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Effect of three energy levels on body weight change, nutrient utilization, rumen biochemical profiles and semen quality in rams

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ABSTRACT

In the present experiment, 21 rams of uniform body weight and age (~ 1 year) were equally divided into 3 groups to find out the effect of 3 levels of energy, viz. control, low and high energy on growth, nutrient utilization, rumen biochemical profiles and semen quality. Body weight gain increased significantly compared to control and low energy. FCR improved significantly in high energy supplemented groups. Intake of DDM, DOM and TDN were significantly higher in high energy and control groups as compared to low energy group, with similar intake of CP and DCP in all groups. N-balance (g/d) was significantly higher in high energy group as compared to low energy group. Fractionation of VFA revealed a shift in propionate with narrower acetate to propionate ratio in high energy group favoring more body weight gain. Significantly higher semen volume (ml), mass activity (0–5 score) and progressive forward motility (%) were observed in optimum energy group compared to low energy group with no added benefit in high energy supplemented group. It is concluded that energy rich diets tend to increase weight gain of young rams with a shift in rumen volatile fatty acid concentration to propionate side. Nutritional strategies for feeding rams primarily reared for semen production have to be formulated critically as supplemented energy is being diverted for body weight gain rather than improving semen quality.

Key words: Digestibility, Energy level, Growth, Semen quality, Sheep, Volatile fatty acids

Under traditional feeding systems, without proper feeding inputs, Indian sheep can achieve 40–50 g ADG in active phase of growth (Singh and Karim 2003). Various feeding strategies were attempted to increase the energy level of ration (Dutta *et al*. 2008). Increasing dietary ME level improved final weight, ADG, FCR in lambs whereas an increase in CP level up to 14% had positive effect (Kioumarsi *et al.* 2008). Energy is one of the important factors responsible for proper utilization of nutrients and thereby the productivity of an animal (Hosseini *et al.* 2008). Rams are fed high energy diets during breeding season, performance testing schemes and also during preparation for shows and sales (Fourie *et al*. 2004). Coulter *et al.* (1997) demonstrated the detrimental effects of high energy diets on the fertility of young bull in the form of lower semen quantity and quality. In this study, energy level of diet was manipulated by varying the proportion of maize grain, soybean meal and wheat bran in concentrate mixture and was offered to sheep in different quantities along with ragi (*Eleucine corocana*) straw. We hypothesized that an increase in energy might result in efficient utilization of rumen ammonia for improved microbial protein synthesis in rumen. A decrease in rumen pH is associated with increased ruminal digestibility of energy which in turn may reduce solubility and degradability of protein sources (Loerch *et al.* 1983). Hence the present study was conducted to assess the effect of different energy levels on nutrient utilization, rumen biochemical parameters and subsequent semen quality in rams.

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MATERIALS AND METHODS

The required quantities of feed ingredients finger millet straw, maize grain, soybean meal, wheat bran, corn starch, mineral mixture and salt were procured from a single source for the entire duration of the experiment. Maize grain and soybean meal were ground in hammer mill and mixed with wheat bran, corn starch (in case of high energy concentrate mixture), mineral-vitamin premix and salt were mixed in horizontal mixer to ensure proper mixing. Three types of concentrate mixture were prepared. Different concentrate mixtures and chaffed finger millet straw were offered to rams in different treatment groups at fixed quantities to ensure maximum consumption.

Rams (21) of uniform body weight (22.17±0.37 kg) were equally divided into 3 groups of 7 each in a completely randomized design and were housed in a well ventilated shed having provision of individual feeding and watering. Rams were fed individually and reared under hygienic and uniform managerial conditions throughout the experiment of 100 days. They were dewormed using broad spectrum anthelmentic (alebendazole) @ 10 mg per kg body weight at the beginning of the trial.

