

Effect of Infrequent Wheat Pasture Supplementation on *In vivo* DM and NDF Digestibility and Nitrogen Retention of Pregnant Beef Cows Consuming Deferred Sorghum

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Abstract: An *In vivo* study was performed using 8 British-breed, eighth-month gestating (420±15 Kg of BW) beef cows to determine the effect of infrequent wheat pasture supplementation on *In vivo* Organic Matter (OM) and Nitrogen Detergent Fiber (NDF) digestibility and Nitrogen Retention (NR). Treatments consisted on an unsupplemented control (Deferred sorghum, DS) and DS plus fresh wheat forage (DSW) offered three times per week. Cows were allotted in a completed randomized design for a period of 17 day. DS intake decreased ($p < 0.05$) with CP supplementation, total OM Intake (OMI) was not different ($p = 0.18$) between treatments while NDF intake was greater ($p < 0.05$) in DS than in DSW. Apparent total-tract OM Digestibility (OMD) was higher ($p < 0.05$) in DSW than in DS and apparent total-tract NDF Digestibility (NDFD) did not differ among treatments. N intake was greater in supplemented than in control cows. Feces and urine N did not differ between treatments ($p = 0.27$) and NR was higher in DSW. NR expressed as percentage of N consumed was not different between treatments. These results suggest that wheat pasture (18.4% CP and 45.6% NDF) can be used as CP supplement for cows consuming low quality forage even if it is offered three times per week.

Key words: Low quality forage, digestibility, protein, nitrogen, cow, supplementation

INTRODUCTION

Feeding low quality forage (Crude Protein, CP <6%) in cow-calf production systems is an usual practice in Argentina and many other regions in the world. Under that condition, CP supplementation to meet animal Metabolizable Protein (MP) requirements is always needed. Requirements could be achieved by supplementing animals with Degraded Intake Protein (DIP) or Undegraded Intake Protein (UIP). The last one is expensive and difficult to provide in extensive systems while DIP supplementation may increase rumen N-NH₃ concentration, allowing ruminal microorganism to meet growth requirements. By increasing bacterial CP synthesis, MP arriving to duodenum is increased and animal requirements could be met. Previous research shows DIP supplementation improve digestibility (Koster *et al.*, 1996; Bohnert *et al.*, 2002a; Currier *et al.*, 2004a), intake (Lintzenich *et al.*, 1995; Currier *et al.*, 2004a; Bohnert *et al.*, 2011) and performance (Bohnert *et al.*, 2002a; Schauer *et al.*, 2005) of animals consuming low quality forages. Another complication in extensive production systems is to implement the supplementation. Decreasing frequency of animals DIP supplementation

have shown minimal effects on performance parameters (Bohnert *et al.*, 2002a; Schauer *et al.*, 2005). However, when supplying great amounts of DIP, the supplementation day may produce N-NH₃ excess that will escape through rumen walls and may be lost as urea in urine excretion contributing to ambient pollution. Furthermore, the amount and kind of supplement fed are important factors to take into account. There are many published works that used high concentration DIP or UIP supplements but none using an annual winter forage (18-20% CP and 40-45% NDF) as DIP supplement. On the contrary, many works in fattening or milk systems used this kind of forage in association with corn silage in order to decrease diet DIP proportion (Bargo *et al.*, 2003; Elizalde *et al.*, 1999; Vogel *et al.*, 1989). We hypothesized that infrequent annual winter forage supplementation improves OM and NDF *In vivo* digestibility because of a higher microbial growth and consequently improves N balance of cows consuming low quality forage. The objective of this work was to evaluate intake, total-tract OM and NDF digestibility and NR of 8 month gestating cows fed deferred sorghum with or without infrequent annual Winter forage supplementation.

