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Degradable protein requirements of beef cattle consuming winter forage hay

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Abstract

A trial was conducted to determine the total rumen degradable protein intake (RDPI) required to maximise the digestible organic matter intake (DOMI) of beef cows consuming low quality grass hay from the northern variation of *Cymbopogon–Themeda* pasture type. Thirty-five pregnant (last trimester) Afrikaner × Simmentaler crossbred cows (517.08 ± 53.06 kg) were stratified according to fasted body weight and allocated to five treatments. A rumen degradable protein (RDP) source, calcium caseinate (90% crude protein on dry matter basis and 100% rumen degradable) was used and mixed with a molasses based concentrate. Treatments provided the following supplemental RDP levels/cow/day from casein: (1) control, 0 g, (2) 180 g, (3) 360 g, (4) 540 g, and (5) 720 g. The cows had *ad libitum* access to low quality grass hay (2.26% CP; 73.94% neutral detergent fibre, NDF). The trial period consisted of 14 d of adaptation, 21 d of the intake study, and 7 d of the digestibility study. No statistically significant influence of RDP on the apparent digestibility of DM, organic matter, or NDF was detected. The grass DM intake, DOMI, and metabolizable energy intake increased in a quadratic manner with increasing supplementary RDP. The single broken-line model predicted DOMI /kg metabolic weight ($BW^{0.75}$) with higher accuracy than the quadratic regression procedure. According to this model, 4.03 g daily RDPI/kg $BW^{0.75}$ or 8.07% RDP of digestible organic matter was required to maximise DOMI of pregnant beef cows consuming winter grassveld hay.

Keywords: digestible organic matter, low quality grass, metabolizable energy, protein intake
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Introduction

Tons of low-quality forages from dry natural pastures and grain and crop by-products are available as feed sources for grazing animals. The ruminant animal can derive energy from cellulose, hemi-cellulose, and pectin in the fibre component of these forages. Pregnant beef cows consuming low quality forages, especially during winter in the summer rainfall regions of South Africa, are subjected to nutrient deficiencies. One of the main factors limiting utilisation of low quality forage by ruminants is nitrogen (N) availability in the rumen. Nitrogen deficiency results in reduced feed intake by limiting the rate of microbial growth and digestion of organic matter (OM) in the rumen and hence slow clearance of digesta in the tract (DelCurto *et al.,* 1990; Freeman *et al*., 1992; Lintzenich *et al.,* 1995; Köster *et al.*, 1996; Basurto-Gutierrez *et al.*, 2003). Lack of N sources for microbes can be overcome with supplements of protein or non-protein nitrogen (NPN) compounds that are degradable in the rumen (Hunter & Siebert, 1987; Mawuenyegah *et al.*, 1997).

Considering the high cost of protein supplementation, it is therefore essential to determine the amount of rumen degradable protein (RDP) required to increase the digestibility and intake of low quality

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roughages in order to optimise animal performance. According to Köster (1995), mature non-pregnant beef cows require 4 g total RDP/kg metabolic weight ($BW^{0.75}$) to maximise digestible organic matter intake (DOMI) from low-quality, tall-grass prairie forage. This recommendation is based on a study conducted on non-pregnant beef cattle consuming low quality roughage produced in the USA. Factors like the physiological stage of the animal and roughage type (physical size and chemical composition) could probably influence these requirements. It is therefore important to determine the RDP requirements and energy intake of pregnant beef cows consuming low quality forage during winter in South Africa. This will verify whether the recommendations of Köster (1995) are applicable for other physiological stages under South African conditions.

Therefore, the purpose of this study was to determine the supplemental RDP (calcium caseinate) required to maximise DOMI and metabolizable energy intake (MEI) in pregnant beef cows consuming low quality hay from the northern variation of the *Cymbopogon–Themeda* pasture type in South Africa. It is expected that more RDP will be required to maximise DOMI and MEI in pregnant beef cows (in their last trimester) particularly for foetal development.

Materials and Methods

All experimental protocols for the management and care of animals complied with the standards required by the NWDARD Animal Ethics Committee (NWAE/24/05/01). Thirty-five pregnant (last trimester) Afrikaner × Simmentaler crossbred cows (average initial live weight of 517.08 kg; SD ± 53.06 kg) were stratified by fasted body weight and allocated to five treatments in a randomized design. The cows were fasted (feed and water) overnight before weighing at the beginning and the end of the trial. Animals were fed in individual pens and had free access to clean water. The trial period consisted of 14 d of adaptation, 21 d of the intake study, and a digestibility study which comprised the last 7 d of the intake study (total 35 d).

