CHANGES IN MILK COMPOSITION, BLOOD AND **RUMEN** METABOLITES IN COWS GRAZING HIGH PROTEIN RYEGRASS SUBSTITUTED WITH ENERGY AND DIFFERENT SOURCES OF PROTEIN

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SUMMARY

Milk responses to feeding diets high in crude protein content, at 2 levels of energy supplementation were studied in 24 Holstein-Friesian multiparous cows over a period of 16 weeks. Cows offered 16 kg dry matter of a high protein ryegrass only (NIL) were compared with cows fed 14 kg dry matter as ryegrass plus 2 kg high protein (50.6%) supplement containing either a highly degradable protein (gluten; 2GL) or a protein of low degradability (fishmeal; 2FM). Giving either diets resulted in a decline in milk, fat and lactose yields compared with the ryegrass diet, though milk protein yield and percentage were not reduced.

The energy content of the ration was increased by replacing 6 kg of ryegrass dry matter with either 6 kg rolled grain (6GR) or 6 kg of a protein/grain mix containing either gluten (6GL) (40.4% CP; 13.3 MJ/kg DM) or fishmeal (6FM) (40.2% CP; 12.0 MJ/kg **DM)** The total amounts of protein offered were similar to diets 2GL and 2FM. In comparison to the high energy, low protein diet (6GR), feeding a protected protein (6FM) stimulated milk and lactose yields, whereas the feeding of a highly degradable protein source (6GL) had a detrimental effect initially on milk and lactose yields. Fishmeal reduced fat% (P < 0.01). Rumen ammonia and plasma urea nitrogen (PUN) reflected protein intakes. Gluten as the protein source with high energy diets caused increases in plasma total protein (P < 0.05), globulin (P < 0.05), glucose (P < 0.01) and PUN (P < 0.01) relative to diet 6GR.

We conclude that excessively high protein contents will have a detrimental effect on production where energy content is restricted. The effects are similar for proteins of very different qualities. Where energy concentration is high, production responses will be different between proteins of different quality.

INTRODUCTION

Milk production is improved by providing cows with a continuous supply of high quality pastures. This is often achieved through the use of high levels of nitrogen fertilisation. This management practise produces crude protein concentrations in the pasture well above the 16% crude protein (CP) required by a mature lactating' cow (NRC, 1989). Levels of 30% CP have been measured in temperate rye and clover pastures (Moss, unpublished).

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Rumen bacteria degrade a portion of feed protein. Satter and Roffler (1975) reported increases in ruminal ammonia from 0.8 to 56.1 mg/100 ml as dietary crude protein increased from 8 to 24%. When diets high in rapidly degradable proteins are fed to dairy cattle, the rate of degradation may exceed the rate of microbial synthesis, resulting in excessive ammonia absorption from the rumen. Increasing ammonia concentration in the rumen can lead to an increase in ammonia in the ruminal veins (Chalmers *et al.*, 1971). Ammonia is synthesised into urea in the liver and excreted (Jordon *et al.*, 1988; Martin and Blaxter, 1964). These two processors are energetically expensive (Martin and Blaxter 1964).

Glucose is the prime nutrient required for mammary gland metabolism. Glucose supplies carbon for lactose synthesis and a significant portion of the energy needed for milk synthesis, and contributes in a significant way to synthesis of milk fat and milk protein (Clark, 1975). Excessive breakdown of protein in the rumen may also reduce the amounts of precursors available for gluconeogenesis. The experiment reported in this paper was conducted to determine the effects of increasing dietary protein content in a diet already supplying 22.8% CP. Two protein sources, a highly degradable protein (gluten) and a rumen protected protein (fishmeal) were compared at two levels of energy intake.

MATERIAL AND METHODS

Twenty-four Holstein Friesian cows in early lactation were offered experimental diets over a period of 16 weeks. They were blocked on calving date and milk yield and randomly allocated within blocks to one of six treatments in a randomised complete block design. During the 3 weeks before treatments were imposed cows were grazing a common pasture supplemented with 5 kg grain/cow.day, and milk yield, protein, fat and lactose percentages were recorded and used as covariates.

