The effects of level of dietary protein on the milk production and rumen physiology of dairy cows fed a diet based on a tropical grass hay

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Abstract

Four rumen-fistulated, multiparous Holstein-Friesian cows in early lactation were offered mixed diets based on rhodes grass hay (*Chloris gayana*) cv. Callide containing 13, 14, 15 or 16% crude protein (CP) on a dry matter basis, in a 4 × 4 latin square design. The estimated undegradable protein concentration in these diets was similar with rumen degradable protein concentration varying. Cows fed a diet containing 13% CP had lower (P = 0.07) milk yields than cows in other treatments (20.4 *vs* 21.9, 22.0 and 22.2 L/d for 13, 14, 15 and 16% CP, respectively). A positive linear relationship was found (P = 0.06) between organic matter intake and dietary CP%. There were negative linear relationships between dietary CP% and digestibilities of dry matter (P = 0.09), organic matter (P = 0.06) and neutral detergent fibre (P = 0.02). Feeding a diet containing 13% CP resulted in significantly lower (P = 0.001) molar proportions (%) of rumen valerate in comparison with other treatments. The molar proportions of isovalerate differed (P = 0.001) between treatments (0.66, 0.78, 0.89 and 1.04%) for 13, 14, 15 and 16% CP, respectively). Dietary protein level had no effect on rates of passage, *in situ* digestion of rhodes grass hay or ratios of allantoin: creatinine in urine.

These data showed that increasing the dietary CP concentration of lactating cows fed diets based on rhodes grass hay increased intakes and altered rumen metabolism. Milk production was not significantly improved at dietary CP concentrations above 14% DM.

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Introduction

The literature contains numerous examples where protein supplementation has increased the milk production of cows fed diets based on tropical grasses (*e.g.* Royal and Jeffery 1972; Stobbs *et al*. 1977; Flores *et al*. 1979; Hamilton *et al*. 1992). The largest increases have been recorded when rumen bypass protein supplements have been fed to grazing cows (*e.g.* Stobbs *et al.* 1977; Flores *et al.* 1979; Hamilton *et al*. 1992). However, in some cases, milk yield has been increased when protein supplements with high rumen degradabilities (dg) such as soyabean meal, casein and sunflower meal have been fed (*e.g.* Royal and Jeffery 1972; Stobbs *et al*. 1977; Moss *et al*. 1992). This is despite the grasses in these experiments having an estimated rumen degradable protein (RDP): metabolisable energy ratio of greater than 12 g/MJ, which is in excess of requirements for lactating cows (ARC 1984; NRC 1989; SCA 1990).

The mechanisms behind these responses in milk production remain unclear. One explanation could be that the undegraded protein (UDP) component of these supplements stimulated milk production. Alternatively, the rumen microflora of cows fed diets based on tropical grasses may benefit from additional RDP supplementation. To examine the second issue further, the present experiment examined the effects on rumen physiology and milk production of cows of increasing dietary crude protein (CP) concentration and RDP by feeding additional soyabean meal.

Materials and methods

Experimental design, animals and treatments

The experiment was conducted at the University of Queensland, Gatton, Australia from July–September 1995. Four rumen-fistulated, multiparous Holstein-Friesian cows (average 75 days

post-partum and 467 kg liveweight) were randomly assigned to diets with different concentrations of CP in a 4 × 4 latin square design. Each 24-d experimental period consisted of a 7-d adjustment period and a 17-d observation period. The cows were individually housed in covered stalls with access to an open pen approximately 50 m^2 in area.

All cows received an isoenergetic mixed ration consisting of rhodes grass hay (*Chloris gayana*) cv. Callide containing 8.9% CP and 72.7% neutral detergent fibre (NDF) [dry matter (DM) basis] and a wheat-based concentrate. Total dietary CP concentration (13, 14, 15 or 16% DM) was altered by substituting soyabean meal for wheat and/or formaldehyde-treated cottonseed meal (FTCM). A similar concentration of estimated UDP was maintained across diets. The diets were formulated to meet the mineral requirements of a 500 kg cow, 120 days into lactation and producing 20 kg of milk per day containing 3.4% milk fat (NRC 1989). The ingredients in and nutrient composition of the diets are shown in Table 1. Diets were offered at 110% *ad libitum* twice-daily at 08.00 h and 17.00 h. Cows had access to water at all times except during milking.

