UNIVERSITY OF GLASGOW

THE EFFECTS OF FEEDING MOLASSES ON RUMEN FERMENTATION, INTAKE AND MILK PRODUCTION

by

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ABSTRACT

The feeding of molasses to dairy cows was reviewed with emphasis on the effects on rumen fermentation, food intake and milk production. Three diary cow experiments and one fistulated wether sheep trial were conducted.

Experiment 1 investigated the maximum feeding of molaferm 20 to mid-lactating dairy cows in a 3 x 3 Latin Square. The three complete diets each contained molaferm 20 at 156, 312 and 468 g/kg DM, respectively. Some cows suffered some scouring when they were fed 468 g/kg DM of molaferm 20, but recovered when they were fed lower levels. Blood concentrations of protein, urea, ß-hydroxybutyrate, Mg and K all were within normal range. Feed intake was increased with each increment of dietary molaferm 20 levels ($p<0.01$). Milk yield in the medium molaferm 20 treatment was higher than in the low treatment ($p<0.01$), but was similar between the high and medium treatments. Milk fat concentration was independent of diets, whereas protein was significantly higher with increment of dietary molaferm 20 levels.

Experiment 2 determined the effects of dietary ERDP and DUP concentrations on the performance of early lactating dairy cows fed 310 g/kg DM of molaferm 20 in a 3 x 3 Latin Square. The three diets (L/L, H/L and H/H) each contained similar concentration of ME but differing levels in ERDP and DUP (93/17, 117/17 and 121/32 (g/kg DM)). No clinical symptoms of the ill health of the cattle were found during the experiment. Treatments showed little effects on blood concentrations of protein, urea, ß-hydroxybutyrate, Mg and K. Feed intake was significantly increased with each increment of CP levels. Milk yield was higher as ERDP increased ($p<0.05$) and further higher as DUP increased ($p<0.01$). Milk fat concentrations was similar between treatments, whereas protein was higher as ERDP increased ($P<0.05$).

Experiment 3 determined the responses of lactating dairy heifers to decreases in dietary FME concentrations produced by addition of unprotected tallow in a 3 x 3 Latin Square. Three complete diets (H_{FME}, M_{FME} and L_{FME}) each contained 310 g/kg DM of molaferm 20 and similar levels of ERDP, DUP and ME but differing in levels of FME (9.4, 8.9 and 8.4 MJ/kg DM). No clinical symptoms of the ill health of the cattle were found during the experiment. Treatments showed little effects on blood concentrations of protein, urea, ß-hydroxybutyrate, Mg and K. Feed intake was slightly lower with decreases in dietary FME concentrations achieved by addition the unprotected tallow. Milk yield in the H_{FME} treatment was significantly lower than in the M_{FME} or L_{FME} treatment ($p<0.01$), while milk concentrations of protein and fat were significantly higher in the H_{FME} ($p<0.05$). Milk concentration of uric acid was lower as decreasing dietary FME, but yield was similar between treatments.

Experiment 4 investigated the effects of dietary ERDP and DUP concentrations on the rumen fermentation of ruminal fistulated wether sheep in a 4 x 4 Latin Square design. Four complete diets (C, CU, CS and CSF) each contained 310 g/kg DM of molaferm 20 and similar ME and FME, but differing in levels of ERDP/DUP (84/17, 109/17, 116/38 and 119/54 g/kg DM). Whole tract digestibilities of DM and OM was similar between treatments, while NDF and hay degradability in the rumen were significantly higher in sheep fed the diets CS and CSF than those fed the diets C and CU. Average ammonia-N in the rumen was significantly higher with each increment of dietary protein levels. However, PD-N output in urine did not respond to the ammonia concentrations. Microbial N supply was similar between the 4 treatments. Total volume of VFAs in the rumen was independent of the diets. Molar percentages of propionic and butyric acids were lower, while acetic acid was higher as ERDP levels increased, but not as DUP increased.

The effects on the microbial protein synthesis and the efficiencies of utilization of ME intake and MP supply were discussed and areas of further research suggested.

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ABBREVIATIONS

ADF	Acid detergent fibre
AIA	Acid insoluble ash
ATP	Adenosine triphosphate
Ca	Calcium
CCMS	Condensed citrus molasses solubles
CF	Crude fibre
CMDS	Citrus molasses distillers solubles
CMS	Condensed molasses solubles
CoEDTA	cobalt ethylenediaminetetra-acetic acid
CP	Crude protein
CSH	Cottonseed hulls
d	day
DE	Digestible energy
dg	Extent of degradation of feed nitrogen
DM	Dry matter
DMI	Dry matter intaken
DOM	Digestible organic matter
DHCE	dry hemicellulose extract
DUP	Digestible undegraded protein
ERDN	Effective rumen degraded dietary nitrogen
ERDP	Effective rumen degraded dietary protein
$ERDP_r$	ERDP requirement
$ERDP_s$	ERDP supply
FME	Fermentable metabolizable energy
FW	Fresh weight
g	gramme
GE	Gross energy
h	hour
HCE	Hemicellulose extract

K	potassium
Kg	kilogramme
k_g	The efficiency of utilization of ME for liveweight gain
k_l	The efficiency of utilization of ME for lactation
k_m	The efficiency of utilization of ME for maintenance
k_n	The efficiency of utilization of MP supply
k_{ng}	The efficiency of utilization of MP supply for liveweight gain
k_{nl}	The efficiency of utilization of MP supply for lactation
k_{nw}	The efficiency of utilization of MP supply for wool growth
l	litre
LHCE	Liquid hemicellulose extract
m	metre
M	Molarity
MCP	Microbial crude protein supply
ME	Metabolizable energy
MEI	ME intake
ME_m	ME for maintenance
Mg	Magnesium
mg	milligramme
MJ	Megajoule
ml	millilitre
mm	Milli-Molarity
MP	Metabolisable protein
MP_g	MP supply for liveweight gain
MP_l	MP supply for lacation
MP_m	MP supply for maintenance
MP_p	MP supply for pregnacy
MP_s	MP supply
MP_w	MP supply for wool gain
MSBP	Molassed sugar beet pulp
N	nitrogen

Na	Sodium
NAN	Non-ammonia-nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NE_{milk}	NE retained in milk
NFE	Nitrogen-free-extract
nm	Nanometre
NPN	non-protein-nitrogen
OM	Organic matter
OMI	Organic matter intake
P	phosphorus
PD	Purine derivatives
r	Correlation coefficient
RDP	Rumen degraded dietary protein
SBP	Sugar beet pulp
s.e.	Standard error
s.e.d.	Standard error of difference
SNF	Solid-not-fat
UDP	Undegraded protein
UMSB	Unmolassed sugar beet pulp
VFAs	volatil fatty acids
WSC	Water soluble carbohydrates
Y_p	milk protein production

CHAPTER 1

Literature Review

1.1 *Introduction*

Molasses is a by-product of both the cane and beet sugar industry and as such its availability is governed by sugar production. Although there are molasses produced by other industries, for example from the citrus fruit and the glucose industry, these products are nowhere near as important as the molasses from the cane and beet industry. Sugar, in particular sucrose , is the major component of molasses and in fact, molasses may be considered as a solution of sugar, plus some glucose and fructose, other organic matter and inorganic material in water.

Molasses is largely produced worldwide each year. In 1982-83, the production by regions was as following (million tonnes), 3.38 in North America, 1.84 in Caribbean, 0.82 in Central America, 7.86 in South America, 3.59 in European Community, 0.72 in other Western Europe, 2.02 in Eastern Europe, 2.67 in USSR. 2.11 in Africa, 0.60 in Middle East, 7.93 in Other Asia and 0.86 in Oceanic (Curtin, 1982). At the present time about 117 million tonnes of sugar are produced, of which about 40% comes from beet and the remainder from cane. This converts to a total world production of about 27 million tonnes of cane molasses and 13 million tonnes of beet molasses. Of this cane molasses, some 21 million tonnes is consumed in the country of origin and 6 million tonnes is exported from 45 producing countries to Europe, the Far East and North America. For beet molasses, most is consumed locally and only about 750,000 tonnes is traded internationally. The EC takes the lion's share of cane molasses at about 3.2 million tonnes, with the UK and the Netherlands the largest consumers of about 0.7 million tonnes each.

The largest consuming industry for molasses is the feed industry and about 60% or more of cane molasses traded is used for animal feeding all over the world. In USA, molasses used for mixed feeds and direct feeding accounted for 81% of total molasses supply (Anonymous 1982). Typically, within the EC user countries, the majority of total molasses supply also goes to the animal feed compounder sector, plus direct consumption on farm. The highest proportion of molasses used in feed industry as total

molasses supply is 95% in Denmark and then 75% in UK, whereas in the EC in general, usage of molasses fell to 58% (Baker, 1979).

Molasses has been fed to cattle for more than a century. One of the earliest documented reports in North America showing the value of cane molasses in cattle feeding was published by Gulley and Carson (1890) (cited by Curtin, 1982). A pioneer work of feeding molasses to lactating cows was reported by Lindsey *et al.* (1907) in the beginning of this century (cited by Curtin, 1982). Since then, extensive research work and feeding practice have demonstrated that molasses is an intake stimulant, binder and dust settler, and an effective carbohydrate source. Therefore molasses is widely used as an energy supplement for animals, especially for ruminant animals in the world.

Molasses is fed to cattle in many ways including topping molasses on forage, replacing forage in complete diets, substituting cereal grains in complete diets and supplementing for grazing cattle. Molasses is also used to mix with sugar beet pulp (SBP) and then molassed SBP (MSBP) is fed to cattle. In most of experiments with dairy cows, however, nutritional value of molasses is investigated by using molasses to replace cereal grains in complete diets. Many studies have shown an equal or greater animal performance when the substitution rates of molasses for cereal grains was up to 100 g/kg DM of total diets for dairy cows (Morales *et al.*, 1989). When molasses is fed at levels varying from 40 to 100 g/kg DM of a concentrate its energy value has been reported to be worth from 75 to 100% the values of maize (Scott, 1953) and to be superior to maize when fed up to 60 g/kg DM of diets (Wing *et al.*, 1988). However, the high sugar concentration of molasses can change the composition of rumen microorganisms and alter the proportion of volatile fatty acids (VFAs) produced in the rumen, resulting in a lower fibre digestion. A very high molasses inclusion in a diet could therefore be detrimental to animal responses (Morales *et al.*, 1989) and furthermore to animal health including loose faeces, ketosis, molasses toxicity.

A number of early studies were summarised in reviews of the feeding of molasses to

dairy cattle by Scott (1953) and recently by Harris and Van Horn (1982). Since 1982, a large quantity of information has been published. In those studies, cane and beet molasses were main types of molasses to have been investigated. The research experiments have been focused on the effects of feeding molasses on rumen fermentation and responses of dairy cows in feed intake and milk production to the feeding of molasses.

1.2 *Molasses types and their nutrient concentrations*

The term molasses initially referred to the final effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane and from sugar beet. Molasses is now generally used to describe any liquid feed ingredient which contains in excess of 43% sugars (Curtin, 1982). Today several types of molasses are recognized, these are cane, beet, citrus, starch molasses and hemicellulose extract (wood molasses).

Cane Molasses Cane molasses is a by-product of the manufacture or refining of sucrose from sugar cane. It usually contains 737 g/kg (700 - 753) of dry matter (DM) and 657 g/kg DM (625 - 714) of water soluble carbohydrates (Standing Committee on Tables of Feed Composition, 1990). Cane molasses is produced in many tropical or subtropical areas where conditions are suitable for sugar cane growth and has been used and investigated extensively in animal feeding, especially in ruminant animals (Morales, *et al.*, 1989).

Beet Molasses Beet molasses is a by-product of the manufacture of sucrose from sugar beet. It usually contains 763 g /kg (753 - 773) of dry matter and 633 g/kg DM (603 - 653) of sugar (Standing Committee on Tables of Feed Composition, 1990). It is produced in temperate areas where conditions are suitable for sugar beet growth and its production is less than cane molasses. Beet molasses has not been as extensively researched (Garrett, *et al.*, 1989) as cane molasses.

Cane and beet molasses are major molasses types used in animal nutrition. In the USA, both together account for over 90% of total supply (Anonymous, 1981). There are other molasses types available in animal nutrition, but their usage is normally localized near the areas of production (Curtin, 1982). The Association of American Feed Control Officials (AAFCO, 1982) describes them as following (from Curtin, 1982).

Citrus Molasses Citrus molasses is the partially dehydrated juices obtained from the manufacture of dried citrus pulp. It must contain not less than 450 g/kg DM of total sugar expressed as invert and its density determined by double dilution must not be less than 71.0 *Brix* (*Brix* is an American term commonly used as an indicator of specific gravity and an approximate representation of total solids).

Hemicellulose Extract Hemicellulose extract (HCE) is a by-product of the manufacture of pressed wood. It is the concentrated soluble material obtained from the treatment of wood at elevated temperature and pressure without use of acids, alkalis, or salts. It contains pentose and hexose sugars, and has a total carbohydrate concentration of not less than 550 g/kg DM.

Starch Molasses Starch molasses is a by-product of the manufacture of dextrose from starch derived from maize or grain sorghum in which the starch is hydrolysed by use of enzymes and/or acid. It must contain not less than 430 g/kg DM of reducing sugars and not less than 500 g/kg DM of total sugars expressed as dextrose. It should contain not less than 730 g/kg of total solids.

The average nutrient concentrations of several types of molasses are presented in Table 1.1. All types have a very high viscosity and contain a high level of sugar but a very low level of crude protein (CP), except sugar beet molasses which has a higher level of CP than the other types. Sucrose consists of a major part of sugars in molasses and other sugars are glucose, fructose and raffinose. The composition of molasses produced in different countries, however, varies because it is influenced by many factors, such

as soil type, ambient temperature, moisture, season of production, variety, production practices at a particular processing plant, and storage variables (Curtin, 1982). Table 1.2 shows some differences between beet molasses and cane molasses in official data of 5 Western European countries.

Cane molasses contains very low level of crude protein (55 g/kg DM). In order to increase its CP concentration, it is mixed with a liquid feed containing a high level of CP. The liquid feed is condensed molasses solubles.

Condensed Molasses Solubles Molasses is widely used in fermentation industries as well for making biochemicals and its evaporated effluents known as condensed molasses solubles (CMS) are available for animals. Since the major part of the carbohydrates from molasses has been fermented, concentrations of protein and ash are relatively increased in CMS (Den Hartog, *et al.*, 1987). In comparison with beet molasses, beet CMS contains a higher level of crude protein (350 g/kg DM) but a relatively lower ME concentration (8.7 MJ/kg DM) and a higher level of minerals (380 g/kg DM). Together with its low dry matter concentration (600 -700 g/kg), beet CMS therefore has a quite low viscosity. The corresponding values of cane CMS are crude protein at 107 g/kg DM, ME 11.7 MJ/kg DM, minerals 163 g/kg DM and DM 600 g/kg. In Europe, the great majority of CMS is based upon beet molasses and there is far less CMS derived from cane molasses based fermentation available. When mixed with any particular cane molasses, beet CMS contributes to easier handling and a better ratio in concentrations between CP and ME of products due to its low viscosity and high CP concentration. In *United Molasses* company of Britain, a series of CMS products have been developed, such as Molaferm 20, 30 and 50 (cane molasses is mixed with 20, 30 or 50% of beet CMS, respectively). The nutritive value of Molaferm 20 is included in Table 1.1.

Table 1.1 *Nutrient concentrations of molasses (g/kg DM)*

	Cane[+]	Beet[+]	Molaferm 20[#]	Citrus[*]	Extract[*]	Starch[*]
DM (g/kg)	737.2	763.2	715.0	650.0	650.0	730.0
GE (MJ/kg DM)	15.2	15.3	--	--	--	--
ME (MJ/kg DM)	--	--	12.2	--	--	--
CP	55.3	136.1	100.6	40.0	5.0	4.0
Sugar	--	631.7	558.0	450.0	550.0	500.0
WSC	656.9	--	--	--	--	--
Ash	99.9	110.6	162.2	60.0	50.0	60.0
Calcium	9.6	1.2	11.2	13.0	8.0	1.0
Phosphorus	1.2	0.4	2.8	1.5	0.5	2.0
Magnesium	4.4	0.1	--	--	--	--
Sodium	1.2	25.0	--	3.0	--	25.0
Potassium	38.6	49.1	55.9	1.0	0.4	0.2

[+] from Standing Committee on Tables of Feed Composition, 1990.

[#] form J. D. Higginbotham, personal communication.

[*] from Curtin, 1982.

Table 1.2 *Some tabulated figures concerning beet and cane molasses*

	U.K. (ADAS,1975)	France (INRA, 1978)	Denmark (Andersen *et al.*, 1979)	Holland (CVB, 1977)	Germany (DLG, 1982)
	Beet molasses/cane molasses				
DM (g/kg)	750/750	775/739	770/700	764/738	770/737
CP (g/kg DM)	47/41	103/56	137/65	140/41	131/50
Ash (g/kg DM)	69/87	116/123	99/117	110/102	108/97
Digestibility (g/kg)					
OM	870/880	890/910	940/870	900/830	860/840
CP	340/350	600/600	710/470	650/---	580/350
NFE	900/900	930/930	980/900	950/870	910/870

from Steg and Van Der Meer (1985).

1.3 *Effect of molasses on rumen fermentation*

During rumen fermentation, short-chain fatty acids and microbial cells are formed from the degradation of feedstuffs and these products serve as sources of energy and protein (Harris and Van Horn, 1982). The addition of carbohydrate sources such as molasses to the rations produces a marked transient appearance of lactic acid (Elsden and Phillipson, 1948). The appearance of lactic acid is associated with a lower rumen pH and a shift in the population of certain bacteria (Harris and Van Horn, 1982), and furthermore affect rumen metabolism.

1.3.1 *Volatile fatty acids and pH*

Molasses and other liquid feeds with a high molasses concentration usually contain a high proportion of soluble carbohydrates (Table 1.1). An increase in soluble carbohydrates in diets has been shown to cause a shift in proportion of volatile fatty acids (VFAs) produced in the rumen (Wing *et al.*, 1988). Because sucrose is a major constituent of sugars in molasses, some studies are discussed in which pure sucrose is used. After intraruminal administration of sucrose, Waldo and Schultz (1960) found that dosages of sucrose resulted in a lower level of acetic acid and a higher level of butyric acid than would normally be observed in the rumen fluid if cattle had been fed the forage used without sucrose. Similar results were obtained by Sutton (1968 and 1969) with glucose and fructose but not with xylose and arabinose as infused sugars. When 60 g/kg DM of sucrose was added to diets with 400 or 600 g/kg DM of grain for dairy cows, Owen *et al.* (1967, Table 1.3) also noted a lower proportion of acetic and a higher proportion of butyric acid in both diets containing respectively 400 and 600 g/kg DM of grains. A further investigation by Kellogg and Owen (1969, Table 3) revealed that increasing sucrose levels from 0 to 90 g/kg DM in diets with 300 and 700 g/kg DM of grain caused a linear increase in the averaged molar proportion of butyric acid and a decrease in propionic acid, but did not show a significant effect on acetic acid in rumen fluid. In a subsequent trial in which sucrose replaced milo (a kind of sorghum) from 0 to 150 g/kg DM of total diets, Kellogg (1969, Table 1.3) reported that the pattern of molar proportion of propionic and butyric acids in the rumen of dairy cows

were similar to that of Kellogg and Owen (1969), but acetic acids increased at 50 and 100 g/kg DM of sucrose.

Table 1.3 *Effects of sucrose on the ruminal VFAs and pH in dairy cattle*

Reference and treatments (g/kg DM)	Molar proportion (%)			Rumen pH
	Acetic	Propionic	Butyric	
Owen *et el.* (1967) (sucrose replaced maize)				
With 40% grain				
control	56.8	25.2	18.0	6.94
60 sucrose	55.1	26.0	18.9	6.82
With 60% grain				
control	56.2	24.4	19.4	6.81
60 sucrose	52.1	27.4	20.5	6.66
Kellogg and Owen (1969) (sucrose replaced maize)				
control	52.0	24.0	16.0	--
30 sucrose	53.5	19.5	19.0	--
60 sucrose	52.0	22.0	19.0	--
90 sucrose	53.5	19.0	22.5	--
Kellogg (1969) (sucrose replaced milo^{+})				
control	51.9	31.7	9.9	6.32
50 sucrose	54.7	30.3	9.5	5.99
100 sucrose	54.7	28.2	10.4	6.10
150 sucrose	53.5	26.2	13.0	6.30

$^{+}$ a kind of sorghum

Molasses inclusion in the diet of dairy cattle causes a similar changes in the pattern of ruminal VFAs as sucrose (Table 1.4). Martin and Wing (1966) reported that additions of molasses from 0 to 126 g/kg DM of total diets slightly increased the molar proportion of butyric and acetic acids and decreased propionic acid in the rumen of dairy steers. In a trial in which citrus molasses distillers solubles (CMDS) replaced maize from 0 to 180 g/kg DM of total diets for dairy cows, Wing *et al.* (1988) observed a linear increase in ruminal acetic acid proportion and a curvilinear decrease in ruminal propionic acid proportion. Change in butyric acid was small with 0, 60 and 120 g/kg DM of CMDS, but it increased dramatically with 180 g/kg DM. In comparison between molassed sugar beet pulp (MSBP) and unmolassed sugar beet pulp (UMSBP) as a major ingredient of concentrates, Beever *et al.* (1988) reported that the VFA concentrations were higher in the rumen of the animals fed the MSBP diet than those fed the UMSBP diet when each concentrate was fed at 11.6 kg DM/d to fistulated cows. MSBP caused a significantly higher percentage of butyric acid and a lower percentage of propionic acid in the rumen than UMSBP. The higher butyric acid proportion could be accounted for totally by increased butyrate levels during the first 1-2 hours post feeding when acetate levels were equally depressed. Huhtanen (1988) substituted barley or sugar beet pulp (SBP) by molasses at the rate of 170 g/kg DM of total diets and observed that the concentration of total VFAs in the rumen of dairy male cattle was not affected by diets. However, feeding molasses increased the molar proportions of butyric acid and propionic acid, but decreased acetic acid.

Data presented in Tables 1.3 and 1.4 shows different effects of molasses or sucrose on rumen pH. Small levels of molasses or sucrose (50-60 g/kg DM) in diets tend to decrease rumen pH (Owen *et al.* 1967, Kellogg 1969 and Wing *et al.* 1988), then pH tend to increase as the levels of molasses or sucrose further increased (Kellogg 1969 and Wing *et al.* 1988). However, Martin and Wing (1966) reported that rumen pH linearly increased as molasses levels increased from 0 to 126 g/kg DM. Huhtanen (1988) reported that the ruminal pH decreased when barley was substituted by molasses at the rate of 170 g/kg DM of total diets, while pH increased when the same amount

Table 1.4 *Effect of molasses on the ruminal VFAs and pH in dairy cattle*

References and treatments (g/kg DM)	Molar proportion (%)			Rumen pH
	Acetic	Propionic	Butyric	
Martin and Wing (1966) (Molasses replaced maize)				
control	62.2	20.7	12.9	6.54
42 molasses	63.5	19.6	12.8	6.62
84 molasses	63.1	19.9	13.1	6.61
126 molasses	63.4	19.4	13.2	6.65
Wing *et al.* (1988) (CMDS replaced maize)				
control	57.5	25.8	16.7	5.95
60 CMDS	58.0	25.9	16.1	5.83
120 CMDS	60.2	22.7	17.0	6.05
180 CMDS	61.5	19.1	19.3	6.19
Huhtanen (1988) (molasses replaced barley or SBP)				
barley	65.2	15.5	16.1	6.33
barley + 170 molasses	62.0	17.1	17.9	6.21
SBP	67.6	17.5	12.8	6.40
SBP + 170 molasses	65.5	18.5	13.7	6.45
Beever *et al.* (1988)[+]				
barley	62.7	19.8	11.9	--
UMSBP	65.8	19.1	11.0	--
MSBP	64.5	17.8	13.6	--

[+] 11.6 kg DM/d of a concentrate was fed which was based on one of those ingredients, respectively.

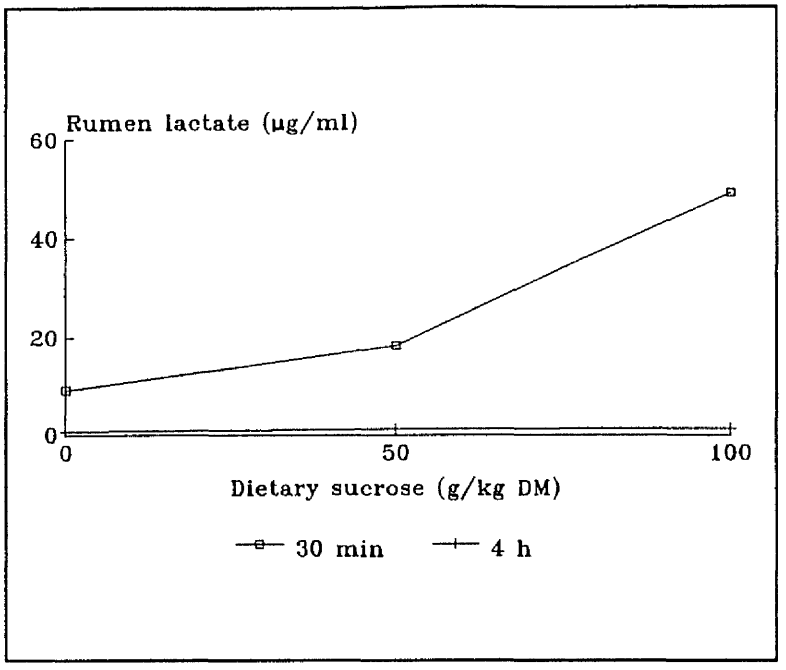

Figure 1.1 The change of the ruminal lactate concentration by feeding sucrose From Kellogg (1969).

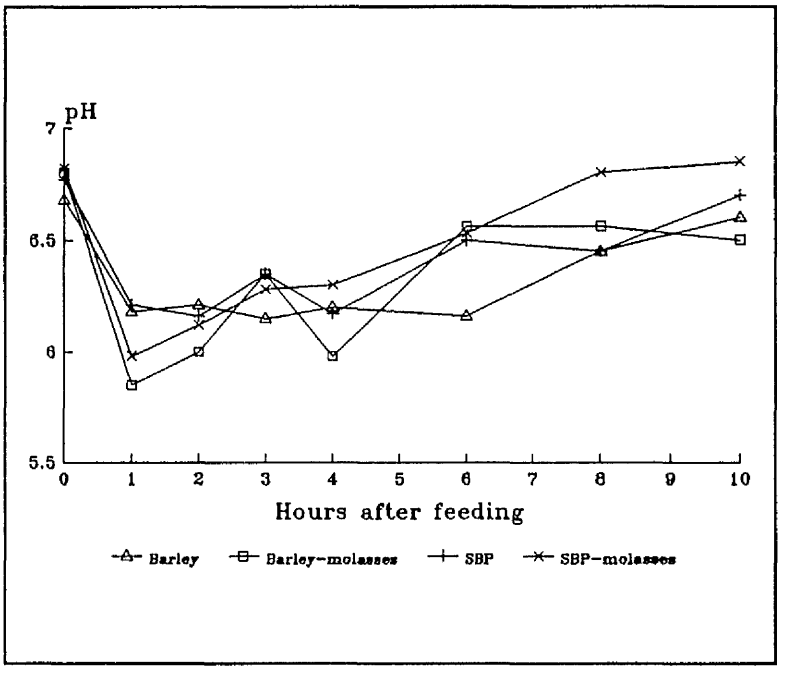

Figure 2.2 The effects of molasses feeding on rumen pH in cattle From Huhtanen (1988).

of molasses was substituted for SBP. Garrett *et al.* (1989) replacing SBP by molasses at the rates of 0, 300 and 600 g/kg DM of total diets, observed a linear decrease in pH (7.01, 7.00 and 6.96) and an increase in lactate (18.78, 23.62 and 22.79 mg/100 ml) in the rumen of dairy steers. Those disagreements could be attributed to differences in sampling times for pH measurement. Data for pH presented in Table 1.3 were obtained at the 4th hour after feeding and in Table 1.4 were averaged from several sampling times after feeding. Kellogg (1969) reported that lactate concentrations largely increased in half an hour after feeding in the rumen of dairy cows fed sucrose from 0 to 100 g/kg DM of total diets and then declined dramatically on all treatments from the first half an hour to the 4th hour after feeding (Figure 1.1). When molasses replaced barley or SBP at the rate of 170 g/kg DM of total diets, Huhtanen (1988) observed a lower pH on molasses treatments than on no molasses treatments in the first hour after feeding, then pH increased on all 4 treatments and the differences disappeared after 3 hours post feeding (Figure 1.2).

In summary, molasses or sucrose inclusion in the diet increases the molar proportion of butyric acid and in general decreases propionic acid, but does not appear to have a consistent effect on acetic acid and pH in the rumen of dairy cattle. Since propionic acid is a glucose precursor, but butyric acid not, increasing butyric acid and decreasing propionic acid by molasses may be associated with decreasing the productive energy value of molasses when high levels of molasses are fed.

1.3.2 *Digestibilities of dietary DM and fibre*

Addition of readily available carbohydrate to forage based diets generally increases the digestion of the total dietary dry matter because of the its higher digestibility (Pate, 1982), but decreases the digestion of the forage dry matter (Burroughs *et al.*, 1949) or the forage fibre fraction (Swift *et al.*, 1947 and Head, 1953).

A number of recent studies have shown various results of DM digestibility when molasses was fed to cattle (Table 1.5). Ahmed and Kay (1975) reported that substitution

Table 1.5. *Effects of molasses on digestibilities of dietary nutrients in dairy cattle.*

References and treatments (g/kg DM)	Digestibility (g/kg)					
	DM	OM	CF	ADF	Cel$^+$	CP(N)
Martin and Wing (1966) (molasses replaced maize)						
control	752	--	--	--	684	749
42 molasses	681	--	--	--	616	735
84 molasses	692	--	--	--	578	729
126 molasses	687	--	--	--	602	725
Ahmed and Kay (1975) (no concentrate fed, molasses replaced dried grass)						
dried grass	742	749	838	--	--	--
250 molasses	773	779	836	--	--	--
500 molasses	763	767	765	--	--	--
Krohn *et al.* (1985) (molasses replaced grass silage)						
control	--	710	--	650	--	--
160 molasses	--	670	--	500	--	--
320 molasses	--	640	--	390	--	--
480 molasses	--	670	--	230	--	--
Huhtanen (1987)[#] (molasses replaced barley or SBP)						
barley	720	740	623	555	664	728
barley + 126 molasses	739	755	640	551	674	738
barley+SBP+63 molasses	730	750	667	592	705	725
SBP	707	730	684	620	722	691
SBP + 124 molasses	724	741	682	622	725	697

Table 1.5. (continued)

References and treatments (g/kg DM)	Digestibility (g/kg)					
	DM	OM	CF	ADF	Cel^{+}	CP(N)
Huhtanen (1988) (molasses replaced barley or SBP)						
barley	730	746	601	615	660	664
barley + 170 molasses	769	778	652	656	705	694
SBP	720	741	725	732	795	610
SBP + 170 molasses	743	757	719	721	769	630
Wing *et al.* (1988) (CMDS replaced maize)						
control	644	660	--	454	--	537
60 CMDS	690	704	--	435	--	520
120 CMDS	624	637	--	436	--	504
180 CMDS	553	570	--	412	--	494
Garrett *et al.* (1989)* (molasses replaced SBP)						
control	752	733	--	633	--	--
300 molasses	775	762	--	581	--	--
600 molasses	787	782	--	413	--	--

$^{+}$ cellulose $^{\#}$ AIA was used as a natural marker * Chromium was used as a marker

of molasses for dried grass at the rate of 250 g/kg DM of total diets increased digestibilities of dry matter and organic matter (OM) by 4% in dairy steers, but further increasing dietary molasses level to 500 g/kg DM resulted in less improvement. Garrett *et al.* (1989) however observed a linear increase in DM and OM digestibilities as molasses replaced SBP from 0 to 600 g/kg DM in total diets for dairy steers fed straw.

A similar result was obtained by Iwuanyanwu *et al.* (1990) in beef heifers fed hay using acid insoluble ash (AIA) as a natural marker when molasses replaced hay at the rates of 0, 29, 58 and 82 g/kg DM. Huhtanen (1987) using AIA as a natural marker in dairy cows, observed an increase in digestibilities of both DM and OM as molasses replaced 124 g/kg DM of SBP or barley in the total diet. There was, however, little effect of molasses on the measurements of retention time or passage rate of particle using Cr-labelled straw or liquid phases using cobalt ethylenediaminetetra-acetic acid (CoEDTA) of the digesta. In a subsequent study with fistulated dairy male cattle, Huhtanen (1988) observed a similar increase in digestibilities of DM and OM when molasses replaced SBP or barley at the rate of 170 g/kg DM of total diets.

In contrast, Wing *et al.* (1988) substituted maize by citrus molasses distillers solubles (CMDS) from 0 to 180 g/kg DM in total diets with cottonseed hulls in fistulated dairy cows. A curvilinear effect of CMDS levels on digestion of dry matter and organic matter was observed. Peak digestibilities occurred near 60 g/kg DM of CMDS and digestibilities on treatments containing over 60 g/kg DM of CMDS were then depressed below the control treatment. A similar result was also reported by Wing and Sklare (1982) using condensed citrus molasses solubles in steers fed cottonseed hulls. Krohn *et al.* (1985) replaced grass silage with molasses from 0 to 480 g/kg DM in total diets and noted that cows fed diets with molasses poorly digested OM in comparison with those fed the control diet without molasses. Beever *et al.* (1988) fed dairy cows either a MSBP or UMSBP diet and reported that ruminal digestibilities of OM on the MSBP diet was significantly lower than on the UMSBP diet (514 *v.* 595 g/kg). The MSBP diet resulted in a higher duodenal OM flow than the UMSBP diet.

Some early studies also showed little advantage of adding molasses to diets on the digestion of total dry matter. Martin and Wing (1966, Table 1.5) substituted maize by molasses with alfalfa hay in dairy steers and observed that the digestibilities of diets with molasses were lower than the diet without molasses. This result agreed with those of Bohman *et al.* (1954) and Hamilton *et al.* (1948). Davis *et al.* (1955) replaced maize

with molasses in diets for dairy heifers and observed little effect of molasses on DM digestibility (cited by Harris and Van Horn, 1982). In a series of digestion trials, Foreman and Herman (1953) measured DM digestibilities of diets containing molasses from 0 to 165 g/kg DM with various forage sources in dairy cattle. Increasing molasses levels showed little effect on DM digestion with alfalfa-brome, but increased DM digestibility with alfalfa hay or timothy. This suggests that effect of molasses on DM digestion may depend on the forage sources.

In the reviews written by Harris and Van Horn (1982) of dairy cattle and Pate (1982) of beef cattle, they both concluded that the feeding of molasses depresses digestibilities of fibre components. Since 1982, a number of studies have been published and some of them are presented in Table 1.5. Wing *et al.* (1988) reported that ADF digestion was significantly depressed due to addition of CMDS. The depression was slight at 60 g/kg DM of CMDS, effects of 60 and 120 g/kg DM were nearly equal and 180 g/kg DM caused further decline. Garrett *et al.* (1989) observed a dramatic decrease in ADF digestibility using Chromium as a marker when molasses replaced SBP at very high levels (300 and 600 g/kg DM) in dairy steers. A similar result was obtained by Krohn *et al.* (1985) in dairy cows. ADF digestibility was depressed by 180% in a diet containing 480 g/kg DM of molasses compared with a diet containing no molasses. Beever *et al.* (1988) using fistulated cows, noted that ruminal cellulose digestibility on a MSBP diet was lower than on an UMSBP diet. The MSBP diet caused a higher duodenal flow of cellulose than the UMSBP diet. The decrease in fibre digestion caused by molasses feeding could be because high levels of easily fermentable carbohydrates in starch concentrates tend to decrease rumen pH and increase VFA concentration in the rumen, resulting in a lower cellulolytic activity (Steg and Van Der Meer, 1985).

Huhtanen (1988, Table 1.5) observed various responses in digestion of fibre when molasses replaced either barley or SBP at the rate of 170 g/kg DM of total diets for dairy cattle fitted with a rumen and duodenal cannula. Molasses improved digestibilities of CF, ADF and cellulose when given with barley, but reduced them when given with

SBP. The result agreed to that obtained by Huhtanen (1987) in dairy cow using AIA as a natural marker when molasses substituted barley or SBP at the rate of 124 g/kg DM of total diets. Foreman and Herman (1953) observed a considerable variability in effect of molasses on digestibilites of CF and cellulose in a series of digestion trials in dairy cattle. With high CF alfalfa hay, 44 or 82 g/kg DM of molasses in total diets increased digestibilities of CF and cellulose, whereas 165 g/kg DM of molasses did not show any advantage. With timothy, there was little effect of up to 165 g/kg DM of molasses. With higher quality of alfalfa, however, digestibilities of CF and cellulose were depressed by increasing molasses levels. Those suggest that effect of molasses on fibre digestion is probably modified by types of concentrate and sources of forages in diets.

In summary, dietary molasses levels affect digestion of DM and OM in diets, but the responses are variable. Adding molasses depresses digestibilities of fibrous components of diets. The decrease is possibly modified by sources of forages and types of concentrates.

1.3.3 *Nitrogen utilization*

Microbial protein synthesis depends on both ammonia and energy available in the rumen (Harris and Van Horn, 1982). If the ammonia and energy could be perfectly balanced to meet the microbial requirement, a maximal yield of microbial protein with little waste could be obtained.

Ammonia is essential as the main source of nitrogen (N) for growth of many bacterial species in the rumen and all species so far studied can utilize it as the main source of N (Bryant, 1974). Many studies have been done to determine the minimum concentration of ammonia in the rumen which provides the maximum rate of synthesis and yield of bacterial protein. A level of 3.5 or 3.6 mmol/l (49 or 50.4 mg/l) of the ammonia in the rumen has been suggested (Satter and Slyter, 1974; Miller, 1982 and Satter, 1982). Beever *et al.* (1988) fed fistulated cows 11.6 kg DM/d of a concentrate

which was based respectively on MSBP, UMSBP and barley with grass silage and noted that ammonia N in the rumen was higher on the MSBP diet than on the UMSBP diet, but lower than on the barley diet (139, 129, 173 mg/l, respectively). When molasses replaced SBP or barley at the rate of 170 g/kg DM of total diets, Huhtanen (1988) reported that molasses with barley increased concentration of ammonia N in the rumen (99.8 *v.* 57.8 mg/l), but not with SBP. However, Wing *et al.* (1988) observed a decrease of ammonia-N concentration in the rumen (from 218 to 192 mg/l) of fistulated cows fed CMDS from 0 to 180 g/kg DM of total diets. Garrett *et al.* (1989) found a linear decrease of ammonia-N in the rumen of dairy steers at slaughter as molasses replaced SBP at the rates of 0, 300 and 600 g/kg DM of total diets, even though urea was added to molasses treatments (2.5 or 5.0 g/kg DM of total diets). The ruminal ammonia concentration can be influenced by many factors, such as frequency of feeding, type of dietary carbohydrate, the extent of dietary protein degradation, and rumen pH (Harris and Van Horn, 1982). Sampling time is also an important factor which contributes to change of the ruminal ammonia concentration. After feeding, ruminal ammonia concentration quickly increases and then declines dramatically after peak as presented in Figure 1.3 (Huhtanen, 1988). Therefore, it is not correct to compare the results of different studies. However, when molasses are fed to cattle at very high levels care should be taken to ensure adequate concentration of ammonia in the rumen for maximum growth of microorganisms due to low CP concentration in molasses.

Fermentable ME (FME) available is another essential factor for growth of the rumen microorganisms. Since molasses contains little lipid, most of ME in molasses is available for use of the rumen bacteria. In the Scottish Agricultural College, the efficiency of converting ME to FME is 0.914. Together with its fast fermentation rate (Johnson, 1976) and then quick provision of energy for microbial growth, which is compatible with ruminal ammonia concentration, this appears to put molasses in a favourable position in terms of synthesis of microbial protein. Beever *et al.* (1988)

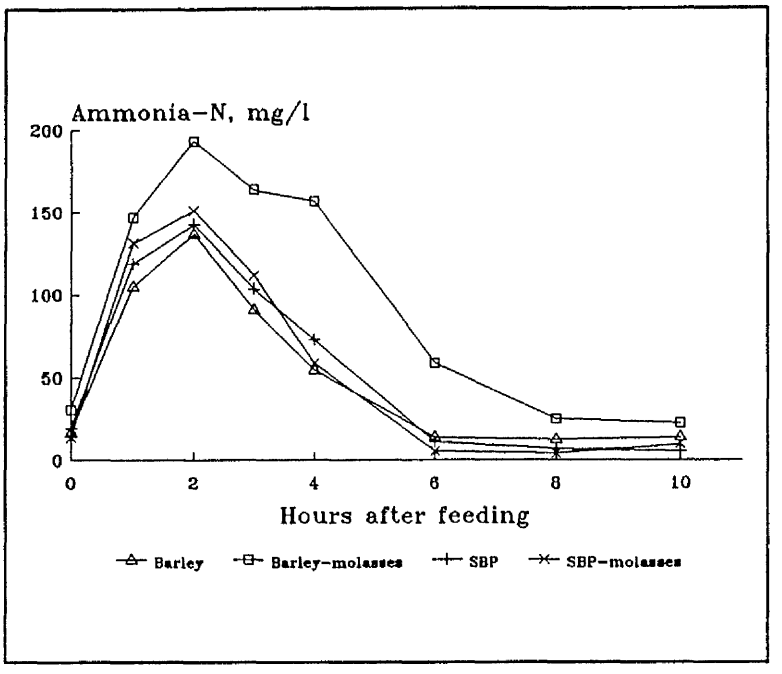

Figure 1.3 The effects of molasses feeding on rumen ammonia concentration in cattle From Huhtanen (1988).

compared the utilization of MSBP and UMSBP or barley with the approximately 200 g CP/kg DM in each of three concentrates in fistulated cows. A markedly higher flow of non-ammonia-nitrogen (NAN) to the small intestine on the MSBP or barley diet than on the UMSBP diet was observed. Consequently, whilst the MSBP or barley diet supplied 34.0 or 33.5 g NAN/kg OMI (organic matter intake) to the intestines, the value for the UMSBP diet was substantially lower (28.8 g NAN/kg OMI). In a trial with fistulated dairy cattle fed diets containing 170 g/kg DM of molasses with either SBP or barley, Huhtanen (1988) reported that the quantity of microbial N entering the small intestine was higher on both diets with molasses than on diets without molasses ($p < 0.05$) and there was a trend towards a higher efficiency of microbial N synthesis on the former than on the latter. Nevertheless, he explained that the increased microbial N production and a trend for the higher efficiency of microbial N synthesis when molasses diets were given may be due to the synergetic effect of different carbohydrates.

