850 g/kg) and 1.5 l of formalin (370 g

formaldehyde/kg) per tonne, and molasses were added at a rate of 20 l/t

as the grass was loaded into the silo. The molasses were applied

undiluted from a modified watering can to each load grass as it was

buckraked into the silo. The molasses used had a DM concentration of

767 g/kg with a sugar content of 520 g/kg DM and a crude protein

content of 51.8 g/kg DM.

The barley used was of a single batch of purchased material.

Prior to feeding or to treatment with formaldehyde reagent, it was

rolled through a 'crimper' to break the grain coat. The grain was

treated with formaldehyde reagent at a rate of 8 l/t. Treatment was

carried out to batches of 20 kg of grain to which the formaldehyde

reagent was added dropwise whilst the grain was mixed in a small

concrete mixer. Mixing was continued for approximately 10 minutes

following the addition of the reagent. Treated grain was allowed to

stand open to the air for at least 3 days before being given to the

cows.

Experimental design and treatments

The experiment was conducted according to a cyclical changeover

design (Davis and Hall, 1969) with six dietary treatments, two blocks

of animals and three, three week experimental periods. In each dietary

treatment silage was given ad libitum together with a rationed amount

of rolled barley supplement. The barley was given at rates of 4.0, 6.5

and 9.0 kg fresh weight per day either in an untreated form or after

treatment with acidified formaldehyde reagent. Food intake and milk yield were recorded daily and body weights were measured on two consecutive days during each week throughout the experiment. Milk samples were taken at the morning and evening milkings on the last 2 days of each experimental period and used to prepare weighted mean bulk samples for analysis.

Chemical analysis

Samples of silage and of untreated and formaldehyde-treated barley taken in each experimental period were analysed as appropriate for oven dry matter, toluene dry matter, ash, total nitrogen, non-protein nitrogen, ammonia, pH, lactic acid, water-soluble carbohydrate, acetic acid, butyric acid and ADF.

Milk samples were analysed for fat, protein, lactose and total solids.

Statistical analysis

Data were analysed by analysis of variance using Edex programme 5A.7 (ARC Unit of Statistics, Edinburgh).

Results

The composition of the diets

The compositions of the silage and the untreated and formaldehyde-treated barley are given in Table 3.4. The silage was well preserved with a low pH and low contents of ammonia and butyric acid. It had a moderately good DOMD value (674 g/kg DM) and a low protein content (108 g/kg DM). The barley samples also were of a rather lower protein content than typically encountered for feeding

barley, the mean value for the treated and untreated samples being 94 g/kg DM.

Food intake

The intakes of DM in concentrates and silage are shown in Table 3.5. With both untreated and formaldehyde treated barley increases in the rate of feeding were associated with a significant (P < 0.05) reductions in silage intake and a significant (P < 0.01) increase in total DM intake. However, at corresponding levels of supplementation there were no significant differences in silage DM intake or total DM intake which were attributable to the effects of the formaldehyde treatment of the grain. For both forms of barley the reduction in silage DM intake per kg barley DM given was 0.28 kg. The intake of energy and crude protein from both silage and concentrate and total diet increased as the rate of supplementation increased with both untreated and formaldehyde treated barley. However, at corresponding levels of supplementation there were no differences in energy and crude protein intake between the untreated and treated barley diets. There was on average an increase of 18.2 and 39.6 MJ ME/d with the medium and high rates as compared with the low rate of supplementation. Corresponding increases in crude protein intake were 110 and 252 g/d respectively.

Milk yield and composition

As shown in Table 3.6, with both the untreated and formaldehyde-treated barley milk yield increased significantly (P < 0.001) as the rate of supplementation was raised from 4.0 to 9.0 kg/d. There was a tendency for this response to be less pronounced with the

Table 3.5. The intake of dry matter (DM), metabolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for cows given silage ad libitum with supplements of untreated barley or formaldehyde-treated barley

	Barley			Treated barley			SED
	4.0	6.5	9.0	4.0	6.5	9.0	
Concentrate intake (kg DM/d)	3.26	5.28	7.18	3.22	5.22	7.33	0.07**
Silage intake (kg DM/d)	9.56	8.99	8.48	9.66	8.76	8.40	0.29*
Total intake (kg DM/d)	12.82	14.27	15.66	12.88	13.98	15.73	0.37**
Concentrate ME intake (MJ/d)	42.4	68.6	93.3	41.9	67.9	95.3	
Silage ME intake (MJ/d)	103.0	96.9	91.4	104.1	94.4	90.6	
Total ME intake (MJ/d)	145.4	165.5	184.7	146.0	162.3	185.9	
Concentrate CP intake (g/d)	308	498	678	302	489	687	
Silage CP intake (g/d)	1028	966	912	1038	942	903	
Total CP intake (g/d)	1336	1464	1590	1340	1431	1590	
ME content of total diet (MJ/kg DM)	11.3	11.6	11.8	11.3	11.6	11.8	
CP content of total diet (g/kg DM)	104	103	102	104	102	101	

Table 3.6. Milk yield and composition, the yield of milk constitutents and the body weight for cows given silage ad libitum with supplements of untreated barley or formaldehyde-treated barley at various rates

	Barley			Treated barley			SED
	4.0	6.5	9.0	4.0	6.5	9.0	
Milk yield (kg/d)	15.46	16.86	17.87	15.52	16.15	17.08	0.37***
Fat (g/kg)	47.4	47.6	45.9	47.1	48.0	45.5	1.6
(g/d)	731	791	809	724	765	771	29
Protein (g/kg)	31.9	33.3	32.9	32.2	33.5	34.0	0.90
(g/d)	490	555	585	499	537	579	16***
Lactose (g/kg)	45.7	45.2	45.5	45.2	45.3	46.0	0.5
(g/d)	709	767	820	704	734	787	20***
Total solids (g/kg)	132.9	133.9	132.4	132.6	135.0	133.3	1.8**
(g/d)	2053	2245	2357	2051	2167	2270	46****
Body weight (kg)	559	573	583	565	569	580	4.6**

treated than with the untreated grain (Figure 3.3) but differences in milk yield between grain treatments did not reach statistical significance (P < 0.05).

There were no statistically significant (P < 0.05) effects of either the level of barley supplementation or the type of barley on milk fat, protein and lactose contents. However, there was a tendency for milk protein content to be higher in animals receiving formaldehyde-treated barley than in the animals receiving the corresponding amount of untreated barley and this effect was most evident at the highest level of supplementation. There were significant (P < 0.001) increases in the yield of milk protein and lactose as the rate of feeding of both untreated and treated barley was increased but these mainly reflected the effects of the supplements on milk yield.

Body weight

There were no significant (P < 0.05) differences in body weight related to the provision of barley in untreated or formaldehyde-treated form (Table 3.6), but body weight was significantly (P < 0.01) increased by the rate of feeding of the grain supplements.

Discussion

In this experiment increasing the rate of supplementation of the diet with barley reduced silage intake but increased total DM intake with associated benefits in ME and crude protein intake. Milk yield was increased as the level of supplementation was raised and there were accompanying reductions in milk fat content and increases in milk protein content. However, at corresponding levels of supplementation
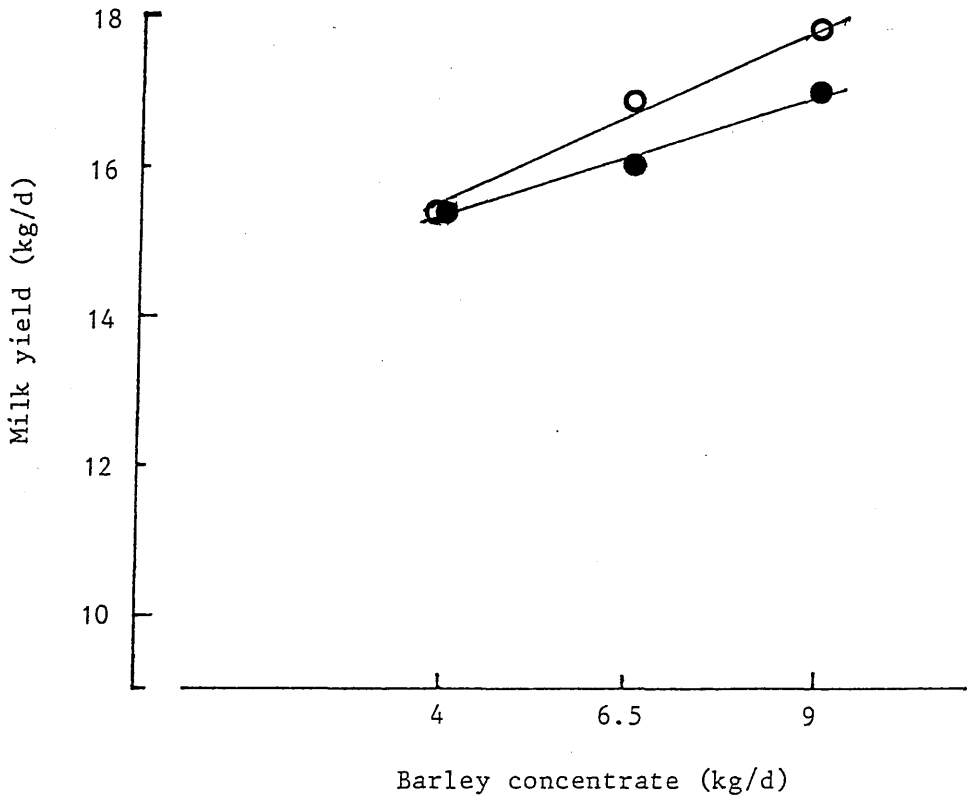
Figure 3.3. The changes in milk yield in response to increased
rates of concentrate feeding in cows given silage
ad libitum with supplements of (O) untreated barley
or (●) treated barley at various rates.

there were no statistically significant differences in silage intake or milk production between the untreated and the formaldehyde-treated barleys. The only trends between the supplements to emerge were a slight negative influence of the formaldehyde treatment on the slope of the relationship between milk yield and concentrate intake (Figure 3.3), and a slight positive influence of milk protein content (Table 3.6).

The reasons why the formaldehyde treatment of the barley was without effect are uncertain but three points should be considered. Firstly, it is possible that the treatment of barley with the acidic-formaldehyde reagent was less effective in reducing the rate of digestion of starch and protein in the rumen than was indicated by polyester bag tests conducted in Experiment 1a. This would effectively reduce the differences in rate of digestion between the untreated and treated barley.

Second, the silage used in Experiment 2 was characterised by an unusually low 'replacement rate' with respect to the barley supplement. For each 1 kg barley DM given silage DM intake was reduced by only 0.28 kg. This is much less than the typical value of 0.5 kg silage DM/kg barley DM reported by Castle (1982). Since the replacement rate was low it is possible that, with this silage, silage intake was not very sensitive to changes in the rate of digestion of barley starch in the rumen.

Third, because of the low N content of the silage and the unusually low N content of the barley, the crude protein content of the total diet was only 101-104 g/kg DM. This should have provided circumstances wherein the cows were sensitive to changes in the duodenal flow of protein, which might arise through the formaldehyde

treatment of barley. However it is possible that there were

deficiencies in the nitrogen supply to the rumen microorganisms and

that these were exacerbated by the cross-linking of the barley protein

resulting from the formaldehyde treatment. In this respect it is

notable that assuming rumen-degradability values of 0.90 and 0.80 for

the N in the silage and the untreated barley (see later; Table 3.29),

the control diets contained only 1.16-1.29 g RDN/MJ dietary ME. The

corresponding figures for the treated barley diets were 0.85-1.10 g

RDN/MJ. This is less than the value of 1.32-1.40 g RDN/MJ ME

recommended by the ARC (1984) as necessary to satisfy the dietary RDN

requirements to achieve maximal rates of microbial protein synthesis in

the rumen.

<u>Experiment 3</u>. Food intake and milk production in cows given silage <u>ad libitum</u> with supplements containing barley or formaldehyde-treated barley with and without fishmeal.

Although it was demonstrated in Experiment 1 that the formaldehyde treatment of barley reduced the rate of ruminal degradation of starch and protein, such treatment in Experiment 2 was without effect on silage intake or milk production. The reasons for this are uncertain but suggested explanations include the possibility: (1) that the effect of the formaldehyde reagent applied at 8 l/t of barley was less than indicated by the incubation studies in Experiment 1 and (2) that the basal diet used in Experiment 2 was inadequate in RDN supply, confounding the potential infuences of the barley treatment on digestion in the rumen. With these possibilities in view the treatment of barley was further examined in the following experiment. In this study the barley was treated with a higher dose rate of formaldehyde reagent to increase the degree of cross-linkage of barley starch and protein, the silage was selected to be higher in protein, and a treatment in which the protein content of the diet was increased by supplementation with fishmeal was included.

<div align="center">Experimental</div>

<u>Animals and their management</u>

The experiment was undertaken with eight Friesian cows; four of the animals were in their 1st lactation and four were in their 2nd-3rd lactations. At the start of the experiment the animals were in the 7-12th weeks of lactation. The 1st lactation cows had an average weight of 479 kg whilst the older cows weighed 554 kg. The

corresponding weights at the end of the experiment were 520 kg and 580 kg.

The animals were housed in a small byre in individual stalls where they were fed and milked at 06.00 and 16.00 daily. Water was available ad libitum and the animals received a proprietary mineral mixture to meet their requirements (Scottish Agricultural Colleges, 1982).

Foods

The silage used was made from a first cutting of a sward of S24 perennial ryegrass in June 1983. The grass was cut with a disc mower and allowed to wilt for approximately 2 hours before harvesting with a precision chop forage harvester set to give a chop length of approximately 20 mm. The grass was treated with formic acid (850 g/l) at a rate of 2.3 l/t during harvesting and ensiled in a 300 t bunker silo.

The barley and the fishmeal were purchased from commercial sources. The barley, prior to feeding or to treatment with formaldehyde reagent, was rolled through a 'crimper' to break the grain coat. The grain was treated with formaldehyde reagent at a rate of 15 l/t. Treatment was carried out as described in Experiment 2.

The fishmeal was purchased as a selected low degradability product with an estimated rumen-degradability value of 45%. The fishmeal was hand mixed with the barley supplement at the time of feeding.

Experimental design and treatments

The experiment was conducted as a duplicated 4 x 4 Latin square with two blocks of animals, one for 1st-lactation and one for multiparous cows. Each block involved 4 animals, 4 treatments and 4

3-week experimental periods. The dietary treatments consisted of silage ad libitum with one of four supplementary feeds. The supplements were: untreated barley (7 kg/d); untreated barley (6 kg/d) and fishmeal (1 kg/d); treated barley (7 kg/d); and treated barley (6 kg/d) and fishmeal (1 kg/d). Feed intake and milk yield were measured daily. Samples of milk were taken at the am and pm milking on the last two days of each experimental period and used to prepare a weighted mean sample for chemical analysis.

Digestibility of silage

To determine the digestible organic matter content of the silage the food was given to four mature wether sheep at a maintenance level of feeding. The animals, which were held in metabolism cages, were given the diet for a 14 day introductory period before a complete faecal collection was made over a period of 7 days.

Intraruminal incubations

The rumen degradability of barley and formaldehyde treated barley were estimated using the polyester bag technique of Mehrez and Orskov (1977). A non-lactating cow fitted with a permanent rumen cannula was used. The cow was given ad libitum access to hay 2 (see Table 3.1). Samples taken at each incubation time were replicated 12 times. Individual samples were analysed for DM and samples from each time period were bulked to provide a single sample for analysis for starch and nitrogen.

Chemical analysis

Samples of silage and of untreated and formaldehyde-treated barley

and fishmeal taken in each experimental period of the feeding experiment as well as the faecal samples from the digestibility experiment and feed and residue samples from the incubation studies were analysed as appropriate for oven dry matter, toluene dry matter, ash, total nitrogen, non-protein nitrogen, ammonia, pH, lactic acid, water-soluble carbohydrate, acetic acid, butyric acid, ethanol, starch and ADF.

Samples of milk were analysed for fat, protein, lactose and total solids.

Statistical analysis

The results were subjected to standard analysis of variance techniques for Latin Square analysis. Squares for heifers and multiparous cows were analysed as replicates and separately.

Results

Composition of foods

The composition of the silage is shown in Table 3.7. The silage was well-preserved with a low pH, low content of butyric acid and a high content of lactic acid. The DOMD value was 675 ± 9.1 (n = 4) g/kg and the crude protein content was 128 g/kg DM. The NPN content of the silage was high but the ammonia levels were satisfactory, indicating that deamination reactions during conservation had been restricted.

The composition of the barley, the fishmeal and the concentrate mixture are given in Table 3.8. The samples of barley were of rather high protein content, 121-124 g/kg DM and therefore did not differ markedly in protein content from the silage. The fishmeal on the other hand contained 638 g protein/kg DM and inclusion of this food in the supplement mixtures increased their protein contents to 196-198 g crude

Table 3.7. The chemical composition of the silage used in Experiment 3

	Silage
DM (g/kg)	220
Organic matter (g/kg/DM)	925
Total N (g/kg DM)	20.5
NPN (g/kg total N)	698
Ammonia N (g/kg total N)	100
pH	3.73
Lactic acid (g/kg DM)	77
Water soluble carbohydrate (g/kg DM)	30
Acetic acid (g/kg DM)	25
Butyric acid (g/kg DM)	2
Ethanol (g/kg DM)	62
Acid detergent fibre (g/kg DM)	316
DOMD (in vivo) (g/kg DM)	675

Table 3.8. The chemical composition of untreated barley and formaldehyde-treated barley, fishmeal and the concentrate mixtures used in Experiment 3.

	Untreated barley	Treated barley	Fishmeal	Untreated barley conc mixture	Treated barley conc mixture
DM (g/kg)	834	831	854	837	834
OM (g/kg DM)	974	975	792	809	803
Total N (g/kg DM)	19.4	19.8	102	31.4	31.8

protein/kg DM.

Degradability of barleys

The rumen degradabilities of the barleys are shown in Table 3.9 and Figure 3.4. As inciated by the extent of degradation after 24 hours of incubation, the formaldehyde reagent reduced DM disappearance by 28.1%, starch disappearance by 18.9% and nitrogen disappearance by 66.5% of the value observed with the untreated barley control

Food intake

The intake of DM in concentrates and silage for the combined groups of cows and heifers are shown in Table 3.10. The formaldehyde treatment of barley increased silage intake and total DM intake; mean values were 0.22 and 0.20 kg/d respectively, though the changes were not statistically significant. More pronounced responses in intake were obtained when fishmeal was included in the diet, and with both untreated and formaldehyde treated barley silage intake and total DM intake responses to fishmeal were statistically significant.

Examination of the food intake results for the cow and heifer groups of animals separately (Table 3.11 and Table 3.12) revealed that whilst the heifers showed no significant ($P < 0.05$) response in silage intake or total DM intake to any of the treatments imposed, the cows showed responses that were especially marked. Thus in the cows formaldehyde treatment of unsupplemented barley was associated with an increase ($P < 0.01$) in silage intake of 0.59 kg/d, though for the diets containing fishmeal, the corresponding response was a non-significant ($P > 0.05$) increase of 0.2 kg/d. Fishmeal inclusion in the cows' diet led to consistent, significant ($P < 0.01$) increases in silage intake.

Table 3.9. The disappearance (%) of dry matter (DM), starch and
total nitrogen from samples of untreated rolled barley (U)
or rolled barley treated with formaldehyde reagent (T)
at a rate of 15.0 l/t

Incubation time (hrs)	DM			Starch		Nitrogen	
	U	T	SEM	U	T	U	T
2.5	43.6	16.6	+2.18	53.4	27.7	27.7	7.6
5.0	59.4	22.4	+2.47	76.1	37.4	46.3	11.9
7.5	72.2	32.7	+1.38	88.1	41.3	56.4	10.4
16.0	80.2	52.8	+1.05	95.9	69.0	74.1	19.1
24.0	83.5	59.7	+1.26	97.5	79.0	84.6	28.3
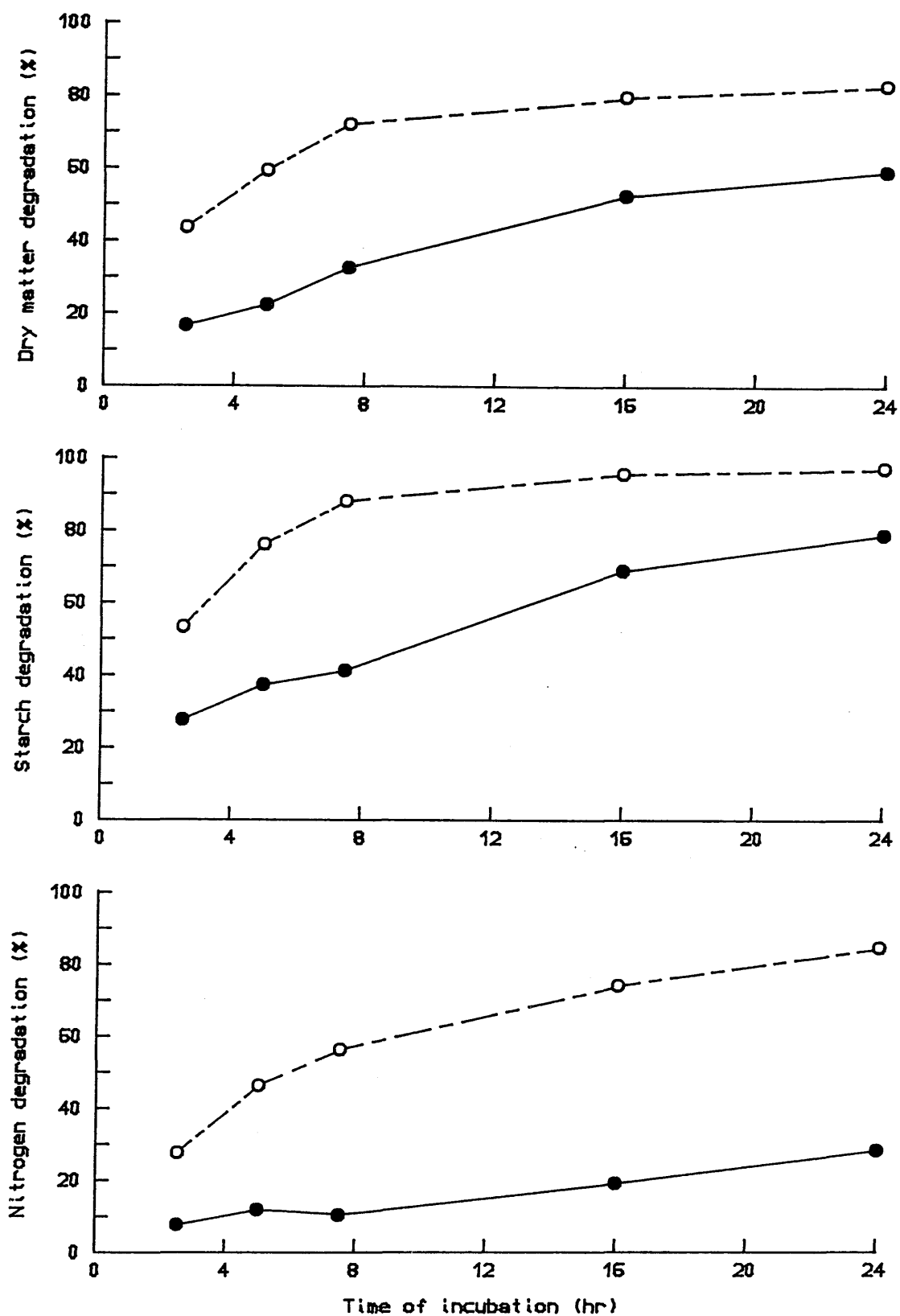
Figure 3.4. The disappearance (%) of dry matter (DM), starch and total nitrogen from samples of untreated rolled barley (O---O) or rolled barley treated with formaldehyde reagent (●——●) at a rate of 15 l/t.

Table 3.10. The intake of dry matter (DM), metbolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for cows and heifers given silage ad libitum with concentrate supplements containing untreated or formaldehyde-treated barley with or without the inclusion of fishmeal

	Untreated barley		Treated barley		SED
	-FM	+FM	-FM	+FM	
Concentrate intake (kg DM/d)	5.85	5.89	5.83	5.87	0.02
Silage intake (kg DM/d)	7.91	8.50	8.11	8.73	0.24*
Total intake (kg DM/d)	13.76	14.39	13.94	14.60	0.24*
Concentrate ME intake (MJ/d)	76.1	74.5	75.8	74.3	
Silage ME intake (MJ/d)	85.4	91.8	87.6	94.3	
Total ME intake (MJ/d)	161.5	166.3	163.4	168.6	
Concentrate CP intake (g/d)	709	1157	721	1168	
Silage CP intake (g/d)	1014	1089	1039	1118	
Total CP intake (g/d)	1723	2246	1760	2286	
ME content of total diet (MJ/kg DM)	11.7	11.6	11.7	11.6	
CP content of total diet (g/kg DM)	125	156	126	157	

Table 3.11. The intake of dry matter (DM), metabolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for cows given silage ad libitum with concentrate supplements containing untreated or formaldehyde-treated barley with or without the inclusion of fishmeal

	Untreated barley		Treated barley		SED
	−FM	+FM	−FM	+FM	
Concentrate intake (kg DM/d)	5.85	5.89	5.83	5.87	0.25 NS
Silage intake (kg DM/d)	8.33	9.51	8.92	9.71	0.27**
Total intake (kg DM/d)	14.18	15.40	14.75	15.58	0.28**
Concentrate ME intake (MJ/d)	76.1	74.5	75.8	75.3	
Silage ME intake (MJ/d)	90.0	102.7	96.3	104.9	
Total ME intake (MJ/d)	166.1	177.2	172.1	180.2	
Concentrate CP intake (g/d)	709	1157	721	1168	
Silage CP intake (g/d)	1067	1218	1143	1244	
Total CP intake (g/d)	1776	2375	1864	2412	
ME content of total diet (MJ/kg DM)	11.7	11.5	11.7	11.6	
CP content of total diet (g/kg DM)	125	154	126	155	

Table 3.12. The intake of dry matter (DM), metbolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for heifers given silage ad libitum with concentrate supplements containing untreated or formaldehyde-treated barley with or without the inclusion of fishmeal

	Untreated barley		Treated barley		SED
	-FM	+FM	-FM	+FM	
Concentrate intake (kg DM/d)	5.85	5.89	5.83	5.87	0.02 NS
Silage intake (kg DM/d)	7.49	7.49	7.29	7.76	0.28 NS
Total intake (kg DM/d)	13.34	13.38	13.12	13.63	0.28 NS
Concentrate ME intake (MJ/d)	76.1	74.5	75.8	75.3	
Silage ME intake (MJ/d)	80.9	80.9	78.7	83.8	
Total ME intake (MJ/d)	157.0	155.4	154.5	159.1	
Concentrate CP intake (g/d)	709	1157	721	1168	
Silage CP intake (g/d)	960	960	934	994	
Total CP intake (g/d)	1669	2117	1655	2162	
ME content of total diet (MJ/kg DM)	11.8	11.6	11.8	11.7	
CP content of total diet (g/kg DM)	125	158	126	159	

With the untreated barley, fishmeal inclusion in the diet was linked with an increase in silage DM intake of 1.18 kg/d, whilst with the treated barley the corresponding value was reduced to 0.79 kg/d.

Tables 3.10, 3.11 and 3.12 also include the results for the estimated intakes of ME and crude protein, together with the calculated compositions of the total diet consumed. Differences in ME intakes between experimental treatments largely reflected the influences of the concentrate supplements on silage intake and therefore differed somewhat between the cow and heifer groups (Table 3.11 and Table 3.12). On average, formaldehyde treatment of barley led to a small increase in ME intake, of approximately 2 MJ/d (Table 3.10). However, much more substantial increases of approximately 5 MJ/d were observed in response to the inclusion of fishmeal in the diets.

Inclusions of fishmeal also had a major influence on the dietary supply of crude protein, on average increasing crude protein intake by approximately 525 g/d (Table 3.10). Thus whilst the energy contents of the total diets consumed varied over a rather narrow range from 11.5–11.7 MJ/kg DM, the protein contents varied from approximately 125 to 156 g/kg DM, depending on the fishmeal inclusion.

Milk yield and composition

The results for milk yield and composition for the combined groups of cows and heifers are shown in Table 3.13. Milk yield was significantly (P < 0.001) increased both by the formaldehyde treatment of the barley supplement and by the inclusion of fishmeal in the diet. The response to formaldehyde treatment of the barley was an increase of 1.55 kg/d with low protein concentrate mixture but the effect was reduced to 1.35 kg/d with the high-protein mixture. Inclusion of

Table 3.13. Milk yield and composition, and the yield of milk
constituents for cows and heifers given silage _ad libitum_ with
concentrate supplements containing untreated or formaldehyde-treated
barley with or without the inclusion of fishmeal

| | Untreated barley | | Treated barley | | SED | |
	-FM	+FM	-FM	+FM		
Milk yield (kg/d)	16.91	18.25	18.46	19.60	0.30	***
Fat (g/kg)	48.3	48.2	46.2	45.6	1.52	NS
(g/d)	804	863	837	874	26.1	NS
Protein (g/kg)	31.8	32.1	32.2	32.7	0.55	NS
(g/d)	537	580	589	635	12.7	***
Lactose (g/kg)	48.9	49.0	48.9	48.6	0.24	NS
(g/d)	827	894	901	951	15.9	***
Total solids (g/kg)	135.7	136.3	134.4	133.6	1.43	NS
(g/d)	2281	2465	2458	2593	32.8	***

fishmeal in combination with untreated barley led to an increase in milk yield of 1.34 kg/d whilst the corresponding value observed with the treated barley supplements was 1.14 kg/d. There were no statistically significant (P < 0.05) effects of the experimental treatments on milk composition but fat content tended to be reduced and protein content tended to be increased with the concentrate mixture containing treated barley.

There were no statistically significant effects on milk fat yield but both the inclusion of fishmeal in the diet and, to a lesser degree, the treatment of barley tended to promote fat production. Statistically significant (P < 0.05) effects on milk protein yield and lactose yield were obtained and both were similarly related to the experimental treatments. Protein and lactose yields were low with the supplement of untreated barley and they were increased to a similar degree by formaldehyde treatment of the grain or by inclusion of fishmeal in the diet. Moreover, the effect observed in response to those dietary manipulations appeared to be additive, so that the increases in protein and lactose yield obtained from formaldehyde-treatment of the grain and the inclusion of fishmeal in the diet were twice those obtained when formaldehyde-treatment or fishmeal supplementation was applied alone. The effects observed on milk total solids yield were statistically significant and reflected the changes in milk fat, protein and lactose production.