Table 1. Composition of experimental diets.

Constituent	Dietary CP (%DM)			
	13	14	15	16
Chemical analysis (DM basis)				
Metabolisable energy (MJ/kg)[1]	9.6	9.8	9.8	9.9
Organic matter (g/kg)	894	901	902	902
Crude protein (g/kg)	130	141	150	162
Neutral detergent fibre (g/kg)	494	493	471	469
Acid detergent fibre (g/kg)	234	225	216	212
Ingredients (g/kg DM)[2]				
Hay	580	580	580	580
Wheat	227	226	226	190
FTCM[3]	98	68	34	1
Soyabean meal	0	49	109	169
Tallow	31	25	19	19
Limestone	18	7	7	7
Dicalcium phosphate	6	6	6	6
Salt	5	5	5	5
Mineral/vitamin premix	1	1	1	1
Molasses (cane)	38	38	38	38

[1] Estimated from NRC (1989) and Minson (1984).
[2] Water was added to all experimental diets (200g/kg as fed).
[3] Formaldehyde-treated cottonseed meal.

Measurements

Feed intake was measured daily at 07.00 h and 16.00 h during the observation period. Samples of feed offered were collected 3 times in each observation period, dried, pooled and then ground through a 1 mm screen. Sub-samples were then analysed for CP (N × 6.25; Leco 2000 Nitrogen Analyser based on AOAC 1970), organic matter (OM; 3 h at 550°C), and NDF and acid detergent fibre (ADF) using the Fibertec system (Fibertec® Tecator AB Hogan as, Sweden) which is based on the methodology of Robertson and van Soest (1981) and van Soest *et al.* (1991). Residues were not analysed for their nutrient content.

Daily milk yields were measured during the observation period using in-line milk meters (Hunday Electronics, Sydney, Australia). Milk samples were taken from consecutive milkings from Day 14 to Day 18 of the observation period and analysed for fat, protein and lactose percentages (Foss Electric Milkscan 605).

Rumen and total tract passage rates of rhodes grass hay were measured according to Shaver *et al*. (1986). Five hundred g of hay was sprayed with a ytterbium (Yb) solution (1.62 g of $YbCl_3$ in 250 ml of deionised water) to provide 1 g of Yb. After spraying, the sample was left to air-dry overnight. The labelled hay was fed by itself at 07.00 h on Day 1 of each observation period. Any refusals (usually less than 10%) were then inserted into the rumen through the cannula. Faecal samples were collected at 4, 8, 12, 18, 24, 30, 36, 48, 60, 72 and 96 h post dosing. These samples were dried (60°C for 120 h) and then ground through a 1 mm screen.

Apparent dry matter digestibility (DMD), organic matter digestibility (OMD) and true NDF digestibility (NDFD) of experimental diets were measured using lanthanum (La) as a marker (Hartnell and Satter 1979a, 1979b; Shaver *et al*. 1986). Faecal samples were collected twice daily from Day 1 to Day 17 of the observation period with at least 10 h between collections, dried (60°C for 120 h) and ground through a 1 mm screen. These samples were analysed for OM and NDF as described previously. Concentrations of Yb in faeces and La in faeces and diets were determined by wet ashing and atomic emission spectroscopy (Spectrophotometer Analytical Instruments Model M+P, Germany). Faecal concentrations of Yb were fitted to the two-compartment model of Grovum and Williams (1973) as described by Shaver *et al*. (1986):

$$y = Ae^{-k_p (t - tt)} - Ae^{-k_2 (t - tt)}$$

where:

y = marker concentration in faeces at time t;

A = biologically undefined;

tt = transit time, *i.e.* the time of first appearance of marker in faeces;

k_p = fractional rate constant for marker movement per hour in the reticulo-rumen; and

k_2 = fractional rate constant for marker movement per hour in the caecum and colon, and possibly mixing in the reticulorumen.

