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Effect of Rearing System on Rumen Development of Balouchi Lamb

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Abstract: The effect of rearing system on rumen development and non-carcass characteristics of Balouchi lambs were studied. Twenty-four male lambs were used in a completely randomized design. Eight lambs remained with their mothers throughout the experiment (NR) and 16 lambs divided 2 groups, ARWF, starter without alfalfa and ARF, starter containing 15% alfalfa and were housed individually. Glucose and BUN were not different significantly ($p>0.05$) between groups. BHBA concentration was higher in artificial rearing whereas, NEFA was higher in natural rearing lambs. Neither DNA content and nor cell size were affected by rearing methods but RNA content and ribosomal Capacity (Cs) were affected by rearing system ($p<0.05$). ARWF lambs showed thickest keratinized layer than NR lambs and NR, thickest than the ARF but other rumen morphological characteristics were not affect by group. Differences between naturally and artificially reared lambs in EBW and non-carcass organs weight except stomach weight (EBW%) and stomach and omasum capacity were not significant ($p>0.05$). The results of this study showed that natural rearing lambs have minimum development of rumen.

Key words: Rearing method, rumen development, lamb, NR, ARWF, EBW

INTRODUCTION

The largest enterprise of Iranian animal agriculture is sheep industry. Sheep numbers in Iran is about 52 million, which amounts to nearly 42% of the available total animal units (Valizadeh *et al*., 2009).

The most of these sheep's are dependent on poor range. Low quality and quantity range could not supply nutrients requirements especially in vital periods, late gestation and lactation. So, sheep production in semi-intensive and intensive systems that are need for better nutrition of ewes resulted to higher birth and weaning weights but, ideally animals should be kept in husbandry systems, which allow them to express natural behaviour. Compared to artificial rearing in intensive systems, natural systems are more beneficial to the welfare of lambs. The lamb will be nursed by its mother, suckle milk, learn to eat roughage at a younger age, have social contact with other animals and have space enough to exercise and play. Most of these factors are absent in artificial lamb rearing systems (Krohn, 2001). The effect of rearing system on performance, welfare and meat quality of lambs and calves were well studies but, there were no complete study related to the effect of different rearing methods on rumen development indices.

Therefore, the objective of the present study was to determine the effect of Natural (NR) vs. Artificial Rearing (AR) of lambs on rumen development indicators.

MATERIALS AND METHODS

The experiment, lasting 6 weeks, was performed on single-born male Balouchi lambs divided into three groups of 8 subjects each. Ewe-reared animals (NR) were maintained with their mothers for the whole experimental period, whereas Artificially Reared lambs (AR) were separated from their dams in three weeks of age and housed in a separate straw-bedded pen. Lambs of AR divided two groups, ARWF, starter without alfalfa and ARF, starter containing 15% alfalfa (Table 1).

Dams and their lambs in group 1 were allowed to graze at pasture for 6 h a day and in evening of day were supplemented with 0.5 kg alfalfa and 0.3 kg concentrate (40% corn, 20% soybean meal, 20% beet pulp and 20% wheat bran). Lambs in groups 2 and 3 were allowed to be with the ewes for 30 min twice daily and weaned at 6 weeks of age.

The animals were weighed at week 0 of experiment (3 weeks of age) and then once a week in the morning before feeding. Average live weight of lambs was 9.8±1.3 kg at week 0.

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Table 1: Experimental diets composition

	Groups		
Feed	NR	ARWF	ARF
Corn	-	58.00	58.00
Soybean meal	-	21.00	21.00
Alfalfa	-	0.00	15.00
Wheat bran	-	15.00	0.00
Beet molasses	-	4.00	4.00
Vit-min supplementation	-	0.40	0.40
White salt	-	0.20	0.20
Limestone	-	1.40	1.40
Chemical composition			
ME (kcal kg^{-1})	-	3.10	3.02
Cp (%)	-	20.00	19.96
NDF (%)	-	15.00	15.00
epNDF (%)	-	8.50	10.57
ADF (%)	-	6.40	8.40
Ca (%)	-	0.65	0.81
P (%)	-	0.37	0.40
Ca:P	-	2.02	1.76

NR: Natural Reared; ARWF: Artificial Reared Without Alfalfa; ARF: Artificial Reared with Alfalfa

Blood samples were taken from the jugular vein at the beginning of the experiment (0 day) and the end of each week (up to week 6). The serum was separated after centrifugation at 1800 g for 10 min and stored at -18°C, until analysis.

