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Effects of intake restriction and realimentation on diet digestion and ruminal fermentation by growing lambs

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A total of 15 lambs weighing 41.6±1.34 kg were used in this experiment to determine the effects of intake restriction and realimentation on nutrient digestibility and ruminal fluid characteristics. During the preliminary and first collection periods, lambs were assigned randomly to one of the three treatments, viz. *ad libitum* intake, 0.90 and 0.80 of *ad libitum* intake. During the second collection period (realimentation period), all lambs were fed *ad libitum*. Total tract digestibility of DM, NDF and ADF were not affected by restriction level, whereas digestion of CP increased ($P<0.01$) for the restricted lambs compared with *ad libitum*. During restriction, fecal N excretion by the 0.80 *ad libitum* was lesser ($P<0.01$) than *ad libitum* lambs. Urinary N excretion was similar between *ad libitum* and the restricted fed lambs. Ruminal pH and ammonia N were similar for both restricting intakes at 2h and 4h post feeding compared with *ad libitum* intake. Total concentration of VFA was lower ($P<0.01$) for 0.80 *ad libitum* than for *ad libitum* at 2 and 4h postfeeding. In realimentation period, no effect was observed in relation to previous feed restriction on pH, ammonia-N and VFA concentrations. The results concluded that a 10 or 20% intake restriction followed by realimentation lead to improvements in ADG and these changes could not be attributed to changes in digestibility or ruminal characteristics.

Keywords: limit-feeding, Realimentation, Digestibility, Nitrogen balance

INTRODUCTION

Possible strategies to reduce the cost of sheep production in Saudi Arabia include restricting feed intake to a level less than *ad libitum* followed by realimentation. Benefits associated with intake restriction and realimentation include improved feed efficiency (Schmidt *et al*., 2005; Al-Selbood, 2009; Abouheif *et al*., 2013), reduced feed costs (Loerch and Fluharty, 1998), improved gain (Schmidt *et al*., 2005; Clark *et al*., 2007; Abouheif *et al*., 2013) and reduced maintenance energy requirements (Kamalzadeh *et al*., 2009). The response varies according to the pattern of restriction and realimentation and stage of development of the lamb (Al-Selbood 2009).

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Feed restriction has been shown to improve digestibility of roughage-based diets (Tyrrell and Moe, 1975). The effects of intake restriction of mixed diets and high concentrate diets have not been researched extensively. Galyean *et al.* (1979) found improved digestibility of diets containing 84% corn when intake was restricted 35%. Murphy *et al.* (1994a) reported increases in DM digestibility in 92% concentrate diet. These researchers postulated that improved nutrient digestibility might contribute to an improvement in feed efficiency. However, Hart and Glimp (1991) and Murphy *et al.* (1994a) reported decreases in DM digestibility when intakes were restricted. This suggests that other mechanisms may be involved in the improvement in feed efficiency with restricted feeding. The objectives of this research were to determine the effects of restricted feeding on 1) nutrient digestibility, 2) nitrogen balance and 3) ruminal fluid characteristics in growing Najdi ram lambs fed high concentrate diet.

MATERIALS AND METHODS

A total of fifteen Najdi ram lambs weighing an average body weight of 41.6±1.34 kg and of approximately 6.5 months old, were selected for this study. The experimental protocol regarding the care and handling of lambs, had been approved by the Ethics Committee of the king Saud University, Riyadh, Kingdom of Saudi Arabia. Lambs were purchased from a local farm; upon arrival, lambs were individually weighed, identified, vaccinated against endemic infectious diseases, injected against internal and external parasites and a recommended dose of vitamins AD_3E injection was given. At the commencement of the metabolic trial, lambs were divided into three groups of similar average live weight. In each group, five lambs were randomly and individually confined in false-bottom metabolic crates to facilitate separate collection of total feces and urine. The trial consisted of a preliminary period of 21 days followed by two consecutive collection periods of 10 days each. During the preliminary and first collection periods (restriction period), each group was assigned randomly to one of the three feeding treatments, viz. *ad libitum* intake, and 0.90 and 0.80 of *ad libitum* intake. Feeding levels of restricted groups were calculated by determining the average DMI of the lambs with *ad libitum* access to feed the previous week and multiplying that average by 0.90 and 0.80 to determine the amounts of feed to offer to lambs in the 0.90 and 0.80 restriction groups, respectively. During the second collection period (realimentation period), all groups of the three feeding treatments were fed *ad libitum*. All lambs were not removed from cages throughout the experiment.

