Chapter 6

CARBOHYDRATE DEGRADABILITY AND ITS EFFECTS ON DIGESTION AND METABOLISM IN DAIRY GOATS

6.1 INTRODUCTION

Postruminal carbohydrate digestion has been considered to be insignificant in ruminant feeding systems. However, at times on certain diets, it is clear that a variable proportion of dietary starch escapes rumen fermentation and is digested and absorbed as glucose from the small intestine. The quantitative estimates of postruminal starch digestion in cattle and sheep on a number of diets have been reviewed (see Armstrong and Beever, 1969; Waldo, 1973).

The major benefits of digestion of starch in the small intestine would be an increase in metabolizable energy from the diet due to the avoidance of fermentative losses of heat and methane and an increase in net energy because glucose is used more efficiently than propionate. Furthermore, postruminal digestion of dietary starch might alleviate conditions associated with high propionate type fermentations, such as reduced feed intake (Anil and Forbes, 1980), soft carcass fat (Garton, et al., 1972), and the low milk fat syndrome (Annison, 1973; Lough, et al., 1983; Emmanuel and Kennelly, 1984).

However, not all readily fermented carbohydrates elicit a high propionate fermentation pattern. Molasses-based diets are a notable example, where fermentation patterns are characterized by relatively low levels of propionate and high levels of acetate and butyrate (Marty and Preston, 1970; Rowe, 1978).

Therefore, it was hypothesized that degradability of different carbohydrate supplements may change both the proportion

of dietary starch digested postruminally and rumen fermentation patterns. These changes would affect the amount and type of energy-yielding nutrients available to the animal which could subsequently alter the productive responses to supplementation.

6.2 EXPERIMENT 1

The objectives of Experiment 1 were to study the rumen degradability of supplemental carbohydrates and their effects on rumen fermentation pattern, postruminal nutrient flow and glucose metabolism in dairy goats. The supplemental carbohydrates chosen for study were sucrose (very rapidly degraded), wheat (moderately rapid degradation) and rice (slowly degraded).

If the general hypothesis discussed above was correct it was expected that: (a) supplementation with sucrose would produce a "molasses-type" fermentation pattern yielding high levels of acetate and butyrate and low levels of propionate, which would reduce glucose entry rates due to insufficient glucogenic precursors, (b) supplementation with wheat would produce a high propionate fermentation pattern but all starch would be degraded in the rumen, and (c) supplementation with rice would yield an intermediate propionate fermentation pattern but large amounts of starch would escape the rumen and be absorbed in the small intestine resulting in a more efficient utilization of starch and an increase in glucose entry rates.

6.2.1 Materials and Methods

6.2.1.1 Animals and Diets

Three Saanen dairy goats (59$\pm$ 3 kg liveweight) in late lactation were fed 3 diets in a Latin Square design. The periods each lasted 16 days. The goats had previously been fitted with both a rumen and abomasal cannula.

A starch-free basal diet was formulated which consisted of (g/kg as fed) 680 oat chaff (screened to remove all oats),

TABLE 6.1 Feeding and experimental schedule in Experiments 1 and 2.

Experiment	Day	Feeding Frequency (equal portions/day)	Function
1	1-9	4	Adjustment period
	10-14	12	Intraruminal infusion of CrEDTA/Ru-P
	13+14	12	Sampling of rumen and abomasal fluid
	15	48	Intra ruminal infusion/sampling of ^{14}C-methane
	16	24	Intravenous infusion/sampling of $NaH^{14}CO_3$+ (2-^{3}H) glucose
2	1-8	2	Adjustment period
	9-15	4	Adjustment period
	16	24	Abomasal infusion of glucose or saline; Intravenous infusion sampling of $Na^{14}CO_3$+ (2-^{3}H) glucose
	17-27	2	Interim
	28-38	4	Adjustment period to abomasal infusion of glucose
	39	24	Intravenous infusion of $NaH^{14}CO_3$+(2-^{3}H) glucose

120 molasses, 100 formal casein, 45 untreated casein, 30 urea, 10 sodium chloride, 3.5 sodium sulphate and 1.5 vitamin/mineral premix (Pfizer Quote 422, N. Ryde., N.S.W.). Animals were treated for gastrointestinal parasites and adapted to the basal diet (plus a mixture of carbohydrate supplements) for 21 days prior to the experiment.

Each day during the experiment, the goats received (as fed basis) 720 g of basal diet and one of the following: (a) 480 g of sucrose, (b) 480 g of cracked wheat, or (c) 480 g of cracked polished rice. The particle sizes of the wheat and rice were similar.

6.2.1.2 Feeding and Experimental Procedures

Feeding frequency was adjusted depending on the type of measurements being taken. A schedule of feeding frequency and experimental procedures is shown in Table 6.1 At least 8 samples were taken for each parameter measured.

Samples of rumen fluid and rumen gas were taken midway between feedings, and samples of abomasal digesta (60ml/sample) were collected at intervals representing approximately every half-hour of the feeding cycle. Infusion and sampling of labelled metabolites in blood have been described in Chapter 3. After completing all three experimental periods, the experiment was repeated again to measure apparent digestibility. Animals were fed their diets for 14 days in 4 equal portions every 6 hr. Total collection of faeces was made over the last 4 days.

Methods of processing, storing and analysing samples have been discussed in Chapter 3.

6.2.2 Results

The DM content (g/100 g) of the basal diet, rice, wheat and sucrose were 85, 90, 92 and 98 respectively. On a DM basis, the basal diet contained (g/100 g) 35 acid detergent fibre (ADF), 4.6 alpha glucose polymers (starch) and 3.4 nitrogen (N). Rice

and wheat contained 0.8 and 3.4 ADF, 78 and 60 starch and 0.9 and 2.2 N, respectively.

Milk yields in the goats did not differ between supplements. Mean values for daily milk yield were 402, 387 and 362 g/day (SEM = 46.5) for supplements of rice, wheat and sucrose, respectively.

6.2.2.1 Rumen Fermentation, Nutrient Flow and Apparent Digestibility

The patterns of fermentation in the rumen of goats fed the basal diet plus the three carbohydrate supplements are shown in Table 6.2. No significant differences were observed in rumen pH or total VFA concentrations between supplements. Compared to wheat or rice, supplementation with sucrose reduced methane production in goats by 40% ($p < 0.05$), rumen fluid concentration of acetate and butyrate by 16 and 35% respectively ($p < 0.01$) and increased propionate concentration by 50% ($p < 0.01$). Although rumen ammonia levels fluctuated widely, there was a trend ($p < 0.09$) towards decreased concentration of ammonia in goats fed the sucrose supplemented diet.

The flow of nutrients through the abomasum are shown in Table 6.3. There were no significant differences in fluid, DM, non-ammonia nitrogen (NAN) or ADF flows between diets. The rice supplemented diet increased starch flow approximately 5-fold ($p < 0.03$) compared to starch flows on the diets supplemented with wheat or sucrose.

Apparent digestibilities of the diets are shown in Table 6.3. Starch, N and ADF digestibility did not differ between diets. The apparent digestibility of DM was decreased by approximately 8% ($p < 0.05$) in goats fed the wheat supplement compared to the sucrose supplement.

6.2.2.2 Entry Rates of Metabolites and Transfer Quotients

In Experiment 1, the entry rates of glucose and CO_2 did not differ between supplements (Table 6.4). The transfer quotient (TQ) differed significantly ($p < 0.05$) between all diets, with

TABLE 6.2 Mean production rates of methane (l/day), concentration (mmol/l) and proportion of volatile fatty acids (VFA), concentration of ammonia (mg NH_3-N/100 ml) and pH in the rumen of goats fed a basal diet supplemented with rice, wheat or sucrose.

