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Effect of replacing a commercial pelleted calf meal with lucerne leaf-meal on performance of neonatal and transitional Holstein heifer calves

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Abstract. A study was conducted to (i) determine *in vitro* ruminal dry matter (DM) fermentation kinetics, effective rumen degradation of DM (ED_{DM}), (ii) estimate energy fractions supply of lucerne leaf-meal (LLM) and composite diets of LLM with commercial pelleted calf meal and also (iii) determine the effects of substituting commercial pelleted calf meal with LLM on the intake, % ruminal nitrogen balance (RNB) and growth of the neonates (21–42 days old) and transition (43–56 days old) Holstein heifer calves. Forty-eight Holstein heifer calves were randomly assigned to three different dietary treatments in a complete randomised design of: (*a*) pelleted concentrate (PEL), (*b*) 65% pelleted concentrate: 35% LLM ($P_{65\ L35}$); (*c*) and 50% pelleted concentrate: 50% LLM ($P_{50\ L50}$). The study comprised of two experiments: neonatal (Experiment 1) and transition (Experiment 2) phases. Lucerne leaf-meal had gross energy of 16.2 MJ/kg and 25% crude protein DM. PEL diet was high in starch and bound protein compared with other diets. Inclusion of LLM in diets increased calcium levels but tended to decrease phosphorus levels. Calves were weaned at the age of 56 days. The feeds were incubated for 0, 4, 10, 18, 24 and 48 h using a Daisy[II] incubator. Rumen fluid was obtained from calves <50 days old. Large Ruminant Nutrition System was used to predict %RNB and energy density of the diets during neonatal and transition phases. Higher mean ED_{DM} levels were found with LLM inclusions whereas fractions *a*, *a* + *b* and *c* did not vary. Neonates on diet C had higher ($P < 0.05$) daily DM and crude protein intakes, %RNB, total digestible nutrients, net energy at maintenance and net energy at gain during neonatal phase. Performance of calves was similar during the transition phase. LLM should be considered as a concentrate replacement in diets of neonates and calves.

Additional keywords: concentrate feed, performance, pre-weaning, ruminal nitrogen balance, transition.

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Introduction

Protein nutrition is an essential component of neonatal dairy heifers. Commercial concentrates that are high in protein are often the main solid feed for dairy calves. Protein-rich forages are also potential protein sources for neonates. Castells *et al*. (2012, 2013), Jones and Heinrichs (2007) and Göncü *et al*. (2010) showed that forages improve rumen development in calves. Castells *et al*. (2012, 2013) reported increased rumen fill, pH, acetate production, and high population of *Ruminococcus albus* in calves supplemented with lucerne (*Medicago sativa*) hay. Lucerne is utilised widely in dairy cattle production and leaf-meals for monogastrics (Guenthner *et al*. 1973; Lindberg *et al*. 1995; Rechulicz *et al*. 2014). Lucerne leaf-meal (LLM) is also a herbal supplement for humans because the leaves are high in essential nutrients and antioxidants (Xie *et al*. 2008) and low in fibre. They are also high in methionine and lysine (Homolka *et al*. 2008). Lucerne hay is, however, low in essential nutrients such as phosphorus, non-fibre carbohydrates such as starch (Mason 1998) and has low digestibility compared with some grasses like tall fescue and red clover (Cherney *et al*. 2004; Homolka *et al*. 2012). Among all other leguminous forages lucerne is peculiar because of its high nutrients and antioxidant levels (Xie *et al*. 2008). However, information on LLM supplementation for optimal feed intake and digestion in pre- and post-weaned dairy calves is limited and inconclusive. Kuan *et al*. (1983) reported that high proportion of LLM in the diet resulted in a linear decrease in the digestibility of dry matter, crude protein and crude fat. However, Lindberg *et al*. (1995) did not report any negative effects on diet digestibility. The low fibre, high β-carotene and protein contents in LLM make it an attractive substitute forage for commercial calf feeding.

The aim of the study was, therefore, to assess nutrient intake, rumen degradability and growth of Holstein heifers during neonatal and weaning periods.

Materials and methods

Study location

The study was conducted at Irene, Animal Production Institute of the Agricultural Research Council of South Africa in 2014. Irene is located at longitude 28°13S, latitude 25°55E, altitude of 1524 m.

