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Lactation Performance of Multiparous Holstein Cows Fed a Restricted Total Mixed Ration Plus Legume and Grass Hay Mixture

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Abstract: This study was done to evaluate the effects of restricting Total Mixed Ration (TMR) and supplying *Eragrostis curvula* and *Medicago sativa* hay mixture adlib on lactation performance of mid-lactation multiparous Holstein cows. Twenty Holstein cows, averaging 598±73 kg body weight and 100 days in milk were assigned to either a 100% TMR diet (control) or a 75% TMR-restricted diet. Cows on the 75% TMR-restricted diet had adlib access to *E. curvula* and *M. sativa* hay mixture (1:1). The experiment included 2 weeks adaptation period and 4 weeks sampling period. Cows were milked twice daily. Total Dry Matter Intake (DMI), DMI as BW%, daily CP intake and intake of net energy for lactation were higher ($p<0.05$) for cows on the 100% TMR than for cows on the 75% TMR. Cows in 75% TMR consumed 12.5% less total DM and 14.2% less kg DM as of BW% than cows in 100% TMR. Intake of NDF was not affected ($p>0.05$) by treatment. Milk yield, milk fat% and yield (kg day^{-1}) did not differ between treatments and averaged 29.2 kg day^{-1}, 3.70% and 1.08 kg, respectively. Feed efficiency ranged from 1.22-1.37 and tended to be higher ($p<0.10$) with 75% TMR diet. Efficiency of nitrogen and phosphorous utilisation was not affected ($p>0.05$) by treatments. Results suggest that TMR restriction to 75% during mid-lactation does not negatively impact milk production.

Key words: Total mixed ration, restricted TMR, dry matter intake, milk yield, cows, South Africa

INTRODUCTION

Confinement feeding systems are widely used on commercial dairy farms where rations are offered as Total Mixed Rations (TMR) consisting of combinations of concentrates (protein, energy and mineral-vitamin premixes) and forages. Economic condition caused by low milk prices and narrow profit margins challenge dairy farmers to find ways to increase farm profitability in order to stay competitive (Muller and Fales, 1998). In addition, environmentally-friendly practices have resulted in an increasing interest in pasture-based dairy farming.

Pasture-based dairying can be competitive in terms of profitability compared with confinement systems (White *et al*., 2002) but supplemental energy needs to be fed to high producing dairy cows on pasture in order to meet genetic potential for lactation performance (Vibart *et al*., 2008). It was reported that the lower milk production from high producing dairy cows consuming only high quality pasture compared with cows consuming a nutritionally balanced TMR was due to a lower DMI and energy intake (Kolver and Muller, 1998).

Some management challenges when pasture is the only forage include low milk production per cow, low milk fat and protein content, variations in production because of climate conditions, difficulty in budgeting pasture availability and inaccurate estimation of total and pasture DMI (Muller and Fales, 1998). Supplementation of pasture with a TMR may reduce these challenges (Bargo *et al*., 2002). Some of the potential advantages of feeding a partial TMR include the provision of a more uniform ration throughout the year and less chance of rumen digestive problems (Bargo *et al*., 2002).

A number of other studies have also compared the performance of dairy cows on TMR's vs. TMR plus pasture (Soriano *et al*., 2001; Bargo *et al*., 2002). Research data is available where TMR is increasingly supplemented with pasture or where TMR is increasingly replaced by high-quality pasture grazing (Washburn *et al*., 2002; White *et al*., 2002; Bargo *et al*., 2002; Fontaneli *et al*., 2005). Results of the study of Vibart *et al*. (2008) showed that the TMR could be replaced by pasture up to the greatest amount of pasture dry matter intake tested (41%). As far as can be ascertained, no study thus far has compared TMR vs. restricted TMR plus *E. curvula* and *M. sativa* forage hay offered in confinement. *E. curvula* hay is available abundantly at low price in South Africa. Combination of *E. curvula* hay with a high nutritional legume such as of *M. sativa* result in a relatively good quality hay mixture which can be used when pasture is