The treatments differed in type of protein fed and level of energy supplement. The first 3 treatments were 16 kg of ryegrass (Lolium perenne) dry matter (DM) on offer (NIL) containing 22.9% CP and 10.6 MJ/kg DM, 2 kg of ryegrass was replaced with a 50.9% CP; 13.3 MJ/kg DM gluten or 50.3% CP; 11.6 MJ/kg DM fishmeal mix; viz. 14 kg DM ryegrass + 2 kg DM gluten/grain mix (2GL) and 14 kg DM ryegrass + 2 kg DM fishmeal/grain mix (2FM). In treatments 4, 5 and 6 the amount of ryegrass offered was reduced and substituted with a grain (11.3% CP; 13.1 MJ/kg DM) or gluten/grain mix (40.4% CP; 13.3 MJ/kg DM) or fishmeal/grain mix (40.2% CP; 12.0 MJ/kg DM); viz. 10 kg DM ryegrass + 6 kg grain (6GR); 10 kg DM ryegrass + 6 kg gluten/grain mix (6GL) and 10 kg DM ryegrass + 6 kg fishmeal/grain mix (6FM). Cows were individually fed their concentrate which was split in half and fed am and pm. Sodium bicarbonate (100 gm) was mixed with the grain in treatment 4 to prevent acidosis.

Pastures were measured twice weekly to determine pre- and post- grazing pasture for dry matter on offer or rejected. Electric tapes were used to control the amount of pasture on offer to the treatment groups.

Milk samples were recorded at the morning and afternoon milking once per week. A composite milk sample was taken from both milkings and analysed for

butterfat, protein and lactose (Fossomatic-Milkoscan 203). Cows were weighed once per week prior to being offered concentrates.

Rumen fluid was sampled via stomach tube prior to and 5 hours after offering the ration in weeks 4, 10 and 15. Approximately 100 ml of ruminal fluid were obtained and an 8 ml sub-sample was taken and acidified with 8 ml of 0.2 N HCL and later analysed for ammonia nitrogen content. Ten ml of rumen fluid was preserved with 3 drops of mercuric chloride and frozen for volatile fatty acid analyses.

Ten millilitres of blood were collected prior to feeding concentrate by jugular puncture on the day of rumen sampling. Blood was decanted into a vial containing lithium heparin. Blood samples were centrifuged at 3000 rpm for 10 minutes. Supernatants were decanted and stored at -20^0 C and later analysed for plasma urea nitrogen (PUN).

RESULTS

HIGH **PROTEIN/RESTRICTED** ENERGY

Substituting 2 kilograms of a ryegrass diet with a gluten (2GL) or fishmeal (2FM) mix resulted in a decline in milk, fat and lactose yields compared to the ryegrass treatment (NIL; Fig 1). The detrimental responses to high protein intake did not occur until the cows had been eating their respective diets for 7 weeks and it was from week 7 onwards that the crude protein in the pasture increased (Table 1). Protein yield and percentage were not changed.

Growth rates of cows fed the NIL, 2GL and 2FM treatments were 0.41, 0.65 and 0.63 kg/cow.day respectively from weeks 5 to 16 (Pc 0.05).

Rumen ammonia concentrations were similar between treatments 5 hours after the morning feed, but prior to the am feed were elevated for cows given fishmeal and slightly depressed for cows given gluten (Table 1). PUN reflected the crude protein intakes over time (Table 1). Feeding a highly degradable protein source such as gluten lead to a significant decline in plasma total protein by week 15. This was mostly due to a decline in the globulin component of the protein.

Rumen volatile fatty acid concentrations generally did not differ during the actively growing phase of ryegrass (week 10), except for N-valeric acid which was higher for the ration containing gluten after am feeding (P < 0.01).