	SUPPLEMENTS			
	Rice	Wheat	Sucrose	SEM
Methane	34.0^{a}	31.8^{a}	19.4^{b}	1.33
Ammonia	13.3^{f}	18.8^{f}	7.6^{e}	1.70
Total VFA	70.0	63.9	70.9	4.48
Acetic	68.0^{a}	67.3^{a}	56.9^{b}	1.15
Propionic	15.3^{a}	17.6^{a}	33.2^{b}	1.71
Butyric	10.6^{a}	10.7^{a}	6.9^{b}	0.32
Isobutyric	1.5^{a}	1.7^{a}	0.9^{b}	0.08
Valeric	$1.2^{a,b}$	1.6^{b}	0.9^{a}	0.10
Isovaleric	3.4	1.4	1.2	0.31
pH	6.6	6.4	6.5	0.04

^{a,b} Means within rows with different superscripts differ ($p < 0.01$)

^{e,f} Means within rows with different superscripts differ ($p < 0.09$)

TABLE 6.3 Mean quantities of fluid, dry matter (DM) alpha-linked glucose polymers (starch), non-ammonia nitrogen (NAN) and acid detergent fibre (ADF) flowing through the abomasum and mean apparent digestibilities (%) of DM, starch, nitrogen (N) and ADF in goats fed a basal diet supplemented with rice, wheat and sucrose.

	SUPPLEMENT			
FLOW THROUGH ABOMASUM	Rice	Wheat	Sucrose	SEM
Fluid (1/d)	14.3	16.1	14.7	0.47
DM (g/d)	637	585	572	28.7
Starch (g/d)	72.2[a]	13.7[b]	11.3[b]	4.89
NAN (g/d)	18.4	16.9	21.9	1.79
ADF (g/d)	126	125	129	16.9
APPARENT TOTAL TRACT DIGESTIBILITY				
DM	75.6[d,e]	71.3[d]	77.2[e]	0.65
Starch	99.3	99.3	99.7	0.11
N	75.4	79.1	75.9	1.78
ADF	48.9	43.3	48.1	4.46

[a,b] Means within rows with different superscripts differ ($p < 0.03$)

[d,e] Means within rows with different superscripts differ ($p < 0.05$)

TABLE 6.4 Entry rates (g Carbon/day) of (2-^{3}H) glucose and carbon dioxide and transfer quotients (glucose Carbon (C) derived from carbon dioxide C) in lactating goats fed a basal diet and supplemented with rice, wheat or sucrose. Mean values and standard error of means.

	SUPPLEMENTS			
	Rice	Wheat	Sucrose	SEM
Glucose	71.6	74.5	80.8	5.02
Carbon Dioxide	268	258	268	6.6
Glucose C from CO_2C(%)	11[a]	13[b]	16[c]	0.4

[a,b,c] Means within rows with different superscripts differ ($p < 0.05$)

the highest TQ measured on the sucrose supplemented diet and the lowest TQ measured on the rice supplemented diet.

6.2.3 Discussion

6.2.3.1 Rumen Fermentation

On the basal diet fed to goats in this study, supplementation with sucrose appeared to stimulate the most efficient fermentation pattern as indicated by the marked decrease in methane production and increased propionate concentration in the rumen (Table 6.2). These results indicated that propionate synthesis reduced the feed energy lost in methane, which is in agreement with the studies on methanogenesis discussed by Demeyer and Van Nevel (1975).

Prediction of methane production based on stoichiometric principles of rumen fermentation (see Leng, 1973) tended to agree with observed values. The observed ratio between methane production on wheat and sucrose supplemented diets was 1.64:1. The predicted ratio based on volatile fatty acid (VFA) proportions was 1.43:1. The differences between predicted values and observed values may be explained by a lack of agreement between VFA concentrations and VFA production rates in the rumen, particularly when diets contain carbohydrate supplements (see Sutton and Morant, 1978).

The trend towards decreased ammonia concentration in the rumen (Table 6.2) of goats supplemented with sucrose compared to wheat or rice suggested that ammonia may have been more efficiently utilized on the sucrose supplemented diet. The lowest concentration of ammonia observed on the sucrose diet was 5.3 mg NH_3-N/100 ml, which was close to the optimum concentration for microbial protein synthesis (5 mg NH_3-N/100 ml), as discussed by Satter and Roffler (1977).

Overall, the fermentation patterns suggested that rumen microbial populations were similar in goats on the wheat and rice supplemented diets but markedly different on the sucrose diet. Based on soluble sugar content in cane molasses (see Paturau, 1982), the level of sucrose fed in the present study was equivalent

to feeding a diet of 70% molasses (as fed basis). Thus, it was expected that the sucrose supplemented diet would produce a "molasses-type" fermentation (high acetate/butyrate and low propionate). In marked contrast however, the sucrose diet fermentation pattern yielded the highest concentration of propionate of the three carbohydrates studied. Marty et al. (1970) also observed that infusion of sucrose into the rumen of cattle increased propionate when the basal diet contained high levels of molasses. The results suggest that soluble sugars are not the major factor which results in low propionate producation on molasses-based diets.

6.2.3.2 Nutrient Flow Through the Abomasum

Flow rates of ADF through the abomasum were similar among supplements (Table 6.3). Based on total ADF intake, the flow rates indicated that approximately 40% of the ADF fraction was digested in the rumen. Since at least 90% of the ADF fraction in the total diets was derived from the basal diet, the similarity among diets in ADF flow rates suggested that interactions between the basal diet and the supplements was small. Thus, most of the differences in fermentation could be directly attributed to the supplements.

Assuming that approximately similar amounts of NAN of dietary or endogenous origin were flowing through the abomasum on the different diets, differences in NAN flows would tend to reflect microbial protein synthesis. Compared to wheat, flow of NAN was not reduced on the sucrose supplemented diet, suggesting that efficiency of microbial protein synthesis was not reduced. This is in contrast to the studies of Al Attar et al. (1976) where microbial protein synthesis was reduced on supplements of sucrose compared to milled barley. Hagemeister et al. (1981) discussed these and other findings and suggested that reduced microbial protein synthesis on diets containing sucrose may be due to the formation of large amounts of lactic acid. However, in the present study, pH was similar among diets, suggesting that if lactic acid was being formed on the sucrose diet, the microbial population was able to utilize it so that pH was not significantly reduced.

Compared to the goats supplemented with wheat, NAN flows were not reduced on the rice supplemented diet. These results appear to be in contrast to the findings of Oldham et al. (1979, cited in

Hagemeister, et al., 1981), where microbial protein synthesis was reduced on diets supplemented with maize (a slowly degraded starch) compared to barley. Possible explanations may be differences in basal diets and microbial populations, or possibly differences in amounts of maize or rice starch escaping the rumen.

The higher flow rates of starch through the abomasum on the rice supplemented diet indicated that rice was more resistant to ruminal degradation than wheat. However the proportion of starch ingested that escaped the rumen (20%) was apparently low compared with previous studies in cattle on sugar cane or molasses-based diets supplemented with rice or rice polishings (Elliott, et al., 1978; Rowe, et al., 1979). These findings suggest that factors associated with the basal diet (microbial population, digesta flows) may markedly affect the degradability of rice starch in the rumen. Feeding sequence may also affect starch degradability. In the studies of Elliott and Rowe and their colleagues, rice was fed once daily and presumably one large feed would tend to allow more starch to escape than frequent small feedings.

The rice starch that escaped rumen fermentation in the animals in the present studies was completely digested but the proportion of starch digested in the small and large intestine was not determined. In sheep fed diets containing high levels of barley or maize in which the amount of starch (g/starch/kg bodyweight$^{.75}$) reaching the duodenum was similar to the amount of starch reaching the abomasum of goats on the rice supplemented diet, at least 95% of the starch entering the small intestine disappeared before reaching the terminal ileum (MacRae and Armstrong, 1969).