To use this model, t must be >tt, as y = 0 when t < tt.

The model was fitted using the NLIN procedure of SAS (1991). Total mean retention time (TMRT) equalled rumen retention time ($1/k_p$) + tt + $1/k_2$.

DMD, OMD and NDFD were estimated as suggested by Faichney (1975) whereby:

Digestibility = 1 – [(concentration of La in feed * DM, OM or NDF concentration)/ (concentration of La in faeces * OM or NDF concentration)]

In situ digestion parameters of rhodes grass hay were determined according to Shaver *et al*. (1986). These observations commenced on Day 9 and concluded on Day 14 of each observation period. Fourteen nylon bags (150 mm × 75 mm; 45 µm pore size), each containing 3 g of hay (ground through 1 mm screen), were attached to chains, placed in the rumen through the cannulae and forced below the rumen mat at 07.00 h on Day 16 of the observation period. Another two bags were prepared but not inserted (0 time bags). Single bags of hay were then removed at 2, 6, 12, 24, 48 and 72 h post insertion. Bags were placed into cold water immediately upon removal. Zero time bags and incubated bags were washed by hand under cold water until the washings from each bag were clear. The bags were dried in a forced-draught oven at 60°C for 72 h, allowed to cool in a desiccator, then weighed. DM disappearance from the samples was fitted to the model of Mertens and Loften (1980) as described by Shaver *et al.* (1986) using the NLIN procedure of SAS (1991):

$DMD_t = a + b(1 - e^{-k_b t})$

where DMD_t = dry matter disappearance at time t;

a = the rapidly digested fraction (that fraction of DM that was removed by washing the 0 time bag);

b = the slowly digested fraction that can be digested at rate k_b;

and a + b = the potentially digested fraction *i.e.* the DM fraction that disappears after 72 h incubation (Shaver *et al*. 1986). Rumen availability of DM (RADM) of the hay and treatment concentrates was calculated using the following formula (NRC 1985):

$RADM = a + b * k_b/(k_b + k_p)$

where a = soluble dry matter;

b = slowly digestible dry matter;

k_b = rate of digestion of b; and

k_p = rate of passage of b through the rumen.

Rumen fluid was collected as scheduled in Table 2. Samples were measured for pH within 30 min of collection and then filtered through 6 layers of muslin and divided into 2 portions of approximately 50 ml. One portion was made alkaline (pH 10) with the addition of 30% NaOH and analysed for volatile fatty acids (VFA) using gas chromatography (Anon. 1971). The other sample was left unaltered as recommended by Nocek *et al*. (1987) and analysed for NH_3 using the phenolhypochlorite colorimetric method of Weatherburn (1967).

The ratio of allantoin:creatinine in spot samples of urine was used as an index of microbial protein synthesis (MPS) based on the methodology of Kolade (1994).

Approximately 100 ml of urine was collected daily using vulval stimulation from each cow between 12.00 h and 18.00 h from Day 8 to Day 15 of the experimental period. These samples were acidified (pH 3) with the addition of 10 ml of 10% H_2SO_4 and then frozen at –20°C until analysed for creatinine using a Sigma Aldrich® creatinine determination kit and for allantoin using the method of Borchers (1977).

Table 2. Rumen fluid sampling schedule.

Day of observation period	Times of rumen fluid sampling (h)
14	00.00, 08.00, 16.00
15	02.00, 10.00, 18.00
16	04.00, 12.00, 20.00
17	06.00, 14.00, 22.00

Statistical analysis

Data were analysed as a 4 × 4 latin square according to Snedecor and Cochran (1967). Differences between treatments were determined using the general linear model (GLM) procedure of SAS (1991) with least square analysis of means. Regressions with single degrees of freedom were used to determine linear and quadratic effects using the GLM procedure of SAS (1991). Effects due to time and time × treatment interactions were included in the analysis of rumen NH_3, pH and VFA. Significance was declared at $P < 0.10$ unless otherwise stated.