HIGH PROTEIN AND ENERGY CONCENTRATES

With 6 kg of concentrate intake the milk responses to an increase in crude protein content were different between protein sources. In comparison to the high energy, low protein content diet (6GR), feeding a protected protein, fishmeal, as the protein source stimulated milk and lactose yields (Fig. 2). In contrast, the feeding of a fermentable protein source, gluten, had a detrimental effect initially on milk and lactose yields, with milk and lactose yields being reduced by 2.1 and 0.10 kg/cow.day respectively from weeks 3 to 9. After week 9 milk yields for treatments 6GR and 6GL were similar.
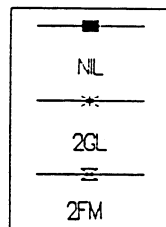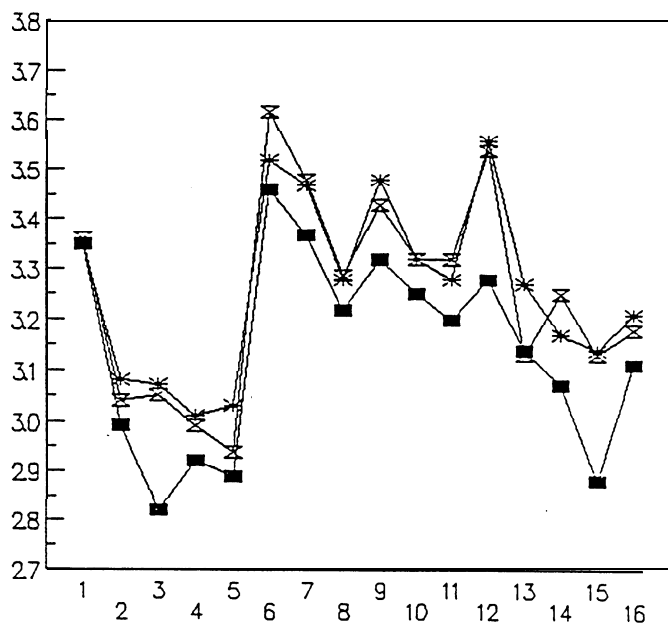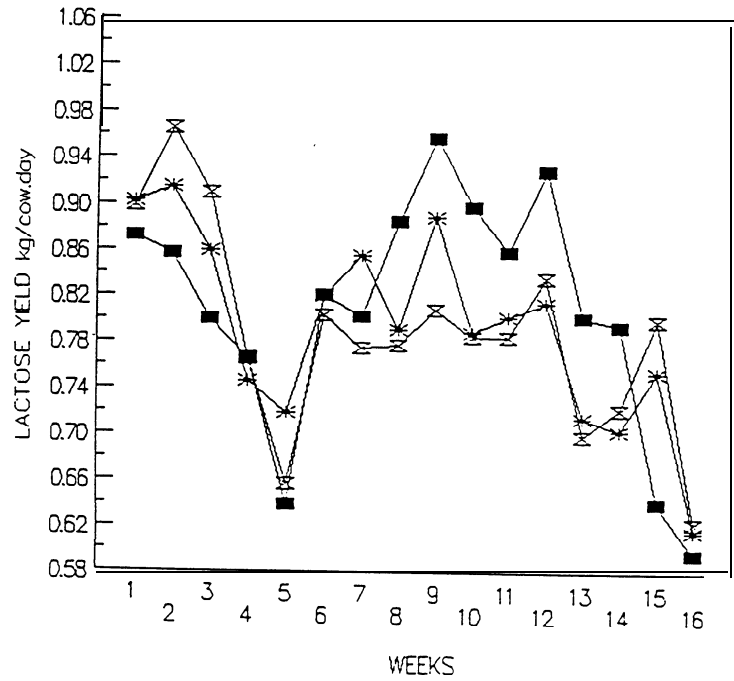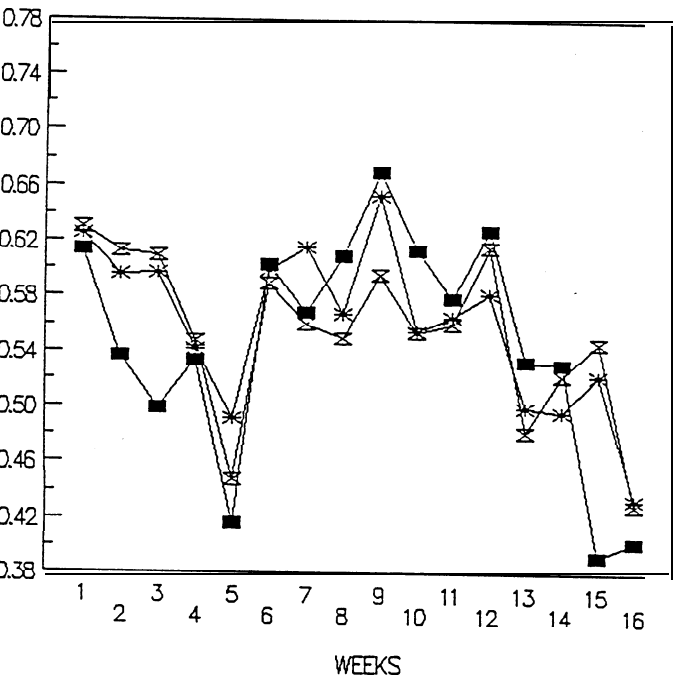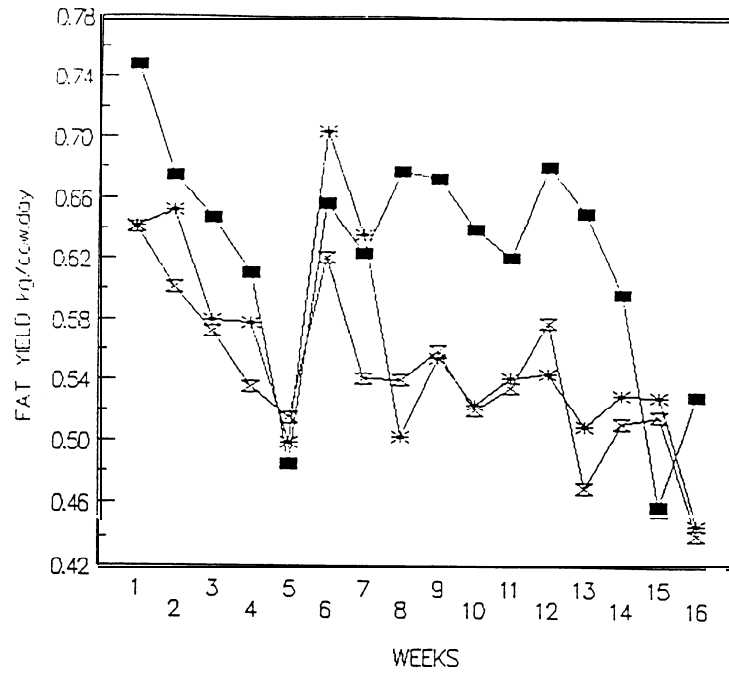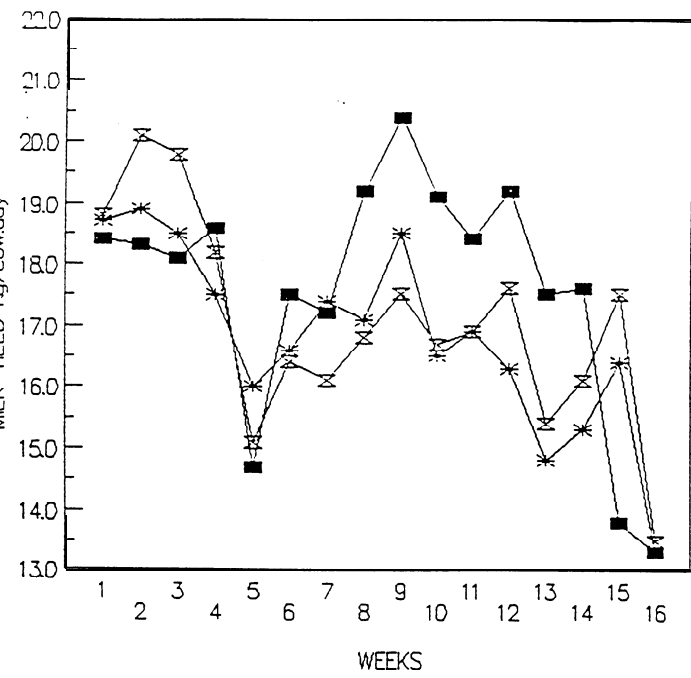
Figure 1. Milk Yield and composition responses to high protein ryegrass (NIL); ryegrass + 2 kg gluten/grain (2GL) or ryegrass + 2kg fishmeal/grain (2FM).