The dormant winter pasture hay of the northern variation of the *Cymbopogon–Themeda* (no. 48b) (Acocks, 1988) pasture type was used. The hay was offered at 130% of the previous five-day average consumption per animal. Based on previous research (DelCurto *et al.*, 1990; Hannah *et al.,* 1991; Köster, 1995), one of the major responses to protein supplementation is increased forage intake thus cows were allowed *ad libitum* consumption of forage rather than to restrict forage intake to the level of unsupplemented cows. The lick and hay were fed twice daily at 08:00 and 14:00. Experimental treatments provided the following supplemental RDP levels/cow/day from casein: (1) control, 0 g, (2) 180 g, (3) 360 g, (4) 540 g, and (5) 720 g. The RDP supplementation in the form of calcium caseinate (90% crude protein (CP) on dry matter basis and 100% rumen degradable; Köster, 1995) was thoroughly mixed with 500 g molasses-based concentrate, divided into two equal portions, and offered before the hay. The control group (0 g casein) also received the 500 g molasses-based concentrate. The mineral premix included in the supplement comprised macro- and micro-minerals to prevent mineral deficiencies (1.50% Ca, 1.0% P, 1.95% Na, 2.39% K, 4.41% Cl, 0.54% S, 0.39% Mg, 3.43 ppm Co, 205.99 ppm Cu, 657.91 ppm Mn, 2.0 ppm Se, 619.12 ppm Zn, 1109.78 ppm Fe, 10.0 ppm I).

Representative feed samples were collected daily at both feeding times. During the intake study, orts were collected each morning, weighed, and a sample was taken per cow for each feeding time. During the digestibility study, faeces were collected each morning, and a 10% faecal representative sample was taken per cow for analyses. The faecal samples were dried at 50 °C for 96 h, then weighed and pooled for each cow to obtain a representative sample. The composite feed, orts, and faecal samples were weighed and milled through a 1-mm sieve. The representative samples (obtained using the quartering method) were placed in airtight, screw-cap sample bottles and securely stored in a dark room at winter room temperature (~18 °C) for subsequent analysis.

A day after the end of the digestibility study (day 36), approximately 35 ml of rumen fluid was obtained from each animal, 3 h after the start of the morning feeding. A vacuum pump and plastic rumen tube were used to extract rumen fluid samples. The samples were strained through four layers of cheese cloth and pH was immediately determined using a portable pH meter.

Chemical composition of feed, orts, and faeces was determined according to the methods prescribed by the AOAC (1995). Samples were dried at 100 °C in a convection oven to a constant mass in order to determine dry matter (DM) content. The OM content was determined by incinerating samples in a muffle furnace at 500 °C for 8 h. Kjeldahl N and neutral detergent fibre (NDF) were determined according to the Van Soest *et al.* (1991) methods. Gross energy (GE) was determined by means of an adiabatic bomb calorimeter.

The SAS (1994), SAS v6.1 model was used to analyse the data using PROC ANOVA in a stratified randomized design. Treatment means that were found to be significantly different (P <0.05) were further subjected to a multiple comparison test using Tukey's test. Furthermore, treatment sums of squares were partitioned into linear, quadratic, and cubic effects of RDP level with orthogonal polynomials and R^2 were calculated. In addition, RDP intake (RDPI) required for maximum DOMI was determined using a single slope, broken-line model (Robbins, 1986) with the NLIN procedure of SAS (1994). The same procedures were followed to determine the RDPI required for maximum MEI.

Results and Discussion

The quality of the natural winter pasture hay used in this study is characterised by a low CP of 2.26% and a high NDF content of 73.94% (Table 1). The degradability of protein in the *Cymbopogon–Themeda* grass hay, as determined by Jacobs (2005), is 67.5%. Research has indicated that microbial growth in the rumen of animals consuming a low protein diet may be restricted by the inadequate supply of ammonia, peptides, and amino acids resulting in reduced rate of cellulose digestion (Redman *et al.*, 1980; Köster *et al*., 1996; McDonald *et al*., 2002). The protein supplement used during the study was casein, which increases the available pool of amino acids and peptides in the rumen (Redman *et al.*, 1980). Miner *et al*. (1990) noted that microbial yield may be enhanced by the availability of growth limiting organic acids supplied by an RDP source that degrades slowly.

Table 1 Chemical composition of grass and supplements

Item		Molasses-based concentrate + RDP (casein)				
		Supplementary rumen degradable protein				
	Grass	0 g	180 g	360 g	540 g	720 g
Dry matter %	92.84	91.48	84.46	85.60	87.05	87.76
Organic matter %	90.82	87.82	89.90	90.70	92.27	92.62
Crude Protein %	2.26	4.04	31.97	37.58	53.25	59.71
Neutral Detergent Fibre %	73.94	23.22	16.22	13.62	10.45	3.79

Generally, CP less than 6–8% in the basal forage is considered to be the threshold value as far as digestion is concerned, since protein supplementation seems to have little benefit on digestion of medium to high quality roughages (Clanton & Zimmerman, 1970). Meissner *et al.* (1991) stated that digestion of cell wall constituents is slow or depressed when NDF content is above 55–60%, as was the case in the present study. The rate of digestion and retention time in the rumen are the major determinants of voluntary intake of poor quality roughages.