All the rams were fed individually in cemented feeding troughs. Rams in group 1 were offered concentrate mixture and finger millet (*Eleucine coracana*) straw as per standards (ICAR 1985) and defined as control. Rams in groups 2 and 3 were offered concentrate and finger millet straw in fixed quantities and termed as low energy (20 % lower than control) and high energy (20 % higher than control). At the offered intakes it was anticipated that rams in all groups received similar amount of CP and 3 levels of energy. The feeding sytem is conventional type involving separate feeding of concentrate and roughage. Hence, accurate control of nutrient intakes like CP and energy was not possible. A metabolism study with a 6-day collection period was conducted on 7 rams from each group for total collection of faeces and urine. This was preceded by a 2-day adaptation period of the rams for faecal and urine bags. Daily feed offered, residues left, faeces voided and urine excreted was recorded on a 24 h basis. Besides feeds and residues, aliquots of faeces were dried at 105°C for 24 h to determine DM. The dry samples of the 6-d collections were pooled for later chemical analysis. Daily fresh samples of faeces (0.005) were preserved with 1: 4 sulphuric acids for N assay. Similarly, aliquots of urine (0.01) were preserved for estimation of N.

About 30 mL of rumen liquor was collected from all animals for 3 consecutive days at 4 h post-feeding through a stomach tube using a vacuum pump. Rumen liquor was strained through 4-layered cheese cloth, and stored in a deep freeze (–20 °C) after adding a drop of mercuric chloride (10%) as a preservative till analyzed.

Samples of feed, faeces and residues were analysed for proximate principles CP, EE, CF and total ash (AOAC 1995). Urinary N content was measured by Kjeldahl method (AOAC 1995). The pH of rumen liquor was recorded promptly using a pre-calibrated digital pH meter. For estimation of individual volatile fatty acids and their proportions, 1 ml rumen liquor and 25% metaphosphoric acid was allowed to stand for 30 min at room temperature and then centrifuged at 5900 g for 15 min. The clear supernatant (10 µl) was used for analysis. Volatile fatty acids (VFAs) were estimated using a gas chromatograph equipped with a flame ionization detector (Cottyn and Boucque 1968). Peaks were identified by comparison with standards i.e. 0.1 % acetic acid, propionic acid and butyric acid. Nitrogen fractions in rumen liquor like ammonia-N (Conway 1957), total-N and TCA ppt.-N (AOAC 1995) were determined.

Semen was collected from all the rams 60 days after the start of experimental feeding with the aid of an electro ejaculator. The ejaculated volume (ml) was recorded directly from the calibrated collection tube. The semen was kept in the warm water bath (37°C) and the mass activity (0–5 score) and individual progressive forward motility (per cent) was assessed (Roberts 1971) within 30 min of the ejaculation. The mass activity was evaluated on a drop of a semen kept on a warm slide and the samples were scored on a scale of 0 (no swirl waves and eddies) to 5 (very fast waves and eddies) by observing under phase contrast microscope fitted with stage warmer. An aliquot (5 µL) of the diluted semen samples in phosphate buffered saline was placed on a pre-warmed glass slide (37°C), covered with a cover slip and per cent progressive forward motile spermatozoa was recorded by 2 independent observers and the values were averaged for each ejaculate.

Data on body weight change, intake, digestibility and rumen parameters was subjected to one- way analysis of variance (Snedecor and Cochran 1994). The statistical software SPSS 8.0 was used for analysis of data and analysis variance assuming for independent constant variance structure with post-hoc Tukey to find the pair-wise significance between treatments.

RESULTS AND DISCUSSION

Initial body weight (kg) of the animals was similar (P>0.05) among 3 different groups (Table 1), but the final body weight (kg) was higher in high energy fed group compared to low energy fed group. Body weight change (kg) was significantly more (P<0.05) in high energy group as compared to control and reduced in low energy group. FCR was better (P>0.05) in high energy group followed by control and low energy. Similar to our study, when lambs were offered 3 energy levels, high energy diet caused improvements in body weight gains and performance when dried fat (tallow) was used as energy source (Sayed *et al.* 2009). A reduction in dietary energy density resulted in reduced growth performance (Beauchemin *et al.* 1995) in lambs and supplementation of high energy improved bodyweight gains (Mohazer *et al.* 2010) in sheep. The weight