In contrast, El Khidir and Thomsen (1982, Figure 1.4) in an *in vitro* trial replaced hay with molasses from 0 to 100% and maintained a 2% level of N by adding urea in all treatments. The structural carbohydrates (hay) induced higher microbial synthesis per unit digestible organic matter (DOM) than simple sugars (molasses). Microbial N linearly decreased from 1.73 to 0.99 mg/100mg DOM in the substrates as molasses/hay rates increased from 0/100 to 100/0 during the 24 hour incubation. Oldham *et al.* (1977) compared N utilisation by substitution of molasses for barley or straw at the rate of 552 g/kg DM of total diets with urea or fish meal in an *in vivo* study in sheep. When all the animals consumed a similar amount of N, with urea the molasses diet significantly decreased N concentrations in duodenal digesta compared with the barley or straw diet (13.2 *v.* 20.2 or 22.6 g/d), but with fish meal the difference was small (17.3 *v.* 18.4 or 19.3 g/d). The concentrations of amino acids in duodenal digesta was also found to be similar to the N concentrations. Several animal studies also showed that in the presence of large amounts of sucrose with sheep (Al Attar *et al.*, 1976) and

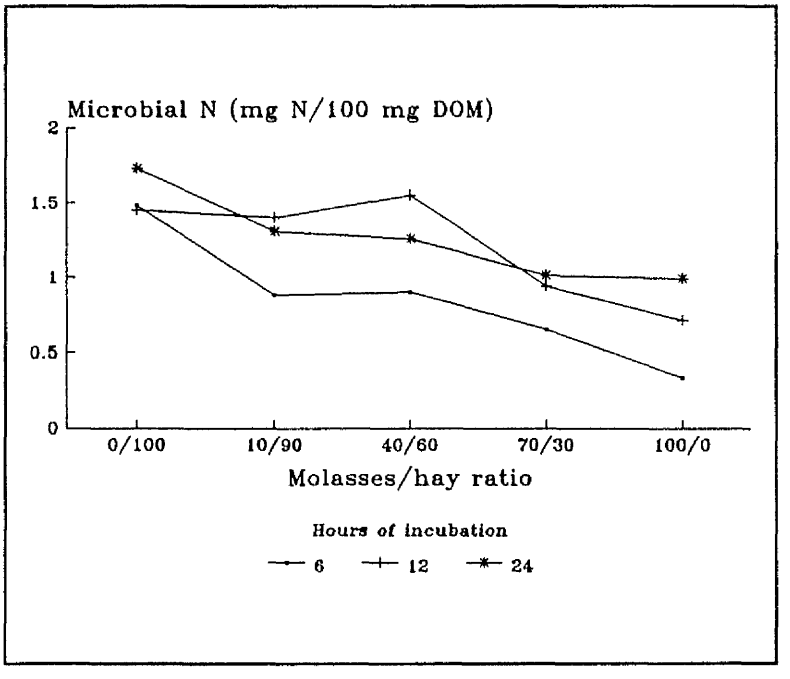

Figure 1.4 The effects of molasses on microbial N synthesis *in vitro* From El Khidir and Thomsen (1982).

sugar beet with cows (Hvelplund and Moller, 1978 and 1980), the efficiency of microbial protein synthesis were found to be low. The low efficiency of microbial protein synthesis when diets with a high proportion of readily fermentable carbohydrates are given may be due to low pH in the rumen (Russel and Domlrowski, 1980), a high production of lactate or a production of propionate via the acrylate pathway with a resultant lower ATP supply (Tamminga, 1979) (cited by Huhtanen, 1988).

Several early studies showed that molasses inclusion in the diet depressed the apparent digestibility of dietary protein (Colovos *et al.*, 1949; Bell *et al.*, 1953 and King *et al.*, 1957). This reduction in beef cattle was reviewed by Pate (1982) to range from 5 to 15% when moderate to high levels of molasses are fed. Martin and Wing (1966, Table 1.5) fed dairy steers molasses from 0 to 126 g/kg DM with maize and alfalfa hay and observed a linear decrease in dietary CP digestibility. Similarly, Wing *et al.* (1988, Table 1.5) substituted maize with CMDS from 0 to 180 g/kg DM in total diets with cottonseed hulls to evaluate responses of dairy cows. Dietary CP digestibility significantly decreased with increased amounts of CMDS. Regression analysis showed that the digestibility was depressed by 0.24% with each increment of percentage unit of CMDS. In steers fed a diet containing 960 g/kg DM of rice straw, White *et al.* (1973) also found that dietary protein was poorly digested as molasses replaced rice straw at the rates of 50, 100 and 200 g/kg DM of total diets. Dietary protein digestion was found lower in sheep as well when molasses replaced barley at the rate of 552 g/kg DM of total diets with fish meal (Oldham *et al.*, 1977) or when 197 g/kg DM of molasses in a diet was compared with 130 g/kg DM in condition that roughage was fed separate from concentrates (Komkris *et al.*, 1965). One interpretation for the decrease in protein digestion when molasses is fed is that molasses partially inhibits the digestion of preformed or microbial protein leaving the rumen (Pate, 1982). However, Huhtanen (1987, Table 1.5) using acid insoluble ash (AIA) as a natural marker, did not note any significant difference in dietary protein digestibility when molasses replaced barley or SBP at the rate of 124 g/kg DM of total diets. In a subsequent trial in which molasses replaced barley or SBP at the rate of 170 g/kg DM with grass silage for dairy male

cattle, Huhtanen (1988, Table 1.5) reported that the both molasses diets led to more extensive digestion of dietary CP than the control diets without molasses.

A number of early studies showed that molasses was less effective in the conversion of urea to protein than cereal grains (Wegner *et al.*, 1941 and Bell *et al.*, 1951). The explanation has been given that the sugar of molasses are absorbed or degraded too rapidly (Reid, 1953), and this would appear to be compatible with the rapid hydrolysis of urea to ammonia upon entering the rumen (Pate, 1982). Bohman *et al.* (1954, Table 1.6) studied interaction between urea and molasses on N partition when maize replaced 20% molasses in a molasses-urea diet for dairy heifers. The cattle fed the diet with maize retained more N than those fed the diet without maize in the basis of the same amount of N intake (8.4 *v.* 6.8g). Faecal N losses were very similar, while less urinary N losses were found in the animals fed the diet with maize than those fed the diet without maize. Oldham *et al.* (1977, Table 1.6) compared N utilisation between a molasses-urea diet and a barley-urea or a straw-urea diet in sheep. A difference was observed in duodenal N passage between diets; in particular less N passed the duodenum when molasses, rather than barley or straw, was fed ($p<0.05$). This difference was fully accounted for by an increased urine N output with the molasses-urea diet. Urine N output for this diet was much greater than for others ($p<0.01$). This resulted in reduced N retention for the molasses-urea diet. The interaction between molasses and urea in the rumen therefore was not beneficial in terms of N supply to the intestines. Similar results were observed by the same author (Oldham *et al.*, 1977) when urea N was replaced by fish meal N. White *et al.* (1973, Table 1.6) reported a similar reduction in N retention when molasses replaced rice straw from 0 to 200 g/kg DM of total diets in steers.

Similarly, Bell *et al.* (1953) in steers and Martin *et al.* (1981) in sheep reported that animals fed molasses-urea diets excreted significantly more N from urine than those fed maize-urea diets. Recently, El Khidir and Thomsen (1982) conducted an *in vitro* trial in which molasses replaced hay from 0 to 100% and all treatments were maintained a

Table 1.6 *Interaction between molasses and urea on N partition*

References	Diet (g/kg DM)		Nitrogen partition (g/d)			
	Molasses	Urea	Intake	Faeces	Urine	Balance
Bohman *et al.* (1954) in dairy cattle	640	22	63.2	29.6	26.8	6.8
	511	20	63.4	29.7	25.5	8.4
Oldham *et al.* (1977) in sheep	552	35	23.4	4.7	18.2	0.5
	0[+]	15	24.9	5.0	9.5	10.8
	0[#]	15	24.0	8.6	10.5	4.7
White *et al.* (1973) in steers	0	20	303	--	--	4.5
	50	20	300	--	--	0.4
	100	20	309	--	--	3.3
	200	20	306	--	--	1.6

[+] Molasses replaced barley.

[#] molasses replaced barley straw.

N level at 20 g/kg DM by addition of urea. The efficiency of microbial N synthesis significantly decreased as molasses inclusion rates increased. Iwuanyanwu *et al.* (1990) in beef heifers noted that addition of molasses to hay-urea diets from 0 to 300g/d linearly decreased the utilization of diets as evidenced by the liveweight gain and feed efficiency. The urea-N, therefore, is less efficiently utilized in forage based diets supplemented with molasses than those supplemented with starch (Pate, 1982 and Harris and Van Horn, 1982) and possibly with forage.

Studies indicated that molasses could provide some protection against ruminal degradation of dietary protein. Masonex (a hemicellulose extract) was suggested to have the function because it contains a polyphenolic fraction which binds with certain proteins to decrease their microbial degradation and increase the amount of dietary protein that escapes ruminal fermentation (Hartnell and Satter, 1975).

Cane molasses may have similar properties to Masonex in providing some protection against ruminal degradation of dietary protein. Chalupa and Montgomery (1979) in an *in vitro* study mixed respectively cane molasses at the rates of 0, 50, 100 and 150 g/kg with a substrate containing 700 g/kg of alfalfa hay and 300 g/kg concentrate mixture, and then added rumen inoculum from a dairy cow to the substrates. After 16 h of fermentation, decreases were observed in concentrations of isobutyrate (0.022, 0.019, 0.020 and 0.016 mM per fermentation) and isovalerate (0.040, 0.030, 0.029 and 0.023 mM per fermentation) as molasses inclusions in substrates increased. A similar depression in concentration of isovalerate (0.08, 0.07 and 0.02% of total VFAs, respectively) was also reported by El Khidir and Thomsen (1982) in an *in vitro* trial in which molasses replaced hay at the rates of 100, 400 and 700 g/kg in substrates. The results from *in vivo* studies showed the similar decreases in the molar proportion of isobutyrate and isovalerate in the rumen as presented in Table 1.7 when molasses was fed to cattle. Since isobutyrate and isovalerate are products of valine and leucine degradation, decreased their production in supplemental fermentations is indicative of decreased proteolysis and/or deamination and suggest that cane molasses might also provide some protection against ruminal degradation of proteins.

In summary, addition of molasses to diets for cattle showed various effects on the microbial N synthesis. Feeding dairy cattle high levels of molasses can decrease the dietary N utilization by depressing the apparent digestibility of dietary CP and the efficiency of converting urea to protein. Molasses, however, may provide some protection against ruminal degradation of protein. When moderate to high levels of molasses are fed to dairy cows, a properly higher dietary protein concentration may be required.

Table 1.7 *The decreases in ruminal concentrations of isobutyrate and isovalerate by molasses feeding*

References	Dietary molasses (g/kg DM)	% of total VFAs	
		isobutyrate	isovalerate
Martin and Wing (1966) in dairy steers	0	--	2.72
	42	--	2.58
	84	--	2.27
	126	--	2.38
Rumsey *et al.* (1971) steers[+]	0	1.8	2.4
	molasses	1.1	0.9
Bond and Rumsey (1973) in beef cattle[#]	Trial 1		
	0	1.2	1.0
	259	0.8	0.8
	Trial 2		
	0	1.0	1.5
	389	0.6	0.9

[+] Molasses was fed *ad libitum*.

[#] A liquid molasses supplement was fed which contained 820 g/kg of blackstrap molasses, 60 g/kg of phosphoric acid and 120 g/kg of water.

1.3.4 *Energy utilization*

Addition of molasses to diets for dairy cattle can result in a decrease in molar proportion of propionic acid and an increase in butyric acid in the rumen as discussed before. Since butyric acid is not a glucose precursor, the productive energy value of molasses can be reduced when high levels of molasses are fed (Pate, 1982). Together with depression of fibre digestion and N utilisation, feeding high levels of molasses could therefore decrease the energy utilisation of total diets. However, few publications are available to describe the energy utilization for milk production in dairy cows.

Lofgreen and Otagaki (1960 a) evaluated the energy value of blackstrap molasses (a kind of cane molasses) for dairy cows by adding 100 or 300 g/kg DM of molasses to a basal diet containing 660 g/kg DM of pineapple bran and 340 g/kg DM of soyabean meal. The addition of 300 g/kg DM of molasses caused a significant decrease in milk concentrations of fat and solid-not-fat, and a lowering of the energy value per unit of milk produced compared with the 100 g/kg DM of molasses (2.24 *v.* 2.37 MJ/kg milk). The NE value of blackstrap molasses for milk production was 3 times lower in the diet containing 300 g/kg DM of molasses than in the diet containing 100 g/kg DM of molasses (2.12 *v.* 6.27 MJ/kg DM). In addition, they suggested that the energy loss of molasses in the diet containing 300 g/kg DM of molasses occurred after absorption because DE values of molasses in both diets were similar (8.90 *v.* 9.32 MJ/kg DM).

Huhtanen (1987, Table 1.8) studied responses of dairy cows to substitution of molasses for either barley or SBP at a level of 120 g/kg DM in total diets. Addition of molasses significantly depressed milk yield but showed little effect on milk concentrations of fat and protein. He calculated the energy balance and efficiency of conversions of ME surplus to maintenance into milk. The inclusion of molasses showed little effect on the calculated energy for maintenance and NE/DE ratio, but significantly decreased the energy values for milk production with both barley and SBP diets. Effect of molasses on the energy efficiency, however, was different between barley and SBP. Molasses inclusion decreased the value with SBP, while increased the value with barley.

In a trial with fattening steers using comparative slaughter technique, Lofgreen and Otagaki (1960 b) determined the NE values for fattening of blackstrap molasses at various levels in beef finishing diets. The NE values of molasses in diets containing 250 or 400 g/kg DM of molasses decreased to only half of the NE value of molasses in the diet containing 100 g/kg DM of molasses (3.48 or 3.23 *v.* 6.35 MJ/kg). In a subsequent trial, Lofgreen (1965) fed beef heifers diets containing respectively 0, 50, 100, 150 and 200 g/kg DM of cane molasses to define the relationship between the

Table 1.8 *Effect of molasses on calculated energy utilization of dairy cows*

	Treatments (+/- molasses)			
	Barley-	Barley+	SBP-	SBP+
DE intake (MJ/d)	210.4	197.4	197.3	198.7
ME intake (MJ/d)	181.0	169.7	169.7	170.9
Energy output (MJ/d)				
Maintenance	48.9	48.8	49.0	48.9
Milk	79.7	74.8	80.1	76.1
Efficiency[+]				
+LVC	0.632	0.660	0.671	0.667
-LVC	0.602	0.622	0.667	0.628
NE/DE	0.546	0.559	0.567	0.565

[+] +LVC, efficiency included live weight change.

-LVC, efficiency excluded live weight change.

From Huhtanen (1987).

levels of molasses in diets and their energy values. In the diets containing 50, 100 and 150 g/kg DM of molasses the NE value for maintenance and NE value for gain were similar, averaging 8.07 and 4.60 MJ/kg DM of molasses. In the diet containing 200 g/kg DM of molasses both values were 10% lower (7.23 and 4.14 MJ/kg DM of molasses). It was concluded that the decline of NE values of molasses occurred in excess of 200 g/kg DM of molasses inclusion. In contrast, Preston *et al.* (1969) conducted a comparative slaughter experiment to estimate the efficiency of ME utilization for fattening in molasses based diets. The efficiency of ME utilization for gain in a diet containing 700 g/kg DM of molasses was higher than in a diet containing 300 g/kg DM of molasses (29.1 *v.* 17.5%) in growing bulls fed forage.

1.3.5 *Metabolic problems*

A problem observed in cattle fed diets containing moderate to high levels of cane molasses is loose faeces which is often associated with diarrhoea. Scott (1953) stated that because of this condition it is important not to set the level of molasses in the diet "too high". The high levels of potassium (K) in cane and beet molasses (see Table 1.1) have been implicated in these digestive disorders. In a trial with lambs fed purified diets, Briggs and Heller (1943) noted that both high concentrations of sugar and K contributed to the laxative property of molasses. Beet molasses is claimed to be more laxative due to its high alkaline concentration and other laxative substance than cane molasses (Morrison, 1959) (cited by Harris and Van Horn, 1982). In addition, Newton *et al.* (1972) reported that the feeding of diets containing 49 g/kg DM of K significantly reduced magnesium (Mg) absorption, but not Mg balance, and temporarily lowered blood serum Mg concentrations.

On grain based diets (particularly sorghum and maize) a substantial proportion of dietary starch may escape microbial degradation (Armstrong, 1974) and in animals fed such diets the rumen fermentation is characterised by a high concentration of propionic acid. The sugars in molasses however are readily fermented in the rumen, leaving no glucose available to the animals from post ruminal carbohydrate digestion and propionic acid concentrations in the rumen of molasses fed animals are usually low (see Table 4) which exacerbates any glucose insufficiency (Cortes *et al.*, 1987) and results in high levels of ketone bodies in the blood. Losada and Preston (1974) suggested that cattle possibly suffered from subclinical ketosis when high levels of molasses were fed (cited by Harris and Van Horn, 1982). Harris (1982) reported that in a few herds in Florida when cows consumed an average of 2-3 kg molasses daily in the condition of free choice, more than 50% of the cows had ketosis before the problem was found and corrected (cited by Harris and Van Horn, 1982). However, Cowan and Davison (1978) did not observe any health problems in lactating cows grazing a pasture due to the feeding of 3 kg/d molasses for 6 months. Miettinen and Huhtanen (1989) substituted molasses for barley or SBP at the rate of 2 kg DM/d with a grass silage for dairy cows

and observed different results. The molasses inclusion in SBP increased the cow blood concentration of beta-hydroxybutyrate by 18%, while in barley showed little effect in a Latin square design with 21 days of each period.

Another problem with molasses feeding is termed as "molasses toxicity" which was first noted in Cuba with the introduction of large scale feed lots for cattle using molasses by Preston and Willis (1974). Losada *et al.* (1971) and Rowe *et al.* (1979) both observed that symptoms of molasses toxicity usually appeared from 6 to 8 days after removal of forage (cited by Cortes *et al.*, 1987). At first there is a refusal to eat molasses and an increase in the shivering reflex. Subsequently, excessive salivation occurs and a tendency to wander around in circles. If untreated the animal becomes comatose and quickly dies (Cortes *et al.*, 1987). Preston (1988) reported that in the first year following the introduction of the molasses/urea fattening system in Cuba, rates of mortality and emergency slaughter in a herd of 10,000 cattle increased from 0.1 and 0.4% (when a forage-based diet was fed) to 1 and 3% respectively, when the diet was changed to high levels of molasses/urea.

Molasses toxicity is related to the nervous symptoms and blindness, indicating damage to the brain and it was subsequently shown (Verdura and Zamora, 1970) (cited by Preston, 1988) that the clinical syndrome was indistinguishable from that of cerebro-cortical necrosis also known as polioencephalomalacia (Edwin *et al.*, 1979). The cause of the necrosis is likely to be a decrease in the energy supply to the brain either due to an absolute deficiency of alimentary thiamine (Edwin *et al.*, 1979) or a deficiency of glucose (Losada and Preston, 1973) (cited by preston, 1988).

Molasses toxicity appears to be precipitated by a low intake of forage in the high-molasses feeding system developed by Preston *et al.* (1967). The most practical preventative measure of molasses toxicity is to assure that all animals consume enough forage. The most practical cure for molasses toxicity is to place the animal on *ad libitum* forage feeding when the initial stages of the condition is observed, because in

the advanced stages the condition is irreversible (Pate, 1982).

1.4 *Effect of molasses on voluntary feed intake of dairy cows*

Dairy cattle physiologically need sufficient fibre intake to satisfy their rumen fermentation and maintain normal milk composition. In practice, optimum fibre intake may be a problem where roughage and concentrate are fed separately or poor quality of roughage is fed. In these condition, feeding molasses is an advantage because of its high sugar concentration (see Table 1.1) and its ability to improve feed intake.

1.4.1 *Replacing cereal grain in complete diets*

A number of studies have shown that replacing cereal grains by molasses up to 100 g/kg DM in complete diets improved total feed intake (Tables 1.9 and 1.10). Wing and Powell (1969) substituted maize by molasses at the rates of 0, 42 or 126 g/kg DM in complete diets with alfalfa. Dairy cows fed the diet containing no molasses consumed less feed than those fed the diets containing molasses, although the data were not analyzed because they were collected by groups and tabulated as average value per cow. Vernlund *et al.* (1980) conducted an extensive feeding trial in which molasses or Masonex (a hemicellulose) replaced maize at the rate of 80 g/kg DM of total diets with variously treated cottonseed hulls (CSH) (regular CSH, pelleted CSH with or without 9 % fat, pelleted untalented CSH with or without 9% fat). The averaged DM intakes on molasses treatments and Masonex treatments were slightly higher than on control treatments without molasses or Masonex. Recently, Wing *et al.* (1988) studied interaction between molasses types and roughage sources in dairy cows. CMDS replaced maize at the rates of 0, 30, 60 and 90 g/kg DM in total diets with maize silage or cottonseed hulls. The effect of CMDS on DM intake was positively linear in both forage based diets. A slight decline in DM intake from 60 to 90 g/kg DM of CMDS made it appear that 60 g/kg DM was optimal. Substitution of maize by molasses, Masonex or Flambeau (a hemicellulose) at the rate of 30 g/kg DM of total diets also showed an increase in total DM intake. Morales *et al.* (1989) conducted a series of trials to estimate interaction between types and amounts of roughage and amounts of

molasses in dairy cows. Total DM intakes varied with various types or amounts of forages or amounts of molasses. The addition of molasses did not show an appreciable effect on DM intakes until 80 g/kg DM of molasses was added.

Table 1.9 *Effect of molasses on DM intakes of cows*

References	Concentrate replaced by molasses	Treatments (g/kg DM)	DM intake (kg/d)
Komkris *et al.* (1965)	concentrate	complete feed	
		130 molasses	13.7
		197 molasses	13.4
		separate feed	
		130 molasses	13.7
		197 molasses	13.0
Wing and Powell (1969)	Maize	0 molasses	22.6
		42 molasses	23.2
		126 molasses	23.0
Verulund *et al.* (1980)	maize	0 molasses	20.0
		80 cane molasses	20.5
		80 wood molasses	20.9
Huhtanen (1987)	barley	0 molasses	16.2
		126 molasses	15.1
	barley & SBP	63 molasses	15.5
	SBP	0 molasses	15.6
		124 molasses	15.6
Wood (1990)	barley	0 molaferm 20+	14.7
		100 molaferm 20	14.9
		195 molaferm 20	15.1

+ a mixture of 80% of cane molasses with 20% of condensed molasses solubles

Table 1.10 *Interaction between molasses and forage on DM intakes of cows when molasses replaced maize*

References	Forage and its concentration (g/kg DM)	Dietary molasses (g/kg DM)	DM intake (kg/d)
Morales (1989)	300 cottonseed hull	0	24.0
		40	24.8
		80	24.9
	350 alfalfa haylage	0	23.2
		40	24.0
		80	22.4
	650 alfalfa haylage	0	20.6
		40	20.2
		80	22.0
	140 cottonseed hull and 350 alfalfa haylage	0	24.5
		40	24.0
		80	22.7
Wing *et al.* (1988)	330 maize silage	0 CMDS[+]	23.3
		30 CMDS	22.3
		60 CMDS	27.0
		90 CMDS	26.5
	340 cottonseed hulls	0 CMDS	23.2
		30 CMDS	23.0
		60 CMDS	26.4
		90 CMDS	25.0
		30 molasses	24.7
		60 molasses	23.1
		30 masonex[#]	26.2
		30 flambeau[*]	27.2

[+] citrus molasses distiller's solubles

[#] a type of hemicellulose extract

[*] a type of hemicellulose from spent sulphite liquor

In contrast, molasses inclusion in excess of 100 g/kg DM in total diets generally shows a negative effect on DM intake. Owen *et al.* (1967) examined responses of dairy cows to addition of 100 g/kg DM of molasses to diets containing 400 or 600 g/kg DM of grain with alfalfa haylage silage or wilted alfalfa silage. The averaged DM intake on molasses treatments was decreased by 7% in comparison with no molasses treatments. Komkris *et al.* (1965, Table 1.9) evaluated interactions between molasses levels and feeding manners on responses of dairy cows fed diets containing pineapple bran and pineapple hay. The results showed interactions were significant for DM intake. Addition of 197 g/kg DM of molasses resulted in a significantly lower DM intake than addition of 100 g/kg DM of molasses when either complete diets were fed or the forages were fed separate from the concentrate. In a trial to estimate NE of molasses in diets containing pineapple bran and soyabean meal, Lofgreen and Otagaki (1960 a) reported that cows fed the diet containing 300 g/kg DM of molasses consumed less feed than those fed the control diet containing no molasses (12.9 *v.* 13.8 kg DM/d), but cows fed the diet containing 100 g/kg DM of molasses had a similar intake (13.9 kg DM/d). The inclusion of 300 g/kg DM of molasses in the total diet was concluded to be too high for dairy cows. Recently, Karalazos and Glouzeljannis (1988) also reported that adding 160 g/kg DM of molasses to replace a concentrate caused a significant decrease in DM intake in dairy cows. Huhtanen (1987, Table 1.9) replaced barley or SBP by molasses at the rate of 124 g/kg DM in total diets to estimate responses of dairy cows. Molasses inclusion tended to decrease grass silage intake in both barley (8.5 *v.* 9.4 kg DM/d) and SBP (9.0 *v.* 9.2 kg DM/d) diets in comparison with the control diets without molasses, and the associated difference in total DM intake was significant in barley, but not in SBP. The lower forage intake on molasses diets is possibly due to metabolic control (Huhtanen, 1987). In the case of molasses diets, the higher fermentation rate of sugars than that of starch or fibre (Sutton, 1980) may increase the concentrations of ruminal acetate, duodenal lactate and hepatic uptake of propionate, which are feedback signals for termination of feeding (Forbes, 1980). However, Wood (1990) did not observe a decrease in total DM intake when dairy cows were fed complete diets containing respectively 0, 100 and 195 g/kg DM of molasses replaced the same amounts of barley.

The effects of molasses on the DM intake are modified by sources and amounts of forages and types of molasses. Owen *et al.* (1967) reported that addition of 100 g/kg DM of molasses depressed DM intake by 12% with alfalfa haylage silage, while showed little effect with wilted alfalfa silage. Similarly, the feeding of 100 g/kg DM of molasses decreased DM intake by 10% in diets containing 400 g/kg DM of grain, but not in diets containing 600 g/kg DM of grain. Vernlund *et al.* (1980) observed various responses in DM intake when dairy cows were fed the diets containing 80 g/kg DM of cane molasses or Masonex with variously treated cottonseed hulls (CSH). In comparison with control diets without molasses, addition of cane molasses increased DM intake with pelleted CSH (19.5 *v.* 20.4 kg/d) and had little effect with regular CSH (21.6 *v.* 21.7 kg/d), but decreased DM intake with pelleted untalented CSH (20.3 *v.* 18.6 kg/d). The feeding of Masonex showed a similar results as cane molasses. Morales *et al.* (1989, Table 1.10) further reported that not only sources but also amounts of forage changed the response in DM intake of dairy cows fed molasses. DM intake was positively related with molasses levels in diets with 300 g/kg DM of CSH, but was negatively related in diets with 140 g/kg DM of CSH and 350 g/kg DM of alfalfa haylage (AH). With 350 g/kg DM of AH, effect on DM intake was small at 40 g/kg DM of molasses, but DM intake was depressed at 80 g/kg DM of molasses. However, with 650 g/kg DM of (AH) effect of 80 g/kg DM of molasses on DM intake was positive.

Wing *et al.* (1988, Table 1.10) found that types of molasses were also a factor to influence DM intake. With CMDS, DM intake was positively related with levels of CMDS and 60 g/kg DM of CMDS caused the highest DM intake in diets with either maize silage or cottonseed hulls. However, with cane molasses, 30 g/kg DM of molasses increased DM intake but 60 g/kg DM of molasses showed little effect. Addition of 30 g/kg DM of Masonex or 30 g/kg DM of flambeau resulted in a greater increase in DM intake than the same level of cane molasses or CMDS. Vernlund *et al.* (1980, Table 1.9) also noted that the addition of 80 g/kg DM of Masonex caused

higher DM intake than the addition of the same level of cane molasses. The similar results in sheep and cattle were also reported by Crawford *et al.* (1978) and Hartnell and Scatter (1978).

1.4.2 *Replacing forage in diets offered the same amount of concentrate*

Supplementing diets with extra molasses based on the same amount of concentrate offered resulted in a significant increase in total DM intake, but generally a decrease in forage intake (Table 1.11). Webb *et al.* (1973) fed dairy cows 0 or 3.72 kg/d of a liquid supplement (containing 60% molasses, 20% ammonium acetate and 20% water) and 6.7 kg DM/d of concentrate and hay *ad libitum*. The feeding of the supplement did not appreciably decrease forage intake, and as a result total DM intake significantly increased. A similar result was reported in the same paper in which concentrate was offered at 14 kg DM/d. Mayne (1989) conducted a trial in which dairy cows were fed 0 or 2 kg/d of molaferm 20, 3 kg/d of a concentrate and grass silage *ad libitum*. Supplementing with molasses significantly increased total DM intake, but decreased silage intake with a mean substitution rate of 0.4 kg silage DM per kg of molasses included in the diet. In order to examine the effect of various quantities of molasses in complete diets for dairy cows, Krohn *et al.* (1985) substituted grass silage with molasses from 0 to 480 g/kg DM of total diets which contained 340 g/kg DM of concentrates. The groups' average DM intake was linearly increased but total forage intake was linearly decreased as levels of molasses increased. The decrease in total forage intake was 0.012 kg with increment of 1 g/kg DM of molasses in the total diets. In a trial in which dairy cows were fed 2.13 kg/d of brewer's grain and grass *ad libitum* and were supplemented pure molasses or molasses/urea *ad libitum*, Berry and Peña (1981) reported that cows consumed 6.15 kg/d of pure molasses or 7.28 kg/d of molasses/urea, and individual grass intakes fell by 2.5 kg DM/d in both supplement treatments in comparison with the control treatment with no molasses.

In summary, replacing cereal grain by molasses up to 100 g/kg DM in total diets results in an increase in total DM intake, but additions in excess of 100 g/kg DM generally

shows various extents of negative effect. However, some dietary factors such as amounts and sources of forage and types of molasses can modify the effect of quantity of molasses on DM intake. Supplementing extra molasses on basis of the same amount of concentrate offered increases DM intake, but generally decreases forage intake. Substitution of molasses for forage therefore should take care of adequate forage intake.

Table 1.11 *Effect of substitution of molasses for forage on feed intake*

References and treatments	Feed intake (kg DM/d)			
	total	concentrate	forage	molasses
Webb *et al.* (1973)				
Trial 1, control	19.80	14.30	5.50	--
supplement+	22.29	14.00	5.60	2.69
Trial 2, control	15.80	6.70	9.10	--
supplement+	19.72	6.70	9.30	3.72
Berry and Peña (1981)				
control	10.39	2.13	8.26	--
pure molasses	13.97	2.13	5.69	6.15
molasses+2.5% urea	15.14	2.13	5.73	7.28
Mayne (1989)				
control	12.90	3.00	9.90	--
+ 0.2 kg Na_2CO_3	13.10	3.20	9.90	--
+ 2 kg molasses	14.10	3.00	9.10	2.00
+ 2 kg molasses & 0.2 kg Na_2CO_3	14.40	3.20	9.20	2.00
Krohn *et al.* (1985)				
control	14.20	4.83	9.37	--
160 g molasses/kg DM	16.73	5.70	8.34	2.69
320 g molasses/kg DM	18.95	6.46	6.45	6.03
480 g molasses/kg DM	19.45	6.63	3.55	9.27

+ a liquid supplement containing 60% molasses, 20% ammonium acetate and 20% water.

1.5 *Responses in milk production of dairy cows fed molasses*

Lactating dairy cows have heavy demands for nutrients in early lactation. Frequently, the cow is unable to consume enough DM to supply the energy for maximum milk production. As a result, concentrate feeding has become a greater part of today's dairy diets than in previous years (Harris and Van Horn, 1982). Thus the feeding of molasses can be of benefit to farmers due to its high energy concentration and its ability to improve feed intake. Effects of molasses on milk production will be discussed here in a number of sections according to the ways in which molasses is fed.

1.5.1 *Replace cereal grain in complete diets*

In an extensive feeding trial with variously treated cottonseed hulls (CSH) (regular CSH, pelleted CSH with or without 9 % fat, pelleted untalented CSH with or without 9% fat), Vernlund *et al.* (1980, Table 1.12) observed similar average milk yields between cows fed control diets and similar complete diets containing 80 g/kg DM of cane molasses or 80 g/kg DM of Masonex substituted primarily for maize meal. The average milk fat concentration was depressed, but the difference did not reach significance. Morales *et al.* (1989, Table 1.13) evaluated effects of molasses on performance of lactating cows fed molasses at the rates of 0, 40 and 80 g/kg DM in complete diets with CSH, alfalfa haylage or both together. In comparison with control treatments without molasses, addition of molasses slightly decreased ($p<0.05$) the average milk yield and milk protein concentration, but increased ($p<0.05$) the average fat concentration. However, the effect of molasses in milk production depends on sources and amounts of forages in the diets.

Wing *et al.* (1988, Table 1.14) examined milk production by replacing maize with CMDS at the rates of 0, 30, 60 and 90 g/kg DM in complete diets with maize silage or cottonseed hulls. Milk yields were positively related with dietary CMDS levels in both forage based diets. With maize silage, milk yield reached the highest level at 60 g/kg DM of CMDS and then declined slightly at 90 g/kg DM of CMDS. Similarly,

Table 1.12 *Effect of molasses on milk production*

References and treatments (g/kg DM)	Milk			
	yield (kg/d)	fat (g/kg)	protein (g/kg)	SNF (kg/d)
Komkris *et al.* (1965) (molasses replaced concentrate)				
complete feed, 130 molasses	15.4	40.5	34.1	86.8
197 molasses	15.4	41.6	33.7	85.2
separate feed, 130 molasses	15.7	41.7	33.6	86.0
197 molasses	14.8	42.3	33.8	86.6
Owen *et al.* (1967) (molasses replaced grain)				
40% grain, control	20.6	40.2	--	88.4
100 molasses	19.7	37.1	--	88.3
60% grain, control	20.5	38.7	--	86.9
100 molasses	19.9	39.4	--	84.0
Wing and Powell (1969) (molasses replaced maize)				
control	23.5	41.7	--	90.6
42 molasses	23.0	41.0	--	89.8
126 molasses	22.9	40.2	--	89.4
Verulund *et al.* (1980) (molasses replaced maize)				
control	20.8	31.3	--	--
80 cane molasses	20.9	28.9	--	--
80 wood molasses	20.8	29.6	--	--

Table 1.12 (continued)

References and treatments (g/kg DM)	Milk			
	yield (kg/d)	fat (g/kg)	protein (g/kg)	SNF (kg/d)
Huhtanen (1987) (molasses replaced barley or SBP)				
barley, control	23.3	49.1	30.6	--
126 molasses	21.9	49.0	31.0	--
barley + SBP + 63 molasses	24.0	48.2	31.4	--
SBP, control	24.4	45.5	30.8	--
124 molasses	23.0	46.4	30.8	--
Wood (1990) (Molaferm 20^{+} replaced barley)				
control	24.2	38.9	31.7	--
100 molaferm 20	21.7	42.7	31.0	--
195 molaferm 20	20.2	40.9	30.5	--

$^{+}$ a mixture of 80% of cane molasses with 20% of condensed molasses solubles

with cottonseed hulls the cows fed diets containing 60 g/kg DM of cane molasses gave 2 kg/d more milk than those fed diets containing no molasses. The addition of 30 g/kg DM of Masonex or flambeau with cottonseed hulls also resulted in an increase in milk yield. However, neither types of molasses nor sources of forages showed significantly effects on milk fat or protein concentrations. They concluded that CMDS and cane molasses each at up to 60 g/kg DM nutritionally were superior to maize. Wing and Sklare (1982) also reported that condensed citrus molasses solubles (CCMS) at 60 g/kg DM of total diets significantly stimulated milk production when dairy cows were fed CCMS at the rates of 0, 30, 60 and 90 g/kg DM in total diets.

Table 1.13 *Interaction between molasses and forages on milk production*

Treatments (g/kg DM) (Molasses replaced maize)	Milk		
	yield (kg/d)	fat (g/kg)	protein (g/kg)
300 cottonseed hull with			
Control	25.8	25.5	32.8
40 molasses	26.8	27.1	31.2
80 molasses	26.7	30.4	33.1
350 alfalfa haylage with			
control	26.3	34.0	32.9
40 molasses	25.7	36.2	33.2
80 molasses	24.4	31.2	31.4
650 alfalfa haylage with			
control	23.2	36.2	31.7
40 molasses	22.6	35.6	32.9
80 molasses	23.6	34.3	30.7
140 cottonseed hull and 350 alfalfa haylage with			
control	27.0	34.8	32.3
40 molasses	24.8	33.6	31.7
80 molasses	25.6	35.2	30.8

From Morals (1989)

In contrast, substitution of molasses for cereal grain in excess of 100 g/kg DM in total diets can be detrimental to milk production (Table 1.12). In a trial in which comparisons were made to evaluate the feeding of molasses in a complete diet as contrasted to feeding the forage separate from the concentrate, Komkris *et al.* (1965) reported that addition of 197 g/kg DM of molasses resulted in a lower milk yield than addition of 130 g/kg DM of molasses when the forage was fed separate from the concentrate, but not when the complete diets were fed. The milk fat and protein concentrations were similar between 4 treatments. Owen *et al.* (1967) investigated effect

Table 1.14 *Interaction between molasses types and forage sources milk production*

Treatments (g/kg DM) (Molasses replaced maize)	Milk		
	yield (kg/d)	fat (g/kg)	protein (g/kg)
330 maize silage with			
control	22.1	33.5	29.6
30 CMDS[+]	22.2	32.1	29.0
60 CMDS	24.3	32.4	28.1
90 CMDS	23.4	34.0	28.6
340 cottonseed hulls with			
control	22.9	28.9	28.9
30 CMDS	23.3	31.3	28.8
60 CMDS	24.2	30.9	28.5
90 CMDS	23.9	30.3	28.1
30 molasses	23.9	27.9	29.5
60 molasses	25.0	34.5	29.4
30 Masonex[#]	24.3	31.1	28.6
30 flambeau[*]	23.4	27.8	28.2

[+] citrus molasses distiller's solubles

[#] a type of hemicellulose extract

[*] a type of hemicellulose from spent sulphite liquor

From Wing *et al.* (1988)

of inclusion of 100 g/kg DM of molasses on lactation performance of dairy cows fed diets containing 400 or 600 g/kg DM of grain with alfalfa haylage or wilted alfalfa silage. The averaged milk yield and fat concentration were significantly depressed by molasses feeding. Since sucrose was found to produce similar results as molasses as reported in the same paper, the authors thus suggested that the influence of molasses is via its sucrose concentration since sucrose is the primary energy source in cane molasses. Wing and Powell (1969) replaced maize with molasses at the rates of 0, 42 and 126 g/kg DM in complete diets containing 300 g/kg DM of chopped alfalfa. Milk

yield and fat concentration were linearly decreased as molasses levels increased. They were significantly different when comparison was made between molasses treatments and no molasses treatment. Huhtanen (1987) substituted barley or SBP with molasses at the rate of 124 g/kg DM in total diets with grass silage to evaluate the lactation performance. The cows fed diets containing molasses produced significantly less milk than those fed diets containing no molasses in both barley and SBP based diets. The feeding of molasses showed little effect on milk concentrations of fat and protein. Recently, Wood (1990) examined the response of dairy cows fed complete diets containing 0, 100 and 195 g/kg DM of molaferm 20 with grass silage. The feeding of molaferm 20 had no significant effects on milk concentrations of fat and protein, but depressed milk yield by 0.02 kg/d with each increment of 1 g/kg DM of molaferm 20 in total diets.

The detrimental effect of molasses inclusion on milk production in excess of 100 g/kg DM in total diets is possibly attributed to both a decrease in dietary fibre digestibility as discussed before and a lower NE value of molasses. Bartsch and Wickes (1978) compared molasses and barley as the energy sources to replace ryegrass-clover hay. When 10% of ME requirement was replaced by molasses or barley, milk yield (12.9 *v.* 13.3 kg/d), fat yield (0.49 *v.* 0.51 kg/d) and protein yield (0.38 *v.* 0.38 kg/d) did not show any significant differences between two treatments. However, when 30% was replaced, molasses significantly decreased milk yield (13.1 *v.* 14.9 kg/d) and milk protein yield (0.39 *v.* 0.46). This confirmed the results as reported by Lofgreen and Otagaki (1960 a) that the NE determined for molasses at 300 g/kg DM of molasses in total diets significantly decreased in comparison with 100 g/kg of molasses (2.13 *v.* 6.27 MJ/kg DM). In a subsequent trial, they also obtained a similar results in beef cattle (Lofgreen and Otagaki, 1960 b) and suggested that the large drop in NE with higher levels of molasses was possibly due to higher heat increment caused by a change in rumen metabolism since TDN and DE values were not significantly depressed to account for the significant loss in NE.

However, effect of molasses on milk fat concentration depends on the types of forages in diets. Wing *et al.* (1988, Table 1.15) observed an interaction between sources of forages and amounts of molasses on milk fat concentration as CMDS replaced maize from 0 to 90 g/kg DM in total diets. As dietary CDMS levels increased, milk fat concentration increased with cottonseed hulls (CSH), but decreased at 30 or 60 g/kg DM of CMDS with maize silage. Similarly, Owen *et al.* (1967) noted that addition of 100 g/kg DM of molasses significantly lowered milk fat concentration in comparison with the no molasses treatment with no molasses (36.6 *v.* 40.0 g/kg) with wilted alfalfa silage, but not with alfalfa haylage silage (39.8 *v.* 38.9 g/kg). Morales *et al.* (1989, Table 1.14) examined the cow response to addition of 0, 40 and 80 g/kg DM of molasses. With 300 g/kg DM of CSH milk fat concentration was linearly increased as dietary molasses levels raised. However, with 350 g/kg DM of alfalfa haylage milk fat concentration increased at 40 g/kg DM of molasses but dramatically declined at 80 g/kg DM, while with 650 g/kg DM of alfalfa haylage, was linearly decreased. Vernlund *et al.* (1980) even found that effect of molasses on milk fat concentration was modified by the ways in which the forage was treated. The addition of 80 g/kg DM of cane molasses increased milk fat concentration (34.2 *v.* 32.7 g/kg) with regular CSH, while decreased (28.4 *v.* 32.7 g/kg) with pelleted CSH.

