

Fish meal supplementation to early lactation Jersey cows grazing ryegrass pasture

E.R. Malleson^{1#}, R. Meeske², L.J. Erasmus¹, W.A. Van Niekerk¹ and R.J. Coertze¹

¹Department of Animal & Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa

²Department of Agriculture, Western Cape, Outeniqua Experimental Farm, P.O. Box 249, George 6530, South Africa

Abstract

This trial was conducted to test the hypothesis that early lactation cows grazing ryegrass pasture and receiving maize and mineral supplementation could respond to additional supplementation with a protein source such as fish meal. Multiparous Jersey cows in early to mid lactation that grazed annual ryegrass pasture in spring were used in a randomised complete block design experiment. In addition to the pasture, cows received 6 kg (as is) of a maize-based supplement, including minerals, fed in two equal portions in the milking parlour. Three groups of 15 cows received a control, a low fish meal or a high fish meal treatment (0, 4 or 8% fish meal replacing maize). Milk yield was measured and milk samples taken fortnightly. A simultaneous study on rumen fermentation was conducted using eight rumen cannulated cows receiving the control and high fish meal treatments in a cross-over design experiment. Ruminal pH and ammonia-N and volatile fatty acid concentrations were measured. Milk yield, 4% fat-corrected milk yield and milk fat and protein percentages of cows on the low and high fish meal treatments (21.9 and 22.1 kg milk/d, 24.1 and 24.2 kg 4% fat corrected milk/d, 4.73 and 4.67% fat and 3.49 and 3.45% protein) were higher than the control (20.5 kg milk/d, 20.4 kg 4% fat corrected milk/d, 3.97% fat and 3.25% protein). The ruminal ammonia-N concentration was higher in the cows on the high fish meal treatment than the control (16.7 vs. 14.2 mg/dL). Fish meal supplementation to cows on ryegrass proved to be profitable.

Keywords: Dairy cattle, cultivated pasture, RUP, maize, milk yield, milk composition

Corresponding author. E-mail: botha.evelyn19@gmail.com

Present address: P.O. Box 30, Silverton 0127, South Africa

Introduction

Many pasture-based dairy farmers in the Southern Cape region of South Africa supplement only maize and minerals to grazing dairy cows as this is seen to be the most economical (Rubin, R., Personal communication, jackrubin@mweb.co.za). Lush pasture, such as ryegrass, has a high content of crude protein (CP) that is highly degradable in the rumen (McCormick *et al.* 1999; 2001b; Bargo *et al.*, 2003). Metabolisable energy (ME) is the first limiting nutrient for cows grazing high quality pasture (Muller & Fales, 1998; Bargo *et al.*, 2002b; Kolver, 2003). However, at higher levels of supplementation and when milk production is high, specific amino acids (AA), particularly methionine (Met) and lysine (Lys), may limit milk production (Kolver *et al.*, 1998b; Muller & Fales, 1998; Kolver, 2003). For a high yielding cow, especially in early lactation, a smaller proportion of the protein is generally supplied by the rumen microbes and more needs to escape rumen degradation than would be the case with lower genetic merit cows (Hongerholt *et al.*, 1998; Santos *et al.*, 1998; Schroeder & Gagliostro, 2000).

Most previous studies, where cows were grazing on various types of pasture and were supplemented with concentrate, were conducted with Holstein cows. Increasing rumen-undegradable protein (RUP), or replacing rumen-degradable protein (RDP) sources with RUP sources, in concentrates has not had a consistent effect on milk production or composition (Carruthers *et al.*, 1997; Santos *et al.*, 1998; Bargo *et al.*, 2003). In several studies RDP sources such as soyabean meal, sunflower meal, urea or rapeseed meal have been replaced with RUP sources such as animal protein blend, fish meal (FM), maize gluten meal, expeller soyabean meal, blood meal, feather meal or heat-treated rapeseed meal (Bargo *et al.*, 2003). Lactation studies with cows grazing pasture that reported an increase in milk production were those of Schroeder & Gagliostro (2000) and Schor & Gagliostro (2001) where the milk response was 6 and 18%, respectively, above the control. (In the former study cows grazed a mixture of lucerne, red clover, orchardgrass and perennial ryegrass. In the latter study cows grazed a mixture of perennial ryegrass, red and white clover and

orchardgrass). Menhaden FM was the RUP source that most frequently increased milk yield compared to soyabean meal and is also ranked highest in essential amino acid (EAA) index, indicating that the type of RUP supplement (AA profile) is more important than the amount of RUP (Santos *et al.*, 1998). Positive responses to RUP supplementation, above that observed with energy, are most likely in early lactation, high yielding, multiparous cows, when high amounts of concentrate grain are fed (Hongerholt & Muller, 1998; Schor & Gagliostro, 2001).

Fish meal was used in this study as a quality protein source since it is recognised as an excellent source of RUP, rich in Lys and Met which are probably the first and second limiting AA for milk yield and milk protein synthesis (Rulquin & Vérité, 1993; Santos *et al.*, 1998; Schroeder & Gagliostro, 2000).

Pastures of kikuyu (*Pennisetum clandestinum*) over-sown with annual ryegrass (*Lolium multiflorum*) are common in the Southern Cape. The former species, adapted to hot climates, is active in summer, complementing the latter, which is used for winter grazing. This trial was conducted on annual ryegrass in spring. The milk yield potential of 500 kg cows grazing ryegrass pasture, with no supplementation, is 16 to 17 litres a day (Dugmore, 1995).

The aim of the study was to determine whether grazing cows that are receiving maize supplementation, with minerals included, would respond to the addition of a high quality protein source, such as FM, to their supplement.