MATERIALS AND METHODS

Animals, treatments and procedure: Eight British-breed, multiparous, eighth-month gestating (ultrasound scan determination, 420±15 Kg live body weight) beef cows were used to determine the effect of infrequent wheat pasture supplementation of deferred sorghum on *In vivo* OM and NDF digestibility and NR. The experiment was conducted in the Pasman Experimental Station (Ministerio de Asuntos Agrarios, Buenos Aires Province, 37°11'S, 62°11'W). Experimental treatments consisted on deferred sorghum (*Sorghum bicolor* L. Moench, DS) coarsely chopped (5-8 cm length) and DS plus fresh wheat forage (DSW) offered three times per week. Animals were kept on individual pens (2.0×1.2 m) with cold water always available. They were randomly allocated to one of the two treatments in a completely randomized assay with four replications. The experiment was performed over 17 day, consisting in a 10 day adaptation period and 7 day of data collection. During adaptation period, cows were fed with a basal diet of coarsely chopped (5-8 cm length) DS and *ad libitum* intake was estimated by difference between supplied and remaining forage. A trace mineral-salt mixture (Composition: 62.9% NaCl, 16% Ca, 12% P, 7.5% S, 0.89% Zn, 0.06% Fe, 0.008% Cu, 0.003% Mg, DM basis) was also daily provided in accordance to recommendations. During the following 7 day cows were fed at 0900 and 1500 h, an amount of forage adjusted to a minimum of a 10% refusal rate. Orts were daily removed and weighted before feeding again and intake for each cow determined. Fresh wheat forage of DSW treatment was harvested by a mechanical reaper and fed to animals on Mondays, Thursdays and Fridays at 50:50 wheat to DS proportion. Wheat amount was estimated to supply animal daily requirements and once DS contribution was discounted, total wheat amount was equally partitioned between the three supplementation days of each week. Deferred sorghum in DS and DSW was always offered *ad libitum* accounting for a minimum of 10% refusal. Feeds chemical composition is shown in Table 1. In average diet composition was 5.95% CP, 70.20% NDF and 1.90 Mcal kg⁻¹ ME for DS and 7.4% CP, 67.4% NDF and 1.97 Mcal kg⁻¹ ME for DSW. On the last 5 day of the adaptation period animals were fitted with harnesses and fecal bags that collected both feces and urine altogether. Twice a day, fecal collection bags were removed and replaced on each cow. Total excreta output (feces and urine) was weighted and mixed together for 3 min to homogenize. Two subsamples (approximately 5% wet weight each other) were taken, one for immediate DM determination and the other frozen (-20°C) until further analyses. Apparent total-tract digestibility was

Table 1: Deferred sorghum and wheat pasture nutritional quality parameters (%)

Parameters	DM	CP	WSC	NFD	ADF	ME
Deferred sorghum	50.12	5.95	1.57	70.20	44.20	1.9
Wheat pasture	22.30	18.39	16.92	45.60	23.70	2.5

DM: Dry Matter; CP: Crude Protein; WSC: Water Soluble Carbohydrates; NFD: Neutral Detergent fiber; ADF: Acid Detergent fiber; ME: Metabolizable Energy (Mcal/kg DM)

calculated by difference between the OM and NDF intakes and OM and NDF excreted in the feces. NR was calculated as N intake (g/d) minus N excreted (g/d, feces and urine). NR was also expressed as percentage of N consumed.

Chemical analysis: Daily samples of DS and wheat pasture were composited by period and daily orts were composited by cow on an equal weight basis (5% as-fed). Thereafter, samples were oven-dried (65°C for 48 h) and ground in a Wiley mill to pass a 1-mm screen. Feeds, orts and feces samples were analyzed for OM (AOAC, 1990), NFD (Robertson and Soest, 1981) and acid detergent Fiber contents, using procedures modified for use in Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY) and N content using micro-Kjeldahl methods (AOAC, 1990). Additionally, Water Soluble Carbohydrates (WSC) by the anthrone method (Pichard and Alcade, 1990) on feed samples were performed.

Statistical analysis: Data of apparent total-tract OM and NDF digestibility and NR were analyzed as a completely randomized design using the GLM procedure of SAS (SAS 1985 Inst. Inc., Cary NC). For all parameters, treatment means were compared using Tukey test. Differences were considered significant when $p < 0.05$.

RESULTS

Chemical composition of diets components is shown in Table 1. DS was typically low quality forage (5.95% CP and 70.2% NDF) while winter grass forage used as supplement was characterized by high CP (18.39%) and low NDF (45.6%). It is important to note that although it was not measured in this assay, CP ruminal degradability of this kind of forage is high (Bargo *et al.*, 2003; Elizalde *et al.*, 1999).