Results

Milk production, nutrient intake and digestibility

The effects of dietary CP concentration on milk production, nutrient intake and digestibility are shown in Table 3. Daily milk yield for the 13% CP diet was lower (P = 0.07) than for the other treatments. A positive linear relationship (P = 0.03) existed between milk yield and dietary CP% and a negative linear relationship (P = 0.07) between milk protein percentage and CP%.

Dietary CP% had no effect on intakes of dry matter (DMI, P = 0.21) or digestible organic matter (DOMI, P = 0.73). A positive linear relationship was found between OMI and CP% (P = 0.06). NDFD was reduced (P = 0.08) at 16% CP. Negative linear relationships were found between CP% and digestibilities of DM (P = 0.09), OM (P = 0.06) and NDF (P = 0.02).

Rumen metabolites

Significant effects of time on rumen NH_3, total VFAs, pH (Figure 1) and VFA molar proportions (data not presented) were found. No significant time × treatment interactions were recorded for NH_3, VFAs or pH. Concentrations of rumen NH_3, total rumen VFAs and molar percentages of individual rumen VFAs are shown in Table 4. Rumen NH_3 concentrations were affected (P = 0.01) by CP% in the diet with 16% > 14% and 15% > 13% CP. The molar proportion of valerate for 13% CP was lower (P = 0.01) than for the other treatments while the proportion for 16% CP was also higher (P = 0.01) than for 14% CP. The molar proportion of isovalerate increased progressively (P = 0.01) with increasing CP%. Dietary CP% had no significant effect on the molar proportions of acetate, propionate or butyrate. Positive linear relationships were found between dietary CP% and rumen ammonia, total rumen VFA concentration, and the molar percentages of valerate, isovalerate, isobutyrate and AB:P [(molar percentage of

Table 3. Milk production, nutrient intakes and digestibilities by cows fed rhodes grass-based diets containing different dietary concentrations of crude protein (CP).

Observation	Dietary CP (%DM)				Significance		Regression	
	13	14	15	16	P[2]	s.e.	Trend[3]	P[4]
Milk production								
Yield (kg/d)	20.4a[1]	21.9b	22.0b	22.2b	0.07	0.45	L[5]	0.03
Fat (%)	3.71	3.53	3.50	3.51	0.60	0.127	NS	NS
Fat yield (g/d)	744	772	752	760	0.81	21.7	NS	NS
Protein (%)	3.09a	3.04b	3.08a	3.04b	0.05	0.020	L	0.07
Protein yield (g/d)	624	661	675	668	0.39	15.0	L	0.08
Intakes (kg/d)								
Dry matter	16.9	17.1	16.9	17.7	0.21	0.29	NS	NS
Organic matter (OM)	15.1	15.4	15.3	16.0	0.16	0.25	L	0.06
Crude protein	2.1a	2.4b	2.5c	2.9d	0.01	0.04	L	0.01
Neutral detergent fibre	8.3	8.4	8.0	8.2	0.11	0.10	NS	NS
Acid detergent fibre	3.9a	3.8ab	3.7b	3.8ab	0.02	0.04	L	0.01
Digestible OM	11.0	11.2	10.6	10.6	0.73	0.45	NS	NS
Digestibility (g/kg)								
Dry matter	732	739	713	675	0.27	22.3	L	0.09
Organic matter	730	727	699	661	0.23	23.4	L	0.06
Neutral detergent fibre	688a	679a	638a	576b	0.08	25.7	L	0.02

[1] Means within rows followed by different letters are significantly different (P < 0.10).
[2] Significance of treatment effect.
[3] Trend of regression.
[4] Significance of regression.
[5] Linear.

acetate + molar percentage of butyrate):molar percentage of propionate] ratio.

Rates of passage, in situ *digestion parameters and urine allantoin:creatinine*

Dietary CP% had no effect on rates of passage or *in situ* digestion parameters. Mean (±s.e.) values for rumen passage rate (K_p), rumen retention time ($1/K_p$), total mean retention time (TMRT), soluble (a) and slowly degraded (b) hay DM, hay rumen DM degradation rate (K_b) and rumen available DM (RADM) were 0.066 ± 0.0128/h, 17.1 ± 2.21 h, 30.54 ± 1.921 h, 17.1 ± 0.82%, 54.6 ± 1.03%, 0.065 ± 0.005/h and 42.9 ± 2.33%, respectively.

