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Milk and blood parameters in fat-tailed sheep

S. Payandeh *et al.*

Patterns of milk production, blood metabolite profile and enzyme activities of two fat-tailed sheep breeds

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This study was conducted to compare milk yield and composition, blood parameters and enzyme activities between two fat-tailed Iranian breeds of sheep (Mehraban and Sanjabi). Two groups of eight ewes per breed were kept indoors and fed a diet based on alfalfa hay (60%) and concentrates (40%). Milk production of the individual ewes was measured and sampled weekly for 11 weeks and blood samples were obtained at 28, 56 and 77 days of lactation. No significant differences between the breeds were evident in average daily milk production and fat and lactose contents, but average milk protein content tended to be higher ($P = 0.05$) in Mehraban than in Sanjabi ewes. However, milk and protein yields were higher ($P < 0.05$) in Mehraban ewes in the last two-thirds of lactation. Plasma lipid profile (triglyceride, cholesterol, HDL- and LDL-cholesterol) was not different between the two breeds at any time of study. At the end of lactation, plasma urea contents and alanine amino transferase activities were higher ($P < 0.05$) either before or after feeding in Mehraban than in Sanjabi ewes, which suggests a more intense metabolic activity in the former probably related to their higher milk and protein production at that stage of lactation.

Additional keywords: ewes, milk composition, plasma parameters.

Milk production, plasma parameters and different enzyme activities were compared in two Iranian fat-tailed sheep breeds (Mehraban and Sanjabi) during lactation. There were few differences in plasma parameters between breeds. However, Mehraban ewes performed better than Sanjabi ewes in the last two-thirds of the lactation period.

Introduction

Although sheep milk is of marginal importance compared with cow milk in quantitative terms (about 21.5% of the total world supply; FAOSTAT 2011), it is of major interest in countries where climatic conditions are not favourable for cattle raising. Sheep have the ability to forage and survive in many areas, where cattle would perform very poorly (Zygyiannis 2006). However, sheep milk is ideal for the production of manufactured dairy products due to the high content of total solids, fat, and protein (Hilali *et al.* 2011). Sheep milk products can provide a profitable alternative to cow milk products owing to their specific taste, texture, typicity and their natural and healthy image (Raynal-Ljutovac *et al.* 2008).

Iran is the country with the largest sheep population in the world after China, India and Australia (FAOSTAT 2011). With 31 recognised breeds, Iran has also one of the highest numbers of sheep breeds in the world according to the Domestic Animal Diversity Information System (www.fao.org/DAD-IS, verified 2 May 2015). Sanjabi and Mehraban are two fat-tailed sheep breeds that are widely kept in the provinces of Kermanshah and Hamadan, respectively, located in the western region of Iran. In this country, sheep milk, besides being used for lamb rearing, is a valuable nutritional food that traditionally has enriched the nomadic shepherd's diet consumed fresh or as cheese, yoghurt and other dairy products (Izadifard and Zamiri 1997; Degen 2007). It is well known that milk yield and composition vary with breed and with the stage of lactation (Sakul and Boylan 1992a, 1992b). Those differences can alter the texture of milk products (Lucey *et al.* 2003). Additionally, knowing breed effects on milk yield and composition is very important to implement breeding selection programs based on the traditional quantitative approach (Carta *et al.* 2009).

Blood biochemical parameters are used to assess the nutritional status of lactating animals and they are known to change according to the physiological status (Nazifi *et al.* 2002; Karapehlivan *et al.* 2007; Piccione *et al.* 2009). The assessment of the relationship between blood biochemical parameters and enzymes activities and patterns of milk production may lead to a better understanding of the ewe's metabolism changes during the lactation period.

The aim of the present work was to study milk yield and composition, plasma metabolites and enzyme activities during lactation in Sanjabi and Mehraban ewes, kept indoors under the same feeding regime and management conditions.