Types of molasses have shown little effect on milk production. Bartley *et al.* (1968) compared the feeding value of dry (DHCE) and liquid hemicellulose extract (LHCE) to liquid cane molasses for lactating cows. The grain mixtures contained respectively 100 g/kg DM of cane molasses, LHCE or DHCE (spray dried). The averaged milk yield for the DHCE, LHCE and cane molasses groups were 18.4, 18.3 and 18.2 kg/d, respectively. No significant differences were detected in any of the parameters measured. In a more extensive trial, Vernlund *et al.* (1980, Table 1.12) observed similar responses in milk yields between cows fed control diets and similar complete diets containing 80 g/kg DM of cane molasses or LHCE substituted primarily for maize meal. No differences between molasses or LHCE could be detected.

1.5.2 *Replacing forage in diets offered the same amount of concentrate*

Several experiments have shown that replacing forages with molasses in diets without altering the amounts of concentrates offered and forages *ad libitum* resulted in an improvement in milk production (Table 1.15). Webb *et al.* (1973) fed cows 6.7 kg/d of a concentrate and brome hay *ad libitum* with or without a liquid supplement (containing 60% of cane molasses, 20% of ammonium acetate and 20% of water). The liquid supplement consumption ranged from 1.26 to 6.26 kg/d and averaged 3.72 kg/d. The cows fed the diet with liquid supplement produced 1.3 kg/d more milk than those fed the control diet, but milk concentrations of fat and protein were similar between two treatments. Berry and Peña (1981) evaluated lactation performance by feeding 2.13 kg DM/day of brewer's grains and chopped grass *ad libitum* with or without *ad libitum* supplement of pure molasses or a mixture of molasses/urea. The cows fed molasses or molasses/urea consumed 6.15 kg DM/d of molasses or 7.28 kg DM/d of the mixture of molasses/urea and produced 11 or 21% more milk than those fed no molasses. Mayne (1989) supplemented 2 kg/d of molaferm 20 to cows fed 3 kg/d of a concentrate and grass silage *ad libitum*. Milk yield was slightly increased with supplement of molasses, but was almost identical when comparison was made between cows fed the control diet with 0.2 kg/d of Na_2CO_3 and with those fed the molasses diet with 0.2 kg/d of Na_2CO_3. Milk concentrations of fat and protein were similar between 4 treatments. Krohn *et al.* (1985) substituted grass silage with molasses at the rates of 0, 160, 320 and 480 g/kg DM with concentrates and barley straw. Milk yield was linearly increased until 320 g/kg DM of molasses and then declined rapidly. Milk concentrations of fat and protein were also increased with each increment of dietary molasses level but dramatically raised at 480 g/kg DM of molasses. The improvement in milk production by supplementing molasses to replace the forages in diets is possibly due to increasing DM intake as discussed before and further increasing total energy intake.

Table 1.15 *Responses of cows to substitution of forage by molasses*

References and treatments (kg DM/d)	Milk		
	yield (kg/d)	fat (g/kg)	protein (kg/d)
Webb *et al.* (1973)			
control	14.9	34.1	31.2
+ 3.72 molasses supplement[+]	16.2	33.6	31.1
Berry and Peña (1981)			
control	6.1	--	--
+ 6.15 molasses	6.8	--	--
+ 7.28 molasses/urea	7.4	--	--
Krohn *et al.* (1985)			
control	21.8	41.4	31.2
+ 160 g/kg DM molasses	24.3	40.0	32.3
+ 320 g/kg DM molasses	25.0	42.1	32.8
+ 480 g/kg DM molasses	22.5	44.8	35.2
Mayne *et al.* (1989)			
control	22.4	39.3	28.7
+ 0.2 Na_2CO_3	22.7	40.7	29.2
+ 2.0 molasses	23.1	38.8	28.2
+ 2.0 molasses + 0.2 Na_2CO_3	22.9	40.6	28.7

[+] containing 60% molasses, 20% ammonium acetate and 20% water.

1.5.3 *Supplementing molasses to dairy cows grazing tropical pastures*

Tropical pastures are capable of producing high DM yields which can support high stocking rates (Chopping *et al.*, 1976). However, milk production is generally low when cows graze the pasture with no supplement (Dale and Holder, 1968), probably because intakes of nutrients are restricted by the low digestibility of tropical pasture

(Hamilton *et al.*, 1970). Energy supplementation therefore is essential for supporting high milk production, which puts molasses in an economically favourable position.

In Australia, supplementing molasses to dairy cows grazing pastures has been reported to increase milk production (Table 1.16). Cowan and Davison (1978) offered 2.1 kg DM/d of maize or 2.2 kg DM/d of molasses to cows grazing in an established green panic and glycine mixed pasture at the stocking rates of 2 or 4 cows/ha for 6 months. Cows responded similarly in the averaged milk yield to the supplements of maize and molasses, but the cows fed supplements produced 1.5 kg/d more milk than those fed no supplement. Milk fat concentration was similar among three treatments. Chopping *et al.* (1980) observed linear increases in milk yield from 7.2 to 9.4 kg/d ($p < 0.01$) and in milk fat concentration ($p < 0.05$), when fresh molasses ranged from 0 to 3.6 kg/d were fed to dairy cows grazing in irrigated couch/pangola pastures for three successive 12 week periods. Over the full experimental period (36 weeks) each kg of fresh molasses fed during lactation increased milk yield by 0.6 litre per day. Davison *et al.* (1986) supplemented 0.5 or 3.5 kg/d of fresh molasses to dairy cows grazing in a well established pasture at the stocking rate of 1.3 cows/ha for 280 days. Milk yield averaged 14.8 and 12.9 kg/d for the high and low molasses groups ($p < 0.01$), respectively. Milk protein concentration was higher, but fat was lower in the high molasses treatment than in the low treatment. Mclachlan *et al.* (1991) supplemented 2.7 kg DM/d of molasses to dairy cows grazing grass-legume pastures and also found that milk yield and SNF concentration were higher with supplement than with no supplement.

Chopping *et al.* (1976) conducted a large experiment to examine effect of supplementing molasses on milk production for three years. Two breeds of cows (Friesian and Jersey) were employed grazing pastures at the two stocking rates (Friesians, 5.9 and 7.9 cows/ha and Jerseys, 7.9 and 9.9 cows/ha) and each cow received either 0 or 3.6 kg/d of fresh molasses. The results showed that molasses supplement was very effective in increasing milk production. In comparison with no

supplement, supplementing molasses increased milk yield by an average of 0.67 kg milk per kg molasses fed with Friesians and 0.39 kg fed with Jerseys. In addition, supplementing molasses generally increased lactation length and milk SNF concentration.

Table 1.16 *Effect of Supplementing molasses to dairy cows grazing pastures*

References and treatments	Yield (kg/d)	Fat		SNF	
		g/kg	kg/d	g/kg	kg/d
Cowan and Davison (1978)					
control	10.3	38.0	0.39	83.8	0.86
+ 2.1 kg DM/d maize	11.8	38.0	0.45	85.1	1.01
+ 2.2 kg DM/d molasses	11.8	37.0	0.44	84.6	1.00
Chopping *et al.* (1980)[+]					
control	7.2	36.0	0.26	82.0	0.62
+ 1.2 kg FW[#]/d molasses	8.1	38.0	0.29	84.0	0.66
+ 2.4 kg FW/d molasses	8.7	40.0	0.33	84.0	0.70
+ 3.6 kg FW/d molasses	9.4	41.0	0.39	85.0	0.82
Davison *et al.* (1986)					
0.5 kg FW/d molasses	12.9	30.3	0.39	87.8	1.13
3.5 kg FW/d molasses	14.8	28.6	0.43	88.9	1.32
McLachlan *et al.* (1991)					
control	10.4	--	--	80.2	0.83
+ 2.7 kg DM/d molasses	11.2	--	--	81.0	0.91
+ 2.7 kg DM/d molasses & 2.5 kg DM/d maize	12.9	--	--	82.4	1.06

[+] units for milk yield or concentrations of fat and SNF was litre/d or g/litre.

[#] Fresh wight.

1.5.4 *Molassed SBP v. unmolassed SBP or barley*

Molasses is usually mixed with sugar beet pulp to maximize palatability due to its high sugar concentration. The rates of addition of molasses normally range from 100 to 400 g/kg DM. This rate in UK has been at 400 g/kg DM for many years (Hemingway *et al.*, 1986 a).

In UK, a numbers of studies have been done to compare nutritive values for dairy cows between molassed SBP (MSBP) and unmolassed SBP (UMSBP) (Table 1.17). In order to determine the cow response in milk production, Parkins *et al.* (1986) conducted a serial trials in which comparisons were made between MSBP and UMSBP at various dietary levels and additional concentrates were fed with extra milk production over certain amounts. When dairy cows were fed MSBP and UMSBP at the rate of 2.6 kg DM/d with hay or at the rate of 2.9 kg DM/d grass silage in the first two trials, MSBP showed equivalent nutritive values to UMSBP in terms of milk yield and composition. However, further increasing dietary MSBP and UMSBP level to 7.0 kg DM/d with silage in the third trial, the cows fed MSBP produced significantly less milk but higher fat concentration than those fed UMSBP.

Hemingway *et al.* (1986 a) compared milk production of dairy cows fed 3.6 kg/d of air-dried SBP containing 0, 200 or 400 g/kg DM of molasses with grass silage and hay. Milk yield and concentrations of fat and protein were little different among three treatments. In a subsequent study, Hemingway *et al.* (1986 b) reported that milk yield was significantly lower but fat concentration was significantly higher in MSBP than in UMSBP when dairy cows were fed respectively MSBP and UMSBP at the rate of 5.0 kg DM/d. The difference in milk yield was increased with further increment of rate of substitution of MSBP for UMSBP as reported by Sutton *et al.* (1988). Dairy cows were fed 11.6 kg DM/d of concentrates which were based on MSBP or UMSBP at two CP levels (125 and 204 g/kg DM, respectively). The animals fed MSBP produced 1.5 kg/d less milk than those fed UMSBP, fat concentration was 1.5 g/kg higher in MSBP than in UMSBP.

Table 1.17 *Comparisons of feeding MSBP and UMSBP or barley for milk production*

References and treatments (kg/d DM)	Yield (kg/d)	Fat		Protein	
		g/kg	kg/d	g/kg	kg/d
Castle (1972)					
MSBP barley					
0 5.0	18.8	40.0	0.75	30.6	0.58
1.7 3.3	19.2	40.6	0.78	30.0	0.58
3.3 1.7	19.1	40.8	0.78	29.8	0.57
5.0 0	19.3	40.6	0.78	30.1	0.58
Castle *et al.* (1981)					
Trial 1, 4.9 barley	20.5	33.2	0.68	31.6	0.65
5.1 MSBP	20.8	36.4	0.76	31.8	0.66
Trial 2, 4.6 barley	17.9	42.4	0.76	31.8	0.57
4.6 MSBP	18.5	42.4	0.78	32.5	0.60
Parkins *et al.* (1986)					
Trial 1, with hay					
2.6 UMSBP	21.8	38.5	0.84	34.4	0.75
2.6 MSBP	21.7	39.4	0.86	34.7	0.75
Trial 2, with silage					
2.9 UMSBP	20.3	38.4	0.78	34.5	0.70
2.9 MSBP	19.8	39.1	0.77	34.8	0.69
Trial 3, with silage					
7.0 UMSBP	20.2	35.1	0.71	34.2	0.69
7.0 MSBP	19.3	37.8	0.73	34.7	0.67
Hemingway *et al.* (1986 a)$^{+}$					
3.6 UMSBP	19.2	42.8	0.82	34.3	0.68
3.6 MSBP 200	19.6	42.5	0.83	35.2	0.69
3.6 MSBP 400	19.3	42.7	0.82	35.4	0.67
Hemingway *et al.* (1986 b)					
5.0 UMSBP	21.6	39.5	0.85	33.7	0.73
5.0 MSBP	20.8	41.2	0.86	33.8	0.71

Table 1.17 (continued)

References and treatments (kg/d DM)	Yield (kg/d)	Fat		Protein	
		g/kg	kg/d	g/kg	kg/d
Sutton *et al.* (1988)[#]					
barley	26.0	36.4	0.95	30.0	0.78
UMSBP	25.9	37.9	0.98	28.7	0.74
MSBP	24.4	39.4	0.96	29.9	0.73
Beever *et al.* (1991)					
5.8 barley	25.9	44.0	1.14	30.0	0.78
5.6 MSBP	25.0	42.5	1.06	30.9	0.77

[+] in air dry basis. MSBP 200, containing 200 g/kg DM of molasses and 800 g/kg DM of SBP. MSBP 400, containing 400 g/kg DM of molasses and 600 g/kg DM of SBP.

[#] 11 kg/d DM of a concentrate was fed which was based on one of those ingredients, respectively.

From literatures discussed above, it is concluded that the nutritive value of MSBP is equivalent to that of UMSBP in terms of milk production when they are fed under certain amounts (such as 3.6 kg/d in air dry basis), but otherwise MSBP is poorer than UMSBP.

However, MSBP can replace barley at higher rate than UMSBP without depressing milk yield. The experiments presented in Table 1.17 showed that when MSBP replaced barley at rate below 5.6 kg DM/d, there were little differences between the nutritive values of these two feeds and they could replace each other on an equal DM basis in diets for milk production (Castle, 1972; Castle *et al.*, 1981 and Beever *et al.*, 1991). However, higher substitution rate of MSBP for barley could decline milk production as reported by Sutton *et al.* (1988). An average of 1.6 kg/d less milk was produced by dairy cows fed MSBP than those fed barley when 11.6 kg DM/d of concentrates were fed which were based on MSBP and barley, respectively.

In summary, molasses is equal or superior to cereal grain for milk production when molasses replaces cereal grains up to 100 g/kg DM in total complete diets, but otherwise in excess of 100 g/kg DM feeding of molasses can be detrimental to milk production. However, the cow response in milk production to the feeding of molasses depends on sources and amounts of forages in diets. Supplementing molasses to dairy cows grazing tropical pastures improves milk production. MSBP shows an equal nutritive value to UMSBP or barley in terms of milk production at below certain amount in diets, but otherwise MSBP is poorer than UMSBP or barley.

CHAPTER 2

A Comparison of Feeding Three Levels of Molasses to Dairy Cows

2.1 *Introduction*

Molasses contains a high level of sugar and is used as an intake stimulant, binder and dust reducer, as well as an energy source. Many studies have shown equal or greater responses in animal performance in comparison with cereal grains when feeding molasses to dairy cows up to 100 g/kg DM in diets (Scott, 1953 and Morales *et al.*, 1989). However, a higher molasses inclusion in the diet can be detrimental to animal response (Morales *et al.*, 1989).

Feeding high levels of molasses may result in a number of health problems including subclinical ketosis (Losada and Preston, 1974), molasses toxicity (Preston and Willis, 1974) and loose faeces, which is often associated with diarrhoea (Scott, 1953). However, molasses toxicity can be prevented and overcomed in the initial stages by allowing cattle to consume sufficient forage (Pate, 1982). Due to the health problem of the cattle and difficult handling when high levels of molasses are fed, the acceptable amount of molasses fed to dairy cows usually is less than 4 kg/d in fresh basis in commercial farms in UK. The lack of information of feeding high levels of molasses may restrict its inclusion in the diet. So far, most of published studies have focused on feeding molasses to dairy cows at less than 200 g/kg DM. Few publications describe the effects on feed intake and milk production of dairy cows fed molasses at more than that level.

In order to investigate maximum levels of molasses which could be mixed with a grass silage, various proportions of molaferm 20 (a mixture of 800 g/kg of cane molasses with 200 g/kg of condensed molasses solubles supplied by *United Molasses*) ranging from 100 to 600 g/kg DM were mixed with silage before this experiment. As the molaferm 20 proportion increased, the mixes became darker and more sticky. However, up to 500 g/kg DM could still be satisfactorily mixed. Therefore the current experiment was designed to evaluate the effects on feed intake, milk yield and composition of mid-lactation dairy cows fed different amounts, 156, 312 and 468 g/kg DM, of molaferm 20 in grass silage-based complete diets.

2.2 *Material and methods*

2.2.1 *Animals and management*

The dairy cattle used in this experiment were British Friesian/Holstein cows. Average liveweight of the animals was 557 kg (s.e., 53.8) and condition score was 2.4 (s.e., 0.58) at start of this experiment. The lactation number varied from second to fifth and the number of days calved ranged from 148 to 171 at start of this experiment.

The cows were group-housed but individually fed *ad libitum* through transponder operated Calan Gates (Broadbent, McIntosh and Spence, 1970). Water was freely available to all animals from water troughs in the cubicle area. Each cubicle measured 2.20 m (length) x 1.15 m (width), and was covered with sawdust which was raked twice daily and renewed weekly. Slurry passages were scraped twice daily during milking. Complete diets were fed once daily at 10.00 h with additional amounts, if necessary, at 17.00 h. Animals were given sufficient feed to ensure a 5 - 10% refusal. The cows were milked twice daily at 07.15 and 13.30 h in a Herring-bone parlour. Standard milking procedures were used, as recommended in commercial practice. No supplementary concentrates were fed during milking.

2.2.2 *Designs and diets*

Three complete diets were evaluated in a changeover design experiment with 3 week experimental periods. Fifteen multiparous mid-lactation cows were blocked into 5 groups, balanced for milk yield, calving date, parity and liveweight, and then randomly allocated to treatments.

The complete diets consisted of a liquid molasses-based supplement, grass silage and proprietary minerals (Table 2.1). The liquid supplement contained respectively 182, 70 and 748 g/kg DM of soyabean meal, fish meal and molaferm 20 (a mixture of 800 g/kg of cane molasses with 200 g/kg of condensed molasses solubles supplied by *United Molasses*). Three treatments of 'low', 'medium' and 'high' therefore contained molaferm 20 at 156 (low), 312 (medium) and 468 (high) g/kg DM, respectively. The

minerals used in the experiment was '*Maxcare Minerals*' (*BP Nutrition International Ltd*) which contained (g/kg) 170 of calcium (Ca), 60 of phosphorus (P), 60 of magnesium (Mg) and 170 of sodium (Na), respectively.

Table 2.1. *The composition of diets (g/kg DM)*

	Treatments		
	Low	Medium	High
Liquid supplement	209	418	627
Silage	776	567	358
Minerals	15	15	15

The silage was made from the first cut of a perennial ryegrass sward harvested on 19 May 1990 with a drum mower. The crop was wilted for 24 hours and then picked up and chopped to an average length of 20 mm with a forage harvester. A silage inoculant ('*Ecosyl*' *ICI plc*) was added at a rate of 3.3 litres/tonne as it was chopped. The silage was ensiled in an unroofed sleeper-walled silo and sheeted with black polythene which was weighted evenly with tyres. Throughout the experiment, the silage was taken from the silo by a block cutter into a feed wagon and then the silage was unblocked and mixed in the wagon. Each diet was mixed in a *Cormall* mixer for approximately 20 minutes and the diets were weighed out into the 0.61 m x 0.61 m silage bins.

2.2.3 *Measurements*

2.2.3.1 *Feedstuffs sampling and feed intake*

Individual DM intake of the cows was recorded on 4 consecutive days of each week. Silage was sampled daily during the experiment and soyabean meal, fish meal and minerals were sampled twice each period for the determination of their oven DM. The silage proportion in diets was adjusted weekly according to its average DM concentration in the previous week. Samples of fresh mixes offered to and refused by cows were taken daily during intake recording periods for the measurement of their

oven DM. The samples were dried in a forced draught oven (*Unitherm* drying oven) at 100 °C for approximately 24 hours.

Bulked samples of silage, molaferm 20, soyabean meal and fish meal were taken in the last 4 days of each period for chemical analysis which was carried out by the Analytical Service Unit of the Scottish Agricultural College, Auchincruive, Ayr. The silage, soyabean meal and fish meal were oven dried at 100 °C to obtain their DM values. The DM concentration of molaferm 20 was determined by freeze drying the sample for 7 days at -10 °C and all subsequent analyses were corrected to a freeze dried basis. The ME concentrations (MJ/kg DM) of the silage, soyabean meal, fish meal and molaferm 20 were estimated using the equations (1 - 4) given below (J. Dixon, personal communications). All the other analyses were carried out using the techniques described by Alexander and McGowan (1966 and 1969).

Grass Silage: *In vivo* OMD% = (*In vitro* OMD% * 0.90725) + 6.033 (1)

DOMD% = *In vivo* OMD% * (OM g/kg) / 1000 (2)

ME (MJ/kg DM) = DOMD% * 0.16 (3)

Concentrate: ME (MJ/kg DM) = (NCGD% x 0.14) + (AHEE% x 0.25) (4)

Where:

OMD = Organic matter digestibility

DOMD = *In vivo* digestible organic matter in dry matter

NCGD = neutral cellulase and gammanase digestibility

AHEE = acid hydrolysed ether extract

2.2.3.2 *Milk yield and composition*

Milk yields of individual cows were recorded daily throughout the experiment. Approximately 10 ml of milk sample was taken daily from the parlour jar of each cow during last 4 days of each period and stored in an airtight plastic bottle containing

preservative ($K_2Cr_2O_7$) for the determination of fat, protein and lactose concentrations. The bottle were inverted to dissolve the tablet and then stored at 4 °C. Large 100 ml samples were collected in the last day of each period for the analysis of milk casein and non-protein-nitrogen (NPN) and stored in preservative-free bottles. All samples were analyzed in the Food Technology Department at the Scottish Agricultural College, Auchincruive, Ayr.

The 10 ml samples were analyzed for protein, fat and lactose using a Foss Electric Milkoscan machine (Model 203). The analyzer is designed for the fully automatic determination of fat, protein and lactose which is displayed simultaneously by digital readout and automatic printer. The principles of the Milkoscan is similar to that of an infra-red spectrometer in that certain basic components are used:

1. source of radiation
2. optical system for focusing energy
3. radiation detector
4. amplifier and readout display

The relative amount of fat, protein and lactose are detected by the absorption of the infra-red light by a specific chemical bond as presented in Table 2.2.

Table 2.2 *Chemical bonds relative to milk constituents*

Constituents	Chemical bond	Structure	Absorption
Fat	Carbonyl group	R-C=O	5.75 nm
Protein	Amino group	R-N-H \H	6.46 nm
Lactose	Hydroxyl group	R-OH	9.60 nm

The 100 ml samples of milk were used in the analysis for casein, NPN and total protein. The method as described by Rowland (1938) was used which determines the nitrogen (N) distribution in milk. The detailed laboratory methods by which total N, non-casein N, NPN, proteose-peptone plus NPN, and globulin N are determined are available in Davis and McDonald (1953). The basis of the analysis for the total protein, casein and NPN is that initially the total protein concentration was determined using a *Kjeldahl Analysis*. Second, the samples were then treated with acetic acid/sodium acetate buffer to precipitate the casein, and the supernatant was analyzed by Kjeldahl Analysis for non-casein N. Third, the milk was treated with trichloroacetic acid to precipitate total true protein and the supernatant was used to determine NPN. Thus, using these figures it is possible to calculate casein N by difference.

2.2.3.3 *Liveweight gains*

Liveweights of the cows were recorded at approximately 13.30 h on Monday, Wednesday and Friday of each week throughout the experiment (scale weighing to nearest 2 kg). The liveweight gain for each cow within each three week period was calculated by linear regression of weight on time on the appropriate 9 liveweight measurements. The slope of the line was taken to be daily liveweight change. Liveweight change was also calculated using the equations as published by AFRC (1990, see Appendix 1) which is based on the ME balance as ME intake minus ME requirements for maintenance and milk production.

2.2.3.4 *Blood parameters*

Blood samples were taken from the caudal vein of each cow at approximately 10.00 h in the last day of each period. The animals were restrained in a cattle-crush during sampling and the blood was collected in evacuated glass tubes using small bore needles (*'Vacutainer'*, Becton-Dickson (UK) Ltd., Wembley, Middlesex). Blood serum was obtained by allowing a clot to form and then centrifuging at 1300 g for 10 minutes to separate the serum. The serum samples were then sent to the SAC Aberdeen Veterinary Investigation Centre where they were analyzed for total protein, albumen, urea, ß-

hydroxybutyrate, Calcium (Ca), Phosphorus (P), Magnesium (Mg) and Potassium (K).

2.2.3.5. *Calculations of energy and protein utilisation*

The energy balance was calculated according to AFRC Technical Committee on Responses to Nutrients, Report Number 5, Nutritive Requirements of Ruminant Animals: Energy (AFRC, 1990). The details of the equations used for calculating energy for maintenance, lactation and liveweight change are presented in Appendix 2. The efficiencies of utilisation of dietary ME for maintenance (k_m), lactation (k_l) and liveweight change (k_g) are presented in Table 2.3.

Table 2.3 *Efficiencies of utilisation of dietary ME for various functions*

	Treatments		
	Low	Medium	High
k_m	0.707	0.716	0.728
k_l	0.624	0.633	0.645
$k_g = k_l$ when gain was positive			
$k_g = k_l/0.80$ when gain was negative			

The efficiencies of conversion of ME surplus to maintenance into milk was calculated by using the equation (5) when liveweight was included and by using the equation (6) when liveweight was excluded.

Energy in milk / (ME intake - ME for maintenance) (5)

Energy in milk / (ME intake - ME for maintenance - ME for gain) (6)

Where the gain was based on the result obtained by linear regression.

The protein utilisation was calculated according to the metabolizable protein (MP) system as published by AFRC Technical Committee on Responses to Nutrients, Report No. 9, Nutritive Requirements of Ruminant Animals: Protein (AFRC, 1992). The

details of the equations used for calculating MP supplied (MP_s) and MP for maintenance (MP_m), lactation (MP_l), liveweight gain (MP_g) and pregnancy (MP_p) are presented in Appendix 3. The net efficiencies of utilisation of absorbed amino acids (k_n), for lactation (k_{nl}) and for liveweight gain (k_{ng}) and the marginal k_n were calculated according to the following equations.

$$k_n = \text{protein retained in milk and gain} / MP_s \quad (7)$$

$$k_{nl} = \text{protein retained in milk} / MP_s \quad (8)$$

$$k_{ng} = \text{protein retained in gain} / MP_s \quad (9)$$

Marginal k_n = [(MP_l + MP_g) in M - in L treatment] / (MP_s in M - in L treatment)

or [(MP_l + MP_g) in H - in M treatment] / (MP_s in H - in M treatment)(10)

Where the gain was based on the result obtained by linear regression.

The ERDP requirement (ERDPr) was calculated according to the equation (11).

$$ERDPr = FME \times Y_{mcp/fme} \quad (11)$$

Where $Y_{mcp/fme}$ = 11 g/MJ.

The efficiencies used in this experiment for calculating RDP, UDP, ERDP, DUP and FME in individual feedstuffs are shown in Table 2.4.

Table 2.4 *The efficiencies for calculating RDP, UDP, ERDP, DUP and FME*[+].

	dg	F1	F2	F3
Silage	0.80	0.84	0.50	0.65
Molaferm 20	0.80	0.88	0.80	0.94
Soyabean meal	0.70	0.91	0.85	0.91
Fish meal	0.45	0.86	0.85	0.91

[+] ERDP = RDP x F1

DUP = UDP x F2

FME = ME x F3

2.2.5 *Statistical analysis*

Fifteen lactating dairy cows were blocked into 5 groups, balanced by milk yield, calving date, parity and liveweight. The cattle from each group were then allocated at random to the 3 treatments in this complete changeover experiment. Linear regression and analysis of variance were carried out using Genstat 5 (Release 1.3, Lawes Agricultural Trust, Rothamsted, UK, 1988). Cows, their groups and periods were used as blocks (period*(group/cow) in analysis of variance for measurements of feed intake, milk production, liveweight gains, concentrations of some blood parameters, energy balance and protein utilization. Degrees of freedom of the residual was 18. Significant differences between treatment means were determined by Duncan's multiple range test.

2.3 *Results*

2.3.1 *Health*

Four of the fifteen cows suffered from scouring when on the high molaferm 20 treatment, but recovered on changing to either the medium or low molaferm 20 treatment. No other clinical symptoms of ill health were found during the experiment.

2.3.2 *Silage quality and chemical composition of diets*

The average silage quality at feeding is presented in Table 2.5. The silage was well preserved and had a moderate concentrations of ME and CP but a low DM level.

Table 2.5 *The mean silage quality at feeding*

DM (g/kg)	151
OM (g/kg DM)	912
CP (g/kg DM)	159
ME (MJ/kg DM)	10.6
ADF (g/kg DM)	315
NDF (g/kg DM)	489
pH	4.1
Ammonia-N as total-N (g/kg)	112

The average chemical composition of diets is presented in Table 2.6. The dietary CP concentration was similar between three treatments. The ME concentration was increased, while the ADF and NDF levels were decreased as the substitution rate of liquid supplement for silage was raised.

Table 2.6 *The mean chemical composition of diets at feeding (g/kg DM, unless otherwise stated).*

	Treatments		
	Low	Medium	High
Crude protein	160	162	163
ME (MJ/kg DM)	11.1	11.5	12.0
ADF	249	188	127
NDF	462	294	200
Calcium	9	11	12
Phosphorus	4	5	5
Magnesium	3	4	4
Potassium	9	14	20
Sodium	4	4	4

Fresh molaferm 20 accounted for 42, 107 and 217 g/kg of total fresh weight in the low, medium and high molaferm 20 treatment, respectively. As proportion of molaferm 20 increased, the fresh mixes became darker and more sticky. There was no drain of molaferm 20 in the bottom of the bin on any treatment. The DM concentrations of the complete diets increased from 186 for the low and 235 for the medium to 318 g/kg for the high molaferm 20 treatment with the increment of substitution rate of liquid supplement for the less DM silage. Fresh feed intake were significantly higher on the low and medium than on the high molaferm 20 treatment (68.8, 68.9 *v.* 58.5 kg/d, s.e.d. 1.99).

Dietary concentrations of FME, RDP, ERDP, UDP, DUP in this experiment calculated by using the efficiencies in section 2.2.3.5 are presented in Table 2.7. The concentrations of FME and DUP were increased, while ERDP was decreased as increment of the substitution rates of the molasses-based liquid supplement for the grass silage.

Table 2.7 *Dietary concentrations of FME, RDP, ERDP, DUP, UDP (g/kg DM, unless otherwise stated).*

	Treatments		
	Low	Medium	High
RDP	122.1	118.2	114.2
UDP	37.4	43.3	49.2
FME (MJ/kg DM)	7.98	9.00	9.99
ERDP	103.9	101.9	99.6
DUP	23.0	30.3	37.7

2.3.3 *Feed intake*

Treatment means of DM intake is presented in Table 2.8. Total DM intake was increased significantly ($p < 0.01$) with each increment of molaferm 20 in the diets. The intakes of silage and molaferm 20 were corresponding to the total DM intake and their concentrations in diets. The individual intake of molaferm 20 in each treatment-period was various and ranged (kg DM/d) from 1.4 to 2.5 in low molaferm 20 treatment, 4.1 to 5.7 in medium and 6.6 to 10.2 in high.

The weekly means of DM intakes of all cows averaged over three treatments was presented in Figure 2.1. After a week of adaptation of molaferm 20, the cows consumed significantly more feed in the second week than in the first week. The DM intake then gradually increased until the fifth week and afterwards were very consistent.

Table 2.8 *The effects of molaferm 20 inclusion levels on feed intake*

	Treatments			s.e.d.
	Low	Medium	High	
Total	12.8[a]	16.2[b]	18.6[c]	1.03
Silage	9.9[a]	9.3[a]	6.7[b]	0.27
Molaferm 20	1.9[a]	4.9[b]	8.6[c]	0.12

abc means with the same or no superscript in the same row are not significantly different (P>0.05).

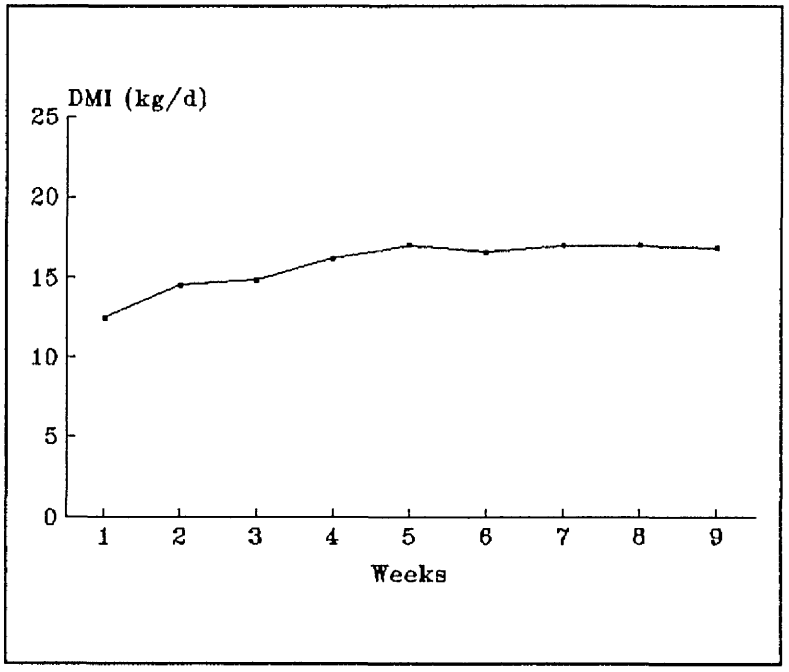

Figure 2.1 Weekly mean of DM intake over three treatments

2.3.4 *Milk yield and composition*

The results are shown in Table 2.9. Milk yield and 4%-fat-corrected yield were significantly increased by adding molaferm 20 up to 312 g/kg DM (medium) ($p<0.01$). No further significant improvement was achieved by the addition of molaferm 20 to 468 g/kg DM (high). Concentrations of fat and lactose in milk were similar between 3 treatments ($p>0.05$), while concentrations of protein and solid-not-fat (SNF) in milk were significantly increased by each increment of molaferm 20 ($p<0.05$).

Table 2.9 *The effects of feeding molaferm 20 on milk yields and composition*

	Treatments			s.e.d.
	Low	Medium	High	
Milk yield (kg/d)	15.5[a]	17.4[b]	17.6[b]	0.53
4%-fat yield (kg/d)	15.3[a]	17.3[b]	17.6[b]	0.91
Milk composition (g/kg)				
Fat	39.6	39.7	40.0	0.70
Protein	31.6[a]	32.7[b]	33.6[c]	0.22
Lactose	44.3	43.9	43.9	0.29

abc means with the same or no superscript in the same row are not significantly different ($P > 0.05$).

The milk constituent yields are showed in Table 2.10. They were significantly increased as addition of molaferm 20 up to 312 g/kg DM in total diets ($p < 0.01$), but were similar ($p > 0.05$) as further increasing molaferm 20 to 468 g/kg DM.

Table 2.10 *The effect of molaferm 20 feeding on milk constituent yields (kg/d)*

	Treatments			s.e.d.
	Low	Medium	High	
Fat	0.61[a]	0.69[b]	0.70[b]	0.037
Protein	0.49[a]	0.57[b]	0.59[b]	0.021
Lactose	0.69[a]	0.76[b]	0.77[b]	0.027

ab means with the same or no superscript in the same row are not significantly different ($P > 0.05$).

Milk concentrations and yields of non-protein-nitrogen (NPN) and casein are presented in Table 2.11. The NPN concentration was similar between the low and medium molaferm 20 treatments ($p > 0.05$), but was significantly increased in the high molaferm 20 treatment ($p < 0.01$). The cows fed the diet containing medium or high molaferm 20 produced significantly higher NPN yield than those fed the diet containing the low

molaferm 20 ($p<0.01$). The effects of the feeding of molaferm 20 on the casein concentrations and yields were similar to the NPN effects.

Table 2.11 *The milk concentrations and yields of NPN and casein*

	Treatments			s.e.d.
	Low	Medium	High	
Composition (g/kg)				
NPN	0.268[a]	0.272[a]	0.285[b]	0.004
Casein	25.17[a]	25.70[a]	26.51[b]	0.388
Yields (g/d)				
NPN	4.169[a]	4.752[b]	4.992[b]	0.167
Casein	390.7[a]	450.3[b]	468.1[b]	20.84

ab means with the same or no superscript in the same row are not significantly different ($P>0.05$).

2.3.5 *Liveweight gain*

Liveweight gains were determined by linear regression of weight on time. The slope of the line was taken to be daily liveweight change. Mean liveweight gains of the animals over 3 periods are presented in Figure 2.2. They were 0.04, 0.99 and 0.86 kg/d (s.e.d., 0.17) of the low, medium and high molaferm 20 treatments, respectively. The gains increased significantly from the low to medium molaferm 20 treatment($p<0.01$), but decreased slightly from the medium to high molaferm 20 treatment ($p>0.05$).

The liveweight gains of the animals were also estimated by the energy balance as also presented in Figure 2.2. They were 0.12, 0.99 and 1.91 kg/d (s.e.d., 0.14) of the low, medium and high molaferm 20 treatments, respectively. The results were very similar to those obtained by the linear regression on the low and medium molaferm 20 treatments, but the gain on the high treatment estimated by the energy balance was double higher than that obtained by the linear regression.

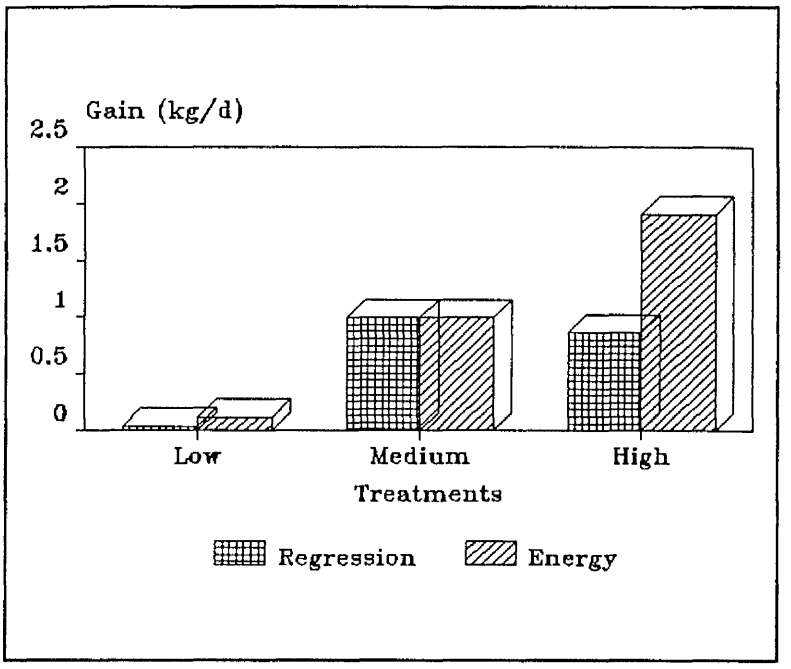

Figure 2.2 Liveweight gains of cows estimated by linear regression and energy balance

2.3.6 *Energy utilisation*

The calculated energy utilization is presented in Table 2.13. Total ME intakes of the cattle were calculated from dietary ME concentrations and total DM intakes. The dietary ME concentration and total DM intake were increased with higher substitution rate of liquid supplement for silage, resulting in a significantly higher ME intake. The animals with higher milk yield and higher liveweight gain in the medium or high molaferm 20 treatment utilized significantly more energy for their milk production and on liveweight gain than those in the low molaferm 20 treatment. ME intake in the low and medium molaferm 20 treatments were all used for maintenance and production, while ME intake was substantially wasted in the high molaferm 20 treatment (ME in excess / ME intake, 0.04 and 0 *v.* 0.21). However, the efficiency of ME conversion surplus to maintenance into milk production was significantly lower with each increment of molaferm 20 level in total diets ($p<0.01$) when estimated liveweight gains by regression of the cattle were included. The efficiency was the highest in the medium molaferm 20 treatment when estimated liveweight gain by regression was excluded because of the highest gain in this group of the animals. The ratio of NE/ME was

significantly lower in the high molaferm 20 treatment than in the medium or low molaferm 20 treatment ($p < 0.05$).

Table 2.13 *ME intake and its partition*

	Treatments			s.e.d.
	Low	Medium	High	
		--- MJ/d ---		
ME Intake	142[a]	186[b]	223[c]	5.23
ME for maintenance	57	57	56	0.57
ME for lactation	77[a]	87[b]	85[b]	3.20
ME for gain	2[a]	43[b]	36[b]	8.07
ME in excess	6[a]	-1[a]	46[b]	10.3
NE for maintenance	40[a]	41[b]	41[b]	0.12
NE for lactation	48[a]	55[b]	55[b]	2.07
NE for gain	1[a]	27[b]	24[b]	5.33
Efficiency				
+ gain	0.565[a]	0.426[b]	0.329[c]	0.032
- gain	0.578[a]	0.640[a]	0.420[b]	0.060
NE/ME	0.627[a]	0.661[a]	0.538[b]	0.035

abc means with the same or no superscript in the same row are not significantly different ($P > 0.05$).

2.3.7 *Protein utilisation*

The calculated protein utilisation is showed in Table 2.14. The metabolisable protein supplied (MP_s) was significantly increased with each increment of dietary molaferm 20 level, which resulted from higher DM intake and also from higher concentrations of DUP and FME in total diets as the substitution rate of liquid supplement for silage increased. MP required (MP_r) was significantly lower in the low molaferm 20 treatment

Table 2.14 *The protein utilization*

	Treatments			s.e.d.
	Low	Medium	High	
		--- g/d ---		
$ERDP_s$	1325[a]	1652[b]	1850[c]	45.4
$ERDP_r$	1120[a]	1605[b]	2040[c]	46.4
ERDP excess	205[a]	47[b]	-190[c]	10.5
MP_s	1054[a]	1582[b]	1958[c]	44.5
MP_r	1022[a]	1335[b]	1343[b]	50.9
MP_m	314[a]	317[b]	318[b]	0.99
MP_l	655[a]	767[b]	796[b]	34.4
MP_g	5[a]	203[b]	181[b]	40.7
MP_p	48	48	48	--
MP excess	32[a]	247[b]	615[c]	65.6
k_n	0.626[a]	0.613[a]	0.499[b]	0.036
k_{nl}	0.621[a]	0.485[b]	0.407[c]	0.026
k_{ng}	0.005[a]	0.128[b]	0.092[b]	0.033
Marginal k_n	0.587	0.019		

abc means with the same or no superscript in the same row are not significantly different ($P>0.05$).

than in the medium or high molaferm 20 treatment due to the significantly lower MPr for lactation and for liveweight gain. As a result, MP was in excess on all the three treatments but particularly on the high molaferm 20 treatment. Therefore, the efficiency

of utilization of MP (k_n) was significantly depressed ($p<0.01$) by the high molaferm 20 inclusion (468 g/kg DM). The efficiency of utilization of MP for lactation (k_{nl}) significantly declined at each increment of molaferm 20 level in total diets ($p<0.01$). When the comparison was made between the low and medium or between the medium and high molaferm 20 treatments, the marginal k_n was very different. The marginal k_n was 0.587 when molaferm 20 was added from 156 to 312 g/kg DM in total diets, but was decreased to nearly zero (0.019) when molaferm 20 was added from 312 to 468 g/kg DM.