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not available and especially on confinement. Researchers hypothesized that cows which receive a correctly formulated but restricted amount of TMR with additional *E. curvula* and *M. sativa* hay offered *ad libitum* in mid-lactation would perform as well as cows receiving a TMR *ad libitum* at this stage. A positive relationship exists between dietary protein content and DMI (Schor and Gagliostro, 2001; Bargo *et al*., 2003) which affect nitrogen excretion and milk production (Mulligan *et al*., 2004). Thus when investigating the effect of reducing TMR, it is important to also investigate the impact on nitrogen excreted. From an environmental point of view, it is advisable to decrease nitrogen and phosphorous excretion per milk unit produced. The objective of this study therefore, was to evaluate the impact of partial replacement of TMR by *E. curvula* and *M. sativa* hay mixture on milk production, milk composition, nitrogen and phosphorous utilization by Holstein cows during mid-lactation.

MATERIALS AND METHODS

The study was conducted at the Animal Production Institute, Irene of the Agricultural Research Council of South Africa. Latitude 25.89907 South and longitude 28.20063 East at 1475 m altitude. Average annual minimum temperature is 14°C with mean annual maximum temperature of 24°C.

Twenty multiparous Holstein cows in 2nd and 3rd lactation, averaging 598±73 kg Body Weight (BW) and 100 Days in Milk (DIM) were used in a completely randomized block design. They were assigned to one of ten blocks of two cows based upon parity, BW and previous milk production. The treatments were either a 100% TMR diet or a 75% TMR plus hay diet (restricted TMR). A TMR diet (Table 1) was formulated to meet nutritional requirement of a 600 kg cow at 120 DIM and yielding 35 kg milk day^{-1} with 3.7% fat and 3.2 CP. The TMR was fed *ad libitum* (100% TMR) or restricted to 75% of TMR. Cows on the restricted diet received TMR at 12:00 h and had *ad libitum* access to chopped grass (*Eragrostis curvular*) legume (*Medicago sativa*) hay (1:1) (Table 2). The same chopped forages were used in preparing the TMR. The 75% TMR restriction was calculated from the average of the 2 weeks TMR consumption during the adaptation period.

The cows were individually housed and fed to monitor individual feed intake. Fresh water was continuously available. The cows were exposed to continuous lighting and were milked at 06:00 and 17:00 h daily in a 10 point DeLaval herring bone parlour equipped with an Alpro Herd management system (DeLaval. (Pty.) Ltd., Heilbron, 9650, South Africa). Animal care was consistent with the guide for the care and use of animals in agriculture research and teaching and animal use was approved by the animal ethics Committee of the Agricultural Research Council of South Africa.

Table 1: Ingredient and chemical composition of the TMR diet (DM basis)

Ingredients (DM%)	Values
Commercial dairy meal 19 CP (%)	53.30
Medico sativa hay	16.90
Eragrostis curvula hay	12.70
Whole cottonseed (linted)	8.40
Cane molasses	8.40
Chemical composition	
OM, DM (%)	92.30
Fat, DM (%)	4.20
CP, DM (%)	15.50
RUP, DM (%)	6.90
NDF, DM (%)	42.80
ADF, DM (%)	21.10
ME, MJ kg^{-1} DM	9.90
NE_L, MJ kg^{-1} DM[a]	6.70
Ca, DM (%)	0.75
P, DM (%)	0.37
K, DM (%)	0.96
Mg, DM (%)	0.28

Table 2: Chemical composition of the forage mixture

Ingredients (%)	Values
Lucerne hay	50.00
Eragrostis curvula hay	50.00
Chemical composition	
DM	90.60
CP, DM (%)	11.40
NDF, DM (%)	57.90
Lignin (NDF) (%)	16.26
ADF, DM (%)	43.00
ME, MJ kg^{-1} M[e]	6.33
NE_L MJ kg^{-1} Dm[e]	4.08

[a]Calculated as Metabolizable Energy (ME) using the database of Van der Merwe and Smith (1991) and converted to NE_L as: ME X 0.67 (NRC, 2001)

Measurements and analyses: Animals were adapted to their diets for 2 weeks and measurements and samples collected over 4 weeks. Cows were weighed and scored using a five point BCS scale; very thin and very fat (Wildman *et al*., 1982) at inception and termination of the experiment.