6.2.3.3 Apparent Digestion

The increase in apparent digestibility of DM on the sucrose supplemented diet compared to the wheat supplemented diet may be related to postruminal digestion of ADF. Increased DM digestibility appeared to be associated with increases in ADF digestibility although changes in ADF digestibility were non significant. Since the amounts of ADF flowing out of the rumen were similar on all diets, the increased digestibility of ADF on the rice and sucrose

supplemented diets may have been due to an increased hindgut fermentation. As discussed previously (see Section 6.2.3.2), approximately 40% of the dietary ADF was digested in the rumen. Total tract apparent digestibility of ADF on the sucrose diet was 48%, suggesting that 17% of the digestible ADF was digested in the hindgut. This value is within the range of cellulose digestibility in the large intestine discussed by Ulyatt et al. (1975).

6.2.3.4 Glucose and Carbon Dioxide Entry Rates

Glucose entry rates did not differ in the goats fed the basal diet supplemented with rice, wheat or sucrose. This contrasts to studies with cattle by Ferreiro et al. (1979) where glucose entry rates increased with the amount of rice consumed. Although these differences may be partly explained by differences in the basal diet and feeding regime, supplementation with rice in the studies of Ferreiro and colleagues resulted in increasing levels of energy intake. Glucose entry rates have been shown to be closely related to digestible energy intake (Judson and Leng, 1968; Herbein, et al., 1978). In the present studies, energy levels were similar between diets and glucose entry rates were constant irrespective of the amount of starch digested in the small intestine. Entry rates of CO_2 also did not differ in goats fed the basal diet supplemented with rice, wheat or sucrose indicating that total amounts of metabolites oxidized were similar among the diets.

6.2.3.5 Transfer of $^{14}CO_2$ to (^{14}C) Glucose

The transfer quotient (TQ) of (^{14}C) glucose SRA/$^{14}CO_2$ SRA during continuous infusion of $NaH^{14}CO_3$ has been discussed as a relative index of gluconeogenesis by Judson and Leng (1973a). The TQ measured in these studies with lactating goats agreed well with values in lactating ewes fed dried grass reported by Wilson et al. (1983). Although problems with crossover of isotopes in the TCA cycle (Krebs, et al., 1966) and futile cycles preclude the use of this TQ as a quantitative measurement of gluconeogenesis (Cridland, 1984), it is useful as it indicates to some extent the source of carbon in glucose synthesis involving CO_2 fixation reactions as distinct from glucose absorbed from the small intestine.

In the present studies, the highest TQ was measured on the sucrose supplemented diet, suggesting more glucose was being synthesized via CO_2 fixation, presumably from propionate. The lowest mean TQ was measured in goats on the rice supplemented diet, suggesting that a lower proportion of glucose came from CO_2 fixation due to rice starch escaping rumen fermentation and being absorbed as glucose from the small intestine.

Since there were no significant differences in glucose entry rates when starch escaped rumen fermentation, the changes in TQ due to these treatments suggested that intestinal glucose absorption suppressed gluconeogenesis. This is in general agreement with the studies of West and Passey (1967) and Lomax et al. (1979) where glucose infusions reduced gluconeogenesis in fasted sheep and lactating cows, respectively.

The TQ measured when goats were fed the wheat supplement was intermediate between the sucrose and rice supplemented treatments. Levels of alpha glucose polymers passing throught the abomasum were similar on both the wheat and sucrose supplemented diets, indicating that virtually all the wheat starch was fermented in the rumen. Therefore, the TQ would have been expected to be very similar between the sucrose and wheat supplements. The results suggest that the markedly different proportions of propionate produced during rumen fermentation on the wheat and sucrose supplemented diets affected the measurement of glucose carbon derived from CO_2 fixation, possibly through metabolic crossover or futile cycling of the isotopic label.

6.3 EXPERIMENT 2

To obtain further insight into the effects of postruminal absorption of carbohydrates on glucose entry rates and transfer quotients, short term studies were undertaken to measure the effects of abomasal infusion of glucose in goats which were consuming a diet yielding a high propionate rumen fermentation pattern.

6.3.1 Materials and Methods

Two dairy goats (non-lactating) were fed 1200 g/day

(as fed basis) of the sucrose-supplemented diet described in Experiment 1. A schedule of feeding frequency is shown in Table 6.1. In the first study goats were abomasally infused with 75 g/day of glucose or an equivalent volume of saline (9 g sodium chloride/l) for periods of 10 hr on each treatment. At the same time, goats received continuous intravenous infusions of (2-^{3}H) glucose and $NaH^{14}CO_3$. Blood samples were taken during the last 6 hr of each respective abomasal infusion treatment.

In the second study, the goats were adapted to abomasal infusions of glucose for 10 days prior to continuous intravenous infusion of labelled metabolites. After measurements were made on the abomasal glucose treatment (10 hr isotope infusion, samples taken over the last 6 hr) the intravenous isotope infusions were continued while the goats were switched to abomasal infusions of saline for a further 10 hr. Samples were again taken over the last 6 hr of the saline treatment.

Methods of infusion, processing, storage and analysis of samples have been discussed in Chapter 3. Differences between abomasal infusions of glucose and saline were compared by paired t-test.

6.3.2 Results

The effect of abomasal infusions of glucose (75g/d) on glucose entry rates in goats fed a sucrose supplemented diet are shown in Table 6.5. Glucose entry rates were increased by 16% ($p < 0.05$) and 9% (nonsignificant) in response to short (10 hr) and long (10 day) term infusions of glucose. There was a significant decline ($p < 0.01$) in the specific radioactivity (SRA) of blood carbon dioxide when glucose was infused for only 10 hr. There were no differences in blood CO_2 SRA between abomasal glucose and saline infusions when goats were adapted to glucose infusions for 10 days prior to measurements. The proportion of glucose carbon derived from carbon dioxide carbon was significantly reduced ($p < 0.05$) in goats abomasally infused with glucose for 10 days compared to abomasal infusions of saline (Table 6.5).

TABLE 6.5 Entry rate (g Carbon/day) of (2-^{3}H) glucose, specific radioactivity (SRA; uCi/g Carbon) of blood carbon dioxide and transfer quotient (glucose carbon (C) derived from carbon dioxide C) in non-lactating goats fed a basal diet supplemented with sucrose and abomasally infused with 75 g/day of glucose or saline for 12 hr or 10 days. Mean values and standard error of mean difference (SE).

	ABOMASAL INFUSIONS					
	12 hr			10 days		
	Saline	Glucose	SE	Saline	Glucose	SE
Glucose	51.2[c]	59.3[d]	1.60	54.1	59.0	0.92
CO_2 SRA	1.0[a]	0.3[b]	0.02	1.0	1.0	0.01
Glucose C from CO_2C(%)	44	17	0.8	19[c]	15[d]	0.4

[a,b] Means within rows with different superscripts differ ($p < 0.01$)

[c,d] Means within rows with different superscripts differ ($p < 0.05$)

6.3.3 Discussion

The studies in Experiment 2 indicated that glucose entry rates only increased slightly in response to exogenous glucose administered abomasally. The increase in glucose entry rates was equivalent to 17% (10 day infusion) or 27% (10 hr infusion) of the total glucose load. By subtracting the glucose load from the entry rate during infusions and comparing this value to the entry rates measured during abomasal infusion of saline, it was estimated that endogenous production of glucose was reduced by 45% in response to the glucose load. This reduction in gluconeogenesis is similar to the reduction (35%) calculated from values of Judson and Leng (1973a) when similar amounts of glucose were intravenously infused into fed sheep.

Abomasal infusion of glucose over a short period of time (10 hr) resulted in a marked decrease in the specific radioactivity (SRA) of blood carbon dioxide, indicating an increased rate of carbon dioxide production in response to unadapted glucose loading. This effect was also observed in fasted sheep by Annison and White (1961) during short term intravenous infusions of labelled (^{14}C) and unlabelled glucose. Their results indicated that during glucose loading, approximately a third of the expired carbon dioxide was derived from reactions involving glucose oxidation.

In contrast, the specific radioactivity of carbon dioxide remained constant between abomasal glucose and saline infusions when goats had been adapted to the glucose load for 10 days. The results suggest that measurements taken in ruminants during short term glucose loadings may lead to erroneous conclusions about intermediary metabolism.