Analysis of the results for the group of cows and the group of heifers separately showed no important differences in the responses in milk yield and composition to the experimental treatment (Table 3.14 and Table 3.15). However, differences in milk yield and composition between the groups were apparent and there was some indication that

Table 3.14. Milk yield and composition, and the yield of milk
constituents for cows given silage _ad libitum_ with concentrate
supplements containing untreated or formaldehyde-treated barley
with or without the inclusion of fishmeal

	Untreated barley		Treated barley		SED	
	−FM	+FM	−FM	+FM		
Milk yield (kg/d)	19.40	21.05	21.12	22.40	0.58	**
Fat (g/kg)	46.4	45.2	43.6	43.0	2.9	NS
(g/d)	886	936	909	940	47.1	NS
Protein (g/kg)	30.7	31.0	31.2	31.8	0.6	NS
(g/d)	600	652	655	708	21.3	**
Lactose (g/kg)	48.5	48.4	48.2	48.0	2.7	NS
(g/d)	941	1021	1016	1077	30.7	**
Total solids (g/kg)	132.3	131.8	130.0	129.6	2.88	NS
(g/d)	2560	2759	2729	2880	56.1	**

Table 3.15. Milk yield and composition, and the yield of milk constituents for heifers given silage ad libitum with concentrate supplements containing untreated or formaldehyde-treated barley with or without the inclusion of fishmeal

| | Untreated barley | | Treated barley | | SED | |
	-FM	+FM	-FM	+FM		
Milk yield (kg/d)	14.43	15.45	15.80	16.80	0.26	***
Fat (g/kg)	50.3	51.2	48.8	48.2	1.61	NS
(g/d)	722	790	765	807	33.2	NS
Protein (g/kg)	32.8	33.1	33.1	33.6	1.07	NS
(g/d)	473	509	523	562	18.0	**
Lactose (g/kg)	49.4	49.6	49.7	49.1	0.43	NS
(g/d)	714	767	788	825	15.0	***
Total solids (g/kg)	139.0	140.7	138.7	137.5	1.19	NS
(g/d)	2001	2170	2185	2306	45.3	***

cows were more responsive than heifers in the yield of milk and of milk constituents. Thus, for example, taking the extremes of the treatments and comparing the untreated low-protein barley concentrate with the treated-barley plus fishmeal concentrate, heifers showed an increase in milk yield of 2.37 kg/d whilst cows showed an increase of 3.0 kg/d. Corresponding values for the increases in fat, protein and lactose yields were 54 and 85 g/d, 89 and 108 g/d and 111 and 136 g/d, respectively.

Discussion

In contrast to Experiment 2, in this experiment treatment of barley with the acid-formaldehyde reagent led to a significant ($P < 0.05$) improvement in milk yield with associated increases in the yield of milk fat and especially protein and lactose. These effects were to a degree linked with an increase in silage intake but the separate analysis of the results for cows and heifers indicated that the changes in intake alone could not explain the observed effect on milk production. In heifers the increases in intake to formaldehyde-treatment of barley were small but the responses in milk production were still substantial suggesting that the dietary treatment was in some way enhancing the supply of nutrients which the cow was deriving from the diet.

As compared with Experiment 2, the protein content of the basal silage plus barley diet used in this experiment was higher, 125 g/kg DM as compared with 101-104 g/kg DM. Assuming rumen-degradabilities of 0.90 and 0.80 for silage and barley N the basal diet was calculated to contain 1.47 g RDN/MJ ME, which is above the range 1.32-1.40 g RDN/MJ ME, recommended by ARC (1984) as being satisfactory to meet the dietary

requirement for maximal rates of microbial protein synthesis in the rumen. However, the corresponding figure for the silage plus treated barley diet was 1.10 g RDN/ME (see Table 3.29). Both cows and heifers responded in milk production to supplementation of the diet with fishmeal but this protein source has a low rumen-degradability and would provide the animals with additional undegraded dietary N (UDN) as well as RDN. Furthermore, fishmeal is rich in methionine and lysine, which have been suggested to be the most limiting amino acids for milk protein synthesis in cows given silage-barley diets (Thomas and Chamberlain, 1984).

The rate of application of acid-formaldehyde used in this experiment was almost double that used in Experiment 2 giving an effective dose of 22.9 g formaldehyde per kg barley crude protein. As judged from the intraruminal incubation studies this dose rate was effective in reducing the rate of degradation of both starch and protein in the rumen (Table 3.9). However, there was little evidence from the figures for disappearance from the polyester bags that the higher rate of application of the reagent was markedly more effective than the lower dose rate used in Experiment 1. Whether this represents the true picture or merely reflects the limitations of the polyester bag technique is difficult to judge.

The similarity of the increases in milk production observed with the formaldehyde-treated barley and with fishmeal supplementation, especially in the heifers where silage intake was not increased, may be argued to provide indirect evidence that the barley treatment did lead to an increased passage of UDN to the small intestine, and that this was the basis of the response in milk production. However, several features of the results suggest that this interpretation is simplistic.

First, the milk yield response to formaldehyde treatment of the barley was as great with the high-protein, fishmeal supplemented diet as it was with the low protein unsupplemented control diet. Second, the increases in silage intake observed in response to the formaldehyde-treated barley argue that the processing of the grain has influences on microbial digestion of forage in the rumen. And finally, formaldehyde-treated barley differed from fishmeal supplements in its tendency to reduce milk fat content (Table 3.13), again implying that the two treatments have differing effects on the nutrient supply derived from the diet.

Experiment 4. Food intake and milk production in cows given silage ad libitum with supplements of barley, treated barley and sodium bicarbonate.

A feature of the results of Experiment 3 was that treatment of the barley supplement with acidified formalin was associated with a response in silage intake in the multiparous cows, but not in the 1st lactation animals, whilst both groups of cows showed increased milk yield. The results suggest that the changes in feed intake and milk yield may in part relate to independent effects of formaldehyde treatment of the grain on the digestion process. For example, the responses in intake may depend heavily on the reduced rate of digestion of barley starch in the rumen, whilst the responses in milk yield may relate more to the rumen-protection of barley protein and increased passage of amino acids to the small intestine. Assuming that the reduced rate of starch digestion in the rumen exerts an influence mainly through an avoidance of low rumen pH, it should be possible to obtain effects on silage intake corresponding to those obtained in Experiment 3 by the dietary inclusion of sodium bicarbonate (Edwards and Poole, 1983). With this hypothesis in mind, an experiment was designed to compare the effects on silage intake of supplements of untreated barley, formaldehyde-treated barley and untreated barley mixed with sodium bicarbonate to act as ruminal buffer.

<div align="center">Experimental</div>

Animals and their management

The experiment was conducted with four Friesian and two Ayrshire cows which at the start of the experiment were in the 17-20th week of

their 2nd-6th lactations. The animals were housed in individual stalls in a cattle byre and milked at 06.00 hours and 16.00 hours in a herringbone milking parlour. The animals were fed twice-daily after milking and water was available ad libitum. The cows received a daily supplement of a commercial mineral mixture sufficient to meet their nutritional requirements (Scottish Agricultural Colleges, 1982).

Foods

The silage was made from a second cut of S24 perennial ryegrass made in July 1983. The grass was cut with a disc mower and allowed to wilt for 2 hours. It was then harvested with a precision-chop forage harvester set to chop at 20 mm lengths. Formic acid (850 g/l) was added at a rate of 3 l/t during harvesting and the crop was ensiled in a 50 t bunker silo.

The barley used for the experiment was purchased from a supplier. The untreated food was rolled before feeding. The treated food was prepared in 20 kg batches through the addition of formaldehyde reagent during mixing of the food in a small concrete mixer, as described in Experiment 2. The rate of application of the formaldehyde reagent was 15 l/t.

The sodium bicarbonate was of food grade (ICI Ltd., Cheshire) and was mixed with the barley immediately prior to feeding.

Experimental design and treatments

The experiment was conducted according to a duplicated Youden Square design with 6 animals, four dietary treatments and four, three-week experimental periods. For the four dietary treatments grass silage was offered ad libitum either without supplement or else with a

supplement of 9.0 kg/d of rolled barley. The barley was given in an untreated form, or treated with formaldehyde reagent, or mixed with 250 g/d of sodium bicarbonate powder. Food intake and milk yield were recorded daily and body weights were measured on two consecutive days each week throughout the experiment. Milk samples were taken at the am and pm milkings on the last 2 days of each experimental period and used to prepare weighted mean bulk samples for analysis.

Chemical analysis

Samples of silage and barley taken in each experimental period were analysed as appropriate for oven dry matter, toluene dry matter, DOMD, ash, total nitrogen, non-protein nitrogen, ammonia, pH, lactic acid, water-soluble carbohydrates, acetic acid, and ADF.

Milk samples were analysed for fat, protein, lactose and total solids.

Statistical analysis

Results were analysed by analysis of variance (Snedecor, 1956).

Results

The composition of the diets

The composition of the silage and of the untreated and formaldehyde-treated barley was given in Table 3.16. The silage was well preserved with a satisfactory lactic acid level and a low pH, though the proportion of ammonia-N in the total N was a little above the desirable level. The protein content of the silage was 149 g/kg DM and the in vitro DOMD value was 666 g/kg DM. The protein contents of the untreated and formaldehyde treated barley samples were similar at

Table 3.16. The chemical composition of the silage and untreated and formaldehyde-treated barley used in Experiment 4.

	Silage	Untreated barley	Treated barley
DM (g/kg)	225[†]	838	832
Organic matter (g/kg DM)	901	978	976
Total N (g/kg DM)	23.9	14.7	14.6
NPN (g/kg total N)	690	–	–
Ammonia N (g/kg total N)	120	–	–
pH	3.90	–	–
Lactic acid (g/kg DM)	88	–	–
Water soluble carbohydrate (g/kg DM)	61	–	–
Acetic acid (g/kg DM)	20	–	–
Acid detergent fibre (g/kg DM)	286	–	–
DOMD (g/kg)	666[§]		

† Toluene distillation (Dewar and McDonald, 1961)

§ Morrison (1972)

approximately 92 g/kg DM.

Food intake

The DM intake observed for the four experimental treatments are shown in Table 3.17. For the animals given the unsupplemented diet the silage intake was 12.63 kg/d but this was reduced ($P < 0.001$) to 7.45 kg/d when the supplement of untreated barley was given. When the barley supplement was treated with formaldehyde, silage intake was increased by approximately 1.17 kg/d and a similar though slightly smaller response was obtained when the untreated barley was supplemented with sodium bicarbonate.

The estimated ME intakes were low for the unsupplemented diet at approximately 135 MJ/d and were increased by approximately 40-52 MJ/d by the allocation of the barley, with the highest values being obtained with the formaldehyde-treated barley. Crude protein intakes did not vary widely between treatments but were again highest for the diet containing the formaldehyde-treated barley.

The estimated ME content of the silage diet was 10.7 MJ/kg DM and its protein content was 149 g/kg DM. The ME contents of the supplemented diets ranged from 11.7 to 11.8 MJ/kg DM and the protein contents were 121 to 123 g/kg DM.

Milk yield and composition

For animals receiving the unsupplemented silage diet, milk yield was 16.36 kg/d (Table 3.18) and significantly higher yields were obtained with each of the supplemented diets. Differences in yield between the various supplement treatments were not significant ($P > 0.05$) though there was a clear trend for higher yields with the

Table 3.17. The intake of dry matter (DM), metabolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for cows given silage ad libitum without supplement or with concentrate supplements containing barley, formaldehyde-treated barley or a mixture of barley and sodium bicarbonate

	Supplement				SED
	None	Barley	Treated barley	Barley + NaHCO$_3$	
Concentrate intake (kg DM/d)	–	7.37	7.35[b]	7.05[b]	0.17
Silage intake (kg DM/d)	12.63[c]	7.45[a]	8.62[b]	8.33[b]	0.35***
Total intake (kg DM/d)	12.63	14.82	15.97	15.38	0.65
Concentrate ME intake (MJ/d)	–	95.8	95.6	91.7	
Silage ME intake (MJ/d)	134.6	79.4	91.9	88.8	
Total ME intake (MJ/d)	134.6	175.2	187.5	180.5	
Concentrate CP intake (g/d)	–	677	671	648	
Silage CP intake (g/d)	1887	1113	1288	1244	
Total CP intake (g/d)	1887	1790	1959	1892	
ME content of total diet (MJ/kg DM)	10.7	11.8	11.7	11.7	
CP content of total diet (g/kg DM)	149	121	123	123	

Means in a line that do not share the same superscript differ significantly (P < 0.05).

Table 3.18. Milk yield and composition, the yield of milk constituents and the body weight for cows given silage <u>ad libitum</u> without supplement or with concentrate supplements containing barley, formaldehyde-treated barley or a mixture of barley and sodium bicarbonate

| | Treatment | | | | SED |
	None	Barley	Treated barley	Barley–NaHCO$_3$	
Milk yield (kg/d)	16.36^a	18.10^b	19.50^b	18.72^b	0.65**
Fat (g/kg)	42.5	44.0	41.8	44.1	1.0 NS
(g/d)	692^a	791^b	804^b	811^b	26.8 **
Protein (g/kg)	31.1	32.3	32.7	31.9	0.8 NS
(g/d)	500^a	575^b	643^c	589^{bc}	27.9 **
Lactose (g/kg)	47.2	46.4	47.3	47.3	0.6 NS
(g/d)	773^a	845^b	923^c	878^{bc}	33.3 **
Total solids					
(g/kg)	127.9^a	129.9^{ab}	129.2^{ab}	130.1^b	0.9 **
(g/d)	2077	2342	2510	2414	82.9 **
Body weight (kg)	526^a	537^{ab}	550^b	541^b	6.66*

Means with unlike letters are significantly different (P < 0.05)

barley-bicarbonate supplement and especially the formaldehyde-treated barley supplement than with the untreated-barley control.

There were no significant effects of the experimental treatment on milk fat, protein or lactose contents but there were small differences in fat and protein contents which led to a statistically significant ($P < 0.01$) difference in total solids content between the unsupplemented diet and the barley-bicarbonate treatment. A notable trend was for the formaldehyde-treated barley supplement to be associated with a reduction in milk fat content and an increase in milk protein content as compared with other supplemented diets.

Each of the supplemented diets gave a greater ($P < 0.01$) production of milk fat, protein, lactose and total solids than the unsupplemented diet of silage alone. Moreover, there were some significant ($P < 0.05$) differences between supplements in the yields of protein and lactose, though not in fat or total solids. As compared with the untreated-barley supplement the treated-supplement gave a higher ($P < 0.05$) yield of both protein and lactose; a similar trend was apparent in comparisons with the barley-bicarbonate supplement though the differences in lactose yield did not reach statistical significance.

Body weight

There were significant ($P < 0.05$) increases in body weight for all the barley supplemented diets as compared with the unsupplemented silage diet (Table 3.18). Differences in body weight associated with the various barley supplements were not statistically significant, though there was a tendency for higher body weights in animals given the formaldehyde-treated barley.

Discussion

In this experiment the rate of 'replacement' of silage by barley supplement was high (cf. Experiment 2). For each 1 kg of barley DM offered, silage DM intake was reduced by 0.70 kg/d. Thus as compared with the diet of silage alone the allowance of the 'control' untreated barley supplement reduced silage intake by 5.18 kg DM/d. Corresponding reductions were also observed with the formaldehyde-treated barley and barley-$NaHCO_3$ supplements but quantitatively the effects were smaller, 4.01 kg DM/d and 4.30 kg DM/d, respectively. Thus for these supplements the rates of replacement were 0.54 kg/kg and 0.61 kg/kg respectively.

It appears reasonable to assume that these effects on forage intake derive in part from the influences of the treated-barley supplement and of the $NaHCO_3$ supplement on fermentation conditions and on the rate of forage digestion in the rumen. Presumably in the one case the effect arises through a reduction in the rate of fermentation of starch whilst in the other they derive from a buffering of the changes in rumen pH which are incidental on starch fermentation. In this respect it should be noted that in this experiment the basal silage-barley diet contained 1.41 g RDN/MJ ME (see Table 3.29) which is supposed to meet the dietary requirement for maximal microbial growth in the rumen (ARC, 1984). The corresponding figure for treated barley silage diet was 1.13 g RDN/ME.

In terms of milk production, however, the results indicated that the effects arising from formaldehyde-treatment of barley did not simply correspond with those obtained from supplementation of the diet with $NaHCO_3$. Although the effects were not statistically significant, there was a trend for a higher milk yield with the treated-barley diet

than with the barley-NaHCO$_3$ diet and this was associated with a reduced milk fat content and increased protein content. As a consequence, whilst the yield of milk fat with the two diets was similar, the yield of protein was about 9% higher with treated barley diet than with the barley-NaHCO$_3$ diet, and the corresponding difference for lactose was 5%. These differences may be attributable to the effects of the formaldehyde treatment on the ruminal digestion of barley protein and the inreased passage of UDN from the rumen to the small intestine.

Experiment 5. Food intake and milk production in cows given grass or
lucerne silages with or without supplements of barley or
formaldehyde-treated barley

There is increasing interest in the United Kingdom in the use of
forage legumes such as white clover and lucerne as silage crops. The
legumes offer several advantages - they are consistently of high
protein contents and they generally promote greater ad libitum intake
of DM than grass crops of corresponding DOMD value (see Thomson, 1984;
Doyle and Thompson, 1985).

The following experiment was designed to investigate the effect of
formaldehyde-treatment of barley supplements on silage intake and milk
production in heifers given high digestibility grass silage or lucerne
silage ad libitum. The grass silage treatments provided a basis for
confirmation of observations made with heifers in Expt 3 whilst the
lucerne silage provided a contrasting forage comparison. In studies
with white clover silage Castle and Watson (1983) reported that the
depression in silage intake in response to barley supplements was
especially large. It therefore seemed likely that if lucerne behaved
similarly it too would provide conditions which would highlight the
influence of formaldehyde-treated barley on silage intake.

Experimental

Animals and their management

The experiment was undertaken using twelve Friesian cows. The
animals were all in their 1st lactation and at the start of the
experiment were 4-10 weeks post-calving. The animals were housed in
individual stalls in a cattle byre and were milked at 06.00 and 16.00

hours each day. Food was given twice-daily after milking and water was available ad libitum. The cows received a daily supplement of a commercial mineral mixture sufficient to meet their nutritional requirement (Scottish Agricultural College, 1982).

Foods

Two silages, one of grass and one of lucerne, were used. Both were made in 50 tonne bunker silos from precision chopped forage which was treated with formic acid (850 g/l) at a rate of 5 l/t during harvesting. The grass silage was made from a first cut late perennial ryegrass sward which was cut on the 29th May (1984) and wilted for 2-3 hours before harvesting. The lucerne silage was from a first cut sward of lucerne (variety Europe), taken at the pre-bud stage on the 28th May (1984) and wilted for 24 hours before harvesting.

The barley used for the experiment was purchased from a supplier. It was rolled before feeding and a portion of each batch prepared was treated with acid-formaldehyde reagent as described in Experiment 2 (page 66). The rate of application of the reagent was 15 l/t.

Experimental design and treatments

The experiment was conducted according to a cyclical changeover design (Davis and Hall, 1969) with twelve animals in two replicate blocks with six animals, six dietary treatments and four four-week periods. The dietary treatment consisted of grass silage or lucerne silage ad libitum without supplement or with supplements of either untreated barley (7.0 kg/d) or formaldehyde-treated barley (7.0 kg/d). Daily food intake and milk yield were recorded and body weights were determined on two consecutive days each week throughout the experiment.

Milk samples were taken at the am and pm milkings on the last two days of each experimental period and bulked to provide weighted mean samples for chemical analysis.

Chemical analysis

Samples of silage and barley taken in each experimental period of the feeding trial as well as the faecal samples from the digestibility experiment were analysed as appropriate for oven dry matter, toluene dry matter, ash, total nitrogen, non-protein nitrogen, ammonia, pH, water-soluble carbohydrates, lactic acid, volatile fatty acids, ethanol, neutral detergent fibre and acid detergent fibre.

Milk samples were analysed for fat, protein, lactose and total solids.

Statistical analysis

Results were analysed by analysis of variance usng Edex programme 5A.7 (ARC Unit of Statistics, Edinburgh).

Results

The composition of the diets

The composition of the silages is given in Table 3.19. The silages were well preserved with a satisfactory pH, low ammonia N concentration and an absence of measurable levels of butyric acid. The two silages were also quite similar in composition with respect to DM, total N and water-soluble carbohydrates. There were, however, differences in the concentration of NDF and ADF between the two silages and reflecting these ADF values there were quite marked differences in DOMD values between silages.

Table 3.19. The chemical composition of the grass silage, lucerne silage, and untreated and formaldehyde treated barley used in Experiment 5.

| | Silage | | Barley | |
	Grass	Lucerne	Untreated	Treated
DM (g/kg)	248[†]	249[†]	831	821
Organic matter (g/kg/DM)	916	891	977	977
Total N (g/kg DM)	25.3	27.2	21.0	21.4
NPN (g/kg total N)	710	680	–	–
Ammonia N (g/kg total N)	80	99	–	–
pH	4.13	4.19	–	–
Lactic acid (g/kg DM)	63	34	–	–
Water soluble carbohydrate (g/kg DM)	78	63	–	–
Neutral detergent fibre (g/kg DM)	523	463	–	–
Acid detergent fibre (g/kg DM)	293	375	–	–
Ethanol (g/kg DM)	51	6	–	–
Acetic acid (g/kg DM)	37	36	–	–
Butyric acid (g/kg DM)	0	0	–	–
DOMD (g/kg DM)	731[§] ±3.8	621[§] ±7.0		

[†] Toluene distillation (Dewar and McDonald, 1961)

[§] Mean ± SE of mean (n = 4)

The composition of the untreated and treated barley samples are shown in Table 3.19. The batches of barley used were virtually identical in composition and were of a relatively high protein content.

Food intake

The results for DM, energy and crude protein intakes are shown in Table 3.20. The consumption of the concentrate allowance was generally complete. However, one cow refused part of both the untreated and treated barley supplement and a second cow refused a small amount of treated barley so that the concentrate intakes were not exactly equal between treatments, the value for the grass silage plus treated barley being slightly lower than intended. Also in two periods with cows given the combination of lucerne silage and untreated barley there were signs of subclinical bloat and it is possible that this may have contributed to reduced DM intakes.

The intake of DM for the unsupplemented lucerne silage was greater ($P < 0.001$) than for the corresponding grass silage treatment, the difference being approximately 1.3 kg/d. This difference was, however, offset by the relatively low ME content of the lucerne crop and ME intake for animals receiving grass silage was slightly higher than for those receiving lucerne.

When the diets were supplemented with untreated barley, silage intake was markedly reduced and did not then differ significantly between the grass and lucerne treatments. Expressed as a 'replacement rate' the change in intake of grass silage with barley supplementation was 0.54 kg/kg whilst the corresponding figure for lucerne silage was 0.86 kg/kg.

When the supplement was formaldehyde-treated barley, grass silage

Table 3.20. The intake of dry matter (DM), metabolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for heifers given grass silage or lucerne silage ad libitum alone or with supplements of untreated barley or formaldehyde-treated barley

	Grass silage			Lucerne silage			SED
	None	Untreated barley	Treated barley	None	Untreated barley	Treated barley	
Concentrate intake (kg DM/d)	-	5.55	5.19	-	5.53	5.69	
Silage intake (kg DM/d)	11.25^b	8.24^a	8.29^a	12.58^c	7.83^a	8.47^a	0.663***
Total intake (kg DM/d)	11.25^a	13.79^{bc}	13.48^{bc}	12.58^b	13.36^{bc}	14.16^c	0.605***
Concentrate ME intake (MJ/d)	-	72.2	67.5	-	71.9	74.0	
Silage ME intake (MJ/d)	131.6	96.4	97.0	124.5	77.5	83.9	
Total ME intake (MJ/d)	131.6	168.6	164.5	124.5	149.4	157.9	
Concentrate CP intake (g/d)	-	758	694	-	726	761	
Silage CP intake (g/d)	1779	1303	1311	2139	1331	1440	
Total CP intake (g/d)	1779	2061	2005	2139	2057	2201	
ME content of total diet (MJ/kg DM)	11.7	12.2	12.2	9.9	11.2	11.2	
CP content of total diet (g/kg DM)	158	149	149	170	154	155	

Means in a line that do not share the same superscript differ significantly (P < 0.05).

intake was reduced to a level corresponding to that found with untreated barley. The replacement rate was 0.57 kg/kg. However, for the lucerne silage the reduction in silage DM intake was less with treated barley than with untreated barley, although the difference did not reach statistical significance (P < 0.05). The replacement rate with the formaldehyde treated barley was 0.74 kg/kg

The estimated ME intakes for the unsupplemented silages were low at 125-132 MJ/d and these were increased by 28-34 MJ/d when the diet was supplemented with barley. Total crude protein intakes varied between diets over a rather narrow range from 1779-2139 g/d. The diets thus contained approximately 150-170 g crude protein/kg DM. Assuming rumen-degradability value of 0.8 for both silage and barley N the control untreated silage and barley diet contained 1.5-1.7 g RDN/MJ ME.

Milk yield and composition

The results for milk yield and composition are given in Table 3.21. For the silage only treatments, there was a higher (P < 0.05) milk yield with the grass than with the lucerne diet. The supplementation of both silages with barley increased (P < 0.05) milk yield as compared with the diets of silage alone. The formaldehyde-treated barley did not increase milk yield significantly more than the untreated barley in animals receiving the grass silage diet but in those receiving the lucerne diet there was a significant (P < 0.05) response in milk yield to formaldehyde treatment of the grain.

There were no significant treatment effects on milk fat content but with the lucerne silage there was a tendency for milk fat content to be increased by barley supplementation and reduced by the

Table 3.21. Milk yield and composition, the yield of milk constituents and the body weight for heifers given grass silage or lucerne silage ad libitum alone or with supplements of untreated barley or formaldehyde-treated barley

	Grass silage			Lucerne silage			SED
	None	Untreated barley	Treated barley	None	Untreated barley	Treated barley	
Milk yield (kg/d)	14.37^{ab}	16.23^{c}	16.12^{c}	13.79^{a}	14.96^{b}	15.91^{c}	0.40***
Fat (g/kg)	45.0^{ab}	44.7^{c}	44.7^{bc}	43.6^{a}	47.0^{bc}	45.0^{bc}	2.0
(g/d)	652^{ab}	726^{c}	716^{bc}	594^{a}	700^{bc}	708^{c}	33.0**
Protein (g/kg)	30.4^{a}	34.8^{c}	34.6^{cd}	30.5^{a}	33.2^{b}	33.4^{b}	0.6***
(g/d)	432^{a}	564^{d}	552^{c}	417^{a}	496^{b}	526^{c}	15.7***
Lactose (g/kg)	48.1^{ab}	48.2^{c}	48.1^{c}	48.1^{a}	48.4^{b}	48.1^{c}	0.45
(g/d)	693^{ab}	784^{c}	779^{c}	665^{a}	726^{b}	769^{c}	21.4***
Total solid (g/kg)	131.3^{ab}	134.9^{d}	135.1^{b}	128.8^{a}	135.5^{b}	133.8^{b}	2.23*
(g/d)	1880^{b}	2190^{d}	2173^{d}	1767^{a}	2023^{c}	2117^{cd}	58.3***
Body weight (kg)	477^{a}	503^{b}	500^{b}	473^{a}	481^{a}	493^{b}	5.3***

Means in a line that do not share the same superscript differ significantly ($P < 0.05$).

formaldehyde treatment of the barley. Milk fat yield did not differ significantly between the diets of unsupplemented silage though the yield tended to be greater for the grass silage. Supplementation of the diets with barley significantly increased milk fat yield ($P < 0.01$) but there was little difference in the response as between untreated and formaldehyde-treated grain.

Milk protein contents were identical for cows receiving the unsupplemented grass and lucerne silages and in both cases they were increased ($P < 0.001$) when the barley supplements were given. Values for the supplemented grass silage were higher ($P < 0.05$) than for the corresponding lucerne silage diets, but there was little effect of the type of barley used. Milk protein yields did not differ significantly ($P < 0.05$) between the unsupplemented silage diets. They were significantly ($P < 0.001$) increased by barley supplements. The effects were generally greater with the grass silage than with the lucerne, and with the untreated barley the difference between corresponding treatment was significant ($P < 0.05$). There was also a significant ($P < 0.05$) response to formaldehyde treatment of the barley but only with the lucerne silage diets.

There were no significant ($P < 0.05$) treatment differences in milk lactose content and, as a consequence, dietary effects on lactose yield were similar to those on milk yield. Most notably lactose yield was increased by the allocation of supplements and, for the lucerne diets, by the formaldehyde treatment of the barley.

Body weight

Body weight was increased significantly by barley supplementation of grass silage and there were no differences between untreated and

formaldehyde-treated barley, but with lucerne silage the untreated barley did not increase body weight significantly (P < 0.05) while the formaldehyde-treated barley did.

Discussion

The results of Experiment 3 indicated that for first lactation cows replacement of supplements of untreated barley with supplements of formaldehyde-treated barley led to no increase in silage DM intake. Consistent with this, in Experiment 5 there were no differences in silage intake between cows receiving the diet of grass silage with supplements of untreated or treated barley. The 'replacement rates' of silage by barley were identical for the two forms of barley given. In contrast, for the animals given the lucerne silage diets, silage DM intake was significantly greater when the supplement was formaldehyde-treated barley than when it was untreated barley.

The reasons for these differences in response between the two silages are uncertain. The silages were very similar in many aspects of chemical composition and both were satisfactory in RDN:ME ratio to meet the cows' dietary requirement for maximal rates of microbial protein synthesis in the rumen (Agricultural Research Council, 1984). The most striking differences between the silages were in their contents of NDF and ADF and in their DOMD values. Clearly the comparative compositional and DOMD values observed are unique to this particular experiment since they reflect the stage of maturity of the grass and lucerne crops at harvesting. However, differences in cell wall content and in the composition of the cell wall between grass and lucerne crops are characteristically observed and like other legumes lucerne typically has a lower DOMD value than grass harvested at a

corresponding stage of maturity (Ministry of Agriculture Food and Fisheries, 1980). Whether the important factors allowing the response in intake to formaldehyde-treated barley to develop with the lucerne silage are related to the chemical composition of the crop or to its relatively low DOMD value is not clear. There is evidence that the rate of breakdown in the rumen of legume crops such as white clover is greater than that of grass and that the difference relates to the physical structure of the forage (Moseley and Jones, 1984). The difference between grass and lucerne crops in the intake response to barley may thus include the interactions between the type of barley given and the microbial breakdown of the forage fibre. Alternatively with the low DOMD lucerne, intake may be enhanced by an increased flow of protein to the duodenum in animals given the formaldehyde-treated barley diet.

Associated with the response in the intake of lucerne silage when untreated barley was replaced by formaldehyde-treated supplements milk yield, protein yield and lactose yield were significantly increased. However, it was notable that similar responses in milk production were not found with the grass silage diet although they had previously been observed with heifers given grass silage in Experiment 3. A contributory factor to this difference was probably the slight refusals of concentrate by some of the cows in this experiment. However, that seems unlikely to provide a total explanation. In Experiment 3 the grass silage was of a lower DOMD value than in Experiment 5 and the total ME intake observed with the silage and barley diets were thus less (154.5 MJ/d as compared with 164.5 MJ/d). This difference in ME intake may be important in modulating the responsiveness of the cows to a change in nutrient supply from the gut induced through treatment of the barley supplement with formaldehyde reagent.