All groups were fed once daily at 09:00 am after discarding the refusals from the previous day; refusals were weighed, sampled for DM determination before discarding. The feeding diet was formed as a pelleted total-mixed ration with a ratio of 75% concentrate: 25% alfalfa hay. Pellets were randomly subsample immediately prior feeding; samples were then composited across the feeding trial period and subsequently ground to pass a 1-mm screen. Feed composites were dried in a forced-air oven at 65 ºC until they attained a constant weight before chemical analyses. The chemical composition (DM basis) was 14.53% CP, 1.16% EE, 24.91% NDF, 14.22% ADF, 0.54% Ca, 0.31% P, and 7.46% ash.

Feed intake of the lambs was recorded daily and live weight at the onset and the termination of each collection period were recorded. Feces voided were collected before feeding in the morning, weighed and a 10% aliquot of total feces was dried at 65 °C for 24h. The dried samples were ground through a 1-mm screen and stored for later analyses. Total daily urine outputs of each lamb was collected in a plastic bucket containing 100ml 6N HCl to prevent nitrogen losses, recorded and a 10% aliquot was sampled. At the end of each collection period, samples of feces and urine of each lamb were composited for chemical analyses. In addition, on the last day of each collection period, rumen fluid was collected via a stomach tube from each animal at 0, 2 and 4h post feeding for measurement of pH values, and ammonia-N and total volatile fatty acids VFA concentrations.

Samples of the diet and feces were analyzed for CP, EE, ash, and moisture according to AOAC (1995). NDF and ADF were determined according to Van Soest *et al.* (1991). VFAs were measured by gas chromatography (model 404, Philips). Ammonia was determined by the distillation method using MgO (AOAC, 1995). Feeding performance, digestibility and nitrogen retention data were analyzed by ANOVA using the GLM procedures (SAS, 2006) according to the following model:

$$Y_{ij} = \mu + \rho_i + e_{ij}$$

where Y_{ij} is the j^{th} observation of the i^{th} restriction level, μ is the common mean, ρ_i is the effect of i^{th} restriction intake level, and e_{ij} is the random error. Data collected at various times for ruminal pH, ammonia-N concentration and total VFA concentration were analyzed by a repeated measurement model. Duncan's multiple range test was used to test for significant differences between means.

RESULTS AND DISCUSSION

The average actual dry matter intakes (DM) during the feed restriction period for the 0.90 and 0.80 *ad libitum* groups were 0.914 and 0.787 of the *ad libitum* intake, respectively. The *ad libitum* DM intake was similar to that predicted by the NRC (1987) (85.5 vs 86 $gkg^{.75}$). The smaller DM intake of the groups submitted to feed restriction resulted from the small amount of feed offered, and its intake was regulated as a function of the DM ingested by the *ad libitum* control

Table 1. Effect of feed restriction and realimentation on digestibility coefficients (%) and nitrogen utilization in Najdi lambs.

Item	Restriction				Realimentation			
	Control	0.90 ad libitum	0.80 ad libitum	SEM	Control	0.90 ad libitum	0.80 ad libitum	SEM
ADG, g d^{-1}	236[a]	218[b]	172[c]	10.13	224[b]	278[a]	269[a]	8.13
DM intake, g d^{-1}	1548[a]	1415[b]	1218[c]	44.33	1550	1478	1529	54.73
Coefficient, %								
DM	71.9	73.5	74.6	1.11	71.9	72.1	70.3	0.55
CP	61.0[c]	65.3[b]	68.2[a]	1.35	61.4	61.0	59.4	0.71
EE	86.6	87.1	86.2	0.68	78.5	86.5	84.2	0.73
NDF	56.9	57.6	59.3	1.75	53.5	53.6	52.8	0.80
ADF	53.1	57.5	56.7	1.51	56.5	55.9	52.3	1.11
Nutritive value								
DCP, %	9.1	9.3	9.5	0.19	8.9	8.8	8.6	0.10
DE, Mcal kg^{-1}DM	3.18	3.25	3.29	0.09	3.19	3.20	3.14	0.05
Nitrogen utilization								
N intake, g d^{-1}	35.7[a]	32.6[b]	28.1[c]	1.02	35.7	34.1	35.3	1.26
Fecal excretion, g d^{-1}	13.3[a]	11.6[ab]	9.5[b]	0.71	14.6	13.3	14.3	0.63
Urinary excretion, g d^{-1}	12.6	12.0	12.2	0.58	11.7	11.9	11.8	0.52
N retained (% intake)	27.4[a]	27.6[a]	22.8[b]	2.54	26.3	26.1	26.0	1.68