The effect of increasing the level of supplemental RDP on the digestibility of the diet by pregnant beef cows is illustrated in Table 2. Protein supplementation during the study resulted in an increase (P <0.01) in grass dry matter intake (DMI), total DMI, and total organic matter intake (OMI). This increased intake with higher levels of RDP supplementation could reduce the digestibility of the diet. McDonald *et al.* (2002) stated that an increase in food intake causes a faster rate of passage of digesta. Accordingly, the digesta is then exposed to the action of microbes and digestive enzymes for a shorter period and there may be a reduction in its digestibility, especially of the slowly-digestible cell wall constituents. In the present study the NDF content of the grass hay, which represents cell wall components, was as high as 74%. Therefore an increased intake could have a detrimental influence on its digestibility. Feed RDP level had no influence (P >0.05) on the apparent digestibility of DM, OM, NDF and GE. This is unexpected as RDP supplementation is related to microbial growth and a subsequent increase in digestion of low quality forage. Hence, there is possibly a counteracting force of increased intake on the rate of passage and digestion (Badyk *et al.*, 2001). The higher (P <0.0001) total DMI with an increasing RDP level could contribute to these findings. These results are consistent with the findings of Nolte (2000), who found that OM digestibility was not affected by increasing levels of supplemental RDP.

Table 2 Effect of increasing level of supplementary rumen degradable protein on digestibility of low quality grass hay during the digestibility study

Item	Supplementary rumen degradable protein					Significance	Significance of contrasts[1]		
	0 g	180 g	360 g	540 g	720 g	P	L	Q	C
Grass DMI (kg/cow/day)	4.57 ± 0.48^{a}	6.61 ± 0.50^{b}	7.67 ± 0.26^{b}	7.14 ± 0.40^{b}	7.31 ± 0.50^{b}	0.0002	0.0001 *0.3075	0.0027 *0.1724	0.2280 *0.0245
Supplementary DMI (kg/cow/day)	0.46 ± 0.00^{a}	0.59 ± 0.00^{b}	0.77 ± 0.00^{c}	0.96 ± 0.00^{d}	1.14 ± 0.00^{e}	0.0001	0.0001 *0.9964	0.0001 *0.0027	0.0001 *0.0008
Total DMI (kg/cow/day)	5.03 ± 0.48^{a}	7.21 ± 0.50^{b}	8.44 ± 026^{b}	8.10 ± 0.40^{b}	8.74 ± 0.37^{b}	0.0001	0.0001 *0.4927	0.0051 *0.1099	0.1469 *0.0266
Total OMI (kg/cow/day)	4.54 ± 0.44^{a}	6.51 ± 0.45^{b}	7.65 ± 0.24^{b}	7.35 ± 0.36^{b}	7.69 ± 0.45^{b}	0.0001	0.0001 *0.4358	0.0034 *0.1356	0.2501 *0.0184
				Apparent digestibility coefficients (%)					
Dry matter	57.60 ± 1.71	61.23 ± 1.34	62.15 ± 1.41	61.28 ± 4.19	61.54 ± 3.97	0.8026	0.3821 *0.0249	0.4272 *0.0205	0.6717 *0.0058
Organic matter	62.07 ± 1.62	65.65 ± 1.25	66.08 ± 1.41	65.50 ± 3.80	65.64 ± 3.56	0.8028	0.3987 *0.0232	0.4210 *0.0210	0.6387 *0.0071
Crude protein	- 5.83 ± 9.67^{a}	46.88 ± 4.22^{b}	52.16 ± 2.71^{bc}	67.19 ± 2.74^{bc}	72.51 ± 2.51^{c}	0.0001	0.0001 *0.6707	0.0001 *0.1106	0.0275 *0.0305
Neutral detergent fibre	61.56 ± 1.83	66.26 ± 1.40	65.32 ± 1.59	64.19 ± 4.19	63.17 ± 3.97	0.8002	0.9006 *0.0005	0.2868 *0.0372	0.5311 *0.0127
Gross energy	58.99 ± 1.68	62.95 ± 1.52	63.48 ± 1.47	65.24 ± 3.38	63.69 ± 3.72	0.5144	0.1577 *0.0630	0.3141 *0.0314	0.9884 *0.0000
				Apparent digestible nutrients (%)					
Digestible OM	56.02 ± 1.49	59.34 ± 1.10	59.89 ± 1.30	59.47 ± 3.41	59.68 ± 3.21	0.7492	0.3182 *0.0323	0.4155 *0.0214	0.6477 *0.0067
Digestible protein	- 0.14 ± 0.26^{a}	2.27 ± 0.23^{b}	2.93 ± 0.20^{b}	5.68 ± 0.22^{c}	7.41 ± 0.38^{d}	0.0001	0.0001 *0.9227	0.4732 *0.0010	0.3904 *0.0014
Digestible NDF	42.04 ± 1.43	45.07 ± 0.90	44.31 ± 1.10	42.43 ± 2.93	40.38 ± 2.74	0.4940	0.3551 *0.0264	0.1436 *0.0674	0.5720 *0.0098
Metabolizable energy (MJ/kg)[2]	8.21 ± 0.25	8.87 ± 0.24	8.98 ± 0.20	9.05 ± 0.53	9.14 ± 0.52	0.4480	0.1003 *0.0850	0.4163 *0.0201	0.6335 *0.0069