Materials and Methods

Forty five multiparous Jersey cows [body weight, 331 ± 29.9 kg; milk yield, 21.4 ± 1.65 kg/d; parity, 4.1 ± 1.53 ; days in milk, 73 ± 28.3 ; (mean \pm s.d.)] from the Outeniqua Experimental Farm (Longitude $22^{\circ}25'$, latitude $33^{\circ}57'$, altitude 190 m), near George in the Southern Cape, South Africa, were used. The long-term (39 y) average rainfall in this area is 725 mm per annum. The mean daily maximum and minimum temperatures during the experimental period of the trial were 21 and 11 °C, respectively. The average milk production of the herd of 326 cows in lactation from which the cows were selected was 17.0 kg/d in August 2005. The higher producing, early to mid lactation, cows from the herd were selected for the trial.

A randomised complete block design was used. Just before the experimental period the cows were blocked according to milk production (of the previous 25 days) and days post calving and within each block were randomly divided into three groups. These three groups were randomly allocated to three experimental treatments.

The cows strip-grazed annual Westerwold ryegrass (*Lolium multiflorum*, Energa cv, fertilised with 56 kg N (limestone ammonium nitrate) per ha after each grazing, and were moved to a new strip twice a day after each milking. The cows were milked at 06:00 and 14:30 in a 20 point Dairy Master (Total Pipeline Industries, 33 Van Riebeeck St., P.O. Box 252, Heidelberg 6665, RSA) swing over milking machine with weigh-all electronic milk meters. The cows grazed all hours (except for the milking times) and clean drinking water was available *ad libitum*. All the cows were grazed together as a single herd to ensure equal pasture allocation. The mean daily pre-grazing pasture allowance was 11 kg dry matter (DM) per cow per day above 3 cm pasture height (based on previous experience with pasture management of the herd), measured with a rising plate meter (Filip's folding plate pasture meter, Jenquip, Rd 5, Fielding, New Zealand). The rising plate meter was calibrated to the pasture used in the trial by measuring three low, medium and high pasture heights and cutting the grass under the plate to 3 cm above the ground. The samples were dried at 60 °C for 72 hours. The linear regression equation, $Y = 62 H - 57$ ($R^2 = 0.4$; $n = 90$) was fitted to the data, where Y is pasture yield (kg DM/ha) and H = pasture height on the rising plate meter.

The average pasture height, before and after grazing, of each pasture strip was measured by taking 100 rising plate meter readings. The post grazing pasture height was used to estimate the pasture intake at 7.6 kg DM/cow/d. This, however, must have been an underestimate as the cows would not likely have been able to produce the amount of milk they did with this pasture intake (based on modelling with CPM Dairy (Version 3.0.7a; Cornell University, Ithaca, NY, University of Pennsylvania, Philadelphia, PA; Willam H. Miner Agricultural Institute, Chazy, NY)). The mean pasture intake of the cows was assumed to be 8.6 kg pasture DM per day, based on the assumption that cows on pasture-concentrate can consume 1.3% of body weight (BW) as neutral detergent fibre (NDF) (Vazquez & Smith, 2000; Bargo *et al.*, 2002b). The expected NDF intake of the cows was calculated as 1.3% of the average BW of all the cows between the beginning and end

of the trial, the NDF consumed in the concentrate subtracted, and then the pasture dry matter intake (DMI) calculated based on the known NDF content of the pasture.

In addition to the pasture the cows each received 6 kg (as is, 5.5 kg DM) of pelleted concentrate per day, divided into two equal portions and fed in the milking parlour. The cows in the three groups each received a different concentrate (Table 1). The pellets of the cows on the control treatment contained no FM. For the two FM treatments some of the maize was replaced by FM: 4% (240 g FM/cow/d) for the low FM treatment and 8% (480 g FM/cow/d) for the high FM treatment. Table 2 shows the chemical composition of the fish meal used. The diets were formulated to be iso-energetic. Megalac (a rumen protected fat; Church & Dwight Co., Inc., 469 N. Harrison St., Princeton, NJ 08543-5297) was added to the latter two treatments to bring the energy to the same level in all three concentrates. The CP concentrations of the diets differed since it is the effect of additional protein that was to be investigated. The molasses was added to facilitate pelleting which was done to increase the palatability.

Table 1 Ingredient composition (% DM) of the concentrate pellets used for the three experimental treatments

Ingredient	Control	Low fish meal	High fish meal
Maize meal	88.75	84.1	78.5
Fish meal (FM)	0	4.0	8
Megalac ¹	0	0.65	1.3
Molasses	6.8	6.8	6.8
MonoCaP	1.3	1.3	1.3
Feed lime	1.8	1.8	1.8
Salt	0.5	0.5	0.5
MgO	0.5	0.5	0.5
Premix ²	0.35	0.35	0.35

¹ Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ).

² Premix (Lactating Cow (Organic); DSM Nutritional Products South Africa (Pty) Ltd.) contained 7.23% Mn, 7.50% Zn, 1.83% Cu, 0.11% Co, 0.14% I, 0.03% Se (1%), 1.28% organic Mn, 2% organic Zn, 0.32% organic Cu, 0.01% organic Se, 5% Rumensin (20%), 3.5% Stafac 500 and provided 96 250 IU of vitamin A, 28 875 IU of vitamin D3, and 577.5 mg of vitamin E/cow/d.

Table 2 Chemical composition of the fish meal used in the concentrates

Ingredient	Control
Dry matter (g/kg as is)	92.3 ^a
Crude protein (g/kg DM)	71.5 ^a
Organic matter (g/kg DM)	84.2 ^a
Fat (g/kg DM)	11.8 ^a
Rumen-degradable protein (RDP, %CP)	38.5 ^a
Rumen-undegradable protein (RUP, %CP)	61.5 ^a
Lysine (g/kg DM)	4.78 ^b
Methionine (g/kg DM)	1.54 ^b

^a Oceana Group Ltd, 7 Coen Steytlen Ave, Cape Town, 8001.