[a,b,c] Means in the same row within treatment bearing different superscripts differed (P<0.01).

group. Average daily gain (ADG) influenced significantly (P<0.01) by the applied feed restriction levels (Table 1). The ADG decreased (P<0.01) by 7.6 and 27.1% for the 0.90 and 0.80 *ad libitum* fed groups as compared with the *ad libitum* control group, respectively. Al-Selbood (2009) and Abouheif *et al*. (2013) have reported that the decrease in ADG during feed restriction is a function of the plane of nutrition, thereby resulting in inadequate intake of nutrients required to sustain normal growth and development. In addition, Neto *et al*. (2011) reported that the growth of an animal could be delayed if any nutrient in the diet is missing, especially if energy and protein availability limit weight gain.

Apparent total tract digestibility of DM, NDF and ADF were not affected (P>0.01) by feeding restriction levels. The non-significant difference between *ad libitum* fed group and feed restricted groups is consistent with the results reported in literature for DM (Reinhardt et al., 1998) and NDF and ADF digestibility (Al-Selbood, 2009). Hart and Glimp (1991) found no differences in diet digestibility with levels of intake restriction less than 70% of *ad libitum* intakes, whereas more severe restriction to higher than 70% of *ad libitum* resulted in increased digestibility coefficients for most dietary constituents. Galyean *et al*. (1979) found that restricting the intake of an 84% corn diet resulted in increased ruminal digestibility, whereas total tract digestion was not affected. In contrast to the latter findings, Murphy *et al*. (1994b) reported that increases in total tract digestibility with restricted feeding were due to lower gut digestion.

Apparent total tract digestion of CP increased (P<0.01) 7 and 11.8% for the lambs receiving 0.90 and 0.80 *ad libitum* intake, respectively, compared with control lambs with *ad libitum* access to feed. The increased digestibility of CP observed for the restricted feeding groups could be attributed to the higher ruminal pH (Table 2) observed on 0.90 and 0.80 *ad libitum* levels. Similar findings were reported by Colucci *et al*. (1989) and Kamalzadeh and Aouladrabiei (2009) who found that digestion of CP in high concentrate diet was higher at low than at high intakes in sheep; this is probably due to an increased efficiency of feed utilization as a result of decreased rumen feed passage. In addition, the improvement in CP digestibility by the 0.80 *ad libitum* was probably due to the reduction in fecal nitrogen output expressed as a percentage of nitrogen intake (33.8 vs 37.2%) compared with *ad libitum* group. Improvements in apparent CP digestion in the present study are in agreement with data reported by Murphy *et al*. (1994b) who found that fecal N excretion decreased linearly with restricted feeding; the reductions in fecal N excretion led to linear increases in apparent total tract N digestion.

Nitrogen intake decreased significantly (P<0.01) by increased level of feed restriction; the decreased N intake resulted from the smaller amounts of ingested DM by the restricted lambs as compared with *ad libitum* control lambs.

Table 2. Effect of feed restriction and realimentation on rumen liquor traits in Najdi lambs.