Both ERDP supply and requirement were significantly increased as a result of higher DM intake caused by increasing substitution rate of liquid supplement for silage. However because of higher FME concentrations in higher molaferm 20 inclusions in diets (see Table 2.5), ERDP was slightly deficient in the high molaferm 20 treatment, while was in excess in the low molaferm 20 treatment.

2.3.8 *Blood parameters*

The results of concentrations of blood parameters are presented in Table 2.15. Treatment mean values of all parameters investigated in this experiment over 3 periods were within normal range, except protein which was slightly higher.

Table 2.15 *The effects of feeding molaferm 20 on concentrations of some blood parameters of cows*

	Treatments			s.e.d.	Normal range
	Low	Medium	High		
Total protein (g/l)	83.9	84.6	84.8	1.77	55-73
Albumin (g/l)	34.0	34.4	34.2	1.02	29-39
BHBA[#] (mmol/l)	0.47	0.51	0.57	0.06	<0.8
Urea (mmol/l)	4.1	4.1	4.3	0.21	2.0-6.6
Calcium (mmol/l)	2.2[a]	2.2[a]	2.4[b]	0.08	2.2-2.6
Phosphate (mmol/l)	1.7	1.6	1.7	0.07	1.7-2.2
Magnesium (mmol/l)	1.0	1.0	1.0	0.04	0.8-1.1
Potassium (mmol/l)	4.5	4.4	4.6	0.17	3.5-5.5

[#] ß-hydroxybutyrate

ab means with the same or no superscript in the same row are not significantly different (P>0.05).

Blood concentrations of Ca were significantly higher on the high molaferm 20 treatment than on the medium or low molaferm 20 treatment ($p<0.05$). The other parameters investigated showed little differences between the 3 treatments ($p>0.05$), although blood concentrations of protein and ß-hydroxybutyrate increased as molaferm 20 level increased. However, the period average concentrations of urea (3.79, 4.21 or 4.45 mmol/l (s.e.d., 0.211) at the 1st, 2nd or 3rd period) and ß-hydroxybutyrate (0.46, 0.44 or 0.65 mmol/l (s.e.d., 0.057)) over three treatments significantly increased ($p<0.05$), while Mg (1.22, 1.05 or 0.76 (s.e.d., 0.041)) decreased ($p<0.01$), as the experiment progressed (Figure 2.3).

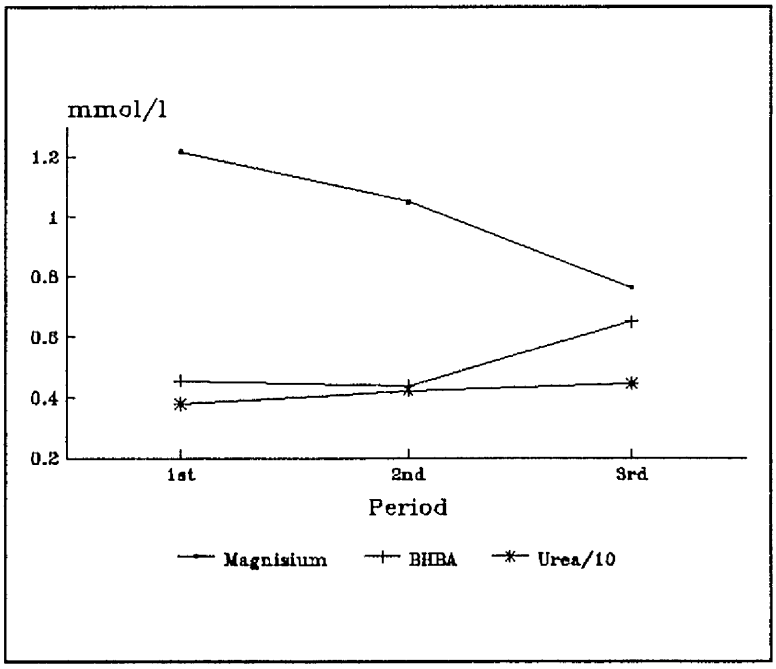

Figure 2.3 Period mean concentrations of some blood parameters over three treatments (urea/10 = blood urea concentration / 10).

2.4 *Discussion*

2.4.1 *Health*

Feeding high levels of molasses to cattle can result in loose faeces (Scott, 1953). In this experiment, four of fifteen cows suffered from scouring on the high molaferm 20 treatment although they recovered when they were fed relatively lower molaferm 20 levels in the next period. Feeding high levels of molasses to dairy cows can also increase an incidence of ketosis (Losada and Preston, 1974). However, in this experiment no clinical symptoms of ketosis were found and all the blood parameters investigated were within the normal range, although blood ß-hydroxybutyrate concentration increased as the dietary molaferm 20 levels increased. It may be that the short feeding (3 weeks) did not allow time for clinical symptoms to develop. This may be supported by the fact that the overall average blood concentrations of ß-hydroxybutyrate and urea over 3 treatments significantly increased ($p < 0.05$) and Mg significantly decreased ($p < 0.01$), as the experiment progressed (Figure 2.4).

2.4.2 *Feed intake*

Molasses is usually used as an intake stimulant and energy source for cattle, due to its higher concentrations of water soluble carbohydrates. In general, addition of molasses up to 100 g/kg DM to diets improves the feed intake of dairy cows, but levels over 100 g/kg DM show more variable results. In this experiment and a similar study of Krohn *et al.* (1985) with sugar beet molasses, total DM intake increased linearly as the levels of molasses increased in the diets. In the condition of free choice of molasses and dried grass, Berry and Peña (1981) reported that cows consumed 6.15 kg DM/day of molasses and total DM intake was significantly higher in comparison with those fed the control diet without molasses. No comparison was made with an equivalent level of cereal/fibre supplement in the current experiment, but there have been some studies available to describe it. With silage *ad libitum*, Mayne (1989) reported that cows which were offered 3 kg/day of concentrate and 2 kg/day of molasses consumed more feed than those which were fed 3 kg DM/day of concentrate without molasses, but not in comparison with those which were offered 5 kg/day of concentrate. Wood (1990) did

not observe a significant difference in feed intake between cows offered 2.9 kg DM/day of molasses and 3.1 kg DM/day of concentrate and those offered 6.0 kg DM/day of concentrate. The studies quoted above showed that when feeding high levels of molasses, feed intake may be improved by the molasses when replacing forage, but not when replacing concentrate.

However, attention should be paid to essential forage intake when high levels of molasses are fed to dairy cows. In this experiment and the studies quoted above in which molaferm 20 replaced forage, increasing molaferm 20 levels in the diets decreased forage intake of dairy cows although the total DM intake was increased. Krohn *et al.* (1985) replaced grass silage with molasses from 0 to 480 g/kg DM of total diets and then found a linear decrease in silage intakes from 7.7 to 1.2 kg DM/day. Berry and Peña (1981) also reported that cows fed a diet with molasses *ad libitum* consumed less dried grass than those fed a diet without molasses (5.7 *v.* 8.3 kg DM/day). Furthermore, high levels of easily fermentable carbohydrates tend to depress rumen pH and increase VFA concentration in the rumen, then resulting in a lower cellulolytic activity (Steg, Honig and Visser, 1985). A decrease in digestibilities of dietary fibre components was reported in reviews by Harris and Van Horn (1982) in dairy cattle and Pate (1982) in beef cattle when high levels of molasses were fed. Both decreases in forage intake and fibre digestion when cattle are fed high levels of molasses may be two of factors which are detrimental to the performance and health of dairy cattle.

2.4.3 *Milk yield and composition*

Milk yield was significantly increased by increasing dietary molaferm 20 levels from 156 to 312 g/kg DM ($p<0.01$), but no further significant improvement was achieved by increasing dietary molaferm 20 levels to 468 g/kg DM. A similar increase in milk yield was recorded by Berry and Peña (1981) when cows were fed on a diet with molasses *ad libitum* compared with the control diet without molasses. Krohn *et al.* (1985) in a similar study with this experiment also reported that milk yield increased

until addition of 320 g/kg DM of molasses, but then decreased as further addition of molasses to 480 g/kg DM.

In current experiment and the trial of Krohn *et al.* (1985), the energy intakes measured were higher as substitution rate of molasses for silage increased. The calculated ME intake of the cows on the low, medium and high molaferm 20 treatments were respectively 142, 186 and 223 MJ/day in this experiment. The calculated intake of metabolisable protein (MP) was also significantly increased as substitution rate of molaferm 20 for silage increased in this experiment (1054, 1582 and 1958 g/d on the low, medium and high molaferm 20 treatment, respectively). However, improvements in milk yield were only achieved by additions of up to 320 g/kg DM of molaferm 20 in both trials. The lack of significant response beyond this inclusion may result from detrimental effects on health (*e.g.*, some scouring on the high molaferm 20 treatment in this experiment), and from reduction of fibre digestibility. Krohn *et al* (1985) reported that digestibility of ADF or NDF were 41 or 51% lower when feeding molasses from 320 to 480 g/kg DM.

A third possible factor may be a decrease in the efficiency of dietary protein utilization when high levels of molasses were included in diets. As discussed in Chapter 1 (Literature Review), many studies have shown that an increase in molasses inclusion in the total diet decreases the dietary CP digestion and possibly depresses the microbial protein synthesis. In this experiment the calculated utilization of dietary protein gave the further evidence for this. The net efficiency of protein utilization (k_n) was significantly decreased (0.617 *v.* 0.510) and the marginal k_n was almost zero (0.019) when dietary molaferm 20 levels increased from 312 to 468 g/kg DM.

The efficiency of energy utilisation may also have decreased at the higher molasses levels. Lofgreen and Otagaki (1960 a) reported that the net energy of molasses was significantly depressed as the molasses levels increased in diets from 100 to 300 g/kg DM. In a subsequent trial in beef heifers, Lofgreen (1965) noted that the decline of NE

values of molasses occurred at inclusions in excess of 200 g/kg DM. Huhtanen (1987) also reported that the calculated energy values for milk production were decreased by addition of molasses to both barley and sugar beet pulp diets. In this experiment the calculated energy utilization of the diets showed the similar results. The efficiency of conversion of ME surplus to maintenance into milk when including the liveweight gain was significantly decreased ($p<0.01$) with each increment of molaferm 20 level in diets. However, when excluding the liveweight gain this efficiency was similar between the low and medium molaferm 20 treatments ($p>0.05$), while significantly decreased when comparison was made between the high and medium or low molaferm 20 treatment ($p<0.01$). The ratio of NE/ME showed the similar pattern. The ME partition also gave the evidence that the efficiency of energy utilization was lower in the high molaferm 20 treatment than in the medium or low molaferm 20 treatment. In the high molaferm 20 treatment 21% of intaked ME was wasted, while the intaked ME in the medium or low molaferm 20 treatments all was almost used for maintenance and production.

Milk protein concentrations significantly responded to dietary molaferm 20 levels in this experiment. This results agreed with those of Krohn *et al.* (1985), which showed a linear increase in milk protein concentrations as molasses replaced grass silage from 0 to 480 g/kg DM. The increase may be associated with more energy intake as molasses substitution rates increased in both experiments. In a study in which 2.9 kg DM/day molasses replaced the same amount of a concentrate with a similar ME concentration with molasses, Wood (1990) reported there was little different in milk protein concentration of dairy cows. A positive correlation coefficients of 0.42 were reported between milk protein concentration and intake of ME by Spömdly (1986) or net energy for lactation by Emery (1978). In this experiment both energy concentrations of diets and feed intake were higher with increasing molasses levels in the diets, which resulted in a higher calculated ME intake of the animals.

The lack of response in milk fat concentrations to dietary molaferm 20 levels in this

experiment disagreed with the results of Krohn *et al.* (1985) who found a linear increase as molasses replaced silage from 160 to 480 g/kg DM. However, when molasses substituted concentrate as reported by Wood (1990) and concentrate or silage as reported by Mayne (1989), there was no evidence of significant differences in milk fat concentrations. A number of Australian studies with grazing cows also showed equivocal effects of feeding molasses (0-3.5 kg/day) on the milk fat concentration. The variable responses in the milk fat concentration to feeding molasses may be due to differences in the basal diets. A number of dietary factors may be responsible, such as amount of roughage, intake and forage:concentrate ratio (Sutton, 1989). Morales *et al.* (1989) reported that the source of roughage was also a factor modifying the effect of molasses on milk fat concentration.

2.5 *Conclusion*

Replacement of grass silage in the diets with molaferm 20 increases ME intake, and enhances milk production and milk protein concentration without depressing milk fat concentration. Lactating dairy cows can consume high molaferm 20 up to 8.6 kg DM/day (468 g/kg DM in total diets), but intakes in excess of 4.9 kg DM/day (312 g/kg DM in total diets) resulting in scouring.

The lack of significant responses in milk production when molaferm 20 was included in diets at excess of 312 g/kg DM could be attributed to a lower dietary protein utilization and a lower molaferm 20 energy efficiency, except the detrimental effect on health. The both factors therefore need to be determined.

CHAPTER 3

The Responses of Dairy Cows Fed a Large Amount of Molasses to Dietary Levels of Effective Rumen Degraded Dietary Protein and Digestible Undegraded Protein

3.1 *Introduction*

Experiment 1 investigated the substitution rates of a liquid molasses-based supplement for grass silage in 3 complete diets which contained similar crude protein (CP) levels. The experiment showed that dairy cows could be fed high molaferm 20 levels in diets up to 468 g/kg DM, but feed intake and milk production showed little advantage at this level compared with 312 g/kg DM of molaferm 20 although the ME intake of the cattle fed the former diet was significantly higher than those fed the latter diet. One of factors responsible for this may be the ability of molaferm 20 to decrease dietary CP utilization in comparison with cereal grains.

As discussed in Chapter 1 (Literature Review), a high molasses inclusion in a diet for cattle can decrease dietary protein utilisation in comparison with cereal grains by three ways: (1) depressing digestibility of dietary CP, (2) decreasing efficiency of conversion of dietary urea to microbial protein and (3) possibly declining the microbial protein synthesis in the rumen. These decreases together with relatively low CP concentration in cane molasses or in molaferm 20 (see Table 1.1) could exacerbate the deficiency of the metabolisable protein for dairy cows fed high levels of molasses. Therefore, the protein requirements of the cattle may be higher than recommended when the animals are fed a large proportion of molasses (Pate, 1982). As a result, an increase in effective rumen degraded dietary protein (ERDP) in the diet containing a high level of molasses may result in a more efficient synchronisation between the nitrogen and energy requirements of the rumen microorganism. This improvement together with a higher digestible undegraded protein (DUP) concentration in the diets would provide more MP for milk production.

Experiment 2 was designed to investigate the responses of dairy cows in early lactation given diets containing 310 g/kg DM of molaferm 20 but different dietary concentrations of ERDP or DUP achieved by adding urea or soyabean meal.

3.2 *Material and methods*

3.2.1 *Animals and management*

The dairy cattle used in this experiment were British Friesian/Holstein cows which all calved in September 1991. Eighteen cattle were used which included 6 heifers and 12 multiparous cows. The mean liveweight of heifers was 503 kg (s.e., 24.2) and cows was 556 kg (s.e., 53.7) and the number of days calved for all the cattle ranged from 22 to 41 at start of this experiment. The lactation number of multiparous cows varied from second to seventh. The weekly average milk yield prior to start of this experiment was 22.4 kg/d (s.e., 1.1) in heifers and 29.9 kg/d (s.e., 2.2) in multiparous cows.

Management of the animals was the same as for experiment 1 (section 2.2.1).

3.2.2 *Design and diets*

Three complete diets were evaluated in a changeover design experiment with 4 week experimental periods. Eighteen cattle were blocked into 6 groups, balanced for lactation number, milk yield, calving date, and liveweight. The animals from each group were then randomly allocated to the treatments. Each treatment included 2 heifers and 4 multiparous cows.

The complete diets were designed to contain 2 levels of ERDP and 2 levels of DUP (Table 3.1). The 3 treatments of low, high and high ERDP concentrations but low, low and high DUP concentrations were referred to as L/L, H/L and H/H, respectively. The diets were based on grass silage, molasses, barley, soyabean meal, urea and minerals (Table 3.2). The molasses used in this experiment was molaferm 20 (a mixture of 800 g/kg of cane molasses with 200 g/kg of condensed molasses solubles) supplied by the *United Molasses*. Urea used was *'ICI Urea'* (*ICI plc*) which contains a guaranteed minimum N level of 46.4 g/kg and minerals used was *'Maxcare Cattle'* (*BP Nutrition International Ltd*) which contains (g/kg) 945 of ash, 170 of Ca, 60 of P, 60 of Mg, and 120 of Na, respectively.

Table 3.1 *The designed dietary concentrations of ERDP and DUP*

	Treatments (Dietary ERDP/DUP)		
	L/L	H/L	H/H
ERDP	Low	High	High
DUP	Low	Low	High

Table 3.2 *The composition of diets (g/kg DM)*

	Dietary ERDP/DUP		
	L/L	H/L	H/H
Silage	460	460	460
Molaferm 20	310	310	310
Barley	192	180	26
Soyabean meal	22	23	189
Urea	1	12	0
Minerals	15	15	15

The silage was made from the first cut perennial ryegrass sward harvested on 16 May 1991 with a drum mower. It was wilted, precision chopped and then mixed with 3.2 litres/tonne of a formic acid additive (*'Add-Safe', BP Nutrition International Ltd*). The silage was ensiled in an unroofed sleeper-walled silo and sheeted with black polythene which was weighted evenly with tyres. Throughout the experiment, the silage was taken from the silo by a block cutter into a feed wagon and then the silage was unblocked and mixed in the wagon.

Before feeding, the diets were completely mixed in a *Cormall* mixer, and then weighed out into the silage bins. To ensure that the urea was completely mixed in diets, it was dissolved in cold water and then the urea water was mixed with concentrates and

molaferm 20 for a while before silage was loaded. Each diet was mixed for approximately 20 minutes.

3.2.3 *Measurements*

3.2.3.1 *Feed intake*

For the details for samplings of feedstuffs and measurements of nutrient concentrations in feedstuffs, please see section 2.2.3.1. The ME concentration of barley was measured by the same method as soyabean meal.

3.2.3.2 *Milk yield and composition*

For the details for samplings of milk and measurements of milk concentrations of fat, protein, lactose and casein, non-protein-nitrogen (NPN), please see section 2.2.3.2.

3.2.3.3 *Liveweight and condition score*

Liveweights and condition scores were recorded at approximately 13:30 h on Monday, Wednesday and Friday of each week throughout the experiment. The Liveweight gain of each cow within each 4 week period was calculated by linear regression of weight on time on the appropriate 12 liveweight measurements. The slope of the line was then taken to be daily liveweight change. The liveweight change was also calculated by using the equations as reported by AFRC (1990, see appendix 1) which is based on the energy balance.

The condition scores of the animals recorded were based on the tail-head system as described by Mulvany (1977).

3.2.3.4 *Blood parameters*

For details for samplings of blood and measurements of blood concentrations of total protein, albumen, urea, ß-hydroxybutyrate, Ca, Mg, K and phosphate, please see section 2.2.3.4.

3.2.3.5 *Calculation of energy and protein utilization*

For details of calculation of energy balance, please see section 2.2.3.5. In this experiment, the efficiencies of utilisation of dietary ME for maintenance (k_m) and lactation (k_l) were 0.727 and 0.644 for the L/L or H/H treatment and 0.723 and 0.640 for the H/L treatment, respectively.

For details of calculation of protein utilization, please see section 2.2.3.5. In this experiment, the following equations were used for estimation of RDP, UDP, ERDP, DUP and FME in barley:

$$RDP = CP * 0.85 \quad (1)$$

$$UDP = CP - RDP \quad (2)$$

$$ERDP = RDP * 0.88 \quad (3)$$

$$DUP = UDP * 0.80 \quad (4)$$

$$FME = ME * 0.94 \quad (5)$$

3.2.4 *Statistical analysis*

Twelve multiparous cows and six heifers were blocked into 6 groups, balanced by lactation number, milk yield, calving date and liveweight. The cattle from each group were then allocated at random to the 3 treatments in this complete changeover experiment. Linear regression and analysis of variance were carried out using a computer package (Genstat 5, Release 1.3, Lawes Agricultural Trust, Rothamsted, UK, 1988). Period, cows and their group were used as blocks (period*(group/cow) in analysis of variance for DM intake, milk production, liveweight gain, blood concentration, energy balance and protein utilization. Degrees of freedom of the residual was 22. Significant differences between treatment means were determined by Duncan's multiple range test.

3.3 *Results*

3.3.1 *Health*

No clinical symptoms of the ill health of the cattle were found during the experiment. The average condition score of the animals was 2.13.

3.3.2 *Feed chemical composition*

The mean silage quality at feeding is presented in Table 3.3. The silage was well preserved and had a high ME concentration and an over moderate CP level.

The mean chemical composition of diets at feeding is shown in Table 3.4. The three diets contained similar concentrations of DM, ME, ADF and minerals, but different CP levels as expected.

The mean dietary concentrations of FME, RDP, UDP, ERDP, DUP at feeding in this experiment calculated by using the efficiencies as presented in sections 3.2.3.5 and Table 2.6 of Chapter 2 are shown in Table 3.5. Their concentrations attained the aim at design of this experiment.

Table 3.3 *The mean silage quality at feeding*

DM (g/kg)	190
OM (g/kg DM)	921
CP (g/kg DM)	162
ME (MJ/kg DM)	11.8
ADF (g/kg DM)	250
NDF (g/kg DM)	385
pH	4.2
Ammonia-N/Total-N (g/kg)	106

Table 3.4 *The mean chemical composition of diets (g/kg DM, unless otherwise stated)*

	Dietary ERDP/DUP		
	L/L	H/L	H/H
DM (g/kg)	329	331	330
ME (MJ/kg DM)	12.0	11.8	12.0
CP	134	158	181
ADF	150	148	142
NDF	219	217	219
Ash	92	92	98
Calcium	9.2	8.4	8.4
Phosphorus	3.9	3.9	3.9
Magnesium	3.7	3.7	3.7
Sodium	4.2	4.2	4.2
Potassium	32.5	32.4	34.9

Table 3.5 *The dietary concentrations of FME, RDP, UDP, ERDP, DUP at feeding (g/kg DM, unless otherwise stated)*

	Dietary ERDP/DUP		
	L/L	H/L	H/H
RDP	108	132	137
UDP	26	26	44
FME (MJ/kg DM)	9.7	9.5	9.6
ERDP	93	117	121
DUP	17	17	32

3.3.3 *Feed intake*

No drain of molaferm 20 was found in the bottom of the bins. Fresh molaferm 20 accounted for averaged 121 g/kg total fresh weight of mixes. The DM concentrations of the complete diets of fresh mixes were very similar between three treatments (329, 331 and 330 g/kg in the L/L, H/L and H/H treatments, respectively). The fresh feed intakes were significantly higher as dietary protein levels increased from the L/L to H/H treatment (51.8, 54.6 and 57.2 kg/d (s.e.d., 1.18), respectively).

Treatment means of total DM intakes are shown in Figure 3.1. They were 17.1, 18.1 18.9 kg DM/d (s.e.d., 0.25) in the L/L, H/L and H/H treatments, respectively. The intakes were significantly higher with increment of dietary ERDP level ($p < 0.001$), and further significantly increased ($p < 0.01$) as the dietary DUP levels were raised. Silage intakes in the L/L, H/L and H/H treatments were respectively 8.0, 8.5 and 8.8 kg DM/d (s.e.d., 0.11) and molaferm 20 were 5.2, 5.5 and 5.8 kg DM/d (s.e.d., 0.08). The averaged molaferm 20 intakes for individual cows during the various treatment periods ranged from 4.4 to 6.6 kg DM/d.

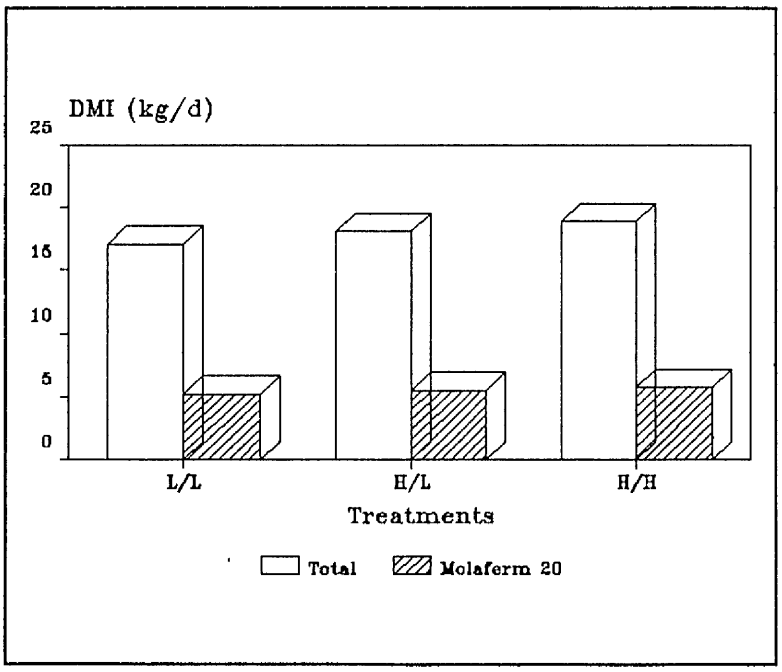

Figure 3.1 The treatment means of total DM intakes of cows.

Weekly means of total DM intakes of all cattle over three treatments are presented in Figure 3.2. The DM intakes were gradually increased and reached the peak at the seventh week (12 weeks since calving) after start of feeding of molaferm 20, and then gradually decreased as the experiment progressed.

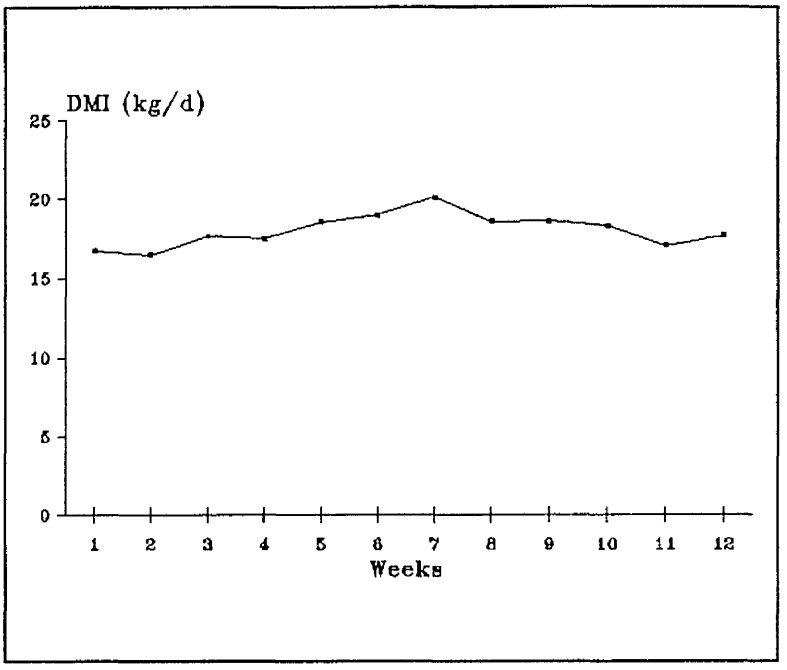

Figure 3.2 The weekly means of DM intakes of all cattle over 3 treatments.

3.3.4 *Milk production*

Milk yields and composition are presented in Table 3.6. Both milk yield and 4%-fat corrected yield were significantly higher by increasing ERDP levels in the diets ($p<0.05$). Milk yield was increased by 0.057 kg/d with each increment of 1 g/kg DM of ERDP derived from urea. Both milk yield and 4%-fat corrected yield were further increased by raising dietary DUP levels ($p<0.001$). Milk yield was increased by 0.162 kg/d with each increment of 1 g/kg DM of DUP in the H/H compared to the H/L treatment. An increase in ERDP level in diets enhanced milk protein concentration ($p<0.05$), but raising DUP concentration showed little effect on milk protein concentration ($p>0.05$). Milk concentrations of fat and lactose were similar between the three treatments ($p>0.05$).

Table 3.6 *Milk yields and composition*

	Dietary ERDP/DUP			s.e.d.
	L/L	H/L	H/H	
Yield (kg/d)				
Milk	19.20[a]	20.57[b]	23.00[c]	0.521
4%-fat milk	23.37[a]	25.26[b]	28.11[c]	0.662
Composition (g/kg)				
Fat	48.68	48.81	48.81	0.724
Protein	33.65[a]	34.31[b]	34.44[b]	0.314
Lactose	47.70	47.89	47.96	0.282

abc means with the same or no superscript in the same row are not significantly different ($p > 0.05$).

Milk constituent yields are shown in Table 3.7. All constituent yields were significantly higher when the dietary ERDP concentrations were increased ($p < 0.05$) and further increased when the dietary DUP concentrations were increased ($p < 0.001$).

Table 3.7 *Milk constituent yields (kg/d)*

	Dietary ERDP/DUP			s.e.d.
	L/L	H/L	H/H	
Fat	0.94[a]	1.01[b]	1.12[c]	0.027
Protein	0.65[a]	0.71[b]	0.79[c]	0.019
Lactose	0.92[a]	0.98[b]	1.11[c]	0.026

abc means with the same or no superscript in the same row are not significantly different ($p > 0.05$).

Milk concentrations and yields of NPN and casein are shown in Table 3.8. Milk NPN concentration was significantly higher ($p<0.001$) as the dietary ERDP level was increased, but no further increase occurred when the dietary DUP concentration was raised. Milk casein concentration was similar between the three treatments ($p>0.05$). However, milk yields of NPN and casein were significantly higher ($p<0.01$) with each increment of dietary protein level.

Table 3.8 *The milk concentrations and yields of NPN and casein*

	Dietary ERDP/DUP			s.e.d.
	L/L	H/L	H/H	
Composition (g/kg)				
Casein	26.00	26.24	26.59	0.319
NPN	0.22[a]	0.27[b]	0.27[b]	0.005
Yields (g/d)				
Casein	499[a]	540[b]	610[c]	16.9
NPN	4.24[a]	5.52[b]	6.19[c]	0.171

abc means with the same or no superscript in the same row are not significantly different ($p>0.05$).

3.3.5 *Liveweight gain*

Liveweight gains of the cows are shown in Figure 3.3. The liveweight gains were determined by linear regression of weight on time. The slope of the line was taken to be the daily liveweight change. The mean liveweight gains of the animals over 3 periods were 0.18, 0.63 and 0.77 kg/d (s.e.d., 0.195) in the L/L, H/L and H/H treatments, respectively. The cows in the H/L or H/H treatment gained more weight than those in the L/L treatment ($p<0.01$), but there was no significant difference in liveweight gains between the H/L and H/H treatments.

The liveweight gains of the cattle were also estimated by the energy balance (Figure

3.3). They were 0.91, 0.89 and 0.88 kg/d (s.e.d., 0.084) for the L/L, H/L and H/H treatments, respectively. The method of estimation gave higher results than the linear regression, especially in the L/L treatment in which the gain obtained by the energy balance was 5 time higher than that obtained by the linear regression. The liveweight gains measured by the energy balance were similar between 3 treatments ($p > 0.05$).

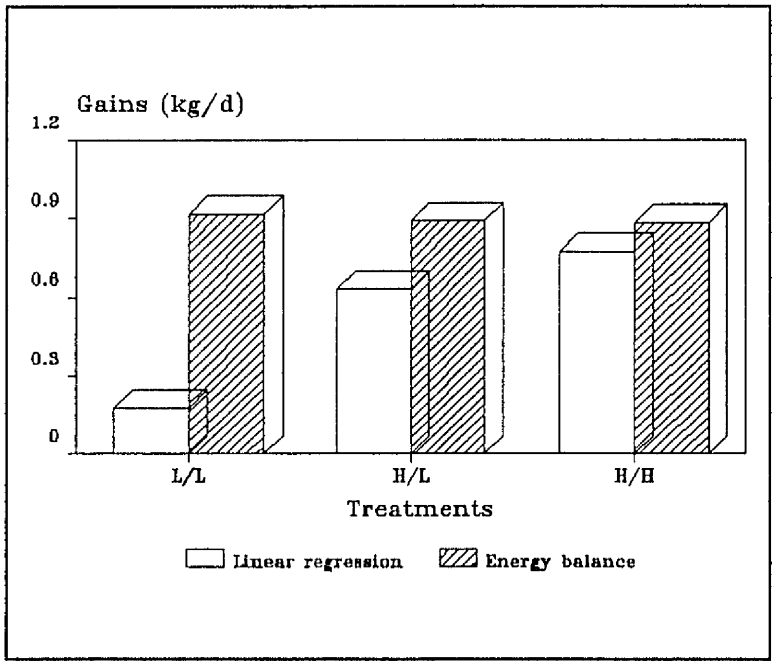

Figure 3.3 The treatment means of liveweight gains of the cattle over 3 periods.

3.3.6 *Energy utilization*

The calculated energy utilization is shown in Table 3.9. The total ME intakes of the cattle were calculated from the dietary ME concentrations and total DM intakes. The ME intakes were significantly higher in the H/H treatment than in the L/L or H/L treatment ($p < 0.01$). The energy retained in milk or liveweight of the animals was estimated from the milk production or liveweight gain. More energy was retained in milk or liveweight as dietary protein levels increased. ME intake was in excess in all the 3 treatments, but the ME was used more effectively with each increment of dietary protein. In the L/L treatment 18% of the ME intake was not utilized, compared to respectively 8% and 6% in the H/L and H/H treatments.

The efficiency of ME conversion surplus to maintenance into milk production was similar ($p > 0.05$) between the three treatments when estimated liveweight gain by regression was included (Energy in milk/(ME intake - ME for maintenance)), but this value was significantly lower ($p < 0.05$) in the L/L treatment than in the H/L or H/H treatment when estimated liveweight gain by regression was excluded (Energy in milk/(ME intake - ME for maintenance - ME for gain)). The ratio of NE/ME was higher in the H/H ($p < 0.01$) or H/L ($p < 0.05$) treatment than in the L/L treatment.

Table 3.9 *ME intake and its partition*

	Dietary ERDP/DUP			s.e.d.
	L/L	H/L	H/H	
		----- MJ/d -----		
ME intake	205[a]	214[a]	227[b]	4.65
ME for maintenance	55[a]	56[b]	56[b]	0.19
ME for lactation	104[a]	113[b]	125[c]	2.77
ME for gain	9[a]	28[b]	33[c]	8.04
ME in excess	37[a]	17[b]	13[b]	7.67
NE for maintenance	40	40	40	0.14
NE for lactation	67[a]	72[b]	80[c]	1.78
NE for gain	5[a]	17[b]	21[b]	5.34
Efficiency				
+ gain	0.447	0.456	0.468	0.016
- gain	0.475[a]	0.554[b]	0.580[b]	0.036
NE/ME	0.546[a]	0.603[b]	0.621[b]	0.025

abc means with the same or no superscript in the same row are not significantly different ($p > 0.05$).

3.3.7 *Protein utilization*

The calculated protein utilization is shown in Table 3.10. Metabolizable protein supply (MP_s) was calculated from dietary ERDP and DUP concentrations and DM intake. MP supply was significantly higher ($p<0.01$) with increment of both dietary ERDP and DUP levels. The MP required for lactation was higher ($p<0.01$) with each increment

Table 3.10 *The protein utilization*

	Dietary ERDP/DUP			s.e.d.
	L/L	H/L	H/H	
		---- g/d ----		
MP supply	1369[a]	1592[b]	1960[c]	37.1
MP requirement	1278[a]	1368[b]	1482[c]	29.5
MP for maintenance	311[a]	313[ab]	314[b]	1.1
MP for lactation	912[a]	984[b]	1100[c]	26.8
MP for gain	7[a]	23[b]	20[ab]	6.3
MP for pregnant	48	48	48	--
MP in excess	91[a]	224[b]	478[c]	35.5
ERDP supply	1590[a]	2118[b]	2287[c]	44.7
ERDP requirement	1825[a]	1891[a]	1996[b]	41.2
ERDP in excess	-235[a]	227[b]	291[c]	14.4
k_n	0.671[a]	0.633[a]	0.571[b]	0.019
k_{nl}	0.666[a]	0.618[b]	0.561[c]	0.018
k_{ng}	0.005[a]	0.014[b]	0.010[ab]	0.004
Marginal k_n	0.395	0.307		

abc means with the same or no superscript in the same row are not significantly different ($p>0.05$).

of dietary protein level, which resulted in a significantly higher total MP requirement (MP_r). However, this increase in MP requirement did not keep in line with the increase in MP supply. Therefore, the MP in excess was significantly higher ($p<0.001$) as dietary protein levels were increased. In the H/H treatment 24% of MP supply was not utilized and 14% in the H/L treatment, compared to 7% in the L/L treatment. As a result, the efficiency of utilization of MP (k_n) was significantly lower in the H/H than in the L/L ($p<0.001$) or in the H/L treatment ($p<0.05$). The efficiency of utilization of MP for lactation (k_{nl}) was significantly decreased with each increment of dietary protein levels. The marginal k_n therefore was higher as increasing dietary ERDP than increasing dietary DUP level.

Both ERDP supply and ERDP requirement depend on the DM intake of the animals. They were both significantly increased with each increment of dietary protein level. Because of high FME concentrations in the diets, however, ERDP supply was deficient for the cattle fed the diet containing the low ERDP, but in excess for the animals fed the diets containing high ERDP.

3.3.8 *Blood parameters*

The results of concentrations of blood parameters investigated in this experiment are presented in Table 3.11. Treatment means of all parameters were within normal ranges, except protein concentration which was slightly higher at the start of the experiment and for all three treatments.

Treatment means of blood concentrations of total protein and urea were higher as dietary protein levels were increased ($p<0.05$). However, the dietary protein concentrations did not show a consistent effect on blood concentrations of albumen, ß-hydroxybutyrate, Ca, Mg, K and phosphate.

Table 3.11 *Blood parameters*

	Start	Dietary ERDP/DUP			s.e.d.	Normal range
		L/L	H/L	H/H		
Protein (g/l)	75.77[a]	79.73[a]	78.99[a]	85.49[b]	2.177	55-73
Albumen (g/l)	31.60	31.17	31.41	32.61	1.076	29-39
Urea (mmol/l)	4.74[ab]	2.74[c]	4.22[b]	5.28[a]	0.304	2.0-6.6
BHBA[#] (mmol/l)	0.66	0.55	0.66	0.56	0.138	<0.8
Calcium (mmol/l)	2.36	2.25	2.28	2.38	0.099	2.2-2.6
Magnesium (mmol/l)	1.17[a]	1.12[ab]	1.07[b]	1.12[ab]	0.036	0.8-1.1
Phosphate (mmol/l)	2.17[a]	2.09[ab]	1.91[b]	2.14[ab]	0.109	1.7-2.2
Potassium (mmol/l)	--	4.85[a]	4.52[b]	4.70[ab]	0.115	3.5-5.5

[+] Personal communication, N. MaCleod

[#] ß-hydroxybutyrate

abc means with the same or no superscript in the same row are not significantly different ($p>0.05$).

Period means of blood concentrations of urea, Mg and K over 3 treatments are showed in Figure 3.4. The urea concentration averaged over three treatments gradually increased ($p < 0.05$), while the concentrations of Mg and K gradually decreased as the experiment progressed. However, the blood concentration of ß-hydroxybutyrate averaged over three treatments did not show a consistent change (0.66, 0.80, 0.33 and 0.65 (s.e.d., 0.125) at start, period 1, 2 and 3, respectively).

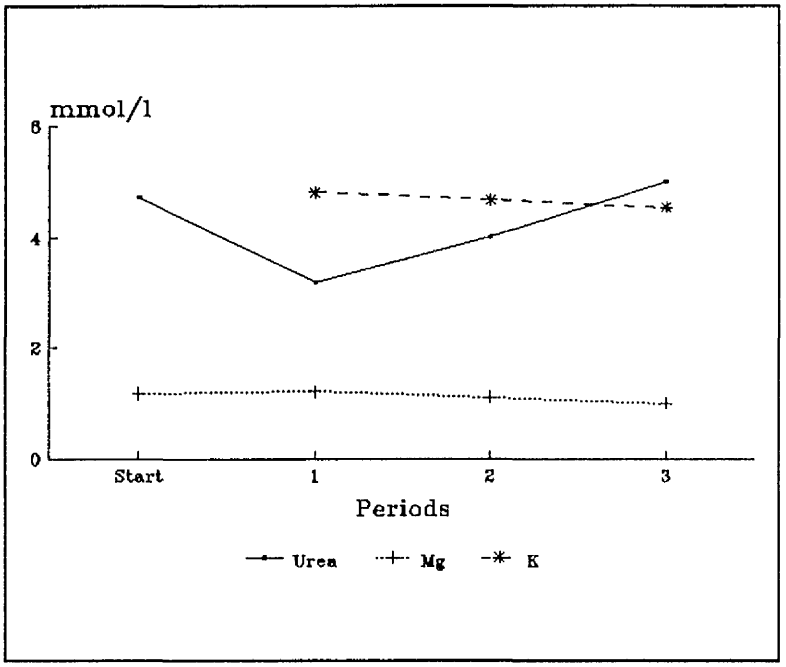

Figure 3.4 The period means of concentrations of some blood parameters over 3 treatments.

3.4 *Discussion*

3.4.1 *Health*

The high concentrations of sugar and K in molasses was reported by Briggs and Heller (1943) to be responsible for laxative property of molasses in an experiment with lambs using purified diets. In experiment 1 mid-lactation cows suffered from some scouring when molaferm 20 was fed at 468 g/kg DM, but recovered when they were changed to a diet containing 312 g/kg DM of molaferm 20. A similar result was observed in this experiment. None of early lactation cows suffered from scouring when molaferm 20

was fed at 310 g/kg DM, and no other clinical symptoms of the ill health of the animals occurred during this experiment as well. Furthermore, the blood concentrations of parameters investigated in this experiment all were within normal range. These evidences may suggest that early lactation cows could be fed molaferm 20 up to 310 g/kg DM for 3 months on grass silage-based diets without any health problems.