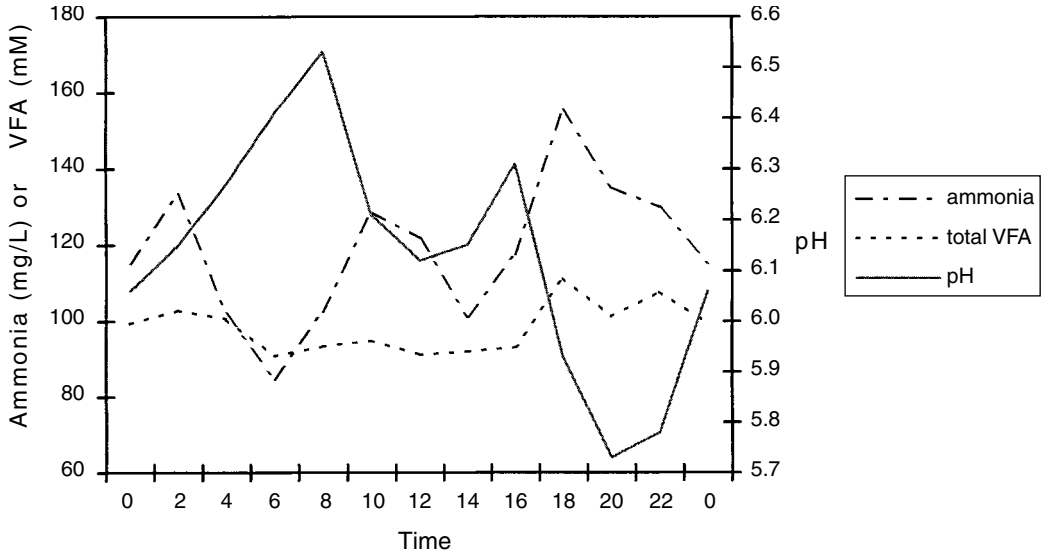

Figure 1. Changes in mean rumen pH and concentrations of ammonia and total volatile fatty acids (VFA) over time.

Table 4. Average concentration of rumen NH_3, pH, concentrations of total volatile fatty acids (VFA), molar proportions of individual rumen VFA, ratio of the molar proportions of (acetate + butyrate): propionate (AB:P), ratio of the molar proportions of acetate: propionate (A:P) and urinary allantoin:creatinine (A:C) ratio for different dietary concentrations of crude protein (CP).

Observation	Dietary CP (%DM)				Significance		Regression	
	13	14	15	16	P[2]	s.e.	Trend[3]	P[4]
NH_3 (mg/L)	94.6a[1]	108.9b	116.8b	144.6c	0.01	4.02	L[5]	0.01
pH	6.13	6.13	6.12	6.18	0.30	0.045	NS	NS
Total VFA (mM)	93.9	99.3	98.5	101.3	0.25	2.97	L	0.02
VFA molar %								
Acetate (A)	68.54	67.78	68.04	67.60	0.61	0.583	NS	NS
Propionate (P)	18.11	18.40	18.82	18.58	0.60	0.385	NS	NS
Butyrate (B)	11.45	11.24	11.26	11.21	0.89	0.248	NS	NS
Valerate	1.07a	1.18b	1.24bc	1.26c	0.01	0.023	L	0.01
Isovalerate	0.66a	0.78b	0.89c	1.04d	0.01	0.044	L	0.01
Isobutyrate	0.59	0.64	0.67	0.74	0.15	0.040	L	0.01
AB:P	4.5	4.4	4.3	4.3	0.60	0.11	L	0.10
A:P	3.9	3.8	3.7	3.7	0.65	0.10	NS	NS
Urine (mg/ml)								
Allantoin (A)	1.64	1.79	1.74	1.54	0.80	0.168	NS	NS
Creatinine (C)	0.44	0.52	0.44	0.44	0.18	0.033	NS	NS
A:C	3.86	3.87	3.91	4.26	0.94	0.569	NS	NS

[1] Means within rows followed by different letters are significantly different ($P < 0.10$).
[2] Significance of treatment effect.
[3] Trend of regression.
[4] Significance of regression.
[5] Linear.