[a,b,c,d,e] Row means with different superscripts differ significantly

[1] L = linear, Q = quadratic, C = cubic*
[2] Metabolizable energy = Digestible energy × 0.8 (McDonald *et al.,* 2002)
* = R^2 values

DMI = Dry matter intake, OMI = Organic matter intake, CPI = Crude protein intake

In contrast with DM, OM, NDF and GE, the apparent digestibility of CP increased in a linear manner (P =0.0001), with a higher RDP content in the diet (Table 2). This could be attributed to the corresponding increase in digestible CP content of the experimental diets with higher RDP inclusion. A negative protein digestibility (-5.8%) was recorded when no RDP was supplemented and there was a substantial increase (46.9%) at 180 g RDP/d. These results were similar to the findings of Church & Santos (1981) and Köster *et al.* (1996), who observed negative N digestibility when wheat straw and low quality, tall-grass prairie forage was fed to cattle, respectively, without protein supplementation. McDonald *et al.* (1981) mentioned that the apparent digestibility of CP is particularly dependent upon the proportion of protein in the feed. The reason for this is that the metabolic faecal N represents a constant tax upon dietary N. If a diet contains a low CP level, as was the case for the 0% supplemental RDP, a negative digestibility coefficient could occur (McDonald *et al.*, 1981) indicating a net loss of N, contributing to the weight loss seen in cows on poor quality winter grazing.

The digestible nutrient content of various diets was related to the apparent digestibility results (Table 2). Accordingly, a non-significant (P >0.05) increase in metabolizable energy (ME) content occurred with an increased RDPI level. The energy intake would be the most important criteria of the effect of different RDPI levels on the utilisation of the low quality roughage fed in this study.

The effect of increasing RDP levels on forage intake by beef cows is presented in Table 3. The grass DMI, total DMI, total OMI, DOMI, and MEI increased in a quadratic manner (P <0.05) with increasing proportions of supplementary RDP. However, according to the multiple comparison test, the largest increase (P =0.0001) in the daily energy intake occurred with a 2.80 g RDPI/kg $BW^{0.75}$ (189 g supplementary RDP/cow/day). This confirms that N is a limiting nutrient in the utilisation of low quality roughages. Köster *et al.,* (1996) observed similar responses in intake parameters with increasing proportions of supplementary RDP (casein) provided to beef cows consuming low quality prairie hay (1.9% CP) and Nolte (2000) on sheep fed wheat straw (3.2% CP).

Scott & Hibberd (1990) and Köster *et al*. (1996) noted that the diminishing responses highlight that the potential to stimulate intake via digestible intake protein (DIP) is limited. In the present study a diminishing and non-significant (P >0.05) response in daily energy intake was observed with more than 2.80 g RDPI/kg $BW^{0.75}$. The limits are probably set largely by characteristics of the forage being consumed (inherent fermentability and protein availability) and the animal's nutrient requirement (Mathis *et al.,* 2000). The digestibility of forage influences the availability of CP to the microbial population and host. Forage digestibility as well as CP content must be considered when predicting intake responses to supplemental protein (DelCurto *et al*., 1990). Köster (1995) stated that once DIP requirements are met, any additional DIP would result in wastage of N, which would narrow the cost:benefit ratio. Excess DIP can result in excessive ruminal ammonia concentration that will be absorbed through the rumen wall, converted to urea in the liver, and excreted in the urine (McDonald *et al.,* 2002). Besides wasting expensive N, the additional ammonia load may also increase the energy cost associated with ammonia detoxification in the liver (Köster *et al.,* 1996).