Forage processing and dietary nutrient composition

Six-week regrowth lucerne was hand-harvested at 5 cm above the ground and air-dried under shade to prevent nutrient loss (Smit 2014) over 7 days, with daily turning to prevent moulding (Mujumdar 1997). After drying, petioles were separated from stems and small twigs were crumbled manually to form LLM and stored in a dry area. Three diets were prepared using mixtures of LLM and a commercial pelleted calf meal concentrate (PEL). The diets were as follows: 100% PEL; 65% PEL with 35% LLM ($P_{65\ L35}$) and 50% PEL with 50% LLM ($P_{50\ L50}$). The nutrient contents of the dietary treatments are presented in Table 1. Lucerne leaf-meal had 95% DM, 90.5% organic matter, 25% crude protein, 0.6% neutral detergent insoluble crude protein, 1.3% acid detergent insoluble crude protein, 16.2 MJ/kg DM gross energy, 1.6% ether extract, 9.5% ash, 22.5% neutral detergent fibre, 7.7% acid detergent fibre, 14.0% hemicellulose, 14.8 cellulose, 0.8% acid detergent lignin, 40.8% non-structural fibre 1.4% calcium, 0.8% phosphorus and 0.2% starch. All the data were expressed on % DM.

Table 1. The chemical composition of lucerne leaf-meal (LLM) and experimental diets (units are in % dry matter except MJ/kg DM for gross energy)

Within rows, means with different letters differ significantly at $P < 0.05$. s.e.m., standard error of the mean

Variable	Treatment			s.e.m.
	PEL	$P_{65\ L35}$	$P_{50\ L50}$	
Dry matter	92.3	92.3	92.6	0.142
Organic matter	91.3	91.4	91.3	0.248
Crude protein	20.4	22.6	22.8	0.626
Neutral detergent insoluble crude protein	3.3a	2.0b	2.2b	0.127
Acid detergent insoluble crude protein	0.8	1.0	1.1	0.091
Gross energy	15.8	15.7	15.8	0.111
Ether extracts	4.7a	2.2b	2.0c	0.070
Ash	8.7	8.6	8.7	0.249
Neutral detergent fibre	34.1	33.4	33.3	2.520
Acid detergent fibre	11.8	13.8	13.0	1.456
Hemicellulose	22.3	19.7	20.3	1.637
Cellulose	9.9c	18.0a	13.4b	0.887
Acid detergent lignin	1.8a	0.4b	0.2b	1.036
Non-fibre carbohydrates	39.7	40.8	40.2	2.674
Non-polar extracts	74.3	74.9	74.7	2.520
Calcium	1.0c	1.7a	1.5b	0.006
Phosphorus	0.6a	0.5b	0.4c	0.012
Starch	13.4a	10.1b	8.5c	0.029

In vitro *degradation*

Ground samples of 0.25 g of the three dietary treatments (PEL, $P_{65\ L35}$ or $P_{50\ L50}$) and LLM were weighed into ash-free and N-free nylon bags (Ankom F57, Ankom Technology Corp., Fairpost, NY, USA) and then heat sealed. *In vitro* micro mineral, macro mineral, reducing and buffer solutions based on ANKOM (Ankom Technology Corp.) procedures were prepared. Rumen fluid was collected from non-experimental dairy Holstein calves aged 50 days old using an oesophageal tube under mild vacuum suction (Tufarelli *et al*. 2010) into a pre-warmed CO_2-filled thermos flask. Rumen fluid was collected before calves were fed.

In vitro fermentation was assessed at 0, 4, 10, 24 and 48 h using a Daisy II incubator (D200, Ankom Technology Corp.). The incubator maintained a constant temperature of 39°C. All samples were analysed in triplicate. Zero-hour bags were washed with water. At termination bags were washed under running water. Samples were placed into a 100°C oven for 48 h, weighed for DM and ashed to determine organic degradability values. The NDF and crude protein disappearance were estimated at 24 and 48 h because these are slowly degradable fractions.

Animals and data collection

Intake and growth study was divided into two experiments: neonatal phase (Experiment 1: involving 24 calves aged 21–41 days) and transition phase (Experiment 2: involving 24 calves aged 42–56 days).