Compared to abomasal infusion of saline, the transfer quotient (TQ) of glucose carbon derived from CO_2 carbon was reduced during abomasal glucose infusions, confirming the effects observed in Experiment 1 between supplements of rice and sucrose.

6.4 CONCLUSIONS

These studies have shown that carbohydrate degradability in

the rumen can markedly affect rumen fermentation patterns and starch flow to the small intestine. Although starch escaping rumen fermentation can provide a source of glucose, glucose entry rates did not increase in lactating goats due to suppression of gluconeogenesis. As Baird (1981) has suggested, maintenance of a constant glucose entry rate in lactating cows even during provision of additional glucose or propionate may indicate that a mechanism is operating during lactation to avoid excessive utilization of glucose by peripheral tissues competing with the mammary gland.

Chapter 7

PRODUCTIVE AND METABOLIC RESPONSES TO POSTRUMINAL SUPPLEMENTS OF PROTEIN AND GLUCOSE IN LACTATING EWES

7.1 INTRODUCTION

In the attempt to improve yield and efficiency of milk production in ruminants, key nutrients have been identified as being limiting for milk production. From studies into mammary metabolism, glucose appears to be of top priority and during early lactation, it is estimated that up to 60-85% of the glucose available to the animal is used for milk synthesis (Annison and Linzell, 1964; Bickerstaffe, et al., 1974).

Amino acids may also be critical for milk production and there is a considerable demand placed on the animal for milk protein synthesis. This area has been the subject of several reviews (Clark, 1975; Oldham, 1981; Mepham, 1982).

Previous studies have compared the response in milk production to abomasal infusions of glucose and/or casein. In view of the importance of glucose in milk production, it is surprising that little or no response is obtained when glucose is infused (Clark, et al., 1973; Vik-Mo, et al., 1974; Ørskov, et al., 1977). In these same studies, positive responses were recorded when casein was infused with glucose and larger responses obtained when higher amounts of casein were infused alone. Dietary intake appeared to be consistently decreased with glucose infusions and intake increased with casein infusions.

Ørskov et al. (1977, 1981) postulated that postruminal protein increased milk yield in cows fed low energy diets by stimulating mobilization of body fat reserves. This effect was not evident

following responses should occur:

(1) at a given level of milk production in ewes on a rumen degradable diet, supplementation with large amounts of protected protein would markedly increase milk yield, but would also increase milk fat secretion and body fat mobilization due to stimulation of growth hormone secretion;

(2) when half the amount of protected protein was replaced with abomasal glucose, milk yield would still be higher than in ewes fed the rumen degradable diet, but fat mobilization and milk fat secretion would be similar due to glucose stimulating insulin secretion, which would balance the lipolytic effects of growth hormone;

(3) when abomasal glucose totally replaced protected protein milk yields would increase slightly compared to ewes fed the rumen degradable diet due to an abundant supply of lactose precursors, but fat mobilization and milk fat secretion would markedly decrease, due to abomasal glucose stimulating insulin secretion.

7.2 MATERIALS AND METHODS

Border Leicester x Merino crossbred ewes between 2.5 - 3.5 years of age were selected from the University flock. All ewes had been mated within three days. At 14 days post-breeding, the ewes' ovaries were examined by endoscopy and 12 ewes were selected which showed evidence of two ovulations. At two monthes gestation, the ewes were surgically fitted with a single T-shaped cannula in the abomasum. The ewes were adapted to the basal diet (Table 7.1) for 1 month prior to lambing. Within 7 days of lambing, the ewes were moved indoors into metabolism cages. All ewes lambed within a 48 hr period. Eight ewes with twin lambs were each held in separate metabolism cages. The other ewes were kept in pens nearby and fed the basal diet.

During the first 3 weeks post lambing, the experimental ewes were fed a ration of 1650 g DM of basal diet plus 75 g formaldehyde-treated casein (formal casein). This was fed in 4 equal portions every 6 hr. During the first two weeks, lambs were kept with the ewes. After this initial period, lambs

when cows fed high energy diets were supplemented with postruminal protein.

Some studies have indicated that postruminal casein infusions increased levels of growth hormone (Oldham, et al., 1978; Barry, 1980). Oldham (1984) suggested that the effects of postruminal protein may be related to casein stimulating growth hormone which subsequently stimulates mobilization of body fat reserves. In contrast, postruminal glucose may stimulate insulin secretion, which may inhibit mobilization of body fat reserves. This theory was first proposed by McClymont and Vallance (1962) as an explanation for reduced milk fat secretion when lactating cows were infused with glucose.

Thus, there is some evidence to suggest that altering dietary protein/energy ratios with postruminal supplements of protein or glucose may affect milk yield and composition through changes in hormonal patterns.

To obtain further insight into the role of postruminal protein and glucose supplements in milk production, lactating ewes with twin lambs were utilized as the experimental model. A rumen degradable diet high in readily fermentable carbohydrates and non-protein nitrogen was fed at a level restricted to approximately 85-90% of the ewes ME requirements (NRC, 1975). Supplements of either glucose infused into the abomasum or formal casein were added at a rate to provide approximately 50% of the ewes estimated glucose entry rates (Wilson, et al., 1983) or 50% of their total protein requirements (NRC, 1975) respectively. Combinations of glucose and casein supplements for the ewes were also compared which were isoenergetic and isonitrogenous but were designed to be either degraded in the rumen or to bypass the rumen and be digested in the small intestine.

The experiments were designed to test the hypothesis that altering the dietary protein/energy ratio through supply of postruminal glucose and/or casein would affect milk production through changes in hormonal patterns. If this hypothesis is correct, the

TABLE 7.1 Composition of basal diet fed to lactating ewes.

Ingredient	Concentration (g/kg DM)
Oaten chaff	331
Corn flour	257
Sucrose	154
Molasses	126
Barley, cracked	47
Urea	47
Dicalcium Phosphate	29
Salt	5.0
Sodium Sulphate	2.7
*Vitamin/Mineral Premix	1.3
	1000

*Pfizer Quote 422 Premix (Pfizer Pty. Ltd., Aust.)

Analysis (DM basis)

Organic Matter (g/kg)	918.0
Total Nitrogen (g/kg)	27.0
Urea Nitrogen (g/kg)	21.6
Gross Energy (MJ/kg)	16.3

TABLE 7.2 Experimental Schedule.

Day	Feeding Frequency (equal portions/day)	Function
1-9	4	Adaptation period
10	4	Begin Cr/Ru markers in diet
12	8	Continuous infusion of (^{3}H) palmitate and ($NaH^{14}CO_3$)
13	8	Continuous infusion of (^{14}C) and (^{3}H) glucose
14	4	Measure milk production; abomasal samples
15	4	Abomasal samples; rumen fluid samples

were housed in metabolism cages adjacent to the ewes and allowed to suckle six times/24 hr for 5 min each time. Water was freely available to all animals at all times.

After 15 days post lambing, the ewes were fitted with portable peristaltic pumps (SIRO pump, Everest Electronics, Seaview, S. Aust.) and adapted to continuous abomasal infusions. At 21 days post lambing, the 8 ewes were allocated to one of four treatments in a replicated 4 x 4 Latin Square. Each period lasted for 15 days including adaptation and sampling. An outline of the experimental procedures within each period is shown in Table 7.2.

The experimental treatments consisted of the following diets (amounts fed daily):

Rumen degradable sucrose and casein (Diet RSC)	: 1650 g (DM) Basal plus 75 g untreated casein and 75 g sucrose.
Abomasally infused glucose and formal casein (Diet AGC)	: 1650 g Basal plus 75 g formal casein and 75 g glucose (abomasal).
Abomasally infused glucose (Diet AG)	: 1650 g Basal plus 150 g glucose (abomasal).
Formal casein (Diet AC)	: 1650 g Basal plus 150 g formal casein.