A linear increase (P <0.001) in the grass DMI and total DMI (grass + lick) as a percentage of body mass was observed (Table 3). The grass DMI as percentage of body mass increased markedly by 3.77 g daily RDP/kg $BW^{0.75}$, which equalled 1.45%. This result was however less than the 1.7% and ± 2.4% recorded by Köster (1995) and Jacobs (2005), respectively. Although not statistically different from 1.3%, the highest figure recorded in the present study was a grass DMI of 1.5% of body mass. The experimental cows used in the present study were in late gestation and this could probably explain the lower grass DMI as a percentage of body mass. According to Forbes (1986), the decrease in food intake that is often seen at oestrus and during late pregnancy is probably due to the high levels of circulating oestrogen, although progesterone acts to protect the animal against this for most of the pregnancy period. Furthermore, there might also be effects of competition for abdominal space which could affect intake during late pregnancy.

The supplementary RDP did not influence the rumen pH substantially (Table 3) and this suggests that pH did not limit the activity of cellulolytic bacteria in the rumen. Erfle *et al*. (1982) observed that rumen pH affects microbial growth rate and microbial protein efficiency. The rumen pH of ruminants consuming predominantly forage diet was near neutrality (± pH 7).

Table 3 Effect of increasing content of supplementary rumen degradable protein on intake and rumen fluid pH in beef cows consuming low quality grass hay during the intake study

Item	Supplementary rumen degradable protein					Significance	Significance of contrasts[1]		
	0g	180g	360g	540g	720g	P	L	Q	C
Grass DMI (kg/cow/day)	4.98 ± 0.40^{a}	6.63 ± 0.43^{b}	7.41 ± 0.24^{b}	6.97 ± 0.30^{b}	7.15 ± 0.42^{b}	0.0004	0.0003 *0.2836	0.0048 *0.1601	0.2108 *0.0283
Grass DMI as % of body mass	0.96 ± 0.10^{a}	1.33 ± 0.12^{ab}	1.45 ± 0.06^{b}	1.41 ± 0.06^{b}	1.54 ± 0.14^{b}	0.0043	0.0006 *0.3000	0.1082 *0.0559	0.2270 *0.0310
Supplemental DMI (kg/cow/day)	0.46 ± 0.00^{a}	0.59 ± 0.00^{b}	0.77 ± 0.00^{c}	0.96 ± 0.00^{d}	1.14 ± 0.00^{e}	0.0001	0.0001 *0.9964	0.0001 *0.0027	0.0001 *0.0008
Total DMI (kg/cow/day)	5.44 ± 0.40^{a}	7.22 ± 0.43^{b}	8.19 ± 0.24^{b}	7.93 ± 0.30^{b}	8.29 ± 0.42^{b}	0.0001	0.0001 *0.4297	0.0059 *0.1226	0.2260 *0.0213
Total DMI as % of body mass	1.05 ± 0.10^{a}	1.44 ± 0.13^{ab}	1.60 ± 0.06^{b}	1.61 ± 0.07^{b}	1.78 ± 0.15^{b}	0.0006	0.0001 *0.4076	0.1557 *0.0374	0.2462 *0.0247
Total OMI (kg/cow/day)	4.54 ± 0.44^{a}	6.51 ± 0.45^{b}	7.65 ± 0.24^{b}	7.35 ± 0.36^{b}	7.69 ± 0.45^{b}	0.0001	0.0001 *0.4358	0.0034 *0.1356	0.2501 *0.0184
Digestible OMI (kg/cow/day)	3.05 ± 0.22^{a}	4.28 ± 0.25^{b}	4.90 ± 0.14^{b}	4.72 ± 0.18^{b}	4.95 ± 0.25^{b}	0.0001	0.0001 *0.4627	0.0015 *0.1455	0.1374 *0.0277
Digestible OMI (g/kg $BW^{0.75}$)	28.03 ± 0.00^{a}	40.44 ± 3.33^{b}	45.45 ± 1.6^{b}	45.07 ± 1.7^{b}	49.30 ± 3.64^{b}	0.0001	0.0001 *0.4515	0.0411 *0.0683	0.1724 *0.0293
CPI (g/cow/day)	128.64 ± 10.12^{a}	352.42 ± 16.67^{b}	470.80 ± 7.49^{c}	673.09 ± 11.88^{d}	850.59 ± 12.73^{e}	0.0001	0.0001 *0.9800	0.8503 *0.0000	0.0447 *0.0020
DCPI (g/cow/day)	-5.78 ± 11.13^{a}	158.93 ± 13.80^{b}	244.55 ± 9.87^{c}	457.77 ± 21.80^{d}	617.66 ± 26.96^{e}	0.0001	0.0001 *0.9488	0.0896 *0.0039	0.6536 *0.0003
RDPI grass (g/day)	80.99 ± 5.23^{a}	107.84 ± 8.52^{b}	116.73 ± 3.88^{b}	107.10 ± 6.05^{ab}	110.55 ± 7.20^{a}	0.0048	0.0070 *0.1724	0.0103 *0.1542	0.1342 *0.0487
RDPI lick (g/day)	18.53 ± 2.49^{a}	189.01 ± 0.00^{b}	289.52 ± 0.00^{c}	509.90 ± 0.00^{d}	681.22 ± 0.00^{e}	0.0001	0.0001 *0.9893	0.0001 *0.0039	0.0001 *0.0002
Total RDPI (g/day)	99.51 ± 5.23^{a}	296.86 ± 8.52^{b}	406.25 ± 3.88^{c}	618.14 ± 5.66^{d}	791.77 ± 7.20^{e}	0.0001	0.0001 *0.9890	0.0265 *0.0007	0.0185 *0.0008
RDPI (g/kg $BW^{0.75}$)	0.91 ± 0.06^{a}	2.80 ± 0.16^{b}	3.77 ± 0.08^{c}	5.90 ± 0.09^{d}	7.85 ± 0.33^{e}	0.0001	0.0001 *0.9577	0.0568 *0.0041	0.1948 *0.0018
ME intake (MJ/cow/day)	44.66 ± 3.29^{a}	64.04 ± 3.77^{b}	73.47 ± 2.15^{b}	71.74 ± 2.7^{b}	75.79 ± 3.86^{b}	0.0001	0.0001 *0.5117	0.0016 0.1307	0.1338 *0.0259
ME intake (MJ/kg $BW^{0.75}$)	0.41 ± 0.04^{a}	0.60 ± 0.05^{b}	0.680 ± 0.02^{b}	0.69 ± 0.03^{b}	0.75 ± 3.84^{b}	0.0001	0.0001 *0.4981	0.0417 0.0620	0.1635 *0.0279
			Rumen fluid pH						
pH	7.00 ± 0.09	7.05 ± 0.07	7.04 ± 0.10	7.07 ± 0.06	6.93 ± 0.08	0.7842	0.6562 *0.0076	0.2870 *0.0443	0.7263 *0.0047