TABLE 1. Plasma and rumen metabolites from cows fed either ryegrass only (NIL), ryegrass + 2 kg gluten/grain mix (2GL) or ryegrass + 2 kg fishmeal/grain mix (2FM).

WEEKS	NIL	2GL	2FM	SIG
TOTAL CRUDE PROTEIN OFFERED (kg/day)				
4	3.4	3.9	3.8	
10	4.2	4.6	4.3	
15	3.6	4.0	4.0	
RUMEN AMMONIA (mg/100 ml)				
4, 10, 15	11.0^{ab}	10.3^{b}	13.9^{a}	**
PLASMA UREA (mmol/l)				
4	5.0^{a}	6.3^{b}	7.5^{b}	**
10	9.2	8.5	8.7	ns
15	7.8^{a}	8.4^{ab}	9.4^{b}	**
PLASMA TOTAL PROTEIN (g/l)				
4	74	75	78	ns
10	77	79	78	ns
15	77^{a}	70^{b}	80^{a}	*

* $P < 0.05$; ** $P < 0.01$

Protein and fat percentages were increased with gluten as the protein source, and fishmeal caused protein % and fat% to be reduced ($P < 0.05$).

Growth rates from weeks 5 to 16 with treatments 6GR, 6GL and 6FM were 0.72, 0.66 and 0.55 kg/cow.day respectively.