^b PICOTag method (Bidlingmeyer *et al.*, 1984).

Representative samples of pasture (cut to 3 cm above the ground as the cows grazed to almost this level) and concentrate (there were negligible feed refusals) were taken weekly and milled through a 1 mm screen. These were analysed at the UP-Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria) for DM (AOAC, 2000, procedure 934.01), ash (AOAC, 2000, procedure 942.05), CP (N was determined using a Leco N analyser, model FP-428, Leco Corporation, St Joseph, MI, USA and CP was calculated as $N \times 6.25$), NDF (Robertson & Van Soest, 1981), acid detergent fibre (ADF; Goering & Van Soest, 1970), *in vitro* organic matter digestibility (IVOMD; Tilley & Terry, 1963 as modified by Engels & Van der Merwe, 1967). Every three pasture samples were composited and these, along with the concentrate samples, were analysed for gross energy (GE; MC – 1000 Modular Calorimeter, Operators Manual), ether extract (EE; crude fat; AOAC 2000, procedure 920.39), Ca (AOAC 2000, procedure 965.09), P (AOAC 2000, procedure 965.17) and AA composition (with the PICOTag method (Bidlingmeyer *et al.*, 1984) using a Waters HPLC with two Model 510 pumps, UV protector Model 440, autosampler Model 712 and Waters Millennium 32 software). Metabolisable energy was calculated with the following formula: ME (MJ/kg DM) = $0.82 \times (GE \times IVOMD)$ (Robinson *et al.*, 2004).

After an adaptation period of 12 days, milk yield was measured in the milking parlour for 46 days. Composite milk samples (ratio 9 mL : 15 mL afternoon : morning milking) were taken every second week and analysed for fat, protein, lactose and milk urea nitrogen (MUN) at Lactolab Pty (Ltd) (ARC, Main rd., Irene, 0062) using the Milkoscan FT 6000 (Foss Electric, Denmark). Fat-corrected milk (FCM) yield was calculated using the following formula: 4% FCM = $0.4 \times \text{kg milk} + 15 \times \text{kg milk fat}$ (NRC, 2001).

At both the beginning and end of the trial the cows were weighed on two consecutive days and the mean of the body weights recorded on these two days was determined. Body condition score (BCS) was determined on the first of these two days using the five point system where 1 is thin and 5 is fat (Wildman *et al.*, 1982).

Concurrent with the milk production study changes in rumen fermentation patterns of cows were determined by using eight Jersey cows fitted with rumen cannulae (with rolled inner flange 10 cm in diameter; Bar Diamond, Inc., P.O. Box 60, Parma, Idaho, USA). These cows grazed, were milked and received concentrate with the cows of the production study. A cross-over design was used. Four of the cows (chosen at random) received the control treatment and four of them the high FM treatment. After an adaptation period of 19 days, the cows were fitted with automated pH meters with data loggers (WTW pH 340i pH meter/ data logger with a WTW SenTix 41 pH electrode) so that the ruminal pH at 10 min intervals throughout the day could be monitored. The electrode was placed in the rumen via the cannula and connected to the data logger that was strapped on like a saddle. The pH in the rumen of each cow was monitored for a total of four days. Samples of ruminal fluid were taken over a period of three days, with at least eight hours between two consecutive sampling times, such that in the end there were samples representing every four hours of the day (00:00, 04:00, 08:00, 12:00, 16:00 and 20:00). From each sample 30 mL of rumen filtrate was preserved with 5 mL 50% H₂SO₄ and frozen for ammonia-N (NH₃-N) analysis (De Bruin, 1995) and 20 mL of rumen filtrate was preserved with 4 mL of 25% H₃PO₄ and frozen for volatile fatty acid (VFA) analysis (Beauchemin *et al.*, 2003). These were analysed for NH₃-N (Broderick & Kang, 1980) and VFA (acetic, propionic, butyric, iso-butyric and valeric acids; Webb, 1994, with modifications) at the UP-Nutrilab. On day 30 of the trial the cows were switched to the opposite experimental treatment (those that were on the control treatment received the high FM treatment and *vice versa*) so that in the end each cow received both treatments and there were eight cows per treatment.

An analysis of variance with the ANOVA model (SAS, 2001) was used to determine the difference between the experimental treatments in terms of milk yield and composition, FCM, change in BW and BCS and mean daily ruminal pH, NH₃-N and VFA. Cows were used as replicates. Significance of difference was determined using Duncan's test (Samuels, 1989). Difference was considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. Tendency was indicated at $P \leq 0.10$.

Results and Discussion

The chemical composition of the pasture (Table 3) was in the range expected for annual ryegrass (Fulkerson *et al.*, 1998; 2005; McCormick *et al.*, 2001b; Marais *et al.*, 2003; Granzin, 2004; Meeske *et al.*, 2006). The main difference between the three experimental treatments was the CP concentration of the supplements: 82, 112 and 146 g/kg for the control, low FM and high FM treatments, respectively (Table 3).

Although the EE increased slightly with the inclusion of FM, the ME of the three concentrates was similar. There is no clear explanation as to why the IVOMD, and hence ME, was higher in the low FM concentrate than the other two.