Table 1. Performance of rams during feeding trial[a]

Atributes	Groups			SEM
	Control	Low energy	High energy	
Initial body weight (kg)	22.2	21.7	22.5	0.37
Final body weight (kg)	28.2^{ab}	26.2^{b}	31.1^{a}	0.63
Body weight change (kg)	6.1^{b}	4.4^{c}	8.6^{a}	0.45
Total DMI (kg)	78.9 ^{b}	72.8^{b}	86.5^{a}	0.59
FCR	13.4	21.7	10.2	2.54

[a] Means having different letters in a row differ significantly (P< 0.05). FCR, feed conversion ratio; SEM, standard error of mean.

gain obtained in the present study was similar to that obtained by Ravikala *et al.* (1995) for Indian sheep. Higher weights gains were observed by Dutta *et al.* (2008) probably due to breed differences in weight gains of Indian sheep as we used Nellore sheep (local).

Intakes of CP and DCP (g/d) were same in all the 3 groups thus ensured similar intake of protein in all the groups. Intake of TDN (g/d) and digestible DM and DOM (g/kg $W^{0.75}$) was significantly higher in high energy diet fed group than low energy group (Table 2). The faecal excretion of nitrogen was more in high energy fed groups compared to low and control. This resulted in lowered CP digestibility in high energy fed group compared to other groups. The digestibilities of DM, OM, EE, CF and NFE did not differ among 3 groups. Urinary excretion of nitrogen was lower (P<0.05) in high energy fed group which resulted in more retention of nitrogen (P < 0.05) compared to low energy fed group. The efficiency with which N absorbed was significantly higher (P<0.05) in the high energy group.

The reason for lower N digestibility in high energy group could be due to poor efficiency with which ruminant animals utilize N and partially attributed to the differences in the pattern of protein and energy absorption following a meal (Kolver *et al.* 1998). Fluharty *et al.* (1999) observed that the source of energy (either concentrate or alfalfa) had positive influence on DM, OM, ADF and NDF digestibilities. Matras *et al.* (1991) reported that N retention was greater in lambs when the rate of energy and N supply to the rumen were synchronized. Increased fecal N in high energy group may be attributed primarily due to more DMI, increased epithelial cell sloughing and metabolic fecal N (Fluharty *et al.* 1999). Decreased urinary nitrogen is an indication that quality of protein absorbed in high energy is superior compared to low energy diet fed group. It also gave further indication that efficiency with absorbed nitrogen utilized at tissue level is higher in high energy groups.

Rumen fermentation parameters: Rumen fluid pH was similar (P>0.05) in all the groups (Table 3). The total and ammonia-N in the rumen fluid were significantly lower in the high energy group as compared to the optimum and low energy fed groups. Other nitrogen fractions like TCA precipitable nitrogen and soluble nitrogen remained similar in all the groups. TVFA concentration (meq/100 ml SRL) was also similar in all groups. Though the acetate level was similar among all the groups, the level of propionate was significantly (P<0.05) higher in high energy group than the optimum and low energy groups. The molar proportion of acetate was significantly (P<0.05) lower and of propionate was higher in high energy group as compared to other groups. The butyrate levels and its molar proportions were significantly (P<0.05) more in low energy group followed by optimum and high energy groups. The acetate: propionate ratio narrowed in high energy group compared to optimum and low energy groups.

The rumen ammonia levels were found more than the threshold values (50 mg/L) that is required to maintain

Table 2. Mean intake and digestibility of nutrients and balance of nitrogen during metabolism trial in various groups[a]