Experiment 6. Food intake and milk production in cows given grass or lucerne silage or a mixture of the two ad libitum with supplements of barley or formaldehyde-treated barley.

In view of the results obtained in Experiment 5 a second experiment was designed to examine the comparative responses of animals given grass or lucerne silage to supplements of formaldehyde-treated as compared with untreated barley. Multiparous cows as distinct from first lactation cows were selected for the study. Also, in addition to diets based on grass silage or lucerne silage, diets containing equal proportions of the two were included to highlight any effect on rumen digestion and thus animal performance which might arise through the mixture of the two forages in the diet (see Moseley and Jones, 1979).

Experimental

Animals and their management

The main experiment was undertaken using 12 Friesian cows. The animals were in their 3rd-7th lactations and were in the 6-10th week of lactation at the start of the experiment. The animals were housed in individual stalls in a cattle byre and were milked at 06.00 and 16.00 hours each day. Food was given twice daily after milking and water was available ad libitum. The cows received a daily supplement of a commercial mineral mixture, sufficient to meet their nutritional requirements (Scottish Agricultural Colleges, 1982).

Additionally two rumen cannulated Friesian cows were used for rumen-degradability studies.

Foods

Two silages, one of grass and one of lucerne were used. The silages were made in 50 tonne bunker silos from precision chopped forage, which was ensiled with the addition of formic acid (850 g/l) at a rate of 2.5 l/t for the grass silage and 5.0 l/t for the lucerne silage. The grass silage was made from a 1st cut sward of late perennial ryegrass mown on the 29th May 1984. The grass was cut and wilted for 2-3 hours before harvesting. The lucerne silage was made from a 3rd cut sward of lucerne (variety Europe) taken on 27th August 1984 and wilted for 24 hours before ensilage.

The barley used for the experiment was purchased from a supplier. It was rolled before feeding and a portion of each batch was treated with acid-formaldehyde reagent as described in Experiment 2 (page 66). The reagent was applied at a rate of 15 l/t.

Design and treatments in the feeding experiment

The main experiment was carried out according to a cyclical change-over design (Davis and Hall, 1969) with twelve animals, two replicate blocks, six dietary treatments and four three-week experimental periods. The dietary treatments consisted of lucerne or grass silage or a 50:50 mixture of the two given ad libitum together with supplements of untreated barley or formaldehyde-treated barley. The 50:50 mixture of silage was prepared each day by intimately mixing equal quantities of silage by hand using a forage fork. Daily food intake and milk production were recorded and bodyweights were determined on two consecutive days each week throughout the experiment. Milk samples were taken from the am and pm milkings on the last two days of each experimental period and used to prepare weighted-mean bulk

samples for analysis.

Degradability of foods

The degradability of untreated barley and formaldehyde treated barley as well as grass and lucerne silages were estimated using two non-lactating cows fitted with permanent rumen cannulas. The first cow was given grass silage with untreated barley in the ratio of 60:40 on a DM basis and thereafter a corresponding mixture of grass silage with treated barley. The second cow was correspondingly given lucerne silage with untreated barley and thereafter lucerne silage with treated barley. All intraruminal incubations were carried out after an initial 14 day establishment period on each diet. The incubations were conducted over a period of 5 days. The rumen pH of the cows was measured at the end of the establishment period for each diet on two consecutive days. Estimates of DM disappearance from Dacron bags were based on 6 observations made at each incubation time and the samples at each time were bulked to give a single sample for analysis of nitrogen.

Incubations for the forage samples were made over 6 time periods from 0 to 24 hours, whilst for barley samples incubations were made over 7 time periods from 0 to 32 hours.

Chemical analysis

Samples of silage and barley taken in each experimental period as well as the faeces samples from the digestibility experiment were analysed as appropriate for oven dry matter, toluene dry matter, ash, total nitrogen, non-protein nitrogen, ammonia, pH, water-soluble carbohydrates, lactic acid, volatile fatty acids, ethanol, neutral detergent fibre and acid detergent fibre.

Milk samples were analysed for fat, protein, lactose and total solids.

Statistical analysis

Results were analysed by analysis of variance using the Edex programme 5A.7 (ARC Unit of Statistics, Edinburgh).

Results

The composition of the diets

The composition of the silages is given in Table 3.22. The silages were well preserved with a satisfactory pH, although the pH of the lucerne silage was 0.4 higher than the grass silage. Both silages had low ammonia-N concentrations and an absence of measurable levels of butyric acid. The grass silage had slightly lower DM and total N contents than the lucerne silage, but the lactic acid content of the grass silage was double that of lucerne silage. Both silages were similar in their concentration of NDF but the lucerne silage was considerably higher in ADF than grass silage. There were quite marked differences in DOMD values between silages reflecting the differences in ADF contents.

The composition of the untreated and treated barley samples are shown in Table 3.22. The batches of barley used were virtually identical in composition and were of a moderate protein content.

Rumen degradability of foods

The results for the disappearance of DM and N from samples of untreated and treated barley are shown in Table 3.23 and Table 3.24. In all instances application of formaldehyde to the barley led to a

Table 3.22. Chemical composition of the grass silage, lucerne silage, untreated barley and treated barley used in Experiment 6.

	Silage		Barley	
	Grass	Lucerne	Untreated	Treated
DM (g/kg)	249[†]	279[†]	822	822
Organic matter (g/kg/DM)	919	906	976	975
Total N (g/kg DM)	25.9	27.9	16.3	16.6
NPN (g/kg total N)	740	610		
Ammonia N (g/kg total N)	90	70		
pH	3.97	4.33		
Lactic acid (g/kg DM)	100	44		
Water soluble carbohydrate (g/kg DM)	14	30		
Neutral detergent fibre (g/kg DM)	481	476		
Acid detergent fibre (g/kg DM)	241	349		
Ethanol (g/kg DM)	50	1		
Acetic acid (g/kg DM)	38	33		
Butyric acid (g/kg DM)	0	0		
DOMD (g/kg DM)	730[§] $\pm$2.7	546[§] $\pm$2.3		

[†] Toluene distillation (Dewar and McDonald, 1961)

[§] Mean $\pm$ SE of mean (n = 4)

Table 3.23. The disappearance (%) of dry matter from untreated barley (UB) and formaldehyde-treated barley (TB) incubated in Dacron bags in the rumen of Cow 1 given a diet of grass silage with untreated barley (G + UB) or grass silage with barley treated with formaldehyde reagent (G + TB) and Cow 2 given a diet of lucerne silage with untreated barley (L + UB) or lucerne silage with barley treated with formaldehyde reagent (L + TB)

Basal diet	Cow 1					Cow 2				
	G + UB		G + TB		SED (n = 6)	L + UB		L + TB		SED (n = 6)
Sample incubated	UB	TB	UB	TB		UB	TB	UB	TB	
Incubation time (hrs)										
2.5	26.8	20.6	39.3	30.2	2.16	40.8	20.9	42.7	22.8	1.30
5.0	40.3	26.9	55.8	40.4	2.05	56.5	29.6	55.7	32.9	1.91
7.5	56.3	41.0	57.6	42.5	2.50	68.8	35.0	65.0	40.1	1.44
16.0	72.5	58.2	70.2	54.0	2.41	70.1	42.8	66.4	41.8	2.05
24.0	83.7	71.1	82.1	67.9	1.74	78.1	47.0	75.9	57.4	2.31
32.0	85.4	77.6	85.6	76.2	1.28	84.3	64.5	86.3	72.7	0.49

Table 3.24. The disappearance (%) of nitrogen from untreated barley (UB) and formaldehyde-treated barley (TB) incubated in Dacron bags in the rumen of Cow 1 given a diet of grass silage with untreated barley (G + UB) or grass silage with barley treated with formaldehyde reagent (G + TB) and Cow 2 given a diet of lucerne silage with untreated barley (L + UB) or lucerne silage with barley treated with formaldehyde reagent (L + TB)

	Cow 1				Cow 2			
	G + UB		G + TB		L + UB		L + TB	
Basal diet								
Sample incubated	UB	TB	UB	TB	UB	TB	UB	TB
Incubation time (hrs)								
2.5	16.4	7.1	20.8	6.6	29.7	6.6	31.0	3.1
5.0	16.4	4.0	27.2	8.4	35.0	8.4	43.5	24.6
7.5	25.7	2.5	27.8	5.6	50.6	7.3	43.5	33.1
16.0	58.2	22.6	49.2	12.8	56.4	8.6	54.1	32.4
24.0	79.9	39.6	71.9	28.8	72.7	6.1	70.6	50.8
32.0	86.1	53.6	85.6	46.9	87.7	29.8	86.8	68.9

pronounced reduction in the rate of both DM and N disappearance indicating an effective cross-linking of both the barley starch and protein. However the effects of the treatment were not entirely consistent in quantitative terms and there were clear indications of interactions between the treatment and the basal diet received by the test animal. For example in Cow 1, which received the grass silage diets, the DM of both the untreated barley and the treated barley were digested more rapidly during the early stages of the incubation when the basal diet contained treated-barley. Similar but less pronounced effects were also apparent for N disappearance. In contrast, in Cow 2 which received the lucerne silage diets, differences in rates of digestion related to the inclusion of treated barley in the basal diet were only clearly apparent for incubations of the treated-barley samples; for the untreated-barley samples differences were small.

Results for the incubations of test samples of the two silages are given in Table 3.25 and in Table 3.26. Again characteristic differences between the foods were evident, and for both DM and N the disappearance rates for the grass silage were greater than for the lucerne silage. There was also evidence indicating the importance of the basal diet given to the cows though as before the effects were not entirely consistent. In the cow given the grass silage diets, inclusion of formaldehyde-treated barley in the diet produced an increase in the rate of digestion of DM for test samples of both grass and lucerne silage, though the effects on N digestion were much less clear cut. On the other hand in the cow given the lucerne silage the effects of basal diet on DM and N digestion rates were quite small.

Table 3.25. The disappearance (%) of dry matter from grass (G) and lucerne (L) silage incubated in Dacron bags in the rumen of Cow 1 given a diet of grass silage with untreated barley (G + UB) or grass silage with barley treated with formaldehyde reagent (G + TB) and Cow 2 given a diet of lucerne silage with untreated barley (L + UB) or lucerne silage with barley treated with formaldehyde reagent (L + TB)

Basal diet	Cow 1					Cow 2				
	G + UB		G + TB		SED (n = 6)	L + UB		L + TB		SED (n = 6)
Sample incubated	G	L	G	L		G	L	G	L	
Incubation time (hrs)										
2.5	35.3	27.8	37.4	31.1	0.50	39.5	31.5	39.6	31.2	0.40
5.0	37.4	31.8	40.2	36.0	0.78	40.3	32.8	42.1	36.2	0.65
7.5	44.9	40.2	46.4	42.0	1.32	43.9	38.5	47.7	42.7	0.77
16.0	51.1	41.7	63.3	54.5	1.13*	60.7	51.4	60.3	48.9	1.37NS
24.0	74.5	59.1	73.7	62.0	1.41	68.2	61.8	67.4	58.8	1.32

Table 3.26. The disappearance (%) of nitrogen from grass (G) and lucerne (L) silage incubated in Dacron bags in the rumen of Cow 1 given a diet of grass silage with untreated barley (G + UB) or grass silage with barley treated with formaldehyde reagent (G + TB) and Cow 2 given a diet of lucerne silage with untreated barley (L + UB) or lucerne silage with barley treated with formaldehyde reagent (L + TB).

Basal diet	Cow 1				Cow 2			
	G + UB		G + TB		L + UB		L + TB	
Sample incubated	G	L	G	L	G	L	G	L
Incubation time (hrs)								
2.5	65.4	55.9	65.4	52.0	73.1	53.9	74.0	54.2
5.0	67.3	57.8	67.3	57.8	74.2	52.7	76.4	60.4
7.5	71.0	63.9	71.0	63.6	75.6	60.0	80.1	66.4
16.0	78.5	71.3	84.1	78.3	85.1	73.7	87.8	73.9
24.0	89.7	84.8	88.0	84.4	87.6	84.0	90.5	85.5

Food intake

The results for DM, energy and crude protein intake are shown in Table 3.27. The consumption of the concentrate allowance was generally complete. Silage DM intakes were similar for all treatments except for a slight increase of approximately 0.50 kg/d when the lucerne silage was supplemented with formaldehyde-treated barley. However, this increase did not reach statistical significance at P < 0.05. As a consequence the total DM intake with the treated barley and lucerne silage treatment was not significantly different from that observed with the other treatments.

The estimated ME intake for grass silage and untreated barley diet was approximately 30 MJ/d higher than for the lucerne silage and untreated barley diet. However, supplementation of lucerne with formaldehyde-treated barley increased the ME intake by approximately 6.0 MJ/d as compared with the untreated barley diet. The estimated ME intake for diets containing the mixtures of silage were intermediate between the grass silage and lucerne silage treatments. Total crude protein intakes differed between diets over a rather narrow range from 2100-2300 g/d. The diets thus contained approximately 140-148 g crude protein/kg DM. Rumen-degradability values for N were estimated from the N disappearance values obtained after 24 hours of incubation in the rumen (Table 3.29). On the basis of these figures the RDN:ME values for the three control diets containing grass silage, mixed silage and lucerne with untreated barley were 1.6, 1.7 and 1.8. In comparison the corresponding values for the diets containing treated barley were 1.3, 1.5 and 1.8.

Table 3.27. The intake of dry matter (DM), metabolizable energy (ME) and the ME and CP contents of the total diet for cows given grass silage or lucerne silage or a mixture of both ad libitum with supplements of untreated barley or formaldehyde-treated barley

	Grass silage		Silage mixture		Lucerne silage		SED
	Untreated barley	Treated barley	Untreated barley	Treated barley	Untreated barley	Treated barley	
Concentrate intake (kg DM/d)	5.72	5.74	5.74	5.73	5.69	5.76	0.03 NS
Silage intake (kg DM/d)	9.34	9.41	9.41	9.18	9.19	9.76	0.639NS
Total intake (kg DM/d)	15.06	15.15	15.15	14.91	14.88	15.52	9.655NS
Concentrate ME intake (MJ/d)	74.4	74.6	74.6	74.5	74.0	74.9	
Silage ME intake (MJ/d)	109.3	110.1	96.0	93.6	80.0	84.9	
Total ME intake (MJ/d)	183.7	184.7	170.6	168.1	154.0	159.8	
Concentrate CP intake (g/d)	583	596	585	595	580	598	
Silage CP intake (g/d)	1512	1523	1582	1543	1603	1702	
Total CP intake (g/d)	2095	2119	2167	2138	2183	2300	
ME content of total diet (MJ/kg DM)	12.2	12.2	11.3	11.3	10.3	10.3	
CP content of total diet (g/kg DM)	139	140	143	143	147	148	

Milk yield and composition

The results for milk yield and composition are given in Table 3.28. The formaldehyde-treated barley did not increase milk yield significantly more than the untreated barley in the animals receiving either the grass silage diets or the diets containing the mixture of the two silages. However in animals receiving the lucerne silage, milk yield was increased by 1.8 kg/d ($P < 0.01$) when formaldehyde-treated as opposed to untreated barley was given.

There were no significant treatment effects on milk fat content but with the grass-silage diets there was a tendency for milk fat content to be reduced by the formaldehyde-treated barley. Milk fat yield did not differ significantly ($P < 0.05$) between treatments although the yield tended to be reduced with the lucerne silage and untreated barley diet.

Milk protein contents were identical for all treatments. However, there was a significant ($P < 0.01$) difference between treatments in milk protein yield, since with the lucerne silage diet the replacement of untreated barley with formaldehyde-treated barley increased milk protein yield by approximately 50 g/d. There were also tendencies for milk protein yield to be increased when untreated barley was replaced by formaldehyde-treated barley with both the grass silage diet and the diet containing the mixture of grass and lucerne silages.

There were no significant ($P < 0.05$) differences in milk lactose content between treatments, but there were significant differences ($P < 0.05$) in milk lactose yield. With the lucerne silage diet formaldehyde treatment of barley increased milk lactose yield by approximately 90g as compared with the untreated barley control.

Table 3.28. Milk yield and composition, the yield of milk constitutents and the body weight for cows given grass silage or lucerne silage or a mixture of both ad libitum with supplements of untreated barley or formaldehyde-treated barley

	Grass silage		Silage mixture		Lucerne silage		SED
	Untreated barley	Treated barley	Untreated barley	Treated barley	Untreated barley	Treated barley	
Milk yield (kg/d)	22.88^b	23.19^b	21.86^b	22.53^b	20.49^a	22.29^b	0.646**
Fat (g/kg)	45.2	43.2	43.0	42.8	40.5	40.1	3.07 NS
(g/d)	1014	992	940	957	831	893	62.6 NS
Protein (g/kg)	30.1^{bc}	30.7^c	29.5^{ab}	30.1^{bc}	29.5^a	29.6^{bc}	0.58 NS
(g/d)	681	704	643	675	604	658	23.9**
Lactose (%) (g/kg)	48.4^b	48.2^b	48.4^b	47.8^b	47.8^a	47.9^b	0.44 NS
(g/d)	1108	1117	1059	1075	979	1068	34.5*
Total solids (%) (g/kg)	131.0^{bc}	129.3^c	128.0^{bc}	128.1^{bc}	125.1^a	124.6^b	3.21 NS
(g/d)	2969	2981	2796	2874	2563	2774	96.9**
Body weight (kg)	533	536	523	530	529	531	6.2 NS

Means in a line that do not share the same superscript differ significantly (P < 0.05).

Table 3.29. The rumen degradability of the barley nitrogen and silage nitrogen (Values are calculated from the disappearance of nitrogen from samples of foods incubated in the rumen for 24 hours)

Rumen degradability	Basal diet					
	Grass silage		Silage mixture		Lucerne silage	
	Untreated barley	Treated barley	Untreated barley	Treated barley	Untreated barley	Treated barley
Untreated barley	0.80	–	0.77	–	0.73	–
Treated barley	–	0.29	–	0.40	–	0.51
Silages	0.90	0.88	0.87	0.87	0.84	0.86

Body weight

There were no significant differences between the treatments in body weight although there were tendencies for the replacement of untreated barley with barley treated with formaldehyde to increase the average body weight.

Discussion

The results of Experiments 3, 4 and 5 indicated that in cows given grass silage diets, replacement of barley with formaldehyde-treated barley supplements increased silage DM intake in multiparous cows but not in heifers. Contrary to this, however, in Experiment 6 no increase in intake of grass silage in response to the treated-barley supplement was observed. The reasons for this are uncertain but a notable difference between the experiments was in the quality of the grass silage used. In Experiments 3 and 4 where multiparous cows showed increases in silage DM intake the silages had DOMD values of 666-675 g/kg whilst in Experiment 6 the corresponding figure was 730 g/kg. As a result of the high quality of the silage in Experiment 6 the ME intake was 184 MJ/d. It is possible that at these ME levels the influence of formaldehyde treatment of the barley supplements on silage intake are diminished or lost.

The results obtained with the lucerne silage provided some support for this view since, as in Experiment 5, the intake of lucerne silage was increased when treated-barley supplements were given. It is tempting to speculate that this difference between the grass and lucerne silages is explainable in terms of the differences in chemical composition between the silages and in their relative rates of digestion in the rumen. The lucerne silage was much higher in ADF than

was the grass silage and the Dacron bag experiments confirmed that
under all circumstances the rate of breakdown of DM in the rumen was
slower for lucerne silage than for grass silage. However, the results
for the effects of barley and treated-barley supplements on the rate of
DM breakdown were anomalous. In the cow given basal diets containing
grass silage there was evidence that the breakdown of lucerne silage DM
was faster when the supplement was treated barley. But in the cow
given basal diets containing lucerne silage differences in the rate of
breakdown of lucerne DM related to the type of supplement given were
not evident.

Whatever the basis of the response in intake to the
formaldehyde-treated barley supplements it was notable that the effects
were not observed with the diet containing mixed grass and lucerne
silage. These diets had ME contents of 11.3 MJ/kg DM and led to an
intake of ca 169 MJ/d (cf Experiments 3 and 4) but DM intake was not
significantly influenced by the type of barley supplement given.

It is, of course, possible that the intake responses to the
treated barley were influenced not only by the energy contents and
rates of ruminal fermentation of the silage but also by the dietary
protein supply. In this respect the Dacron bag experiments indicated
that the mean rumen degradability values for the N in the dietary
components used in the feeding experiment were as given in Table 3.29.
On the basis of these figures the RDN:ME values for the three control
diets containing grass silage, mixed silage and lucerne silage with
untreated barley were 1.6, 1.7 and 1.8. In comparison, the
corresponding values for the diets containing treated barley were 1.3,
1.5 and 1.8. These values are all at or above the requirement
advocated by ARC (1984) as the minimum to ensure maximal microbial

protein synthesis in the rumen, though in the case of the grass silage and mixed silage diets the adequacy was marginal.

Associated with the response in the intake of lucerne silage when untreated barley was replaced by formaldehyde-treated barley milk yield, protein yield and lactose yield were significantly ($P < 0.05$) increased. However, similar responses were not observed with the diet containing grass silage or grass and lucerne silage where DM intake was unaffected by the type of supplement given. These results are similar to those obtained with first lactation cows in Experiment 5 and imply that the cows did not respond to the change in the pattern of nutrient absorption from the gut which would have been induced by the treated-barley. Whether the lack of response is related to the level of ME supply in the diets or to the modulating effects of dietary deficiencies of RDN on rumen microbial protein synthesis and thus protein passage to the small intestine requires further consideration and is discussed later.

Experiment 7. Food intake and milk production in cows given silage with barley supplements treated with different rates of acid-formaldehyde reagent.

The results of earlier experiments indicated that responses in silage intake and milk production to the formaldehyde treatment of barley grain are most likely to occur in multiparous cows given silage of moderate or low digestibility, such as lucerne silage. The dose rate of the formaldehyde reagent necessary to induce the response is not well defined. In Experiment 2 a dose rate of 8 l/t was without beneficial effects on production but the experimental conditions were adverse in that the basal diet was low in protein. In subsequent experiments a treatment rate of 15 l/t was preferred though the effects of lower application rates were not tested.

The following experiment was designed to investigate the effect of increasing dose rate of acid-formaldehyde reagent on food intake and milk production in cows given low digestibility lucerne silage containing high levels of rumen degradable nitrogen.

Experimental

Animals and their management

The experiment was undertaken using eight Friesian cows weighing 480-649 kg. The animals were in their 2nd-4th lactation and at the start of the experiment were 5-7 weeks post-calving. The animals were housed in individual stalls in a cattle byre and were milked at 06.00 and 16.00 hours each day. Food was given twice-daily after milking and water was available ad libitum. The cows received a daily supplement of a commercial mineral mixture sufficient to meet their nutritional

requirements (Scottish Agricultural College, 1982).

Foods

The lucerne silage used was made in a 50 tonne bunker silo from precision chopped forage which was treated with formic acid (850 g/l) at a rate of 5 l/t during harvesting. The silage was made from 3rd cut sward of lucerne (variety Europe) taken on 27th August 1984 and wilted for 24 hours before ensilage.

The barley used for the experiment was purchased from a supplier. It was rolled before feeding and a portion of each batch was treated with acid-formaldehyde reagent as described in Experiment 2 (page 66). The reagent was applied at rates of 8.0, 11.5 and 15.0 l/t.

Design and treatment in the feeding experiment

The main experiment was conducted according to a duplicated 4 x 4 Latin Square design with eight animals, two replicate blocks of four animals with four treatments and four three-week periods. The dietary treatments consisted of lucerne silage ad libitum with supplements (7.0 kg/d) of untreated barley, or barley treated with formaldehyde-reagent at rates of 8 l/t, or 11.5 l/t, or 15 l/t. Daily food intake and milk yield were recorded and body weights were determined on two consecutive days each week throughout the experiment. Milk samples were taken at the am and pm milkings on the last two days of each experimental period and bulked to provide weighted mean samples for chemical analysis.

Degradability of foods

The degradability of untreated barley and barley treated with different levels of formaldehyde reagent were estimated using two

non-lactating cows fitted with permanent rumen canulas. Both cows were fed lucerne silage with untreated barley in the ratio 60:40 on a DM basis. Intraruminal incubations were carried out after an initial 14 day establishment period. The incubations were conducted over a period of 7 days. Estimates of DM disappearance from Dacron bags were based on 8 observations, 4 with each cow, made at each incubation time. Samples of each time were bulked to give a single sample for analysis of starch and nitrogen. The incubations were made over 6 time periods from 0 to 40 hours.

Chemical analysis

Samples of silage and barley taken in each experimental period of the feeding experiment as well as incubated barley residues were analysed as appropriate for oven dry matter, toluene dry matter, ash, total nitrogen, non-protein nitrogen, ammonia, pH, water soluble carbohydrates, starch, lactic acid, volatile fatty acids, neutral detergent fibre and acid detergent fibre.

Milk samples were analysed for fat, protein, lactose and total solids.

Statistical analysis

Results were analysed by analysis of variance.

Results

The composition of the diets

The composition of the silage is given in Table 3.30. The silage was well preserved with a satisfactory pH and ammonia N concentration. The level of crude protein was high, 171 g/kg DM as was the ADF

Table 3.30. The chemical composition of the lucerne silage, untreated barley and the barley treated with acid-formaldehyde reagent at levels of 8.0, 11.5 or 15.0 l/t used in Experiment 7.

	Silage	Untreated barley	Treated barley (l/t)		
			8	11.5	15
DM (g/kg)	294[†]	847	846	834	835
Organic matter (g/kg DM)	872	978	976	978	979
Total N (g/kg DM)	27.4	17.1	17.0	17.0	16.9
NPN (g/kg total N)	649	–	–	–	–
Ammonia N (g/kg total N)	108	–	–	–	–
pH	4.26	–	–	–	–
Lactic acid (g/kg DM)	40				
Water soluble carbohydrate (g/kg DM)	51				
Neutral detergent fibre (g/kg DM)	408				
Acid detergent fibre (g/kg DM)	349				
Acetic acid (g/kg DM)	34				
Butyric acid (g/kd DM)	0				
DOMD (g/kg DM)	536[§] ±7.36				

[†] Toluene distillation (Dewar and McDonald, 1961)

[§] Mean ± SE of mean (n = 3)

content. The latter was reflected in the DOMD value.

The composition of the untreated barley and the barleys treated with the different dose rates of acid-formaldehyde reagent are shown in Table 3.30. The batches of barley were virtually identical in composition and were of a moderate protein content.

Rumen degradability of barleys

The results for the disappearance of DM, starch and nitrogen from samples of untreated and treated barleys are shown in Table 3.31, Table 3.32, Table 3.33 and Figure 3.5, Figure 3.6 and Figure 3.7. In all instances application of formaldehyde to the barley led to pronounced reductions (P < 0.05) in the rate of disappearance of DM, starch and N, indicating an effective cross-linking of barley starch and protein. So far as DM was concerned there were sigificant (P < 0.05) differences in the rate of disappearance depending on the level of formaldehyde treatment. The differences were more pronounced between the 8.0 and 11.5 1/t rates than between the 11.5 and 15 1/t rate of application, and were generally greater during the early stages of incubation (Table 3.32). Consistent with this, small differences in the rate of starch degradation associated with the rate of formaldehyde application were evident particularly in the period up to 24 hours of incubation. More pronounced effects of the increasing rate of application were apparent for the disappearance of N, and these differences were quite marked throughout the total incubation period (Table 3.33). In terms of the rumen-degradability of N, estimated from the disappearance values measured after 24 hours of incubation (Table 3.33), the values for the untreated barley and for the samples treated at 8.0, 11.5 and 15.0 1/t were 0.71, 0.34, 0.29 and 0.20 respectively.

Table 3.31. The disappearance (%) of dry matter from untreated barley and barley treated with acid-formaldehyde reagent at levels of 8.0, 11.5 or 15.0 l/t incubated in Dacron bags in the rumen of the cows given a diet of lucerne silage with untreated barley.

Incubation time (hrs)	Untreated barley	Treated barley (l/t)			SED
	0	8	11.5	15	
2.5	30.0	25.6	23.6	23.5	2.18
5.0	47.4	36.9	32.3	33.2	3.46
7.5	53.3	41.4	38.7	39.0	3.33
16.0	64.3	55.2	48.9	49.1	2.14
24.0	75.1	63.5	60.4	62.1	2.38
40.0	79.2	78.0	73.2	73.4	1.46

Table 3.32. The disappearance (%) of starch from untreated barley and barley treated with acid-formaldehyde reagent at levels of 8.0, 11.5 or 15.0 l/t incubated in Dacron bags in the rumen of the cows given a diet of lucerne silage with untreated barley.

Incubation time (hrs)	Untreated barley	Treated barley (l/t)		
	0	8	11.5	15
2.5	39.0	28.2	25.0	26.6
5.0	63.9	44.9	40.6	37.8
7.5	72.8	52.3	48.8	49.1
16.0	80.9	70.3	62.5	63.2
24.0	90.8	81.0	77.8	79.3
40.0	93.3	93.3	89.4	90.1

Table 3.33. The disappearance (%) of nitrogen from untreated barley and barley treated with acid-formaldehyde reagent at levels of 8.0, 11.5 or 15.0 l/t incubated in Dacron bags in the rumen of the cows given a diet of lucerne silage with untreated barley.