Ewes were gradually adapted to glucose infusions into the abomasum by increasing delivery rate and/or concentration of glucose over a 6 day period until the required level was reached. Throughout the experiment, lambs were fed almost entirely on milk so that their weight gains would reflect milk production. It was however, necessary to provide restricted roughage as chopped straw. Lambs consumed less than 1 kg/head of straw during the entire experiment of 11 weeks duration.

Samples from one of the ewes during a glucose entry rate infusion was lost and it was necessary to calculate a missing value (see Snedecor and Cochran, 1967). Because of problems with low feed intake on Diet AG (abomasal glucose) and other practical difficulties with obtaining abomasal samples, digesta flows were not obtained on all animals. During digesta flow studies, ewes not consuming all feed offered were replaced by the spare ewes and

in this way it was possible to obtain six measurements of digesta flows for each dietary treatment. Assuming that period effects would be negligible since intake was held constant and since the data were only used for estimation of nutrient flows (rather than a test of a hypothesis), the digesta flow rates were analysed as a completely random design (4 treatments, 6 animals/treatment).
Methods of sampling and analytical procedures are described in Chapter 3. Glucose recycling was calculated as the difference in glucose entry rates as measured by (U-^{14}C) and (2-^{3}H) glucose (see Judson and Leng, 1972).

7.3 RESULTS

During isotope infusions, animals were fed equal portions of their ration every 3 hr. The specific radioactivity of glucose and palmitate when at plateau varied by ± 7% of mean specific radioactivity, indicating that animals were in a relatively steady state. Furthermore, duration of the infusions (10 hr) and sampling period (6 hr) would have minimized any cyclical fluctuations associated with feeding. This point was verified by Armenanto et al. (1984) who found that mean glucose entry rates were similar when dairy cattle were fed every 2 or 12 hr if infusions and sampling period covered an entire feeding cycle.

Separation of plasma fatty acids on the basis of saturation (argentation thin layer chromotography) was used to estimate possible interconversion of palmitate during the infusion. Low levels of activity (1-2% of total activity) were associated with the monoenoic fraction, of which the majority would have been oleate (C18:1). Assuming equal amounts of activity in stearate and oleate (see West and Annison, 1964) interconversion could have amounted to approximately 2-4% of the specific radioactivity of palmitate. This amount of interconversion is more than the values reported by Leat and Ford (1966) but less than the values reported by West and Annison (1964).

In the ewes fed Diet AG, the abomasal infusion of 150 g/day of glucose often resulted in marked reductions in intake of the basal diet. Only two of the eight animals in the experiment

TABLE 7.3 Daily milk yield, milk composition and lamb growth in lactating ewes fed a basal diet and supplemented with sucrose and untreated casein (RSC), abomasally infused glucose and formal casein (AGC), abomasally infused glucose (AG) or formal casein (AC). Mean values and standard error of means (SEM).

	RSC	AGC	AG	AC	SEM
MILK YIELD (g)	1213^{b}	1435^{c}	866^{a}	1736^{d}	23.4
MILK FAT YIELD (g)	94^{e}	93^{e}	75^{e}	120^{f}	9.3
MILK FAT %	7.6^{f}	6.4^{e}	9.1^{g}	$6.7^{e,f}$	0.54
MILK PROTEIN YIELD (g)	60^{b}	73^{c}	43^{a}	89^{d}	3.8
MILK PROTEIN %	5.1	5.3	5.7	5.3	0.90
LAMB DAILY GAIN (g)	128^{b}	$186^{b,c}$	7^{a}	221^{c}	27.1

^{a,b,c,d} Means within rows with different superscripts are different ($p < 0.01$)

^{e,f,g} Means within rows with different superscripts are different ($p < 0.05$)

remained on full feed during infusion of the high level of glucose. Therefore, the specific values obtained from ewes fed Diet AG are not regarded as a reliable estimate of milk production and metabolism on high levels of glucose due to the overall reduction in energy intake.

7.3.1 Milk Production and Lamb Growth

The results for milk yield, milk constituents and lamb growth are shown in Table 7.3.

Compared with the control diet of rumen degradable sucrose and casein supplements (RSC), abomasal glucose and casein supplements (AGC) increased milk yield of the sheep by 18% ($p < .01$) and further addition of formal casein (AC) increased milk yield by 43% ($p < .01$) compared to the controls ewes supplemented with RSC. Milk yield of the sheep was reduced by 30% ($p < .01$) on abomsal glucose (AG) compared to controls (RSC). Milk fat yield did not differ between the sheep supplemented with RSC and AGC, but was increased by 28% ($p < .05$) in the sheep on diet AC compared with the control (RSC). Compared with the control (RSC), milk protein yield was increased by 22% ($p < .01$) in ewes supplemented with abomasal glucose and casein (AGC) and by 48% ($p < .01$) with abomasal casein alone (AC). There were only small changes in milk protein content, but milk fat content was 16% less ($p < .05$) on abomasal glucose and casein (AGC) and 20% higher ($p < .05$) on abomasal glucose relative to ewes fed the control diet (RSC).

Lamb growth rates reflected milk yields of the ewes. Weight gains were highest on treatment AC and lowest on treatment AG. The extremely low mean weight gains in lambs on treatment AG is related to weight loss in some lambs on this treatment. Compared with the control (RSC), growth rates were increased by 70% ($p < .01$) with the formal casein supplement (AC).

7.3.2 Rumen Fermentation Products, Digesta Flow and Hormones

Samples of rumen fluid taken midway between feedings revealed no significant differences between diets in fermentation products. Mean concentrations of total VFA and ammonia (standard deviation

TABLE 7.4 Daily flow of organic matter (OM) and non-ammonia nitrogen (NAN) through the abomasum of lactating ewes supplemented with sucrose and untreated casein (RSC), abomasally infused glucose and formal casein (AGC), abomasally infused glucose (AG) or formal casein (AC).

	RSC	AGC	AG	AC	SEM
OM Flow (g/day)	838^{a}	930^{b}	822^{a}	996^{c}	26.2
NAN Flow (g/day)	31.0^{a}	37.1^{b}	27.9^{a}	50.4^{c}	1.28

^{a,b,c,d} Means within rows with different superscripts differ ($p < 0.01$)

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TABLE 7.5 Plasma concentrations of growth hormone and insulin in lactating ewes supplemented with sucrose and untreated casein (RSC), abomasally infused glucose and formal casein (AGC), abomasally infused glucose (AG) or formal casein (AC).

	RSC	AGC	AG	AC	SEM
Growth Hormone (ng/ml)	5.1^{e}	8.0^{f}	9.7^{f}	8.2^{f}	1.21
Insulin (ng/ml)	2.1	3.1	1.6	2.5	0.58

^{e,f} Means within rows with different superscripts differ ($p < 0.09$)

in parentheses) were 98 (± 16) umol/l and 24 (± 7) mg NH_3-N/100 ml respectively. Molar proportions of the major VFA were: acetate, 53 (± 4)%; propionate, 36 (± 4)% and butyrate, 10 (± 3)%.

The results of OM and NAN flows through the abomasum of sheep are shown in Table 7.4. Compared to ewes fed diet RSC, flows of OM did not differ on diet AG, but were increased by 11 and 19% ($p < .01$) when ewes were fed diets AGC and AC respectively. The flow of NAN was increased slightly 11% ($p < .05$) in sheep fed diet RSC compared to diet AG. Compared to controls (RSC), NAN flows were increased by 20 and 63% ($p < .01$) in ewes consuming diets AGC and AC respectively.

The effect of dietary treatments on plasma hormone levels in the lactating ewes are shown in Table 7.5. There were no significant differences in plasma insulin levels between ewes fed the different diets although ewes on diet AG tended to have relatively lower levels and ewes on diet AGC relatively higher levels. Growth hormone levels increased by 57-60% in ewes fed diets AGC and AC compared to diet RSC ($p < .09$). The highest levels of growth hormone were observed on ewes fed diet AG although levels did not significantly differ from ewes fed diets AGC and AC.