Atributes	Groups			SEM
	Control	Low energy	High energy	
Body weight (kg)	26.7[b]	26.8[b]	31.6[a]	0.64
Metabolic bodyweight (kg $W^{0.75}$)	11.8 [b]	11.8 [b]	13.3 [a]	0.21
Intake of nutrients				
CP (g/day)	112.90	107.40	116.18	1.21
DCP (g/day)	75.16	71.58	69.55	1.10
TDN (g/day)	547.30[a]	489.41 [b]	578.48 [a]	9.76
DDM (kg $W^{0.75}$)	48.6 [a]	42.7 [b]	48.5[a]	0.88
DOM (kg $W^{0.75}$)	45.7 [a]	40.6 [b]	46.2[a]	0.80
Digestibility (%)				
DM	68.0	68.5	68.5	0.70
OM	69.0	70.1	69.6	0.69
CP	66.6 [a]	66.6 [a]	59.9 [b]	0.05
EE	68.4	65.8	79.0	3.40
CF	60.2	62.8	57.3	1.44
NFE	73.4	74.6	73.4	0.59
Nitrogen (N) balance(g/d)				
Nitrogen intake	18.1[ab]	17.2[b]	18.6[a]	0.19
Nitrogen excreted				
N faeces	6.0[b]	5.7[b]	7.5[a]	0.20
N Urine	5.4[a]	6.9[a]	3.4[b]	0.43
N retained	6.7[a]	4.6[b]	7.7[a]	0.44
N retained (% N intake)	37.0[ab]	26.6[b]	41.6[a]	2.23
N retained (% N absorbed)	55.5 [ab]	39.7[b]	69.5 [a]	3.76

DDM, digestible dry matter; DOM, digestible organic matter; [a] Means having different letters in a row differ significantly (P< 0.05).

Table 3. Rumen fermentation profiles in three experimental groups[a]

Atributes	Groups			SEM
	Control	Low energy	High energy	
pH	6.7	6.7	6.6	0.02
NH_3-N (mg/100 ml)	21.5 [a]	20.7 [a]	15.7 [b]	0.84
Total-N	48.2[ab]	50.6[a]	40.9 [b]	3.40
TCA P- N	5.9	7.0	4.7	0.53
S-N	18.0	19.8	20.5	0.72
Volatile fatty acids (VFA) (mmol/ 100 ml)				
Total	7.9	7.4	8.0	0.07
Acetate	5.4	5.3	5.0	0.07
Propionate	1.8[b]	1.7[c]	2.8[a]	0.06
Butyrate	0.7[b]	0.8[a]	0.7[b]	0.01
Acetate (%)	68.2[a]	67.9[a]	62.7[b]	0.66
Propionate (%)	22.6[b]	21.3[b]	28.4[a]	0.78
Butyrate (%)	9.2[b]	10.8[a]	8.8[b]	0.25
Acetate: propionate	3.0[a]	3.2[a]	2.2[b]	0.11

NH_3-N, ammonia nitrogen; total-N, total nitrogen; TCA P- N, trichloro acetic acid precipitable nitrogen; S-N, soluble nitrogen;[a] Means having different letters in a row differ significantly (P< 0.05).