Nine animals (three per treatment) were slaughtered at 63 days of age. The entire digestive apparatus was removed from the carcass, the ruminal compartment was separated (the reticulo-rumen remained together), emptied, washed clean, drained of excess water and weighed. Samples (approximately, 1 cm^2) were collected from the dorsal, ventral, caudal dorsal and caudal ventral blind sacs and pillar and atrium ruminis area.

Tissue samples were obtained within 30 min of death, placed in individual containers and fixed immediately in a 10% formaldehyde solution for subsequent measurements. In lab tissue samples dehydrated in a series of ethanol solutions from 70-100%. The material was sectioned with an automatic microtome, at 6 μm thickness and after the slides are dried, stained with hematoxylin mixture and Eosin. The material was observed under a light microscope (Olympus BX-51) at 20 and 40X. Digital images of stained sections were taken using an Olympus BX-51 camera (DP 11) and measurements were made using image analysis computer software (DP2-BSW Version 1.3).

Papillary height was defined as the distance from the tip to the base of the papillae and papillary width was defined as the average width of the base, middle and tip of the papillae.

Papillae length and width were used to estimate surface area per cm^2 of each rumen section. Denseness of papillae for each of the samples was determined using digital images, from Scanning Electron Microscopy (SEM, VEGA TESCAN, Czech Republic).

Surface area of papillae per surface area of each ruminal section is presented as the Surface Area Ratio (SAR) and was calculated using the following methodology. Papillae were considered to be cylindrical in shape with one closed end. Therefore, Eq. 1, used to calculate lateral area of papillae, was based on the surface area of a cylinder plus the area of a circle; Eq. 2 was used to calculate the average SAR of each section of the rumen by multiplying the average surface area of the papillae in each section by the average denseness or number of papillae per unit area in that section.

$$\text{Surface area of papillae } (cm^2) = 2\times r\times\pi\times L + \pi\times r2 \quad (1)$$

Where:
r = Radius (cm)
L = Length (cm)

$$\text{SAR} = \text{Average surface area of papillae in section X} \times \text{Average papillae denseness in section X} \quad (2)$$

where, X can be caudal, ventral, or dorsal.

For extract of total RNA and DNA used of Trizol RNA Prep 100 kit and Accuprep Genomic DNA Extraction Kit; Cat No: K-3032, respectively. Ribosomal capacity, i.e., the capacity for protein synthesis and cell size was calculated as the ratio of RNA to protein and protein to DNA, respectively (Tesseraud *et al*., 1996).

The Proc Mixed program of SAS 9.1 was used to analyze measurements. Because, blood parameters were measured over the time, a repeated measures approach using ANOVA with mixed linear models in SAS 9.1 was used. Difference between means was tested for significance using Duncan test.

RESULTS AND DISCUSSION

As shown in Table 2 glucose and BUN were not different significantly ($p>0.05$) between groups however, this factors high in artificial reared lambs. Concentration of plasma glucose by week of study (Fig. 1) declined with advancing age. Concentrations were typical of non-ruminants initially (96.4 mg dL^{-1}) but reached a nadir at 63.5 mg dL^{-1} by weeks 9 of age. Plasma glucose approached concentrations typical of mature ruminants post-weaning and did not differ between treatments.

Changes in blood glucose concentration with advancing age are in response to reduced glucose in erythrocytes rather than a response to maturing ruminal function and diets (Quigley *et al*., 1991).

Plasma concentrations of BHBA were significantly affected by group ($p = 0.001$). AR compares with NR lambs maintained higher concentrations of blood BHBA (0.27, 0.55 and 0.40 mmol L^{-1} for NR, ARWF and ARF, respectively).

Table 2: Effects of rearing system on blood metabolites

	Groups				Effect	
Measurements	NR	ARWF	ARF	SEM	Group	Time
Glucose (mg dL^{-1})	74.02	85.82	84.99	21.23	0.90	0.13
BUN (mg dL^{-1})	4.72	6.64	7.50	1.37	0.42	0.22
BHBA (mmol L^{-1})	0.27^{c}	0.55^{a}	0.40^{b}	0.03	0.001	0.001
NEFA (mmol L^{-1})	0.359^{a}	0.221^{b}	0.230^{b}	0.029	0.015	0.87