Incubation time (hrs)	Untreated barley	Treated barley (l/t)		
	0	8	11.5	15
2.5	20.9	14.4	8.2	5.0
5.0	30.9	14.6	12.1	5.0
7.5	34.2	15.3	12.4	3.5
16.0	54.1	23.7	17.8	9.3
24.0	71.5	34.3	29.3	19.9
40.0	81.1	70.0	57.5	49.8
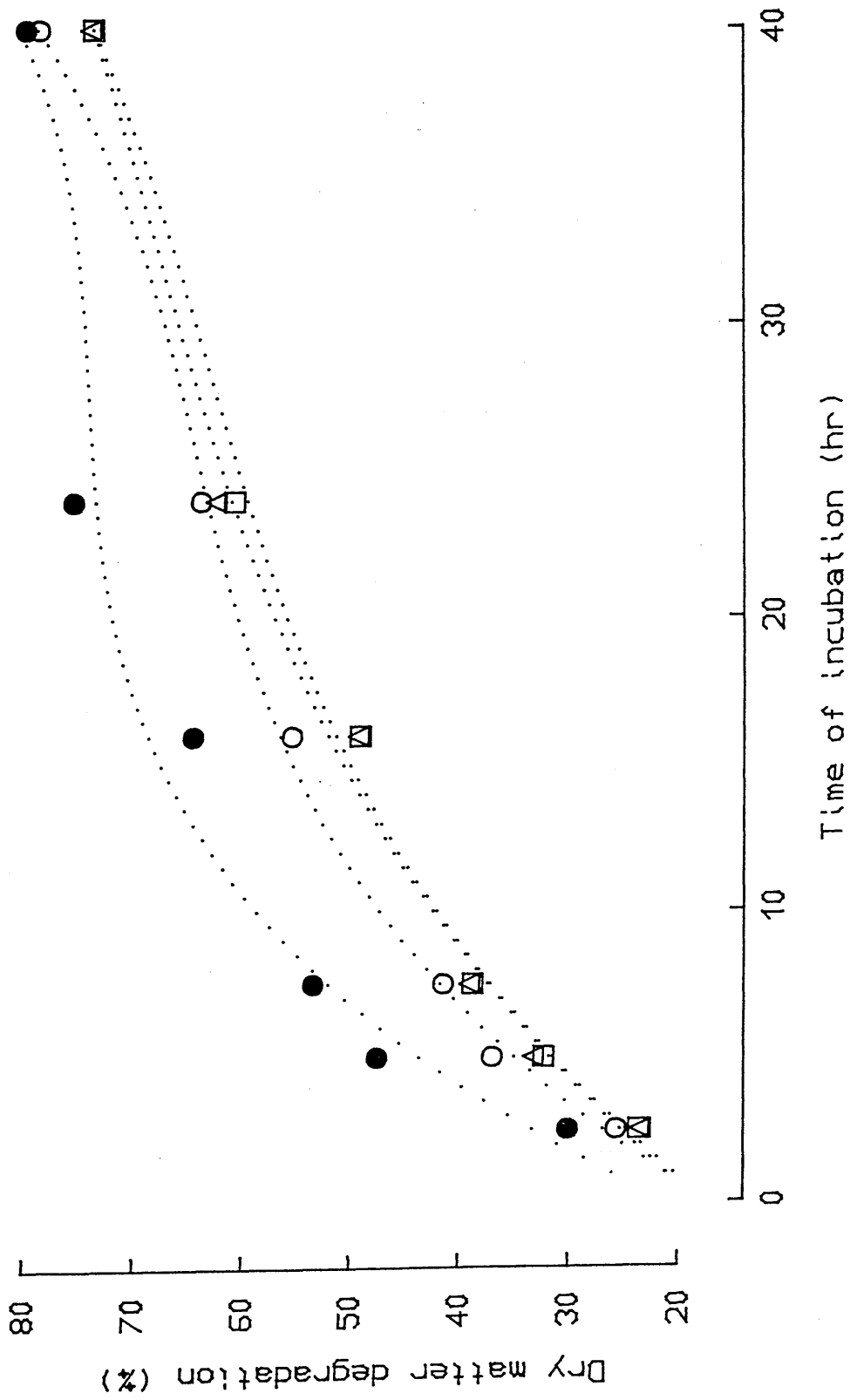
Figure 3.5. The disappearance (%) of dry matter from samples of untreated barley (●), or barley treated with formaldehyde reagent at rates of 8 l/t (◉), 11.5 l/t (□) and 15 l/t (▲).
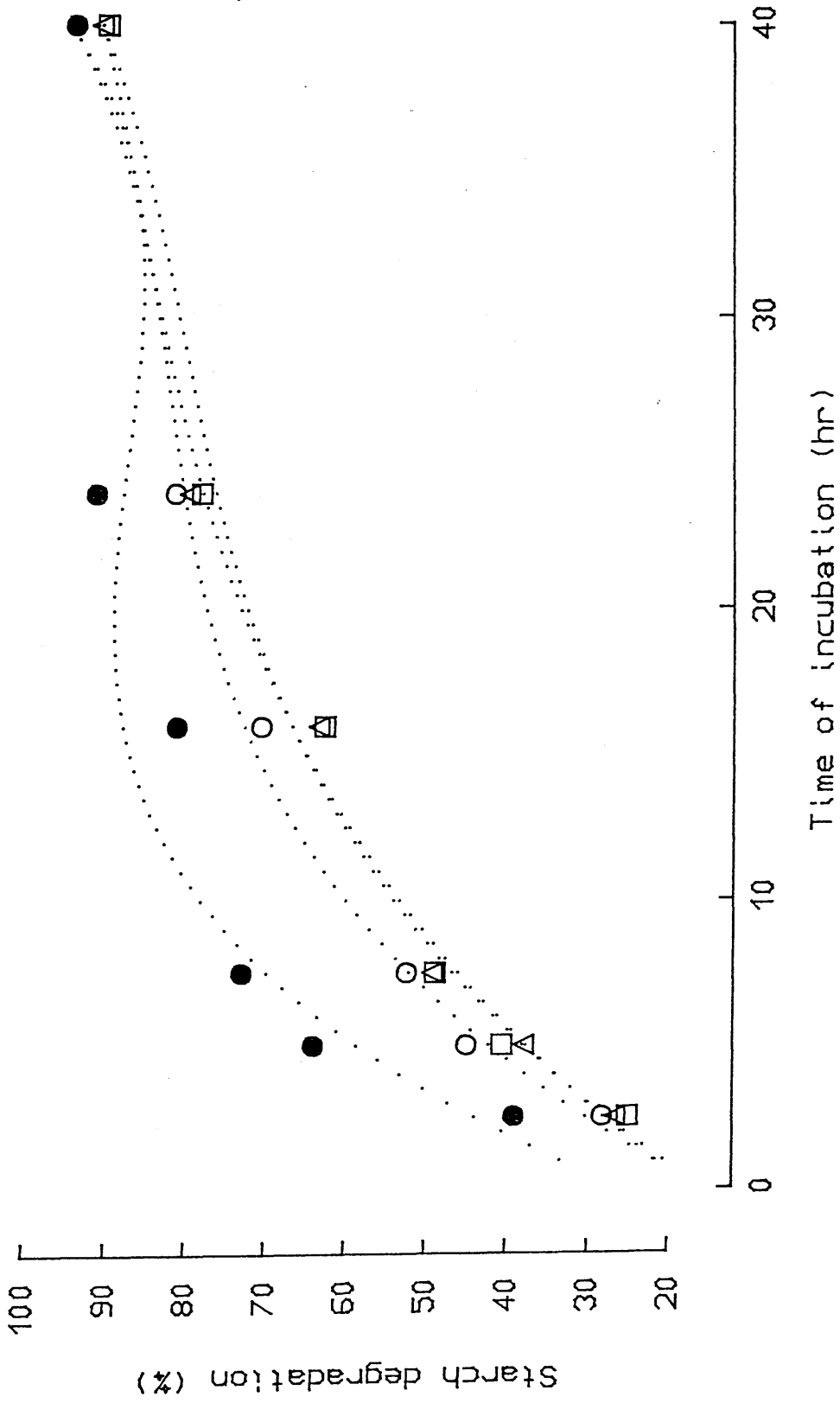
Figure 3.6. The disappearance (%) of starch from samples of untreated barley (●), or barley treated with formaldehyde reagent at rates of 8 l/t (O), 11.5 l/t (◻) and 15 l/t (△).
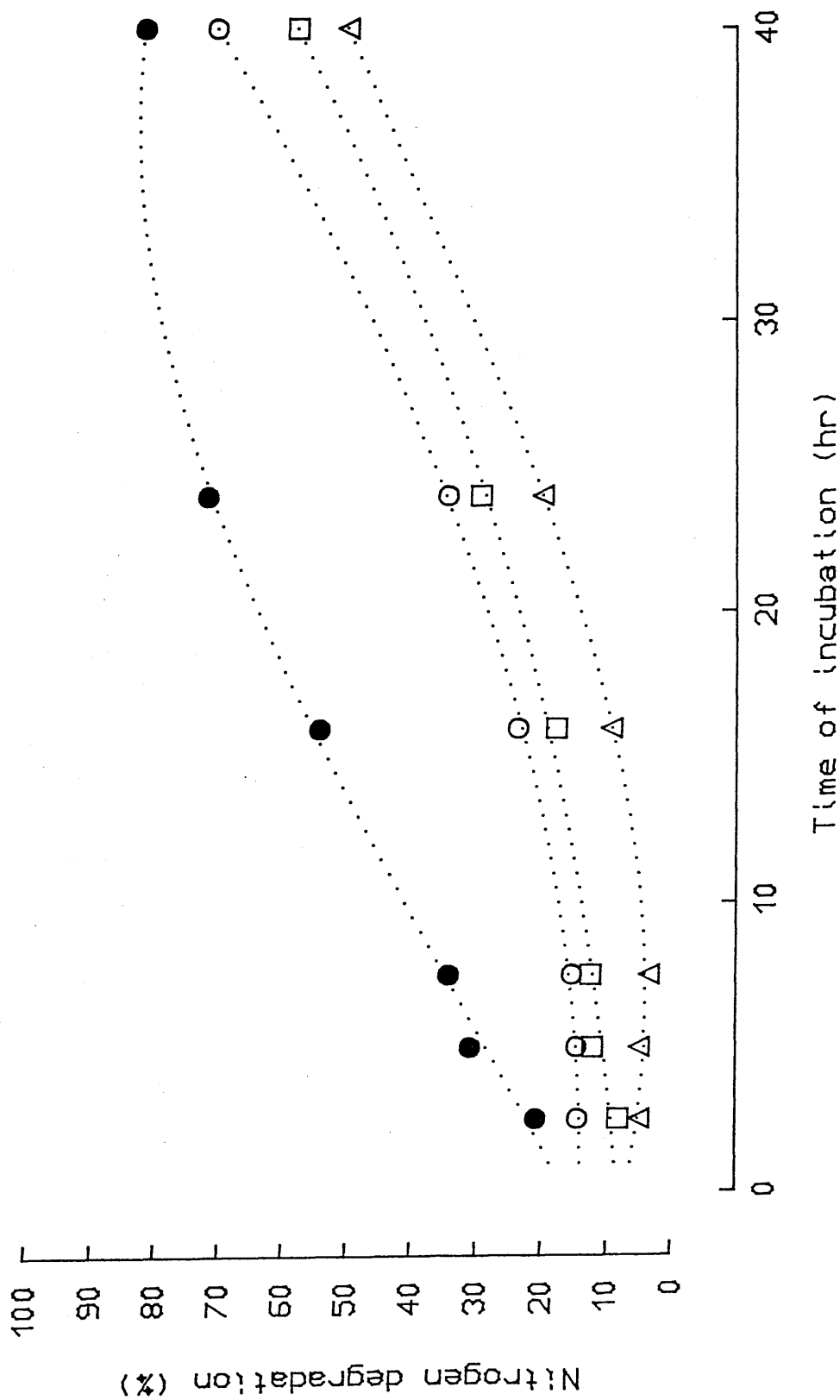
Figure 3.7. The disappearance (%) of nitrogen from samples of untreated barley (●), or barley treated with formaldehyde reagent at rates of 8 l/t (◉), 11.5 l/t (▣) and 15 l/t (△).

Food intake

The results for DM, energy and crude protein intakes are shown in Table 3.34. Consumption of concentrate allowances was virtually complete, small, occasional refusal being observed in some cows given the barley treated at the high rates of formaldehyde application. There were no significant (P < 0.05) differences between treatments in silage intake or total DM intake but there was a consistent trend for intakes to be increased by approximately 0.5 kg DM/d between cows given the barley supplements treated at 11.5 and 15.0 l/t.

The estimated ME intake for the control untreated barley diet and for the diet containing barley treated at a rate of 8.0 l/t were identical but both were approximately 3.3 MJ/d less than those for the cows receiving barley treated with the higher rates of formaldehyde application. Total crude protein intakes differed between diets over a rather narrow range from 2851-2935 g/d, the diets thus containing approximately 151-152g crude protein/kg DM. Rumen-degradability values for the silage N were assumed to be similar to those observed in Experiment 5 (Table 3.29). On this basis the degradability value was 0.84 for animals receiving the diet of silage with untreated barley and 0.86 for those receiving the corresponding diet with barley treated at 15 l/t. The rumen-degradability values for barley N were estimated from the N disappearance after 24 hours in the Dacron bag studies (Table 3.33). Values of 0.71, 0.34, 0.29 and 0.20 were obtained for the control untreated sample and for the barley treated at 8.0, 11.5 and 15 l/t respectively. On the basis of these values the dietary RDN:ME ratios for the four corresponding diets were 2.00, 1,80, 1.80 and 1.76 respectively.

Table 3.34. The intake of dry matter (DM), metabolizable energy (ME) and crude protein (CP) and the ME and CP contents of the total diet for cows given silage ad libitum with concentrate supplements containing barley or formaldehyde-treated barley at various rates

		Treated barley (l/t)			SED
	Control	8	11.5	15	
Concentrate intake (kg DM/d)	5.92	5.91	5.80	5.83	0.03 NS
Silage intake (kg DM/d)	12.97	12.98	13.54	13.48	0.32 NS
Total intake (kg DM/d)	18.89	18.89	19.34	19.31	0.03 NS
Concentrate ME intake (MJ/d)	77.0	76.8	75.4	75.8	
Silage ME intake (MJ/d)	111.3	111.4	116.2	115.7	
Total ME intake (MJ/d)	188.3	188.2	191.6	191.5	
Concentrate CP intake (g/d)	633	628	616	616	
Silage CP intake (g/d)	2221	2223	2319	2308	
Total CP intake (g/d)	2854	2851	2935	2924	
ME content of total diet (MJ/kg DM)	10.0	10.0	9.9	9.9	
CP content of total diet (g/kg DM)	151	151	152	151	

Milk yield and composition

There were no statistically significant (P < 0.05) effects of
dietary treatment on milk yield but the feeding of barley treated with
formaldehyde at any rate consistently increased mean milk yield, by
0.85 - 1.34 kg/d (Table 3.35). This change in yield was associated
with a consistent, though non-significant (P < 0.05) reduction in milk
fat content, and milk fat yield was on average reduced by 2.5% of the
control value (Table 3.35). There were no significant changes in milk
protein content related to diet but there was a trend for protein
content to be increased by all diets containing formaldehyde treated
barley. Consequently milk protein yield was significantly (P < 0.05)
increased by the treated-barley diets. There were no changes in milk
lactose content related to diet, but as a consequence of increasing
milk yield with barley treated with formaldehyde the milk lactose yield
increased by approximately 50 g/d compared to untreated barley control
diet. Again these increases were not statistically significant at the
P < 0.05 level.

Body weight

There were no significant differences between the treatments in
body weight although there were tendencies for the replacement of
untreated barley with barley treated with formaldehyde to increase the
average body weight.

Discussion

The results of Experiment 2 indicated that replacement of a
supplement of untreated barley with barley treated with formaldehyde at
a rate of 8 l/t led to no increase in silage DM intake. Consistent

Table 3.35. Milk yield and composition, the yield of milk constituents and the body weight for cows given silage _ad libitum_ with supplements of untreated barley or barley treated with acid-formaldehyde reagent at levels of 8.0, 11.5 or 15.0 l/t

| | Treated barley (l/t) | | | | SED |
	Control	8	11.5	15	
Milk yield (kg/d)	21.36	22.42	22.21	22.74	0.57
Fat (g/kg)	40.9	38.3	38.4	37.1	1.6
Fat yield (g/d)	864	843	847	839	42.1
Protein (g/kg)	28.5	28.9	29.4	29.0	0.4
Protein yield (g/d)	602	642	652	657	17.0*
Lactose (g/kg)	48.7	49.1	49.1	48.6	0.3
Lactose yield (g/d)	1043	1098	1090	1104	32.4
Total solids (g/kg)	125.1	123.4	123.5	121.5	1.7
Total solids yield (g/d)	2657	2738	2737	2751	81.8
Body weight (kg)	548.4	550.4	550.4	556.9	3.36

with this, in Experiment 7 there were no differences in silage intake between cows receiving the diets with supplements of untreated barley and barley treated at a rate of 8 l/t. However, the treatment of barley with formaldehyde reagent at 11.5 and 15.0 l/t rates increased dry matter intake by approximately 0.5 kg/d. There were differences in the disappearance of barley DM in the rumen between the 8.0 l/t rate of treatment and the higher rates and more pronounced differences for the disappearance of N. These differences may contribute to the varying effects of the supplements on silage intake.

There were consistent increases in milk yield when barley treated with formaldehyde reagent was given though no differences in milk yield attributable to the application rate of the reagent were evident. This result contrasts with findings of Experiment 2 that the lack of response in milk yield in that experiment may be related to low protein content of the diet given. In earlier experiments there was a tendency of milk fat content to be reduced by formaldehyde treatment of barley with both high and low rates of application and this reduction was more marked in Experiment 7, resulting in a reduction in milk fat yield of approximately 2.5% of the control level (Table 3.35). As observed previously milk protein content and milk protein and lactose yields tended to increase when formaldehyde treated barley was given but there was no indication that these effects were related to application rate of the formaldehyde reagent.

In summary there was some evidence from this experiment that the effects of formaldehyde treated barley on silage intake may vary with the rate of application of the formaldehyde reagent. However, the effects of the treatment of the grain on milk yield and composition were independent of the reagent application rate within the range 8.0 - 15.0 l/t.

Experiment 8. The importance of palatability in the regulation of
intake of grass and lucerne silages.

The results of Experiment 5 indicated that the DM intakes of cows
given lucerne silage were considerably higher than those of animals
given grass silage. This finding is consistant with those of other
workers who have studied the voluntary intake of grass and legume crops
given fresh or conserved by drying or by ensilage (see Butler et al.,
1968; Thomson, 1979; Greenhalgh, 1981; Reed, 1981; Castle et al., 1983;
Chamberlain et al., 1984). This difference in intake between grass and
legume crops has generally been attributed to differences between the
forages in their rate of microbial breakdown in the rumen (see
Greenhalgh, 1981). However, it was noted in Experiment 5 that the cows
appeared to relish the lucerne silage and this raised the question of
whether part of the difference in intake between silages was related to
the palatability of the foods.

The importance of palatability as a factor influencing voluntary
food intake in ruminants is a matter on which the evidence is
inconclusive. The predominant view has been that palatability plays
little part in intake regulation where only a single food is on offer
(Balch and Campling, 1962). However, the importance of palatability in
some circumstances was demonstrated unequivocally by Greenhalgh and
Reid (1971) using an approach where a mixed diet of straw and dried
grass was given partly by normal feeding and partly by introduction to
the animals via a rumen cannula. This approach, which was also adopted
in the experiment described below, does not impair the digestion of the
food (Bailey and Balch, 1961; Greenhalgh and Reid, 1967) and allows
palatability effects on intake to be separated from the digestion of

food and the metabolism of absorbed nutrients.

<center>Experimental</center>

Animals and their management

Eight intact and 5 rumen cannulated sheep weighing approximately 65 kg were used. The animals were held in metabolism cages in an experimental animal house and had free access to drinking water and to mineralised salt blocks.

Experimental Plan

The intact sheep were divided into two groups of four animals, one given grass silage (582 g DM/d) and the other given lucerne silage (594 g DM/d) in two meals each day at 09.00 hours and 16.00 hours. When they had been established on the diets the animals underwent a stabilization period of 14 days before complete faecal collections were made over a 7-day period for the determination of the digestibility of dietary organic matter.

The five rumen cannulated sheep were used in a second part of the experiment which was conducted according to a Youden square design with 5 animals, 4 treatments and 4 14-day experimental periods. The experimental treatments were based on three diets: one of grass silage alone; one of lucerne silage alone; and one of a mixture of grass silage and lucerne silage in equal parts. For the grass silage diet, and likewise for the lucerne silage diet, half the silage was consumed via the mouth whilst the other half was given via the rumen cannula. For the diet of grass silage plus lucerne silage there were two treatments; for one treatment the grass silage was consumed through the mouth and the lucerne silage was given via the rumen canula, whilst for

the other treatment the lucerne silage was consumed and the grass
silage was given through the cannula. In practice the procedure for
each treatment was as follows. At 09.00 hours each day a quantity of
silage equivalent to half the previous day's total DM intake was given
via the rumen cannula in a coarsely minced form designed to simulate
the effects of mastication. The sheep were then offered unminced
silage ad libitum in an amount equal to 1.15 of that eaten on the
previous day; no further food was given after refusals were taken at
17.00 hours. The procedure was repeated the following day. The two
silages used in the experiment were a perennial ryegrass (Lolium
perenne) silage made from grass cut at an early stage of growth and
minimum wilted (~ 2 hours) before ensilage with 2.6 l formic acid/t as
additive, and a lucerne (v. Europe) silage cut at the first appearance
of flower and wilted for 24 hours before ensilage with 6.6 l formic
acid/t as additive.

Chemical analysis

Samples of silage and faeces were analysed as appropriate for dry
matter, ash, total nitrogen, non-protein nitrogen, water-soluble
carbohydrates, lactic acid, acetic acid, butyric acid, neutral
detergent fibre (NDF) and acid detergent fibre (ADF).

Statistical analysis

Results were analysed by analysis of variance (Snedecor, 1956)
intake values being based on the last 7 days of each experimental
period.

<u>Results</u>

<u>Composition and digestibility of the silages</u>

The analysis of the two silages are shown in Table 3.36. The grass silage was lower in DM content than the lucerne silage and also had a substantially higher organic matter content, possibly reflecting some soil contamination of the lucerne crop. The silages had high and similar nitrogen contents and in both cases more than half of the nitrogen was in non-protein form; the ammonia content of the lucerne crop was low, 79 g/kg total N but the value for the grass silage was twice as great indicating that significant amino acid deamination may have occurred during storage in the silo. However, as judged from the concentrations of lactic acid, acetic acid and butyric acid in the grass silage there was no evidence of clostridial activity and the material was apparently well-preserved with a low pH typical of that found in corresponding silages which have been made at the Hannah Institute. The pH of the lucerne silage was higher than that of the grass and correspondingly the concentration of lactic acid was lower but again the material was well-preserved and showed no signs of aerobic instability on removal from the silo. For the lucerne silage the neutral detergent fibre (NDF) values were lower and the acid detergent fibre (ADF) values were higher than for the grass silage, but the differences were relatively small. However, the maturity of the lucerne crop was evident from the coefficients of digestibility of organic matter measured <u>in vivo</u>; the value determined for the lucerne silage was 0.677 ± 0.003 (mean with SEM, n = 4) whilst that for the grass silage was 0.833 ± 0.002, giving DOMD values of 576 ± 2.9 and 754 ± 2.9 for the lucerne and grass silages respectively.

Table 3.36. The chemical composition and digestibility
of the grass silage and lucerne silage

	Grass silage	Lucerne silage
DM (g/kg)[†]	226	280
OM (g/kg DM)	890	834
Total N (g/kg DM)	27.9	26.7
NPN (g/kg total N)	663	547
NH_3N (g/kg total N)	158	79
pH	4.13	4.50
Lactic acid (g/kg DM)	103	36
Water soluble carbohydrate (g/kg DM)	26	26
Acetic acid (g/kg DM)	29	19
Butyric acid (g/kg DM)	1	0
Neutral detergent fibre (NDF, g/kg DM)	470	461
Acid detergent fibre (ADF, g/kg DM)	294	319
DOMD (g/kg DM)	754 ± 2.9	576 ± 2.9

† By the method of Dewar and McDonald (1961)

Intake of silages

The sheep quickly became accustomed to the intraruminal feeding procedure and no practical difficulties with the technique were experienced excepting the minor problem of exactly balancing the dietary proportions of grass and lucerne silages when one was eaten and the other was given via the cannula. Analysis of the results showed that when grass silage was eaten and lucerne silage was given through the cannula the lucerne silage was 0.52 of the total DM intake; when lucerne was eaten and grass was given through the cannula the corresponding value was 0.53. The DM, OM and N intake of the sheep are given in Table 3.37. There were significant ($P < 0.05$) differences in intake between the diet consisting solely of grass silage and the diets containing lucerne silage but intake was not significantly affected by the proportion of lucerne in the diet. Animals had similar total DM intakes regardless of whether grass silage was eaten and lucerne silage was given through the rumen cannula or vice versa. Calculations of digestible organic matter intakes from the observed OM intakes and the digestibilities of organic matter determined for the individual silages showed that the intakes of digestible organic matter were virtually identical for the diet consisting solely of grass silage or lucerne silage. However, there appeared to be some synergistic effects between the silages since for the mixed diets the DOM intake tended to be increased, though the effect was not statistically significant ($P > 0.05$).

Discussion

The results of Experiment 8 demonstrate three points clearly. First, it is apparent that whilst the digestibility of the lucerne

Table 3.37. The intake of dry matter (DM), organic matter (OM) and
digestible organic matter (DOM) in sheep receiving diets of grass
silage, lucerne silage or an equal mixture of the two with
half of each diet being ingested by mouth and half
being administered through a rumen cannula

Experimental treatment	DM intake (g/d)	Nitrogen intake (g/d)	OM intake (g/d)	DOM intake (g/d)
Grass silage, eaten and administered through cannula	809	22.5	719	596
Grass silage eaten, lucerne silage administered through cannula	1060	28.9	918	686
Lucerne silage eaten, grass silage administered through cannula	1003	27.3	862	644
Lucerne silage eaten and administered through cannula	1038	27.3	865	586
SED	80.9*	2.2	71.0	57.7

* P < 0.05, statistical significance of treatment effect by F test.

silage was 24% less than the grass silage the sheep consumed more lucerne silage DM than grass silage DM. This result is in agreement with the results of Experiment 5 with the diet of silage alone and is similar to those previously reported in other comparisons between grass and legume food (Butler et al., 1968; Thomson, 1979; Greenhalgh, 1981, Reed, 1981; Castle et al., 1983; Chamberlain et al., 1984; Thomson et al., 1985). Second, it is apparent from the comparison between the mixed grass silage/lucerne silage diets that administration of either the grass or the lucerne through the rumen cannula had no effect on intake. This argues that there was no difference in palatability between the two foods and that intake was regulated through some gut-fill or metabolic mechanism (see Baile and Forbes, 1974).

Third, the results show that the response in intake to the inclusion of lucerne in the diet was not linear and that the animals were achieving their maximum levels of DM intake with the mixed-silage diet. Expressed in other terms this result suggests important synergistic effects between the silages as the DOM intake with the mixed diet tended to be higher than those with the diets of grass silage or of lucerne silage given alone.

This type of synergism was not observed in Experiment 6 when mixtures of grass and lucerne silage were given to cows with supplements of barley or treated barley. In that experiment the ME intakes of the cows given the mixed silage diets were virtually exactly intermediate between those obtained with the diets containing grass silage or lucerne silage (see Table 3.27). Synergistic effects between grass and legume foods have been reported previously, however, most notably in the experiments of Moseley and Jones (1979). These workers found that in sheep given dried ryegrass or red clover, or a 2:1

mixture of the two, DM and DOM intake was higher with the mixture than with either of the components. The mechanism underlying this effect has not been fully established but an important indication obtained by Moseley and Jones (1979) was that the mixed grass and clover diet led to a beneficial effect on the rate of passage of OM through the rumen. They hypothesised that the combination of forages had led to undefined modifications in rumen environment with associated improvements in rumen microbial activity. A similar hypothesis can be put forward to explain the present observations though it is not possible to specify the nature of the potential changes in ruminal conditions or the associated microbiological responses that would underlie the observed effect on the animal's food intake.

The observed synergism between the silages is potentially important nutritionally and requires further, more detailed investigation.

SECTION IV

GENERAL DISCUSSION

Responses in milk production to formaldehyde treatment of barley

supplements

Milk yield

In six of the seven experiments reported here, treatment of barley

supplements with the acid-formaldehyde reagent led to an improvement in

milk yield compared with an untreated barley control diet. In

Experiment 3 the mean response in milk yield was a statistically

significant ($P < 0.05$) increase of 1.3-1.5 kg/d or an improvement of

approximately 8.0-8.5% of the control yield. In Experiment 4 the

response did not reach significance ($P < 0.05$) but again was

approximately 1.4 kg/d or an inrease of 7.7% of the control yield. In

Experiment 5, with the diet of lucerne silage, the response was 1.0

kg/d ($P < 0.05$) or 6.4% of the control, and a corresponding increase of

1.8 kg/d ($P < 0.05$) or 8.8% of the control was found with the lucerne

silage in Experiment 6. In Experiment 7 the response was 1.4 kg/d ($P >$

0.05) or 6.5% of the control when the barley was treated with

formaldehyde reagent at the high level of 15 l/t, and 1.0 kg ($P > 0.05$)

or 5% of the control when the rate of application was at the low level

of 8 l/t. In contrast to these results, in two experiments the effects

of the formaldehyde treatment were found to be negative or small. In

Experiment 2 milk yield was changed by a mean of -0.5 kg/d or -2.9% of

the control value when barley was treated with formaldehyde reagent.

Similarly with the grass silage diet in Experiment 5 yield was altered

by -0.1 kg/d or -0.7%, whilst with grass silage in Experiment 6 the

corresponding values were +0.3 kg/d or +0.8%.

The results of the experiments showing both the positive and the negative responses are shown in Figure 4.1. Calculated as a weighted mean value for all the experiments the response to the treatment of the grain was 0.9 kg/d or 4.7% of the control milk yield.

Milk composition and yields of milk constituents

In none of the experiments undertaken were there statistically significant effects of formaldehyde-treated barley on milk composition, but there were consistent trends in the compositional changes. Notably milk fat content tended to be reduced and milk protein content tended to be increased without changes in lactose content. The effects on milk fat content were generally small except in Expt 7, when fat contents were reduced by a maximum of almost 4 g/kg compared to the control. The influence of the formaldehyde treated barley on milk composition is summarised in Figures 4.2 and 4.3. Calculated as a weighted mean value for all the experiments the response to the treatment of the grain was -2.4 g/kg for fat, +0.38 g/kg for protein and +0.12 g/kg for lactose, or a reduction of 4.7% for fat, an increase of 1.3% for protein and an increase of 0.2% for lactose as compared with the control treatments.