Materials and methods

This study was carried out in the Animal Research Station of Agriculture College of Razi University, Kermanshah, Iran. The research protocol was approved by the Animal Care and Use Committee of Razi University. Sixteen pregnant ewes at their second and third lambing, carrying one fetus, were selected 1

month before lambing from a synchronised pure flock. All animals were clinically healthy and free from internal and external parasites. Ewes were divided into two groups of eight animals, balanced in parity (2.5 ± 0.53 and 2.4 ± 0.92 in Sanjabi and Mehraban groups, respectively; $P = 0.74$). The two groups were kept in an enclosed facility as a single flock and fed the same diet without grazing at all. Diet was based on alfalfa hay (60% DM) and a concentrate mix (40% DM) consisting of maize grain (26% DM), soybean meal (9% DM), wheat bran (3.5% DM), and minerals and vitamin (1.5% DM). Chemical composition of the diet was (DM basis): crude protein 13.9%, neutral detergent fibre 36.1%, acid detergent fibre 25.6%, calcium 0.61%, phosphorus 0.37%, and calculated metabolisable energy 10.0 MJ/kg (NRC 1985). The ration was fed in two equal amounts at ~0730 hours and 1730 hours. Daily feed amounts (as-fed basis) offered until Week 6 and from Week 7 onwards were calculated according to the recommendations of NRC (1985): 2.26 and 1.67 kg/day, respectively, in Sanjabi ewes, and 1.95 and 1.46 kg/day, respectively, in Mehraban ewes. Visual observation of the troughs revealed that feed offered daily was almost completely eaten from day to day. Water was provided *ad libitum* to all ewes for the entire period.

Ewes' bodyweight (BW) was recorded after lambing and the last day of the experiment. Milk yield was determined throughout 24-h periods at weekly intervals up to 77 days of lactation (DIM), starting 1 week after parturition. Lambs were separated from their dams at 1630 hours on the day before each test day and bottle-fed. On the test day, ewes were milked twice by hand at 0630 hours and 1630 hours, and individual milk was sampled at each milking. The morning samples were kept refrigerated at 4°C, then they were mixed with the corresponding evening samples and the resulting subsamples were immediately taken to the laboratory for analysis. Milk fat, protein, lactose and solids not fat were determined using a Milko-Scan (Foss Electric, Hillerød, Denmark).

Jugular blood samples of all ewes were collected in heparinised tubes before and 6 h after morning feeding at 28, 56 and 77 DIM. Plasma was separated by centrifugation (750g for 15 min and 4°C) and preserved at -80°C for further analysis. Commercial kits (ELITech France and Pars Azmoon Co., Tehran, Iran) and a Hitachi 902 Chemistry Analyser (Hitachi, Tokyo, Japan) were used to determine glucose, urea, uric acid, creatinine, triglycerides, total cholesterol, HDL- and LDL-cholesterol concentrations, and alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities.

This study was conducted in a completely randomised design. Data of parity, BW, and BW change were analysed using the *t*-test procedure of SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Data on milk yield and composition, and blood metabolites contents and enzymes activities were analysed by repeated-measurements analysis using the MIXED procedure of SAS 9.1. The statistical model included the fixed effects of breed, sampling time (when appropriate), week of lactation and their interactions, and the

random effects of sheep within a breed, assuming an autoregressive + RE model. Statistical significance was declared at $P < 0.05$.

Results and discussion

Sanjabi ewes were heavier ($P < 0.05$) at lambing (59.5 ± 5.32 kg vs 45.2 ± 7.57 kg in Mehraban ewes) and at the end of the experimental period (53.8 ± 6.93 kg vs 42.9 ± 6.54 kg in Mehraban ewes). The percentage of BW change tended to be higher ($P = 0.08$) in Sanjabi ($-9.5 \pm 5.67\%$) than in Mehraban ($-4.6 \pm 4.59\%$) ewes. This trend could be explained by differential changes in the weights of total digestive tract contents and visceral organs as lactation progressed. Mehraban ewes might have had a higher weight of total digestive tract contents due to a higher feed consumption relative to BW (offered feed was 3.2% vs 2.8% of BW in Sanjabi ewes, in the second part of the lactation), and higher visceral organ weights due to a higher metabolic activity, related to their higher milk yield in the last two-thirds of lactation (Tables 1 and Table 2).

Average daily milk yield and milk component contents and yields were not different ($P > 0.05$) between the two breeds (Table 1). Protein percent tended to be higher ($P = 0.05$) in Mehraban ewes as well as the solids not fat content ($P = 0.06$). However, breed $\times$ week interaction was significant ($P < 0.05$) in all measured traits, except for fat yield. As shown in Table 2, milk and protein yields were higher ($P < 0.05$) in Mehraban ewes in the last two-thirds of the lactation period. Raynal-Ljutovac *et al.* (2008) indicated that the total protein content in sheep milk ranges from 4.75% to 7.20%; therefore, both Mehraban and Sanjabi breeds with 6.98% and 6.69% of milk protein, respectively, could be considered as breeds with high milk protein content. Milk fat and milk lactose contents of both breeds were within the range of 3.60% to 9.97% for fat and 4.11% to 5.51% for lactose reported by Raynal-Ljutovac *et al.* (2008).