Table 3: Effects of rearing system on molecular indices of rumen tissue

	Groups				
Measurements	NR	ARWF	ARF	SEM	p-value
RNA (μg g^{-1})	2631.60^{b}	3399.60^{a}	3359.90^{a}	286.92	0.03
DNA (μg g^{-1})	156.40	144.97	132.60	39.87	0.77
Pr (mg g^{-1})	410.00	400.07	406.87	19.89	0.82
Cs ($\times 10^{-3}$)	6.41^{b}	8.5^{a}	8.25^{a}	0.60	0.01
Cell size ($\times 10^{3}$)	2.92	2.79	3.07	0.65	0.87

NR: Natural Reared; ARWF: Artificial Reared Without Alfalfa; ARF: Artificial Reared with Alfalfa. Values in the same row with different superscripts are significantly different

Increases in blood BHBA were closely related to availability of fermentable starter. Concentration of blood BHBA in artificial reared animals especially in ARWF likely reflected high production of butyrate in the rumen with subsequent metabolism to it by ruminal epithelium. Butyrate is ketogenic in young animals (Sutton *et al*., 1963).

Lambs in natural rearing system maintained lower levels of BHBA, probably due to marginal ruminal fermentation of ingested forage, milk entering the rumen via backflow, incomplete esophageal groove closure, or lower tract fermentation of digesta (Quigley *et al*., 1991).

Blood BHBA increased significantly with advancing age and markedly increased from weeks 3-6 (Fig. 2).

Induction of ketogenesis by ruminal mucosa may result from exposure to dry feed intake. This suggestion is in agreement with findings of Manns and Boda (1966), who reported that metabolism of butyrate by ruminal wall of young Iambs increased with age and presumably, dry feed intake.

Sutton *et al*. (1963) reported that metabolic activity of ruminal epithelium is induced by presence of ruminal VFA, especially butyrate. Early grain feeding and consequent VFA production possibly caused an increase in metabolic activity of ruminal epithelium, thereby increasing production of a larger amount of BHBA per unit feed intake.

Blood concentration of NEFA affected by group and was high in NR animals although glucose was lower in these lambs. This observation is agreed with Trenkle and Kuhlemeir (1966), who reported an inverse relationship between glucose and NEFA, especially under conditions of fasting. High milk feeding and minimal dry feed intake in ewe-reared lambs may have increased

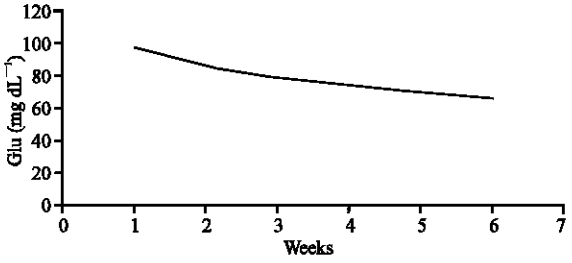

Fig. 1: Changes in glucose concentration over time

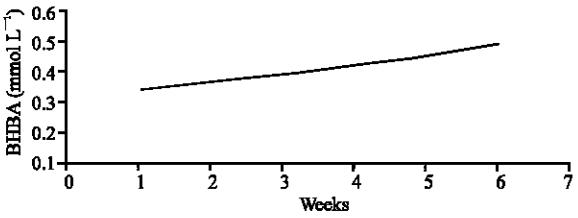

Fig. 2: Changes in BHBA concentration over time

NEFA during this period due to increase flow of milk high-chain fatty acids into liver (Baldwin *et al*., 2004).

Molecular characteristics of rumen tissue samples in experimental groups are shown in Table 3. Neither DNA content and nor cell size were affected by rearing methods. Unlike the DNA content and cell size, RNA content and ribosomal capacity (Cs) of AR groups significantly ($p<0.05$) higher than that of naturally reared (NR group).

Based on our knowledge there aren't any data related to molecular approaches in rumen development studies but, Estornell *et al*. (1994) indicate that addition of a supplementary energy source to a well-balanced diet improves growth and protein synthesis in growing rats. This change could be attributed to an increase in the ribosomal activity for protein synthesis in basal layer of epithelium. It be seems that high production in butyrate, increase energy source for epithelial cells and cause to high cell division.

Morphological characteristics of rumen wall except of thickness of keratinized layer were not affected by rearing methods of lambs (Table 4).