The total diets of the cows on all three treatments were adequate in all the main nutrients (assuming an average pasture intake of 8.6 kg DM/cow/d). The CP (192, 203 and 216 g/kg DM for the control, low FM and high FM diets, respectively) was adequate for a production of more than 20 kg milk a day (NRC, 2001). Including FM in the concentrate increased both RDP and RUP in the diet, based on the assumption that the RUP of the maize was 43% of CP (NRC, 2001) and the RUP of the FM was 62% of CP (Table 2). Each successive FM level would have supplied an additional 90 g RUP/d to diet. The diets contained 6.7 g Lys/kg DM and 0.7 g Met/kg DM (94 g Lys and 10 g Met per day) for the control treatment, 7.4 g Lys/kg DM and 1.1 g Met/kg DM (105 g Lys and 16 g Met per day) for the low FM treatment and 7.8 g Lys/kg DM and 1.8 g Met/kg DM (111 g Lys and 25 g Met per day) for the high FM treatment. Thus, both Lys and Met increased as the level of FM in the concentrate increased, especially Met. The high FM treatment comes closest to the ideal Lys : Met ratio of 3.0 : 1 (NRC, 2001).

Table 3 Chemical composition (mean ± s.d.) of the concentrate fed and the ryegrass pasture grazed by the cows during the trial

Nutrient ¹	Concentrate ³			Pasture
	Control	Low FM	High FM	
DM (g/kg as is)	919	915	914	137 ± 36.0 ⁴
ME (MJ/kg DM) ²	12.9	13.3	12.9	11.3 ± 0.42 ⁵
OM (g/kg DM)	932	912	904	866 ± 14.4 ⁴
CP (g/kg DM)	82	112	146	262 ± 32.3 ⁴
NDF(g/kg DM)	112	118	127	463 ± 32.3 ⁴
ADF(g/kg DM)	37	37	40	256 ± 14.6 ⁴
IVOMD (g/kg DM)	924	951	913	802 ± 33.4 ⁴
EE (g/kg DM)	17	23	30	32 ± 4.5 ⁵
Ca (g/kg DM)	15.5	20.3	23.0	5.2 ± 1.03 ⁵
P (g/kg DM)	5.6	7.5	8.7	4.1 ± 0.26 ⁵
Ca : P	2.77 : 1	2.71 : 1	2.64 : 1	1.28 : 1 ± 0.214 ⁵

¹ DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility; EE – Ether extract.

² ME = 0.82 x (GE x IVOMD) (Robinson *et al.*, 2004).

³ n = 1; ⁴ n = 9; ⁵ n = 3.

The effect of including different levels of FM in the diets of Jersey cows is presented in Table 4. The cows on the low FM treatment responded by producing 7% more milk, yielding 28% more milk fat, yielding 13% more milk protein and producing 18% more 4% FCM than the cows on the control treatment. Milk yield, 4% FCM yield and milk fat, protein and lactose percentages of cows on the low and high FM treatments were higher than the control (P < 0.01). There was no additional benefit to the higher level of FM as the two FM treatments did not differ from each other in terms of the above parameters (P > 0.10).

In the review of Bargo *et al.* (2003) it was stated that there is an average increase in milk production of 0.8 kg/d for each 100 g/d of RUP. Since the low FM diet supplied an estimated additional 90 g RUP/d, the milk response was approximately 1.5 kg milk per 100 g additional RUP supplementation, almost double the

level reported by Bargo *et al.* (2003). This could be related to the excellent AA profile of FM since the AA profile of the RUP supplement is more important than the amount of RUP (Santos *et al.*, 1998).

Since all the cows grazed the same pasture and similar amounts of ME were supplied by the respective concentrates, the cows on the three experimental treatments must have had a similar total ME supply in which case the ME must have been adequate for the higher level of production. Hence ME was not limiting for the cows on the control treatment indicating that another nutrient, such as CP, RUP or AA, could have been limiting, hence the response. The lack of response to the higher level of FM was probably because ME once again became the first limiting nutrient.

Apart from the effect of increased CP, the milk response of the cows on the two FM treatments over the control could be due to the increased supply of EAA, especially Lys and Met, to the small intestine. Santos *et al.* (1998) stated that FM consistently increased the proportion of Lys in the EAA flowing to the duodenum when supplied at greater than 4% of diet DM but not if less than 4%, and brought the ratio of Lys to Met, as % EAA, at the duodenum close to the recommended level. Xu *et al.* (1998) found increased milk production (39 vs. 34 kg/d) when cows were fed a blend of blood meal, FM and meat and bone meal as an AA source or ruminally protected Lys and Met compared to maize distillers grains as a control. Robinson *et al.* (1995) and Wu *et al.* (1997) also found increased milk yield with supplementation of ruminally stable Met and Lys while there have also been cases where responses were small and inconsistent (Rulquin *et al.*, 1993). Supplementing FM (vs. solvent soyabean meal) increased milk yield and 3.5% FCM yield in the trial

Table 4 Effect of fish meal (FM) supplementation on mean milk yield, milk composition, body weight and body condition score¹ of cows consuming an estimated 8.6 kg ryegrass pasture DM and 5.5 kg supplement DM/d (n = 15)

Parameter	Experimental treatment ²			s.e.m. ³
	Control	Low FM	High FM	
Milk yield (kg/d)	20.5 ^a	21.9 ^b	22.1 ^b	0.34
4% FCM ⁴ (kg/d)	20.4 ^a	24.1 ^b	24.2 ^b	0.47
Fat (%)	3.97 ^a	4.73 ^b	4.67 ^b	0.132
Fat yield (kg/d)	0.81 ^a	1.03 ^b	1.03 ^b	0.028
Protein (%)	3.25 ^a	3.49 ^b	3.45 ^b	0.051
Protein yield (kg/d)	0.67 ^a	0.76 ^b	0.76 ^b	0.014
Lactose (%)	4.59 ^a	4.78 ^b	4.79 ^b	0.019
MUN ⁵ (mg/dL)	16.80	17.43	17.93	0.440
BW ⁶ beginning (kg)	327	338	327	6.2
BW end (kg)	371	387	369	7.6
BW change (kg)	+44	+49	+42	3.7
BCS ⁷ beginning	2.1	2.1	2.2	0.06
BCS end	2.5	2.3	2.4	0.07
BCS change	+0.4 ^a	+0.2 ^{ab}	+0.2 ^b	0.06

¹ Five-point system where 1 is thin and 5 is fat (Wildman *et al.*, 1982).