As a result of the changes in milk yield and composition there were alterations in the yield of milk fat, protein and lactose. The results of the experiments are summarised in Table 4.1 and Figure 4.4. In several experiments the yield of constituents broadly followed the increases in milk yield. However, there was a consistent trend for the response in fat yield to be less and the response in protein yield to be greater than would be anticipated from the changes in milk yield.
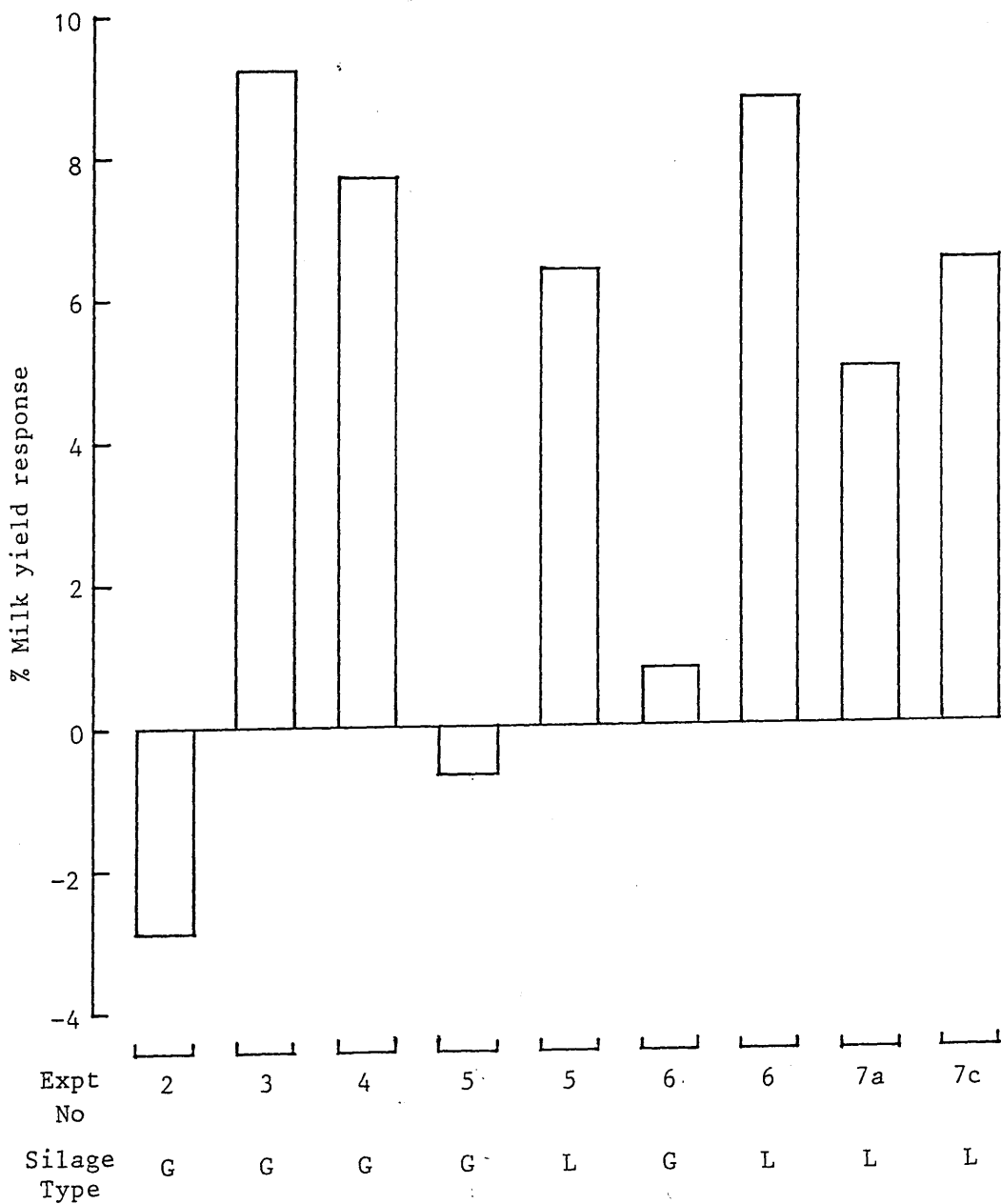
Figure 4.1. Percentage response in milk yield for the cows given diets containing grass silage (G) or lucerne silage (L) with treated barley or untreated barley supplements. (Data are for experiments 2 to 7)
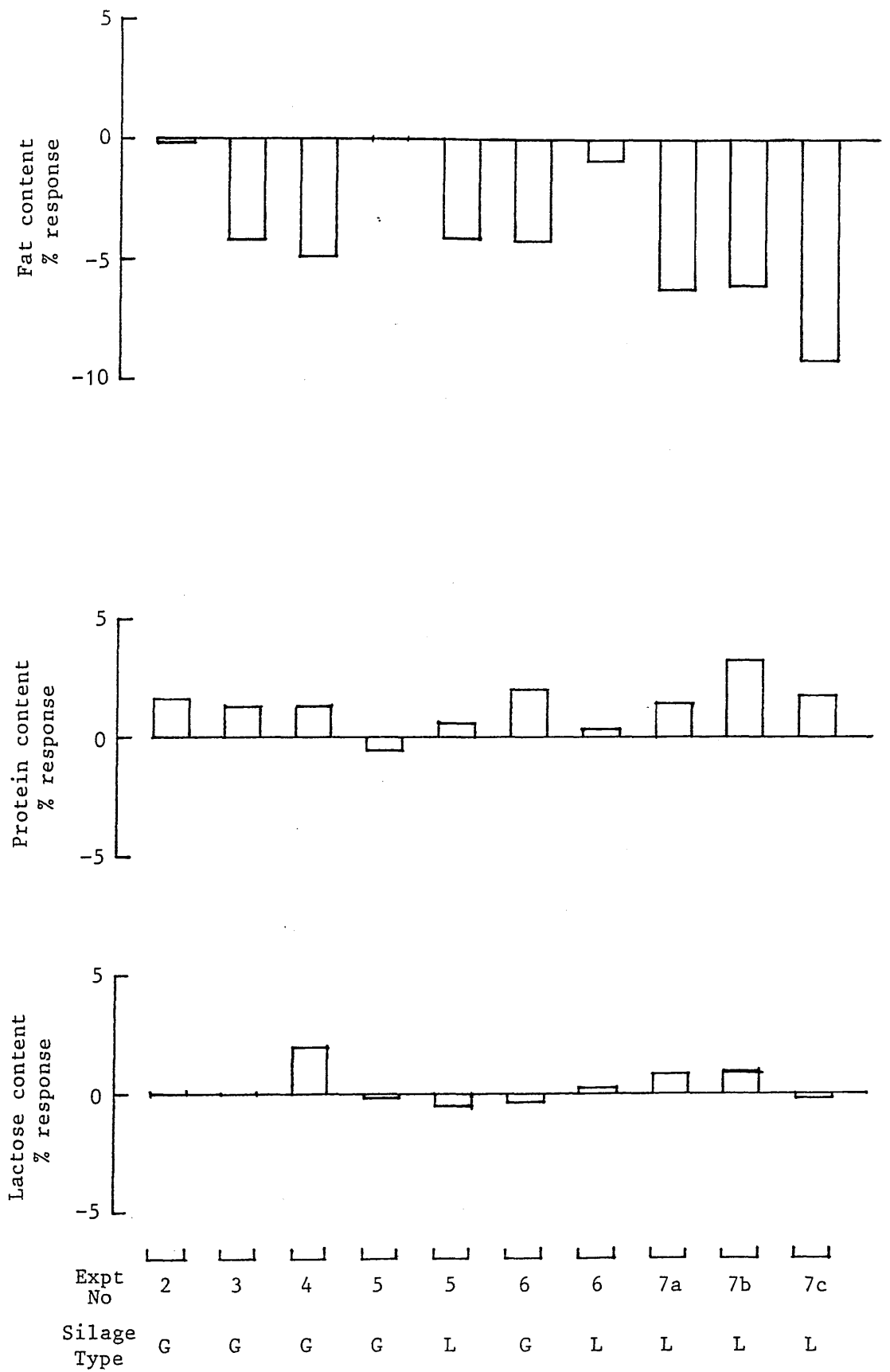
Figure 4.2. Percentage response in fat, protein and lactose content in the milk of cows given diets containing grass silage (G) or lucerne silage (L) with treated barley or untreated barley supplements. (Data are for experiments 2 to 7)
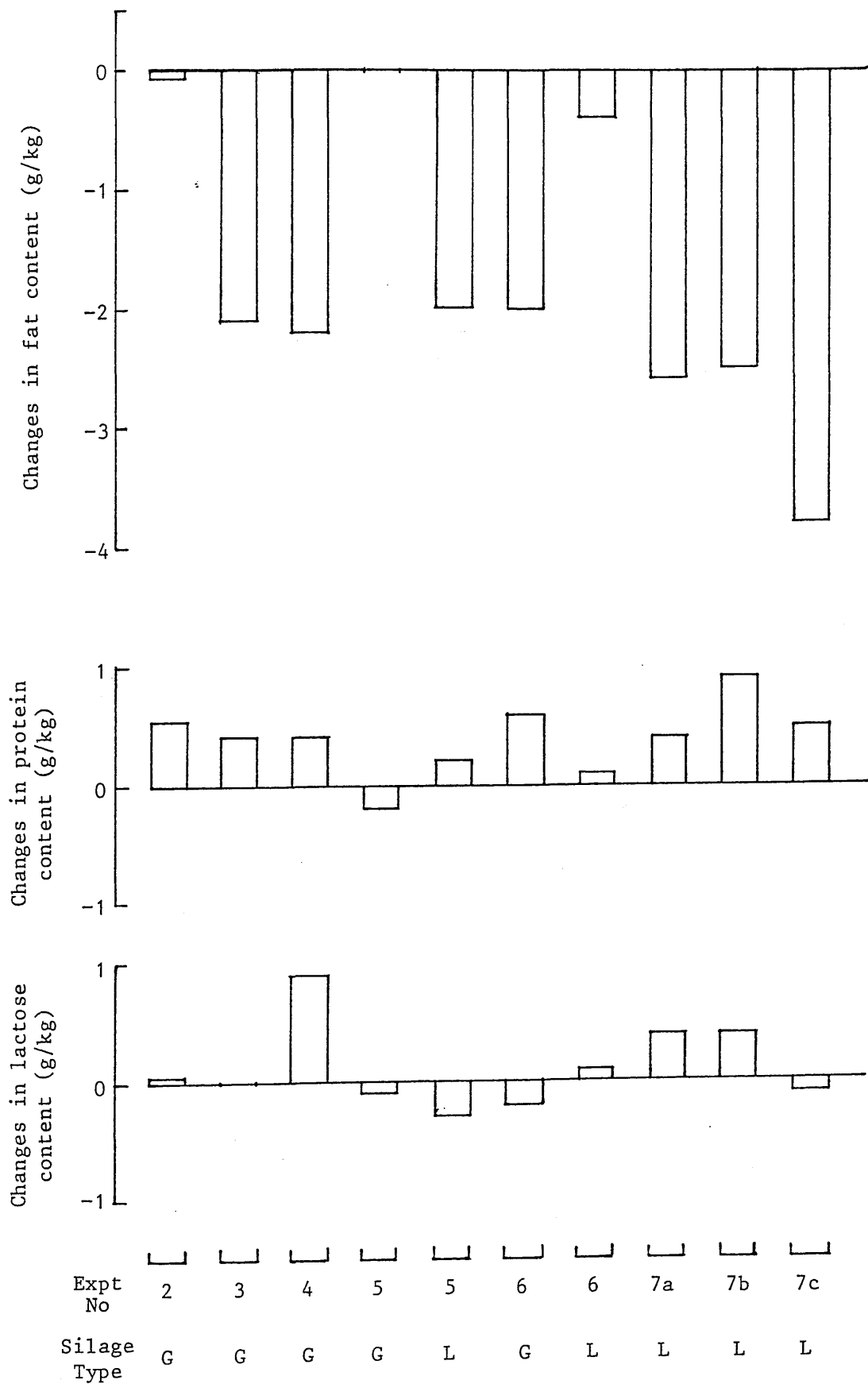
Figure 4.3. Changes in milk fat, protein and lactose contents (g/kg) for cows given diets containing grass silage (G) or lucerne silage (L) with treated barley or untreated barley supplements. (Dat are for experiments 2 to 7)

Table 4.1. The response of milk yield (kg/d or %), fat yield (g/d or %), protein yield (g/d or %), lactose yield (g/d or %) to the formaldehyde treatment of barley compared to the untreated barley control.

Expt No	Type of silage	Milk response		Fat response		Protein response		Lactose response	
		kg/d	%	g/d	%	g/d	%	g/d	%
2	Grass	-0.48	-2.9	-24	-3.0	- 5	- 0.9	-24	-3.1
3	Grass	+1.55	+9.2	+33	+4.1	+52	+ 9.7	+74	+8.9
4	Grass	+1.40	+7.7	+13	+1.6	+68	+11.8	+78	+9.2
5	Grass	-0.11	-0.7	-10	-1.4	-12	- 2.1	- 5	-0.6
5	Lucerne	+0.95	+6.4	+ 8	+1.1	+30	+ 6.0	+43	+5.9
6	Grass	+0.31	+0.8	-22	-2.2	+23	+ 3.4	+ 9	+0.8
6	Lucerne	+1.80	+8.8	+62	+7.5	+54	+ 8.9	+89	+9.1
7a	Lucerne	+1.06	+5.0	-21	-2.4	+40	+ 6.6	+55	+5.3
7b	Lucerne	+0.85	+4.0	-17	-2.0	+50	+ 8.3	+47	+5.8
7c	Lucerne	+1.38	+6.5	-25	-2.9	+55	+ 9.1	+61	+5.8
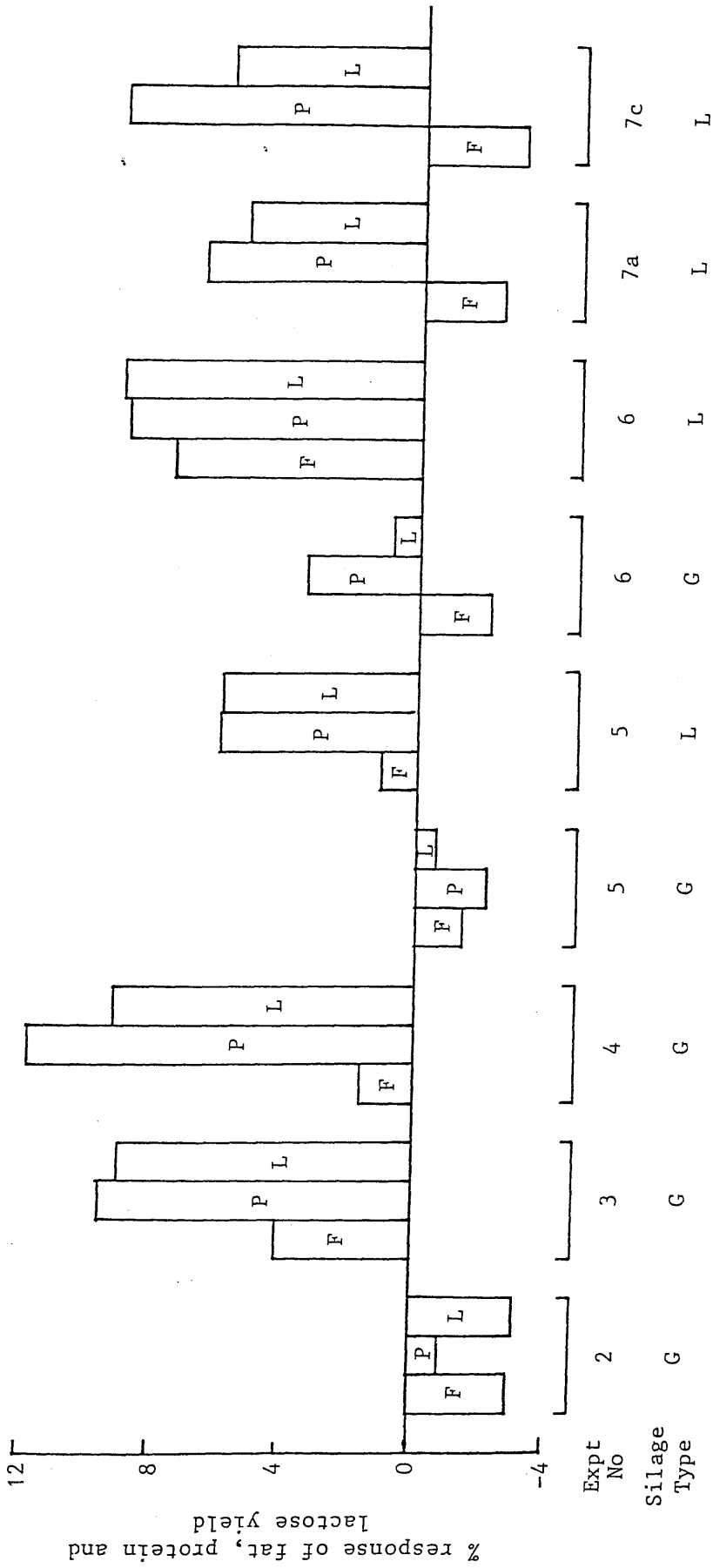
Figure 4.4. Responses in the yield of fat (F), protein (P) and lactose (L) (% of control value) for the cows given diets containing grass silage (G) or lucerne silage (L) with treated barley or untreated barley supplements. (Data are for experiments 2 to 7)

In Experiments 6 and 7 positive responses in the yield of milk protein and lactose were accompanied by negative responses in the yield of milk fat, and in Experiment 7 these appeared more pronounced at the higher levels of formaldehyde application. Taking the results of all the experiments together the responses in fat yield ranged from -3 to +7.5% of the control milk fat yield but none reached statistical significance at $P < 0.05$. Calculated as a weighted mean value for all observations the response in fat to formaldehyde treatment of the barley supplements was 2.2 g/d or +0.08% of the control fat yield. Corresponding changes in protein yield ranged from -2.1% to 11.8% of the control yield. In most experiments the yield was statistically greater ($P < 0.05$) than the control value. The largest response was observed in Experiment 4 when the cows were given a higher rate of concentrate (9 kg/d) than otherwise used. Calculated as a weighted mean value the response in protein was +35 g/d or +6.1% of the control value. Finally, the changes in lactose yield ranged from -3.1 to +9.2% of the control lactose yield. As for protein it was positive in most experiments and statistically significant ($P < 0.05$) changes were obtained in Experiments 3, 4, 5 and 6. Calculated as a weighted mean value across all experiments the response to the formaldehyde treatment of the grain was +44 g/d or an increase of 4.8% of the control yield.

Basis of responses in milk production

Dry matter intake

The responses in milk production described above can in part be explained by the effects of formaldehyde treatment of the barley on total DM intake, an effect exerted in the main through the influence of the treatment on silage intake. Only with the grass silage treatments

in Experiment 5 was there any evidence of differences in concentrate intake between treated and untreated barley. In that case barley treatment led to small but important refusals of barley, and that almost certainly contributed to the negative effect of formaldehyde-treated grain on milk yield (see Figure 4.4). The reasons for the small refusals of the treated-grain are not apparent.

In the experiments in which diets containing grass silage were used, substantial responses in silage intake were observed in some instances, notably in Experiment 4. In other instances, in particular in Experiments 2 and 6, and with the heifers used in Experiment 3 and 5 the effects on silage DM intake were small or negative (Table 4.2). In contrast, effects of the barley treatment on silage intake were observed almost invariably when the diet was based on lucerne silage. The responses were on average 5.7% of the control intake. They were evident in both cows and heifers and at levels of formaldehyde treatment of barley ranging from 11.5-15 l/t (Table 4.2).

The variability in response in silage DM intake in animals given grass silage diets appears to depend on a number of factors. In Experiment 2 where negative effects on intake were observed the diets were low in nitrogen and this probably led to the adverse intake effect; this is discussed further below. It should be pointed out, however, that the level of formaldehyde treatment used for the grain in that experiment was 8 l/t. In Experiment 7, grain treated at that level did not induce an increase in the intake of lucerne silage although increases in intake were observed when the reagent was applied at higher levels of 11.5 and 15 l/t. In Experiment 3 the intake response observed for cows was quite substantial (+7.1% of the control) but a similar response was not obtained for the heifers used. This

Table 4.2. A summary of the results for the responses (% of control) for silage dry matter intake together with information on the composition of the diets and on the absolute levels of dry matter intake (as % of body weight) achieved

Expt No	Type of silage	Animal	Diet CP (g/kg DM)	Silage DOMD (g/kg)	Level of concentrate (kg DM/d)	Total DMI as % B.Wt.	Response in silage intake (% of control)	Level of formaldehyde (l/t)
2	Grass	Cows	104	674	3.22	2.28	+1.7	8
2	Grass	Cows	102	674	5.22	2.46	-2.6	8
2	Grass	Cows	101	674	7.33	2.71	-0.9	8
3	Grass	Cows	126	675	5.83	2.60	+7.1	15
4	Grass	Cows	123	666	7.35	2.90	+15.7	15
6	Grass	Cows	140	730	5.74	2.83	+0.7	15
3	Grass	Heifers	126	675	5.83	2.62	-2.7	15
5	Grass	Heifers	149	731	5.19	2.70	+0.6	15
6	Lucerne	Cows	148	546	5.76	2.92	+6.2	15
7a	Lucerne	Cows	151	536	5.91	3.43	0.0	8
7b	Lucerne	Cows	152	536	5.80	3.51	+4.4	11.5
7c	Lucerne	Cows	151	536	5.83	3.47	+3.9	15
5	Lucerne	Heifers	154	621	5.69	2.87	+8.2	15

probably reflects the stage of maturity of the animals and their ability to increase intake in response to a given stimulus. Distinctions between cows and heifers in their intake response have been reported in a number of previous studies (Cressman et al., 1977; Poos et al., 1979; Thomas et al., 1981). In Experiment 5 also the heifers showed little intake response where the diet was based on grass silage. This again may be seen as a comment on the characteristics of the animals but it should be pointed out that with the lucerne used in the experiment, which was of low DOMD value, improvements in intake were observed. Furthermore, the grass silage used in Experiment 5 was of especially high DOMD value (730 g/kg) and with a similar silage in Experiment 6 cows also showed no improvement in silage intake when formaldehyde-treated grain was given. With these high digestibility silages the cows may have been close to their maximum achievable DM intake and there may have been little scope for the improvements normally associated with the formaldehyde treated barley to become manifest.

The largest responses in silage intake observed in the series of experiments was in Experiment 4 where the diets were supplemented with quite a high level of concentrate (7.35 kg DM/d). It may be argued that this response was related to the adverse effect of the high concentrate level on rumen pH and on the rate of ruminal digestion of forage. Where ruminal pH and cellulolysis are substantially depressed by the supplementary barley there is greatest scope for formaldehyde-treated barley to produce an increase in intake. Consistent with this, similar responses in intake were observed in Experiment 4 with formaldehyde treatment of barley and with sodium bicarbonate additions to the barley. However, the evidence available

from Experiment 6, which it must be acknowledged was from dry cows given lower levels of concentrate than that used in Experiment 4, suggests that the effects of the barley treatment on rumen pH were small (Figures 4.5 and 4.6). Similarly, whilst there was evidence that in cows given grass silage the treated barley increased the rate of forage digestion in the rumen, the effects were modest (see Figures 4.7 and 4.8). Moreover, they were not observed with the lucerne-based diet, implying that there may be some variability in response from one silage to another.

Nitrogen supply and utilization
Rumen-degradability

The evidence deriving from the Dacron bag studies conducted in Experiments 1a, 1b, 3, 6 and 7 consistently showed that treatment of rolled barley with the acid-formaldehyde reagent led to a pronounced reduction in the ruminal degradability of barley protein (Figure 4.9). This effect was observed with both rolled and ground barley and at levels of application of the reagent ranging from 8-15 l/t. There were differences in the effectiveness of the treatment from experiment to experiment and within experiments depending on the details of the treatment. In Experiment 7, for example, there was clear evidence that the degradability of N was decreased as the dose rate of application of the reagent was increased, and in Experiment 1 there was some evidence that the effectiveness of the treatment might be reduced when the barley was finely ground. Somewhat surprisingly, however, there was no general relationship between the rate of formaldehyde application g/kg barley CP and the observed rumen-degradability value (Figure 4.10).