The keratinized layer was 62 and 20% thickest in ARWF than in ARF and NR lambs, respectively ($p<0.05$). The tissue alterations in the fore-stomach have been shown to be influenced by VFA during carbohydrate fermentation (Tamate *et al*., 1962; McGavin and Morrill, 1976). The feeding condition, which involves rapid fermentation in the rumen could cause harmful changes in the rumen mucosa such as rumen parakeratosis (Orskov, 1976).

Table 4: Effects of rearing system on rumen morphological characteristics

	Groups				Effect	
Measurements	NR	ARWF	ARF	SEM	Group	Sac
Papillae height (μm)	1398.590	1412.430	1539.430	66.440	0.370	<0.0001
Papillae width (μm)	219.730	273.690	301.130	23.670	0.180	<0.0001
Epithelium (μm)	62.090	46.350	63.160	5.520	0.200	0.01
Keratinized layer (μm)	12.130^{a}	14.530^{a}	8.950^{b}	0.630	0.018	0.0006
Muscular layer (μm)	757.150	709.190	1034.600	94.730	0.160	<0.0001
Rumen wall (μm)	1094.200	1044.810	1190.220	91.030	0.570	<0.0001
Papilla density (No/cm^2)	111.520	112.780	108.770	0.950	0.110	0.17
SAR (cm^2)	1.153	1.156	1.159	0.002	0.170	<0.0001

NR: Natural Reared; ARWF: Artificial Reared Without Alfalfa; ARF: Artificial Reared with Alfalfa. Values in the same row with different superscripts are significantly different

Table 5: Effects of rearing system on non-carcass organs weight and capacity

	Groups				
Measurements	NR	ARWF	ARF	SEM	p-value
Empty Body W. (EBW, kg)	15.725	14.445	15.675	0.61	0.39
Stomach W. (SW, EBW%)	3.14^{b}	3.33^{ab}	3.81^{a}	0.124	0.05
Rumen W. (EBW%)	2.34	2.38	2.55	0.08	0.10
Rumen W. (SW%)	67.96	68.68	66.92	1.68	0.63
Omasum W. (EBW%)	0.56	0.52	0.58	0.05	0.67
Omasum W. (SW%)	16.15	15.73	15.21	1.34	0.79
Stomach Capacity (SC, g)	3352.5^{b}	3227.0^{b}	5097.0^{a}	106.98	0.0007
Rumen Capacity (SC%)	89.5	90.49	92.21	0.72	0.07
Omasum Capacity (SC%)	7.95^{a}	6.9^{a}	4.7^{b}	0.51	0.01
Heart W. (EBW%)	0.42	0.47	0.49	0.03	0.17
Liver W. (EBW%)	1.69	1.64	1.76	0.19	0.82
Lungs W. (EBW%)	0.89	1.09	1.15	0.14	0.29
Kidneys W. (EBW%)	0.38	0.36	0.42	0.03	0.39

NR: Natural Reared; ARWF: Artificial Reared Without Alfalfa; ARF: Artificial Reared with Alfalfa. Values in the same row with different superscripts are significantly different

Mgasa *et al*. (1994) in a study on male goats verified that animals fed hay plus a pelleted ration consisting mainly of alfalfa showed faulty keratinization (dyskeratosis) of the *stratum corneum* in most parts of the rumen. Cells of the *stratum* appeared rounded and vacuolated, some without nucleus. Their work showed that goats on concentrates had much more pronounced development of rumen papillae and keratinization of the *stratum corneum* compared to those on green forage pellets, indicating that the structure of the forestomach is under the influence of the diet.

In the current experiment, differences observed in papillary width and height and thickness of muscular layer and rumen wall were not significant ($p>0.05$) among treatment but animals fed starter diet containing alfalfa exhibited thickest muscular layer and rumen wall. The papilla density and SAR were did not differ among groups ($p>0.05$).

Table 5 shows, the effect of rearing methods on non-carcass characteristics. Empty Body Weight (EBW) were similar for three groups of lambs but stomach weight (EBW%) and capacity of artificial reared animals were significantly ($p = 0.05$) higher than natural reared lambs. Omasum capacity of natural reared lambs was higher than other groups ($p = 0.01$) probably because more intake of milk. The other non-carcass organs weight and capacity were not significantly ($p<0.05$) affected by rearing methods. Slight difference of weight and capacity of stomach compartments between groups showed that natural rearing lambs have minimum physical development of rumen.

CONCLUSION

In over all, it was concluded that natural rearing of lambs in intensive system that be done in this study haven't considerable differences in any morphological, molecular and biochemical categories and cause to minimum development of rumen than artificial rearing methods.

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