	Restriction				Realimentation			
Item	Control	0.90 ad libitum	0.80 ad libitum	SEM	Control	0.90 ad libitum	0.80 ad libitum	SEM
Rumen liquor, pH								
0 h	6.14^{A}	6.33^{A}	6.35^{A}	0.11	6.32^{A}	6.27^{A}	6.35^{A}	0.19
2 h	5.73^{B}	5.88^{B}	5.81^{B}	0.16	5.61^{B}	5.41^{B}	5.50^{B}	0.12
4 h	5.54^{B}	5.64^{B}	5.43^{B}	0.17	5.51^{B}	5.44^{B}	5.42^{B}	0.13
Total VFA (mM)								
0 h	25.3^{C}	24.7^{B}	24.1^{B}	3.66	26.2^{B}	27.1^{B}	29.1^{B}	3.85
2 h	46.2^{aB}	42.6^{abA}	39.1^{bA}	4.07	49.3^{aA}	50.4^{aA}	48.6^{aA}	4.56
4 h	52.7^{aA}	49.3^{abA}	41.8^{bA}	4.62	50.5^{aA}	48.0^{aA}	49.4^{aA}	4.28
Ammonia nitrogen (mg/100ml)								
0 h	15.3	13.8	13.1	2.87	15.2	14.7	14.2^{B}	3.86
2 h	18.3	16.7	16.3	3.03	17.4	18.0	18.1^{AB}	4.11
4 h	17.7	15.9	16.2	3.15	19.9	18.6	19.7^{A}	4.48

^{a,b} Means in the same row within treatment bearing different superscripts differed (P<0.01).
^{A,B,C} Means in the same column bearing different superscripts differed (P<0.01).

During feed restriction, fecal nitrogen excretion by the 0.80 *ad libitum* lambs was lesser (P<0.01) than that by the *ad libitum* lambs. Conversely, fecal N by the 0.90 *ad libitum* lambs was similar to both *ad libitum* and 0.80 *ad libitum* lambs. Over both levels of feed restriction, fecal N output as percentage of N intake by the 0.90 (P>0.01) and 0.80 (P<0.01) *ad libitum* were reduced 4.6 and 9.4% relative to *ad libitum* lambs, respectively. This is in line with Al-Mamun *et al*. (2007) and Kamalzadeh and Aouladrabiei (2009) who found that fecal N loss was significantly lower in limited-fed rams than control ones. Clark *et al*. (2007) reported that when cattle were submitted to a 20% reduction in DM ingestion they reduced the normal sustainable ruminal fermentation and altered N losses through the feces. According to Murphy *et al*. (1994b), N excretion in the feces is directly related to the N ingestion by the animal, and one of the most effective strategies to reduce N excretion is to manipulate its dietary intake. Therefore, the implementation of feed restriction strategy may be an efficient tool to reduce environmental pollutions by reducing N in the feces without altering animal's performance. Urinary N excretion was similar (P>0.01) between *ad libitum* and the restricted fed lambs. Nitrogen retained as percentage of N intake by 0.80 *ad libitum* lambs was lesser (P<0.01) than those by control and 0.90 *ad libitum* lambs, whereas N retained percentage by control was similar (P>0.01) to 0.90 *ad libitum* lambs. The reduction value for N retained percentage by 0.80 *ad libitum* lambs was 16.8% compared to *ad libitum* lambs. Pereira *et al*. (2007) and Taylor-Edwards *et al*. (2009) reported that smaller N ingestion resulted in smaller N retention in the animal. Generally, a positive N retention was an indication that protein was retained in the lamb's body, resulting conditions in which there was no weight loss in the lambs when energy requirements were met (Neto *et al*., 2011). This finding does not confirm the data of Kamalzadeh *et al.* (2009), who found a negative N balance in feed restricted sheep on low quality roughage compared with control. However, this discrepancy was probably related to the feeds quality differences from the latter study and this trial.