DS intake decreased ($p = 0.0002$) with CP supplementation about 27% (Table 2). This was associated with the addition of pasture intake which was 1.91 kg OM/day in average, for supplemented animals. In contrast, total OM Intake (OMI) intake was not different between treatments being 9.83 kg OM/day in average which represents 2.3% of body weight. As designed, CP

Table 2: Feed intake, NDF intake, CP intake and apparent digestibility in beef cows in their two last months of gestation fed deferred sorghum or supplemented with wheat pasture

Items	Treatments			
	DS	DSW	SEM	p-values
OM intake (kg/day)				
Total OMI (Kg/day)	9.94 ^a	9.72 ^a	0.18	0.4230
Total OMI (g/kg BW/day)	23.67 ^a	23.14 ^a		
Deferred Sorghum	9.94 ^a	7.80 ^b	0.19	0.0002
Wheat pasture	0.00 ^b	1.91 ^a	0.03	<0.0001
NDF intake (kg/day)	6.98 ^a	6.35 ^b	0.13	0.0140
NDF intake g/kg BW	16.62 ^a	15.12 ^b		
CP intake (kg/day)	0.59 ^b	0.82 ^a	0.04	0.0004
Digestibility				
OM	51.70 ^b	57.8 ^a	3.02	0.199
NDF	55.10 ^a	60.3 ^a	2.09	0.084

Means in a row with different letter (s) differ ($p < 0.05$). SEM: standard error of the mean; DS = Deferred Sorghum, DSW = DS plus fresh wheat forage 3 times a week

Table 3: Nitrogen retention of eighth-month gestating cows fed deferred sorghum with or without wheat pasture (g/day)

Items	Treatments			
	DS ^a	DSW	SEM	p-values
N intake	94.4b	129.9a	1.62	<0.0001
Feces and urine N	79.1a	89.0a	5.75	0.267
N retention ^b	16.3b	40.9a	5.90	0.026
N retention/N intake	0.16a	0.25a	0.05	0.246

^aDS = Deferred Sorghum, DSW = DS plus fresh wheat forage 3 times a week. ^bN retention = N intake - N output (Fecal + Urine N). Means in a row with different letter (s) differ ($p < 0.05$). SEM: Standard Error of the mean

DSW, being 0.59 and 0.82 kg day⁻¹ for DS and DSW, respectively. On the contrary, NDF intake was greater in DS than in DSW.

Apparent total-tract OMD was higher ($p < 0.05$) in DSW than in DS being 51.7 and 57.8%, respectively. On the contrary, apparent total-tract NDFD did not differ among treatments and was 57.7% in average.

Effects of dietary treatments on NR are shown in Table 3. N intake was 28% greater in DSW than in DS as expected. On the contrary, feces and urine N did not differ between treatments, resulting in a 150% higher NR in DSW. NR expressed as percentage of N consumed was not different between treatments, showing no better efficiency in supplemented animals.

DISCUSSION

When CP is supplemented to ruminants fed low-quality forages (CP <6% and NDF >60%) an increase on forage and total OMI is expected (DelCurto *et al.*, 1990; Beaty *et al.*, 1994; Koster *et al.*, 1996; Bandyk *et al.*, 2001; Bohnert *et al.*, 2011). On the contrary, in the current study forage intake decreased and total OMI was not different when CP was supplemented to animals fed DS (CP 5.6% and NDF 70.2%). Likewise, Salisbury *et al.* (2004) reported a forage intake 14% greater for control weathers than