Rumen ammonias were similar pre-feeding for treatments 6GR and 6GL and both were significantly below treatment 6FM for the entire experiment (Table 2). Samples taken at 5 hours after the commencement of feeding showed an increase in rumen ammonia with the increase being greatest for cows eating the ration containing gluten and smallest for those consuming fishmeal. The 3 treatments did not differ in their effects on rumen pH either pre-feeding or 5 hours after feeding.
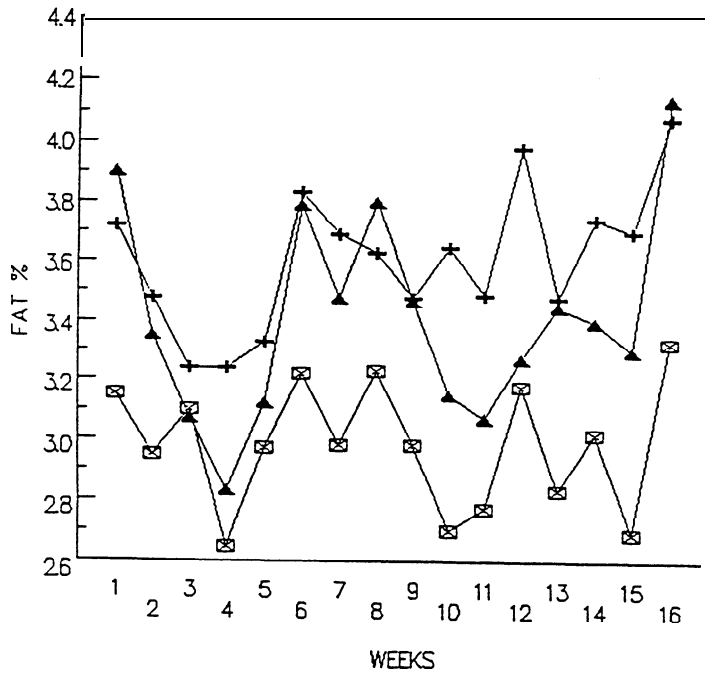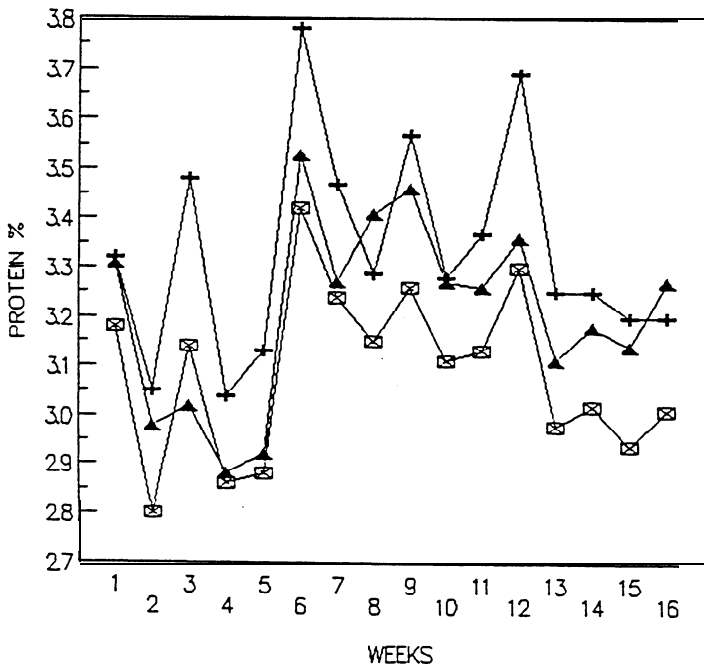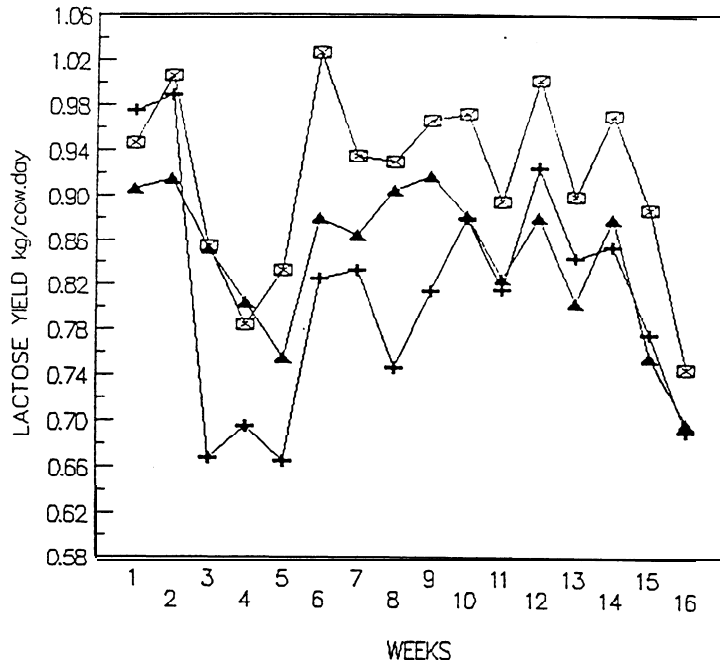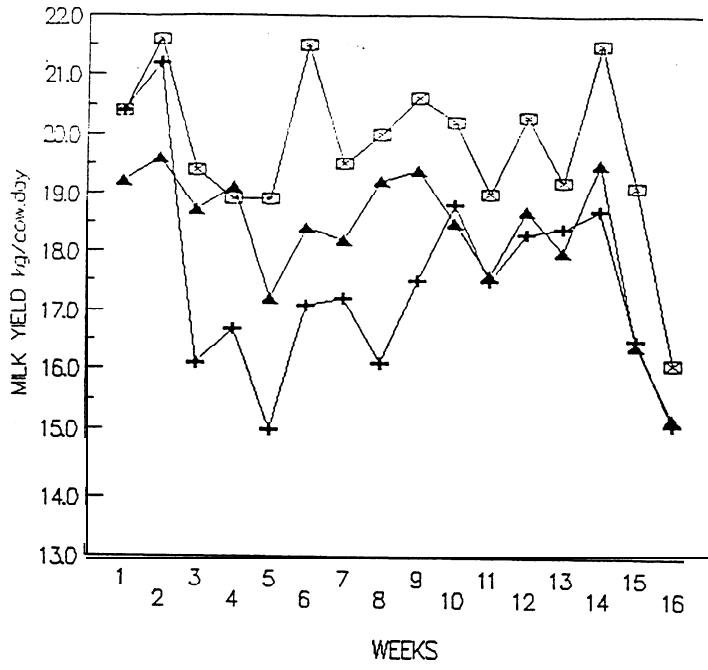
Figure 2. Milk Yield and composition with increasing crude protein content and different protein sources in a ryegrass/ grain ration.