[a,b,c,d,e] Row means with different superscripts differ significantly
[1]L = linear, Q = quadratic, C = cubic; * = R^2 values
DMI = Dry matter intake, OMI = Organic matter intake, CPI = Crude protein intake, DCPI = Digestible crude protein intake, RDPI = Rumen degradable protein intake,
ME - Metabolizable energy = Digestible energy X 0.8 (McDonald *et al*., 2002)

The single slope, broken-line model suggested by Köster *et al*. (1996) was also used in the current study to estimate the RDP requirements since it yields lower estimates than the polynomial regression procedure (Baker, 1986). Generally, the quadratic regression procedure yields larger values because it predicts requirements where a maximum response is obtained. Considering the high cost of protein supplementation and the reduced magnitude of incremental improvements in DOMI as the maximum response is approached, the single slope broken-line model seems to be a more cost-effective approach (Baker, 1986; Robbins, 1986). Furthermore, the single slope broken-line model (Figure 1) predicted DOMI/kg $BW^{0.75}$ from RDPI/kg $BW^{0.75}$ with a higher accuracy ($R^2 = 0.45$) than the quadratic regression procedure ($R^2 = 0.07$). According to this model, 4.03 g daily RDPI/kg $BW^{0.75}$ was required to maximise DOMI of pregnant beef cows consuming winter grass hay from the grassveld type studied.