Work with silage diets has indicated that the rate of formaldehyde
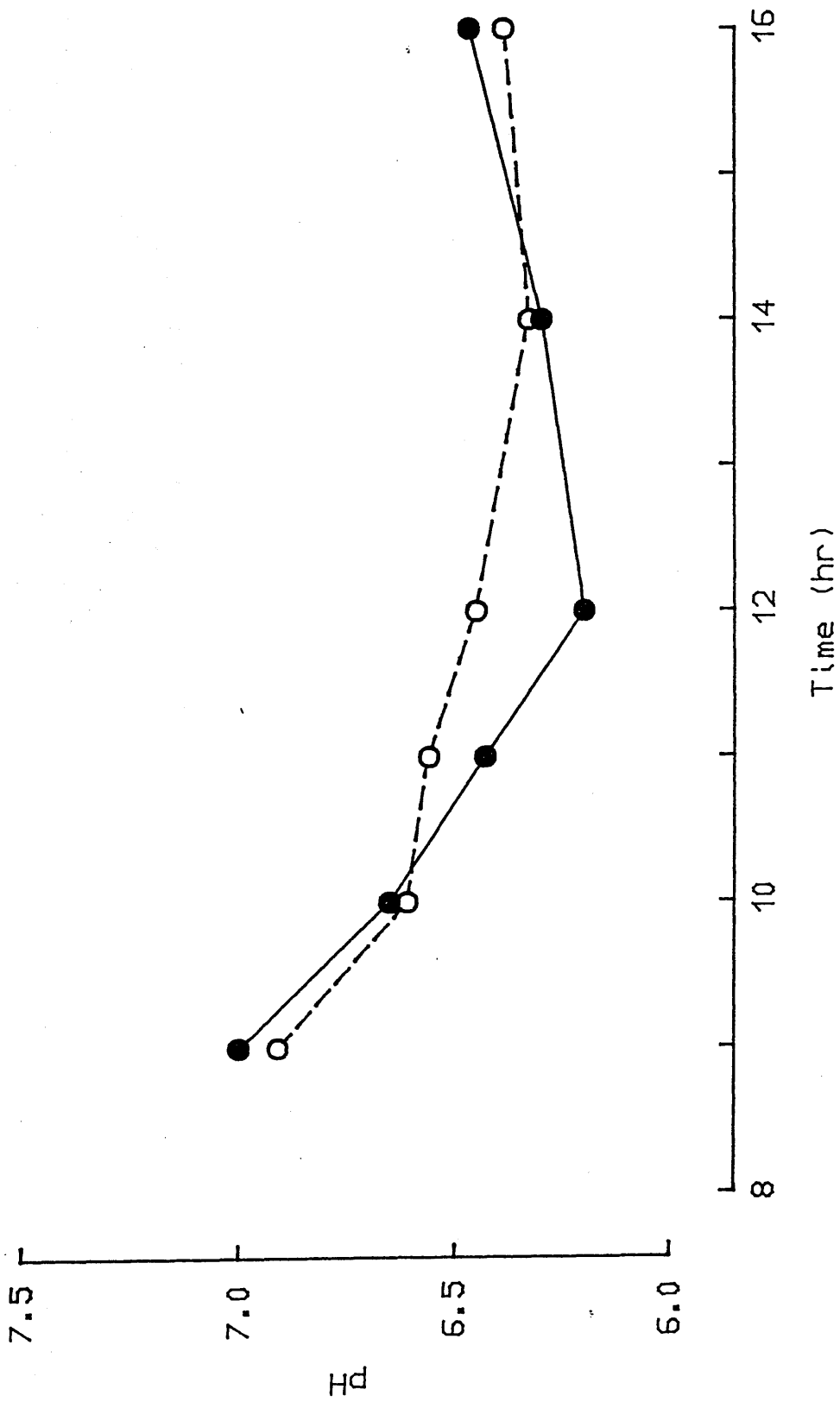
Figure 4.5. The pH of rumen liquor in cows given diets of grass silage with untreated barley (O---O) or formaldehyde-treated barley (●——●) in Experiment 6. (Values are means of two sets of observations)
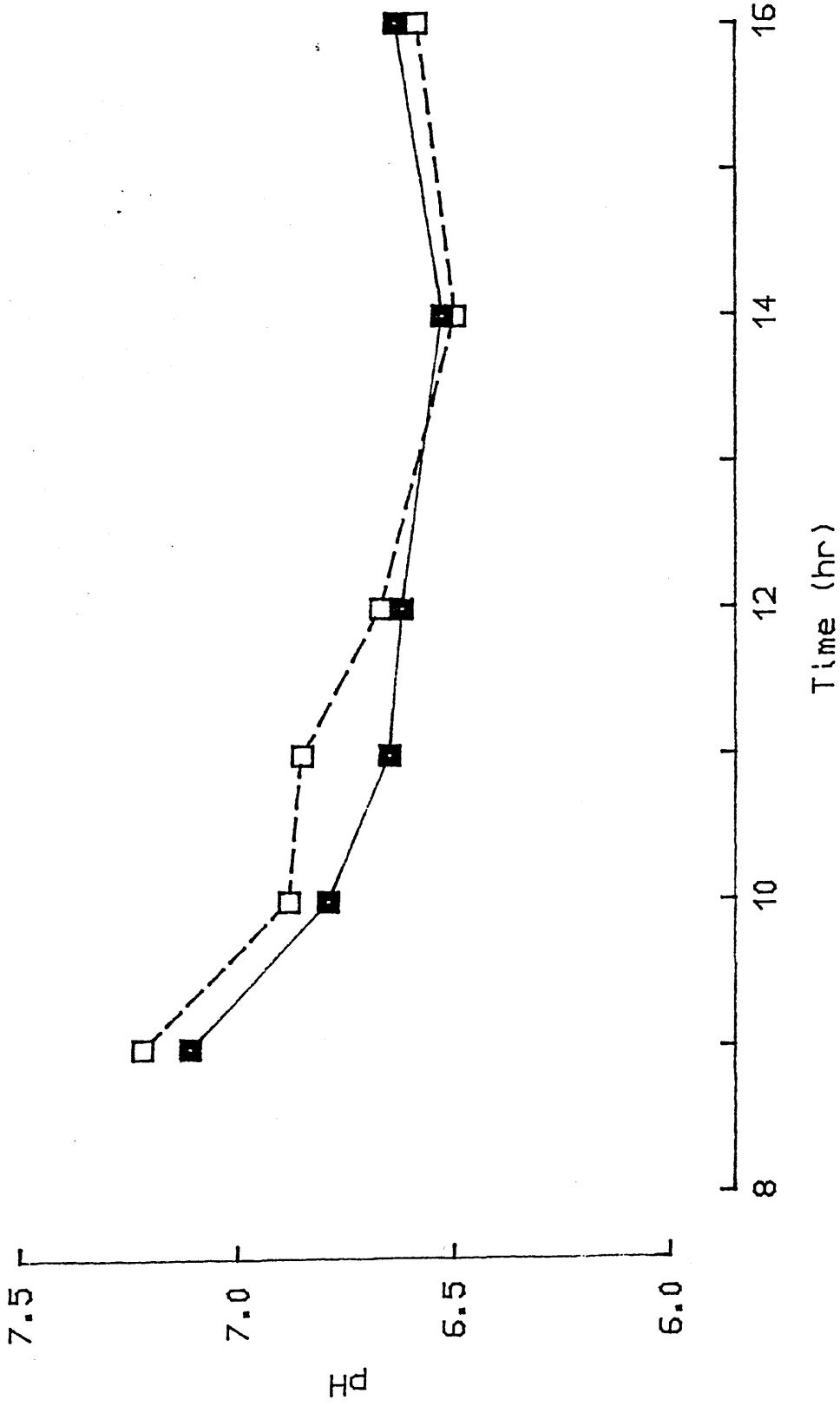
Figure 4.6. The pH of rumen liquor in cows given diets of lucerne silage with untreated barley (□--□) or formaldehyde-treated barley (■——■) in Experiment 6. (Values are means of two sets of observations)
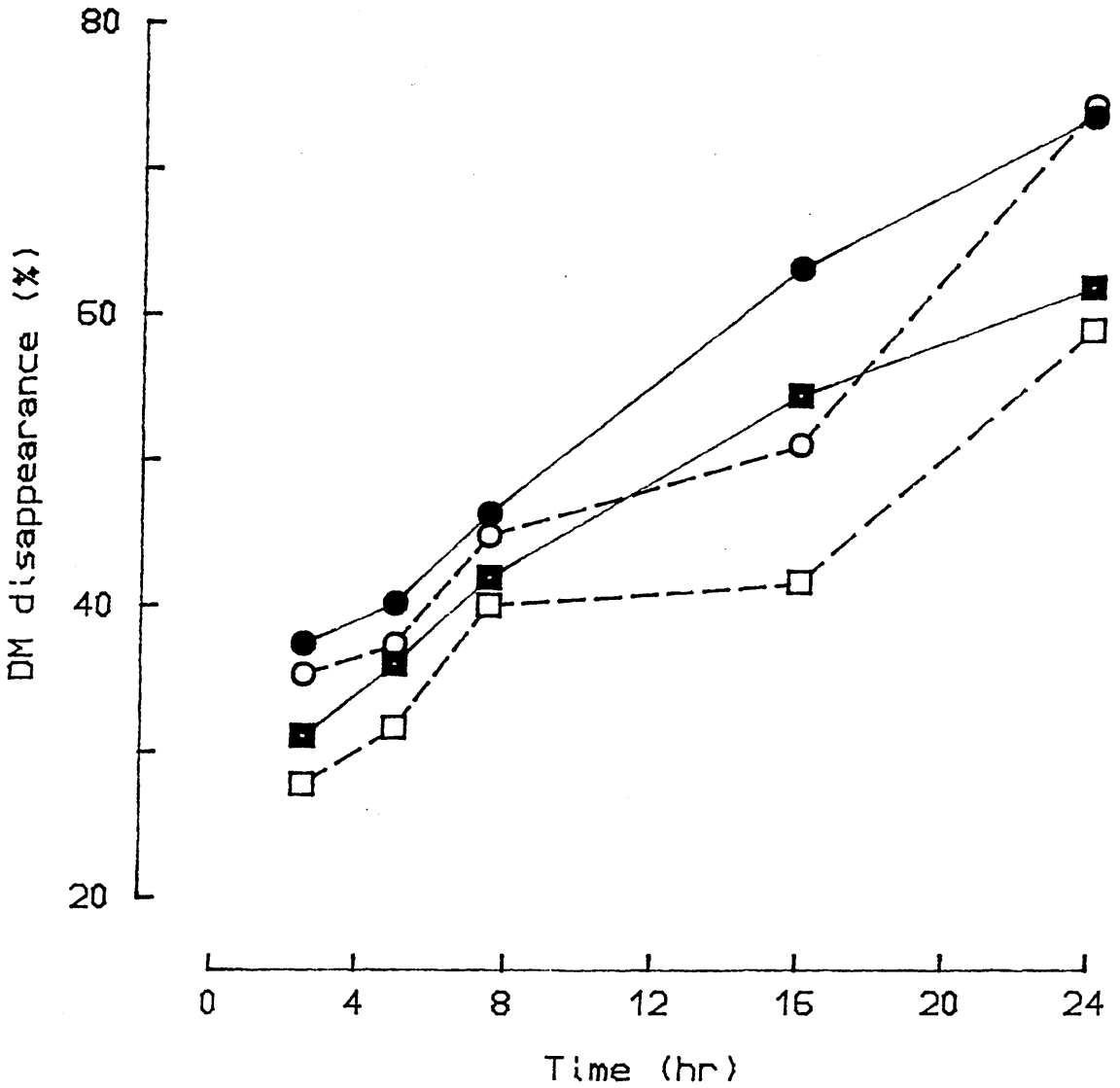
Figure 4.7. The disappearance (%) of dry matter from grass (O, ●) and
lucerne (□, ■) silage incubated in Dacron bags in the rumen
of a cow given a diet of grass silage with untreated barley
(----) or grass silage with barley treated with acid-
formaldehyde reagent (——).
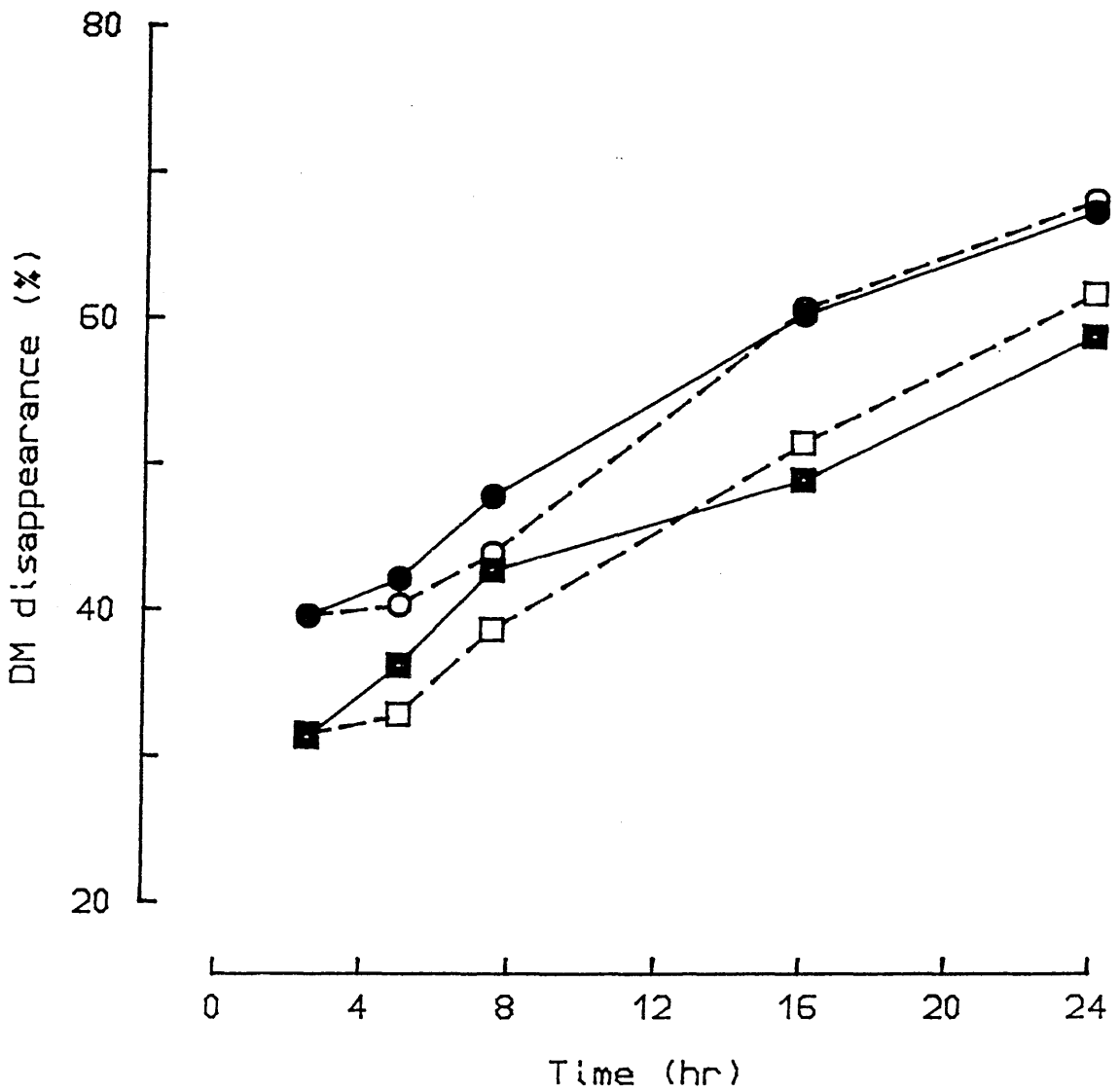
Figure 4.8. The disappearance (%) of dry matter from grass (O, ●) and
lucerne (□, ■) silage incubated in Dacron bags in the rumen
of a cow given a diet of lucerne silage with untreated barley
(----) or lucerne silage with barley treated with acid-
formaldehyde reagent (——).
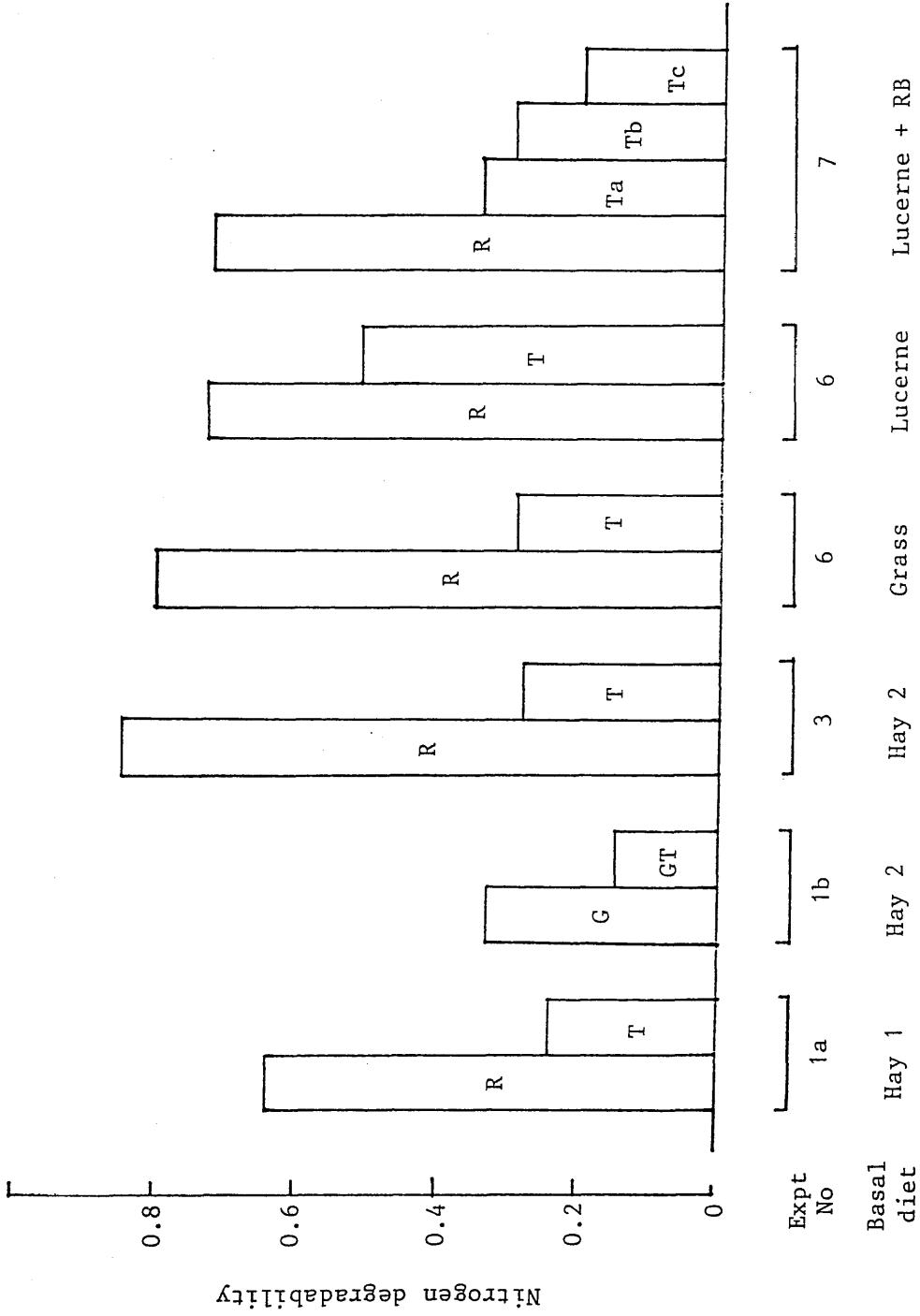
Figure 4.9. Values for the rumen-degradability of nitrogen for rolled barley (R) or ground barley (G) or barley treated with acid-formaldehyde reagent (T). (Data are for experiments 1, 3, 6 and 7)
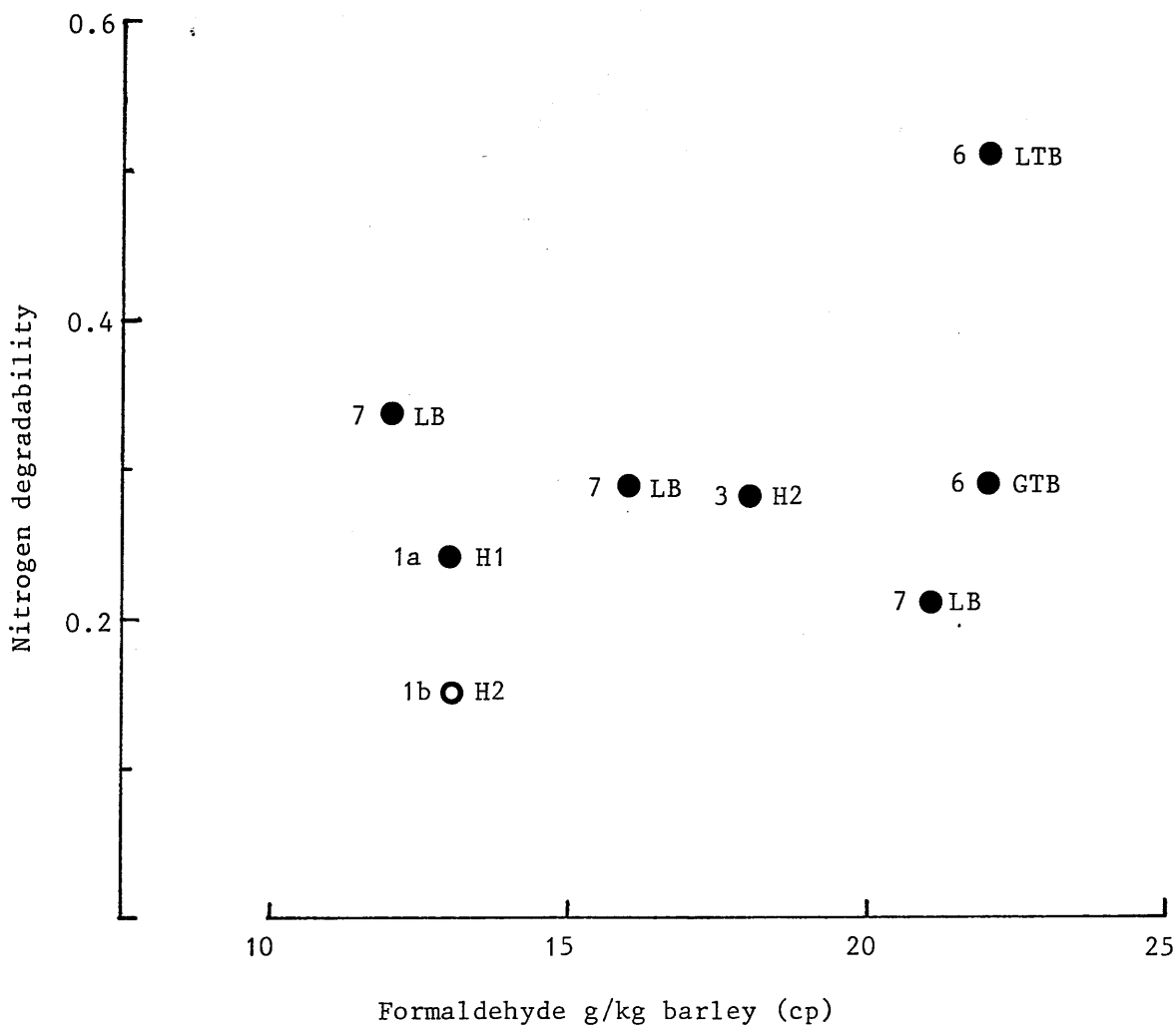
Figure 4.10. The effect of rate of application of acid-formaldehyde reagent
(g/kg crude protein) on the rumen-degradability of nitrogen in
samples of rolled barley (●) and ground barley (O). (Data are
from incubations in animals given diets of Hay (1), Hay (2),
grass silage-treated barley (GTB), lucerne silage-treated
barley (LTB) or lucerne silage-untreated barley (LB)

application to the herbage before ensiling to obtain protection of
protein was in the range of 20 to 35 g formaldehyde/kg crude protein
(see Thomas and Thomas, 1985). However with vegetable protein
concentrates such as soyabean meal rates of formaldehyde application
of 6 g/kg CP or above are necessary to achieve satisfactory
cross-linking of protein and substantially reduced rumen breakdown
(Barry, 1976; Rooke et al., 1983). The rates of application used for
the barley samples used in these experiments were 13, 18, 25, 17, 22,
12, 16 and 21 g/kg barley CP respectively; all the rates of application
are within the range reported to be effective for vegetable protein
concentrates (Barry, 1976).

It was evident from the experiments that the nature of the diet
given to the cows had an important influence on the degradability
values that were observed in the Dacron bag tests. The data suggest
that the measured degradability values were influenced by the protein
content of the basal diet given to the cow (Experiments 1 and 3) and by
the type of barley (untreated or formaldehyde treated) and the type of
silage (grass or lucerne) used to formulate the basal diet (Experiment
6) (see Figure 4.9).

A problem encountered in the interpretation of the degradability
curves for barley and for the silage samples used in the experiment was
the derivation of a single degradability value for use in ration
computation. Recent studies on the determination of ruminal
degradability of protein concentrate foods using the Dacron bag
technique (ARC, 1984) have recommended that the curves for
disappearance of N with time be coupled with estimates of the outflow
rate of food from the rumen to calculate an appropriate degradability
value. The principles of this procedure have been discussed by Ørskov

(1982). In essence the approach is to fit the observed disappearance value to a disappearance curve for N using an equation of the form

$$P = a + b (I - e^{-ct})$$ (1)

where p is the amount degraded at time (t), a, b, c and e are constants in the exponential equation, a can be interpreted as the rapidly-soluble fraction, b the amount which will degrade, c the fractional-rate constant at which the fraction described by b will be degraded per hour. Outflow rate has been estimated for specific protein concentrate foods by measuring the rate of passage of chromium-mordanted food particles from the rumen. The data have been summarized by using a mathematical expression of the form

$$P = a + \frac{bc}{c + k}$$ (2)

where a, b and c are the constants from the equation (1) describing degradation, and k is the fractional outflow rate. Based on this approach, ARC (1984) have provided values for the degradability of N for a variety of protein concentrate foods for cows at outflow rates ranging from $0.02-0.08h^{-1}$. They have also provided similar degradability/outflow rate data for cereal foods and forages, though for these foods there is no routine method for determining outflow and the basis of the ARC (1984) computation is not readily apparent.

In the Dacron bag studies conducted here the degradability curves obtained for untreated barley were similar in form to those described for protein concentrates by ARC (1984) and could therefore be fitted to equation (1). However, the disappearance curves for treated barley were almost linear and showed little evidence of a plateau over the incubation periods studied. The standard mathematical treatment was therefore inappropriate. Also, in the absence of information on the

outflow rate of the cereal particles from the rumen, there was no clear basis on which to assess the incubation time most appropriate to calculate the degradability value from the disappearance curve. A similar problem was encountered in relation to the silage samples.

The approach adopted therefore was to select an arbitrary incubation time and to use that time for calculation of the degradability values for all food samples. Thus the values used below in the interpretation of the experiments are correct relative to each other but their absolute accuracy may be open to debate. The incubation time adopted was 24 hours. This time was selected on the basis that the degradability values determined for untreated barley and for silage samples at this time were close to those recommended for ration formulation by ARC (1984). The degradability values for the food used in Experiments 1-7 are summarised in Table 4.3.

RDN supply

The dietary RDN supply and the animals' requirement for RDN calculated as 1.4 g N/MJ ME intake (ARC, 1984) are given for each experimental treatment in Table 4.4 together with the animals' status with respect to RDN supply. In Experiment 2 all treatments were deficient with respect to RDN supply, the deficiency being greater with the treated barley than with the untreated barley. In contrast in Experiment 7 all treatments were more than adequate in RDN supply, and the excess was quite substantial. In Experiments 3, 4, 5 and 6 the adequacy of RDN supply varied with the dietary treatment. With diets based on grass silage of crude protein below 160 g/kg DM and barley supplements, the diet contained adequate amounts of RDN. However, when barley was replaced by formaldehyde-treated barley in these diets the

Table 4.3. Rumen-degradability of nitrogen for the foods used in
Experiments 1 to 7. Values are based on a 24h period of
intraruminal incubation.

Expt. No.	Incubated food	Basal diet	Degradability
1a	Untreated barley	Hay 1	0.64
1a	Treated barley (8 l/t)	Hay 1	0.24
1b	Untreated ground barley	Hay 2	0.33
1b	Treated ground barley (8 l/t)	Hay 2	0.15
3	Untreated barley	Hay 2	0.85
3	Treated barley (15 l/t)	Hay 2	0.28
6	Untreated barley	Grass silage + UB	0.80
6	Treated barley (15 l/t)	Grass silage + TB	0.29
6	Untreated barley	Lucerne silage + UB	0.73
6	Treated barley (15 l/t)	Lucerne silage + TB	0.51
7	Untreated barley	Lucerne silage + UB	0.71
7	Treated barley (8 l/t)	Lucerne silage + UB	0.34
7	Treated barley (11 l/t)	Lucerne silage + UB	0.29
7	Treated barley (15 l/t)	Lucerne silage + UB	0.20
6	Grass silage	Grass silage + UB	0.90
6	Grass silage	Grass silage + TB	0.88
6	Lucerne silage	Lucerne silage + UB	0.84
6	Lucerne silage	Lucerne silage + TB	0.86
3	Fishmeal*	Hay	0.45

* Thomas, P.C. (Personal communication)

Table 4.4. The dietary RDN supply (g/d) and the requirement for RDN calculated from the results of ME intake in Experiments 2 to 7

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	RDN supply (g/d) ‡	RDN requirement (g/d) §	Status (g/d)
2	Grass	Untreated barley	3.26	179	204	-25
2	Grass	Untreated barley	5.28	204	232	-28
2	Grass	Untreated barley	7.18	227	259	-32
2	Grass	Treated barley	3.22	143	204	-61
2	Grass	Treated barley	5.22	159	227	-68
2	Grass	Treated barley	7.33	182	255	-73
3	Grass	Untreated barley	5.85	244	233	+11
3	Grass	Treated barley	5.83	189	241	-52
3	Grass	Untreated barley + FM	5.89	286	248	+38
3	Grass	Treated barley + FM	5.87	226	252	-26
3*	Grass	Untreated barley	5.85	231	220	+11
3*	Grass	Treated barley	5.83	170	216	-46
3*	Grass	Untreated barley + FM	5.89	254	217	+37
3*	Grass	Treated barley + FM	5.87	203	223	-20

Table 4.4. (continued)

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	RDN supply (g/d)	RDN requirement (g/d)	Status (g/d)
4	Grass	None	-	272	135	+137
4	Grass	Untreated barley	7.37	247	245	+2
4	Grass	Treated barley	7.35	212	263	-51
4	Grass	Untreated barley + NaHCO$_3$	7.05	262	253	+9
5*	Grass	None	-	256	132	+124
5*	Grass	Untreated barley	5.55	285	236	+49
5*	Grass	Treated barley	5.19	214	230	-16
5*	Lucerne	None	-	294	126	+168
5*	Lucerne	Untreated barley	5.53	263	209	+54
5*	Lucerne	Treated barley	5.69	261	221	+40
6	Grass	Untreated barley	5.72	294	257	+37
6	Grass	Treated barley	5.74	240	259	-19
6	Grass/lucerne mixture	Untreated barley	5.74	290	239	+51
6	Grass/lucerne mixture	Treated barley	5.73	252	235	+17
6	Lucerne	Untreated barley	5.69	277	216	+61
6	Lucerne	Treated barley	5.76	284	224	+60

Table 4.4. (continued)

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	RDN supply (g/d)	RDN requirement (g/d)	Status (g/d)
7	Lucerne	Untreated barley	5.92	377	264	+113
7a	Lucerne	Treated barley (8 1/t)	5.91	337	263	+74
7b	Lucerne	Treated barley (11.5 1/t)	5.80	343	268	+75
7c	Lucerne	Treated barley (15 1/t)	5.83	343	268	+75

* Heifers.

‡ RDN supply (g/d) value taken from disappearance of N from dacron bags after 24h incubation.

§ RDN requirement = 1.4 x ME intake MJ/d for the silage-concentrate mixture or 1.0 x ME intake MJ/d for the silage alone (ARC, 1984).

reduction in supply of RDN was such that the animals' requirements for RDN (ARC, 1984) were no longer satisfied. For diets containing grass or grass and lucerne silage of higher crude protein content (160-170 g/kg DM), the RDN requirement was met when both untreated and treated barley supplements were given.

Where the RDN status of the diet was inadequate the shortfall in supply was generally small, less than approximately 50 g/d except in Experiment 2. As has been pointed out above, there may be significant errors in the determination of absolute values for the degradability of dietary N. Also, as indicated by ARC (1984), there is appreciable uncertainty about the value for the rate of synthesis of microbial protein which should be adopted for calculation of RDN requirement (the range indicated by ARC varies with diet from 1.34-1.40 g N/MJ ME). As a consequence the calculated RDN deficiencies should be interpreted with some caution. However, assuming they are correct the data for RDN supply indicate that the deficiencies observed in the diets containing formaldehyde-treated barley could be corrected through a small daily supplement of vegetable protein. The need would be met by a supplement of approximately 600 g/d of soyabean meal, for example. Alternatively, expressed in other terms, the data indicate that for dietary adequacy to be maintained in rations containing the treated supplements the protein content of the basal silage being given should be at least 165 g/kg DM.

Duodenal amino acid supply and amino acid utilization

Table 4.5 summarises the results of the experiments for the calculated supply of UDN and microbial N to the duodenum, for the supply of tissue amino acid nitrogen, and for the utilization of amino

Table 4.5. The dietary UDN supply (g/d), tissue amino acid nitrogen (AA-N) supply, milk nitrogen loss and tissue N deposition in Experiments 2, 3, 4, 5, 6 and 7

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	Duodenal supply UDN (g/d)‡	Duodenal supply Mic AA-N (g/d)§	Tissue AA-N (g/d)§§	Milk N loss (g/d)	Tissue N deposition (g/d)
2	Grass	Untreated barley	3.26	35.0	143.0	93.5	76.8	-24.3
2	Grass	Untreated barley	5.28	30.6	162.9	101.6	87.0	-26.4
2	Grass	Untreated barley	7.18	27.2	181.8	109.7	91.7	-23.0
2	Grass	Treated barley	3.22	71.3	114.5	97.5	78.2	-21.7
2	Grass	Treated barley	5.22	69.9	127.3	103.5	84.2	-21.7
2	Grass	Treated barley	7.33	72.2	145.8	114.5	90.8	-17.3
3	Grass	Untreated barley	5.85	40.0	186.4	119.0	94.0	-16
3	Grass	Treated barley	5.83	109.0	151.2	136.5	111.0	-15.5
3	Grass	Untreated barley + FM	5.89	94.0	198.5	153.6	102.2	+10.4
3	Grass	Treated barley + FM	5.87	160.0	201.8	190.0	111.0	+38.0
3*	Grass	Untreated barley	5.85	36.2	175.8	111.3	74.1	-0.2
3*	Grass	Treated barley	5.83	94.8	136.0	121.2	82.0	-2.2
3*	Grass	Untreated barley + FM	5.89	84.7	173.6	135.6	90.0	+8.6
3*	Grass	Treated barley + FM	5.87	142.9	178.2	168.6	88.1	+43.5

Table 4.5. (continued)

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	Duodenal supply UDN (g/d)	Mic AA-N (g/d)	Tissue AA-N (g/d)	Milk N loss (g/d)	Tissue N deposition (g/d)
4	Grass	None	-	29.9	107.7	72.2	78.4	-44.2
4	Grass	Untreated barley	7.37	39.4	196.2	123.7	90.1	-5.4
4	Grass	Treated barley	7.35	101.4	210.0	163.5	100.8	+22.7
4	Grass	Untreated barley + NaHCO$_3$	7.05	40.7	202.2	127.5	92.3	-3.8
5*	Grass	None	-	28.6	105.6	70	67.7	-33
5*	Grass	Untreated barley	5.55	44.8	188.8	123	88.4	-2.4
5*	Grass	Treated barley	5.19	106.8	184.0	153	86.5	+29.5
5*	Lucerne	None	-	48.2	100.8	78	65.4	-23.4
5*	Lucerne	Untreated barley	5.53	66.1	167.2	122	77.7	+8.3
5*	Lucerne	Treated barley	5.69	91.2	176.8	141	82.4	+22.6
6	Grass	Untreated barley	5.72	41.2	205.6	129.6	106.7	-15.9
6	Grass	Treated barley	5.74	99.0	207.2	160.7	110.3	+11.4
6	Grass/Lucerne mixture	Untreated barley	5.74	56.7	191.2	130.1	100.8	-9.0
6	Grass/Lucerne mixture	Treated barley	5.73	90.1	188	146.0	105.8	+1.5

Table 4.5. (continued)

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	Duodenal supply UDN (g/d)	Mic AA-N (g/d)	Tissue AA-N (g/d)	Milk N loss (g/d)	Tissue N deposition (g/d)
6	Lucerne	Untreated barley	5.69	72.3	173	128.8	94.7	-4.5
6	Lucerne	Treated barley	5.76	84	179	138.1	103.1	-3.7
7	Lucerne	Untreated barley	5.92	80	211	153	94.4	+18.6
7a	Lucerne	Treated barley (8 l/t)	5.91	119	210	173	100.6	+32.4
7b	Lucerne	Treated barley (11.5 l/t)	5.80	127	214	179	102.2	+36.8
7c	Lucerne	Treated barley (15 l/t)	5.83	125	214	178	103.0	+35.0

* Heifers

‡ UDN values taken from disappearances of N from dacron bags after 24h incubation.

§ Microbial N = 1.4g N/MJ ME for silage-barley diets and 1.0 g N/MJ ME for silage alone (ARC, 1984).

§§ Tissue N calculated assuming a digestibility of 0.70 for duodenal AA-N (Microbial N = 0.80 AA-N; UDN = 1.0 AA-N) and an efficiency of utilization of 0.75 (ARC, 1980).

acid nitrogen for milk protein synthesis and tissue deposition. In all cases the data have been calculated using the factors proposed by ARC (1980, 1984) (see Table 4.5). As indicated above for RDN the calculations should be interpreted with some caution in view of the uncertain accuracy of the estimates of rumen-degradability of dietary N and the questions about the appropriate efficiency which should be adopted for the synthesis of microbial protein in the rumen.