During the realimentation period, ADG increased (P<0.01) in the previously feed restricted lambs relative to *ad libitum* fed lambs; the 0.90 and 0.80 *ad libitum* lambs averaged 22.1% higher daily gain than control lambs. This is in line with results of Mahouachi and Atti (2005) and Abouheif *et al*. (2013). However, this superior body gain could not be attributed to DMI because intake values were not different (P>0.01) between the previously restricted and *ad libitum* groups, but possibly due to the better feed efficiency of the realimented lambs and/or the decreased heat production during the restriction and its continuation during refeeding (Yambayamba *et al*., 1996). Homem *et al*. (2007) reported that during the compensatory growth, the animal's metabolism continues to adjust to low food ingestion while the animals are not restricted; the base energetic metabolism of the animal remains low and increases slowly, adjusting to the new regimen. Thus, energy and protein use become more efficacious while the energetic needs for growth remain low, which could explain the greater weight gain in these animals. Similarly, Kamalzadeh *et al*. (2009) found that sheep subjected to feed restriction reduced their energy need for maintenance by about 29% compared to the control; after realimentation, these reduced maintenance requirements during restriction only persisted at the initial stages of realimentation and temporarily resulted in comparatively more energy for gain. The compensatory growth effect on nutrients digestibility and N utilization has been observed in several studies (Al-Selbood, 2009; Neto *et al*., 2011). In the present study, it was observed that DMI, digestibility coefficients and nitrogen utilization by the realimented lambs were similar (P>0.01) to the *ad libitum* lambs. This was expected because the lambs ingested similar amounts of diet.

It might be expected that decreases for substrate available for fermentation when intake is restricted would lead to higher ruminal pH. However, means ruminal pH were similar (P>0.01) for both restricting intakes at 2h and 4h postfeeding compared with *ad libitum* intake (Table 2). Similar findings have been reported by Hart and Glimp (1991), Murphy *et al*. (1994a,b) and Clark *et al*. (2007) that animals limit-fed high concentrate diets had similar ruminal pH as animals fed high concentrate diets *ad libitum*. Ruminal pH in *ad libitum* and restricted-fed groups was reduced (P<0.01) with time after postfeeding by an average of 7.3% at 2h postfeeding and remained unchanged (P>0.01) up to 4h postfeeding. In realimentation period, no effect was observed (P>0.01) in relation to the previous feed restriction level on pH values in comparison to control lambs.

Ruminal ammonia concentrations were similar (P>0.01) for all levels of restriction and post feeding times during both restriction and realimentation periods. The lack of change in ruminal ammonia concentration with level of intake is consistent with the findings of Hart and Glimp (1991). On the other hand, Clark *et al*. (2007) found that ruminal ammonia concentration was greater for the 80% restriction fed cattle than that of *ad libitum* cattle at 0.25 and 4h post feeding but similar between treatments at 8h through 24h post feeding. Murphy *et al*. (1994a) found that ruminal ammonia concentrations decreased with increasing level of intake and that concentrations throughout the day increased linearly with restricted feeding. It seemed that lambs fed on both levels of restriction had sufficient energy content in their meals, which stimulated microbial synthesis to utilize available ruminal ammonia levels as those fed the *ad libitum* level. This probably indicated by the smaller fluctuations in ruminal pH.

Total ruminal concentration of VFA was affected (P<0.01) by level of feed restriction; concentration of VFA was lower for 0.80 *ad libitum* than for *ad libitum* feeding levels at 2 and 4h post feeding. Clark *et al.* (2007) found that steers assigned to *ad libitum* treatment had greater total ruminal VFA concentration than the 80% feed restricted steers, in contrast with Galyean *et al*. (1979), Hart and Glimp (1991) who observed minimal effects of level of feeding on total concentration of VFA. In this trial, it is presumed that this effect was related to the availability of the substrate in the rumen; the fermented substrate appeared to be greater by *ad libitum* fed lambs than by lambs restricted to 80% of *ad libitum* feeding level. In realimentation period, no effect was observed (P>0.01) in relation to the previous feed restriction level on total concentration of VFA values in comparison to *ad libitum* lambs.

CONCLUSIONS

The results of this trial concluded that mild feed restriction is a viable alternative to *ad libitum* feeding; a 10 or 20% reduction in DMI followed by realimentation lead to improvements in ADG by DMI-restricted lambs, but have no effect on most nutrients digestibility. Therefore, the mechanism for improved ADG with restricted intake must not lie on changes in diet digestibility or ruminal characteristics. Possibly, a reduction in the size of liver (Al-Selbood, 2009; Abouheif *et al*., 2013) reduces the maintenance requirement of the lambs and leaves more energy for gain. The mechanism can be speculated on, but still to be elucidated.

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