Experiment 1: calves were randomly allocated to the three diets at 21 days old, fed 4 L of milk/day, and assessed for nutrient intake and growth until 42 days old. Experiment 2: 24 calves aged 42 days were randomly allocated to the three diets and milk supply reduced to 2 L/calf.day. Nutrient intake and growth were assessed until weaning at 56 days old. Calves in both experiments were individually housed in 2×5-m^2 concrete-floored pens without bedding with *ad libitum* access to fresh water throughout the experimental periods. Milk and solid feed consumption were recorded daily. Bodyweight was recorded weekly before the morning feeding. Milk contained 3.8% fat, 3.5% protein and 4.8% lactose.

Simulation of LLM and diets

Large Ruminant Nutrition System (LRNS) model of the University of California-Davis (LRNS version 1.0.31, 2014) was used to predict energy nutrient density supply and balances of the three diets of LLM and commercial concentrates. The LRNS is based on the Cornell Net Carbohydrate and Protein System Version 5.0.40 (Fox *et al*. 2004). Level 1 solutions of the LRNS model were used to predict % ruminal nitrogen balance (RNB) (Table 2) and diet concentrations (Table 3). During the transition phase, diets were not predicted for energy density because the diets and chemical composition were similar to the diets used in neonatal phase and only quantity varied. The LRNS predictions were based on tropical climatic conditions of 22°C, 30% relative humidity and wind-speed of zero for calves housed in a 10-m^2 cubicle with no exposure to storms and sunlight.

Table 2. Effect of replacing calf starter pellets with lucerne leaf-meal on diet intake, % ruminal nitrogen balance, feed conversion ratio and growth of pre-weaned and transition Holstein heifer calves
Within rows, means with different letters differ significantly at $P < 0.05$. RNB, ruminal nitrogen balance; s.e.m., standard error of the mean

Variable	Treatment			s.e.m.
	PEL	$P_{65\ L35}$	$P_{50\ L50}$	
Neonatal phase				
Intake of solid feed (g/calf.day)				
Dry matter	506b	538b	634a	2.95
Crude protein	96b	113ab	133a	59.61
Starch	66a	54b	57ab	32.97
Total solid feed and milk intake[A] (g/calf.day)				
Dry matter	905b	938b	1033a	295.4
Crude protein	236b	253b	273a	59.61
Predicted % RNB (% of required)	61.3c	110.3b	126.7a	1.00
Initial weight (kg/calf)	35.2	41.1	37.1	8.46
Final weight (kg/calf)	44.9	52.8	53.6	9.79
Average daily gain (kg/calf.day)	0.46b	0.56ab	0.79a	0.16
Feed conversion ratio	1.97a	1.68b	1.31c	0.73
Transition phase				
Intake of solid feed (g/calf.day)				
Dry matter	1094	1114	1143	283.3
Crude protein	208b	235a	240a	58.19
Starch	142a	112b	103b	29.96
Total solid feed and milk intake[A] (g/calf.day)				
Dry matter	1294	1318	1343	283.30
Crude protein	278b	305a	310a	58.19
Predicted % RNB (% of required)	59.7c	119.7b	142.0a	1.89
Initial weight (kg/calf)	47.2	55.4	58.3	8.95
Final weight (kg/calf)	57.3	66.2	69.8	8.70
Average daily gain (kg/calf.day)	0.78	0.83	0.88	0.23
Feed conversion ratio	1.66	1.59	1.53	0.43

[A]Calves were fed 4 L of milk during neonatal and 2 L/day of milk during transition phase; therefore, milk DM intakes were 400 g/calf.day and 200 g/calf.day, respectively and CP intakes of 140 g/calf.day and 70 g/calf.day, respectively.