supplemented ones although total DM Intake (DMI) was 6.4% higher for supplemented weathers. Other researchers (Swanson *et al.*, 2000; Sletmoen *et al.*, 2000; Bohnert *et al.*, 2002a; Currier *et al.*, 2004b) also reported no differences in forage DMI and an increase on total DMI when CP was supplemented. The largest increase on cows forage OM and total OMI reported by Koster *et al.* (1996) occurred at decreasing rates ranging from 180-720 g DIP d⁻¹. Researchers mentioned the inherent fermentability of the diet and the protein requirements of the animal as the probable cause for this limited to response. In this respect, important is to note that the basal forage CP in this work was 1.9%, very much lower than the forage used in our study (5.9%). In the same way, Swanson *et al.* (2000) suggested that the lack of response observed on forage and total OMI may have happened because forage DIP (6.7% CP, 3.9% DIP) was enough to maintain ruminal fermentation, so additional DIP didn't improve ewes forage intake. It has been stated that DIP requirement depend on basal diet fermentability (NRC, 1985) so in this kind of forage where microbial biomass yield is very low DIP requirement is low. In this regards, although CP rumen degradability of DS was not measured, it seems probably that it is adequate to achieve bacterial DIP requirement. Assuming that DIP content of DS is similar to published data, forage DIP contribution would be approximately 410 g day⁻¹ while cows DIP requirement was 413 g day⁻¹, calculated by supposing a microbial efficiency of 8% of TND as NRC recommend for cows consuming low-quality diets. Thereafter, sufficient basal forage CP could be one reason for the lack of increase observed on animal intake.

Another aspect that should be note is the high intakes recorded in this study that were 2.3% of BW for eighth month gestation cows in average. Koster *et al.* (1996) reported 0.6% of BW for control to a maximum of 1.5% in supplemented cows while Sletmoen *et al.* (2000) reported values from 1.1-1.6% of BW for control and supplemented last three month gestation cows. In the same way, predicted intake using NRC (update 2000) equation which takes into account ADF and CP content is 7.84 kg day⁻¹ or 1.87% of BW both higher than recorded in the current experiment. In association with these findings, Bohnert *et al.* (2002a) and Bohnert *et al.* (2011) proposed as a possible explanation for the lack of intake response with CP supplementation the daily NDF Intake (NDFI) Mertens (1985, 1994), Bohnert *et al.* (2002a) suggested that DMI is maximized when NDFI is approximately 12.5 g kg⁻¹ BW and Ferrell *et al.* (1999) estimated a maximum forage NDFI without supplementation of approximately 1.2% BW. Nevertheless, Bohnert *et al.* (2011) in one of the

experiments reported, also found OMI higher than expected ranging from 2.40-2.67% of BW and NDFI of 19.2 g/kgBW/d in average. Likewise, Bohnert *et al.* (2002b) reported intakes of low quality forage from 2.07-2.38% of BW when two CP sources and two frequencies effects were studied. They also reported an increase in NDFI with CP supplementation and suggested it was a result of the high NDF concentration in the supplements (approximately 57% DM basis) which was similar to the supplement use in our study (45.6% DM basis). In the present study, NDFI in unsupplemented cows was 16.6 g/kgBW/d (Table 2) and represents 1.7% BW, both higher than those of the references so intake would not be expected to increase with CP supplementation. Another reason for the decreased observed on forage intake with CP supplementation may be the frequency of supplementation. In the current study CP was supplemented only three times a week and there is evidence that it could affect the CP effect on intake. For example, Beaty *et al.* (1994) and Farmer *et al.* (2001) reported a decreased on forage and OMI as supplementation frequency decreased. Similarly, Bohnert *et al.* (2002a) reported a decrease on straw DMI, total DMI and NDFI from daily to every 3 or 6 day supplementation but in much lesser extent than the increased recorded because of CP supplementation. On the other hand, Farmer *et al.* (2004) reported no effect of supplementation frequency, when steers consuming prairie hay were supplemented daily or on alternative days with increasing amounts of urea in the supplement. Therefore, although frequency may affect intake response the magnitude of frequency effect seems to be smaller compared to CP supplementation effect. In our study, the observed lack of response on intake is more probably resulting from a combination of the above-mentioned factors rather than a principal effect of one.