TABLE 2. Rumen ammonia (mg/100 ml) for cows fed different levels and sources at the same level of energy supplementation.

		6GR	6GL	6FM	SIG
WEEK 10	AM	10.6	13.9	15.1	ns
	PM	17.9^a	23.2^b	21.4^{ab}	*
WEEK 15	AM	12.7^a	13.2^a	22.9^b	*
	PM	35.3	52.0	37.5	ns
WEEKS 4,10,15 AM		8.4^a	10.1^a	13.8^b	**

* $P<0.05$; ** $P<0.01$

TABLE 3. Plasma metabolites measured in cows eating ryegrass substituted with 6kg grain (GR), gluten/grain (6GL) or fishmeal/grain (6FM)

WEEKS	6GR	6GL	6FM	SIG
PLASMA UREA NITROGEN mmol/l			
10,15	5.74^a	8.13^b	9.20^b	**
TOTAL PROTEIN g/l			
10,15	75.7^a	83.0^b	78.6^{ab}	*
ALBUMIN g/l			
10,15	36.4	36.8	35.3	NS
GLOBULIN g/l			
10,15	39.4^a	46.2^b	42.6^{ab}	*
A/G			
10,15	0.92^a	0.81^b	0.86^{ab}	**
GLUCOSE mmol/l			
4,10,15	3.55^a	3.94^b	3.51^a	**

* $P<0.05$; ** $P<0.01$

Volatile fatty acids measured in weeks 10 and 15 were not affected by treatment, except with gluten as the protein source higher I- and N-valeric acids post-feeding and higher caproic acid pre-feeding were measured (P < 0.05).

Plasma metabolites are shown in table 3. PUN concentrations reflected the crude protein intake. Gluten used as the protein source (6GL) caused increases in plasma total protein, globulin and glucose relative to treatment 6GR. The albumin to globulin ratio was significantly lowered when grain was replaced with a gluten/grain mix. At similar energy intakes, treatments 6GR and 6FM did not differ in plasma total protein, albumin, globulin, A/G ratio and glucose.

ENERGY AND PROTEIN INTERACTION

Milk, fat, protein and lactose yields responded to an increase in energy content for cows eating high protein rations containing fishmeal. When gluten was fed as the protein source, a milk response to increase in energy intake did not occur until week 9 (Fig. 1 and 2). At the same level of crude protein content an increase in metabolizable energy content lead to an overall increase of 1.0 l/cow.day for the gluten rations and 2.6 l/cow.day for fishmeal (Fig 1. and 2). A significant interaction (P < 0.01) occurred between protein source and level of energy content for fat%. An increase in energy content caused an increase in fat% with gluten as the protein source as compared to a decrease with fishmeal. The fat percentages recorded for treatments 2GL, 2FM, 6GL and 6FM were 3.26, 3.23, 3.60 and 2.98% respectively. The highest yields of milk, protein and lactose were achieved with treatment 6 FM.