As the experiment progressed, the period means of blood urea concentration over three treatments was significantly increased, while Mg decreased. This result was similar to Experiment 1. However, the period mean of blood ß-hydroxybutyrate concentration over three treatments did not show a consistent increase in this experiment as the experiment progressed, which disagreed to results obtained in Experiment 1 with mid-lactation cows. One of factors caused the disagreement may be attributed to the different lactation stage of cows between two experiments.

3.4.2 *Feed Intake*

Addition of molasses to the diets of dairy cattle in excess of 100 g/kg DM to replace cereal grains can cause various decreases in total DM intake as discussed in Section 1.4.1. These decreases are modified by many dietary factors including molasses types. In this experiment, however, total DM intakes of cows were within the normal range as observed at Crichton Royal Farm of the Scottish Agricultural College. The weekly means of DM intake over the 3 treatments were consistent with stage of lactation. The intake was gradually increased until the seventh weeks (12 weeks since calving) and then declined down towards to end of the experiment (Figure 3.2). Furthermore, the DM intake in this experiment was higher than prediction DM intake (Figure 3.5) estimated by using the equations as reported by ARC (1980) (for details of equations, please see Appendix 4). These evidences may suggest that feeding early lactation cows at 310 g/kg DM of molaferm 20 shows little detrimental to total DM intake in this experiment on the grass silage-based complete diets for three months.

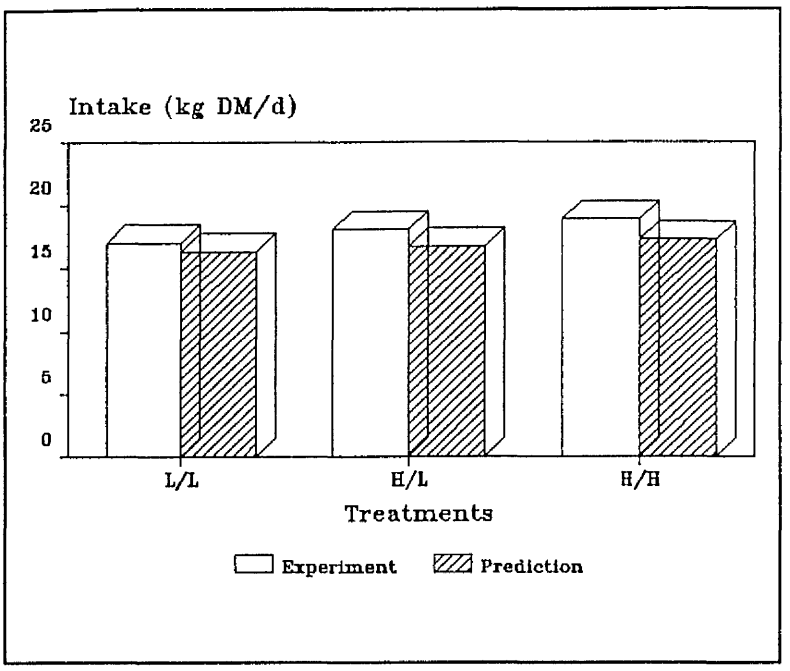

Figure 3.5 Comparison of DM intake between the experiment and prediction.

Low protein concentration of the feed depresses voluntary feed intake of the ruminant (Forbes, 1986). The low dietary protein concentration may cause the ERDP deficiency and subsequently depress the activity of the rumen microflora, which in return results in a lower rate of digestion of cellulose. In this experiment, ERDP requirement (ERDPr) was calculated by the equation (ERDPr = FME x 11) as reported by AFRC (1992). According to the calculation the cows fed the control diet suffered from ERDP deficiency (Table 3.10). Addition of urea increased the dietary ERDP concentration and enhanced total DM intake by 0.042 kg/d with each increment of 1 g/kg DM of dietary ERDP derived from urea. This result supported those obtained by Wohlt and Clark (1978) and Wohlt *et al.* (1978) in dairy lactation cows fed no molasses. In those two experiments the DM intakes of dairy cows were positively related to addition of urea to a control diet containing either 92 or 135 g/kg DM of crude protein. Wohlt *et al.* (1978) also observed a significant increase in the ruminal ammonia-N concentration and DM digestibility by the addition of urea. The urea acts primarily by increasing rates of digestion and passage after absorption from the intestines and secretion in saliva (Forbes, 1986).

However, there is no benefit to performance of dairy cows by addition of urea when a control diet contains certain amount of ERDP. Burroughs *et al.* (1975) summarized 17 experimental comparisons in 9 experiments and reported that benefits were obtained from addition of urea in nearly all experiments when the control diets contained less than 110 to 120 g/kg DM of CP, but otherwise no benefits occurred when the control diets contained more natural protein. However, a positive response in DM intake occurred in this experiment with increment of dietary ERDP by addition of urea to the control diet containing 134 g/kg DM of CP. The reason for this increase will be discussed in next section. Furthermore, ERDP intake in excess of the requirement of the rumen microorganisms may cause surplus of ammonia in the rumen and then increase the burden of the liver to metabolize it to urea. A significantly higher blood urea concentration by an increase in dietary ERDP level was evident in this experiment. Therefore, feeding diets containing too much ERDP possibly cause a deleterious effects on health and fertility of cows and result in a loss of energy (Twigge and Van Gils, 1988).

An increase in dietary DUP concentration by addition of soyabean meal to the diet containing 158 g/kg DM of CP resulted in a significantly higher total DM intake in this experiment. This result agreed those obtained by Wohlt *et al.* (1978) and Wohlt and Clark (1978) with similar treatments with this trial but no molasses inclusion in diets. Sloan *et al.* (1988 a) fed dairy cows different levels of fish meal and soyabean meal to achieve various RDP and UDP concentrations in diets containing no molasses and reported that an increase in dietary UDP enhanced silage intake, but had no effect on concentrate intake. In a subsequent study, Sloan *et al.* (1988 b) investigated responses of dairy cows to various UDP concentrations in diets containing 50 to 100 g/kg DM of molasses by addition of urea and fish meal/soyabean meal. They reported that dairy cows fed high UDP diets consumed more concentrate than those fed low UDP diets, while silage intake was similar between two groups of cattle. The reason that an increase in dietary DUP concentration increases the total DM intake is not quite clear, but possibly an increase in DUP by adding soyabean meal or fish meal results in a

better balance between protein and energy. Preston and Leng (1984) reported that an imbalance of protein/energy ratio in the absorbed nutrients decreased the intake of the basal diet and correction of the imbalance would undoubtedly lead to a more efficient utilization of the feed. Egan and Moir (1964) compared the effects of duodenal infusion of casein, urea or phosphate (as control) into sheep on a low protein forage (6.5 g/kg DM). Casein had a rapid effect and within a few hours intake was increased. Egan (1965) concluded that casein alleviated a protein deficiency and thereby stimulated the rate of removal of metabolites by tissues and thus stimulated intake.

3.4.3 *Milk Production*

The high milk fat concentration (48 g/kg) produced by cows fed all three diets could be due to the other factors rather than the high molaferm 20 inclusion in this experiment, since no evidence has shown that the feeding of high levels of molasses results in a high increase in milk fat concentration as discussed in Chapter 1 (Literature Review). In fact, the silage used in this experiment was very good and had a high ME concentration (11.8 MJ/kg DM) and the average milk fat concentration in the whole herd at Crichton Royal Farm in the year when this experiment was conducted was higher (45 g/kg) than normal.

Burroughs *et al.* (1975) summarized responses of dairy cows fed no molasses to addition of urea to 17 basal diets in 9 experiments and reported that benefits were obtained from addition of urea in nearly all diets containing less than 110 to 120 g/kg DM of protein, but otherwise no benefits occurred with diets containing higher levels of proteins. This conclusion was supported by Wohlt *et al.* (1978) and Wohlt and Clark (1978) in dairy cows fed a restricted amount of concentrates containing no molasses and maize silage containing 81 g/kg DM of CP *ad libitum*. A significant response in milk yield was observed when urea at the rate of 15 g/kg DM of concentrate was added to the control concentrate containing 92 g/kg DM of CP, while no significant difference in milk yield occurred when the same amount of urea was added to the control concentrate containing 135 g/kg DM of CP. Cameron *et al.* (1991) further reported that

addition of urea at the rate of 7.5 g/kg DM to the control diet containing 149 g/kg DM of CP had no significant effect on OM digestion in the rumen and microbial N passage to the small intestine. As a result, milk yield and milk concentrations of fat and protein were similar between 2 treatments.

However, in this experiment milk yield and milk protein concentration significantly responded to addition of urea to the control diet containing 134 g/kg DM of CP. Milk yield was increased by 0.057 kg/d with each increment of 1 g/kg DM of ERDP derived from urea. Since dietary DUP concentration was the same between L/L and H/L treatments, lower milk production in the treatment L/L must be due to the low dietary ERDP concentration. The ERDP concentration in the L/L diet was deficient which might restrict the microbial growth (Table 3.10). As a result, less microbial protein was available for the cows, which in turn resulted in a lower MP supply and further a lower milk production. However, the net efficiency of utilisation of MP supply (k_n) was similar between 3 treatments, while the efficiency for lactation (k_{nl}) was lower as dietary ERDP level was increased. This was because of a very low MP supply in the L/L treatment.

The disagreement of this experiment with the above studies was probably due to the high molaferm 20 inclusion in the diets in this trial. As discussed in Section 1.3.3., in comparison with cereal grains a high molasses inclusion in a diet for cattle can decrease N utilisation in three ways. First, feeding molasses depresses digestibility of dietary CP. The decrease was reviewed by Pate (1982) in the range of 5 to 15% when moderate to high levels of molasses are fed to cattle. Wing *et al.* (1988) reported that CP digestibility was negatively related with dietary molasses levels in dairy cows. Second, feeding molasses decreases the efficiency of converting dietary urea to microbial protein. This decrease was due to higher N output in urine (Oldham *et al.*, 1977), which implicates that higher ammonia-N escapes from the rumen into blood, leaving less ammonia-N available for the microbial growth. Last, feeding molasses possibly declines the microbial protein synthesis. The lower efficiency of microbial protein

synthesis may be due to low pH in the rumen (Russel and Domlrowski, 1980) or a high production of lactate via the acrylate pathway with a resultant lower supply of adenosine triphosphate (ATP) (Tamminga, 1979 cited by Huhtanen, 1988). A high molasses inclusion in a diet for cattle could devalue the dietary protein, leaving less ERDP available for the microbial synthesis. Therefore, in this experiment an increase in dietary ERDP concentration by adding urea significantly increased DM intake and milk production. The dietary protein concentration should be higher than recommended when high levels of molasses are given to dairy cows.

Due to limited body reserves, protein-deficient diets quickly influence the nutritional and productive status of cows in early lactation. Microbial synthesis in the rumen is incapable of providing sufficient MP to the high producing dairy cows. The importance of utilizing bypass protein (DUP) is that it can escape degradation by rumen microorganisms and be utilised more efficiently in the intestinal tract of the host animals. Therefore, the ratio of degradable to undegradable protein supplements can be altered to determine the ratio necessary to support high milk production (Roffler *et al.*, 1978). In this experiment, dietary ERDP concentrations were similar between the H/L and H/H treatments and were in excess in both treatments. If the protein source is ignored in application of the metabolizable protein system as reported by AFRC (1992), the significantly higher milk production in the treatment H/H than H/L must be due to higher dietary DUP concentration in the former than latter. Milk yield was increased by 0.162 kg/d with each increment of 1 g/kg DM of DUP in the treatment H/H surplus to H/L. However, the net efficiency of utilization of MP (k_n) and the efficiency for lactation (k_{nl}) were significantly lower as dietary DUP increased (Table 3.10), which indicates that a higher amount of MP might not be utilized in the treatment H/H than H/L ($p<0.001$).

This result agreed to those obtained by Wohlt and Clark (1978) and Wohlt *et al.* (1978) with similar treatments with this experiment but no molasses inclusion in diets. When dairy cows were fed molasses at 50 to 100 g/kg DM of total diets, Sloan *et al.* (1988

a) reported that dairy cows given a concentrate containing high UDP level produced more milk ($p < 0.05$) than those fed a concentrate containing low UDP level, while milk concentrations of fat and protein were similar between 2 groups of cows. Recently, a number of studies have been carried out in USA to estimate the effects of dietary protein degradability on milk production by using various sources of proteins including soyabean meal and fish meal (Wohlt *et al.*, 1991; Keery *et al.*, 1993 and Roseler *et al.*, 1993). The results were inconsistent. Keery and Amos (1993) explained that many factors contribute to inconsistent responses of early lactation dairy cows to an increase in dietary UDP concentration, such as insufficient intakes of RDP and ruminal fermentable OM, sufficient UDP in the control diet and utilisation of cows with low yield potential or past peak yield.

3.5 *Conclusion*

The feeding of 310 g/kg DM of molasses had no detrimental effect on the health of early-lactation dairy cows in the grass silage-based diets. The results of the experiment showed a significant response in food intake and milk production to an increase in dietary ERDP and a greater response to an increase in dietary DUP when the cows were fed this high level of molasses.

The increases in food intake with increment of dietary ERDP and DUP may implicate a greater microbial growth in the rumen of the cattle and then a greater fibre digestion. This needs to be determined.

CHAPTER 4

The Effects of Dietary Fermentable Metabolizable Energy Levels on Milk Production and Milk Uric Acid Concentration of Dairy Heifers Fed a Large Amount of Molasses

4.1 *Introduction*

A high molasses inclusion in a cattle diet can depress the energy utilisation of molasses as discussed in Chapter 1 (Literature Review). The first experiment of this study also showed that feeding molaferm 20 at 468 g/kg DM significantly decreased the calculated efficiencies of energy utilization of the diet in comparison with 312 g/kg DM. However, molasses contains a large proportion of non-structural carbohydrates (sugars) which have fast fermentation rates in the rumen (Johnson, 1976). When a diet is formulated to have a low fermentable metabolizable energy (FME) concentration, addition of a large amount of molasses may partially make up the deficiency of the energy requirement of the microorganisms in the rumen of dairy cattle.

Unprotected tallow is widely used as a feed ingredient to meet the energy requirement of high milking dairy cows. Since its hydrolysed products (free fatty acids) in the rumen can inhibit microbial activity and dietary fibre digestion, its inclusion in a diet for dairy cows has been extensively investigated. Ørskov and Ryle (1990) stated that the dietary inclusion of unprotected lipids up to 70 g/kg DM is acceptable for the ruminant. In addition the digesta outflow rate from the rumen could be increased when a large amount of molasses is fed. This could shorten the retention time of the free fatty acids from hydrolysis of unprotected tallow in the rumen and minimize the negative effect of the tallow on dietary fibre digestion and microbial activity.

Previous experiments have shown that molasses could be fed to dairy cows up to 312 g/kg DM of total diets without adverse effect (Experiment 1) and a CP level at 160 g/kg DM in the diet containing 310 g/kg DM of molasses was satisfactory for feed intake and milk production of dairy cows (Experiment 2). The current experiment was therefore designed to evaluate the effects of dietary FME concentrations produced by altering dietary levels of unprotected tallow on feed intake, milk production and milk uric acid concentration of dairy cows fed diets containing 310 g/kg DM of molasses and 160 g/kg DM of CP.

4.2 *Material and methods*

4.2.1 *Animals and management*

The dairy cattle used in this experiment were British Friesian/Holstein first lactation heifers. Average liveweight of the animals was 521 kg (s.e., 47.4), condition score was 2.37 (s.e., 0.25) and the number of days calved ranged from 42 to 87 (average 69 (s.e., 16.8)) at the start of the experiment. The weekly average milk yield prior to start of this experiment was 22.1 kg/d (s.e., 2.3).

For details of management of the animals, please see section 2.2.1.

4.2.2 *Design and Diets*

Prior to start of the experiment, all animals were fed a complete diet containing (g/kg DM) 310 of molaferm 20, 460 of grass silage, 70 of soyabean meal, 145 of barley and 15 of mineral supplement for adaptation of molaferm 20 for a week. Then three complete diets were evaluated in a changeover design experiment with 4 week experimental periods. Fifteen lactating dairy heifers were blocked into 5 groups, balanced for milk yield, calving date and liveweight, and then randomly allocated to the treatments.

The complete diets consisted of grass silage, soyabean meal, barley, oat feed, urea, mineral supplement and tallow-molaferm 20 blends (Table 4.1). The mineral supplement used in this experiment was '*Maxcare Minerals*' (*BP Nutrition International Ltd*). The tallow-molaferm 20 blends were mixtures unprotected tallow with molaferm 20 (molaferm 20 is a mixture of 800 g/kg of cane molasses with 200 g/kg of condensed molasses solubles) supplied by *United Molasses*. The unprotected tallow in the blends was a tallow mixture from cattle, pig and horse. The three blends were Nil blend (ordinary molaferm 20), Low blend (low amount of tallow mixed with molaferm 20) and High blend (high amount of tallow mixed with molaferm 20), respectively. The three diets each contained one of the blends and therefore had different FME concentrations but the similar ME levels. The three treatments are referred as H_{FME}

Table 4.1 *Composition of diets (g/kg DM)*

	Treatments		
	H_{FME}	M_{FME}	L_{FME}
Silage	460	460	460
Nil blend	310	--	--
Low blend	--	312	--
High blend	--	--	323
Soyabean meal	65	70	75
Barley	130	72	15
Oat feed	20	70	110
Urea	--	1	2
Minerals	15	15	15

(high dietary FME concentration), M_{FME} (medium dietary FME concentration) and L_{FME} (low dietary FME concentration), respectively. In order to keep the same molaferm 20 concentration (310 g/kg DM) in the three diets, the dietary proportions of blends were calculated according to total sugar concentrations in the blends. The fatty acid profile of the tallow are shown in Tables 4.2.

The silage was made from the first cut of a perennial ryegrass sward harvested on 18-19 May 1992 with a drum mover. The crop was wilted for 24 hours and then picked up and chopped to an average length of 20 mm with a forage harvester. A silage inoculant (*'Ecosyl' ICI plc*) was added at a rate of 3.0 litres/tonne. The silage was ensiled in an unroofed sleeper-walled silo and sheeted with black polythene which was weighted evenly with tyres.

Before feeding, the diets were separately mixed respectively in a *Cormall* mixer, and weighed out into silage bins. To ensure that the urea was completely mixed in diets, it was dissolved in cold water and then the urea water was mixed with concentrates and

the tallow-molaferm 20 blends for a while before silage was loaded. The each diet was mixed for approximately 20 minutes.

Table 4.2 *Fatty acid profile of the tallow*[+]

Fatty acids	Proportion (%)
Lauric (C_{12})	1
Palmitic (C_{16})	25
Palmitoleic ($C_{16:1}$)	4
Stearic (C_{18})	18
Oleic ($C_{18:1}$)	41
Linoleic ($C_{18:2}$)	8
Linolenic ($C_{18:3}$)	1

[+] Data supplied by *United Molasses*

4.2.3 *Measurements*

4.2.3.1 *Feed intake and composition*

For the details for sampling of feedstuffs and measurements of nutrient concentrations in feedstuffs, please see section 2.2.3.1. The ME concentrations of barley and oat feed were measured by the same method as soyabean meal.

4.2.3.2 *Milk yield and composition*

For the details of sampling of milk and measurements of milk concentrations of fat, protein, lactose and casein, non-protein-nitrogen (NPN), please see section 2.2.3.2.

Milk samples for analysis of uric acid concentration from individual cows were taken into plastic bottles containing no preservative in the last two days of each period and then stored in a freezer. The measurement of uric acid concentration was carried out using the techniques described by Marsili, Ostapenko, Simmons and Green (1981) at

Department of Food Science and Technology of the Scottish Agricultural College, Auchincruive, Ayr.

4.2.3.3 *Liveweight gain and condition scores*

For the details of measurements of liveweight gain and condition score of the cattle, please see section 3.2.3.3.

4.2.3.4 *Blood parameters*

For details of sampling of blood and measurements of blood concentrations of total protein, albumen, ß-hydroxybutyrate (BHBA), urea, Ca, Mg, K and phosphate, please see section 2.2.3.4.

4.2.3.5 *Calculations of energy and protein utilisation*

For details of calculation of the energy balance, please see section 2.2.3.5. The efficiencies of utilization of dietary ME for maintenance (k_m), Lactation (k_l) and liveweight gain (k_g) in this experiment are shown in Table 4.3.

Table 4.3 *The efficiencies of utilization of dietary ME for various functions*

	Treatments		
	H_{FME}	M_{FME}	L_{FME}
k_m	0.719	0.718	0.716
k_l	0.636	0.635	0.633

$k_g = k_l$ when gain was positive

$k_g = k_l/0.80$ when gain was negative

For details of calculation of protein utilization, please see section 2.2.3.5. The efficiencies used in the Scottish Agricultural College for calculations of RDP, UDP, ERDP, DUP and FME in individual feedstuffs are shown in Table 4.4.

Table 4.4 *The efficiencies for calculations of RDP, UDP, ERDP, DUP and FME*[+]

	dg	F1	F2	F3
Silage	0.80	0.84	0.50	0.65
The blends	0.80	0.88	0.80	--
Soyabean meal	0.70	0.91	0.85	0.91
Barley	0.85	0.88	0.94	0.80
Oat feed	0.80	0.88	0.94	0.80
Urea	1.00	0.80	--	--

[+] ERDP = RDP x F1 DUP = UDP x F2 FME = ME x F3

FME concentrations in the blends were calculated by the equation FME = 0.9 x ME - ME_{fat} where ME_{fat} = 35 KJ/g of fat (AFRC, 1992)

4.2.4 *Statistical analysis*

Five groups of 3 dairy heifers were allocated to the 3 x 3 Latin Square Design experiment with 4 week experimental periods. Linear regression and analysis of variance were carried out by using Genstat 5 (Release 1.3, Lawes Agricultural Trust, Rothamsted, UK, 1988). The dairy cattle, their group and period were used as blocks (period*(group/cow) in analysis of variance for measurements of feed intake, milk production, milk uric acid concentration, liveweight gain, concentrations of blood parameters, energy balance and protein utilisation. Degree of freedom of the residual was 18. Significant differences between treatment means were determined by Duncan's multiple range test.

4.3 *Results*

4.3.1 *Health*

No clinical symptoms of the ill health of the heifers were found during the experiment. The average condition score of the animals was 2.50 (s.e., 0.41).

4.3.2 *Silage quality and chemical composition of blends and diets*

The average chemical compositions of tallow-molaferm 20 blends at feeding are presented in Table 4.5. The concentrations of DM, OM, ME and oil was increased but CP was decreased with each increment of tallow levels.

The average silage quality at feeding is presented in Table 4.6. The silage was well preserved and had high concentrations of DM, ME and CP.

The average chemical compositions of the diets at feeding are shown in Table 4.7. CP and ME levels were similar between diets and NDF and ADF were slightly increased as the tallow levels raised. The oil concentration derived from the supplemented tallow were 0, 12 and 24 g/kg DM in the H_{FME}, M_{FME} and L_{FME} diets, respectively.

Dietary concentrations of RDP, UDP, ERDP, DUP and FME calculated by using the efficiencies in Section 4.2.3.5 in this experiment are presented in Table 4.8. ERDP and DUP concentrations in diets were similar between diets, while FME was 0.5 MJ/kg DM lower with each increment of dietary tallow levels.

Table 4.5 *The chemical composition of tallow-molaferm 20 blends*

	Nil blend	Low blend	High blend
Dry Matter (g/kg)	688	691	693
Organic Matter (g/kg DM)	895	909	915
CP (g/kg DM)	107	102	96
ME (MJ/kg DM)	11.9	12.7	13.4
Oil (g/kg DM)	0	33	64

Table 4.6 *The mean silage quality at feeding*

DM (g/kg)	234
OM (g/kg DM)	906
CP (g/kg DM)	168
ME (MJ/kg DM)	11.6
ADF (g/kg DM)	263
NDF (g/kg DM)	423
pH	4.1
Ammonia-N / Total-N (g/kg)	53

Table 4.7 *The mean chemical composition of diets (g/kg DM, unless otherwise stated)*

	Treatments		
	H_{EME}	M_{EME}	L_{EME}
CP	160	159	159
ME (MJ/kg DM)	11.6	11.5	11.4
NDF	243	269	289
ADF	145	158	168
Oil	29	41	53
Ca	9	9	9
P	4	4	4
Mg	4	4	4
Na	4	4	4
K	30	29	29

Table 4.8 *The dietary concentration of RDP, UDP, ERDP, DUP and FME*

	Treatments		
	H_{FME}	M_{FME}	L_{FME}
RDP (g/kg DM)	136	135	135
UDP (g/kg DM)	34	34	34
FME (MJ/kg DM)	9.4	8.9	8.4
ERDP (g/kg DM)	109	108	108
DUP (g/kg DM)	23	23	23

4.3.3 *Feed intake*

The liquid feeds did not separate from the complete diets. The fresh blends of tallow-molaferm 20 accounted for 159, 160 and 164 g/kg of total fresh weight of mixes as the dietary tallow levels increased. The average DM concentrations of the complete diets of fresh mixes were very similar between the 3 treatments (372, 373 and 374 g/kg in the H_{FME}, M_{FME} and L_{FME} treatments, respectively). The fresh feed intakes showed no significant difference between the 3 treatments (46.2, 47.4, 48.3 kg/d (s.e.d., 2.02), respectively).

Treatment means of total DM intakes of the cattle are shown in Figure 4.1. Total DM intakes of the heifers on the H_{FME}, M_{FME} and L_{FME} treatments were respectively 18.1, 17.4 and 17.4 (s.e.d., 0.62) kg/d; DM intakes of the tallow-molaferm 20 blends were 5.7, 5.5 and 5.7 (s.e.d., 0.20) kg/d; DM intakes of the grass silage were 8.3, 8.0 and 8.0 kg/d (s.e.d., 0.28). The cattle fed the medium or low FME diet containing medium or high tallow consumed slightly less feed than those given the high FME diet containing no tallow, but the difference did not reach significance ($p>0.05$). Period means of DM intake of the animals over three treatments are shown in Figure 4.2 and were 17.2, 17.7 and 18.0 (s.e.d., 0.75) kg/d in the 1st, 2nd and 3rd period, respectively. The mean intakes of all cattle was increased as the experiment progressed, but the difference was not significant ($p>0.05$).

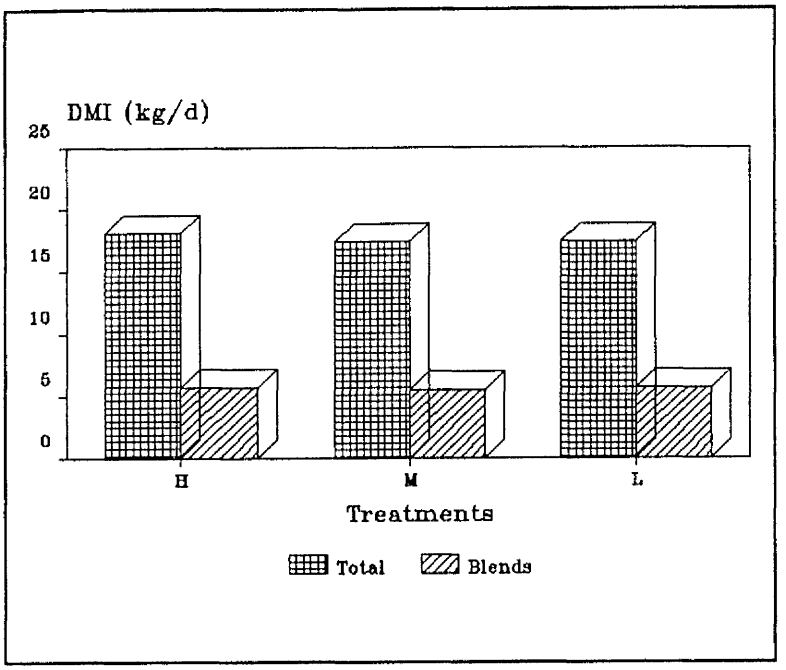

Figure 4.1 DM intakes between the H_{FME} (H), M_{FME} (M) and L_{FME} (L) treatments

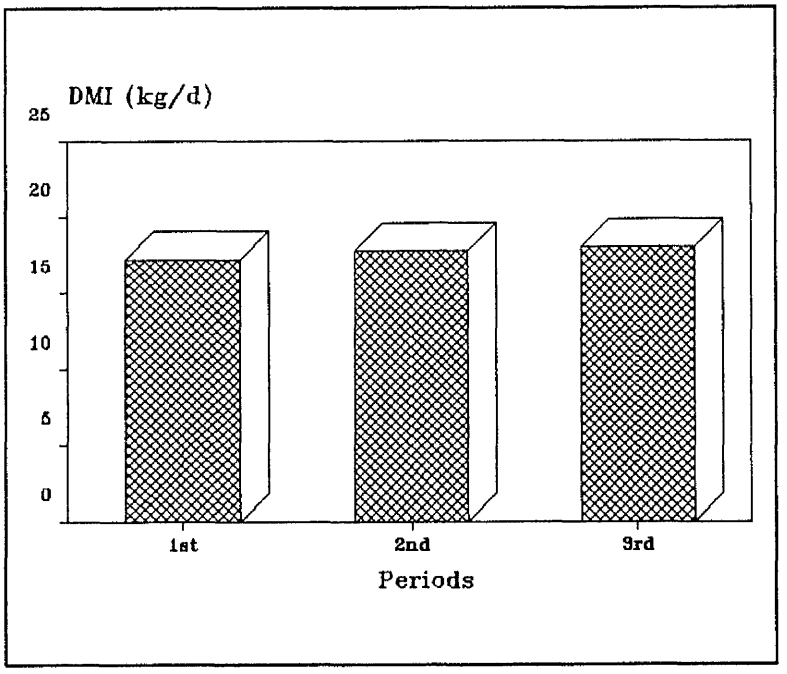

Figure 4.2 The period means of total DM intakes of all cattle over 3 treatments.

4.3.4 *Milk production*

Milk yields and composition of the heifers are shown in Table 4.9. The cattle fed the high FME diet produced significantly lower milk yields and 4%-fat corrected yields than those fed the medium or low FME diet ($p < 0.05$). However, milk concentrations of fat and protein were significantly lower ($p < 0.05$) as each decrease in the dietary FME concentration. Milk concentrations of NPN and lactose was similar between 3 treatments ($p > 0.05$).

Table 4.9 *Milk yield and composition*

	Treatments			s.e.d.
	H_{FME}	M_{FME}	L_{FME}	
Yield (kg/d)				
Total	18.2^{a}	19.3^{b}	19.4^{b}	0.26
4%-fat yield	21.1^{a}	22.3^{b}	21.9^{b}	0.33
Composition (g/kg)				
Fat	46.7^{a}	46.4^{ab}	45.4^{b}	0.57
Protein	33.5^{a}	32.0^{b}	31.3^{b}	0.36
Lactose	47.3	46.9	47.1	0.21
NPN	0.25	0.27	0.26	0.01

ab means with the same or no superscript in the same row are not significantly different ($p > 0.05$).

Milk constituent yields are shown in Table 4.10. Yields of fat and lactose were significantly lower in the H_{FME} treatment than in the M_{FME} or L_{FME} treatment ($p < 0.05$), while protein yield was similar between the three treatments ($p > 0.05$).

Table 4.10 *Milk constituent yields (kg/d)*

	Treatments			s.e.d.
	H_{FME}	M_{FME}	L_{FME}	
Fat	0.85[a]	0.89[b]	0.88[b]	0.013
Protein	0.61	0.61	0.60	0.011
Lactose	0.86[a]	0.90[b]	0.91[b]	0.014

ab means with the same or no superscript in the same row are not significantly different ($p>0.05$).

4.3.5 *Liveweight gain*

Liveweight gains of the heifers estimated by linear regression are presented in Figure 4.3. They were 0.52, 0.58 and 0.85 kg/d (s.e.d., 0.174) in the H_{FME}, M_{FME} and L_{FME} treatments, respectively. There was no significant difference in gains between the 3 treatments ($p>0.05$). The liveweight gains of the cattle were also estimated by the energy balance (Figure 4.3) and they were 1.23, 0.84 and 0.81 kg/d (s.e.d., 0.191) in the H_{FME}, M_{FME} and L_{FME} treatments, respectively. The cattle fed the high FME diet were predicted to have gained more liveweight than those fed the diet containing medium or low FME ($p<0.05$).

The two methods gave similar results in the L_{FME} treatment, but the energy balance resulted in a higher gain than the linear regression in the M_{FME} or H_{FME} treatment, especially in the H_{FME} treatment the gain estimated by the energy balance was 2 time higher than by the linear regression.

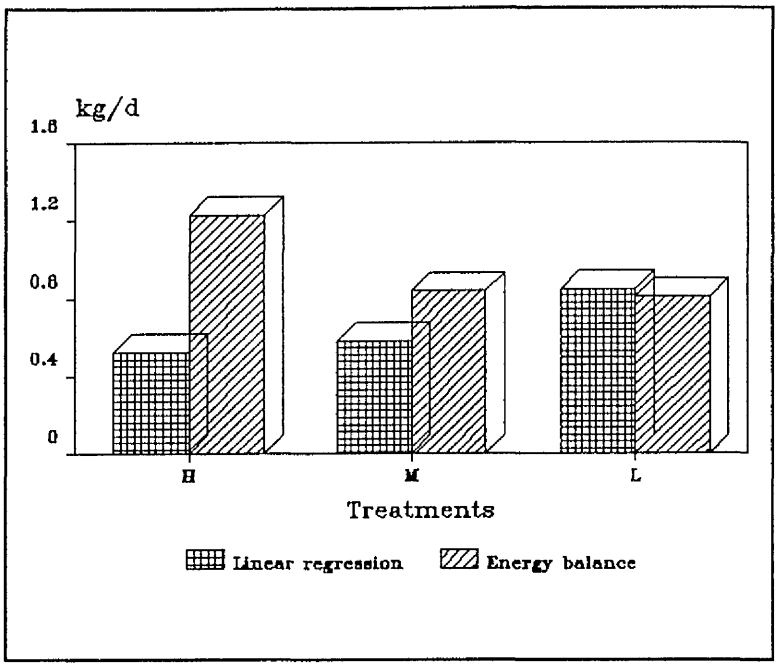

Figure 4.3 Liveweight gains of the animals obtained by lineal regression and energy balance between the H_{FME} (H), M_{FME} (M) and L_{FME} (L) treatments.

4.3.6 *Milk uric acid concentration and yield*

Milk uric acid concentration and yield are shown in Table 4.11. Milk uric acid concentration was lower as the dietary FME concentration declined. The decreases in PM milk and total milk (PM + AM) were significant ($p<0.05$), while in AM milk were not ($p>0.05$). However, milk yields of uric acid were all similar in either AM, PM or total milk ($p>0.05$), which was due to higher milk yield in the M_{FME} or L_{FME} treatments than in the H_{FME} treatment (19.7 or 19.4 *v.* 18.3 kg/d, the mean values in the last two days of each period).

Table 4.11 *Effect of dietary FME levels on milk concentration and yield of uric acid*

	Treatments			s.e.d.
	H_{FME}	M_{FME}	L_{FME}	
Concentration (mg/kg)				
In AM milk	39.0	37.9	38.8	1.01
In PM milk	36.4^{a}	32.4^{b}	33.2^{b}	1.17
Weighted Mean	38.1^{a}	35.8^{b}	37.0^{ab}	0.82
Yield (mg/d)				
In AM milk	460	469	486	17.2
In PM milk	241	234	227	10.7
Total	701	703	713	20.4

ab means with the same or no superscript in the same row are not significantly different ($p>0.05$).

4.3.7 *Energy utilization*

The calculated energy utilization is presented in Table 4.12. A short term treatment period (4 weeks) in this changeover experiment resulted in a high standard error of difference (s.e.d.) in ME for gain. Another factor which can influence the accuracy of the calculated efficiency of energy utilization was the unprotected tallow due to its negative effect on the dietary fibre digestion in the rumen. However, the result may still give some useful information because ME for gain only accounted for a small part of total ME intake (from 11 to 19%) and the tallow was included in diets at small amounts and in addition the high molasses inclusion could shorten the retention time of fatty acids from hydrolysis of the tallow in the rumen, which may minimize the negative effect of unprotected tallow on the fibre digestion and microbial activity in the rumen of the cattle.

Table 4.12 *The calculated ME partition and energy efficiency*[+]

	Treatments			s.e.d.
	H_{FME}	M_{FME}	L_{FME}	
	MJ/d			
ME intake	210	200	198	7.19
ME for maintenance	54	54	54	0.21
ME for lactation	97[a]	103[b]	103[b]	1.34
ME for gain	23	25	37	9.00
ME in excess	36[a]	18[ab]	4[b]	13.7
NE for maintenance	39	39	39	0.15
NE for lactation	62[a]	65[b]	65[b]	0.85
NE for gain	14	16	23	5.74
Efficiency[+]				
+ gain	0.397	0.445	0.451	0.037
- gain	0.466	0.537	0.607	0.095
NE/ME	0.578	0.600	0.641	0.039

[+] ab means with the same or no superscript in the same row are not significantly different ($p>0.05$).

The liveweight gain was based on the result obtained from linear regression.

Total ME intake was higher in the H_{FME} treatment than in the M_{FME} or L_{FME} treatment, but the difference was not significant ($p>0.05$). ME partitions for lactation ($p<0.05$) and for liveweight gain ($p>0.05$) were lower in the cows fed the high FME diet containing no tallow than in those fed the medium or low FME diet containing medium or high tallow. As a result, a significant higher amount of ME was wasted in the H_{FME} treatment than in the M_{FME} or L_{FME} treatment (ME in excess / ME intake, 0.17 *v.* 0.09 or 0.02). Therefore, feeding the high FME diet containing no tallow caused decreases in the efficiencies of ME conversion surplus to maintenance into milk production and in the ratio of NE/ME than feeding the medium or low FME diet containing medium or high tallow, although the differences were not significant ($p>0.05$).

4.3.8 *Protein utilization*

The calculated protein utilization is presented in Table 4.13. ERDP was in excess in all three treatments, although ERDP requirements were significantly higher ($p<0.05$) as dietary FME concentrations increased. Because MP supply depends on both microbial protein and DUP, a decrease in dietary FME concentration resulted in a lower microbial protein supply and hence a significantly lower MP supply in the M_{FME} or L_{FME} than in the H_{FME} treatment. However, MP requirements for maintenance, lactation, liveweight gain and pregnancy were not significantly different between three treatments ($p>0.05$).

Table 4.13 *Protein utilization*[+]

	Treatments			s.e.d.
	H_{FME}	M_{FME}	L_{FME}	
		g/d		
ERDP supplied	1973	1878	1879	67.5
ERDP required	1871[a]	1703[b]	1608[b]	63.7
ERDP in excess	102[a]	175[b]	271[c]	6.34
MP supplied	1689[a]	1558[b]	1494[b]	57.6
MP required	1315	1329	1361	54.0
MP for maintenance	303	302	302	1.24
MP for lactation	851	853	840	15.6
MP for gain	113	124	171	42.4
MP for pregnancy	48	48	48	--
MP in excess	374[a]	229[ab]	133[b]	90.5
k_n	0.571	0.627	0.677	0.062
k_{nl}	0.504	0.547	0.562	0.026
k_{ng}	0.072	0.082	0.112	0.026

[+] ab means with the same or no superscript in the same row are not significantly different ($p>0.05$).

The liveweight gain was based on the result obtained from linear regression.

Therefore, MP excess was significantly lower ($p < 0.05$) with each decrease of dietary FME concentration (MP excess / MP supply, 0.22, 0.15 and 0.9 in the H_{FME}, M_{FME} and L_{FME} treatments, respectively). As a result, the net efficiency of utilization of MP supply (k_n), for lactation (k_{nl}) and for gain (k_{ng}) were all higher as the dietary FME concentration depressed, but the differences did not reach significance ($p > 0.05$).

4.3.9 *Blood parameters*

Treatment means of concentrations of blood parameters investigated in this experiment are shown in Table 4.14. All of them were within normal range of SAC Veterinary Service.

The treatment means of concentrations of total protein, albumen, phosphate and magnesium all remained similarly between the start of and on the experiment ($p > 0.05$), and all also were similar between the experimental treatments ($p > 0.05$). Concentrations of ß-hydroxybutyrate and urea were decreased ($p < 0.05$), and calcium and potassium were increased ($p < 0.05$) after the cattle were fed molaferm 20 in comparison with the start of the experiment, while all were similar between the experimental treatments ($p > 0.05$).

Period means of ß-hydroxybutyrate and K are shown in Figure 4.4. The blood concentration of ß-hydroxybutyrate was lower after feeding on the experimental diets ($p < 0.05$), but was significantly higher when the animals were on the experiment for 12 weeks ($p < 0.01$). The K concentration was increased at first and then slightly declined as experiment progressed. The period means of other parameters investigated in this experiment did not show consistent changes as the experiment progressed.

Table 4.14 *The concentrations of some blood parameters*

	Start	Treatments			s.e.d.	Normal range
		H_{FME}	M_{FME}	L_{FME}		
			g/litre			
Total protein	71.0	70.2	70.0	69.3	1.61	55-73
Albumen	28.0	29.3	29.2	29.0	0.80	29-39
			mmol/litre			
Urea	4.35[a]	3.82[b]	3.90[b]	4.01[ab]	0.22	2.0-6.6
BHBA[+]	0.65[a]	0.59[ab]	0.56[ab]	0.53[b]	0.05	<0.8
Calcium	2.41[a]	2.54[ab]	2.49[ab]	2.60[b]	0.07	2.2-2.6
Magnesium	0.94	1.00	1.00	0.97	0.03	0.8-1.1
Phosphate	1.97	2.02	1.92	2.00	0.08	1.7-2.2
Potassium	3.93[a]	4.51[b]	4.17[ab]	4.52[b]	0.19	3.5-5.5

[+] ß-Hydroxybutyrate

ab means with the same or no superscript in the same row are not significantly different ($p>0.05$).