Table 3. Predicted energy density of the calf diets using Large Ruminant Nutrition System (LRNS) for Experiment 1
Within rows, means with different letters differ significantly at $P < 0.05$. TDN, total digestible nutrients; ME, metabolisable energy; NEm, net energy at maintenance; NEg, net energy at gain; s.e.m., standard error of the mean

Variable	Treatment			s.e.m.
	PEL	$P_{65\ L35}$	$P_{50\ L50}$	
Apparent TDN (%DM)	79.0c	80.0b	82.7a	0.333
ME (Mcal/kg DM)	2.9	2.9	3.0	0.530
NEm (Mcal/kg DM)	1.9b	1.9b	2.0a	0.003
NEg (Mcal/kg DM)	1.3b	1.3b	1.4a	0.003

Chemical analyses

Dry matter of ingredients and diets was determined according to AOAC (2000) (Procedure 930.15). Ash contents and organic matter were determined according to AOAC (2000) (Procedure 942.05) at 550°C for 8 h. Ether extract was determined according to AOAC (2006) (Procedure 2003.05). Crude protein was determined using the Kjeldahl procedure (AOAC 2000) (Procedure 968.06). Gross energy values of the feed samples were determined by combustion in an adiabatic bomb calorimeter (PARR model 2081). Starch was determined by a modification of the method of Holm *et al.* (1986) as cited by Hall (2000). Calcium was determined according to Giron (1973) using a Perkin Elmer atomic spectrophotometer. Phosphorus was assayed according to AOAC (2000) (Procedure 965.17). Neutral detergent fibre and acid detergent fibre were determined according to Van Soest *et al.* (1991) and acid detergent lignin was determined according to Goering and Van Soest (1970). Acid detergent insoluble crude protein and neutral detergent insoluble crude protein (Licitra *et al.* 1996) were determined by measuring the crude protein content of the acid detergent fibre and neutral detergent fibre residue by Kjeldhal analysis and contents were expressed as a percentage of total N (Van Soest *et al.* 1991). All samples were analysed in triplicates.

Non-linear procedures (PROC NLIN) in SAS (2009) were used to estimate *in vitro* degradation kinetics in the rumen. Data were fitted into exponential model without lag time (Ørskov and McDonald 1979) to determine the rate constants and potential degradation according to the exponential model:

$$PD = a + b(1 - e^{-ct})$$

Table 4. Diet *in vitro* DM degradation at different times of incubation in nylon bags and constants in the exponential equation: PD = $a + b(1 - e^{-ct})$ (Ørskov and McDonald 1979)

Within rows, means with different letters differ significantly at $P < 0.05$. DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; CP, crude protein; ED, effective degradation; kp, passage rate in the rumen at 0.05/h for forage diets and 0.08/h for pellets; s.e.m., standard error of the mean

Variable	Incubation time (h)	Treatment PEL	$P_{65\ L35}$	$P_{50\ L50}$	s.e.m.
DM (OM)	0	53.6 (53.9)	56.6 (58.4)	55.6 (57.6)	1.975 (2.424)
(%)	4	63.0 (65.2)	62.3 (64.4)	62.1 (64.6)	2.225 (2.597)
	10	69.3 (72.0)	71.6 (75.2)	73.6 (77.8)	2.552 (3.099)
	18	76.3b (79.8b)	77.8a (82a)	78.5a (82.9a)	0.805 (1.058)
	24	84.1b (90.9)	88.3a (95.8)	88.9a (94.3)	0.555 (1.759)
	48	85.4 (92.6)	89.3 (97.2)	92.2 (97.7)	4.060 (4.320)
NDF (%)	24	83.5b	88.3a	88.9a	1.334
	48	83.6	89.8	92.5	8.270
CP (%)	24	71.7	82.9	82.3	7.621
	48	77.0b	88.3a	85.6a	1.319
		Degradation kinetics of dry matter			
a (%)	–	32.5	37.9	39.7	3.992
a + *b* (%)	–	86.3	93.4	94.4	3.816
c (/h)	–	0.08	0.06	0.06	0.018
ED (%)	–	83.6b	88.0a	92.0a	3.298

where PD is the potential degradability after time '*t*', *a* is the soluble fraction (%; fraction washed out at $t = 0$; this value resulted from the incubation of 0 h bags, *b* is the insoluble but degradable fraction after time '*t*', *c* is the rate of degradation of slowly degradable fraction *b* and *t* is the incubation time (h).