DISCUSSION

Milk, lactose and fat yields are reduced if protein content is increased by 2.7% without an increase in energy content for cows grazing ryegrass containing 22.9% CP. Irrespective of the protein source there was a detrimental effect on milk production. Feeding a protected protein (fishmeal) increases the amount of amino acids being absorbed at the small intestine as compared to a highly degradable protein source (gluten), but this increase is not evident in the milk responses. The amino acids are probably broken down to carbon skeletons and ammonia. Gluten is rapidly broken down in the rumen to ammonia and volatile fatty acids as indicated by the results. If the bacteria have insufficient energy for making optimal use of the ammonia, then excess ammonia is absorbed through the rumen mucosa (Jordon et al., 1983 and Payne, 1977). Excess blood ammonia produced from both protein rations is transported to the liver and converted 'to urea (Jordon et al., 1983) and excreted by the kidneys. Energy is required to excrete this ammonia, hence less is available for milk production. It is interesting to note that a partitioning of energy occurred with the high protein treatments (2GL and 2FM) and energy was channelled into liveweight gain.

The increase in milk yield when 6 kg grain was substituted for 6 kg ryegrass suggests an increase in efficiency of energy use. Satter and Roffler (1975) reported reduced ruminal ammonia concentrations as dietary crude protein is lowered. There is a decrease in transport of ammonia into ruminal veins with a decrease in rumen ammonia (Chalmers et al., 1971). This is evidenced in this experiment by a decline in

PUN'. Less energy is required to excrete **PUN** and can be used for milk production and Iiveweight gain, and the replacement of pasture with grain means more metabolizable energy is available for milk production

If energy content is increased in high protein rations, milk performance will be improved. For cows eating high protein rations containing either fishmeal or gluten, additional energy lessened the detrimental effects with feeding gluten and led to a substantial increase in milk yield with fishmeal. Spain *et al.* (1990) had a similar response to fishmeal, with milk yields increased at high levels of fishmeal when energy intake increased from low to high levels. PUN utilisation in sheep was increased when carbohydrate was added to the diet (Houpt 1959). The provision of starch in the diet helps alleviate ammonia absorption as they provide carbon skeletons for microbial synthesis (Houpt, 1959; Payne, 1977), and lowers pH within the rumen, thus preventing absorption of ammonia (Payne, 1977).

The type of protein source added to a high energy ration was shown to be important. Fishmeal is a better protein source than a highly rumen degradable protein such a gluten. Forster et *al.* (1983) also found that by feeding a resistant protein at the same protein concentration as a degradable protein, greater milk production may be achieved. Clark (1975) showed that increased protein flowed at the duodenum with feeding protected protein and this resulted in an increase in production of milk. If energy is high an, increase in amino acid absorption at the small intestine through feeding fishmeal stimulates milk and lactose yields. Gluconeogenic amino acids from the small intestine provide major substrates for glucose synthesis (Clark 1975). An increase in amino acid absorption may increase glucose synthesis, hence an increase in milk yield. The incorporation of gluten into a high energy diet will initially reduce milk and lactose yields. It appears that the animal takes several weeks to adapt to the high amounts of ammonia production.

The response to fishmeal fed in a diet of high energy concentration was in milk and lactose yields, not in protein or fat percentages. Spain *et* al. (1990) reported higher milk fat percentages for cows fed corn gluten rather than fishmeal at high energy intakes. Data of Oldham et *al.* (1985) and Pennington and Davis (1975) indicated that dietary inclusion of fishmeal or cod liver oil decreased milk fat%. Rumen acetic and butyric acids were not significantly affected by feeding gluten or fishmeal, indicating that fishmeal did not reduce milk fat% by altering rumen fermentation. Therefore, some other factor is present in fishmeal that affects milk fat%. This compound may be residual oil in fishmeal.

Plasma glucose was significantly high for cows consuming ration 6GL. Possibly high blood ammonia concentrations prevented insulin from being produced and hence decreased the plasma glucose utilisation. This is consistent with the theories of Fernandez *et al.* (1988) and Visek (1984). One of the clinical changes in blood chemistry with ammonia toxicity is severe hyperglycaemia (Payne, 1977).

CONCLUSION

Milk and milk constituents yields are reduced when dietary crude protein concentration is increased from 22.9 to 25.6% without an increase in energy concentration in the diet, and effects are similar for widely different protein types.

An increase in energy to protein ratio in the diet by feeding additional grain leads to improvements in milk and lactose yields, the improvement being greater for proteins with a high level of rumen undegradable protein.

ACKNOWLEDGMENTS

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