Figure 1 shows milk, fat, protein and lactose yields from Weeks 2 to 11. The shape of the lactation curves was in agreement with that commonly observed in meat-wool sheep breeds (Pulina *et al.* 2009). The lactation peak occurred in the second week of lactation in both breeds, as previously reported by Izadifard and Zamiri (1997) in the Mehraban and Ghezel breeds, which are also Iranian fat-tailed Iranian sheep, but in contrast to Nezamidoust *et al.* (2013) who observed peak yield at ~6 weeks after lambing in Makui breed, another Iranian fat-tailed sheep breed. The rate of decline in milk yield after the fourth week was greater in Sanjabi ewes (~25% from 28 to 56 DIM vs nil in Mehraban ewes; Table 2), indicating that this breed has a lower lactation persistency. Protein and lactose yields sharply declined between Weeks 2 and 4 in Sanjabi and Mehraban ewes. However, both traits remained higher ($P < 0.05$) in Mehraban ewes from that point until the end of the experimental period. Fat yield did not differ between breeds nor change during the experimental period. Rhind *et al.* (1992) working with two sheep breeds pointed out

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that differences in the pattern of milk production could be explained by changes in insulin and growth hormone profiles and their relative contents as lactation progresses.

Plasma metabolite concentrations in Sanjabi and Mehraban breeds before and after feeding at 28, 56 and 77 DIM are shown in [Table 3](#). The plasma metabolite concentrations observed in both breeds were similar to those previously reported in Iranian fat-tailed sheep by [Eshratkhah *et al.* \(2008\)](#) and [Nazifi *et al.* \(2005\)](#). The only blood metabolite whose content was different between breeds was uric acid, being higher ($P < 0.05$) in Mehraban ewes. However, several significant breed $\times$ week, breed $\times$ time of sampling, and breed $\times$ week $\times$ time of sampling interactions were observed. Blood urea concentration was higher ($P < 0.05$) in Mehraban than in Sanjabi ewes at 28 DIM before feeding and at 77 DIM before and after feeding. Plasma creatinine concentration was higher ($P < 0.05$) in Mehraban than in Sanjabi ewes at 28 DIM before and after feeding. In Sanjabi ewes, blood glucose concentration was higher ($P < 0.05$) before than after feeding. In Mehraban ewes, total, HDL- and LDL-cholesterol contents in blood were higher ($P < 0.05$) before than after feeding. The observed differences between breeds and time of sampling suggest adaptations in the metabolic activity depending on the availability of substrates and the energy status of the animals as reported, respectively, by [Gaál *et al.* \(1993\)](#) and [Gow *et al.* \(1981\)](#) both working with sheep. However, the results obtained in the present work disagree with those of [Nazifi *et al.* \(2005\)](#) who did not find significant diurnal variations of blood metabolite concentrations in Iranian fat-tailed sheep.

Within each breed, blood plasma metabolite contents followed similar trends before and after feeding ([Table 3](#)) as lactation progressed. Except for blood urea and creatinine, those trends were similar in both breeds. Blood glucose and HDL had a quadratic pattern of change with a maximum at 56 DIM. Blood uric acid had also a quadratic pattern of change, but with a minimum at 56 DIM. Blood LDL rose from 28 to 56 DIM and then remained unchanged. Blood triglycerides markedly decreased from 56 DIM. Blood urea had a quadratic pattern of change with a maximum at 56 DIM, whereas blood cholesterol and creatinine rose from 28 to 56 DIM and then remained unchanged in Sanjabi ewes. On the contrary, blood cholesterol had a quadratic pattern of change with a maximum at 56 DIM, blood urea rose from 28 to 56 DIM and then remained unchanged, and blood creatinine did not change in Mehraban ewes. Our results partially agree with those reported in previous works, which in turn are contradictory. The results obtained by [Antunovic *et al.* \(2011\)](#) showed a positive linear trend of blood glucose and triglyceride contents from 20 to 60 DIM, but they did not observe differences in blood urea and cholesterol contents in the same period. On the contrary, [Sobiech *et al.* \(2008\)](#) found an increase of blood urea concentration from 28 to 70 DIM, but they did not observe significant changes in blood glucose, triglyceride and cholesterol concentrations. Moreover, [Piccione *et al.* \(2009\)](#) did not observe changes in blood urea,