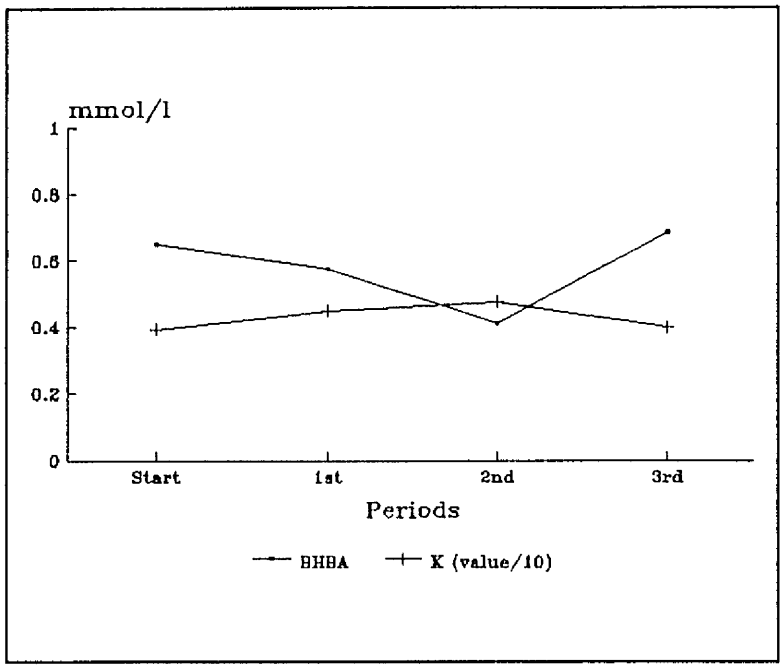

Figure 4.4 The period means of blood concentrations of K (result/10) and ß-hydroxybutyrate

4.4 *Discussion*

4.4.1 *Health*

A high molasses inclusion in a dairy cattle diet is often associated with loose faeces as reviewed by Scott (1953) and also resulted in some metabolism disorders including ketosis as discussed in Chapter 1 (Literature Review). However, the previous experiments with mid-lactation cows and with early lactation cows did not observe scouring from the animals fed 310 g/kg DM of molaferm 20 for 2 or 3 months. A similar result was obtained in this experiment. None of the early lactation dairy heifers suffered from scouring when molaferm 20 was fed at 310 g/kg DM. No other clinical symptoms of the ill health of the animals occurred during the experiment and the blood concentrations of parameters investigated all were within the normal range of SAC Veterinary Service. These evidences implicate the early lactation dairy heifers could be fed molaferm 20 up to 310 g/kg DM on a grass silage-based diet for 3 months without any health problem.

However, in this experiment the period mean of blood concentration of ß-hydroxybutyrate was significantly increased when the animals were fed molaferm 20 for more than 2 months. The detrimental effect of molasses might gradually occur as early lactation dairy heifers are fed molaferm 20 at a rate of 310 g/kg DM on a grass silage-based diet for a long term (more than 3 months).

4.4.2 *Feed intake*

The feed intake of dairy cattle is depressed by a high substitution rate of molasses for cereal grains in a diet as discussed in Chapter 1 (Literature Review). However, in this experiment the DM intakes of heifers were satisfactory when molaferm 20 was fed at a rate of 310 g/kg DM. The period means of DM intakes of all cattle were consistent with stage of lactation and were gradually increased as the experiment progressed (Figure 4.2). Furthermore, the treatment means of DM intake in this experiment were higher than the treatment means of DM intake predicted using the equation as reported by ARC (1980). The predicted DM intakes were 16.0, 16.2 and 16.2 kg/d in the H_{FME}, M_{FME} and L_{FME} treatments (Figure 4.5), respectively. These evidences suggest that feeding dairy heifers molaferm 20 at 310 g/kg DM on the grass silage-based diets shows no detrimental effect on the total DM intake for 3 months. Indeed, the intakes of all 3 diets were higher than predicted.

Free fatty acids from hydrolysis of unprotected fats in the rumen can inhibit fibre digestion and microbial activity, possibly by coating the feed particles and preventing bacterial attachment (Ørskov and Ryle, 1990). Many studies have shown that feeding unprotected tallow resulted in various decreases in digestibilities of dietary OM and fibre in the total tract of cattle (Murphy and Morgan, 1983 and Steele, 1984) and in total DM intake (Murphy and Morgan, 1983; Steele, 1984 and Robinson and Burgess, 1990). The effect of tallow on DM intake depends on many dietary factors including amounts of concentrates fed (Clapperton and Steele, 1983) and source of forage (Smith *et al.*, 1993). However, some recent studies in US showed little detrimental effect of tallow on digestibilities of dietary DM, OM and fibre when tallow was supplemented

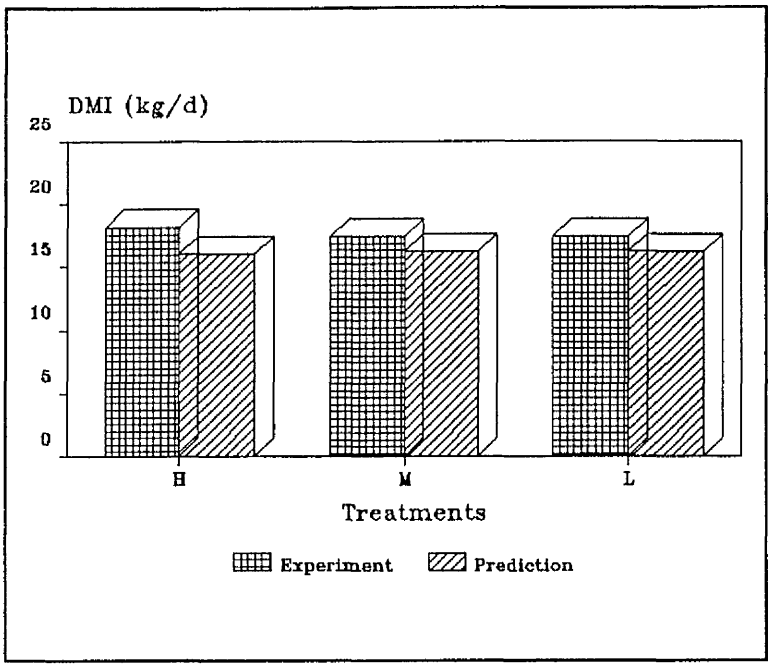

Figure 4.5 Comparison of the DM intake in the H_{FME} (H), M_{FME} (M) or L_{FME} (L) treatment between the experiment and prediction.

at the rates of less than 50 g/kg DM with high oil maize (Elliott *et al.*, 1993) or whole soyabeans (Schauff *et al.*, 1992). In this experiment DM intake was similar between the three treatments ($p>0.05$), although the cattle fed no tallow consumed 0.7 kg DM/d more than those fed tallow. The lack of significant response in DM intake could be because the tallow was included in the diets at small amounts (12 or 24 g/kg DM of oil derived from the tallow in the low or high tallow diet (M_{FME} or L_{FME})). Ørskov and Ryle (1990) reported that the unprotected lipids fed to the ruminant up to 70 g/kg DM still are acceptable. In addition the high molaferm 20 inclusion (310 g/kg DM) in the diets could shorten the retention time of free fatty acids from hydrolysis of the tallow in the rumen and then minimized the negative effect of the tallow on fibre digestion and feed intake.

4.4.3 *Milk production*

In early lactation, dairy cows have a large requirement for energy, but intake of energy may be limited by capacity of DM intake. Addition of fat source, such as tallow, to the

diet may improve the energy status of high producing dairy cows. However, the effects of substitution rates of unprotected tallow for cereal grains depends on the amount of the tallow in the diet. When the rate was less than 50 g/kg DM, addition of the tallow increased the energy intake and then increased the milk yield in various levels (Clapperton and Steele, 1983; Murphy and Morgan, 1983; Elliott *et al.*, 1993 and Smith *et al.*, 1993). In contrast, when the rate was higher, feeding the tallow showed negative effects on milk yield (Banks *et al.*, 1980; Clapperton and Steele, 1983 and Robinson and Burgess, 1990).

In this experiment, the oil concentration contributed from the unprotected tallow was 12 or 24 g/kg DM in the low or high tallow diet (M_{FME} or L_{FME}). The low tallow inclusions in the diets containing a high level of molaferm 20 were expected to have little negative effect on the ruminal fermentation. In fact, the feed intake was not impaired by feeding the tallow. However, addition of the tallow depressed the dietary FME concentrations because the diets were formulated to similar ME levels in this experiment. The lower dietary FME concentrations together with the tallow inclusion could result in lower microbial synthesis in the rumen. This was supported by the milk uric acid concentration. The cattle fed the medium or low FME diet containing low or high tallow had a significantly lower uric acid concentration in milk than those fed the high FME diet containing no tallow ($p<0.05$). The lower uric acid levels in milk indicated lower microbial synthesis in the rumen. Boggs *et al.* (1987), replacing ground ear maize by unprotected tallow at a rate of 75 g/kg DM in steers fed 100 g/kg DM of molasses, reported that microbial N flow at duodenum was lower in animals fed tallow than those fed no tallow (51 *v.* 69 g/d).

However, the milk yield was significantly higher in the animals fed the medium or low FME diet than those fed the high FME diet ($p<0.01$). Although milk concentrations of fat ($p<0.05$) and protein ($p<0.01$) were lower in the former animals than the latter animals, fat yield was still significantly higher ($p<0.05$). The growing heifers, their low milk production and the short term feeding (4 weeks), of course, could influence

the potential of negative effect of the tallow on milk production in this experiment as the diets contained similar ME. If ignored these factors, the ruminal microbial activity in the cattle fed the medium or low FME could be impaired less than expected. This leaves an interesting question. What is the minimum FME concentration in a diet to support the maximum microbial synthesis? Is the 8.4 MJ/kg DM of FME in the L_{FME} diet containing 310 g/kg DM of molaferm 20 sufficient to support a satisfactory microbial growth? Indeed, the calculated efficiencies of utilization of MP (k_n) and for lactation (k_{nl}) and the efficiencies of utilization of ME and the ratio of NE/ME were highest in the L_{FME} treatment, although the differences were not significant ($p>0.05$).

Milk fat concentration was negatively related with dietary oil levels in this experiment ($p<0.05$, $r=-0.95$). The fat concentration was declined by 0.05 g/kg of milk with each increment of 1 g/kg DM of oil derived from the tallow. Many previous studies have shown various decreases in this parameter when cereal grains were replaced by unprotected tallow at 49 g/kg DM (Wrenn *et al.*, 1978), at 65 and 85 g/kg DM (Robinson and Burgess, 1990) or at 25 and 50 g/kg DM with high oil maize (Elliott *et al.*, 1993). In contrast, some other studies showed this parameter was increased by 1 to 6 g/kg of milk when unprotected tallow was fed (Clapperton and Steele, 1983; Steele, 1984 an Schauff *et al.*, 1992). The contradictory responses could be from different diets used in the experiments, because many dietary factors can influence milk fat concentration, such as amount of roughage, intake and forage:concentrate rate (Sutton, 1989).

Unlike milk fat, the effects of feeding unprotected tallow on milk protein concentration is very consistent. The experiments quoted above indicated decreases in the protein concentration when the tallow was fed to dairy cows, except the studies conducted by Wrenn *et al.* (1978) and Schauff *et al.* (1992) who observed that this parameter was unchanged as the tallow was fed. In this experiment, milk protein concentration was also significantly depressed by feeding the tallow ($p<0.01$). The decrease was 0.09 g/kg of milk with each increment of 1 g/kg DM of oil derived from the tallow. The

higher protein concentration compensated the lower milk yield in the cattle fed no tallow. The milk protein yield therefore was similar between the three treatments ($p > 0.05$).

4.5 *Conclusion*

Lactation dairy heifers could be fed molaferm 20 up to 310 g/kg DM without clinical ill health in grass silage-based diets for 3 months. Dietary FME concentration of 8.4 MJ/kg DM could be satisfactory for microbial growth in the rumen of the animals fed this high level of molaferm 20 and feed intake and milk production were not impaired by decreases in dietary FME levels achieved by addition of unprotected tallow (up to 24 g/kg DM). The ruminal microbial protein synthesis and fibre digestion need to be determined when addition of unprotected tallow to decrease FME in diets containing high levels of molaferm 20.

CHAPTER 5

The Effects of Dietary Concentrations of ERDP and DUP on Rumen Fermentation and Microbial Protein Synthesis in Wether Sheep Fed a High Level of Molasses

5.1 *Introduction*

The dairy cattle experiments in this study have evaluated the effects of feeding high levels of molaferm 20 (156 to 468 g/kg DM in total diets) on feed intake and milk production. Since molaferm 20 contains a high sugar level, feeding those high levels of molaferm 20 could influence rumen fermentation of the animals. However, most published studies described the effect on rumen fermentation by feeding molasses at less than 200 g/kg DM in total diets and there is little information on the effect at higher levels.

The non-structural carbohydrates in molasses are fermented very fast in the rumen (Johnson, 1976 and Ørskov and Ryle, 1990), but this together with the low crude protein concentration of molasses (*e.g.*, 40 g/kg DM in cane molasses) may depress the utilization of dietary protein in comparison with cereal grains. The underlying cause may be an imbalance between supplies and requirements of nitrogen and energy for the rumen microorganisms when a large amount of molasses is fed. The dietary protein requirement may therefore be higher than recommended for those diets (Pate, 1982). Indeed, the previous experiment reported in Chapter three has observed positive responses in DM intake and milk production of dairy cows fed diets containing 310 g/kg DM of molasses to increases in dietary ERDP or DUP levels. The increases in DM intake with raised dietary ERDP concentrations achieved by supplementing diets with urea and soyabean meal suggest a higher levels of microbial activity in the rumen, resulting in a higher rate of fibre digestion.

A 4 x 4 Latin Square experiment was therefore designed to evaluate the rumen fermentation and microbial protein synthesis in wether sheep fed the similar diets as those in Chapter Three with cows, but an extra diet was added in which some soyabean meal was replaced by fish meal to further increase dietary DUP concentration. In order to compare with these high sugar diets, all animals were then fed a conventional ration containing more fibre but no molasses following the completion of the Latin Square design experiment.

5.2 *Material and methods*

5.2.1 *Animals and management*

Three Suffolk and one Finn Dorset male wether sheep each fitted with a permanent rumen cannula were used in this experiment. The animal were born from 1985 to 1989 and their liveweights ranged from 55.0 to 79.0 kg (mean 67.0; s.e., 9.9) at start of the experiment.

The sheep were individually housed and fed in metabolism crates which were fitted with a slatted floor. A container was placed under the floor to collect urine. Each animal was fitted with a faecal collection harness to which a polyethylene faeces collection bag was attached. Feed was offered twice daily in equal meals at 09.00 and 17.00 hours. Fresh drinking water was continuously available.

5.2.2 *Design and diets*

Four complete diets was used in a 4 x 4 Latin Square design experiment with 3 week experimental periods. Following completion of the Latin Square, all the animals were immediately fed a conventional sheep ration containing no molasses but higher fibre concentration for an extension period of 3 weeks.

In the Latin Square design the diets were fed to the sheep at amounts ranged from 1420 to 1500 g DM/d according to their liveweights. The ingredients and their proportions in the complete diets are shown in Table 5.1. The four diets here were referred to respectively as C (control), CU (control + urea), CS (control + soyabean meal) and CSF (control + soyabean meal and fish meal), and the four treatments correspondingly as C, CU, CS and CSF. The first three diets were similar to those used in Experiment 2 with dairy cows, but a part of silage was replaced by barley straw (260 g/kg DM of total diets) to decrease the dietary CP concentrations. The fourth diet was designed to increase dietary DUP concentration by substitution of fish meal for a part of soyabean meal in diet CS.

Table 5.1 *Composition of diets (g/kg DM)*

	Treatments			
	C	CU	CS	CSF
Silage	200	200	200	200
Barley straw	260	260	260	260
Molaferm 20	310	310	310	310
Barley	192	180	26	16
Soyabean meal	22	23	189	110
Fish meal	0	0	0	89
Urea	1	12	0	0
Minerals	15	15	15	15

The molasses product used was Molaferm 20 (a mixture of 800 g/kg of cane molasses with 200 g/kg of condensed molasses solubles) supplied by *United Molasses*. The barley straw used was untreated but chopped to an average length of 40 mm. The soyabean meal, fish meal, barley, urea and minerals supplement were purchased from normal sources.

The silage was made from the 3rd cut of a perennial ryegrass sward. The crop was wilted for 24 hours and then picked up and chopped to an average length of 20 mm with a forage harvester. A silage inoculant (*'Ecosyl' ICI plc*) was added at a rate of 3.0 litres/tonne as it was chopped. Prior to the experiment, the silage used throughout the experiment was mixed and stored in plastic bags at a rate of about 30 kg/bag, and then frozen at -20 °C. The frozen silage was defrosted one day before it was used. The average quality of silage at feeding is presented in Table 5.2.

Table 5.2 *The average silage quality at feeding*

DM (oven, g/kg)	235
OM (g/kg DM)	906
CP (g/kg DM)	231
ME (MJ/kg DM)	10.8
ADF (g/kg DM)	263
NDF (g/kg DM)	423
pH	3.6
Ammonia-N/Total-N (g/kg)	156

Before feeding, the diets were completely mixed in a mixer and then weighed out into two plastic bags in equal weights for feeding at 09.00 and 17.00 hours. To decrease the viscosity of molaferm 20 and dissolve the urea, the same amount of hot water as molaferm 20 was added when the diets were mixed. The mean chemical composition of the diets at feeding is shown in Table 5.3.

Following the completion of the Latin Square design experiment, a conventional sheep ration containing no molasses was immediately fed to all 4 sheep for a 3 week extension period. The ration consisted of (g/kg DM) 791 of ryegrass hay (chopped to an average length of 40 mm) and 209 of a proprietary sheep concentrate based on cereal grain and its by-products (*BOCM Silcock Ltd*, Hampshire) and was fed at an amount of 1250 g DM/d. The animals were fed twice daily in two equal meals at 09.00 and 17.00 hours by tipping the concentrate on the hay. The mean chemical composition of the ration (here referred as P5) is also shown in Table 5.3.

Table 5.3 *The chemical composition of the diets (g/kg DM, unless otherwise stated)*

	Diets				
	C	CU	CS	CSF	P5
DM	546	547	547	548	825
OM	896	896	889	876	936
CP	123	154	178	202	81
ME (MJ/kg DM)	10.7	10.5	10.7	10.8	9.2
ADF	203	202	206	198	334
NDF	329	327	323	298	598
Oil	19	19	19	25	16
Ca	8.0	8.0	8.5	13.2	6.1
P	2.9	2.9	3.4	6.2	3.1
Mg	3.7	3.7	4.0	3.9	2.2
Na	6.1	6.1	6.1	7.1	1.9
K	22.4	22.3	25.4	26.8	13.7

5.2.3 *Measurements*

5.2.3.1 *Feed intake and composition*

Feed given to and refused by the sheep were recorded every morning before feeding in the last week of each period. Silage was sampled twice weekly and barley straw, hay, molaferm 20, barley, soyabean meal, fish meal and the proprietary concentrate were sampled twice each period for the determination of their DM. Samples of fresh mixes offered were taken daily and samples of refusal were taken twice weekly for the measurement of their oven DM. The samples were dried in a forced draught oven (*Unitherm* drying oven) at 100 °C for approximately 24 hours.

For chemical analysis, bulked samples of silage were taken 3 times, samples of barley

straw, hay, molaferm 20, barley, soyabean meal, fish meal and the proprietary concentrate were taken twice, and samples of fresh mixes offered were taken daily and samples of refusal were taken twice in the last week of each period. The chemical analysis was carried out at the Analytical Service Unit of the Scottish Agricultural College, Auchincruive, Ayr. For determinations of DM concentration of molaferm 20 and the ME concentrations of molaferm 20, fish meal, soyabean meal and silage, please see Section 2.2.3.1 of Chapter 2. The ME concentration of barley was measured by the same method as soyabean meal and the ME concentrations of barley straw and grass hay were determined by the equations given below.

In vivo OMD% = (*In vitro* OMD% * 1.207) - 10.21 (1)

DOMD% = *In vivo* OMD% * (OM g/kg DM) / 1000 (2)

ME (MJ/kg DM) = DOMD% * 0.15 (3)

Where:

OMD = Organic matter digestibility

DOMD = Digestible organic matter in dry matter

5.2.3.2 *Whole tract digestion*

Total faeces was collected daily in the last 6 days of each period and sampled over each 3 day period. The samples were dried to a constant weight at 60 °C in a forced draught oven (*Unitherm* drying oven) for determination of their DM concentration, and then milled for further measurements of their OM and NDF concentrations. The analysis was carried out at the Analytical Service Unit of the Scottish Agricultural College, Auchincruive, Ayr. The apparent digestibilities of DM, OM and NDF were then calculated by the following equation,

Digestibility = nutrient absorbed / intake (4)

5.2.3.3 *In sacco rumen hay degradability*

The dacron bags used (supplied by *Henry Simons*, Stockport, Cheshire) were made with double stitching and rounded shoulders to prevent any lodging of food residues post incubation. The bags were approximately 150 mm x 60 mm with a pore size of 40 μm.

Approximately 3 - 4 g of fresh washed hay (chopped to a length of 10 mm) was placed into each weighed bag. The bags were reweighed and were then securely tied with 25 cm of nylon cord, after twisting the neck of the bag. Sets of four bags were then tied to an appropriate bung with the individual bag strings sheathed in polythene tubing. They were immediately placed into the rumen prior to the morning feeding and incubated for 24 hours. Two sets of 4 bags (total 8 bags) were examined in the last 2 days of each period.

After removal from the rumen the bags were rinsed in running tap water and then cold water washed in a pre-set automatic washing machine (*Zanussi Model Z915T*, programme B). They were then dried in an oven at 60 °C for at least 48 hours and reweighed. The loss of contents in bags during washing was adjusted by washing bags which just contained chopped hay. The hay degradability were calculated by the following equation:

$$\text{Degradability} = \text{hay disappeared} / \text{hay given} \qquad (5)$$

5.2.3.4 *Urinary purine derivatives*

Total urine was collected and sampled daily in the last 6 days of each period. No preservative was added to the samples of urine. The surfaces on which the urine came into contact were thoroughly washed with 0.1% of hyperchlorite solution (Deosan - 11% w/w available chlorine) in order to minimise the possible contamination of the urine. These surfaces were subsequently rinsed with fresh water. A 2 ml fresh urine sample was taken and were immediately frozen at -20 °C for analysis.

The total purine derivatives (PD) in urine was determined by using the method as

described by Borchers (1977). The principle of the method is as following,

(1) Hypoxanthine and xanthine are first converted to uric acid by xanthine oxidase.

(2) Uric acid is then converted to allantoin by uridase.

(3) Total allantoin is then measured as total purine derivatives (PD) which contain actual allantoin and the allantoin converted from other 3 purine derivatives.

Reagents: Glycine buffer (0.67 M, pH 8), 5.029 g of glycine per 100 ml of distilled water.

Xanthine oxidase solution, 5 units of the enzyme in 12.0 ml glycine buffer.

Uricase solution, 5 units of the enzyme in 12.0 ml glycine buffer.

2,4 Dinitrophenylhydrazine solution (0.1%), 1.33 g of 2,4 Dinitrophenylhydrazine per 1000 ml of HCl (2M).

Working standards, 1.000 g allantoin was exactly weighed out and made up to 1000 ml with distilled water. 6 standards were then made up by using above solution with distilled water and the standards contained allantoin at 0, 10, 20, 30, 40 and 50 mg/l, respectively.

1 ml of urine sample was diluted to 50 ml of a flask by using distilled water. The pH of the diluted sample was adjusted to 8.0 in a pH meter (*Corning*). 2.5 ml aliquot of urine was incubated at room temperature with 0.150 ml of xanthine oxidase for 2 hours, followed by a further 2 hours incubation with uricase. The total allantoin was then determined by addition of 0.5 ml NaOH (0.6M). The mixture was then heated in boiling water bath (100 °C) for 12 minutes. 1 ml of 2,4 Dinitrophenylhydrazine solution was then added to each tube and heating continued for 4 minutes. Tubes were cooled to room temperature in cold water. Into each tube 5 ml NaOH (2.5M) was

added to make the solution alkali and tubes stood at room temperature for 10 minutes. The standards were taken through the same procedures as samples.

Optical density (OD) of each sample and standard was read at 520 nm in a spectrophotometer (*Stasar II, Gilford Instrument Laboratories INC.*, Oberlin, Ohio 44074). Distilled water was used to adjusted OD to zero before reading. The allantoin concentration in each sample was obtained by comparison with standards.

5.2.3.5 *Rumen pH, VFAs and ammonia*

Samples of rumen liquor were taken from each sheep via the cannula using a flexible polythene tube fitted to suction equipment at 0.00, 1.30, 3.00, 4.30, 6.00 and 7.30 hours post feeding in the last day of each period.

A liquor sample of 8 ml was taken using a wide bore pipette and placed in individual centrifuge tubes. A 2 ml of preservative (15% w/w metaphosphoric acid) was added to each sample and mixed well. The sample was then centrifuged for 10 minutes at 3000 rmp in a pre-cooled centrifuge (4 °C). The supernatant was then removed using a disposable pasteur pipette and frozen at -20 °C in 7 ml Bijoux bottle and 1.5 ml microtubes for further analysis.

pH

The pH of each sample of fresh liquor was measured immediately after it was taken from the rumen by using a portable pH meter (*Cranwell UK*, Brentwood, Essex). The meter was calibrated using standard buffer solution of pH 7.0 at 25 °C.

Volatile fatty acids

Reagents: Pivalic acid solution, 0.22 g of pivalic acid per 100 ml of distilled water.

Oxalic acid solution, 5.67 g of oxalic acid per 500 ml of distilled water.

Standard solution, The solution contained the following acids (Molarity), acetic (0.05), propionic (0.02), butyric (0.02), isobutyric (0.002), valeric (0.002) and isovaleric (0.002).

The frozen supernatant of preserved sample was defrosted, if necessary it was spun down at 1000 rpm at 4 °C. A 0.5 ml of sample or standard was pipetted into a 1.5 ml microtube and then into each tube of sample 0.2 ml of 1.1 M NaOH was added and into each tube of standard 0.2 ml of distilled water. A 0.1 ml of pivalic acid solution and 0.4 ml of oxalic acid solution were pipetted into each tube of sample or standard, respectively. Each tube was then capped and mixed well and centrifuged at 3000 rmp in a refrigerated centrifuge (pre-cooled to 4 °C) for 10 minutes. The supernatant from each tube was removed into an autosampler vial and then the vial was capped. The sample was ready for gas chromatography (GC) analysis.

In analysis 1 µl of sample was injected into a glass GC column (2 mm x 2 mm ID packed with 4% Carbowax on Carbopack B-DA 80/120 mesh (*Supelco inc.*)). The measurement was carried isothermally at 170 °C using N_2 as the carrier gas at a flow rate of 24 ml/minute. An electronic integrator was used to quantify peak areas.

Ammonium

Reagents: Caustic phenol solution, 1000 ml of solution contained 1.5 g of sodium hydroxide (AR), 12.5 g of phenol (AR) and 0.0625 g of sodium nitroprusside.

Buffer solution, 1000 ml of solution contained 2.5 g of sodium hydroxide (AR), 1.87 g of anhydrous Na_2HPO_4 (AR), 1.59 g of $Na_3PO_4.12H_2O$ and 20 ml of sodium hyperchlorite.

Blank solution, 1000 ml of solution contained 30 g of Metaphosphoric acid (Flake - approximately 60% HPO_3).

Working standards, 7.7592 g of pre-dried $(NH_4)_2SO_4$ was exactly weighed out and made up to 500 ml with Blank solution. 7 working standards were then made up using above solution with Blank solution and the standards contained ammonia at 0, 60, 120, 180, 240, 300, 360 mg/l, respectively.

The frozen supernatant of preserved sample was defrosted, if necessary it was spun down at 1000 rpm at 4 °C. All the samples and standards were diluted 1 to 10 into 1.5 ml microtubes using a diluter (*Hook and Tucker III*). Once diluted the samples and standards should be mixed on the vortex mixer. 20 µl of the samples and standards was transferred into a 96 well microplate by positive displaced pipettor and 80 µl of caustic phenol reagent was then added to each well using an 8-way pipettor. The microplate was mixed using programme 1 on the microplate reader (*Dynatech* MR5000) and 200 µl of buffer was added to each well before a second mixing stage. The microplate was then left to incubate at room temperature for 1 hour. The microplate was then read at 510 nm using programme 2 and the ammonia concentration of the rumen liquor was calculated using the microplate reader (*Dynatech* MR5000).

5.2.3.6 *Liveweight*

The liveweight of each sheep was recorded at start of the experiment and end of each period.

5.2.3.7 *Calculations of energy and protein utilisation*

The energy balance was calculated according to AFRC Technical Committee on Responses to Nutrients, Report Number 5, Nutritive Requirements of Ruminant Animals: Energy (AFRC, 1990). The details of the equations used for calculations of energy for maintenance and liveweight gain are presented in Appendix 6. The efficiencies of utilization of dietary ME for maintenance (k_m) and liveweight gain (k_g) are calculated from estimated equations for each diet and are shown in Table 5.4.

Table 5.4 *Efficiencies of utilization of dietary ME for various functions*

	Diets				
	C	CU	CS	CSF	P5
k_m	0.708	0.706	0.708	0.710	0.680
k_g (mean)	0.481	0.473	0.478	0.484	0.488

The protein utilization was calculated according to metabolizable protein (MP) system as reported by AFRC Technical Committee on Responses to Nutrients, Report Number 9, Nutritive Requirement of Ruminant Animals: Protein (AFRC, 1992). The details of the equations used for calculations of MP supply (MP_s) and MP for maintenance (MP_m), fleece growth (MP_w) and Liveweight gain (MP_g) are presented in Appendix 7. The ERDP requirement ($ERDP_r$) was calculated according to the equation (6).

$$ERDP_r \text{ (g/d)} = FME \times Y_{mcp/fme} \quad (6)$$

Where $Y_{mcp/fme}$ = 10 g/MJ

The concentrations of FME, ERDP and DUP in individual feedstuffs used in this experiment are shown in Table 5.5.

Table 5.5 *The concentrations of FME, ERDP and DUP in individual feedstuffs*

	FME (MJ/kg DM)	ERDP (g/kg DM)	DUP (g/kg DM)
Silage	8.7	159	29
Straw (barley)	7.0	25	7
Barley	12.7	91	14
Molaferm 20	11.5	65	13
Soyabean meal	12.7	293	135
Fish meal	12.0	303	312
Urea	0	2320	0
Hay (ryegrass)	8.0	32	16
Concentrate	9.2	115	35

The dietary concentrations of FME, ERDP and DUP are then presented in Table 5.6.

Table 5.6 *The dietary concentrations of FME, ERDP and DUP*

	Diet				
	C	CU	CS	CSF	P5
FME (MJ/kg DM)	9.8	9.7	9.8	9.8	8.3
ERDP (g/kg DM)	84	109	116	119	49
ERDP/FME (g/MJ)	8.57	11.24	11.84	12.14	5.90
DUP (g/kg DM)	17	17	38	54	20

2.2.4 *Statistical analysis*

Four wether sheep each fitted with a permanent rumen cannula were allocated to a 4 x 4 Latin Square design experiment with 3 week experimental periods. Following the completion of the Latin Square, a 3 week extension period (referred as "P5") was carried out by feeding the 4 animals a conventional sheep ration. Analysis of variance was carried out by using Genstat 5 (Release 1.3, Lawes Agricultural Trust, Rothamsted, UK, 1988). In the Latin Square experiment, the period or sheep was used as blocks for measurements of all parameters. Degree of freedom of the residual was 9. Significant differences between treatment means were determined by Duncan's multiple range test. In the extension period, the results were compared with those obtained from treatment C and mean of all four treatments in the Latin Square. The sheep was used as blocks for comparison of all parameters.

5.3 *Results*

5.3.1 *Whole tract digestibilities of DM, OM and NDF*

Whole tract digestibilities of DM, OM and NDF are shown in Table 5.7. Supplementing the control diet with urea or protein showed no benefit for digestion of DM and OM. The digestibility of DM or OM was similar between the four treatments ($P > 0.05$), although increased dietary protein levels slightly raised OM digestion. In

contrast, increases in dietary ERDP and DUP concentrations by addition of soyabean meal or soyabean meal and fish meal significantly raised NDF digestibility ($p<0.05$), while supplementing with urea to enhance dietary ERDP concentration showed no effect on NDF digestion.

5.3.2 *Ruminal hay degradability*

The hay degradability in the rumen is shown in Table 5.8. After 24 hour incubation, approximately 25% of hay DM had disappeared from dacron bags. Diet CS supported the highest hay degradability, and followed by diet CSF. Supplementing with urea only showed a slightly higher hay degradability than the control diet ($p>0.05$).

Table 5.7 *Whole tract digestibilities of DM, OM and NDF (g/kg)*

	Diets				s.e.d.
	C	CU	CS	CSF	
DM					
Intake (g/d)	4124	4373	4164	4117	156.0
Faeces (g/d)	1124	1147	1115	1128	58.0
Digestibility	0.73	0.74	0.73	0.73	0.013
OM					
Intake (g DM/d)	3720	3948	3737	3674	142.9
Faeces (g DM/d)	962	983	945	909	54.6
Digestibility	0.74	0.75	0.75	0.75	0.013
NDF					
Intake (g DM/d)	1587	1588	1685	1639	116.2
Faeces (g DM/d)	512	521	478	445	34.3
Digestibility	0.67^{a}	0.67^{a}	0.72^{b}	0.73^{b}	0.018

^{ab} means with the same or no superscript in the same row are not significantly different ($p<0.05$)

Table 5.8 *Ruminal hay degradability*

	Diets				s.e.d.
	C	CU	CS	CSF	
Before incubation (g DM)	2.977	2.953	2.849	2.794	0.0420
After incubation (g DM)	2.313	2.250	2.040	2.037	0.0865
Degradability	0.223^{a}	0.238^{ab}	0.284^{c}	0.271^{bc}	0.0198

^{abc} means with the same or no superscript in the same row are not significantly different ($p<0.05$)

5.3.3 *Faecal water concentration and daily urine output*

The faeces produced by the sheep fed the high level of molasses (310 g/kg DM) in this experiment was darker than normal faeces. However, the high molasses inclusion did not result in loose faeces. The faeces did not stick together. Faecal water concentration and daily urine output are presented in Table 5.9. The various protein sources did not influence the faecal water concentration ($p>0.05$). Supplementing the control diet with protein resulted in slightly higher daily urine output ($p>0.05$).

Table 5.9 *Faecal water concentration and daily urine output*

	Diets				s.e.d.
	C	CU	CS	CSF	
Fresh faeces output (g/d)	1164	1204	1172	1134	78.8
DM faeces output (g/d)	375	382	372	376	19.6
Faecal water conc. (g/kg)	677	683	682	666	10.6
Urine output (g/d)	2428	2465	2754	2644	279

5.3.4 *Ruminal NH_3-N and pH*

The average NH_3-N concentrations in the rumen liquor of sheep fed diets C, CU, CS and CSF at various sampling times post feeding were 63.5, 81.4, 90,4 and 112.9 mg/l (s.e.d, 14.85), respectively. The concentrations were higher as each increment of dietary CP, but the differences only reached significance between treatments CSF and C ($p < 0.01$). The changes of ruminal NH_3-N concentrations at various sampling times post feeding are shown in Figure 5.1. This parameter increased sharply at 1.30 hours post feeding in all 4 treatments, and then dropped down quickly towards to 7.30 hours after feeding for treatments C and CU whilst the decline was much slower for treatments CS and CSF.

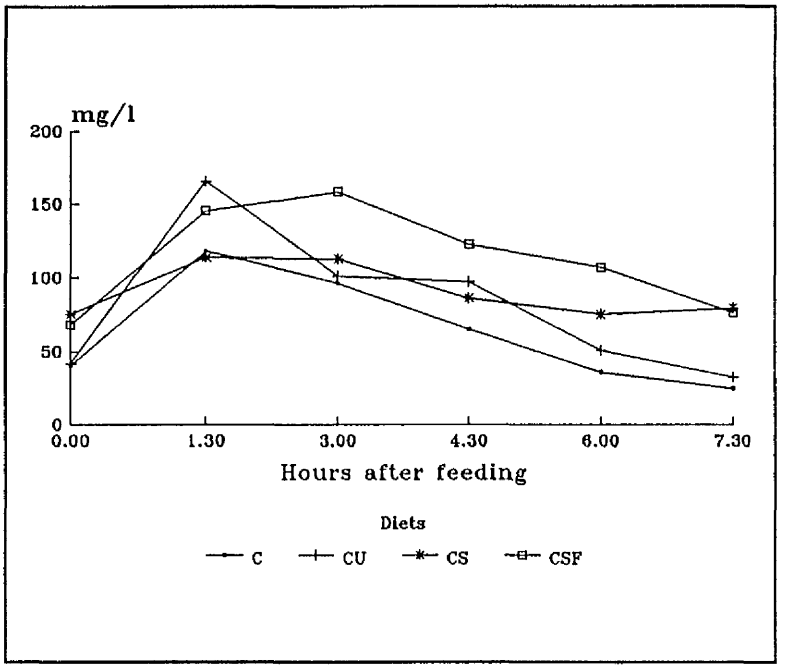

Figure 5.1 The NH_3-N concentrations in the rumen liquor post feeding

The average pH in the rumen liquor of sheep fed diets C, CU, CS, CSF at various sampling times post feeding were 6.40, 6.49, 6.62 and 6.47 (s.e.d., 0.06), respectively. The feeding of diet CS resulted in a significantly higher pH than other three diets ($p < 0.05$). The changes of pH in the rumen liquor at various sampling times post feeding are shown in Figure 5.2. The patterns of the parameter was similar in all four

treatments. The pH value were sharply depressed at 1.30 hours post feeding and then gradually recovered. The pH in the rumen of sheep fed diet C was significantly lower than those fed diet CS at 1.30 hours post feeding ($p<0.05$), but otherwise were similar between the four treatments at other 5 sampling times.

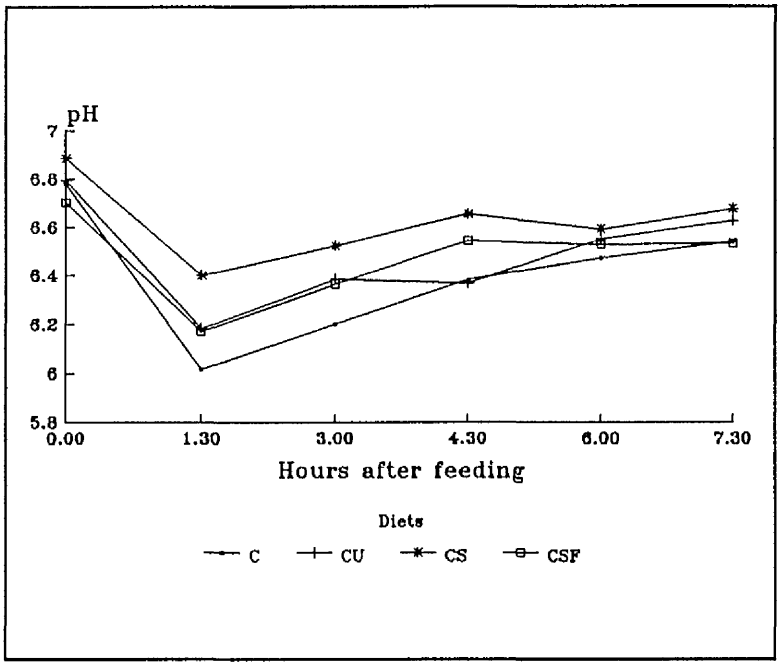

Figure 5.2 The pH in the rumen liquor of sheep post feeding

5.3.5 *Urinary purine derivative*

In analysis, urinary hypoxanthine, xanthine and uric acid all were converted to allantoin and then allantoin was measured as the total purine derivatives (PD). PD thus included actual allantoin and allantoin converted from other three purine derivatives.

The results of PD-N are shown in Table 5.10. The weighed mean PD-N concentration in urine of sheep fed diet CSF was lower than those fed the other three diets but a significant difference only occurred between treatments CU and CSF ($p<0.05$). The increases in dietary ERDP levels achieved by supplementing the control diet with urea or protein had little effects on total PD-N yield (mg/d), PD-N yield mg/kg metabolic weight, PD-N yield mg/kg digestible organic matter (DOM) intake or PD-N yield

mg/MJ FME intake ($p>0.05$). In contrast, the increases in dietary ERDP levels significantly depressed PD-N output as expressed as mg per g effective rumen degraded N (ERDN) intake ($p<0.05$). The latter parameter was similar between treatments CU, CS and CSF ($p>0.05$). Using the factors proposed by Chen, Kyle, Ørskov and Hovell (1991), *i.e.* ratio of purine-N/total-N in mixed rumen microbes of 0.116 and purine digestibility of 0.83, the microbial N supply to the animals fed diets C, CU, CS and CSF was calculated to be 14.0, 14.7, 14.2 and 14.0 g N per day (s.e.d., 1.06), respectively. This parameter was independent of diets ($p>0.05$).

Table 5.10 *Urinary purine derivative nitrogen (PD-N) concentration and yield*[+]

	Diets				s.e.d.
	C	CU	CS	CSF	
Concentration (mg/l)	527[ab]	554[a]	507[ab]	462[b]	39.7
Yield (mg/d)	1214	1272	1230	1212	91.6
Yield/DOM (mg/kg DM)	1259	1226	1251	1283	72.9
Yield/$kg^{0.75}$ (mg/kg)	52	53	52	51	4.0
PD-N/ERDN (mg/g)	74[a]	56[b]	53[b]	54[b]	4.4
PD-N/FME (mg/MJ)	101	100	101	103	5.9

[+] [abc] means with the same or no superscript in the same row are not significantly different ($p<0.05$)

5.3.6 *Ruminal volatile fatty acids*

The average total volumes of volatile fatty acids (VFAs) and molar proportion of each individual VFA in the rumen at various sampling times post feeding are shown in Table 5.11. The total volumes of VFAs in the rumen liquor averaged from 6 samplings post feeding did not significantly differ ($p>0.05$) between four treatments. This value was the lowest in sheep fed diet CS and higher in sheep fed diet CU or CSF. The total volumes of VFAs at various sampling times post feeding is presented in Figure 5.3. They all reached peak at 1.30 hours post feeding and then gradually declined for all 4 treatments.