Individual milk yield was recorded daily. Weekly samples of TMR, hay mixture and orts, within cow were collected and pooled by treatment and frozen pending chemical analyses.

Dry matter was determined by oven drying at 60°C for 48 h. Dried TMR were ground and analysed for Organic Matter (OM) by ashing in a muffle furnace at 600°C for 6 h. Crude Protein (CP) was determined according to AOAC (2000) method 968.06 and ether extract according to AOAC (2000) procedure 920.39. Calcium (Ca), potassium (K) and Magnesium (Mg) were determined according to Giron (1973) using a Perkin elmer atomic spectrophotometer. Phosphorus (P) was assayed according to AOAC (2000) procedure 965.17. Neutral Detergent Fibre (NDF) was determined according to Van Soest *et al.* (1991) and Acid Detergent Fibre (ADF) according to Goering and van Soest (1970). Non-Fibre Carbohydrate (NFC) was calculated from the assayed nutrients (Hall, 1998). Dried refusals were only analysed for CP and NDF. The DM, CP and NDF of the forage mixture were determined with the above mentioned methods.

A milk analysis was done at Lacto Lab (Pty.) Ltd., Irene using infrared analyser (4000 Foss Electric, Hillerod, Denmark). Composite milk samples were prepared from consecutive morning and afternoon milking once weekly and analyzed for fat, CP, lactose and Milk Urea Nitrogen (MUN). Faecal grab samples were collected from all cows at approximately 11:00 on day 26 or 27th and dried at 60°C in a forced-air oven for 96 h. The faecal samples were ground to pass a 1 mm Wiley mill screen and analyzed for DM, N (Kjeldahl method) (AOAC, 2000) and Phosphorus (P) was assayed according to AOAC (2000) procedure 965.17.

Urine samples were collected from cows by mechanical stimulation of the vulva, immediately after collecting fecal grab samples. A sub-sample of 15 mL was preserved in 60 mL 0.072 N H_2SO_4 and stored at -20°C pending analysis (Broderick *et al*., 2008) for N and P. Total Manure excretion (Me) was determined using equations of Nennich *et al.* (2005) and urine excretion as described by Nennich *et al.* (2006), respectively based on DMI and MUN:

$$\text{Me, kg day}^{-1} = [\text{DMI}\times 2.63\ (\pm 0.10)]+9.4\ (\pm 2.8) \quad (1)$$

$$\text{Urine excretion, kg day}^{-1} = [\text{MUN}\times 0.563\ (\pm 0.115)]+17.1\ (\pm 2.0) \quad (2)$$

Statistical analysis: Analysis of a Completely Randomized block Design (CRD) with repeated measurements was used to test for differences between treatments. The data was normally distributed with homogeneous treatment variances. Treatment means were separated using Fisher's protected t-test and mean differences considered significant at $p<0.05$ and tendencies were noted at $p\leq 0.10$.

RESULTS AND DISCUSSION

Intake, milk yield, milk composition and efficiency of feed conversion to milk: Dry matter intake and milk production are shown in Table 3 and 4, respectively. Total DMI was higher (p = 0.008) for cows on the 100% TMR than for cows on the 75% TMR as reported by Bargo *et al.* (2002) and Vibart *et al.* (2008) when they compared 100% TMR to restricted TMR plus pasture. Cows on the restricted diet consumed all partial TMR plus 61% of the forage mixture.