Effective degradability (ED_{DM}; %) was calculated from the aforementioned parameters (*a*, *b* and *c*) assuming a fractional passage rate (*kp*) of 5%/h for the forage diets and 8%/h for the PEL:

$$ED = a + b(c/(c + kp))$$

Statistical analyses

All the data on degradation, energy fractions, nutrient intake from milk and solid feed, and growth were subjected to analysis of variance using GLM procedures in Minitab Statistical Software, Version 17 (Minitab 2010) and covariance analysis was done using initial bodyweight of the calves as covariate. Treatment means were compared using a Fishers' least significant difference and significant differences were declared at $P < 0.05$. Fitted non-linear regression degradation parameters and ED were analysed as completely randomised design with diet as a factor using analysis of variance procedures in SAS (2009).

Results and discussions

All the diets had similar crude protein contents ($P > 0.05$). However, diets containing LLM tended to have higher crude protein contents, which were not statistically significant at 5% level. The minimum crude protein required to support rumen bacterial growth for dairy calves is 18% (NRC 2001; Drackley *et al*. 2002). All the diets in the present study were above the minimum crude protein requirements. Bach (2014) noted that a starter feed above 20% crude protein positively influences growth of the calves after weaning. The neutral detergent fibre contents across all dietary treatments were higher than the recommended neutral detergent fibre content for dairy calf starter of 15–25% (Davis and Drackley 1998), depending on the digestibility of the feed. The LLM fibre in the present was highly degradable and, therefore, would not impact on nutrient and hence availability. The diets were also low in acid detergent lignin which, according to Goering and Van Soest (1970) and Buxton *et al*. (1996), would not negatively affect digestibility. There were no differences observed in acid detergent insoluble crude protein in both dietary treatments. Sniffen *et al*. (1992) reported that acid detergent fibre-bound N represents the portion of protein in a feedstuff that is unavailable for use by the animal because it is completely indigestible. Additionally, Van Soest (1994) stated that as the concentration of acid detergent fibre-bound N increases, total N digestibility decreases. This negative association has been observed in several studies (Weiss *et al*. 1986; Van Soest 1994). In addition, an increase in inclusion of LLM in the diets tended to decrease neutral detergent fibre and acid detergent lignin contents of the diets. Therefore, there would be more ruminal microbe attachment to the feed particles, as well as an increase in microbial growth and enzyme activity or intestinal activity (McAllister *et al*. 1994; McSweeney *et al*. 2001).

In vitro DM digestibility estimates increased with incubation time for all diets and no differences ($P > 0.05$) were observed during the 48 h of incubation (Table 4). The higher *In vitro* DM digestibility in the LLM substituted diets may probably be due to more cell contents in the diets, which are almost completely digestible and are not affected by lignin concentration in cell walls (Van Soest and Moore 1965). Greater organic matter degradation observed with LLM substituted diets at 24 h incubation may result from an increase in microbial protein synthesis (Verbic and Babnik 1997). This is based on the view that microbial protein synthesis is highly dependent on the availability of the rumen degradable organic matter. It has been reported that the rate of N and carbohydrates degradation, especially from the mixture of

forage and concentrates, increases the efficiency of microbial protein synthesis due to an improved rumen environment (Karshi and Russell 2001). *In vitro* neutral detergent fibre and crude protein degradation values were higher in LLM substituted diets at 24 and 48 h than Diet PEL. LLM substituted diets had higher effective degradability values than Diet PEL. Concentrate feeding for calves has risk of acidosis (Nocek 1997). However, the results of the present study showed that LLM substituted diets were more digestible in the rumen compared with PEL. Thus, LLM may provide more digestible energy, but have higher risk of acidosis compared with PEL.

The predicted metabolisable energy densities of the diets during the neonatal phase were similar and within the range of 2.9–3.2 Mcal/kg DM as recommended by NRC (1989) for dairy calves (Table 3). These results are similar to those reported by Chester-Jones and Broadwater (2009) with a mean of 2.7 Mcal/kg DM and are within the recommendations by NRC (1989). Higher total digestible nutrients, net energy at maintenance and net energy at gain were observed with LLM substituted diets compared with diet PEL.