Table 5.11 *The average total concentration and molar proportions of VFAs in the rumen*

	Diets				s.e.d.
	C	CU	CS	CSF	
Total (mm/l)	75.0	80.0	70.0	79.2	5.11
Molar percentage (%)					
Acetate	60.2^{a}	63.4^{b}	62.0^{ab}	63.5^{b}	1.48
Propionate	26.1^{a}	23.3^{b}	24.7^{ab}	23.7^{b}	1.08
Butyrate	11.7^{a}	11.3^{ab}	10.3^{b}	10.3^{b}	0.55
Isobutyrate	0.69^{a}	0.62^{a}	1.06^{b}	0.84^{c}	0.066
Valerate	0.90	1.03	1.10	1.00	0.102
Isovalerate	0.43^{a}	0.36^{a}	0.79^{b}	0.62^{c}	0.056

^{abc} means with the same or no superscript in the same row are not significantly different ($p<0.05$)

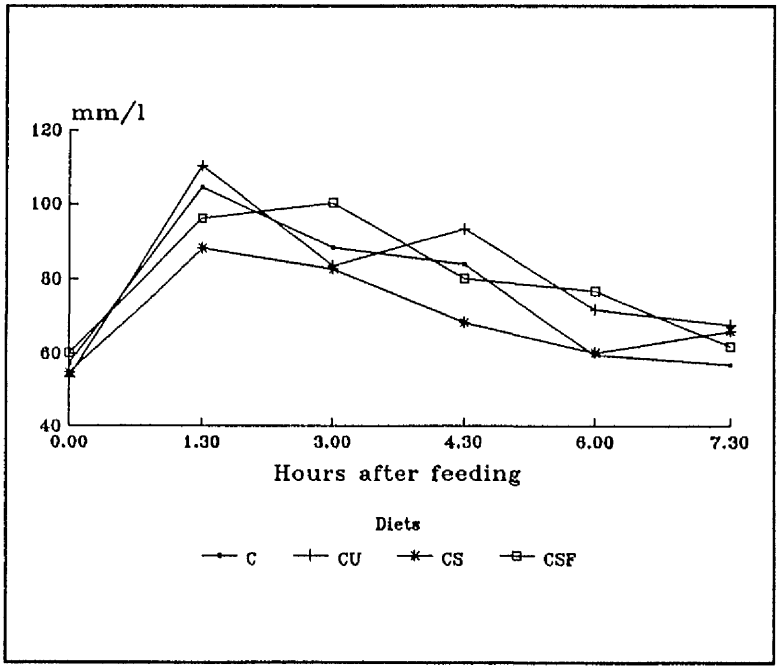

Figure 5.3 Total volume of VFAs at various sampling times post feeding

Supplementing the control diet with urea or protein significantly increased molar proportion of acetic acid ($p<0.05$) by 2 or 3%, but at the cost of propionic acid ($p<0.05$). There were no significantly different in molar proportions of these two acids between treatments CU, CS and CSF ($p>0.05$). The ratio of acetate/propionate was therefore lower in treatment C than treatments CU, CS and CSF (2.31, 2.72, 2.51 and 2.68 (s.e.d., 0.214), respectively), but the difference did not reach significance. The molar proportion of butyrate in treatment C was higher than in treatment CS or CSF ($p<0.05$). The feeding of diet CS resulted in highest molar percentages of isobutyric and isovaleric acids ($p<0.05$) and followed by diet CSF ($p<0.05$). The molar proportion of valeric acid was similar between four treatments ($p>0.05$).

The molar proportions of individual VFA in the rumen liquor at various sampling times post feeding are respectively presented from Figure 5.4 to 5.9. The molar percentage of acetic acid was depressed after feeding and then gradually increased, while propionic acid showed the reverse response for all four treatments. The molar proportion of butyric acid was higher in the sheep fed diets C and CU before feeding, and then decreased initially before increasing post feeding. In contrast, this parameter was lower for diets CS and CSF before feeding and then increased and afterwards decreased post feeding. The changes in molar proportions of valeric acid, isovaleric acid and isobutyric acid after feeding were very consistent for all four treatments. The molar percentages of isovaleric acid and isobutyric acid decreased immediately after feeding, whereas valeric acid showed an increase.

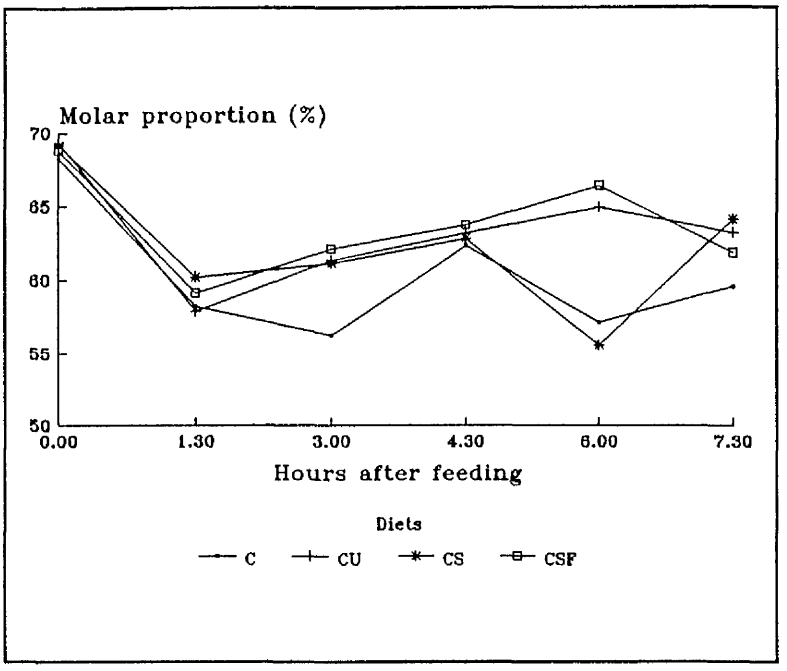

Figure 5.4 The molar proportion of acetic acid post feeding

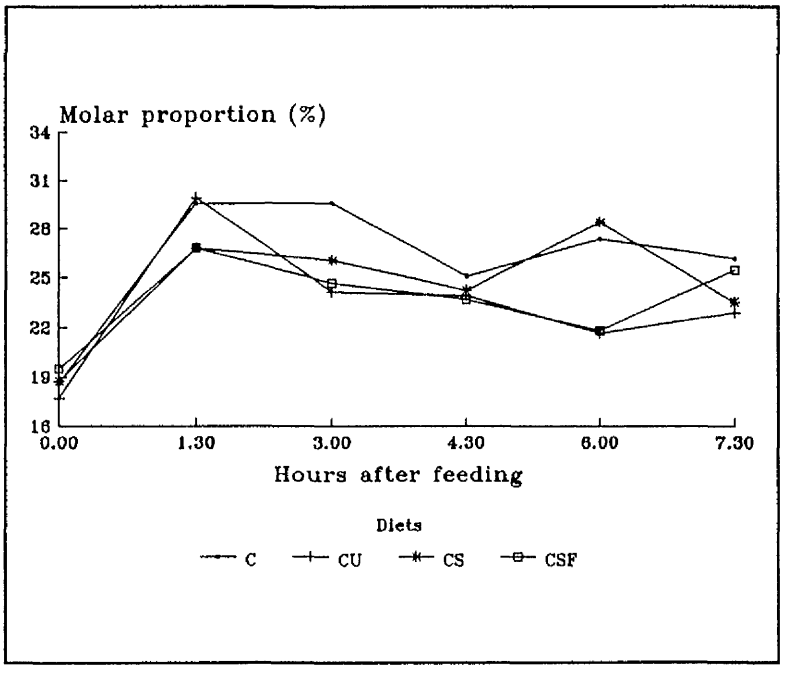

Figure 5.5 The molar proportion of propionic acid post feeding

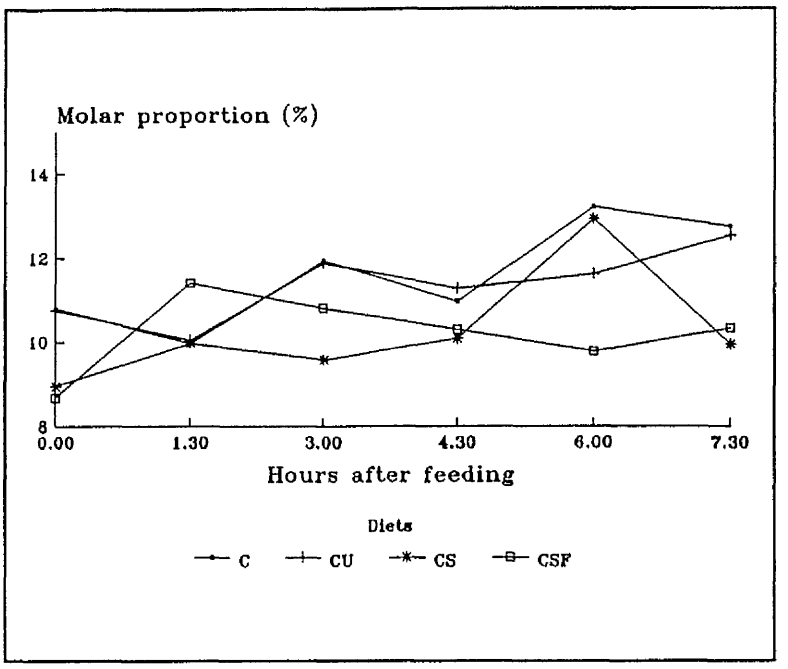

Figure 5.6 The molar proportion of butyric acid post feeding

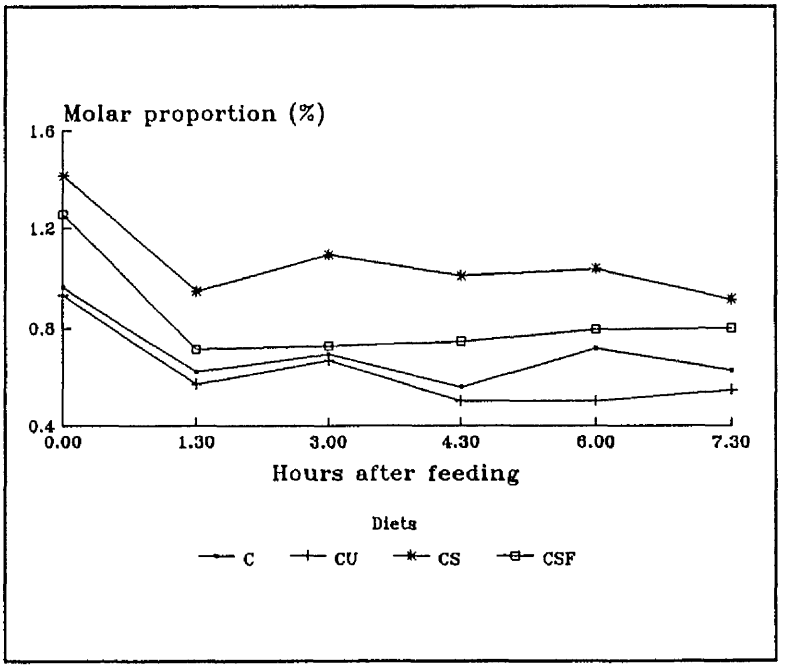

Figure 5.7 The molar proportion of isobutyric acid post feeding

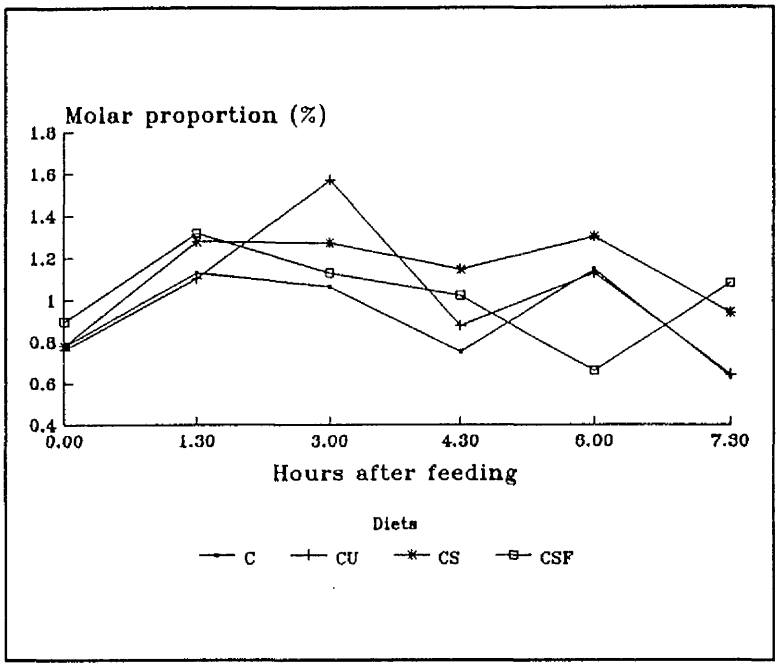

Figure 5.8 The molar proportion of valeric acid post feeding

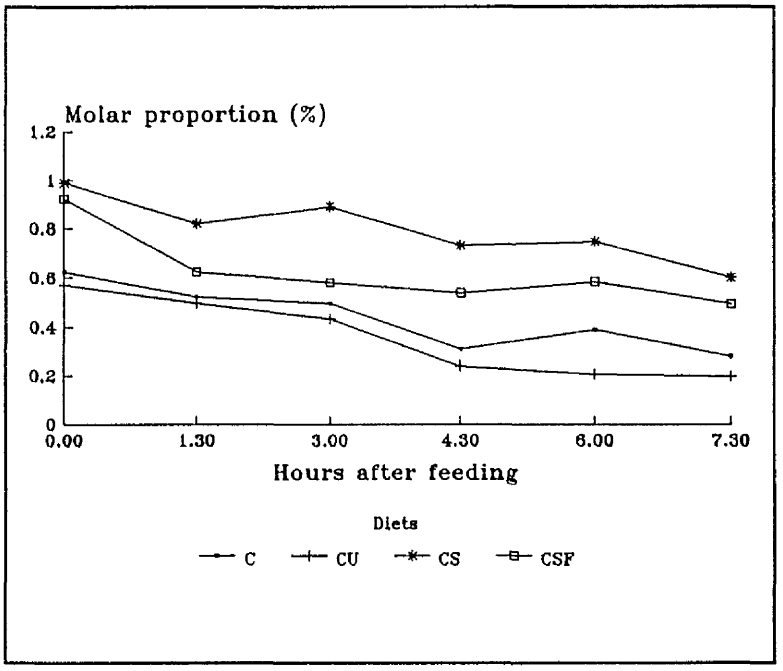

Figure 5.9 The molar proportion of isovaleric acid post feeding

5.3.7 *Calculated energy balance*

The daily ME intake (14.7, 15.6, 14.8 and 14.7 MJ/d, (s.e.d., 0.69)) and ME partitioned for maintenance were similar (average 8.7 MJ/d) between the four treatments ($p > 0.05$). ME partitioned for liveweight gains were increased from 1.3 to 5.4 MJ/d as the dietary protein levels raised, but the parameter did not significantly differ between treatments due to a great standard error of difference (s.e.d.) (2.57) caused by the factors including the small number of animals for each treatment (4 sheep), the short term feeding (3 weeks) in the changeover design and the abnormal body condition of the fistulated sheep. ME intakes exceeded ME requirements for all 4 treatments, although the excess was decreased with each increment of dietary protein levels (4.7, 3.1, 2.5 and 0.5 MJ/d (s.e.d., 2.64) for diets C, CU, Cs and CSF, respectively).

5.3.8 *Calculated protein utilization*

The calculated protein utilization is shown in Table 5.12. The ERDP supply to the sheep fed diets supplemented with urea or protein was significantly higher those fed the control diet ($p < 0.05$), but was similar between those fed the three supplemented diets ($p > 0.05$). The ERDP intake was therefore calculated to be deficient in the animals fed the control diet and was in excess in those fed the other three diets.

Calculated MP supply to the animals was significantly increased with increments of both dietary ERDP levels and DUP concentrations ($p < 0.01$). MP partitioned for maintenance, fleece growth and liveweight gain were similar between the four treatments ($p > 0.05$), although MP for gain was 5 times higher in treatment CSF than C. This was owing to a great standard error of difference (s.e.d.) (12.2) in MP partitioned for gain. The reasons were discussed in the previous section. However, MP requirement was exceeded in all 4 treatments.

Table 5.12 *The calculated protein utilization*

	Diets				s.e.d.
	C	CU	CS	CSF	
	g/d				
ERDP supply	116[a]	159[b]	161[b]	163[b]	5.03
ERDP requirement	136	143	137	136	5.14
ERDP excess	-20[a]	16[b]	24[c]	27[d]	0.77
MP supply	97[a]	116[b]	140[c]	161[d]	4.35
MP requirement	69	82	82	90	15.6
MP for maintenance	53	53	52	53	1.08
MP for fleece growth	12	15	15	16	2.76
MP for gain	4	14	15	21	12.2
MP excess	28[a]	34[a]	58[ab]	71[b]	14.2

[abcd] means with the same or no superscript in the same row are not significantly different ($p<0.05$)

5.3.9 *All parameters evaluated in extension period (P5)*

All parameters investigated in this extension period (referred as "P5") are compared with those obtained in treatment C and mean values of 4 treatments (referred as "mean") in the Latin Square design experiment. They are presented in Table 5.13.

Digestibilities of DM, OM and NDF in P5 all were significantly lower ($p<0.001$) in P5 in comparison with treatment C or mean. The decreases in P5 ranged from 18% for DM or OM digestibility to 22% for NDF digestibility. The DM disappearance rate of grass hay in the dacron bags suspended in the rumen of sheep was also 15% or 30% lower in P5 than in treatment C ($p<0.05$) or mean ($p<0.01$).

Table 5.13 *The comparison of parameters measured in P5 with those obtained for diet C and mean of 4 diets in Latin Square*

	Diets			s.e.d.
	C	Mean+	P5	
Digestibilities DM	0.73[a]	0.73[a]	0.62[b]	0.006
OM	0.74[a]	0.75[a]	0.63[b]	0.005
NDF	0.67[a]	0.70[b]	0.56[c]	0.010
Hay DM degradability	0.23[ab]	0.26[a]	0.20[b]	0.014
Rumen pH	6.50[a]	6.40[a]	6.23[b]	0.056
Rumen NH_3-N (mg/l)	63.5[a]	87.1[b]	32.2[c]	7.70
Rumen VFAs (mm/l)	75.0	76.0	75.1	4.02
molar percentage (%)				
Acetate	60.2[a]	62.3[a]	68.5[b]	1.14
Propionate	26.1[a]	24.5[a]	21.2[b]	0.79
Butyrate	11.7[a]	10.9[a]	9.0[b]	0.36
Isobutyrate	0.69[a]	0.80[b]	0.41[c]	0.032
Valerate	0.90[a]	1.01[a]	0.59[c]	0.055
Isovalerate	0.43[a]	0.55[b]	0.23[c]	0.019
Urine PD-N excretion				
Content (g/kg)	527	510	571	26.4
Yield (mg/d)	1214[a]	1232[a]	558[b]	53.9
mg/kg DOM	1259[a]	1246[a]	792[b]	67.1
$mg/kg^{0.75}$	52[a]	52[a]	23[b]	2.9
mg/g ERDN	74[a]	57[b]	69[a]	4.5
mg/MJ FME	101[a]	100[a]	65[b]	5.4
Faecal water content (g/kg)	677[a]	677[a]	592[b]	9.61
Urine output (g/d)	2428[a]	2573[a]	1042[b]	211

+ Mean for diets C, CU, CS and CSF in the Latin Square.

[abc] means with the same or no superscript in the same row are not significantly different ($p < 0.05$)

Fresh faeces outputs were similar (1088, 1164 or 1168 g/d (s.e.d., 51.8)), while DM faeces outputs were significantly higher ($p<0.01$) in P5 than in treatment C or mean (442, 375 or 376 g/d (s.e.d., 12.6)). Therefore fresh faeces contained significantly less water ($p<0.01$) in P5 than in treatment C or mean. Urine output in P5 was only half that ($p<0.01$) in diet C or mean.

The average NH_3-N concentration in the rumen liquor at various sampling times after feeding was proportional to dietary ERDP intake and was significantly lower in P5 than in diet C ($p<0.01$) or mean ($p<0.001$). The average pH was also significantly lower ($p<0.01$) in P5 than in diet C or mean. The changes of NH_3-N concentration and pH at various sampling times after feeding in P5 (Figure 5.10) showed similar tendencies as those obtained from Latin Square experiment. The NH_3-N concentration was dramatically increased at 1.30 hours after feeding and then continuously dropped down towards 7.30 hours, while pH was sharply decreased at 1.30 hours post feeding and maintained to 3.00 hours and then went up.

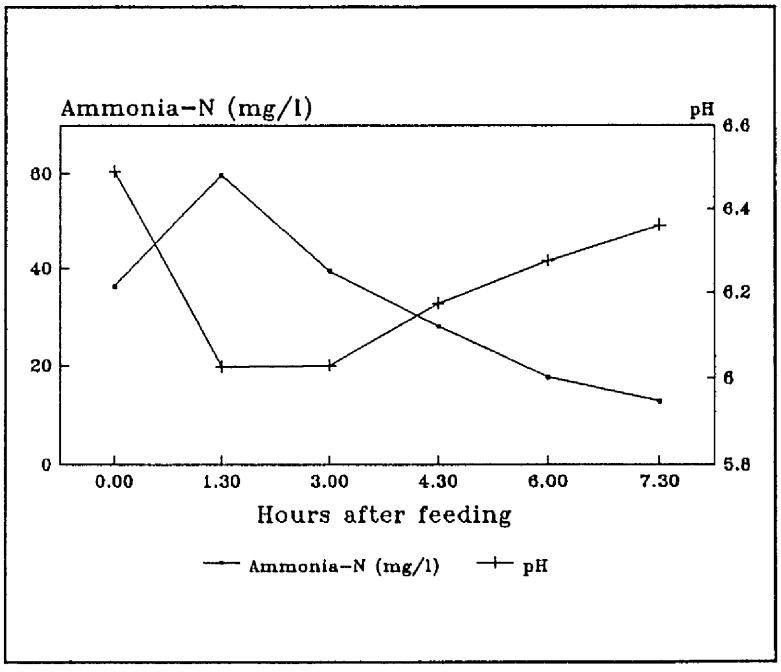

Figure 5.10 NH_3-N concentrations and pH of the rumen liquor post feeding in P5

The average total volume of VFAs in the rumen liquor at various sampling times post feeding was similar ($p > 0.05$) between P5, treatment C and mean. However, the molar proportions of individual major acids in P5 were different from treatment C and mean. The molar percentage of propionic and butyric acids were significantly lower ($p < 0.01$) for P5 than for treatment C or mean, but acetic was significantly higher ($p < 0.01$). The ratio of acetate/propionate was significantly higher ($p < 0.05$) for P5 (3.39) than for treatment C (2.31) or mean (2.56). The average molar proportions of all three minor acids (isobutyric, isovaleric and valeric) were significantly lower ($p < 0.01$) for P5 than for treatment C or mean.

The changes of molar proportions of individual acids at various sampling times after feeding in P5 are presented in Figures 5.11 and 5.12. The molar percentage of acetic acid declined at 1.30 hours and then gradually increased towards 7.30 hours, but propionic reached a peak at 1.30 hours and then gradually decreased. The feeding of the conventional ration did not resulted in a great changes in butyric acid. However, the feeding led to continuous decreases in the molar percentages of isobutyric and isovaleric acids. The molar proportion of valeric acids reached the peak at 3.00 hours after feeding and then fell.

The urinary purine derivatives (PD) were measured by total allantoin following the hypoxanthine, xanthine and uric acid were converted to allantoin. The PD-N concentration in urine of sheep fed the conventional diet was slightly higher ($p > 0.05$) than those fed treatment C or mean, but the PD-N yield was significantly lower ($p < 0.001$). The PD-N yield expressed as per kg DOM intake, as per kg metabolic weight and as per MJ FME intake all were significantly lower ($p < 0.001$) in P5 than in treatment C or mean. However, the urinary PD-N output as per g ERDN intake was similar between P5 and treatment C ($p > 0.05$) but higher than mean ($P < 0.05$). Using the factors proposed by Chen *et al.* (1991) as described before, the microbial N supply to the sheep fed the conventional ration was calculated to be 5.8 g/d (s.e., 1.0) and was less than half that for treatment C (14.0 g/d) or mean (14.2 g/d).

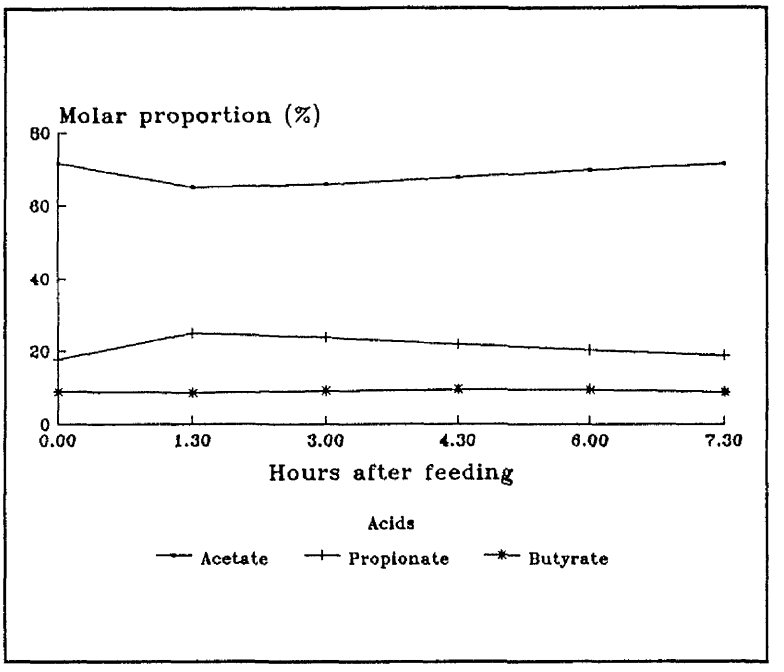

Figure 5.11 The molar proportions of major acids in the rumen liquor post feeding

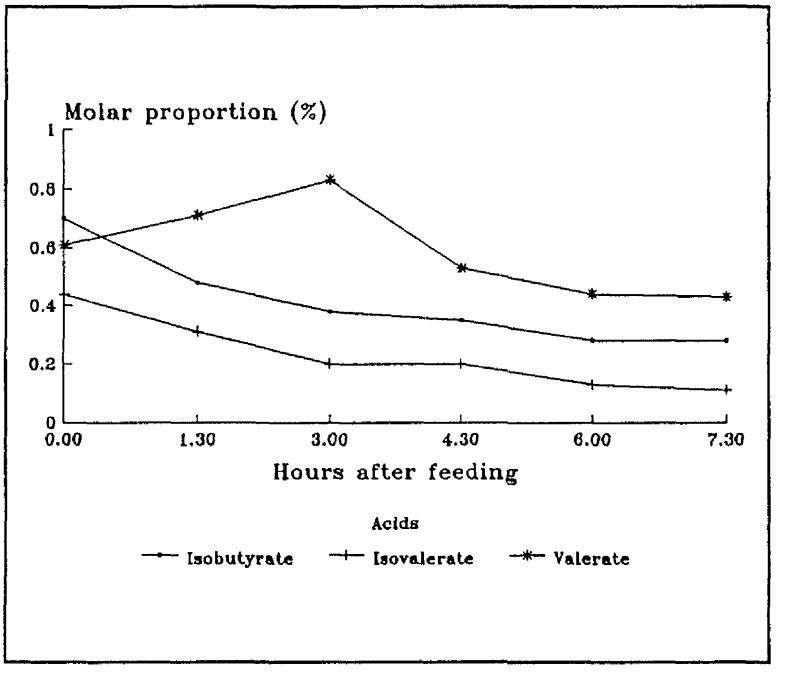

Figure 5.12 The molar proportions of minor acids in the rumen liquor post feeding

Total ME intake of sheep fed the conventional ration (10.6 MJ/d (s.e., 0.48)) was significantly lower than those fed the molasses diets in the Latin Square experiment ($p<0.001$). The protein utilization was also calculated. The low ERDP concentration in the conventional ration resulted in a lower ERDP supply (57 g/d (s.e., 3.3)) and subsequently an ERDP deficiency (39 g/d (s.e., 2.2)). Calculated MP supply to the sheep thus was significantly lower ($p<0.001$) for P5 (59 g/d (s.e., 2.9) than in treatment C or mean (129 g/d).

5.4 *Discussion*

5.4.1 *Effects of ERDP and DUP in molasses diets*

No response in total VFAs concentrations in the rumen to supplementing urea or true protein in the current experiment agreed with the previous studies conducted by Hume, Moir and Somers (1970) with sheep, Cameron *et al.* Clark (1991) with dairy cows and Keery *et al.* (1993) with steers. In contrast, Klusmeyer, McCarthy and Clark (1990) reported that replacing 80 g/kg DM of maize by soyabean meal in total diets increased total VFAs concentrations in the rumen of dairy cows from 98.6 to 112.7 mm/l. They also found that supplementing soyabean meal increased the average molar percentages of acetate and decreased propionate, but did not affect butyrate and isobutyrate. The results also differed from those obtained in the current experiment. However, lack of responses in the average molar percentages of isobutyrate and isovalerate to supplementing urea in the current experiment agreed with the previous studies (Hume *et al.*, 1970 and Cameron *et al.*, 1991). The significant increases in the molar proportions of isobutyrate and isovalerate when the soyabean meal and fish meal were supplemented in the current experiment probably resulted from the deamination of dietary amino acids in the rumen.

Purine derivatives (PD) in the urine of ruminant animals originate mainly from the degradation of absorbed microbial nucleic acids and the daily excretion is directly related to the daily amount of purines absorbed (Chen, Hovel, Ørskov and Brown, 1990; Verbic, Chen, MacLeod and Ørskov, 1990). Daily PD excretion therefore

provides a measurement of the supply of microbial nucleic acids and hence microbial protein (since the nucleic acid and protein concentrations in microbial mass are corrected) to the animals (Rys, Antoniewicz and Maciejewicz, 1975; Chen, Ørskov and Hovell, 1991). Therefore in this experiment total PD in the urine of sheep was measured as an indicator of ruminal microbial synthesis.

The metabolisable protein (MP) system for assessing the protein requirement of ruminants (AFRC, 1992) considers separately the amounts required for microbial activity in the rumen and the amino acid requirements of the animal tissue. The amino acids presented to the small intestine of ruminants are derived mainly from microbial protein synthesized in the rumen and dietary protein that has escaped degradation in the rumen. The supply of microbial protein depends on the supply of ERDP to the rumen bacteria and the intake of FME. The dietary ERDP can be broken down to ammonia in the rumen and subsequently the ammonia is served as the main source of N for microbial growth. Therefore, the ammonia continuously available in the rumen is essential to achieve maximum efficiency of microbial synthesis. Several *in vitro* studies have shown maximum microbial growth to occur when the NH_3-N concentration was 50 to 80 mg/l (Allison, 1970; Annison, 1975 and Satter and Slyter, 1974). Hume et al. (1970) observed *in vivo*, that microbial growth attained a maximum level when rumen NH_3-N concentration reached approximately 90 mg/l. In contrast, Miller (1973) found a considerably higher values of approximately 290 mg/l. Results of an *in vivo* study reported by Okorie, Buttery and Lewis (1977) indicated that maximal protein synthesis was achieved when the rumen NH_3-N concentration reached 50 mg/l; an observation consistent with *in vitro* observations of Satter and Slyter (1974). Schaefer, Davis and Bryant (1980) concluded that many of the predominant species of rumen bacteria can attain 95% of their maximal growth in the presence of 14 mg/l of NH_3-N. In the Latin Square feeding of this experiment, the feeding of all 4 diets resulted in reasonably high NH_3-N concentrations (mean values for all sampling times) in the rumen of sheep from 63.5 to 112.9 mg/l, although the concentrations were lower in the rumen liquor taken at 7.30 hours post feeding in sheep fed diet C (24.9 mg/l) or those fed diet CU (32.3

mg/l). Hence, it is unlikely that the rate of ruminal microbial protein synthesis for the low ERDP diet (diet C) was limited by ammonia supply in the rumen. This was supported by the fact that PD-N yields in the urine of sheep fed the four diets were similar ($p > 0.05$) as expressed as either total yield per day or total yield per kg DOM intake or total yield per kg metabolic weight. In fact the calculated microbial N supply to the animals fed diets C, CU, CS or CSF was very similar (14.0, 14.7, 14.2 or 14.0 g N per day (s.e.d., 1.06)).

The NH_3-N concentrations in the rumen of sheep were directly proportional to dietary ERDP concentrations. The mean concentrations was nearly double for diet CSF than for diet C. Since the microbial synthesis was similar between the four treatments, the excess NH_3-N in the rumen of sheep fed high ERDP diets was probably absorbed from the rumen into blood and then excreted mainly in urine as urea. This resulted in a significantly lower proportion of PD-N as total ERDN intake or as total calculated MCN production ($p < 0.01$) in treatments CU, CS and CSF than in treatment C.

Microbial protein synthesis can occur in the rumen on diets in which urea is the only N source, however, efficiency of microbial growth may be limited by a deficiency of preformed amino acids (Stern and Hoover, 1979). Some studies have shown that microbes can derive 30 - 50% of their N directly from amino acids or peptides (Kempton and Leng, 1979 and Redman, Kellaway and Leibholz, 1980). Salter, Daneshvar and Smith (1979) examined the origin of the N incorporated into rumen bacteria in steers fed diets containing protein and urea and concluded that methionine and phenylalanine may be limiting for bacterial growth on diets low in protein and high in NPN. Demeyer (1981) reported that some microbial species have a specific requirement of methionine. Therefore in the current experiment supplementing diets with soyabean meal and especially fish meal might be expected to achieved higher rates of microbial synthesis in the rumen compared with diet C or CU. However, no improvement in microbial synthesis was observed with supplementing diets with soyabean meal and fish meal in comparison with diet C and CU. The lack of the

response in microbial synthesis to increased dietary ERDP levels could be due to the reasonable high protein concentration in diet C (123 g/kg DM). This is supported by the NH_3-N concentrations in the rumen and also consistent with results obtained by Hume *et al.* (1970) that microbial protein production was increased when sheep fed diets containing N from 0.95 to 1.82% (11.5% of crude protein), but no further improvement was found when dietary N concentration was raised to 3.29% (20.6% of CP).

Responses in the whole tract apparent digestibilities of DM and OM in this experiment accorded with response in the efficiency of microbial growth as measured by urinary PD excretion. Supplementing the control diet with protein or urea showed little effect on the DM and OM digestion. In contrast, the whole tract digestibility of NDF and hay degradability in the rumen of sheep fed diets supplementing with soyabean meal or fish meal were significantly higher than those fed the control diet or diet supplementing with urea. Some previous studies showed the similar results. McAllan and Smith (1983) observed a greater rate of fibre digestion when fish meal rather than urea provided the N source. Silva and Ørskov (1988) fed mature male sheep with the control diet containing untreated barley straw and urea (13 g N/kg DM of straw) and found that supplementing straw with fish meal at 100 g/kg DM of total diet consistently improved the degradability of straw from 8 to 72 hours of incubation, while supplementing with soyabean meal at 100 g/kg DM had no effect. Kropp, Johnson Males and Owen (1977) noted that substitution of N from soyabean meal with N from urea significantly depressed the digestion of cellulose in steer. In this experiment increases in NDF digestion and hay degradation without raised total microbial growth in the rumen of the sheep fed diet CS or CSF might be associated with the higher concentrations of branched-chain fatty acids (isobutyrate and isovalerate) in the rumen probably arising from deamination of dietary amino acids. The molar proportions of these two acids in the rumen of sheep fed diet CS or CSF was significantly higher than those fed diet C or CU. The higher concentrations might result in a greater growth of cellulolytic bacteria and possibly with decreases in growth of other microorganism, since growth

rate of cellulolytic bacteria is dependent on the presence of branched-chain fatty acids except of ammonia (Allison, Bryant and Doetsch, 1958).

The dietary DUP escapes the rumen degradation and then is broken down into amino acids by enzymes secreted by the host animals. Dietary DUP directly serves the host animal as source of amino acids for their maintenance and production. In this experiment, the liveweight gains of the sheep (15, 56, 68 or 98 g/d (s.e.d., 48) in sheep fed diets C, CU, CS or CSF) were directly proportional to the dietary DUP concentrations. Since the supplies of microbial protein and ME to the host animals were similar between the four treatments, the higher gains in sheep fed diet CS or CSF could be owing to their higher DUP intakes. However, there were no significantly differences in liveweight gains due to a great standard errors of difference (s.e.d.) between the four treatments. This could be attributed to the small number of animals in each treatment (4 sheep), the short term feeding (3 week) in the changeover design and the abnormal body condition of the fistulated animals. The fourth factor responsible to this could be that the sheep used were mature and hence their slow growth could limit the potential of effect of dietary DUP concentration on the liveweight gain.

5.4.2 *Conventional ration v. molasses diets*

The loose faeces is often observed in ruminants fed diets containing moderate to high levels of molasses, which is often associated with diarrhoea (Scott, 1953). In this experiment the sheep fed diets containing 310 g/kg DM of molasses did not suffer from scouring, as a similar result as observed in all 3 previous dairy cow experiments, but their faeces were darker, looser and stickier than that of sheep fed the conventional ration in extension period (P5). The mean water concentration in fresh faeces of sheep fed the former diets was 13% higher than that of sheep fed the latter ration. The laxative property of molasses was reported by Briggs and Heller (1943) in a trial with lambs fed purified diets, to be attributed to its higher concentrations of both sugar and potassium.

The addition of carbohydrate sources such as glucose or molasses to rations of ruminants produces a marked transient appearance of lactic acid (Elsden and Phillipson, 1948 and Kellogg, 1969). The appearance of lactate is associated with a low rumen pH and a shift in the population of certain bacteria (Harris and Van Horn, 1982). In ruminants fed molasses the rumen fermentation is characterised by a low molar proportion of propionic acid as discussed in Chapter One. However, the results obtained from this experiment were opposite to the findings. Rumen pH values for the conventional ration without molasses were significantly lower than for the diets containing 310 g/kg DM of molasses. The molar proportion of propionic acid in the former was 13% lower than in the latter ($p<0.01$). The disagreement may be due to the different ingredient and chemical compositions between the molasses diets and the conventional ration and might also be attributed to the period effect in this experiment as the conventional ration was fed only in period 5. Even in Latin Square, the molar percentage of propionic acid as total VFAs significantly fluctuated (26, 24, 22 and 26% (s.e.d, 1.3) in the 1st, 2nd, 3rd and 4th period, respectively).

The lower ERDP concentration for the conventional ration (P5) resulted in a significantly lower NH_3-N concentration in the rumen than for the molasses diets. The average ruminal NH_3-N concentration (32.2 mg/l) at various sampling times post feeding in sheep fed the conventional ration in P5 was lower than recommended levels which support maximum microbial synthesis in the rumen as discussed in the previous section. The parameter was even lower than the value of 20 mg/l from 6.00 hours after feeding (Figure 5.10) suggesting the minimum concentration for the optimum microbial growth. The PD-N output in urine of sheep fed this ration was significantly lower ($p<0.001$) than those fed the higher ERDP diets in the Latin Square design experiment as expressed as either mg/d or mg/DOM or mg/$kg^{0.75}$. Using the factors proposed by Chen *et al.* (1991) as discussed before, calculated microbial N supply to the animals fed the conventional ration was only 5.8 g/d (s.e., 1.0), half that obtained from Latin Square experiment. However, when PD-N output was expressed as proportion of ERDN intake, there was no significant difference between P5 and treatment C or mean

suggesting that ERDP supply limited the microbial growth for the conventional ration (P5). Therefore, the lower microbial growth in the rumen of the animals fed the conventional ration resulted in a lower rate of hay degradability in the rumen or whole tract digestibilities of DM, OM and NDF ($p < 0.001$).

5.5 *Conclusion*

The results from Latin Square indicated that feeding 310 g/kg DM of molaferm 20 did not result in clinical symptoms of ill health of the animals for 3 months. The dietary concentrations of ERDP and DUP at 84 and 17 g/kg DM could satisfy the requirements of microbial growth when high levels of molaferm 20 was fed. However, supplementing DUP, but not ERDP, stimulated fibre digestion in the rumen suggesting that the UK Metabolisable Protein system may be in error for diets containing high sugar levels.

CHAPTER 6

General discussion

The feeding of high levels of molasses to ruminants can result in some metabolic disorders and subsequently affect the animal performance. Some cattle did suffer some scouring in experiment 1 when they were fed the diet containing 468 g/kg DM of molaferm 20, but otherwise no clinical ill health of cows or sheep was observed and all blood parameters investigated in cattle were within normal range when the animals were fed molaferm 20 at 310 g/kg DM or below. Therefore, in the following discussion the animals would be assumed to be healthy and the attention will be paid to effects of the feeding molaferm 20 on the microbial growth in the rumen and the efficiencies of utilization of energy and protein.

6.1 *Effects on microbial growth in the rumen*

Molaferm 20 contains 715 g/kg of dry matter, of which 780 g/kg DM is the inverted sugar. Almost all its ME is derived from the non-structural carbohydrates and so it is fermented very quickly in the rumen. (Johnson, 1976 and Ørskov and Ryle, 1990). The conversion rate of ME to FME in molaferm 20 is 0.914 based on the metabolisable protein (MP) system as applied at the Scottish Agricultural College. The feeding of molaferm 20 could have a major effect on the microbial growth and subsequently the food intake and milk production.

Molasses has been demonstrated to be less effective in conversion of dietary urea to the microbial protein in comparison with cereal grains, since a higher N loss in urine was observed in the ruminants fed molasses. However, in terms of conversion of dietary ERDP derived from protein to microbial protein the results were not consistent. Beever *et al.* (1988) feeding molassed sugar beet pulp (SBP) compared with unmolassed SBP and Huhtanen (1988) replacing barley or SBP with molasses, noted a higher microbial growth. In contrast, Oldham *et al.* (1977) and Al Atter *et al.* (1976) both *in vivo* and El Khidir and Thomsen (1982) *in vitro* found a lower microbial protein synthesis when molasses was supplemented to the basal diets. Nevertheless, the feeding of molasses has definitely shown lower digestibilities of dietary fibrous components, which may implicate a lower cellulolytic bacteria activity in the rumen.

Although the FME in molaferm 20 was not directly determined in this study, the higher urea concentrations in blood in the cows fed high levels of molaferm 20 might give evidence that the microbial growth in the rumen was lower. In experiment 1 (Chapter 2) the treatment means of blood urea concentrations were higher as the substitution rate of the molaferm 20-based liquid supplement for the grass silage increased. In experiments 1 and 2 (Chapter 2 and 3), the period means of blood urea concentrations were significantly increased as the experiments progressed. The higher blood urea concentrations suggest that less N might be captured in the rumen and more N loss from urine.

In experiment 4 (Chapter 5) when molaferm 20 was a major source of FME in the Latin Square design, total PD-N excretion in urine of the sheep failed to respond to the increases in dietary levels of ERDP derived from either urea or soyabean meal or/and fish meal. The lack of response could be the relatively high dietary ERDP concentration in the control diet, but may also be attributed to the fast fermentation rate of molaferm 20 in the rumen of the sheep. The fast fermentation rate may result in a quick disappearance of the energy derived from molaferm 20 from the rumen after feeding, leaving less FME available for the microbial growth afterwards. Indeed, the PD-N output in urine as per g ERDN intake (PD-N/ERDN) in sheep fed the conventional ration in the extension period was higher than mean value of those fed the molaferm 20 diets in the Latin Square design (69 *v.* 59 mg/g), although the former ration contained substantially less FME and ERDN than the latter diets.