Table 3: Dry matter, CP, NDF and NE_L intake of cows on the 100 and 75% TMR plus forage mixture

Diets	Treatments (TMR) 100%	75%	SEM	p-value
Total diet				
Forage:concentrate	30:70	42:58	-	-
Dry matter intake				
TMR (kg day^{-1})	24.80	18.1	2.2	-
Forage mixture (kg day^{-1})	-	3.7	-	-
Total DMI (kg day^{-1})	24.80^b	21.7^a	0.656	0.008
Total forage (kg day^{-1})	7.45^a	9.10^b	0.29	0.002
Forage (BW%)	1.25^a	1.59^b	0.054	0.002
Total DMI (BW%)	4.2^b	3.6^a	0.11	0.008
Crude protein intake				
TMR (kg day^{-1})	3.66	2.66	0.12	-
Forage mixture (kg day^{-1})	-	0.42		-
Total (kg day^{-1})	3.66^b	3.08^a	0.085	0.001
NDF intake			-	
TMR (kg day^{-1})	9.17	6.51	0.31	-
Forage mixture (kg day^{-1})	-	2.14	-	-
Total (kg day^{-1})	9.17	8.65	0.317	0.275
NE_L intake				
TMR (MJ day^{-1})	174	130	-	-
Forage mixture (MJ day^{-1})	-	13	-	-
Total (MJ day^{-1})	174^b	143^a	4.85	0.003

[ab]Means in the same row with different superscripts differ ($p\leq 0.05$)

Table 4: Milk yield, milk composition and efficiency of feed conversion to milk of cows on the 100 and 75% TMR plus forage mixture

Milk production and composition	Treatments (TMR) 100%	75%	SEM	p-value
Milk (kg day^{-1})	29.50	27.80	1.2050	0.36
Milk fat (%)	3.53^a	3.89^b	0.0673	0.01
Milk fat (kg day^{-1})	1.05	1.15	0.0500	0.47
Milk CP (%)	3.26^a	2.95^b	0.0800	0.04
Milk CP (kg day^{-1})	0.98	0.84	0.0400	0.10
Milk lactose (%)	4.53	4.41	0.0500	0.17
MUN (mg dL^{-1})	16.90^a	14.30^b	0.5700	0.01
Efficiency				
ECM[1]	29.80	28.90	1.3500	0.11
FE[2]	1.20	1.33	0.0500	0.10

[ab]Means in the same row with different superscripts differ ($p\leq 0.05$); [1]Energy Corrected Milk: ECM = ((0.327×kg milk)+(12.95×kg fat)+(7.2×kg protein)) (Orth, 1992); [2]Feed Efficiency: FE = ECM/DMI

Intake of the forage mixture by the later group constituted 0.64% of BW and hence, higher (p = 0.002) total forage intake as of BW%. Cows in 75% TMR consumed 12.5% less total DM and 14.2% less kg DM as percentage of BW than cows in 100% TMR. Intake of NDF was not affected by treatment (p>0.05). Intake of ME was higher for cows on the full TMR diet resulting in more (p<0.05) NE_L compared to the restricted diet. Restricting TMR also reduced intake of crude protein (3.7 vs. 3.1). Milk yield (Table 4) did not differ (p>0.05) between treatments and averaged 29.2 kg day^{-1}; also milk fat concentration and yield were similar (p>0.05) averaging

3.70 and 1.08% kg day^{-1}, respectively. Thus, despite the higher DMI and NE_L supply in the 100% TMR treatment, milk yield remained unaffected. Milk protein concentration and yield, respectively were lower ($p \leq 0.05$) and tended to be lower (p = 0.10) for the 75% TMR (Table 4) diet.

The differences in milk protein were attributed to differences in daily protein intake. Reduced total protein intake lowers metabolizable protein available for milk protein synthesis (NRC, 2001). Milk lactose was similar in both treatments and averaged 4.47%. Lactose concentration in milk usually is not affected or is only slightly altered by dietary manipulation (Casper *et al.*, 1990; Kim *et al.*, 1991) which is confirmed in the present study. Milk urea nitrogen was lower (p = 0.001) in the 75% TMR group.

Reduced MUN is typically found with decreased dietary CP and/or decreased by pass protein. Factors that affect MUN include dietary CP (%), dietary CP intake, proportion of RDP and RUP and protein/energy ratio in the diet (Nousiainen *et al.*, 2004; Roseler *et al.*, 1993; Baker *et al.*, 1995).