No significant linear or quadratic relationships were found between these parameters and CP%. Urinary allantoin:creatinine ratio was not significantly different between treatments (Table 4).

Discussion

Rumen parameters

Rumen degradable protein supplements such as soyabean meal (dg = 0.65; NRC 1989) provide a source of ammonia, branched chain fatty acids (BCVFA) and amino acids (Johnson *et al*. 1994) which enables rumen microflora to maximise their utilisation of carbohydrates. Therefore, it would be expected that changes in the rumen concentrations of these parameters would be closely related to the intake of protein supplements with a high rumen degradability. The average rumen NH_3 concentration of cows fed 13% CP was 94.6 mg/L with concentrations rising to 144.6 mg/L when cows were fed 16% CP. Similar increases in rumen NH_3 concentrations have been noted previously when protein supplements of high rumen degradability have been fed to cows grazing tropical grasses (Stobbs *et al*. 1977; Moss *et al*. 1992). All recorded rumen NH_3 concentrations in the current study exceeded the minimum NH_3 concentration for maximum rumen OM fermentation, based on recommendations of 25–85 mg/L (Satter and Slyter 1974; Mehrez *et al*. 1977; Kang-Meznarich and Broderick 1980; Boniface *et al*. 1986; Morrison *et al*. 1988; Kennedy *et al*. 1992). This is consistent with the observation that dietary CP% had no effect on the *in situ* DM digestion of rhodes grass hay in the current experiment, and would suggest that a rumen NH_3 concentration of 90 mg/L is adequate to meet the requirements of rumen microflora of lactating cows fed a diet based on rhodes grass hay.

The other effects on the rumen environment observed with increasing CP% in the current study were a linear increase in total VFA concentration, a linear decline in AB:P ratio and an increase in the molar proportions of valerate, isovalerate and isobutyrate. This is not unexpected as the proportions and concentrations of valerate and BCVFA are usually directly related to the degradability of a protein source as they result from the oxidative deamination and decarboxylation of valine, isoleucine and leucine (Andries *et al*. 1987; Stritzler *et al*. 1992). Similar increases in the rumen concentrations of valerate, isovalerate and isobutyrate have been noted previously when RDP supplements have been fed to lactating cows (*e.g.* Stobbs *et al*. 1977; Santos *et al*. 1984; Johnson *et al*. 1994; Delagarde *et al*. 1997). BCVFA supplementation has been shown to stimulate growth of rumen bacteria, especially cellulolytic bacteria (Andries *et al*. 1987) resulting in increased rumen VFA concentration (Hemsley and Moir 1963). However, based on the lack of a treatment effect on the *in situ* digestion of rhodes grass hay in the current study, the increase in VFA concentration observed here would appear to be caused more by the deamination of additional dietary peptides as reported by Klusmeyer *et al* (1990) and Aldrich *et al*. (1993), than by an increase in forage carbohydrate digestion *per se*.

The decline in rumen AB:P ratio as CP intake increased has been reported previously (Johnson *et al*. 1994) and could be due to a number of factors in the current study. There was an unexpected decline in the intake of ADF with increasing dietary CP%. This may have promoted a change in rumen fermentation, decreasing the ratio of AB:P (Annison and Bickerstaffe 1974). Alternatively, Teather *et al*. (1980) observed increased growth rates of *Selenomonas* spp., a proportionate-producing species in the rumen, with an increase in rumen BCVFA concentration. Regardless of the mode of action, the increase in ATP yield from the end products of rumen fermentation with an increase in rumen propionate production as opposed to acetate is well documented (*e.g.* Hungate 1966; Leng 1982).

Intake and digestibility

As shown in the present study, increasing protein concentrations in diets of lactating cows usually result in an improvement in intake (SCA 1990). Poos *et al*. (1979) observed that the DMI of lactating cows increased by 11% when the CP concentration in a mixed diet was increased from 12% to 15% DM by adding soyabean meal. In the present study, OMI increased by approximately 12% when dietary CP was increased from 13% to 16%. It has been suggested that these positive responses in intake to protein supplementation are stimulated hormonally by a lactating cow's responses to an improved intestinal amino acid supply (Oldman 1984). This is unlikely in the current study, as the diets were formulated to the same concentration of UDP. In addition, MPS, as indicated by the allantoin:

creatinine ratio in urine, was not increased as dietary protein concentration was raised.