7.3.3 Entry Rates of Glucose, Palmitate and Carbon Dioxide

The estimates of glucose, palmitate and carbon dioxide entry rates are shown in Table 7.6. Compared to the controls (RSC) glucose entry rates (^{14}C or ^{3}H) increased by 17% ($p < .07$) in ewes supplemented with abomasal casein (AC) and decreased by 10-11% in ewes supplemented with abomasal glucose (AG). Glucose entry rates in ewes fed diet AGC tended to be intermediate between ewes on diets RSC and AC. Palmitate entry rates were fairly similar in ewes fed diets RSC, AGC and AG. Although palmitate entry rates were approximately 30% higher in ewes supplemented with formal casein (AC), the difference did not reach significance below the 10% level ($p < .13$). Compared to ewes fed the control diet (RSC), entry rates of carbon dioxide were reduced ($p < .02$) by 25 and 39% in ewes supplemented with abomasal glucose (AG) and formal

TABLE 7.6 Entry rates (g Carbon/day) of glucose (^{14}C and ^{3}H), palmitate and carbon dioxide; estimate of glucose recycling and transfer quotients (CO_2 Carbon (C) from glucose; Glucose C from CO_2) in lactating ewes supplemented with sucrose and untreated casein (RSC), abomasally infused glucose and formal casein (AGC), abomasally infused glucose (AG) or formal casein (AC).

	RSC	AGC	AG	AC	SEM
(2-^{3}H) Glucose	90.4^{e}	$97.7^{e,f}$	80.1^{d}	105.8^{f}	5.43
(U-^{14}C) Glucose	83.6^{e}	$91.4^{e,f}$	75.4^{d}	97.6^{f}	4.87
Glucose Recycling (%)	7.3	6.2	5.8	7.6	0.84
Palmitate	39.3	36.8	37.7	50.0	5.46
Carbon Dioxide	405^{b}	$337^{a,b}$	247^{a}	303^{a}	30.7
CO_2 C from Glucose C (%)	10.2^{e}	11.9^{f}	13.4^{g}	10.6^{e}	0.67
Glucose C from CO_2 C (%)	17.6^{d}	11.4^{b}	6.9^{a}	13.7^{c}	0.10

^{a,b,c,d} Means within rows with different superscripts differ ($p < 0.02$)

^{e,f,g} Means within rows with different superscripts differ ($p < 0.07$)

casein (AC) respectively.

The results for glucose recycling, and transfer quotients of carbon between glucose and CO_2 are also shown in Table 7.6

Dietary treatments had no significant effect on glucose recycling, which averaged approximately 7% in all sheep across treatments. The oxidation rate of glucose (CO_2 carbon from glucose carbon) was similar in sheep fed the control diet (RSC) or supplemented with formal casein (AC). Compared to controls (RSC), glucose oxidation rates tended to increase ($p < .07$) by 17 and 31% in ewes fed diets AGC and AG respectively.

The proportion of glucose carbon derived from CO_2 represents a relative index of gluconeogenesis from propionate. Compared to controls (RSC), the apparent proportion of glucose derived from CO_2 was reduced by 22, 35 and 61% in ewes fed diets AC, AGC and AG respectively. Each value was significantly different from the other values ($p < .02$).

7.4 DISCUSSION

In the present experiment, feed intakes were restricted in an attempt to overcome the effects of bypass protein potentially increasing energy intake.

Overall, the values for glucose and CO_2 entry rates and transfer quotients, when expressed on an OM intake basis, agreed well with the values reported by Wilson *et al.* (1983) for lactating ewes fed dried grass. Although there is little information in the literature on palmitate entry rates in lactating ewes, the values reported in fed, lactating goats (Yamdagni and Schultz, 1969) and fed, lactating cows (Jackson, *et al.*, 1968; Konig, *et al.*, 1984) were fairly similar to those obtained in this experiment when expressed on a metabolic body weight basis.

7.4.1 Milk Yield and Composition

The increases in milk yield and lamb body weight gains observed in ewes on treatment AGC (compared to ewes on treatment RSC)

demonstrated that if the same amount of supplemental nutrients (glucose and casein) could be supplied postruminally rather than being degraded in the rumen, milk production increased. Compared to controls (RSC), ewes supplemented with abomasal glucose and casein (AGC) secreted an extra 13 g of milk protein which was associated with an extra 38 g protein (NAN x 6.25) flowing through the abomasum, indicating an efficiency of utilization of 34% for the extra protein supply. The lack of change in milk fat secretion between treatments RSC and AGC was associated with similar palmitate entry rates. Thus, the hypothesis was accepted that when both protein and glucose were supplied postruminally to lactating ewes, milk production would increase without increases in fat mobilization.

When additional formal casein was supplied in place of abomasal glucose, milk production of the ewes increased by 21% (treatment AC vs AGC). Milk fat secretion increased by 29% which was associated with a 36% increase in palmitate entry rate in the ewes on treatment AC. Thus the results appeared to support the hypothesis that high levels of bypass protein would increase milk yield and milk fat secretion but would also stimulate fat mobilization. However, the interaction between protein and energy appeared to be more complex than a simple fat mobilization mechanism in the animal to balance additional protein supply. In comparison to the AGC treatment, an additional 83 g of protein flowed through the abomasum in sheep on treatment AC, but only an extra 16 g protein were secreted in milk, indicating an efficiency of utilization of only 19% for the additional protein supplied. This was considerably lower than the 34% efficiency of utilization for additional protein supplied in ewes supplemented with abomasal glucose and casein (AGC) compared to the rumen degradable supplement (RSC), suggesting that the additional protein available to ewes on treatment AC was being used for other purposes besides milk protein.

7.4.2 Glucose Metabolism

The increased glucose entry rates in ewes fed high levels of formal casein (AC) suggest that a portion of the protein was being used for glucose synthesis. Further evidence to support this was the reduced incorporation of CO_2 carbon into glucose carbon in ewes

on treatment AC compared to controls (RSC), suggesting that a relatively larger proportion of glucose was being synthesized without CO_2 fixation reactions. The known pathways for gluconeogenesis from glucogenic amino acids in casein, particularly glutamine and serine support this suggestion. However if increased palmitate entry rates reflected increased lipolysis, then there would also be an additional source of glucose available in the form of glycerol released from adipose triglycerides. Assuming that palmitate constitutes 25 molar % of adipose tissue triglycerides (Christie, 1981), glycerol could have accounted for approximately 20% of the increase in glucose entry rates observed in ewes on treatment AC compared to the controls (RSC).

Compared to ewes fed the degradable supplements (RSC), therewas a moderate increase in glucose entry rates when ewes were supplemented with abomasal glucose and casein (AGC) which may reflect the additional glucose supplied postruminally. However, rates of glucose oxidation tended to increase in ewes on treatment AGC, suggesting that the extra glucose made available from abomsal glucose infusion was being used as an energy source. This effect was also evident in ewes on treatment AG, where high levels of abomasal glucose were associated with high rates of glucose oxidation. If blood CO_2 entry rates are an index of the amounts of absorbed organic nutrients which are oxidized, the marked decrease in CO_2 entry rates when ewes were supplemented with high levels of formal casein (AC) suggests that less nutrients were oxidized compared to ewes fed the control diet (RSC).

From estimates of glucose and CO_2 entry rates and rates of glucose oxidation, it was possible to calculate the amounts of glucose oxidized. The proportion of glucose oxidized was 46%, 41%, 41% and 30% for ewes on treatments RSC, AGC, AG and AC respectively. Thus, these results indicate that ewes fed high levels of formal casein (AC) had the highest glucose entry rates but the lowest proportion of glucose oxidized, suggesting the most efficient utilization of glucose. As suggested by Oldham (1984), increasing entry rates of fatty acids may serve as an alternate energy source for oxidation and spare glucose for lactose synthesis.