Energy-corrected milk, however was similar (p = 0.11) between treatments. Feed Efficiency (FE) ranged from 1.22-1.37 and tended (p = 0.10) to be higher in 75% TMR when compared to 100% TMR. Vibart *et al.* (2008) also reported a similar trend when TMR was partially substituted with pasture. Feed conversion efficiencies were 1.54, 1.43, 1.46 and 1.27 with 55, 70, 80 and 100% TMR, respectively in the 1st study and 1.62, 1.54, 1.65 and 1.33 in the 2nd study, respectively. Controversially, Bargo *et al.* (2002) reported entire-lactation gross feed efficiencies (3.5% FCM) of 1.25, 1.23 and 1.37 for lactating cows fed pasture plus concentrate, pasture plus TMR (approximately, 70% TMR and 30% pasture) and TMR, respectively.

Dry matter intake and milk yield were also compared within weeks and results are shown in Table 5 and 6. During week 1, no differences in total daily DMI were observed but DMI tended ($p<0.1$) to be higher in the 100% TMR while it was consistently higher ($p<0.05$) in the 100% TMR group from week 2 until the end of the experimental period. Weekly milk yield was not affected ($p>0.05$) by treatments from week 1 until week 4 (Table 5).

Body Weight (BW) and Body Condition Score (BCS): Body Weight (BW) and Body Condition Score (BCS) changes are shown in Table 7. Body weight change and body weight change as of BW% did not vary; averaging -1.5 kg and -0.25%, respectively suggesting that restricting TMR to 75% had no significant impact on body weight. Contrary, Vibart *et al.* (2008) reported that cows on full TMR lost 15 kg of BW and cows receiving the 55, 70 and 85% TMR plus pasture gained, respectively 19, 18 and 22 kg. Cows on the full TMR, however produced more milk.

Table 5: Total dry matter intake during weeks 1-4 of cows on100 and 75% TMR plus forage mixture

Dry matter intake (kg day^{-1}) (weeks)	Treatments (TMR) 100%	75%	SEM	p-value
1	25.1	22.2	0.995	0.068
2	24.7^a	21.5^b	0.691	0.009
3	24.8^a	21.5^b	0.937	0.047
4	25.3^a	21.4^b	0.933	0.015

Table 6: Milk yield during weeks 1-4 of cows on 100 and 75% TMR plus forage mixture

Milk yield (kg day^{-1}) (weeks)	Treatments (TMR) 100%	75%	SEM	p-value
1	29.5	29.6	1.54	0.95
2	29.9	26.4	1.64	0.17
3	29.6	27.3	1.71	0.36
4	28.7	27.7	0.91	0.47

Table 7: Body weight and body condition score of cows on the 100 and 75% TMR

Parameters	Treatments (TMR) 100%	75%	SEM	p-value
Body weight				
Mean BW (kg)	600.0	575	19.3	0.39
BW change (kg)	4.2	-6.6	5.66	0.21
BW change (%)	0.7	-1.2	0.98	0.18
Energy from BW gain (Mcal kg^{-1})	21.4	-	-	-
Energy from BW loss (Mcal kg^{-1})	-	26.5	-	-
Body condition score				
Mean BCS	2.4	2.225	0.104	0.34
BCS change	0.0	-0.15	0.091	0.34

^{a,b}Means in the same row with different superscripts differ ($p \leq 0.05$)

The 100% TMR diet was formulated to support 35 kg day^{-1} but cows produced 16% less milk than predicted (29.5 kg day^{-1}). Energy from BW changes (Gain or loss) was reported (Table 7) as either extra energy required or added to that provided by diet, depending on BW gain or loss throughout the study, respectively (daily BW gain = 5.10 Mcal kg^{-1} BW whereas, average daily BW loss = 4.02 Mcal kg^{-1} BW added to that provided by dietary intake) (NRC, 2001). Results indicate that while cows in 100% TMR had 21.4 Mcal kg^{-1} (0.76 day^{-1}) surplus energy to replenish body reserves, cows in 75% TMR have mobilised a total of 26.5 Mcal kg^{-1} (0.94 day^{-1}) from reserve to support milk production. The difference in DMI appeared to be the major factor responsible for a lower energy supply in the 75% TMR group.