² Experimental treatment: Control = supplement containing no fish meal (FM); Low FM = supplement containing 4% FM; High FM = supplement containing 8% FM.

³ Standard error of mean.

⁴ FCM – 4% fat-corrected milk; ⁵ MUN – Milk urea N; ⁶ BW – body weight; ⁷ BCS – body condition score.

^{a,b} Means in the same row with different superscripts differ (P < 0.05).

of Broderick (1992) where cows were fed lucerne silage. In the trial of MacDonald *et al.* (1998), where cows grazing pasture and receiving maize silage supplementation were supplemented with urea, SBM, or FM, the response was greater and more consistent for the cows receiving FM than SBM and there was no response to urea. The RUP supply was also greater from the FM than the SBM and zero from the urea, while the RDP supply was greatest from the SBM.

There is also a possibility that pasture intake was higher in the cows on the fish meal treatments, as predicted by the CPM Dairy model (Version 3.0.7a; Cornell University, Ithaca, NY, University of Pennsylvania, Philadelphia, PA; Willam H. Miner Agricultural Institute, Chazy, NY). This would have contributed to the higher milk production as well as being driven by the higher production since cows appear to consume feed to meet their energy demands (NRC, 2001). Some other studies also reported DMI and pasture intake for cows receiving RUP supplementation (Donaldson *et al.*, 1991; Schor & Gagliostro, 2001) while other studies reported no difference in DMI (Santos *et al.*, 1998; Bargo *et al.*, 2003).

The response in milk fat and protein percentages may be partly due to the increased CP in the diet. In the study by McCormick *et al.* (2001a) milk fat (3.34 vs. 3.11%) and protein (3.42 vs. 3.27%) percentages were increased when Holstein cows grazing annual ryegrass-oat pastures were fed a high CP supplement (22.8% CP) vs. a moderate CP supplement (16.6% CP), while RUP sources (maize gluten meal-blood meal mixture) had no effect. The response could also be attributed to the increased flow of EAA to the small intestine as this is known to increase milk protein yield (Rulquin & Vérité, 1993). Supplementation with a protein source rich in EAA increases milk protein yield especially when maize (low in Lys) is fed and even with pasture of high N content (Rulquin & Vérité, 1993). Robinson *et al.* (1995) and Xu *et al.* (1998) found supplementing rumen protected Lys and Met to increase milk fat and protein percentages and yield. Increased milk protein concentration and yield have been the most consistent response to supplementing ruminally protected Met and Lys (Rulquin *et al.*, 1993; Robinson *et al.*, 1995; 1998; 1999). Supplementing FM (vs. solvent soyabean meal) increased milk protein percentage and yield in the trial of Broderick (1992). Some studies have shown no effect on milk fat (Robinson *et al.*, 1999) or even a tendency to drop (Rulquin *et al.*, 1993). Schor & Gagliostro (2001) found no effect of blood meal supplementation on milk fat concentration. Feeding FM could reduce milk fat percentage mainly due to high concentrations of unsaturated long-chain fatty acids in FM or a reduction in acetate : propionate ratio in ruminal fluid negatively affecting milk fat (Schroeder & Gagliostro, 2000). There was, however, no difference in ruminal acetate : propionate ratio between the control and high FM treatments in the present study (Table 5). The possibility that the Megalac could have contributed to the milk fat response cannot be ruled out.

The lactose response is in agreement with the results of Tesfa *et al.* (1995) with cows grazing a mixture of meadow fescue, timothy and red clover. The milk lactose was lower in cows supplemented with a cereal by-product based concentrate (12.4% CP) than in cows given additional N, in the form of urea or rapeseed meal (non-heat treated or heat treated), in the concentrates (15.0 to 15.6% CP). Robinson *et al.* (1995) found increased milk lactose when ruminally protected Lys and Met were fed. There is, however, no biological explanation as to why the lactose percentage should have increased.

There was no treatment effect on MUN ($P > 0.10$; Table 4). The values were within the range of 12 to 18 mg/dL, suggested by Linn & Olsen (1995) and De Villiers *et al.* (2000) as indicative of a balanced ration and still below 20 mg/dL where reproductive performance of the cow could start being negatively affected (De Villiers *et al.*, 2000).

There was no effect on change in BW ($P > 0.10$). The cows on the control treatment had a higher increase in BCS than the cows on the high FM treatment ($P < 0.05$; Table 4). This could be because absorbed protein may induce mobilisation of body fat (Schor & Gagliostro, 2001). In the study of Schroeder & Gagliostro (2000) body fat mobilisation was possibly enhanced by RUP feeding. There was no difference between treatments in changes in BCS or BW in the study by Jones-Endsley *et al.* (1997) where the amount of CP in the concentrate was increased or in the study by Hongerholt & Muller (1998) where the RUP in the concentrate was increased.

The mean daily ruminal pH (Table 5) did not differ between the control and high FM treatments ($P > 0.10$), as was expected since changing the level and source of protein did not affect ruminal pH in previous studies (Carruthers & Neil, 1997; Jones-Endsley *et al.*, 1997; Bargo *et al.*, 2001; 2003). Although the pH varied throughout the day, it was never below 5.8, the level at which cows start experiencing sub-clinical acidosis (Graf *et al.*, 2005).