The results for Expt 2 indicated quite clearly that all the diets used were deficient in amino acid supply, judged in terms of the negative value obtained for tissue N deposition. These did not differ greatly between diets but tended to be less negative for the diets containing the formaldehyde-treated barley. At the other end of the scale, in Expt 7, all dietary treatments led to substantial positive N balance, again the balance tending to be increased with the formaldehyde-treated barley diets. In the other experiments tissue nitrogen balances varied with dietary treatments in a rather complex way. Two factors contributed to this. In some instances, large increases in amino acid supply to the duodenum arising from an increase in UDN supply with the formaldehyde treated barley were substantially offset by associated reductions in the duodenal supply of microbial amino acids arising because of restrictions on RDN supply and microbial protein synthesis in the rumen. The results of Expt 3 provide an example of these effects. Furthermore, in some instances an increase in duodenal total amino acid was accompanied by a response in the secretion of milk protein whilst in others milk production changed little and the response was mainly in tissue nitrogen deposition. This effect can be seen in Expt 3 in the comparison between the diet containing silage and treated-barley and the diet containing untreated

barley and fishmeal (Table 4.5). In other instances, for example the treated barley diet in Expt 4, an increased absorption of amino acids from the duodenum was accompanied by an improvement both in milk protein synthesis and tissue protein deposition.

A possible explanation for these differential responses in milk and tissue protein synthesis could lie in the effects of the dietary treatments on ME supply. But so far as can be judged from the results available, this is not the case, or at least the relationships are not simple. Data on dietary ME supply and utilization from each of the experiments are summarised in Table 4.6. As can be seen for most of the experimental treatments the cows were in positive energy balance and for many treatments the positive balance was quite large. In instances, such as those in Expt 3, where a change in duodenal amino acid supply was linked with differential responses between treatments in the increase in tissue nitrogen deposition relative to the increase in milk protein secretion, there was no evidence of differences between treatments in ME intake. Differences in ME utilization simply reflected the responses in milk and tissue nitrogen utilization already pointed out. In comparison, in Expt 4 where the treated-barley supplement led to an increase in duodenal amino acid supply linked with parallel improvements in milk and tissue protein synthesis, the treated barley did enhance ME supply through effects on silage DM intake.

Changes in milk composition and in the yields of milk constituents

A feature of the experiments was that the milk yield responses to the formaldehyde-treated barley supplements were associated with differential responses in milk fat, protein and lactose secretion. There was, in fact, rather little effect of the dietary treatment on

Table 4.6. The dietary ME supply, ME requirements for maintenance and milk production, and energy status

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	ME intake (MJ/d)	ME requirement for: Maintenance†	ME requirement for: Milk production‡	ME status	ME above maintenance
2	Grass	Untreated barley	3.26	145.2	55.7	82.9	+6.8	89.7
2	Grass	Untreated barley	5.28	165.5	56.7	91.1	+17.7	108.8
2	Grass	Untreated barley	7.18	184.7	57.4	94.6	+32.7	127.3
2	Grass	Treated barley	3.22	146.0	56.2	82.8	+7.0	89.8
2	Grass	Treated barley	5.22	162.3	56.4	87.8	+18.1	105.9
2	Grass	Treated barley	7.33	185.9	57.2	90.9	+37.8	128.7
3	Grass	Untreated barley	5.85	166.1	56.3	103.6	+6.2	109.8
3	Grass	Treated barley	5.83	172.1	56.3	109.3	+6.5	115.8
3	Grass	Untreated barley + FM	5.89	177.2	56.3	111.1	+9.8	120.9
3	Grass	Treated barley + FM	5.87	180.2	56.3	115.4	+8.5	123.9
3*	Grass	Untreated barley	5.85	157.0	51.4	82.1	+23.5	105.6
3*	Grass	Treated barley	5.83	155.0	51.4	88.7	+14.6	103.6
3*	Grass	Untreated barley + FM	5.89	154.4	51.4	89.0	+14.4	103.1
3*	Grass	Treated barley + FM	5.87	159.1	51.4	93.7	+14	107.7

Table 4.6. (continued)

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	ME intake (MJ/d)	ME requirement for: Maintenance	Milk production	ME status	ME above maintenance
4	Grass	None	-	134.6	53.3	82.9	-1.6	81.3
4	Grass	Untreated barley	7.37	175.2	54.1	93.9	+27.2	121.1
4	Grass	Treated barley	7.35	187.5	55.1	99.2	+33.2	132.4
4	Grass	Untreated barley + NaHCO$_3$	7.05	180.5	54.4	97.4	+28.7	126.1
5*	Grass	None	-	131.6	49.6	75.2	+6.8	82.0
5*	Grass	Untreated barley	5.55	168.6	51.6	87.3	+29.7	117.0
5*	Grass	Treated barley	5.19	164.5	54.2	86.5	+26.6	110.3
5*	Lucerne	None	-	125.5	49.3	71.0	+5.2	76.2
5*	Lucerne	Untreated barley	5.53	149.4	49.9	81.8	+17.7	99.5
5*	Lucerne	Treated barley	5.69	157.9	50.8	85.0	+22.1	107.1
6	Grass	Untreated barley	5.72	183.7	53.8	119.9	+10	129.9
6	Grass	Treated barley	5.74	184.7	54.0	119.0	+11.7	130.7
6	Grass/Lucerne mixture	Untreated barley	5.74	170.6	53.1	111.1	+6.4	117.5
6	Grass/Lucerne mixture	Treated barley	5.73	168.1	53.6	114.3	+0.2	114.5

Table 4.6. (continued)

Expt No	Type of silage	Type of concentrate	Level of concentrate (kg DM/d)	ME intake (MJ/d)	ME requirement for: Maintenance	Milk production	ME status	ME above maintenance
6	Lucerne	Untreated barley	5.69	154.0	53.5	100.6	-0.1	100.5
6	Lucerne	Treated barley	5.76	159.8	53.7	109.0	-2.9	106.1
7	Lucerne	Untreated barley	5.92	188.3	54.9	105.3	+28.1	133.4
7a	Lucerne	Treated barley (8 l/t)	5.91	188.2	55.1	107.5	+25.6	133.1
7b	Lucerne	Treated barley (11.5 l/t)	5.80	191.6	55.1	107.1	+29.4	136.5
7c	Lucerne	Treated barley (15 l/t)	5.83	191.5	55.6	107.1	+28.8	135.9

* Heifers

+ Assuming the efficiency of utilization of ME for maintenance (Km) 0.55 MJ/kg $W^{0.75}$ (MAFF, 1980).

ME for milk production = Net energy in milk x 1.60 (MAFF, 1980)

the secretion of total energy in milk and thus the increases in
production did not represent any major repartition in energy use
between milk secretion and body tissue deposition (Figure 4.11).
Rather, they represented a repartition in energy use between the
individual milk constituents: in Expt 7, for example, the improvement
in lactose and protein secretion was balanced by a reduction in the
energy secretion in fat. Effects of this type have been reported in
other studies most clearly in the experiments of Sutton (1984) which
involved comparisons between diets containing different proportions, 60
or 90%, of either starchy or fibrous concentrates (see Table 4.7).
These secretory effects are thought to be due to the influences of diet
on the composition of the mixture of nutrient substances absorbed as
products of digestion and a similar explanation probably applies in the
present experiments. The increases in milk protein secretion seem
likely to relate to a change in the amount or possibly composition of
the amino acid mixture absorbed from the duodenum when the cows were
receiving treated-barley as compared with untreated-barley supplements.
There is a large body of results that show improvements in milk protein
content and yield to the intraabomasal infusion of casein or of amino
acid mixtures (Schwab et al., 1976; Clark et al., 1977; Broderick et
al., 1971; Rogers et al., 1979) and as shown by the summary of Thomas
and Chamberlain (1984), the increases in protein yield are linked with
corresponding changes in lactose yield and milk yield.

The limited increases in milk fat yield and in particular the
negative effects on fat yield, such as observed in Expt 7, could be
related either to the effects of the treated-barley supplements on the
proportion of VFA formed in the rumen or on the passage of starch to
the duodenum. It is well established that intraruminal infusions of
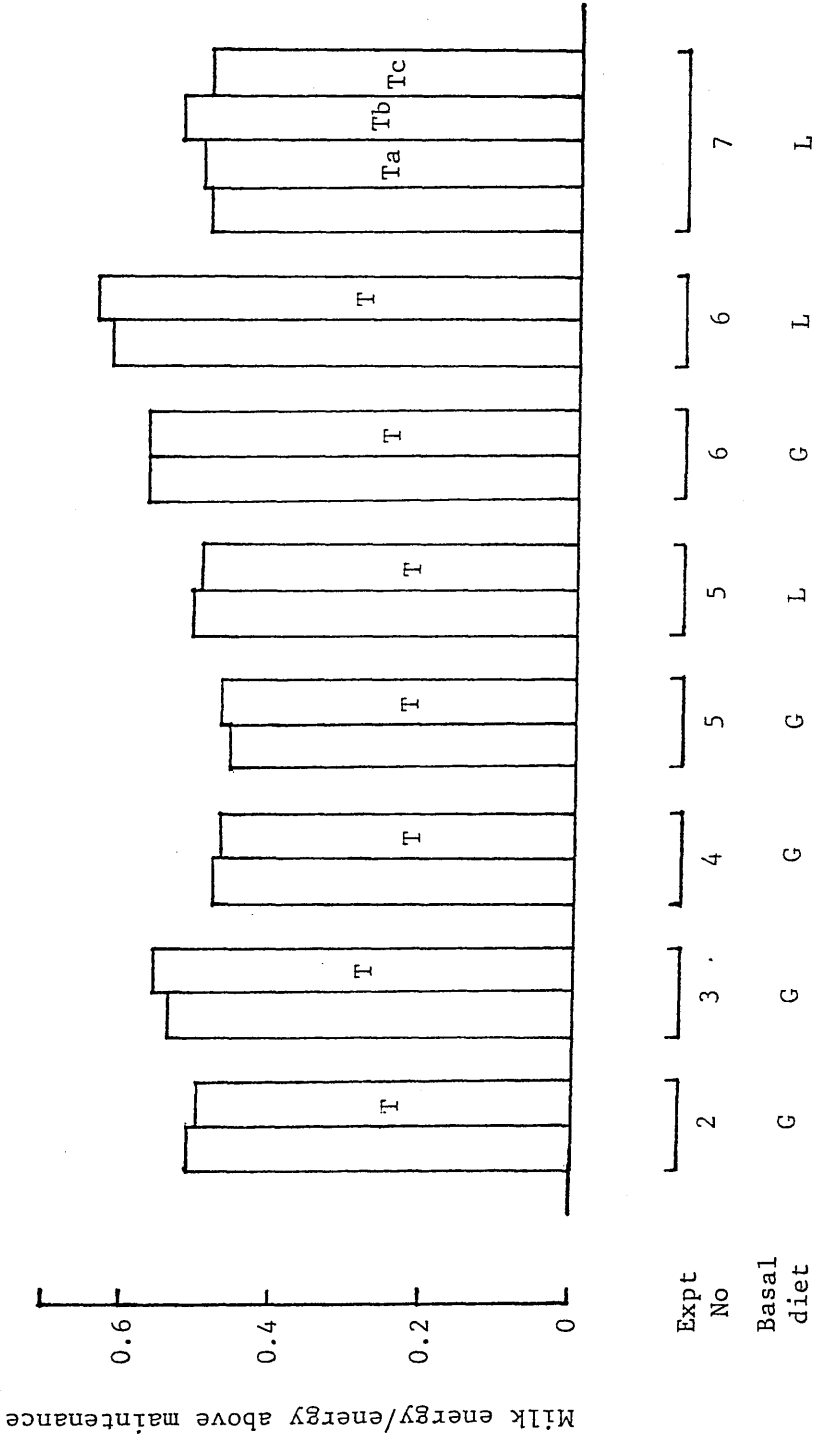
Figure 4.11. The ratio of energy output in milk to the energy intake above maintenance for cows given diets containing grass silage (G) or lucerne silage (L) with treated barley (T) or untreated barley (U) supplements. (Data are for experiments 2 to 7)

Table 4.7. The effects on milk yield, the content and yield of fat, and acetate plus butyrate to propionate ratio in the rumen of Friesian cows in mid-lactation on various diets

Expt[†]	Type and proportion in diet[‡]	Acid detergent fibre (ADF) (g/kg dry matter)	Milk yield (l/day)	Fat content (g/kg)	Fat yield (kg/day)	Rumen ratio: acetate + butyrate to propionate
A	Forage to concentrate					
	600 g barley/kg	195	21.4	36.9	0.78	5.0
	750 g barley/kg	150	23.5	30.9	0.71	3.8
	900 g barley/kg	108	24.8	21.4	0.54	2.7
B	Type of cereal					
	600 g rolled barley/kg	212	16.1	44.9	0.73	4.9
	900 g rolled barley/kg	112	20.6	20.6	0.42	1.9
	600 g ground maize/kg	191	18.9	40.4	0.76	4.8
	900 g ground maize/kg	88	15.6	29.7	0.46	2.7
C	Carbohydrate in concentrates					
	600 g starchy/kg	193	24.7	41.8	1.03	4.4
	800 g starchy/kg	136	31.0	21.8	0.68	2.6
	600 g fibrous/kg	235	24.7	42.6	1.05	4.7
	800 g fibrous/kg	188	25.0	37.9	0.95	3.9

Table 4.7. (continued)

Expt	Type and proportion in diet	Acid detergent fibre (ADF) (g/kg dry matter)	Milk yield (l/day)	Fat content (g/kg)	Fat yield (kg/day)	Rumen ratio: acetate + butyrate / propionate
D	Level of intake: 750 g barley/kg diet					
	155 MJ digestible energy (DE)/day	150	19.2	33.1	0.64	4.4
	190 MJ DE/day	150	23.5	30.9	0.71	3.8
	237 MJ DE/day	150	23.7	25.3	0.60	2.4
E	Meal frequency (meals/day)					
	700 g barley/kg: 2	162	19.7	32.6	0.64	4.5
	700 g barley/kg: 6	162	20.2	39.2	0.79	4.8
	900 g barley/kg: 2	102	23.0	17.9	0.42	2.2
	900 g barley/kg: 6	102	21.4	29.7	0.62	2.4

† A and D, Broster, Sutton and Bines (1979); B, Sutton, Oldham and Hart (1980); C, Sutton, Bines, Napper, Willis and Schuller (1984); E, Sutton, Hart and Broster (1982).

‡ Digestible energy intake (MJ/day) for the total diet was approximately equal within each experiment except D, and was approximately 180 to 190 MJ/day for A, C and E, and 160 MJ/day for B.

After Sutton (1984)

propionic acid (Rook and Balch, 1961; Rook et al., 1965; Chalmers, 1979; Chalmers et al., 1980) or intraabomasal infusion of glucose (see Rogers et al., 1979) lead to reductions in milk fat content and fat yield. On the basis of the information available it is not possible to comment on the effects of formaldehyde-treatment of grains on rumen VFA proportions. However, in recent studies with sheep fitted with duodenal cannulas (van Ramshorst, 1986), formaldehyde treatment of barley has been shown to increase the flow of starch to the small intestine. In animals receiving barley and dried grass diets providing approximately 211g starch/d, the passage of starch to the duodenum was 7.8 g/d with untreated barley and 16.4 g/d when the barley was formaldehyde treated. In both cases there was no loss of starch in faeces, and the data suggest that treatment of the barley improved glucose uptake in the small intestine by more than double. Presumably similar effects in the cows used in these experiments would at least in part account for the dietary effects on milk fat secretion.

Conclusions and practical implications

The results of the experiments presented show that treatment of barley with acidified formaldehyde reagent leads to a reduction in the rate of rumen-degradation of barley protein and starch. Where treated supplements were given to dairy cows in replacement for untreated supplements, there were improvements in silage intake and milk yield. The latter were linked particularly with increases in the yield of milk protein and lactose; effects on the yield of milk fat were variable and in some instances negative.

In the course of the experiments, a number of qualifications on the use of the grain treatment were identified. The results indicated

that whilst application of the formaldehyde reagent at rates of 8 to 15
l/t were effective in reducing the rumen-degradation of barley protein
and starch and improving milk yield, improvements in food intake were
not obtained at the 8 l/t rate. There were also significant influences
arising from the chemical composition of the basal diets that were
supplemented. Formaldehyde-treated barley did not lead to improvements
in performance in cows given low protein diets or in cows receiving
very highly digestible silages. In practice it would seem inadvisable
to expect responses to grain treatment in animals receiving silages
with DOMD values above 700 g/kg or with diets containing less than 120g
crude protein/kg DM. In the latter case, it is clear that formaldehyde
treatment of grain should be accompanied by dietary supplementation
with protein sources which can be used to correct potential
deficiencies in dietary RDN supply. The use of similar supplements
with basal diets containing higher levels of protein may also be
justified where the reduced degradability of the protein in
formaldehyde-treated barley leads to dietary RDN supplies becoming
marginal.

REFERENCES

Agricultural Research Council (1980) The Nutrient Requirement of

  Ruminant Livestock, Commonwealth Agricultural Bureaux; Farnham

  Royal, Slough.

Agricultural Research Council (1984) The Nutrient Requirement of

  Ruminant Livestock. Supplement 1. Commonwealth Agricultural

  Bureaux; Farnham Royal, Slough.

Appleman, R. and Delouche, J.C. (1958) Behavioural, physiological and

  biochemical responses of goats to temperature, 0 to 40°C. J.

  Anim. Sci., 17: 326-335.

Armstrong, D.G. and Prescott, J.H.D. (1971) Amount, physical form and

  composition of feed and milk secretion in the dairy cow. In

  Lactation, pp. 349-378. Ed. I.R. Falconer. Butterworths; London.

Arnold, G.W. (1970) Regulation of food intake in grazing ruminants.

  In Physiology of Digestion and Metabolism in the Ruminant, pp.

  264-276. Ed. A.T. Phillipson. Oriel Press; Newcastle, England.

Baile, C.A. and Forbes, J.M. (1974) Control of feed intake and

  regulation of energy balance in ruminants. Physiological Reviews

  54: 160-214.

Baile, C.A., Mayer, J. and Mclaughlin, C. (1969) Feeding behavior of

  goats: ruminal distension, ingesta dilution, and acetate

  concentrations. Am. J. Physiol., 217: 397-402.

Baile, C.A. and Pfander, W.H. (1967) Ration density as a factor

  controlling food intake in ruminants. J. Dairy Sci., 50: 77-80.

Balch, C.C. (1952) Factors affecting the utilization of food by dairy

  cows. 6. The rate of contraction of the rumen. Br. J. Nutr., 6:

  366-375.

Balch, C.C. (1971) Proposal to use time spent chewing as an index of the extent to which diets for ruminants possess the physical property of fibrousness characteristic of roughages. Br. J. Nutr., 26: 383-392.

Balch, C.C. and Campling, R.C. (1962) Regulation of voluntary food intake in ruminants. Nutr. Abst. Rev., 32: 669-686.

Ballard, F.J., Hanson, R.W. and Kronfeld, D.S. (1968) Gluconeogenesis and lipogenesis in tissue from ruminant and non ruminant animals. Fed. Proc., 28: 218-231.

Banks, W., Clapperton, J.L., Ferrie, M.E. and Wilson, A.G. (1976) Effect of feeding fat to dairy cows receiving a fat-deficient basal diet. 1. Milk yield and composition. J. Dairy Res., 43: 213-218.

Banks, W., Clapperton, J.L. and Steele, W. (1982) Feeding fat to dairy cows. Report of the Hannah Research Institute (1982), pp. 75-83.

Baraton, Y. and Pflimlin, A. (1978) Document ITEB, No. 78.121 cited by Twigge and Van Gils (1984).

Barker, S.B. and Summerson, W.H. (1941) The colorimetric determination of lactic acid in biological material. J. Biol. Chem., 137: 535-554.

Barry, T.N. (1976) The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation. Proc. Nutr. Soc., 35: 221-229.

Barry, T.N., Cook, J.E. and Wilkins, R.J. (1978) The influence of formic acid and formaldehyde additives and type of harvesting machine on the utilization of lucerne silages. 1. The voluntary intake and nitrogen retention of young sheep consuming the silages with and without intraperitoneal supplements of DL-methionine. J. agric. Sci., Camb., 91: 701-715.

Barry, T.N., Fennessy, P.F. and Duncan, S.J. (1973) Effect of formaldehyde treatment on the chemical composition and nutritive value of silage. N.Z. J. Agric. Res., 16: 64-68.

Baumgardt, B.R. (1970) Control of feed intake in the regulation of energy balance. In Physiology of Digestion and Metabolism in the Ruminant, pp. 235-253. Ed. A.T. Phillipson. Oriel Press; Newcastle, England.

Baumgardt, B.R. and Peterson, A.D. (1971) Regulation of feed intake in ruminants. 8. Caloric density of diets for young growing lambs. J. Dairy Sci., 54: 1191-1194.

Beever, D.E., Coelho Da Silva, J.F., Prescott, J.H.D. and Armstrong, D.G. (1972) The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass. Sites of digestion of organic matter, energy and carbohydrate. Br. J. Nutr., 28: 347-356.

Beever, D.E., Thomson, D.J. and Cammell, S.B. (1976) The digestion of frozen and dried grass by sheep. J. agric. Sci., Camb., 86: 443-452.

Beever, D.E., Ulyatt, M.J., Thomson, D.J., Cammell, S.B., Austin, A.R. and Spooner, M.C. (1980) Nutrient supply from fresh grass and clover fed to housed cattle. Proc. Nutr. Soc., 39: 66A.

Bhattacharya, A.N. and Warner, R.G. (1968) Influence of varying rumen temperature on central cooling or warming and on regulation of voluntary feed intake in dairy cattle. *J. Dairy Sci.*, 51: 1481-1489.

Bines, J.A. (1979) Voluntary food intake. In *Feeding Strategy for High Yielding Dairy Cow*, pp. 23-48. Ed. W.J. Broster and H. Swann, H. Granad Publishing; London.

Blaxter, K.L. (1962) The Energy Metabolism of Ruminants. Hutchinson; London.

Blaxter, K.L. (1966) The feeding of dairy cows for optimal production. George Scott Robertson Memorial Lecture, Queen's University Belfast.

Blaxter, K.L., Reineke, E.P., Crampton, E.W., Petersen, W.E. (1949) The role of thyroidal materials and of synthetic goitrogens in animal production and an appraisal of their practical use. *J. Anim. Sci.*, 8: 307-352.

Blaxter, K.L., Wainman, F.W. and Wilson, R.S. (1961) The regulation of food intake by sheep. *Anim. Prod.*, 3: 51-61.

Blaxter, K.L. and Wilson, R.S. (1962) The voluntary intake of roughages by steers. *Anim. Prod.*, 4: 351-358.

Blaxter, K.L. and Wilson, R.S. (1963) The assessment of a crop husbandry technique in terms of animal production. *Anim. Prod.*, 5: 27-42.

Broderick, G.A., Kowalczyk, T. and Satter, L.D. (1971) Milk production response to supplementation with encapsulated methionine per os or casein per abomasum. *J. Dairy Sci.*, 53: 1714-1721.

Broster, W.H. (1976) Plane of nutrition for the dairy cow. In Recent Advances in Animal Nutrition, pp. 271-285. Ed. H. Swan and W.H. Broster. Butterworths; London.

Brown, L.D., Hillman, D., Lassiter, C.A. and Huffman, C.F. (1963) Grass silage vs hay for lactating dairy cows. J. Dairy Sci., 46: 407-410.

Butler, G.W., Rae, A.L. and Bailey, R.W. (1968) Influence of pasture species on aspects of animal production. N.Z. J. Agric. Sci., 3: 8-12.

Campling, R.C. (1966) A preliminary study of the effect of pregnancy and of lactation on the voluntary intake of food by cows. Br. J. Nutr., 20: 25-39.

Campling, R.C. (1966a) The intake of hay and silage by cows. J. Br. Grassl. Soc., 21: 41-48.

Campling, R.C. (1966b) The effect of concentrates on the rate of disappearance of digesta from the alimentary tract of cows given hay. J. Dairy Res., 33: 13-23.

Campling, R.C. and Balch, C.C. (1961) Factors affecting the voluntary intake of food by cows. 1. Preliminary observations on the voluntary intake of hay, of changes in the amount of the reticulo-ruminal contents. Br. J. Nutr., 15: 523-530.

Campling, R.C. and Freer, M. (1966) Factors affecting the voluntary intake of food by cows. 8. Experiments with ground, pelleted roughages. Br. J. Nutr., 20: 229-244.

Campling, R.C., Freer, M. and Balch, C.C. (1961) Factors affecting the voluntary intake of food by cows. 2. The relationship between the voluntary intake of roughages, the amount of digesta in the reticulo-rumen and the rate of disappearance of digesta from the alimentary tract. Br. J. Nutr., 15: 531-540.

Campling, R.C., Freer, M. and Balch, C.C. (1962) Factors affecting the voluntary intake by cows. 3. The effect of urea on the voluntary intake of oat straw. Br. J. Nutr., 16: 115-124.

Campling, R.C., Freer, M. and Balch, C.C. (1963) Factors affecting the voluntary intake of food by cows. 6. A preliminary experiment with ground, pelleted hay. Br. J. Nutr., 17: 263-272.

Campling, R.C. and Lean, I.J. (1983) Food characteristics that limit voluntary intake. In Nutritional Physiology of Farm Animals, pp. 457-475. Ed. J.A.F. Rook and P.C. Thomas. Longman; London and New York.

Campling, R.C. and Murdoch, J.C. (1966) The effects of concentrates on the voluntary intake of roughages by cows. J. Dairy Res., 33: 1-11.

Carr, S.B. and Jacobson, D.K. (1967) Intraruminal addition of mass or removal of rumen contents on voluntary intake of the bovine. J. Dairy Sci., 50: 1814-1818.

Castle, M.E. (1982) Feeding high-quality silage. In Silage for Milk Production, pp. 127-150. Ed. J.A.F. Rook and P.C. Thomas. National Institute for Research in Dairying; Reading.

Castle, M.E., Gill, M.S. and Watson, J.N. (1983a) Silage and milk production: a comparison between three rates of high-protein concentrate supplementation of grass silages of two digestibilities. Grass & Forage Sci., 38: 135-140.

Castle, M.E., Reid, D. and Watson, J.N. (1983) Silage and milk production: studies with diets containing white clover silage. Grass & Forage Sci., 38: 193-200.

Castle, M.E., Reid, D. and Watson, J.N. (1984) Silage and milk production: a comparison between supplements of barley and soybean meal offered with white clover silage. Grass & Forage Sci., 39: 287-289.

Castle, M.E., Retter, W.C. and Watson, J.N. (1979) Silage and milk production: comparisons between grass silages of three different chop lengths. Grass & Forage Sci., 34: 293-301.

Castle, M.E. and Watson, J.N. (1975) Silage and milk production. A comparison between barley and dried grass as supplements to silage of high digestibility. J. Br. Grassl. Soc., 30: 217-222.

Castle, M.E. and Watson, J.N. (1976) Silage and milk production. A comparison between barley and groundnut cake as supplements to silage of high digestibility. J. Br. Grassl. Soc., 31: 191-195.

Castle, M.E. and Watson, J.N. (1979) Silage and milk production. A comparison between soya, groundnut and single-cell protein as silage supplements. Grass & Forage Sci., 34: 101-106.

Chalmers, J.S. (1979) Dietary factors influencing the composition of cows milk with special reference to milk protein. PhD Thesis, University of Glasgow.

Chalmers, J.S., Thomas, P.C. and Chamberlain, D.G. (1980) The effect of intraruminal infusions of propionic acid on milk composition in cows given silage diets. Proc. Nutr. Soc., 39: 27A.

Chamberlain, D.G. and Thomas, P.C. (1982) Effect of intravenous supplements of L-methionine on milk yield and composition in cows given silage-cereal diets. J. Dairy Res., 49: 25-28.

Chamberlain, D.G., Thomas, P.C. and Quig, Jan (1986) Utilization of silage nitrogen in sheep and cows: amino acid composition of duodenal digesta and rumen microbes. Grass & Forage Sci., 41: 31-38.

Chamberlain, D.G., Thomas, P.C., Wilson, Wilma D., Kassem, M.M. and
Robertson, S. (1984) The influence of the type of carbohydrate in
the supplementary concentrate on the utilization of silage diets
for milk production. In Proc. 7th Silage Conf., pp. 37-38
(Abstr.). Ed. F.J. Gordon and E.F. Unsworth. Queen's University;
Belfast.

Clancy, M., Wangsness, P.J. and Baumgardt, B.R. (1977) Effect of
silage extract on voluntary intake, rumen fluid constituents, and
rumen motility. J. Dairy Sci., 60: 580-590.

Clapperton, J.L. and Smith, E.J. (1983) Personal communication.

Clapperton, J.L. and Steele, W. (1983) Fat supplementation in animal
production - ruminants. Proc. Nutr. Soc., 42: 343-350.

Clark, J.H., Spiers, H.R., Derrig, R.G. and Bennink, M.R. (1977) Milk
production, nitrogen utilization and glucose synthesis in
lactating cows infused postruminally with sodium caseinate and
glucose. J. Nutr., 107: 631-644.

Claypool, D.W., Pangborn, M.C. and Adams, H.P. (1980) Effect of
dietary protein on high-producing dairy cows in early lactation.
J. Dairy Sci., 63: 833-837.

Conrad, H.R., Baile, C.A. and Mayer, J. (1977) Changing meal patterns
and suppression of feed intake with increasing amounts of dietary
non-protein nitrogen in ruminants. J. Dairy Sci., 60: 1725-1733.

Conrad, H.R., Pratt, A.D. and Hibbs, J.W. (1964) Regulation of feed
intake in dairy cows. 1. Change in importance of physical and
physiological factors with increasing digestibility. J. Dairy
Sci., 47: 54-62.

Cook, C.W., Mattox, J.E. and Harris, L.E. (1961) Comparative daily consumption and digestibility of summer range forage by wet and dry ewes. J. Anim. Sci., 20: 866-870.

Coombe, J.B. and Tribe, D.E. (1963) The effects of urea supplements on the utilization of straw plus molasses diets by sheep. Aust. J. agric. Res., 14: 70-92.

Coop, I.E. and Drew, K.R. (1963) Maintenance and lactation requirements of grazing sheep. Proc. N.Z. Soc. Anim. Prod., 23: 53-62.

Cottrill, B.R., Osbourn, D.F., Wilkinson, J.M. and Richmond, P.J. (1976) The effect of dietary pH and nitrogen supplementation on the intake and utilization of maize silage by young calves. Anim. Prod., 22: 154-155.

Cottyn, B.G. and Boucque, C.V. (1968) Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. J. Agric. Food Chem., 16: 105-107.

Cowan, R.T., Read, G.W., Greenhalgh, J.F.D. and Tait, C.A.G. (1981) Effect of feeding level in late pregnancy and dietary protein concentration during early lactation on food intake, milk yield, liveweight changes and nitrogen balance of cows. J. Dairy Res., 48: 201-212.

Crabtree, J.R. and Williams, G.L. (1971) The voluntary intake and utilisation of roughage-concentrate diets by sheep. 2. Barley and soya bean meal supplementation of hay diets. Anim. Prod., 13: 83-92.

Cressman, S.G., Grieve, D.G., Macleod, G.K. and Young, L.G. (1977) Influence of dietary protein concentration on milk production by dairy cattle during early lactation. J. Dairy Sci., 60 (Suppl. 1): 68.

Culpin, S. (1962) The feeding value of silage and barn-dried hay. J. Br. Grassl. Soc., 17: 138-140.