Nitrogen and phosphorous excretion: Nitrogen (N) and Phosphorous (P) excretion data are shown in Table 8. Effect of restricting TMR on N and P utilization was

Table 8: Urinary and faecal Nitrogen (N) and Phosphorous (P) excretion of cows on the 100 and 75% TMR plus forage mixture

	Treatments (TMR)			
Excretions	100%	75%	SEM	p-value
Nint (g day^{-1})	585^a	493^b	13.984	0.001
Pint (g day^{-1})	91.96^a	76.95^b	2.2300	0.000
Urine (kg day^{-1})	22.06	21.46	0.1990	0.204
Faeces (kg day^{-1})	9.03^a	7.93^b	0.1970	0.008
Faecal N (g day^{-1})	231	199	6.7840	0.190
Urinary N (g day^{-1})	212	166	14.800	0.120
Total Ne (g day^{-1})	443^a	368^b	17.500	0.003
Faecal P (g day^{-1})	66.2^a	54.0^b	2.7840	0.050
Urinary P (g day^{-1})	1.2	1	0.1550	0.450
Total Pe (g day^{-1})	67^a	55^b	2.7840	0.040
Ne/Nint (%)	75.78	75.49	2.9400	0.960
Pe/Pint (%)	73.5	72.2	0.0130	0.600

$^{a, b}$Means in the same row with different superscripts differ (p<0.05); Ne: Nitrogen excretion; Pe: Phosphorous excretion; Nint: Nitrogen intake; Pint: Phosphorous intake

evaluated with reference to total dietary intake. Intake of both N and P were lower (p = 0.001) in 75% TMR. Faecal DM was higher (p<0.05) with 100% TMR. Estimated mean excreted urine in 100% TMR (22.1 kg day^{-1}) and 75% TMR (21.5 kg day^{-1}) were similar to data reported by Sannes *et al.* (2002) (21.8-25.7 kg day^{-1}) and Broderick (2003) (20.8-27.3 kg day^{-1}) entailing metabolic stability. Nennich *et al.* (2005) reported a mean urine excretion of 24.1 kg day^{-1}. Urinary and faecal N did not vary (p>0.05) between treatments but total Ne was higher (p<0.05) in 100% TMR. In the study of Hristov *et al.* (2004), high Nitrogen intake did not have effects on fecal and urine N, as observed in this study but controversially, total N excretion was not affected. Kebreab *et al.* (2002) reported a greater and exponential effect of Nitrogen intake on urinary nitrogen with limited effects on faecal N excretion. From an environmental point of view, it is advisable to decrease N excretion per milk unit produced. In this sense, improved milk production decreases the partial contribution of maintenance N requirements (Rotz, 2004) which directly helps to improve the N utilisation efficiency and decrease N excretion per litre of milk (Arriaga *et al.*, 2009).

Intake of P, faecal P and total P were higher (p<0.05) in 100% TMR but no differences in Urinary P were observed. Proportion of N and P excretion relative to N and P intake (Ne/Nint and Pe/Pint) did not differ (p>0.05) between treatments and averaged, respectively 75.6 and 72.8% suggesting that restriction of TMR did not significantly affect the efficiency of N and P utilisation.

CONCLUSION

Partial substitution of TMR with *M. sativa* and *E. curvula* hay supplied adlib lowered total DMI, crude protein intake and NE_L. Milk yield and feed efficiencies were similar in both treatments although, daily milk production on 75% TMR was about 1.7 kg less then the full TMR, feed efficiencies were better on the restricted diet. Results suggest that restricting the TMR to 75% does not significantly impact milk production in mid-lactation. However, further studies are necessary to determine the long term effects of restricting energy and N intake during mid-lactation on subsequent lactation and longevity.

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