Table 5 Effect of fish meal (FM) supplementation on mean daily ruminal pH, ammonia-nitrogen (NH₃-N) and volatile fatty acid (VFA) concentrations of cows consuming an estimated 8.6 kg ryegrass pasture dry matter (DM) and 5.5 kg supplement DM/d (n = 8)

Parameter	Experimental treatment ¹		s.e.m. ²
	Control	High FM	
pH	6.14	6.08	0.022
NH ₃ -N (mg/dL)	14.2 ^a	16.7 ^b	0.405
Total VFA (mmol/L)	123.4 ^b	115.3 ^a	0.67
Acetate (mol/100 mol)	66.1	65.4	0.377
Propionate (mol/100 mol)	18.5	18.5	0.308
Butyrate (mol/100 mol)	13.5	14.2	0.183
Acetate : propionate	3.61 : 1	3.56 : 1	0.080 : 1

¹ Experimental treatments: Control = supplement containing no fish meal (FM); High FM = supplement containing 8% FM.

² s.e.m. - standard error of mean.

^{a, b} Means in the same row with different superscripts differ (P <0.05).

The mean daily ruminal NH₃-N concentration was higher for the cows on the high FM treatment than the control (P <0.05), both well above the minimum level of 5 mg/dL for maximum microbial protein synthesis (Satter & Slyter, 1974) and within the range expected for cows on pasture-concentrate diets (Bargo *et al.*, 2003). The higher ruminal NH₃-N concentration for the cows on the high FM treatment is to be expected due to the higher CP of the diet. Previous studies (Carruthers *et al.*, 1997; Jones-Endsley *et al.*, 1997; Bargo *et al.*, 2001) reported higher NH₃-N concentrations when the concentrate contained more CP. The ruminal NH₃-N, as well as the MUN, levels indicate that RDP was adequate, hence the deduction that the milk response may have been linked to RUP.

The VFA levels were in the expected range for cows on a pasture-concentrate feeding system (Kolver *et al.*, 1998a; Schor & Gagliostro, 2001; Bargo *et al.*, 2002a; 2002c; 2003). The concentration of total VFA in the ruminal fluid was higher for cows on the control treatment than the high FM treatment (P <0.05). There was no treatment effect on molar proportions (mol/100 mol total VFA) of acetate, propionate or butyrate or on the acetate : propionate ratio (P >0.10) although butyrate tended to be higher for the cows on the high FM treatment (P <0.10). The acetate concentration (81.5 and 75.4 mmol/L) was higher in the control cows (P <0.05). Differing the level and source of protein in the concentrate has been reported not to affect the total VFA or molar proportions of individual VFA (Bargo *et al.*, 2001; Schor & Gagliostro, 2001).

These results indicate that cows grazing ryegrass and receiving maize and mineral supplementation respond in terms of milk yield and composition to up to 240 g FM a day, above which there is no additional response, indicating that energy might become the first limiting nutrient again.

In order for FM inclusion in the supplement to be profitable the additional revenue from the milk response would need to be greater than the additional feed cost. This was found to be the case during this trial illustrating that fish meal supplementation to cows on ryegrass can be profitable even though FM is expensive. The amount of additional profit made depends on the magnitude of the production response and the milk price.

Conclusions

High producing, multiparous Jersey cows in early to mid lactation grazing annual ryegrass pasture and receiving 6 kg as is (5.5 kg DM) a day of maize-based supplement, respond to the inclusion of FM in their supplement up to 240 g (as is) FM per day, above which there is no additional response. At this level of FM cows responded by producing 18% more FCM than control cows. This response can lead to increased profit. Additional protein needs to be included in the supplement, preferably of high quality (low degradability and

good AA composition) to complement the highly degradable protein of the pasture. Future research could investigate other levels of FM, in the region of 240 g FM per day, to establish the optimal level. Further research should also be conducted using different breeds as well as different stages of lactation.

Acknowledgements

The authors would like to thank all the people who helped with the execution of the project including the staff of the Outeniqua Experimental Farm, George and the UP-Nutrilab, University of Pretoria, as well as the Western Cape Animal Production Research Trust and Western Cape Department of Agriculture for funding the project.