In recent years, many countries have published their own new national protein requirement systems for ruminants to overcome the limitations of digestible crude protein (DCP) system. The microbial protein synthesis is central to all the systems and the microbial crude protein (MCP) production were proposed which are presented in Table 6.1. In experiment 4 of this study, the total PD-N was measured in the urine of sheep fed the diets containing 310 g/kg DM of molaferm 20. The MCN production in the rumen of sheep was then calculated according to the proposals reported by Chen

Table 6.1 *The ruminal microbial protein synthesis in experiment 4 compared with those in new systems of 8 countries*

	Protein Systems (7 Countries)							Diets (experiment 4)				
	F(88)	D	NKJ	US	AUS	NL	IDWP	C	CU	CS	CSF	s.e.d.
MCP/RDP	0.90	0.95	v	0.90	0.8-1.0	v, >1.0	0.8-1.0	0.66[a]	0.49[b]	0.49[b]	0.50[b]	0.033
MCP/DOM	0.126	0.161	0.165	0.140	0.095-0.170	ns	ns	0.091	0.087	0.090	0.093	0.005
MCP/ME (g/MJ)	8.1	10.1	10.3	9.6	6.1-11.0	ns	(9-11)	6.0	6.0	6.0	6.1	0.35

F(88) - *The French "Protein Digested in the Intestine" system*

D - *The West German "Crude Protein Flow at the Duodenum" system*

NKJ - *The Nordic AAT-PBV system*

US - *The US NRC "Absorbed Protein" system*

AUS - *The Australia "Apparently Digested Protein Leaving the Stomach" system*

NL - *The Dutch "Digestible Protein in Intestine" system*

IDWP - *The UK "Metabolisable Protein" system*

v - variable; ns - not stated

ab means with the same or no superscript in the same row are not significantly different (P>0.05)

The protein systems are from AFRC (1992)

et al. (1991) (the purine digestibility was 0.83 and the ratio of purine-N/total-N in mixed rumen microbes was 0.116).

$$\text{Total microbial N (MCN) (g/d)} = (\text{PD-N} / 0.83) / 0.116 \quad (1)$$

$$\text{Total MCP (g/d)} = \text{MCN} \times 6.25 \quad (2)$$

The results are also presented in Table 6.1. Compared with the 7 protein systems, the efficiency of microbial protein synthesis was very low in experiment 4 when a large amount of molaferm 20 was fed (310 g/kg DM). MCP production as proportion of RDP intake in sheep fed the control diet was about 70% of predicted values from the protein systems and 50% in those fed the diets supplemented with urea or protein. The similar results were obtained in comparison of experiment 4 with the 7 protein systems when the MCP production were expressed as proportion of either digestible organic matter (DOM) intake or ME intake.

In conclusion, the feeding high levels of molaferm 20 appeared to depress microbial protein synthesis in the rumen of sheep and cattle. This decrease was found to be about 30% in comparison with anticipated in sheep fed the diets containing 310 g/kg DM of molaferm 20. The lower microbial growth could be partially attributed to the metabolic disorder when high levels of molasses are fed. In experiment 4 the faeces produced by the sheep fed the molaferm 20 diets was softer than those fed the conventional ration. The Tamminga's suggestion that feeding molasses could resulted in a lower ATP supply for microbial growth might also be the part of answer to the lower microbial protein synthesis.

6.2 *Effects on energy utilization*

The feeding of high levels of molasses can depress dietary energy utilization in many ways, except its detrimental effect on the animal health. First, molasses reduces the digestibilities of DM and dietary fibrous components, particularly when low quality roughages were fed. The feeding of molasses is also associated with the lower dietary

CP digestion. This decrease was ranged from 5 to 15% when moderate to high levels of molasses were fed (Pate, 1982). Second, the feeding of molasses results in an increase in the molar proportion of butyric acid and a decrease in propionic acid. Since butyric acid is not a glucose precursor, molasses could decline the dietary energy utilization. Finally increases in molasses inclusion in diets is associated with depression of molasses energy. Lofgreen and Otagaki (1960) reported that the net energy value of molasses could be decreased by 3 times when dairy cows were fed a diet containing 300 g/kg DM of molasses in comparison with a diet containing 100 g/kg DM of molasses.

In this study the above parameters were not directly determined, but there were some evidences to show that the efficiency of energy utilization was low when the cows were fed high levels of molasses. In experiment 1 since the ME intakes were substantially different between the groups of cows at various periods within the same treatment (*e.g.*, 174, 190 and 195 MJ/d at the 1st, 2nd and 3rd periods in the medium molaferm 20 treatment), the relationship between the NE retained in milk and the ME intake was investigated into the treatment-periods (9 pairs of data). The results are presented in Figure 6.1. When the parameters were expressed as MJ per kg metabolic liveweight, the NE retained in milk was curvilinearly correlated with the ME intake. As the molaferm 20 inclusion in diets increased from 156 to 312 g/kg DM, the ME intake was raised from 1.20 to 1.60 MJ/kg $^{0.75}$ and the NE retained in milk was correspondingly higher (0.42 *v.* 0.47 MJ/kg$^{0.75}$). However, when the inclusion further increased to 468 g/kg DM, the ME intake was further higher (1.87 MJ/kg$^{0.75}$) but the NE retained in milk did not respond (0.46 MJ/kg$^{0.75}$). This result was different from that obtained by Moe and Tyrrell (1975) who summarized several experiments in which cows were fed diets containing 400 to 700 g/kg DM of maize silage and 600 to 300 g/kg DM of concentrate. The NE retained in milk (MJ/kg$^{0.75}$) was linearly correlated with the ME intakes ranged from 0.67 to 1.88 MJ/kg$^{0.75}$.

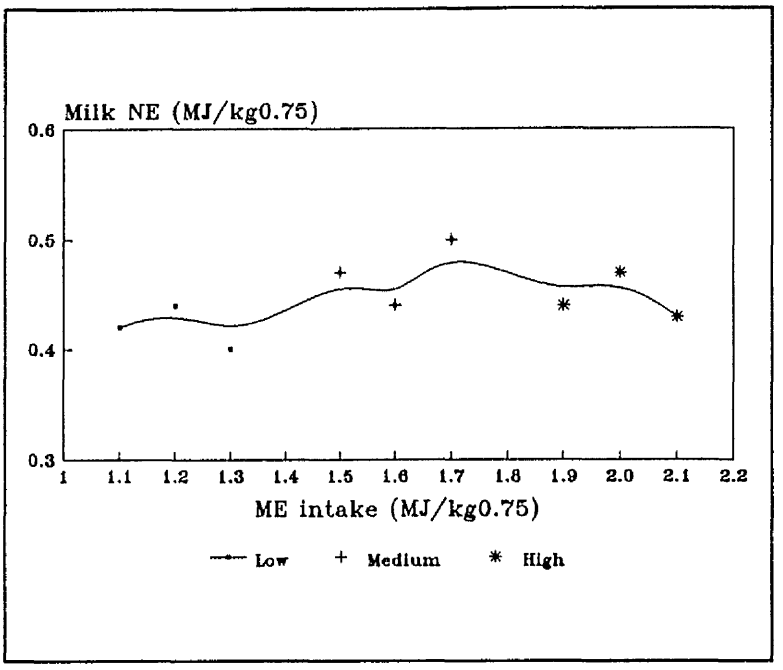

Figure 6.1 The relationship between NE in milk (MJ/kg$^{0.75}$) and ME intake (MJ/kg$^{0.75}$) in experiment 1 (Each treatment had 3 values from the 3 periods)

The efficiency of utilization of ME intake for milk production has now been well quantified (average value of 0.6) and has been shown to remain constant over a wide range of conditions such as different animal production potentials or feeding levels (Coulon and Rémond, 1991). This efficiency value accords with that obtained by Moe and Tyrrell (1975) in computer model in which the maintenance ME was assumed as 0.46 MJ/kg$^{0.75}$. However, in this study the efficiency of utilization of ME intake for milk production (NE_{milk} / (MEI - ME_m)) in all three dairy cow experiments was calculated to be lower than this value (Figure 6.2). In experiment 1, the efficiency (0.57) was close to 0.60 when cows were fed the diet containing 156 g/kg DM of molaferm 20 and then was linearly decreased ($p<0.01$) when the animals were fed higher levels of molaferm 20. The efficiency was nearly half (0.33) when molaferm 20 was fed at 468 g/kg DM. In experiments 2 and 3, the efficiencies in all treatments (310 g/kg DM of molaferm 20 in diets) were 22 to 33% lower than 0.60. Even when cows were fed the diet containing high ERDP and DUP in treatment H/H of experiment 2 (121 and 32 g/kg DM), this value was still only 0.47.

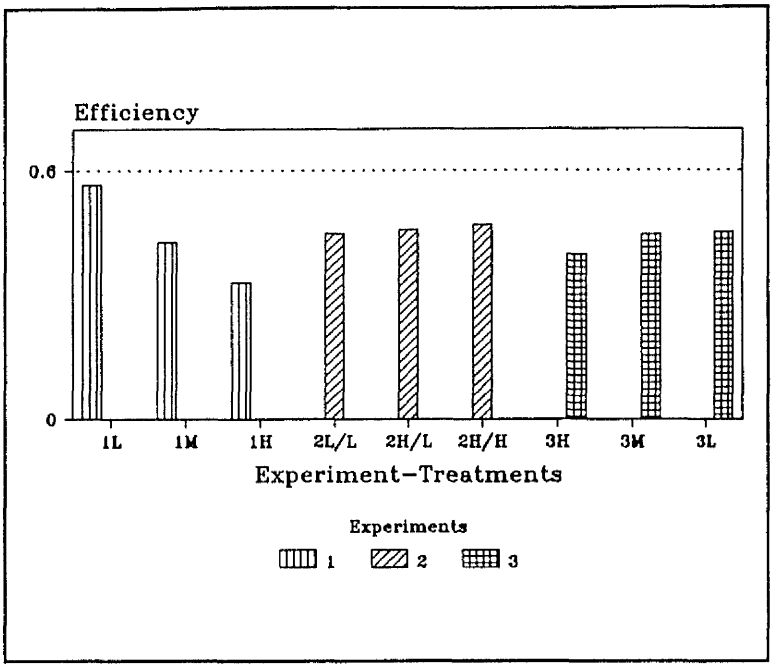

Figure 6.2 The efficiency of ME utilization for lactation ($NE_{milk}/(MEI-ME_m)$) in three dairy cow experiments

(Names in X axis represent respectively; experiment 1, treatments low, medium and high molaferm 20 (1L, 1M and 1H); experiment 2, treatments L/L, H/L and H/H (2L/L, 2H/L and 3H/H); experiment 3, treatments H_{FME}, M_{FME} and L_{FME} (3H, 3M and 3L).

In experiment 2 with early lactation cows and experiment 3 with lactating dairy heifers, the efficiency of ME utilisation for lactation was similar ($p>0.05$) between all 6 treatments and was ranged from 0.40 to 0.47 when cows were fed diets containing 310 g/kg DM of molaferm 20. It is interesting to note that the efficiency obtained from medium molaferm 20 treatment in experiment 1 (0.42) with mid-lactation cows (the diet contained 312 g/kg DM of molaferm 20) fell into this range. This may indicate that the efficiency of ME utilization was mainly influenced by molaferm 20 inclusions in diets rather than the lactation stage or dietary ERDP and DUP concentrations (experiment 2) or dietary FME concentration (experiment 3).

In experiment 1, the ratio of NE/ME was significantly higher in the cows fed the diet

containing 156 or 312 g/kg DM of molaferm 20 than those fed the diet containing 468 g/kg DM of molaferm 20. The lower ratio in the high molaferm 20 treatment may implicate a higher loss in energy after absorption. This supported the result obtained by Lofgreen and Otagaki (1960). The NE value of molasses for lactation was significantly lower when cows fed a diet containing high molasses than those fed a diet containing low molasses, but the DE values of molasses in both diets were similar. They therefore suggested that the energy loss of molasses occurred after absorption when high levels of molasses were fed.

The predicted milk yields in all three dairy cow experiments were calculated by using the equations based on the energy balance reported by ARC (1980, the details of equations are presented in Appendix 7). The results are presented in Figure 6.3. All the predicted milk yields were higher than the actual production between treatments within each experiment. In experiment 1, the cows fed the diet containing 468 g/kg DM of molaferm 20 produced substantially less milk than predicted (7.4 kg/d less), while those fed the diet containing 312 g/kg DM of molaferm 20 produced 1.6 kg/d less. The corresponding value for cattle fed the 156 g/kg DM of molaferm 20 was 5.0 kg/d. The more effective utilisation of ME in the medium molaferm 20 treatment was due to a higher liveweight gain of the animals. The less milk yield produced than predicted was ranged from 0.4 to 6.1 kg/d in experiment 2 and from 0.5 to 5.4 kg/d in experiment 3. The results indicate that the ME was less well utilized than predicted for milk production when high levels of molaferm 20 were fed to dairy cows.

It is concluded that the feeding of high levels of molaferm 20 depress the efficiency of utilisation of ME intake. The lower efficiency was mainly caused by the dietary molaferm 20 levels rather than lactation stage or dietary ERDP and DUP levels or dietary FME concentrations. The unanswered question is where the ME is lost after absorption. The lower molar proportion of propionic acid in the rumen and the detrimental effects on the animal health could be the part of the answer when a large amount of molasses was fed, but there could be something else to be answered.

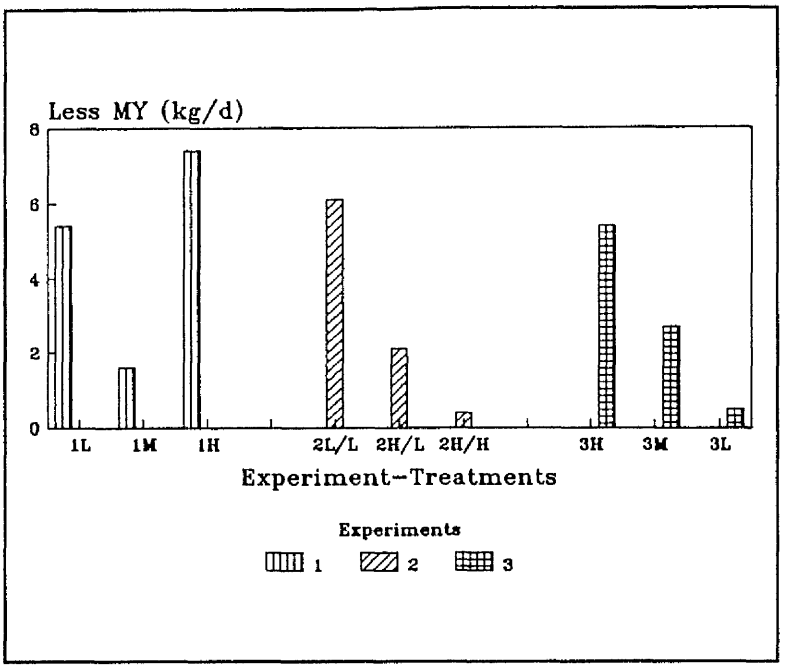

Figure 6.3 The less milk produced than predicted in three dairy cow experiments (For details of names in X axis, please see Figure 6.2)

6.3 *Effects on protein utilization*

The feeding of high levels of molasses can depress the dietary nitrogen utilization as discussed in Chapter 1. In this study, since molaferm 20 contains a low level of CP thus the effects of the CP from molaferm 20 on dietary protein utilization could be small. The effect may partially be attributed to its energy aspect as discussed in the above sections and may also be associated with its other aspects, such as high concentration of ash, especially K (57 g/kg DM). Although in this study the attention was not paid to those properties of molaferm 20, there still were some evidences to show that the cattle fed the high levels of molaferm 20 achieved a lower efficiency of utilization of dietary protein than anticipated when normal diets were fed.

The UK metabolisable protein (MP) system proposed that net efficiency of utilization of MP supply (k_{nl}) for milk production is 0.68 in dairy cows (AFRC, 1992). In this study, the k_{nl}s were lower than this value and ranged from 0.407 to 0.666 in all 3 dairy cow experiments (Figure 6.4). In experiment 2 with early lactating dairy cows fed 310

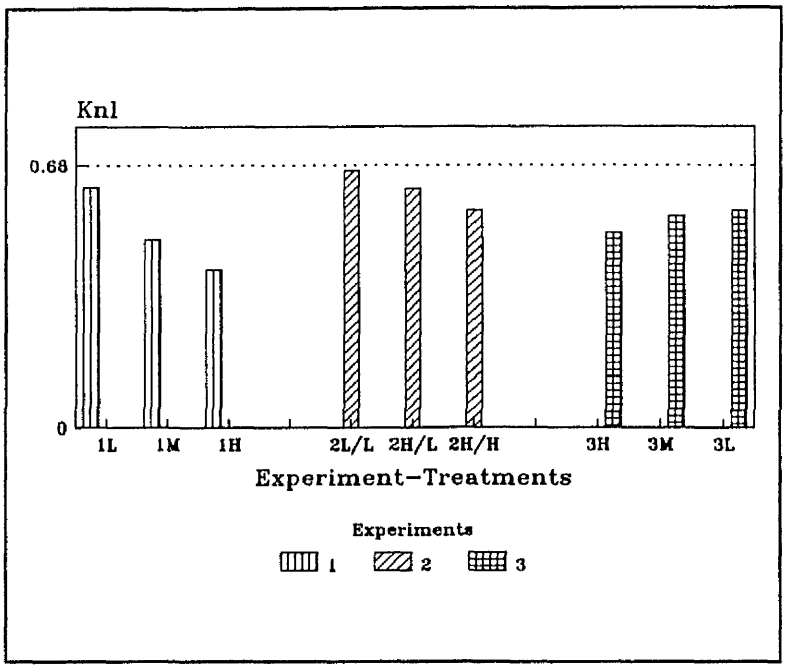

Figure 6.4 The k_{nl}s obtained in cow experiments compared with that (0.68) reported by AFRC (1992) (For details of names in X axis, please see Figure 6.2)

g/kg DM of molaferm 20, k_{nl} was close to 0.68 in the L/L treatment (0.666), but was decreased as increasing in dietary ERDP and DUP levels (0.618 and 0.561 in the H/L and H/H treatments, respectively). In experiment 3 with early lactating heifers fed the diets containing the same level of molaferm 20 and similar ERDP and DUP to treatment H/L in experiment 2, the K_{nl}s was even lower than those obtained from experiment 2 (ranged from 0.504 to 0.562). The differences could mainly be due to different physiological stage of the animals.

In experiment 1 the k_{nl} was close to 0.68 in mid-lactation dairy cows fed the diet containing 156 g/kg DM of molaferm 20 (0.62) and then was linearly declined (0.49 or 0.41 in cattle fed molaferm 20 at 310 or 468 g/kg DM) with each increment of dietary concentrations of molaferm 20 (also presented in Figure 6.4). Since the molaferm 20 intakes were various between groups of cows in different periods within each treatment (*e.g.*, 4.7, 6.2 or 6.4 kg DM/d in periods 1, 2 or 3 in the high

molaferm 20 treatment), the relationship of molaferm 20 intake and K_{nl} was also investigated into period treatments (9 pairs of data). The K_{nl} was negatively correlated to the molaferm 20 intake (Figure 6.5). The regression equation was:

$$Y = 1.035 - 0.106\ X \qquad (3)$$

where $Y = k_{nl}$; X = Molaferm 20 intake (kg DM/d)

With each increase in intake of 1 kg DM/d molaferm 20, the k_{nl} was decreased by 0.106. Actually, when molaferm 20 intake was 6.4 kg DM/d in 3rd period of the high molaferm 20 treatment, the k_{nl} was only 0.368.

In order to test the MP system, Webster (1992) investigated the relationship between MP supply and milk protein production (Y_p) by using the data from four institutions (the former National Institute for Research in Dairying, the former Grassland Research Institute, the Boxworth Experimental Husbandry Farm and the Agricultural Research Institute of Northern Ireland). The slope obtained from the overall regression equation was 0.199. In this study the milk protein yield was also positively correlated with the MP supply when the data from all 3 dairy cow experiments were applied to the MP system (Figure 6.6). The overall regression equation was:

$$Y_p\ (g/d) = 330 + 0.186 \times \text{MP supply (g/d)}\ \ (r=0.61) \qquad (4)$$

The slope in this study was lower than that obtained by Webster (1992), indicating a lower efficiency of utilization of MP supply for milk protein production in dairy cattle fed the diets containing high levels of molaferm 20.

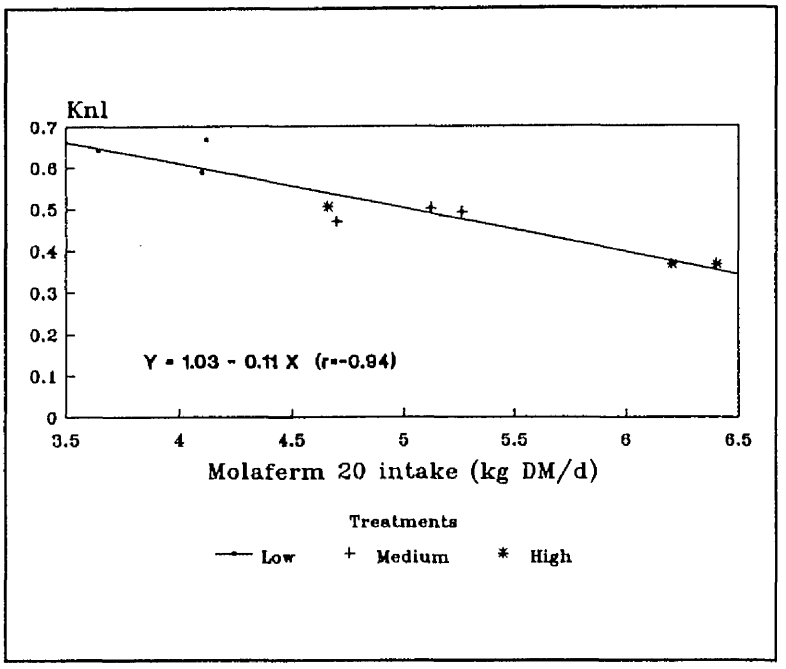

Figure 6.5 The relationship between molaferm 20 intake and the k_{nl} in experiment 1

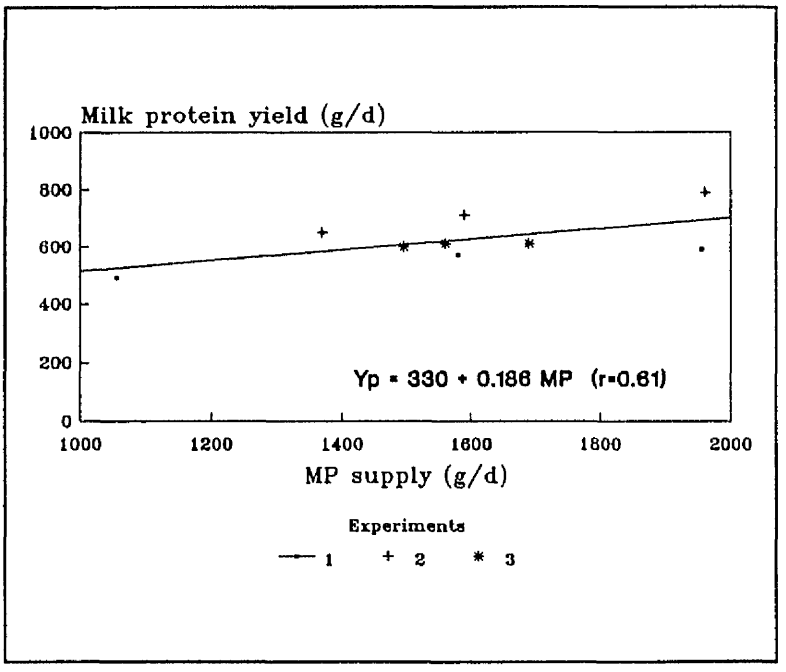

Figure 6.6 The relationship between MP supply and milk protein yield (Y_p) in dairy cow experiments

It is concluded that the efficiency of utilisation of MP supply for milk production (k_{nl}) was depressed when high levels of molaferm 20 were fed in this study. The decrease in k_{nl} resulted from the feeding of high molaferm 20 (310 g/kg DM) was not be made up by increasing dietary ERDP or DUP concentrations in experiment 2 or increasing dietary FME concentrations in experiment 3. Nevertheless, the increases in both dietary concentrations of ERDP and DUP raised the milk production in experiment 2. This could be a commercial advantage.

6.4 *Further areas of research*

The feeding of 310 g/kg DM of molaferm 20 to lactating dairy cows and fistulated sheep did not resulted in ill health of the animals for 3 months. However, the period means of blood concentrations of ß-hydroxybutyrate and urea were increased and Magnesium decreased as the experiment progressed in dairy cow experiments and the fresh faeces contained more water in sheep fed molaferm 20 in comparison with sheep fed no molasses. Therefore the long term consequence of feeding high levels of molaferm 20 on health and productivity of cows given grass silage needs to be determined. The experiment should be designed as a continuous feeding of high levels of molaferm 20 for a whole winter feeding period rather than changeover. The blood concentrations of ß-hydroxybutyrate, urea, Mg and K should be measured to determine any subclinical symptoms of ill health.

The substantially high concentrations of minerals in molaferm 20 (total ash, 162.2 g/kg DM) could influence the metabolism of some major minerals and trace elements when a large amount of molaferm 20 is fed. For example the high K concentration can depress the Mg absorption as reported by Newton *et al.* (1972). However, little investigation has been carried out into this area. The experiments on metabolism of minerals, especially trace elements, should therefore be conducted to determine if there is any disorder of the metabolism.

The rumen dilution rate should be determined when high levels of molaferm 20 are fed.

The rumen dilution rate have been demonstrated to correlated positively with microbial growth (Isaacson *et al.*, 1975; Harrison *et al.*, 1976 and Kennedy *et al.*, 1976). If the feeding of large amounts of molaferm 20 could short the retention time of digesta in the rumen, this would be an advantage for microbial growth. At meantime, if possible, measuring the efficiency of conversion of molaferm 20 energy to ATP in the rumen. The ATP available in the rumen is essential for microbial protein synthesis. This would help to explain Tamminga's suggestion that degradation of rapidly fermentable carbohydrates may result in the formation of lactic acid as an end product or the production of propionate via the inefficient acrylate pathway and in consequence the final ATP yield may be reduced. Subsequently the studies should investigate any substantial change of the ruminal bacteria composition and then microbial protein synthesis based on the feeding of high levels of molaferm 20.

The feeding of a high level of molasses was observed to decrease the NE value of molasses in comparison with the feeding of a lower level. Since the DE values of molasses in both diets were similar Lofgreen and Otagaki (1960 a) suggested that the energy loss of molasses at high conclusion occurred after absorption. In experiment 1 the ratio of NE/ME was also found significantly lower in dairy cows fed 468 g/kg DM of molaferm 20 than those fed less molaferm 20. An energy metabolism experiment should be carried out to detect where the energy goes by using a calorimeter and slaughter technique.

The microbial protein synthesis in sheep and the efficiency of utilization of MP supply for lactation (k_{nl}) were found to be lower than predicted in this study when high levels of molaferm 20 were fed. Further experiments should be conducted to detect the effects of feeding high levels of molaferm 20 together with various dietary ERDP and DUP concentrations on microbial growth in the rumen, the flow rate of MCP and dietary DUP to the abomasum, the flow rates of amino acids to duodenum and the absorption of essential and nonessential amino acids in the small intestine. The experiments could further help to explain the low efficiency of utilization of MP supply.

Finally, in experiment 3 the decreases in dietary FME concentrations produced by addition of unprotected tallow did not impaired the feed intake and milk production of dairy heifers when 310 g/kg DM of molaferm 20 was fed. A further experiment needs to be undertaken to investigate the microbial growth and dietary fibre digestion in the rumen and whole tract when the similar diets are fed.

SUMMARY

1. The inclusion of molasses in dairy cow diets was reviewed, with emphasis on the effects on rumen fermentation, feed intake and milk production. The review led to the design for this study on the feeding of higher levels since most of published research studies had determined the nutritive value of molasses at less than 200 g/kg DM inclusions in diets. Three dairy cow experiments and one fistulated wether sheep trial were conducted.

2. The initial experiment was designed in 3 x 3 Latin Square to investigate the maximum molaferm 20 feeding to mid-lactating dairy cows. The three complete diets were fed which contained different levels of grass silage and a molaferm 20-based liquid supplement. Molaferm 20 concentrations in three diets were 156, 312 and 468 g/kg DM, respectively. The ERDP concentration was similar, but ME and DUP levels were increased with each increment of the substitution rates of the liquid supplement for silage. Some cows suffered from scouring in the high molaferm 20 treatment, but recovered in either the medium or low molaferm 20 treatment after they went to next period. Those fed 312 g/kg DM of molaferm 20 or below did not suffer from the scouring. Treatment means of blood concentrations of total protein, albumen, urea, ß-hydroxybutyrate, Ca, phosphate, Mg, K all were within normal range, while period means of blood concentrations of urea and ß-hydroxybutyrate were increased and Mg decreased as the experiment progressed. Feed intake was significantly increased with each increment of dietary molaferm 20 levels. ME intake and MP supply were proportional to the feed intake and their dietary concentrations. Milk yield produced by cows fed molaferm 20 at 312 g/kg DM was significantly higher those fed 156 g/kg DM, but was similar between the high and medium molaferm 20 treatment. Milk concentrations of fat and lactose were independent of dietary molaferm 20 levels, whereas protein, NPN and casein were significantly higher with increment of dietary molaferm 20 levels. However, the calculated efficiencies of utilisation of ME intake and MP supply were significantly decreased as molaferm 20 conclusions in diets increased. The

results of the experiment indicated that the lactating dairy cows could be fed molaferm 20 up to 312 g/kg DM without adverse effects on health and animal performance.

3. The lack of response to an increase in ME intake in above trial when 468 g/kg DM of molaferm 20 was fed could partially be due to that the high molaferm 20 inclusion decreased dietary protein utilization. In a 3 x 3 Latin Square design experiment early lactating dairy cows were fed three diets (L/L, H/L and H/H) which contained 310 g/kg DM of molaferm 20 and similar concentrations of ME but differing levels in ERDP and DUP (93/17, 117/17 and 121/32 (g/kg DM), respectively). No clinical symptoms of the ill health of the cattle were found during the experiment. Treatment means of blood concentrations of total protein, albumen, urea, ß-hydroxybutyrate, Ca, phosphate, Mg, K all were within normal range, while period means of blood concentration of urea was increased and Mg and K decreased as the experiment progressed. Feed intake was significantly increased with each increment of CP levels. Milk yield was higher as ERDP increased ($p < 0.05$) and further higher as DUP increased ($p < 0.01$). Milk concentrations of fat, lactose and casein were similar between treatments, whereas protein and NPN was higher as ERDP increased ($P < 0.05$) but no further improvement was achieved as DUP increased. The efficiency of utilisation of ME intake for milk production and the ratio of NE/ME was higher in the treatments H/L and H/H than L/L ($p < 0.05$). The efficiency of utilization of MP supply for lactation (k_{nl}) was lower as each increment of dietary CP protein. The results showed a significant response in feed intake and milk production to an increase in dietary ERDP and a greater response to an increase in dietary DUP when the early-lactating cows were fed the diets containing 310 g/kg DM of molaferm 20.

4. The third experiment was designed in a 3 x 3 Latin Square to determine the effects of dietary FME concentrations produced by addition of unprotected

tallow in lactating dairy heifers fed diets containing similar levels of ME. Three complete diets were fed each containing 310 g/kg DM of molaferm 20 and similar ERDP and DUP levels to the H/L treatment in experiment 2. ME concentrations were similar but FME concentrations were different in the diets (H_{FME}, M_{FME} and L_{FME}; 9.4, 8.9 and 8.4 MJ/kg DM, respectively). No clinical symptoms of the ill health of the cattle were found during the experiment. Treatment means of blood concentrations of total protein, albumen, urea, ß-hydroxybutyrate, Ca, phosphate, Mg, K all were within normal range, while period means of blood concentration of ß-hydroxybutyrate was increased and K decreased as the experiment progressed. Feed intake was slightly lower with decreases in dietary FME concentrations achieved by addition of unprotected tallow. Milk yield produced by cows fed the H_{FME} diet was significantly lower than those fed the M_{FME} or L_{FME} diet ($p<0.01$), while milk concentrations of protein and fat were significantly higher in the former than in the latter ($p<0.05$). Milk concentrations of lactose and NPN were similar between 3 treatments. Milk concentrations of uric acid was lower as decreasing dietary FME levels, but yields were similar between three treatments. The efficiencies of utilization of ME intake and MP supply for milk production did not significantly differ between treatments. The results showed that when dairy heifers were fed 310 g/kg DM of molaferm 20, the dietary FME concentration of 8.4 MJ/kg DM could be satisfied for microbial growth. Feed intake and milk production were not impaired by decreases in dietary FME concentrations achieved by addition of unprotected tallow (up to 24 g/kg DM).

5. The results in experiment 2 implicated a higher microbial growth as an increase in dietary ERDP levels. The last experiment in a 4 x 4 Latin Square was therefore designed to detect the effects on rumen fermentation and microbial growth in ruminally fistulated wether sheep fed the similar diets as those in experiment 2, but an extra diet was added which contained higher DUP. Four complete diets were fed each containing 310 g/kg DM of molaferm 20 and

similar ME and FME, but differing in levels of ERDP/DUP (C, CU, CS and CSF; 84/17, 109/17, 116/38 and 119/54 g/kg DM, respectively). Whole tract digestibilities of DM and OM was similar between treatments, while NDF and hay degradability in the rumen were significantly higher in sheep fed the diets CS and CSF than those fed the diets C and CU. Average pH value in the rumen of sheep fed the diet CS was higher than those fed other diets. Average ammonia-N in the rumen was significantly higher with each increment of dietary protein levels. However, PD-N output in urine did not respond to the ammonia concentrations and Microbial N supply was similar between 4 treatments. Total volume of VFAs in the rumen was independent of the diets. Molar percentages of propionic and butyric acids were lower, while acetic acid was higher as ERDP levels were increased, but no further effects were observed as DUP were increased. Molar proportions of isobutyric and isovaleric acids were higher in the rumen of sheep fed the diets CS and CSF than those fed the diets C and CU. Following the completion of the Latin Square, all sheep were fed a conventional ration containing no molasses and lower ME (9.2 MJ/kg DM) and ERDP/DUP (49/20 g/kg DM) in an extension period. All the parameters investigated in Latin Square were determined in this period and compared with those obtained from Latin Square. The results from the Latin Square indicated that dietary ERDP and DUP concentrations at 84 and 17 g/kg DM could be satisfied for the total microbial growth. Microbial N supply and whole tract digestion of DM and OM did not respond to increases in dietary ERDP or DUP when 310 g/kg DM of molaferm 20 was fed. However, Whole tract digestibility of NDF and ruminal hay degradability were significantly higher in sheep fed diets supplemented with soyabean meal and fish meal than those fed the control diet or the diet supplemented with urea.

6. It was concluded that dairy cows could be fed molaferm 20 up to 310 g/kg DM without adverse effect on animal health in grass silage-based complete diets for 3 months. The feeding of the high levels of molaferm 20 decreased the

microbial protein synthesis and both efficiencies of utilization of ME intake and MP supply for milk production. Nevertheless, when 310 g/kg DM of molaferm 20 was fed, increases in dietary ERDP and DUP raised the dietary fibre digestion and then increased feed intake and milk production. The milk production could also be increased with supplement of unprotected tallow when cows were fed this level of molaferm 20.

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APPENDICES

APPENDIX 1

The equations used to estimate liveweight change ($\triangle W_p$) (AFRC, 1990):

$$\triangle W_p \text{ (kg/d)} = \{[0.25 * (P1/P2 + P3/P4)^2 - (P1 * P3 - MEI) / (P2 * P4)]^{0.5} - [0.5 * (P1/P2 + P3/P4)]\} * k_g$$

Where:

MEI = ME intake (MJ/d)

$P1 = 1 + 0.018 * (Y * EV_l * k_m) / (k_l * E_m)$

$P2 = 0.018 * EV_g * k_m / E_m$

$P3 = E_m / k_m + Y * EV_l / k_l$

$P4 = EV_g = 27.36$ (MJ/d)

E_m = energy for maintenance (MJ/d)

$= 0.53 * (W / 1.08)^{0.67} + 0.0043 * W$

EV_l = energy retained in milk (MJ/d)

$= 1.509 + 0.0406 * F$

W = cow liveweight (kg)

F = fat concentration of milk (g/kg)

Y = milk yield of the cow (kg/d)

k_m = the efficiency of ME usage for maintenance

k_l = the efficiency of ME usage for lactation

k_g = the efficiency of ME usage for liveweight gain

APPENDIX 2

The equations used to estimate the ME requirements of maintenance (ME_m), lactation (ME_l) and liveweight change (ME_g) (AFRC, 1990):

ME_m (MJ/d) = $[0.53 * (W/1.08)^{0.67} + 0.0091 * W] / k_m$

ME_l (MJ/d) = $[(1.509 + 0.0406 * F) * Y] / k_l$

ME_g (MJ/d) = $(27.36 * G) / k_g$

Where:

W = cow liveweight (kg)

F = fat concentration of milk (g/kg)

Y = milk yield of the cow (kg/d)

G = liveweight gain of the cow (kg/d) obtained by linear regression

k_m = the efficiency of ME usage for maintenance

k_l = the efficiency of ME usage for lactation

k_g = the efficiency of ME usage for liveweight gain

APPENDIX 3

The equations used to estimate the metabolisable protein (MP) supply (MP_s) and requirements (MP_r) for maintenance (MP_m), lactation (MP_l), liveweight change (MP_g) and pregnancy (MP_p) (AFRC, 1992):

MP_s (g/d) = (0.68 * MCP + DUP) * DMI

MCP (g/d) = (FME * $Y_{mcp/fme}$) where $Y_{mcp/fme}$ = 11 g/MJ

If ERDP < FME * $Y_{mcp/fme}$, then MCP = ERDP

MP_r (g/d) = MP_m + MP_l + MP_g + MP_p

MP_m (g/d) = 2.706 * $W^{0.75}$

MP_l (g/d) = 1.471 * (P - N) * YLD

MP_g (g/d) = 1.695 * G * (168.07 - 0.16869W + 0.0001633W^2) * (1.12 - 0.1223G)

MP_p (g/d) = 16 when weeks from conception is 20

28	24
48	28
78	32

Where:

DMI = DM intake of the cow (kg/d)

MCP = microbial crude protein supply (g/d)

W = liveweight of the cow

P = milk protein concentration (g/kg)

N = milk NPN x 6.38 (g/kg)

YLD = milk yield of the cow

G = liveweight gain of the cow (kg/d) obtained by linear regression

APPENDIX 4

ARC Technical Review No. 2 proposed that estimates of DM intake (DMI) should be based on an average daily intake of 135 g/kg$W^{0.75}$ over the whole lactation, for a cow with a lactation yield of 5000 kg of fat corrected milk. Adjustments were introduced for the effect of yield and month of lactation. The equation was then as followings (ARC, 1980):

$$DMI\ (kg/d) = [0.135 * W^{0.75} + 0.2 * (Y - Y_{5000}(n))] * F$$

Where:

W = cow liveweight

Y = milk yield (kg/d) in lactation week n

F = 1.08

$Y_{5000}(n)$ = average milk yield (kg/d) for lactation week n when total lactation yield = 5000 kg = an^be^{-cn}

a = 21.4

n = number of lactation weeks

b = 0.2

e = natural logarithm = 2.71828

c = 0.04

APPENDIX 5

The equations (AFRC, 1990) used to calculate the energy utilization are illustrated as the followings:

$$E_m \text{ (MJ/d)} = 0.251 * (W / 1.08)^{0.75} + 0.007 * W \quad (1)$$

$$EV_g \text{ (MJ/d)} = 4.4 + 0.32 * G \quad (2)$$

$$k_m = 0.35 * q_{mi} + 0.503 \quad (3)$$

$$k_f = 0.78 * q_m - 0.006 \quad (4)$$

$$k_g = \{k_f / (L - 1)\} * \{[1 - (k_f / k_m)^{L-1}] / [1 - (k_f / k_m)]\} \quad (5)$$

$$\text{Where } L = (MEI * k_m) / E_m \quad (6)$$

Where:

E_m = energy required for maintenance

W = liveweight (kg)

G = liveweight gain (kg/d)

EV_g = energy value of liveweight gain

q_m = ME / GE

k_m = efficiency of utilization of dietary ME for maintenance

k_f = efficiency of utilization of dietary ME for growth at twice maintenance

k_m = efficiency of utilization of dietary ME for growth

MEI = ME intake (MJ/d)

APPENDIX 6

The equations (AFRC, 1992) used to calculate the protein utilization are illustrated as the followings:

$$MCP\ (g/d) = 10 * FME \quad (1)$$

(if dietary ERDP $\leq$ 10 * FME, then MCP = dietary ERDP)

$$MP_s\ (g/d) = 0.6375 * MCP + DUP \quad (2)$$

$$MP_m\ (g/d) = 2.1875 * W^{0.75} \quad (3)$$

$$MP_w\ (g/d) = 11.54 + 0.3846 * NP_g \quad (4)$$

$$MP_g\ (g/d) = 1.695 * NP_g \quad (5)$$

$$\text{Where } NP_g = G * (160.4 - 1.22 * W + 0.0105 * W^2 \quad (6)$$

$$k_n = (MP_w + MP_g) / MP_s \quad (7)$$

$$k_{nw} = MP_w / MP_s \quad (8)$$

$$k_{ng} = MP_g / MP_s \quad (9)$$

Where:

MCP = microbial crude protein supply

W = liveweight (kg/d)

G = liveweight gain (kg/d)

MP_s = MP supply

MP_m = MP for maintenance

MP_w = MP for growth of fleece

MP_g = MP for liveweight gain

APPENDIX 7

The equations used for calculation of predicted milk yield (Y_p) are listed below (ARC, 1990):

$$Y_p \text{ (kg/d)} = [(0.25(Q1/Q2 + Q3/Q4)^2 - (Q1*Q3\text{-}MEI)/(Q2*Q4)]^{0.5} - (0.5(Q1/Q2 + Q3/Q4))] * k_l \quad (1)$$

Where:

$$Q1 = 1 + 0.018 * (\Delta W * EV_g / k_g) * k_m/E_m \quad (2)$$

$$Q2 = 0.018 * EV_l * k_m/E_m \quad (3)$$

$$Q3 = (E_m/k_m) + (\Delta W * EV_g/k_g) \quad (4)$$

$$Q4 = EV_l$$

ΔW = Liveweight gain (kg/d)

MEI = ME intake (MJ/d)

For details of EV_g, k_g, k_m, E_m and EV_l, please see Appendix 1