Curran, M.K. and Holmes, W. (1970) Prediction of the voluntary intake of food by dairy cows. 2. Lactating grazing cows. Anim. Prod., 12: 213-224.

Curran, M.K., Wimble, R.H. and Holmes, W. (1970) Prediction of the voluntary intake of food by dairy cows. 1. Stall-fed cows in late pregnancy and early lactation. Anim. Prod., 12: 195-212.

Davey, R.J. and Wellington, G.H. (1959) Studies on the use of hormones in lamb feeding. 1. Production performance. J. Anim. Sci., 18: 64-84.

Davies, H.L. (1962) Intake studies in sheep involving high fluid intake. Proc. Austr. Soc. Anim. Prod., 4: 167-171.

Demarquilly, C. (1973) Chemical composition, fermentation characteristics, digestibility and voluntary intake of forage silages. Changes compared to the initial green forage. Annales de Zootechnie, 22: 1-35.

Deswysen, A., Vanbelle, M. and Focant, M. (1978) The effect of silage choplength on the voluntary intake and rumination behaviour of sheep. J. Br. Grassl. Soc., 33: 107-115.

Dewar, W.A. and McDonald, P. (1961) Determination of dry matter in silage by distillation with toluene. J. Sci. Food Agric., 12: 790-795.

Dinusson, W.E., Andrews, F.N., Beeson, W.M. (1950) The effects of stilbetrol, testosterone, thyroid alteration and spaying on the growth and fattening of beef heifers. J. Anim. Sci., 9: 321-330.

Dobson, A. and Phillipson, A.T. (1968) Absorption from the ruminant forestomach. In Handbook of Physiology. Alimentary Canal, 1968, Sect. 6, Vol. V, pp. 2761-2774. Am. Physiol. Soc.; Washington D.C.

Donefer, E., Lloyd, L.E. and Crampton, E.W. (1963) Effect of varying alfalfa : barley ratios on energy intake and volatile fatty acid production by sheep. J. Anim. Sci., 22: 425-428.

Dulphy, J.P., Bechet, G. and Thomson, E. (1975) Effect of physical structure and conservation quality of grass silages on their intake. Annales de Zootechnie, 24: 81-94.

Egan, A.R. (1965) The nutritional status and intake regulation in sheep. 4. The influence of protein supplements upon acetate and propionate tolerance of sheep fed on low quality chaffed oat hay. Aust. J. agric. Res., 16: 473-483.

El-Shazly, K., Dehority, B.A. and Johnson, R.R. (1961) Effect of starch on the digestion of cellulose in vitro and in vivo by rumen microorganisms. J. Anim. Sci., 20: 268-273.

Emery, R.S. and Brown, L.D. (1961) Effect of feeding sodium and potassium bicarbonates on milk fat, rumen pH and VFA produced in the rumen. J. Dairy Sci., 44: 1899-1902.

Ettala, Elsi and Lampila, M. (1978) Factors affecting voluntary silage intake by dairy cows. Annales Agriculturae Fenniae, 17: 163-174.

Farhan, S.M.A. and Thomas, P.C. (1978) The effect of partial neutralization of formic acid silages with sodium bicarbonate on their voluntary intake by cattle and sheep. J. Br. Grassl. Soc., 33: 151-158.

Ferguson, K.A. (1958) The influence of thyroxine on wool growth. Proc. N.Z. Soc. Anim. Prod., 18: 128-140.

Ferguson, K.A. (1975) In <u>Digestion and Metabolism in Ruminants</u>, pp.448-464. Ed. I.W. McDonald and A.C.I. Warner. University of New England Publishing Unit; Armidale, New South Wales.

Fisher, L.J. (1972) Response of lactating cows to the intravenous infusion of amino acids. <u>Can. J. Anim. Sci.</u>, 52: 377-384.

Fogerty, A.C. and Johnson, A.R. (1980) Influence of nutritional factors on the yield and content of milk fat: protected polyunsaturated fat in the diet. In <u>Factors Affecting the Yield and Contents of Milk Constitutents of Commercial Importance</u>, pp. 96-104. International Dairy Federation, Bulletin 125.

Forbes, J.M. (1970) The voluntary food intake of pregnant and lactating ruminants: a review. <u>Brit. Vet. J.</u>, 126: 1-11.

Forbes, J.M. (1977) Development of a model of voluntary food intake and energy balance in lactating cows. <u>Anim. Prod.</u>, 24: 203-214.

Freer, M. and Campling, R.C. (1963) Factors affecting the voluntary intake of food by cows. 5. The relationship between the voluntary intake of food, the amount of digesta in the reticulo-rumen and the rate of disappearance of digesta from the alimentary tract with diets of hay, dried grass or concentrates. <u>Br. J. Nutr.</u>, 17: 79-88.

Garstand, J.R., Thomas, C. and Gill, M. (1979) The effect of supplementation of grass silage with fishmeal on intake and performance by British friesian calves. <u>Anim. Prod.</u>, 28: 423 (Abstr.).

Gengler, W.R., Martz, F.A., Johnson, H.D., Krause, G.F. and Hahn, L. (1970) Effect of temperature on food and water intake and rumen fermentation. <u>J. Dairy Sci.</u>, 53: 434-437.

Gill, M. and Ulyatt, M.J. (1977) The effect of supplementation with protein, energy and L-methionine on the digestion of silage by sheep. J. agric. Sci., Camb., 89: 43-51.

Goering, H.K. and Van Soest, P.J. (1970) Forage fibre analysis. USDA Agriculture Handbook, No. 379: pp. 1-20.

Gordon, F.J. (1979) The effect of protein content of the supplement for dairy cows with access ad libitum to high digestibility, wilted grass silage. Anim. Prod., 28: 183-189.

Gordon, F.J. (1980) The effect of silage type on the performance of lactating cows and the response to high levels of protein in the supplement. Anim. Prod., 30: 29-37.

Greenhalgh, J.F.D. (1981) The contribution of legumes to the nutrition of ruminants. In Legumes and Fertilizers in Grassland Systems. Winter Meeting, Br. Grassl. Soc., Dec. 1981.

Greenwood, C.T. (1970) Starch and glycogen. In The Carbohydrate, Vol. IIB, pp. 471-513. Ed. W. Pigman and D. Horton. Academic Press; New York and London.

Grenet, E. (1970) Size and structure of plant particles in the omasum and faeces of cattle. Annales de Biologie Animal, Biochimie, Biophysique, 10: 643-657.

Grimbleby, F.H. (1956) The determination of lactose in milk. J. Dairy Res., 23: 229-237.

Hadjipieris, G. and Holmes, W. (1966) Studies on feed intake and feed utilization by sheep. 1. The voluntary feed intake of dry, pregnant and lactating ewes. J. Agric. Sci., 66: 217-223.

Halliwell, G. (1957) Cellulolysis by rumen microorganisms. J. Gen. Microbiol., 17: 153-165.

Harb, M.Y. and Campling, R.C. (1983) Effects of the amount of barley and time of access to grass silage on voluntary intake, eating behaviour and production of dairy cows. Grass & Forage Sci., 38: 115-119.

Harris, C.E. and Raymond, W.F. (1963) The effect of ensiling on crop digestibility. J. Br. Grassl. Soc., 18: 204-212.

Hemsley, J.A. and Moir, R.J. (1963) The influence of higher volatile fatty acid on the intake of urea-supplemented low quality cereal hay by sheep. Aust. J. agric. Res., 14: 509-517.

Hill, F.W. and Dansky, L.M. (1954) Studies of the energy requirements of chickens. I. Effect of dietary energy level on growth and feed consumption. Poultry Sci., 33: 112-119.

Huffman, C.F. (1959) Summer feeding of dairy cattle. A Review. J. Dairy Sci., 42: 1495-1551.

Huida, L. (1982) Gas chromatographic determination of water and ethanol in silage by internal standard method. J. Agric. Soc., Finland, 54: 137-143.

Hungate, R.E. (1966) The Rumen and its Microbes. Academic Press; New York.

Hutchinson, K.J. and Wilkins, R.J. (1971) The voluntary intake of silage by sheep. II. The effects of acetate on silage intake. J. Agric. Sci., 77: 539-543.

Hutton, J.B., Hughes, J.W., Newth, R.P. and Watanabe, K. (1964) The voluntary intake of the lactating dairy cow and its relation to digestion. Proc. N.Z. Soc. Anim. Prod., 24: 29-42.

Ittner, N.R., Kelley, C.F. and Guilbert, H.R. (1951) Water consumption of Hereford and Brahman cattle and the effect of cooled drinking water in a hot climate. J. Anim. Sci., 10: 742-751.

Jarret, I.G. (1948) J. Counc. Sci. Industr. Res. Aust., 2 I: 311.

Jones, G.M., Stephens, C. and Kensett, B. (1975) Utilisation of starea, urea or soyabean meal in complete rations for lactating dairy cows. J. Dairy Sci., 58: 689-695.

Kay, M. and Scott, L. (1984) Fishmeal supplementation of grass silage diets for growing stock. Anim. Prod., 38: 551 (Abstr.).

Kelly, N.C. and Thomas, P.C. (1975) The effect of intravenous infusions of methionine on the voluntary intake of grass silage by sheep. J. Br. Grassld. Soc., 30: 237-239.

Kirby, P.S. and Chalmers, A.J. (1982) A comparison of extracted soyabean and fishmeal as proten supplements for growing beef cattle given grass silage ad libitum. Anim. Prod., 34: 386 (Abstr.).

Kirby, P.S., Chalmers, A.J. and Hannam, D.A.R. (1983a) Fishmeal supplementation of grass silage diets for fattening British Friesian steers. Anim. Prod., 36: 538 (Abstr.).

Kirby, P.S., Chalmers, A.J. and Clark, W.A. (1983b) A comparison of formaldehyde-treated soya meal and two types of fishmeal as protein supplements for growing beef cattle given silage ad libitum. Anim. Prod., 36: 538 (Abstr.).

Kirton, A.H. and Barton, R.A. (1958) Liveweight loss and its components in Romney ewes subjected to L-thyroxine therapy and a low plane of nutrition. Part II. Effects on some non-carcass components of liveweight. J. Agric. Sci., 51: 282-288.

Kronfeld, D.S. (1969) Ketone body metabolism, its control, and its implications in pregnancy toxaemia, acetonaemia, and feeding standards. In Physiology of Digestion and Metabolism in the Ruminant, pp. 566-583. Ed. A.T. Phillipson. Oriel Press; Newcastle, England.

Laird, R., Leggate, A.T. and Castle, M.E. (1979) The effect of supplementary protein on the performance of dairy cows offered grass silage ad libitum. Anim. Prod., 29: 151-156.

Lambourne, L.J. (1964) Stimulation of wool growth by thyroxine implantation. II. Feed intake of grazing Merino wethers treated repeatedly with thyroxine. Aust. J. agric. Res., 15: 676-697.

Lancaster, R.J. and Wilson, R.K. (1975) Effect on intake of adding sodium bicarbonate to silage. N.Z. J. Exp. Agric., 3: 203-206.

Lyons, T., Caffrey, P.J. and O'Connell, W.J. (1970) The effect of energy, protein and vitamin supplementation on the performance and voluntary intake of barley straw by cattle. Anim. Prod., 12: 323-334.

MacRae, J.C. and Armstrong, D.G. (1968) Enzyme method for the determination of α-linked glucose polymers in biological materials. J. Sci. Food Agric., 19: 578-581.

Majdoub, A., Lane, G.T. and Aitchison, T.E. (1978) Milk production response to nitrogen solubility in dairy rations. J. Dairy Sci., 61: 59-65.

McCullough, T.A. (1969) A study of factors affecting the voluntary intake of food by cattle. Anim. Prod., 11: 145-153.

McDonald, P., Macpherson, H.T. and Watt, J.A. (1963) The effect of histamine on silage dry matter intake. J. Br. Grassl. Soc., 18: 230-232.

McLeod, D.S., Wilkins, R.J. and Raymond, W.F. (1970) The voluntary intake by sheep and cattle of silages differing in free-acid content. J. agric. Sci., Camb., 75: 311-319.

Mehrez, A.Z. and Orskov, E.R. (1977) A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. agric. Sci., Camb., 88: 645-650.

Ministry of Agriculture Food and Fisheries. (1980) Bulletin 433. The
    Nutrient Requirements of Ruminant Livestock. MAFF; London.

Montgomery, M.J. and Baumgardt, B.R. (1965) Regulation of food intake
    in ruminants. 1. Pelleted rations varying in energy
    concentration. J. Dairy Sci., 48: 569-574.

Moore, L.A., Thomas, J.W. and Sykes, J.F. (1960) The acceptability of
    grass legume silage by dairy cattle. Proc. 8th Intern. Grassl.
    Congr., Reading. pp. 701-704.

Moose, M.G., Ross, C.V. and Pfander, W.H. (1969) Nutritional and
    environmental relationships with lambs. J. Anim. Sci., 29:
    619-627.

Morgan, D.J. and L'Estrange, J.L. (1977) Voluntary feed intake and
    metabolism of sheep when lactic acid is administered in the feed
    or intraruminally. J. Br. Grassl. Soc., 32: 217-224.

Morris, J.G. (1958) Drought feeding studies with cattle and sheep.
    1. The use of native grass hay (bush hay) as the basal component
    of a drought fodder for cattle. Queensland J. Agric. Sci., 15:
    161-180.

Moseley, G. and Jones, J.R. (1979) Some factors associated with the
    difference in nutritive value of artificially dried red clover and
    perennial ryegrass for sheep. Br. J. Nutr., 42: 139-147.

Moseley, G. and Jones, J.R. (1984) The physical digestion of
    perennial ryegrass (Lolium perenne) and white clover (Trifolium
    repens) in the foregut of sheep. Br. J. Nutr., 52: 381-390.

Mould, F.L. and Ørskov, E.R. (1984) Manipulation of rumen fluid pH
    and its influence on cellulolysis in sacco, dry matter degradation
    and the rumen miroflora of sheep offered either hay or
    concentrate. Anim. Feed Sci. Technol., 10: 1-14.

Mould, F.L., Ørskov, E.R. and Mann, S.O. (1983) Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. Anim. Feed Sci. Technol., 10: 15–30.

Murdoch, F.R. and Hodgson, A.A. (1979) Response of high-producing dairy cows fed alfalfa hay and corn silage to supplemented protein and urea. J. Dairy Sci., 62: 1752–1757.

Murdoch, J.C. (1964) Some factors affecting the intake of roughage by sheep. J. Br. Grassl. Soc., 19: 316–320.

Murdoch, J.C. and Rook, J.A.F. (1963) A comparison of hay and silage for milk production. J. Dairy Res., 30: 391–397.

Nelson, B.D., Ellzey, H.D., Morgan, E.B. and Allen, M. (1968) Effects of feeding lactating dairy cows varying forage-to-concentrate ratios. J. Dairy Sci., 51: 1796–1800.

Neumark, H., Bondi, A. and Volcani, R. (1964) Amines, aldehydes and keto-acids in silages and their effect on food intake by ruminants. J. Sci. Food Agric., 15: 487–492.

Oldham, J.D. (1984) Protein-energy interrelationships in dairy cows. J. Dairy Sci., 67: 1090–1114.

Oldham, J.D. and Alderman, G. (1982) Recent advances in understanding protein-energy interrelationships in intermediary metabolism in ruminants. In Protein and Energy Supply for High Production of Milk and Meat, pp. 33–63. Published for the United Nations by Pergamon Press, Oxford.

Oltjen, R.R., Davis, R.E. and Hiner, R.L. (1965) Factors affecting performance and carcass characteristics of cattle fed all-concentrate rations. J. Anim. Sci., 24: 192–197.

Ørskov, E.R. (1980) Recent advances in knowledge relating to the new concepts of protein evaluation for ruminants. In Proc. 4th Study Conf., Scottish Agricultural Colleges, 16-27 December 1980, pp. 45-53. Scottish Agricultural Colleges; Aberdeen.

Ørskov, E.R. (1982) Protein Nutrition in Ruminants. Academic Press; London.

Ørskov, E.R. and Fraser, C. (1975) The effects of processing of barley-based supplements on rumen pH, rate of digestion and voluntary intake of dried grass in sheep. Br. J. Nutr., 34: 493-500.

Ørskov, E.R., Soliman, H.S. and Macdearmid, A. (1978) Intake of hay by cattle given supplements of barley subjected to various forms of physical treatment or treatment with alkali. J. agric. Sci., Camb., 90: 611-615.

Osbourn, D.F., Cammell, S.B., Terry, R.A. and Outen, G.E. (1975) Forage composition and the conduct of digestion trials for the comparative evaluation of laboratory procedures. J. Br. Grassl. Soc., 30: 101-105.

Østergaard, V. (1979) Strategies for concentrate feeding to attain optimum feeding level in high yielding dairy cows. Beretning fra statens Husdyrbrugs forsog 482, National Institute of Animal Science; Copenhagen.

Owen, J.B., Davies, D.A.R., Miller, E.L. and Ridgman, W.J. (1967) The intensive rearing of lambs. 2. Voluntary food intake and performance on diets of varying oat husk and beef tallow content. Anim. Prod., 9: 509-520.

Owen, J.B., Davies, D.A.R. and Ridgman, W.J. (1969) The control of voluntary food intake in ruminant. Anim. Prod., 11: 511-520.

Owen, J.B. and Ridgman, W.J. (1968) Further studies of the effect of dietary energy content on the voluntary intake of pigs. Anim. Prod., 10: 85-91.

Palmquist, D.L. and Jenkins, T.C. (1980) Fat in lactation rations: Review. J. Dairy Sci., 63: 1-14.

Palmquist, D.L. and Moser, E.A. (1981) Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. J. Dairy Sci. 64: 1664-1670.

Parrott, C., Loughead, H., Hale, W.H. and Theurer, B. (1968) Effect of concentrate level on digestible energy intake with steers. J. Anim. Sci., 27: 1173-1174.

Peterson, A.D. and Baumgardt, B.R. (1971) Food and energy intake of rats fed diets varying in energy concentration and density. J. Nutr., 101: 1069-1974.

Pettyjohn, J.D., Everett, J.P. and Mochrie, R.D. (1963) Responses of dairy calves to milk replacer fed at various concentrations. J. Dairy Sci., 46: 710-714.

Polan, C.E., Miller, C.N. and McGilliard, M.L. (1976) Variable dietary protein and urea for intake and producton in Holstein cows. J. Dairy Sci., 59: 1910-1914.

Poos, M.I., Bull, L.S. and Hemken, R.W. (1979) Supplementation of diets with positive and negative urea fermentation potential using urea or soyabean meal. J. Anim. Sci., 49: 1417-1426.

Purser, D.B. and Moir, R.J. (1966) Rumen volume as a factor involved in individual sheep differences. J. Anim. Sci., 25: 509-515.

Ragsdale, A.C., Thompson, H.J., Worstell, D.M. and Brody, S. (1950) Environmental physiology with special reference to domestic animals. 9. Milk production and feed and water consumption responses of Brahman, Jersey and Holstein cows to changes in temperature, 50° to 105°F and 50° to 8°F. Missouri Univ. Agr. Exptl. Sta. Res. Bull. 460.

Reid, C.W.S., Ulyatt, M.J. and Munro, J.A. (1977) The physical breakdown of feed during digestion in the rumen. Proc. N.Z. Soc. Anim. Prod., 37: 173-175.

Reik, R.F., Hardy, M.H., Lee, D.H.K. and Carter, H.B. (1950) The effect of the dietary plane upon the reactions of two breeds of sheep during short exposures to hot environments. Aust. J. Agric. Res., 1: 217-230.

Rogers, G.L., Bryant, A.M. and McLeay, L.M. (1979) Silage and dairy cow production. III. Abomasal infusions of casein, methionine and glucose, and milk yield and composition. N.Z. J. Agric. Res., 22: 533-541.

Ronning, M. and Laben, R.C. (1966) Response of lactating cows to free-choice feeding of milled diets containing from 10 to 100% concentrates. J. Dairy Sci., 49: 1080-1085.

Rook, J.A.F. (1976) Nutrition influences on milk quality. In Principles of Cattle Production, pp. 221-236. Ed. H. Swan and W.H. Broster. Butterworths; London.

Rook, J.A.F. (1983) Nutritional imbalances. In Nutritional Physiology of Farm Animals, pp. 369-414. Ed. J.A.F. Rook and P.C. Thomas. Longman; London and New York.

Rook, J.A.F. and Balch, C.C. (1961) The effects of intraruminal infusions of acetic, propionic and butyric acids on the yield and composition of the milk of the cow. Br. J. Nutr., 15: 361-369.

Rook, J.A.F., Balch, C.C. and Johnson, V.W. (1965) Further observations on the effects of intraruminal infusions of volatile fatty acids and of lactic acid on the yield and composition of the milk of the cow. Br. J. Nutr., 19: 93-99.

Rooke, J.A., Brookes, I.M. and Armstrong, D.G. (1983) The digestion of untreated and formaldehyde-treated soya-bean and rapeseed meals by cattle fed a basal silage diet. J. agric. Sci., Camb., 100: 329-342.

Rooke, J.A., Norton, B.W. and Armstrong, D.G. (1982) The digestion of untreated and formaldehyde-treated soya-bean meals and estimation of their rumen degradabilities by different methods. J. agric. Sci., Camb., 99: 441-452.

Schwab, C.G., Satter, L.D. and Clay, A.B. (1976) Response of lactating cows to abomasal infusion of amino acids. J. Dairy Sci. 59: 1254-1270.

Shamoon, S.A. (1984) Amino acid supplements for ruminant farm livestock with special reference to methionine. PhD Thesis, University of Glasgow; Scotland.

Smith, E. Jill and Clapperton, J.L. (1981) The voluntary food intake of sheep when silage juice is infused into the rumen. Proc. Nutr. Soc., 40: 22A (Abstr.).

Snedecor, G.W. (1956) Statistical Methods. Iowa State University Press; Iowa.

Somogyi, M. (1945) A new reagent for the determination of sugars. J. Biol. Chem., 160: 61-68.

Sriskandarajah, N., Ashwood, A. and Kellaway, R.C. (1980) Effects of rolling and alkali treatment of barley grain supplements on forage intake and utilization by steers and lactating cows. J. agric. Sci., Camb., 95: 555-562.

Stewart, C.S. (1977) Factors affecting the cellulolytic activity of rumen contents. Appl. Environ. Microbiol., 33: 497-502.

Stiles, D.A., Bartley, E.E., Kilgore, G.L., Boren, F.W., Ott, R.L. and Clark, J.G. (1968) Comparative value of alfalfa pasture, bromegrass pasture, or alfalfa hay for lactating dairy cows. J. Dairy Sci., 51: 1620-1624.

Storry, J.E. and Brumby, P.E. (1980) Influence of nutritional factors on the yield and content of milk fat: protected non-polyunsaturated fat in the diet. In Factors Affecting the Yields and Contents of Milk Constituents of Commercial Importance, pp. 105-125. International Dairy Federation, Bulletin 125.

Sutton, J.D. (1984) Feeding and milk fat production. In Milk Compositional Quality and its Importance in Future Markets, pp. 43-52. Ed. M.E. Castle and R.G. Gunn. Occ. Publ. Br. Soc. Anim. Prod., No. 9.

Sutton, J.D., Oldham, J.D. and Hart, I.C. (1980) Products of digestion, hormones and energy utilization in milking cows given concentrates containing varying proportions of barley or maize. In Energy Metabolism, pp. 303-306. Ed. L.E. Mount. Butterworths; London.

Tayler, J.C. and Wilkins, R.J. (1976) Conserved forage - complement or competitor to concentrates. In Principles of Cattle Production, pp. 343-364. Ed. H. Swan and W.H. Broster. Butterworths; London.

Terry, R.A., Tilley, J.M.A. and Outen, G.E. (1969) Effect of pH on cellulose digestion under in vitro conditions. J. Sci. Food Agric., 20: 317-320.

Theurer, C.B. (1970) Role of feed intake in altering performance of finishing beef cattle. In Fourth Annual Beef-Swine Nutrition Seminar, Sept. 29, 1970. Kansas City, Missouri.

Thomas, C., Aston, K., Daley, S.R. and Hughes, P.M. (1982) A comparison of red clover with grass silage for milk production. Anim. Prod., 34: 361.

Thomas, C., Aston, K., Tayler, J.C., Daley, S.R. and Osbourn, D.F. (1981) Milk production from silage. 1. The influence of an additive containing formaldehyde and formic acid on the response of lactating heifers and cows to supplementary protein. Anim. Prod., 32: 285-295.

Thomas, C., Gill, M. and Austin, A.R. (1980) The effect of supplements of fishmeal and lactic acid on voluntary intake of silage by calves. Grass & Forage Sci., 35: 275-279.

Thomas, C. and Thomas, P.C. (1985) Factors affecting the nutritive value of grass silage. In Recent Advances in Animal Nutrition, pp. 223-256. Ed. W. Haresign and D.J.A. Cole. Butterworths; London.

Thomas, C. and Wilkinson, J.M. (1975) The utilization of maize silage for intensive beef production. 3. Nitrogen and acidity as factors affecting the nutritive value of ensiled maize. J. agric. Sci., Camb., 85: 255-261.

Thomas, J.W., Moore, L.A., Okamoto, M. and Sykes, J.F. (1961) A study of factors affecting rate of intake of heifers fed silage. J. Dairy Sci., 44: 1471-1483.

Thomas, P.C. (1983) Milk protein. Proc. Nutr. Soc. 42: 407-418.

Thomas, P.C. and Chamberlain, D.G. (1982) Silage as a food-stuff. In Silage for Milk Production, pp. 63-102. Ed. J.A.F. Rook and P.C. Thomas. Technical Bulletin No. 2. National Institute for Research in Dairying; Reading.

Thomas, P.C. and Chamberlain, D.G. (1984) Manipulation of milk composition to meet market needs. In Recent Advances in Animal Nutrition, pp. 219-243. Ed. W. Haresign and D.J.A. Cole. Butterworths; London.

Thomas, P.C., Kelly, N.C., Chamberlain, D.G. and Chalmers, J.S. (1980) Some aspects of energy and protein utilization in ruminants given silage diets. In Energy Metabolism, pp. 357-361. Ed. L.E. Mount. Butterworths; London.

Thomas, P.C., Kelly, N.C. and Wait, M.K. (1976b) The effect of physical form of a silage on its voluntary consumption and digestibility by sheep. J. Br. Grassl. Soc., 31: 19-22.

Thompson, H.J., Worstell, D.M. and Brody, S. (1949) Environmental physiology with special reference to domestic animals. 5. Influence of temperature 50° to 105°F, on water consumption in dairy cattle. Missouri Univ. Agric. Exptl. Sta. Res. Bull. 436.

Thomson, D.J., Beever, D.E., Haines, M.J., Cammell, S.B., Evans, R.T., Dhanoa, M.S. and Austin, A.R. (1985) Yield and composition of milk from Friesian cows grazing either perennial ryegrass or white clover in early lactation. J. Dairy Res., 52: 17-31.

Trigg, T.E., Parr, C.R., Day, A.M. and Parsons, B.W. (1982) Effect of milk production and energy metabolism of abomasal infusions of protein to lactating, pasture-fed dairy cows. In Energy Metabolism of Farm Animals, pp. 42-45. Ed. A. Ekern and F. Sundstøl. Agricultural University of Norway; Aas, Norway.

Troelson, J.E. (1967) Systems for estimating the nutritional quality of hay for domestic ruminants. PhD Thesis. University of Saskatchewan;

Troelson, J.E. and Bigsby, F.W. (1964) Artificial mastication - a new approach for predicting voluntary forage consumption by ruminants. J. Anim. Sci., 23: 1139-1142.

Troelson, J.E. and Campbell, J.B. (1968) Voluntary consumption of forage by sheep and its relation to the size and shape of particles in the digestive tract. Anim. Prod., 10: 289-296.

Tulloh, N.M. (1966) Physical studies of the alimentary tract of grazing cattle. IV. Dimensions of the tract in lactating and non-lactating cows. N.Z. J. Agric. Res., 9: 999-1008.

Twigge, J.R. and Van Gils, L.G.M. (1984) Practical aspects of feeding protein to dairy cows. In Recent Advances in Animal Nutrition, pp. 201-217. Ed. W. Haresign and D.J.A. Cole. Butterworths; London.

Tyrrell, H.F. and Reid, J.T. (1965) Prediction of the energy value of cow's milk. J. Dairy Sci., 48: 1215-1223.

Ulyatt, M.J. (1965) The effects of intraruminal infusions of volatile fatty acids on food intake of sheep. N.Z. J. Agric. Res., 8: 397-408.

Ulyatt, M.J., Blaxter, K.L. and Mcdonald, I. (1967) The relations between the apparent digestibility of roughages in the rumen and lower gut of sheep, the volume of fluid in the rumen and voluntary feed intake. Anim. Prod., 9: 463-470.

Van Dooren, P. (1972) Survey on the chemistry of the tanning of proteins by formaldehyde (Internal Report, Clunies Ross Laboratory). CSIRO Division of Animal Production; Sydney, Australia.

Van Horn, H.H., Zomata, C.A., Wilcox, C.J., Marshall, S.P. and Harris, B. (1979) Complete rations for dairy cattle. VIII. Effect of percent and source of protein on milk yield and ration digestibility. J. Dairy Sci., 62: 1086-1093.

Van Ramshorst, H. (1986) Personal communication.

Villavicencio, E., Rusoff, L.L., Girouard, R.E. and Waters, W.H. (1968) Comparison of complete feed rations to a conventional ration for lactating cows. J. Dairy Sci., 51: 1633-1638.

Virtanen, A.I. (1969) On nitrogen metabolism in milking cows. Fed. Proc., 28: 232-240.

Waldo, D.R., Miller, R.W., Okamoto, M. and Moore, A. (1965) Ruminant utilization of silage in relation to hay, pellets, and hay plus grain. I. Composition, digestion, nitrogen balance, intake and growth. J. Dairy Sci., 48: 910-916.

Waterhouse, A., Laird, R. and Holliday, R.J. (1983) A response to protein supplementation of grass silage for growing and finishing cattle. Anim. Prod., 36: 503 (Abstr.).

Wilkins, R.J., Hutchinson, K.J., Wilson, R.F. and Harris, C.E. (1971) The voluntary intake of silage by sheep. 1. Interrelationships between silage composition and intake. J. agric. Sci., Camb., 77: 531-537.

Wilkins, R.J., Fenlon, J.S., Cook, J.E. and Wilson, R.F. (1978) A further analysis of relationships between silage composition and voluntary intake by sheep. Proc. 5th Silage Conf., pp. 34-36.

Wilson, G.F. (1978) Effect of water content of Tama ryegrass on voluntary intake of sheep. N.Z. J. Exp. Agric., 6: 53-54.

Wohlt, J.E. and Clark, J.H. (1978) Nutritional value of urea versus preformed protein for ruminants. 1. Lactation performance of dairy cows fed corn-based diet containing varying amounts of supplemented nitrogen from urea and/or soyabean meal. J. Dairy Sci., 61: 902-915.

Wong, D.I.J., Thomas, P.C. and Chamberlain, D.G. (1983) Unpublished observations.