References

- AOAC, 2000. Official methods of analysis (17th ed.). Volume I. Association of Official Analytical Chemists, Inc. Maryland, USA.
- Bargo, F., Rearte, D.H., Santini, F.J. & Muller, L.D., 2001. Ruminant digestion by dairy cows grazing winter oats pasture supplemented with different levels and sources of protein. *J. Dairy Sci.* 84, 2260-2272.
- Bargo, F., Muller, L.D., Delahoy, J.E. & Cassidy, T.W., 2002a. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. *J. Dairy Sci.* 85, 1777-1792.
- Bargo, F., Muller, L.D., Delahoy, J.E. & Cassidy, T.W., 2002b. Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *J. Dairy Sci.* 85, 2948-2963.
- Bargo, F., Muller, L.D., Varga, G.A., Delahoy, J.E. & Cassidy, T.W., 2002c. Ruminant digestion and fermentation of high-producing dairy cows with three different feeding systems combining pasture and total mixed rations. *J. Dairy Sci.* 85, 2964-2973.
- Bargo, F., Muller, L.D., Kolver, E.S. & Delahoy, J.E., 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. *J. Dairy Sci.* 86, 1-42.
- Beauchemin, K.A., Yang, W.Z., & Rode, L.M., 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. *J. Dairy Sci.* 86, 630-643.
- Bidlingmeyer, A.B., Cohen, S.A. & Tarvin, T.L., 1984. Rapid analysis of amino acids using pre-column derivatization. *J. Chrom.* 336, 93-104.
- Broderick, G.A., 1992. Relative value of fish meal versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. *J. Dairy Sci.* 75, 174-183.
- Broderick, G.A. & Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63, 64-75.
- Carruthers, V.R. & Neil, P.G., 1997. Milk production and ruminal metabolites from cows offered two pasture diets supplemented with non-structural carbohydrates. *N. Z. J. Agric. Res.* 40, 513-521.
- Carruthers, V.R., Neil, P.G. & Dalley, D.E., 1997. Effect of altering the non-structural: structural carbohydrate ratio in a pasture diet on milk production and ruminal metabolites in cows in early and late lactation. *Anim. Sci.* 64, 393-402.
- De Bruyn, T.D., 1995. The nutritional value of bana grass, greengold and pennaris for sheep. MSc (Agric) dissertation, University of Pretoria, South Africa.
- De Villiers, P., Petzer, I.M. & Erasmus, L.J., 2000. The use of milk recording information as a herd management tool. Chapter 8 in Dairy herd improvement in South Africa. Livestock business division, Irene, 0062. pp. 53-64.
- Donaldson, R.S., McCann, M.A., Amos, H.E. & Hoveland, C.S., 1991. Protein and fiber digestion by steers grazing winter annuals and supplemented with ruminant escape protein. *J. Anim. Sci.* 69, 3067-3071.
- Dugmore, T.J., 1995. Characteristics of common roughages for dairy cows in Kwazulu-Natal. In: Dairying in Kwazulu-Natal. Kwazulu-Natal Department of Agriculture, Private Bag X9059, Pietermaritzburg, 3200, South Africa. pp 157-162.
- Engels, E.A.N. & Van der Merwe, F.J., 1967. Application of an *in vitro* technique to South African forages with special reference to affect certain factors on the results. *S. Afr. J. Agric. Sci.* 10, 983-995.
- Erasmus, L.J., Smith, W.A. & Cronje, P.B., 2000. Feeding the lactating dairy cow to express its genetic potential. Chapter 9 in Dairy herd improvement in South Africa. Livestock Business Division, Irene 0062, South Africa. pp. 67-79.

- Fulkerson, W.J., Slack, K., Hennessy, D.W. & Hough, G.M., 1998. Nutrients in ryegrass (*Lolium* spp.), white clover (*Trifolium repens*) and kikuyu (*Pennisetum clandestinum*) pastures in relation to season and stage of regrowth in a subtropical environment. *Aust. J. Exp. Agric.* 38, 227-240.
- Fulkerson, W.J., McKean, K., Nandra, K.S. & Barchia, I.M., 2005. Benefits of accurately allocating feed on a daily basis to dairy cows grazing pasture. *Aust. J. Exp. Agric.* 45, 331-336.
- Gehman, A.M., Bertrand, J.A, Jenkins, T.C. & Pinkerton, B.W., 2006. The effect of carbohydrate source on nitrogen capture in dairy cows on pasture. *J. Dairy Sci.* 89, 2659-2667.
- Goering, H.K. & Van Soest, P.J., 1970. Forage Fibre Analysis (Apparatus, reagents, procedure and some applications). Agricultural Handbook No. 379. A.R.S., U.S. Dept. of Agric., USA.
- Graf, C.M., Kreuzer, M. & Dohme, F., 2005. Effects of supplemental hay and corn silage versus full-time grazing on ruminal pH and chewing activity of dairy cows. *J. Dairy Sci.* 88, 711-725.
- Granzin, B.C., 2004. Effects of supplement grain type and level of feeding on the milk production of early-lactation Holstein-Friesian cows grazing temperate ad tropical pastures. *Aust. J. Exp. Agric.* 44, 735-743.
- Hongerholt, D.D. & Muller, L.D., 1998. Supplementation of rumen-undegradable protein to the diets of early lactation Holstein cows on grass pasture. *J. Dairy Sci.* 81, 2204-2214.
- Hongerholt, D.D., Varga, G.A. & Muller, L.D., 1998. Effects of feeding dried grass pasture and a grain ration differing in rumen undegradable protein at two feeding frequencies on nutrient digestibility and microbial protein synthesis during continuous culture. *Anim. Feed Sci. Technol.* 74, 1-13.
- Jones-Endsley, J.M., Cecava, M.J. & Johnson, T.R., 1997. Effects of dietary supplementation on nutrient digestion and the milk yield of intensively grazed lactating dairy cows. *J. Dairy Sci.* 80, 3283-3292.
- Kolver, E.S., 2003. Nutritional limitations to increased production on pasture-based systems. *Proc. Nutr. Soc.* 62, 291-300.
- Kolver, E.S., Muller, L.D., Varga, G.A. & Cassidy, T.J., 1998a. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *J. Dairy Sci.* 81, 2017-2028.
- Kolver, E.S., Muller, L.D., Barry, M.C. & Penno, J.W., 1998b. Evaluation and application of the Cornell Net Carbohydrate and Protein System for Dairy Cows fed diets based on pasture. *J. Dairy Sci.* 81, 2029-2039.
- Linn, J.G. & Olsen, J.D., 1995. Using milk urea nitrogen to evaluate diets and reproductive performance of dairy cattle. Proceedings of 4-State Applied Nutritional and Management Conference. Minnesota Extension, Dept. Anim Science, Univ. Minnesota, St. Paul MN. pp. 155-167.
- MacDonald, K.A., Penno, J.W., Kolver, E.S., Carter, W.A. & Lancaster, J.A., 1998. Balancing pasture and maize silage diets for dairy cows using urea, soybean meal or fishmeal. Proceedings of the New Zealand Society of Animal Production 58, 102-105.
- Marais, J.P., Goodenough, D.C.W., de Figueiredo, M. & Hopkins, C., 2003. The development of a *Lolium multiflorum* cultivar with a low moisture content and an increased readily digestible energy to protein ratio. *Aust. J. Agric. Res.* 54, 101-106.
- McCormick, M.E., French, D.D., Brown, T.F., Cuomo, G.J., Chapa, A.M., Fernandez, J.M., Beatty, J.F. & Blouin, D.C., 1999. Crude protein and rumen undegradable protein effects on reproduction and lactation performance of Holstein cows. *J. Dairy Sci.* 82, 2697-2708.
- McCormick, M.E., Ward, J.D., Redfearn, D.D., French, D.D., Blouin, D.C., Chapa, A.M. & Fernandez, J.M., 2001a. Supplemental dietary protein for grazing dairy cows: effect on pasture intake and lactation performance. *J. Dairy Sci.* 84, 896-907.
- McCormick, M.E., Redfearn, D.D., Ward, J.D. & Blouin, D.C., 2001b. Effect of protein source and soluble carbohydrate addition on rumen fermentation and lactation performance of Holstein cows. *J. Dairy Sci.* 84, 1686-1697.
- Meeske, R., Rothauge, A., Van der Merwe, G.D. & Greyling, J.F., 2006. The effect of concentrate supplementation on the productivity of grazing Jersey cows on a pasture based system. *S. Afr. J. Anim. Sci.* 36, 105-110.
- Muller, L.D. & Fales, S.L., 1998. Supplementation of cool-season grass pastures for dairy cattle. In: Grass for Dairy Cattle. Eds Cherney, J.H. & Cherney, D.J.R., CAB International, Oxon, UK. pp. 335-350.
- NRC, 2001. Nutrient Requirements of Dairy Cattle (7th rev. ed.). National Academy Press, Washington, D.C., USA.