Although the use of digestibility markers is subject to criticism, the use of inert rare earth elements such as lanthanum is well accepted in the literature (*e.g.* Kotb and Luckley 1972; Hartnell and Satter 1979a). Diurnal fluctuations in faecal marker concentrations have been observed; however, collecting faecal grab samples at least 10 h apart over 7 days compensates for this, resulting in digestibility coefficients comparable with using total faecal collection (Kotb and Luckley 1972).

The decline in digestibility as CP% rose is in contrast to previously observed improvements in digestibility when protein concentrations were increased in mixed diets fed to lactating cows (Poos *et al*. 1979; Oldman 1984). Some reductions in the digestibility would be expected as intakes increase, although in the current experiment, the magnitudes of these changes are greater than previously observed. Morgan and Stakelum (1987) calculated a reduction in OMD of 1.4 percentage units per unit increase in feeding level expressed as a multiple of maintenance (approximately 6–9 kg DM; NRC 1989) which is approximately equivalent to a decline of 0.15–0.25 percentage units of OMD per kg increase in DMI. Robinson *et al.* (1987) recorded a decrease in NDFD of approximately 1.0 percentage unit for every kg increase in DMI. These values compare with the 8.3 percentage units reduction in OMD and 13.4 percentage units reduction in NDFD per kg increase in DMI in the current study. This may indicate that the OMD and NDFD of diets based on rhodes grass fall rapidly with an increase in intake at high feeding levels. The exact reasons for this decline in digestibility in the current study are unclear as neither rate of digestion nor passage of hay differed between treatments. The decline in OMD with increasing OMI resulted in DOMI being similar between treatments.

Milk production

Recommended CP intake for a 467 kg cow producing 22 L/d of milk (3.5% butterfat) while maintaining weight is 2385 g/d (NRC 1989). The results of the current study agree with these recommendations in that milk yields were significantly lower when the diet contained 13% CP (CP intake of 2.1 kg/d), but were not significantly improved when dietary CP concentration was higher than 14% (CP intake of 2.4 kg/d). Raising dietary CP concentration from 13% to 14% resulted in a 7.4% increase in milk production, while raising CP above 14% gave only minor additional increases (*i.e.* 0.5% for 15% CP, 1.4% for 16% CP). The exact digestive and physiological reasons for this pattern of response remain unclear. The change in the proportions of end-products of rumen fermentation would appear to be a factor. If the hepatic uptake of gluconeogenic VFA (propionate, valerate and isovalerate) mirrored rumen concentrations, this could have increased the supply of precursors available for gluconeogenesis and increased glucose supply to the mammary gland promoting increased lactose synthesis (Kronfeld 1982). It would appear that, in the current study, when diets were formulated to 14% CP and above, lactose synthesis at the mammary gland no longer limited milk yield.

It is difficult to compare the findings of the current study with earlier grazing studies where lactating cows were supplemented with protein meals with high rumen degradabilities (*e.g.* Royal and Jeffery 1972; Stobbs *et al*. 1977; Moss *et al*. 1992). Pasture intakes were not reported in these studies, making it difficult to estimate dietary protein concentrations from the data presented.

The findings of this current study suggest that little of the milk production responses in previous grazing experiments were caused by an improved rumen digestibility of tropical grasses. As a consequence, improved MPS and hence improved microbial amino acid flows to the small intestine seem unlikely. Due to the design of the current study, no conclusions can be drawn regarding the effects on milk production of the UDP fraction of highly degradable protein supplements fed in previous studies. An increase in the energy content of the end-products of rumen fermentation may contribute to higher milk yields when highly rumen degradable protein supplements are fed, as energy intake is a major limitation to the milk yield of cows grazing tropical pastures (Reeves *et al.* 1996).

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