Overall, glucose entry rates appeared to be closely associated with milk yields in ewes on all diets. Using the values for glucose entry rates and milk yield measured in ewes on treatment AG as base values, additional milk yield and glucose entry rate was calculated for the other diets. For each additional 100 g milk yield, glucose entry rates increased by 7.5, 7.7 and 7.4 g in ewes on treatments RSC, AGC and AC respectively.

These figures agree well with the relation between mammary uptake of glucose and milk yield in goats (7.6 g glucose uptake/100 g milk) and cows (7.2 g glucose uptake/100 g milk) observed by Annison and Linzell (1964) and Hartmann and Kronfeld (1973) respectively, suggesting that nearly all additional glucose entry over 80 g glucose carbon/day (glucose entry rate for AG ewes) was taken up by the mammary gland for milk synthesis.

7.4.3 Estimates of Efficiency of Energy Utilization

Calculations were made to estimate the efficiency of ME utilization for production of milk energy. The ME content of the basal diet was calculated in the same way as in experiments reported in Chapter 5 (see Section 5.4.3). Since the basal diet was very similar in formulation to the basal diet fed to sheep and goats in experiments reported in Chapter 5, it was assumed that the ewes digested the OM to the same extent as the Angoras and Merinos (81% OM digestibility).

The maintenance ME requirements for the ewes (45 kg liveweight) were calculated from estimates reported by NRC (1975). The energy value of milk constituents was calculated from values used by Cowan *et al.* (1981). It was assumed that milk lactose percentage did not differ between diets and that the concentration of milk lactose was 5.8% (Cowan, *et al.*, 1981).

The calculated total energy (MJ) secretion in milk was 6.2, 6.7, 4.8 and 8.4 MJ for ewes on treatments RSC, AGC, AG and AC respectively. The calculated efficiency of utilization of productive ME for milk energy was 45, 48, 34 and 60% for ewes on treatments RSC, AGC, AG and AC respectively. Compared to control ewes (RSC),

efficiency of energy utilization was increased by 7% when ewes were on treatment AGC, indicating a slight increase in efficiency when glucose and casein were supplied postruminally rather than being degraded in the rumen. In contrast, efficiency increased by 33% when ewes were on treatment AC compared to controls (RSC), indicating a substantial improvement in efficiency of energy utilization when ewes were supplemented with high levels of formal casein.

This increased efficiency was associated with an extra 2.2 MJ of milk energy secreted in ewes fed formal casein (AC) compared to ewes fed the degradable supplements (RSC). The apparent increase in body fat mobilization (palmitate entry rates) may help to explain the additional energy secreted in milk from ewes supplemented with high levels of formal casein (AC). Compared to control ewes (RSC), palmitate entry rates were increased by 11 g carbon/day in ewes on treatment AC. Assuming that palmitate represented approximately 20% of the total plasma free fatty acids and that fatty acids contained approximately 75% carbon, then an additional 73 g/day of fatty acids were available in ewes on treatment AC compared to the controls (RSC). Assuming an energy value for fat of 39.5 kJ/g, then approximately 2.9 MJ (73 x 39.5) of extra energy was made available to ewes fed formal casein (AC) due to body fat mobilization. This figure agrees well with the additional energy secreted in milk (2.2 MJ), suggesting an efficiency of body fat utilization for milk energy of 76% (2.2 MJ/2.9 MJ).

7.4.4 Protein/Energy Interrelationships

Other researchers have reported similar indirect and direct responses to postruminal casein infusions. Ørskov *et al.* (1977) found that cows in negative energy balance produced more milk and went further into negative energy balance when supplemented with postruminal casein. However, later studies (Ørskov, *et al.*, 1981) indicated that this response occurred only in animals that were in substantial negative energy balance already, while cows in only slight negative energy balance did not respond to bypass protein in the same way. Konig *et al.* (1984) reported direct evidence for palmitate entry rates increasing in response to low levels of casein infusion, although the effect was not significant when casein

levels were doubled. At the higher levels of casein, glucose entry rates were significantly elevated. Other studies (Lindsay and Williams, 1971; Ranawana and Kellaway, 1977; Clark, et al., 1977) have also observed variable increases in glucose entry rates in response to postruminal casein infusion.

A possible explanation for the responses observed by Ørskov and colleagues may be related to the basal protein supply. Kaufmann (1982) emphasized that a deficiency of energy for the cow would also result in a deficiency of energy for the rumen microbes. Inadequate energy intake could reduce the capacity for microbes to utilize nitrogen for protein synthesis, thereby reducing the protein supply to the small intestines. Thus, both protein and energy supply might be low when cows exhibit substantial negative energy balance. Under these conditions, postruminal protein would markedly increase the protein:energy ratio and energy reserves would be drawn upon to balance the protein.

Kaufmann's reasoning suggests that with increased digestible energy intake, both energy and protein (microbial) supply would increase. Under these conditions, the same amount of supplemental postruminal protein would cause a smaller increase in the protein/energy ratio compared to the low energy diet so that requirements for mobilized energy to balance protein would be relatively less. In absolute terms, the demands for milk protein might be closer to being met on the high energy intake which could supply more microbial protein. Thus, additional postruminal protein may exceed the demand for milk protein, and alternatively supply energy through gluconeogenesis. So besides the smaller relative increase in the protein/energy ratio on the high energy diet compared to the low energy diet, additional glucose from excess protein would further lower the "metabolic" protein/energy ratio which should reduce the demand for mobilized energy.

The results of Konig et al. (1984) suggest that these mechanisms might be operating in response to altered protein/energy ratios. Assuming that energy and microbial protein supply was constant from the basal diet, the low level of casein infusion increased milk protein secretion and elevated fat mobilization (palmitate entry rate). Compared to the low level, higher levels of casein infusion

caused no further increase in milk protein secretion and fat mobilization decreased while glucose entry rate increased. It appeared that protein demands were met at the low level and further increases in protein supply caused energy requirements to be balanced by glucose rather than mobilized fat.

A somewhat similar situation may have occurred in the present experiment, but interpretation is difficult due to low levels of significance for palmitate entry rates when casein replaced glucose (AC vs AGC). In contrast to the results of Konig et al. (1984), milk protein secretion continued to increase with increasing casein supply, although efficiency of utilization decreased. Glucose entry rates also increased with increasing formal casein which is similar to Konig's results. In contrast to Konig's data, palmitate entry rates appeared to be higher in ewes on the high casein (AC) than on the low casein (AGC) treatment. It is possible that the provision of abomasal glucose (AGC) kept palmitate entry rates from rising, which would agree with the observations of West and Passey (1967), who found an inverse relationship between intravenous infusion of glucose and palmitate entry rates. An additional explanation for the difference in response of palmitate entry rates to increasing casein between the present experiment and Konig's study is to be found in the data for milk protein secretion. While Konig's results indicate that protein demand for milk protein secretion was met at the low level of casein, demand apparently had not been met at the low level in the present study. The decreased efficiency of protein utilization and increase in glucose production in ewes fed the high level of casein (AC) suggest that demand was being met somewhere between the casein supplied on treatments AGC and AC. Continued demand for protein by ewes on treatment AC might explain the increase in palmitate entry rates which was necessary to balance energy with protein supply and additional supply of protein over demand may have contributed to the increased glucose entry rates.

The generally low level of response in milk yield to postruminal glucose supplements (AGC and AG) might be explained by the rumen fermentation pattern associated with the basal diet. The high levels of propionate may have provided sufficient glucogenic precursors to meet the demands for glucose in the lactating ewes. A much different

response to glucose might have been observed if the basal diet had produced a fermentation pattern with high levels of acetate and low levels of propionate. Tyrrell et al. (1979) observed a marked increase in the retention of energy from ruminally infused acetate on a diet with a high glucogenic potential (60% maize grain and 40% lucerne hay) compared to a diet with a lower glucogenic potential (lucerne hay alone). Although these results and the results of Ørskov et al. (1969) suggest that additional glucogenic precursors (propionate) direct energy towards tissue deposition rather than milk secretion, more research is needed on the milk yield response to postruminal glucose supplements under conditions where rumen fermentation patterns yield very high amounts of acetate and very low amounts of propionate, as is observed on straw and molasses-based diets. Under these conditions, glucose may be the limiting nutrient and milk responses might be higher on a supplement of abomasal glucose and casein (AGC) compared to abomasal casein alone (AC).