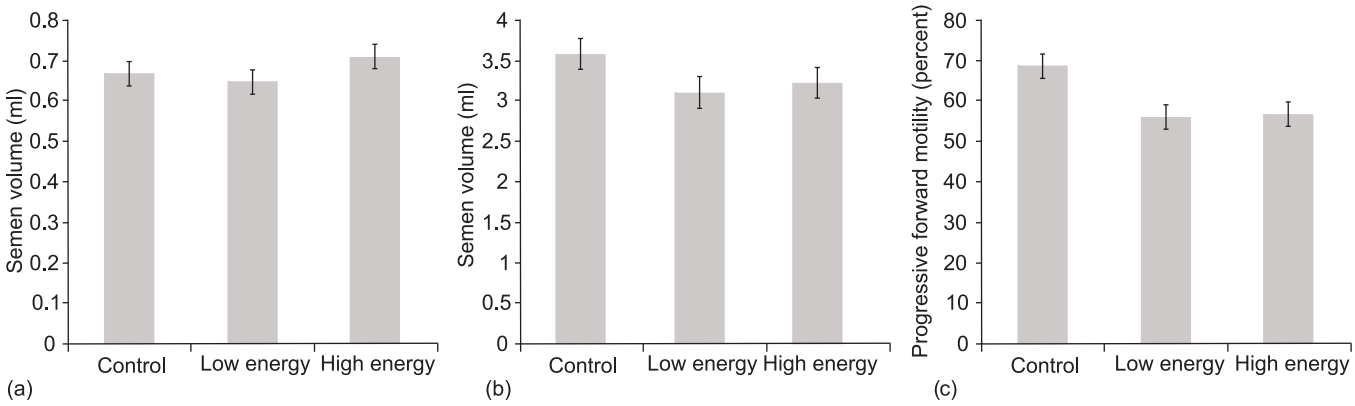

Fig. 1. Effect of feeding of three levels of energy with similar intake of protein on ram semen quality (a: semen volume, ml per ejaculate), b: mass activity in 0–5 scale) and c: progressive forward motility, percent).* P<0.05.

maximum microbial protein synthesis (Satter and Slyter 1974). The synchrony between release of energy and protein might have resulted in lower ammonia concentration in high energy group as compared to other 2 groups. A decreased acetate and butyrate concentrations accompanied by an increase in propionate concentration was reported (Bartley *et al.* 1979) in steers fed moderate to high energy diets. Generally diets that contain a rich source of starch would be expected to increase propionate and decrease acetate proportions in the rumen (Bergman 1990). Melaku (2004) observed that fiber level of feedstuff had an influence on molar proportion of acetate and propionate. In the present study, the high energy diet contained low CF which might have contributed to low rumen acetate level. The increase in glucogenic precursors spared the need for glucogenic amino acids, thus permitting a greater rate of growth and feed conversion efficiency (MacRae and Lobley 1986). Chamberlain *et al.* (1985) and Huhtanen (1988) reported an increase in the ratio of butyrate when barley was provided to grass silage-fed cattle and this was attributed to high protozoal counts. Energy metabolism pathways and feed energy utilization efficiency in ruminants could be influenced by the differences in the molar proportions of rumen fermentation end products. In this regard, higher molar proportions of propionate in rams supplemented with more energy may be hypothesized to improve energy utilization through reduced methane production in the rumen (Wolin and Miller 1988). Indeed, a positive relationship was reported between higher proportion of propionate and efficiency of energy utilization efficiency (van Houtert 1993).

Semen evaluation: Semen volume (ml) of rams from optimum (0.67±0.02), low (0.65±0.03) and high (0.71±0.03) dietary energy fed rams were similar. The mass activity (0–5 score) of spermatozoa was significantly (P<0.05) higher in optimum (3.57±0.19) than low (3.1±0.19) dietary energy fed rams. However the mass activity of the low energy fed group was similar to high (3.2±0.19) dietary energy fed rams. The progressive forward motility (%) was higher (P<0.05) in optimum (68.71±3.02) than low (55.67±4.33) dietary energy diet fed rams. However the progressive forward motility (%) of spermatozoa of the low energy fed group was similar to high (56.29±4.41) dietary energy fed rams (Fig. 1).

Supplementation of dietary energy in excess of the recommended level did not improve semen quality. In this experiment, nitrogen intake in different groups was same in high and control groups (Table 2), indicating that differences in semen quality were mainly due to energy consumption. However, progressive forward motility of spermatozoa was significantly affected by varying the optimum dietary energy level. Though, the effect of feeding higher dietary energy levels on semen quality remains controversial (Coulter *et al.*1997, Mwansa and Makarechian 1991, Chase *et al.* 1993), it may adversely influence semen quality by affecting thermoregulatory mechanism of the testis (Coulter *et al.* 1997). It was also documented that during breeding season males may lose approximately 15% of the body weight. Hence during breeding season, the breeding males may be daily supplemented with slightly higher energy (about 5% more than the optimum) diets. Further, nutritional strategies have to be critically formulated depending on breeding load on rams.

The present study revealed that the marginal increase in dietary energy level had significantly improved the body weight gains. The extra supplemented energy was partitioned towards body weight gain, resulting in lowered rumen ammonia levels and a shift in acetate: propionate ratio. However the shift did not positively influence on semen quality. This study suggested critical formulation of nutritional strategies for sheep destined for semen production.

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