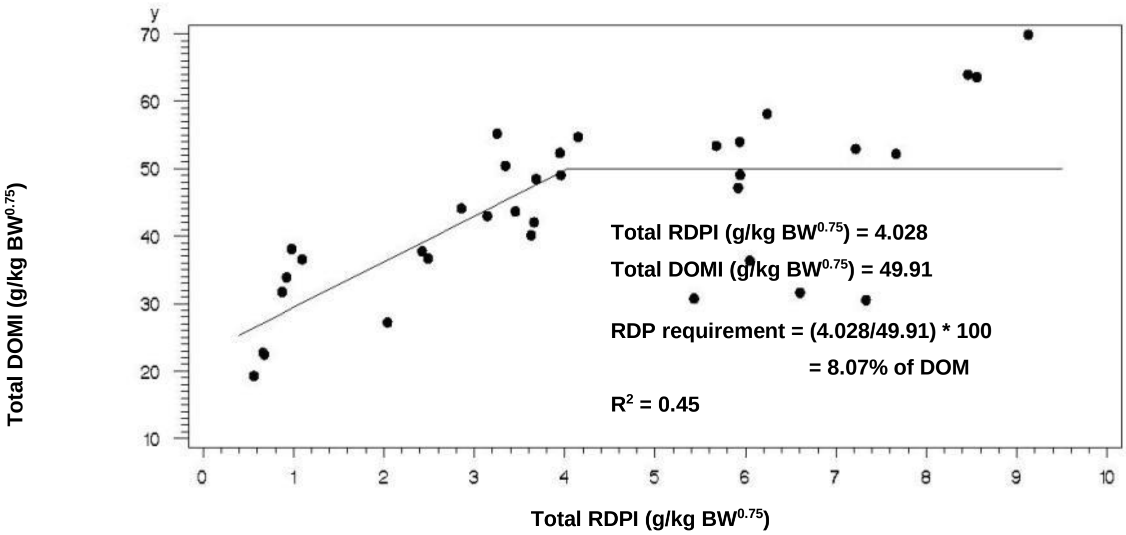

Figure 1 Daily rumen degradable protein intake (RDPI) required to maximise digestible organic matter intake (DOMI) of pregnant beef cows using a single slope broken-line model

Expression of the required RDP as a percentage of digestible organic matter (DOM) is essential as the amount of RDP required to maximize DOMI will vary with the inherent digestibility of the forage (Köster, 1995; Mathis *et al.*, 2000). According to the single slope broken-line model (Figure 1), it was estimated that 8.07% RDP of DOM would be required to maximise total DOMI (49.91 g DOMI/kg $BW^{0.75}$) of pregnant beef cows consuming low quality hay fed in the present study (4.03 g total RDPI/kg $BW^{0.75}$). This corresponds to some extent with the predictions of Köster *et al.* (1996), who found that 11.1% of DOM (4.01 g total RDPI/kg $BW^{0.75}$) would be required to maximise total DOMI (36.15 g/kg $BW^{0.75}$) of low quality prairie hay (1.9% CP) fed to non-pregnant beef cows. In contrast, Van der Merwe (2010) found that pregnant beef cows consuming winter grass hay from the false grassveld type (sour veld in the eastern parts of South Africa – 4.91% CP) required 9.36% RDP of DOM (3.63 g total RDP/kg $BW^{0.75}$) to maximise total DOMI (38.66 g/kg $BW^{0.75}$). The 8.07% RDP of DOM in the present trial is similar to the value of 9.36% found by van der Merwe (2010). In a study by Nolte *et al.* (2003), the single slope broken-line model predicted the total daily DOMI as 27.01 g/kg $BW^{0.75}$, with an associated total RDP requirement of 11.6% of DOM (3.30 g/kg $BW^{0.75}$) for sheep fed wheat straw (3.2% CP). The differences in the results could be attributed to differences in forage quality and the type and physiological status of the animal. Wilson & Kennedy (1996) stated that physical characteristics of the fibre particles such as tissue origin, shape, buoyancy, and specific gravity can play a role by affecting comminution, digesta load, digestive weakening, and ease of passage.

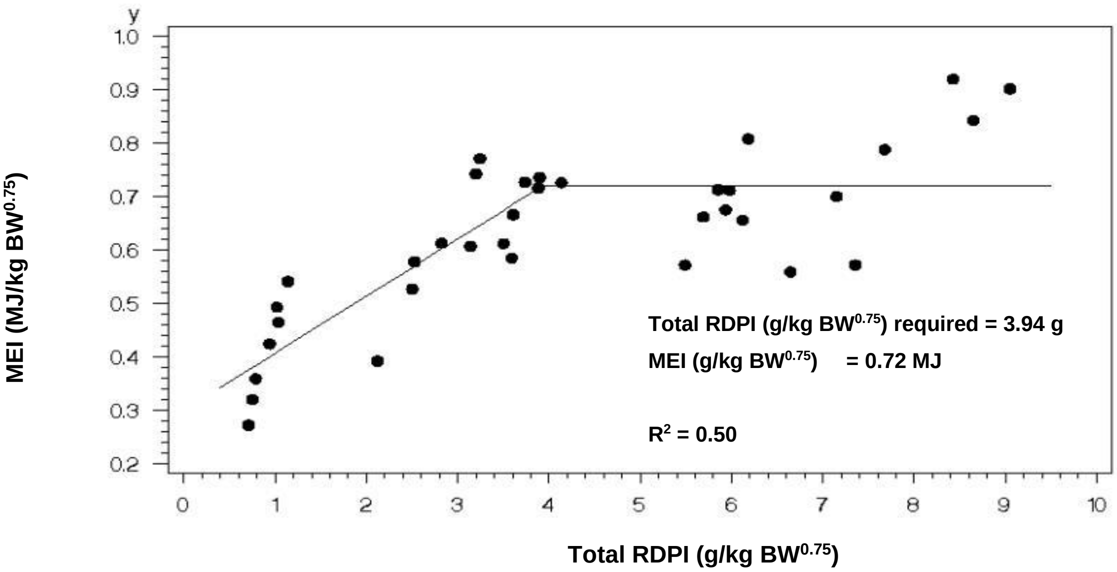

Figure 2 Daily rumen degradable protein intake (RDPI) required to maximise metabolizable energy intake (MEI) of pregnant beef cows using a single slope broken-line model