7.4.5 Hormonal Responses

Plasma growth hormone concentrations tended to be elevated on treatments AGC, AG and AC compared to the control (RSC). The elevation in ewes supplemented with high levels of abomasal glucose (AG) could be explained as a response to lowered energy intake due to reduced intake of the basal diet, and agrees with the general relationship between energy status and growth hormone as discussed by Trenkle (1981). The elevated levels observed in ewes supplemented with high levels of formal casein (AC) might be associated with the increased rate of fat mobilization. Peel et al. (1982b) found that palmitate entry rates were increased when exogenous growth hormone was administered to lactating cows. However, a similar increase in growth hormone levels was observed in ewes supplemented with abomasal glucose and casein (AGC), suggesting that growth hormone might have increased in AGC and AC ewes in response to abomasal casein supplements. Oldham et al. (1978; 1982) and Barry (1980) also observed increases in growth hormone levels in response to formaldehyde treated casein and soybean meal or abomasal infusion of casein. Of particular interest is the fact that Oldham et al. (1982) observed an increase in growth hormone without any effect on production, suggesting that protein in the intestine may have elicited the response. The relationship

between postruminal protein and growth hormone is unclear and other researchers have observed no change in growth hormone in response to abomasally infused casein (Gow, et al., 1979; Konig, et al., 1984). However, as observed by Vernon (1982), growth hormone influences tissue responses to insulin, suggesting an interrelationship exists between these two hormones. By calculating the growth hormone: insulin ratio on the various treatments, a different pattern emerged compared to observing growth hormone levels alone. The mean ratios were 2.4:1, 2.6:1, 6.0:1 and 3.3:1 for treatments RSC, AGC, AG and AC respectively. Although there was only an 8% difference in the ratios between ewes on treatment RSC and AGC, there was a 28% difference when ewes on treatment AC were compared to controls (RSC). These results suggest that there was a relatively larger growth hormone response in ewes fed high levels of formal casein (AC) compared to ewes supplemented with both abomasal glucose and lower levels of casein (AGC).

Insulin levels did not significantly differ between treatments. Lowest levels were observed on treatment AG, which would tend to reflect the low energy intake of the ewes when supplemented with large amounts of abomasal glucose. It was hoped that this experiment might reveal insight into whether insulin was the cause of the low milk fat syndrome. McClymont and Vallance (1962) demonstrated that large intravenous doses of glucose could reduce milk fat secretion and suggested that increased insulin secretion was the cause. Although milk fat secretion did not differ significantly between ewes on RSC, AGC and AG treatments, milk fat % was significantly different between all diets.

Although interpretation should be cautious due to lack of significant differences in insulin levels, a relationship appeared to exist between milk fat % and insulin levels. As insulin level decreased, milk fat % tended to increase. When treatments were ranked in decreasing order for milk fat % the rankings were AG, RSC, AC and AGC. When treatments were ranked in decreasing order for insulin level, the rankings were exactly opposite (AGC, AC, RSC and AG). Thus, these results suggest that the "insulinogenic theory" for explaining low milk fat diets may be valid. However, the present study seemed to indicate that glucose per se does not cause the low milk fat syndrome since neither milk fat secretion nor milk fat

% were reduced in ewes receiving abomasal glucose (AG and AGC) to the degree that is observed on low milk fat diets. Other studies with abomasal glucose infusions also showed only slight changes in milk fat content (Vik-Mo, et al., 1974; Clark, et al., 1973).

Horino et al. (1968) and Bines and Hart (1984) showed that propionate was highly insulinogenic and intraruminal propionate infusions have been shown to reduce milk fat secretion (Lough, et al., 1983; Emmanuel and Kennelly, 1984).

Recent studies by Istasse and Ørskov (1984) compared continuous and pulse infusion of propionate into the rumen to simulate continuous and twice daily feeding regimes. Continuous infusion caused insulin levels to remain constant, but pulse infusion caused plasma insulin concentration to increase 3-fold and propionic acid concentration to increase 6-fold compared to levels prior to the pulse being administered. However, milk fat content was similar on both treatments.

In the present study, feeding regimes were similar and rumen propionate (36% molar) and acetate (53% molar) concentrations were similar on all treatments, thereby eliminating these variables as major contributing factors to changes in milk fat content. Overall, it appears that the low milk fat syndrome is not yet fully understood and appears to be due to a combination of dietary and hormonal factors.

7.4.6 Estimates of Microbial Protein Synthesis and Rumen Degradability of Formal Casein

From the results obtained for digesta flows on the different diets, estimates were made of microbial protein synthesis. The following assumptions were made: (1) On RSC and AG treatments, 10% of the dietary nitrogen (including supplemental untreated casein in RSC) that was not urea nitrogen escaped rumen fermentation, (2) endogenous nitrogen contributed 4 g N to the total NAN flow, (3) all other NAN was microbial in origin. Efficiency of microbial protein synthesis was calculated from apparently digestible organic matter (ADOM) (see Hagemeister, et al., 1981).

AG: (1) 1515 g OM intake (1650 g DM x 92% OM) - 822 g OM out = 693 g ADOM.

(2) 27.9 g NAN - 4.0 (endogenous) - 0.9 (dietary) = 23.0 g microbial NAN.

(3) Microbial efficiency = 23.0/693 = .0332 = 20.7 g microbial protein/ 100 g ADOM.

RSC: (1) 1652 g OM intake (1800 g DM x 92% OM) - 838 g OM out = 814 g ADOM.

(2) 31.0 g NAN - 4.0 (endogenous) - 2.0 (dietary) = 25.0 g microbial NAN.

(3) Microbial efficiency = 25.0/814 = .0307 = 19.2 g microbial protein/ 100 g ADOM.

Based on the previous assumptions, calculated efficiency of microbial protein sythesis was slightly lower than the mean value of 22.1 g reported by Hagemeister *et al.* (1981). If the endogenous nitrogen contribution was only half of what was assumed, then microbial efficiency would have been similar to their values. What is evident is that efficiency was not abnormal on a semi-purified diet containing very high levels of urea. It is also apparent that although NAN flow was increased by 11% on diet RSC vs diet AGC, apparent microbial efficiency was not improved by supplementation with untreated casein. Although the *in vitro* studies of Maeng *et al.* (1976) and Hespell and Leedle (1983) have shown microbial growth to increase when casein partially replaced urea as a nitrogen source, there are probably sufficient peptides and amino acids available *in vivo* from endogenous sources and lysed bacteria.

Estimates of the rumen degradability of formal casein were calculated as the difference between NAN flows in ewes on treatment AG and the AGC or AC treatments divided by the amount of casein nitrogen supplied in AGC (10.8 g) or AC (21.6 g). Estimated degradabilities of formal casein were 14.8% in ewes on treatment AGC and 0% on AC, for mean value of 7% degradability. This agrees well with the previous estimate of 1% degradability measured during incubation of formal casein in nylon bags suspended in the rumen reported in Chapter 5.

7.5 CONCLUSION

In conclusion, the marked increases in milk production in response to protected protein appear to be due to effects on both protein and energy status. Although glucose should be able to be used for both oxidation and synthesis of lactose and non-essential amino acids, the increased production and efficiency associated with protein may be related to a higher potential for metabolic flexibility. Essential and non-essential amino acids can contribute to milk protein synthesis, gluconeogenesis, oxidation and may also increase mobilization of body fat reserves. These effects may be related to the different endocrine responses elicited by abomasal casein, compared to abomasal glucose.