Apparent total-tract OMD increased 12% when wheat forage was added to DS. Similarly, Bohnert *et al.* (2011) reported an increase in OMD of 20% in C4 and 9% in C3 low quality forage when soybean meal was supplemented to steers and similarly 18% in C4 and 7% in C3 low quality forage when soybean meal was supplemented to weathers. Currier *et al.* (2004a) reported an increased on total-tract OMD and NDFD in contrast with our results were NDFD was not different among treatments. Also, Bohnert *et al.* (2002a) reported an increased on apparent total tract OMI and NDFD with CP supplementation with higher increase in NDFD when UIP was added compared to DIP ($p < 0.05$). These researchers conclude that forage digestibility increase in response to CP supplementation regardless CP degradability or supplementation frequency. Also suggested that the

lower NDFD with DIP supplementation could be associated to the large amounts of DIP offered on the supplementation day that could affect the rumen function. Other researchers have also reported the increase on OM degradability of low quality forage when CP is added (Beaty *et al.*, 1994; Bandyk *et al.*, 2001). It has been suggested that the increase on OM degradability is the result of a great N availability for ruminal microbial grow (Russell *et al.*, 1992). Nevertheless, Currier *et al.* (2004b) reported no differences on apparent total tract OM disappearance neither NDF disappearance from the stomach of low quality forage with the addition of urea or biurets supplement every day or every 2 day but observed that duodenal bacterial N flow increased with CP supplementation. In the same way, Bohnert *et al.* (2002b) noted no effect of CP source neither frequency of supplementation on OM and NDF disappearance from the stomach but also observed higher microbial protein arriving to duodenum in supplements animals. Lintzenich *et al.* (1995) reported that apparent ruminal OM or NDF degradability doesn't increase when steers were supplemented with alfalfa but reported that true ruminal OMD (corrected for bacterial OM) increase with CP supplementation, so as total-tract OMD. A possible explanation for these discrepancies may be the quality of the basal forage. In this regard, CP and NDF content in addition to the kind of respiration pathway (C3 or C4) of the basal forage may affect CP supplementation responses. Anyway, it is generally observed that there is an improvement on nutrient availability just by improve on OM degradability, total tract OMD and/or microbial grow when CP is supplemented to animals consuming low quality forage.

Daily N intake was higher (28%) in control than in supplemented animals in concordance with many other authors that reported higher CP intake because of treatments design and/or because of higher intakes. NR increased with CP supplementation, although it was positive in both, supplemented and unsupplemented cows. Similarly, Bohnert *et al.* (2011) reported N balance of 0.023 and 0.123 g/kg BW/d for supplemented and control animals (overage for C3 and C4 species). It is important to note that both forage species had CP contents similar to DS use in this work (6.3 and 5.7% for low-quality C3 and C4 grass hay). Similar works (Lintzenich *et al.*, 1995; Bohnert *et al.*, 2002a; Currier *et al.*, 2004a) have reported an increase on N balance but they also reported negative controls. The negative balances observed by these authors are probably the result of the smaller CP of the basal forage used in those experiments. It was 2.8% in Lintzenich *et al.* (1995), 5% in Bohnert *et al.* (2002a) and 4% in Currier *et al.*

(2004a), consequently animals had smaller N intakes (g/kg of BW). Fecal and urinary N excretion has been reported to increase in ruminants consuming low-quality forage supplemented with CP (Bohnert *et al.*, 2002a; Currier *et al.*, 2004a). Lintzenich *et al.* (1995) reported more N fecal losses but no increased on urine losses when different forms of lucerne hay was added to low quality forage. In our study fecal and urine losses were collected altogether and contrary to the reports aforementioned, the N content was not different with CP supplementation. In consequence, as supplemented animal consumed more N and excrete the same that controls, N balance was higher. The efficiency of N used expressed as NR/N intake was not different when cows were supplemented with CP. The overall response to CP supplementation indicated that supplemented animals have more NR but have not improve efficiency of N use respect to unsupplemented controls.

CONCLUSION

DS intake decreased when wheat pasture was supplemented, there was a substitution of supplement by forage, so total OMI was not different among treatments. In this work forage intake was maximized before CP was added. Apparent total-tract OMD increased with CP supplementation from 51.7-57.8%, although apparent total-tract NDFD doesn't improve (57.7% in average). The N balance was higher in supplemented animals although they were not more efficient in N use. These results suggest that supplementing cows consuming low quality forage with wheat pasture (18.4% PC and 45.6% NDF) improve nutrients supply by improving total OMD and NR.

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