The single slope broken line model indicted that 3.94 g total daily RDPI/kg $BW^{0.75}$ (297 g supplemental RDP/500 kg per pregnant cow) was required to maximise the MEI from winter grassveld hay to 0.72 MJ/kg $BW^{0.75}$. The latter is equivalent to 76.13 MJ/cow/day, and this corresponds to the NRC (1984) requirements of 76.55 MJ/cow/day for mature (500 kg) pregnant (in the last trimester) beef cows. According to the single slope broken-line model, a moderate relationship (R^2 = 50) existed between RDPI and MEI.

Table 4 Effect of increasing levels of supplementary rumen degradable protein on body mass changes of beef cows consuming low quality grass hay

Item	Supplementary rumen degradable protein					Significance	
	0g	180g	360g	540g	720g	P	[1]Q
Initial mass (kg)	550.57 ± 17.74	526.00 ± 28.38	516.00 ± 10.89	507.14 ± 10.75	485.71 ± 23.32	0.2258	0.9195 *0.0003
Final mass (kg)	500.57 ± 16.26	499.43 ± 28.39	500.29 ± 12.94	480.86 ± 10.35	466.14 ± 23.88	0.6482	0.5221 *0.0129
Mass change (kg)	-50.00 ± 4.05^{a}	-26.57 ± 7.17^{ab}	-15.71 ± 2.56^{b}	-26.29 ± 7.92^{b}	-19.57 ± 5.22^{b}	0.0021	0.0158 *0.1267
Final mass as % of initial mass	90.93 ± 0.65^{a}	94.89 ± 1.50^{ab}	96.90 ± 0.58^{b}	94.90 ± 1.27^{ab}	95.91 ± 1.20^{b}	0.0141	0.0319 *0.1128

[a,b] Row means with different superscripts differ significantly
[1] Q = quadratic, * = R^2 values

The body mass changes of the cows during the experimental period are illustrated in Table 4. The cows in all the treatments experienced a loss of body mass. The body mass loss decreased quadratically with increasing amounts of RDP and was minimised at 3.77 g daily RDPI /kg $BW^{0.75}$. This body mass loss was however substantially less than that of the control group. In agreement, DelCurto *et al*. (1990) found that protein supplementation during gestation minimised body mass loss in mature beef cows grazing tall-grass prairie. Furthermore, these mass changes were recorded over a short period of time and should be interpreted with caution.

Conclusions

According to the multiple comparison test, a statistically significant increase in DOMI and MEI from the low quality hay occurred when the daily RDPI of pregnant cows was increased to 2.80 g/kg $BW^{0.75}$ (297 g total RDPI/500 kg cow/day). Thereafter a non-significant and diminishing increase in energy intake occurred. However, a substantial increase in grass DMI and decrease in body mass loss were observed with a 3.77 g daily RDPI/kg $BW^{0.75}$ (406 g total RDPI/500 kg cow/day). These findings were supported by predictions with the broken-line model that 3.94 g total daily RDPI/kg $BW^{0.75}$ (417 g total RDPI/500 kg pregnant cow/day) was needed to maximise MEI (76 MJ ME/500kg cow/day) from winter forage hay of the northern variation of the *Cymbopogon–Themeda* veld type. This means that 8% RDP of DOM is needed to maximise energy intake and supply in the requirements of beef cows during the last trimester of gestation.

Casein as an RDP source was used in the present study. Therefore, in an effort to reduce costs of supplementary feeding, it is important to investigate the potential to substitute amino acid N with non-protein N (urea) in RDP supplements for pregnant beef cows consuming the low quality forage used in the present study.

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Authors' contributions
HJvdM (late) conceived and designed the study, supervised the implementation of the study, and the interpretation of the data, approved the draft manuscript; MAB (ORCID: 0000-0001-7457-2368) conducted the study, interpreted the analysed data, and drafted the original manuscript; CHMdB (ORCID: 0000-0001-8601-462X) co-supervised the implementation of the study and interpretation of the data, revised the manuscript, and approved the final version.

Conflict of interest declaration
The authors declare no conflict of interest.

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