Average daily solid feed consumption during the neonatal phase was lower than the 680 g per calf recommended by NRC (2001) at ~41–42 days of age (Table 2). However, higher ($P < 0.05$) intakes of DM, crude protein, neutral detergent fibre and acid detergent fibre were observed in calves fed Diet $P_{50\ L50}$ during neonatal and transition phases and this may be ascribed to the differences in ruminal development and thus the calf's metabolic and structural capacity to accommodate and digest solid feed (Khan *et al*. 2007). However, higher starch intake by calves on Diet PEL might be due to high starch content of the feed (13.4%) presented in Table 1. In addition, the higher average daily crude protein and neutral detergent fibre intakes during neonatal phase in calves fed Diet $P_{50\ L50}$ were the function of higher starter feed consumption. These findings are supported by higher average daily gain and better feed conversion ratio observed in calves on Diet $P_{50\ L50}$. However, Quigley *et al*. (2000) reported higher feed conversion ratio values of 3.10 in Holstein calves fed on a calf starter ration.

During the neonatal and transition phase, the DM and crude protein intakes were within the recommended range for the dairy calves as reported by Van Amburgh and Drackley (2005); however, a lower ($P < 0.05$) crude protein intake was observed with calves fed a 50% LLM inclusion during the transition phase. Total DM intake and feed conversion ratio for the solid feed during the transition phase did not differ across treatments ($P > 0.05$). It was reported that feeding a fibre source to young dairy calves was necessary because it improved rumen health (Thomas and Hinks 1982). This is also supported by recent literature that inclusion of forage in the diet of the calves during the pre-weaning period improved rumen health (Khan *et al.* 2011; Castells *et al.* 2012, 2013; Montoro *et al.* 2013). The higher ($P < 0.05$) average daily gain observed for LLM substituted diets than calf starter concentrate pellets (PEL) during the pre-weaning phase could be attributed to a better development of the rumen. This higher average daily gain observed during neonatal phase may be an indication that calves would reach puberty by 12 months and bred to calve by 22 months. Kertz *et al*. (1979) stated that high starter intake before weaning helps to ensure intake and sustain a desirable growth rate after weaning which improves the age at first calving. The results of the present study, indicate greater growth rate with LLM inclusion and they are higher than the target growth rate of 0.7 kg for Holstein for the first 12-week period recommended, as reported by (Dawson 2006).

Calves on LLM diets had higher ($P < 0.05$) DM intakes and average daily gain. The higher DM intake of lucerne results in higher protein intakes hence saving of protein concentrates (Kirilov 2001). The PEL diet had lower ($P < 0.05$) RNB values than LLM substituted diets. This is a serious hindrance for early growth as calves require more protein for muscle accretion and immune development. The slower growth for calves on this diet confirmed these observations. During the transition phase, a higher ($P < 0.05$) RNB was observed in calves on Diet $P_{50\ L50}$ and this indicates opportunities to improve the efficiency of N utilisation by rumen microbes. However, a lower value ($P < 0.05$) of RNB observed with Diet PEL indicates lower rumen fermentation, lower microbial yield and insufficient rumen-degradable N relative to carbohydrate supply (Maglione and Russell 1997). Ruminal N limitation can decrease microbial flow from the rumen (NRC 1985; Russell *et al*. 1992), depress fibre fermentation (Russell *et al.* 1992) and reduce DM intake (NRC 1987; Van Soest 1994).

Chester-Jones and Broadwater (2006) reported the highest average daily calf starter intakes of 0.09, 0.51, 1.12 and 2.18 kg for periods 1–14, 15–28, 29–42 and 43–56 days, respectively, for Holstein heifer calves. These results are higher than those of the present study at 21–42 and 43–56 days with intake values of 0.91–1.03 and 1.29–1.34 kg/calf.day, respectively. The higher the quality, the faster the rate of digestion and the higher the potential intake of the LLM.

Conclusions

The results of the present study indicate that nutrient composition, effective degradability of DM and energy density improved with higher LLM inclusion in the calf diet. This was confirmed by improved diet intake, % ruminal N balance and growth of Holstein heifer calves on LLM substituted diets. However, there is a need for further studies to investigate rumen microbial dynamics of a rapidly degradable LLM, production of volatile fatty acids and determine the effects of LLM on rumen papillae development and the outflows of microbial protein to the lower gut of neonates fed higher proportions of LLM.

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