- Robertson, J.B. & Van Soest, P.J., 1981. The analysis of dietary fiber in food. Eds. James, W.P.T. & Theander, O., Dekker, New York, USA.
- Robinson, P.H., Fredeen, A.H., Chalupa, W., Julien, W.E., Sato, H., Fujieda, T. & Suzuki, H., 1995. Ruminally protected lysine and methionine for lactating dairy cows fed a diet designed to meet requirements for microbial and postruminal protein. *J. Dairy Sci.* 78, 582-594.
- Robinson, P.H., Chalupa, W., Sniffen, C.J., Julien, W.E., Sato, H., Watanabe, K., Fujieda, T. & Suzuki, H., 1998. Ruminally protected lysine or lysine and methionine for lactating dairy cows fed a ration designed to meet requirements for microbial and postruminal protein. *J. Dairy Sci.* 81, 1364-1373.
- Robinson, P.H., Chalupa, W., Sniffen, C.J., Julien, W.E., Sato, H., Fujieda, T., Watanabe, K. & Suzuki, H., 1999. Influence of postruminal supplementation of methionine and lysine, isoleucine, or all three amino acids on intake and chewing behavior, ruminal fermentation, and milk and milk component production. *J. Anim. Sci.* 77, 2781-2792.
- Robinson, P.H., Givens, D.I. & Getachew, G., 2004. Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolisable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and *in vitro* determinations. *Anim. Feed Sci. Technol.* 114, 75-90.
- Rulquin, H. & Vérité, R., 1993. Amino acid nutrition of dairy cows: productive effects and animal requirements. In: *Recent Advances in Animal Nutrition*. Eds Garnsworthy, P.C. & Cole, D.J.A., Nottingham University Press, UK. pp. 55-77.
- Rulquin, H., Pisulewski, P.M., Vérité, R. & Guinard, J., 1993. Milk production and composition as a function of postruminal lysine and methionine supply: a nutrient-response approach. *Livest. Prod. Sci.* 37, 69-90.
- Samuels, M.L., 1989. *Statistics for the Life Sciences*. Collier MacMillan Publishers, London, UK.
- Santos, F.A.P., Santos, J.E.P., Theurer, C.B. & Huber, J.T., 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. *J. Dairy Sci.* 81, 3182-3213.
- SAS, 2001. *Statistical Analysis Systems user's guide: Statistics Version 8*. SAS Institute Inc. Cary, N.C., USA.
- Satter, L.D. & Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.* 32, 199-208.
- Schor, A. & Gagliostro, G.A., 2001. Undegradable protein supplementation to early-lactation dairy cows in grazing conditions. *J. Dairy Sci.* 84, 1597-1606.
- Schroeder, G.F. & Gagliostro, G.A., 2000. Fish meal supplementation to grazing dairy cows in early lactation. *J. Dairy Sci.* 83, 2899-2906.
- Tesfa, A.T., Virkajärvi, P., Tuori, M. & Syrjälä-Quist, L., 1995. Effects of supplementary concentrate composition on milk yield, milk composition and pasture utilization of rotationally grazed dairy cows. *Anim. Feed Sci. Technol.* 56, 143-154.
- Tilley, J.M.A. & Terry, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassld. Soc.* 18, 104.
- Vazquez, O.P. & Smith, T.R., 2000. Factors affecting pasture intake and total dry matter intake in grazing dairy cows. *J. Dairy Sci.* 83, 2301-2309.
- Webb, E.C., 1994. Synthesis of long chain fatty acids in ruminants and their effects on meat quality. PhD thesis, University of Pretoria, South Africa.
- Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F. & Lesch, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65, 495-501.
- Wu, Z., Fisher, R.J., Polan, C.E. & Schwab, C.G., 1997. Lactational performance of cows fed low or high ruminally undegradable protein prepartum and supplemental methionine and lysine postpartum. *J. Dairy Sci.* 80, 722-729.
- Xu, S., Harrison, J.H., Chalupa, W., Sniffen, C., Julien, W., Sato, H., Fujieda, T., Watanabe, K., Ueda, T. & Suzuki, H., 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. *J. Dairy Sci.* 81, 1062-1077.