triglycerides and cholesterol contents between samples taken at 2, 5 and 15 weeks postpartum. Again, the increases of blood triglyceride, cholesterol and HDL contents between 28 and 56 DIM found in the present work agree well with the results presented by [Nazifi *et al.* \(2002\)](#) in Iranian fat-tailed sheep. It is worth mentioning that values obtained at 28 DIM were not necessarily representative of the blood metabolite concentrations in the first days after parturition, as changes in blood metabolite contents during the first month of lactation reported by different authors are contradictory ([El-Sherif and Assad 2001](#); [Carcangiu *et al.* 2007](#); [Karapehlivan *et al.* 2007](#); [Sobiech *et al.* 2008](#)).

Plasma enzyme activities in Sanjabi and Mehraban ewes before and after feeding at 28, 56 and 77 DIM are shown in [Table 4](#). No significant differences ($P > 0.05$) were found in blood ALP, AST and LDH activities between both breeds; however, blood ALT activity was higher ($P < 0.05$) in Mehraban than in Sanjabi ewes at 77 DIM. All blood enzyme activities showed significant breed $\times$ time of sampling interactions. In Sanjabi ewes, blood AST and ALT activities were lower ($P < 0.05$) before than after feeding. In both breeds, blood ALP activity was higher ($P < 0.05$) and blood LDH activity was lower ($P < 0.05$) before than after feeding. Blood AST, ALT and ALP activities were lower and LDH activity was higher in both breeds than those previously reported in Iranian fat-tailed sheep by [Nazifi *et al.* \(2005\)](#). Moreover, contrary to the results obtained in the present work, those authors did not find significant diurnal variations of blood enzyme activities.

Patterns of change in blood enzyme activities as lactation progressed were similar in both breeds either before or after feeding, except for ALT ([Table 4](#)). Blood AST and ALP activities showed negative and positive linear trends, respectively. Blood ALT activity had a quadratic pattern of change in Sanjabi ewes with a maximum at 56 DIM, whereas there was a positive linear trend in Mehraban ewes. Blood LDH activity had a clear quadratic pattern of change before feeding in both breeds, with a maximum at 56 DIM, but there were not clear trends in the values obtained after feeding. As observed in blood metabolite concentrations, there seemed to be an inflection point at 56 DIM in the blood enzyme activity values. Our results disagree with those reported in previous works, in which no differences have been found in blood ALT, AST and LDH activities between 20 and 60 DIM ([Antunovic *et al.* 2011](#)), and in blood ALT, AST and ALP activities between 28 and 70 DIM ([Sobiech *et al.* 2008](#)).

Conclusion

The present work gives data on production traits and blood metabolite concentrations and enzyme activities in Sanjabi and Mehraban sheep breeds during lactation. Mehraban ewes performed better than Sanjabi ewes in the last two-thirds of lactation. There were fewer differences in blood metabolite concentrations and enzyme activities between breeds than within breeds as lactation progressed. Plasma urea contents and ALT activity values at the end of the experimental period suggested a more intense

metabolic activity in Mehraban ewes probably related to their higher production of milk at that stage of lactation.

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Fig. 1. Production of milk and milk components in Sanjabi (●) and Mehraban (▲) ewes during the experimental period.

Table 1. Milk yield and composition in Sanjabi and Mehraban ewes

s.e., standard error of the mean

Productive traits	Sanjabi	Mehraban	s.e.	P-value		
				Breed	Week	Breed × Week
Daily milk yield (g/day)	406	505	102	0.35	0.00	0.001
Fat (%)	5.15	5.39	0.37	0.54	0.00	0.001
Protein (%)	6.69	6.98	0.14	0.05	0.00	0.05
Lactose (%)	4.60	4.62	0.01	0.19	0.00	0.001
Solids not fat (%)	12.31	12.61	0.15	0.06	0.00	0.01
Fat yield (g/day)	19.47	28.44	7.19	0.23	0.18	0.35
Protein yield (g/day)	26.98	35.18	7.12	0.27	0.00	0.005
Lactose yield (g/day)	18.69	23.30	4.67	0.34	0.00	0.002
Energy value of milk (Mj/kg milk) ^A	4.01	4.07	0.12	0.61	0.00	0.001

^APredicted with the equation of Brett *et al.* (1972). $E = 0.0328 F + 0.0025 D + 2.203$; where F is fat concentration (g/kg milk) and D is day of lactation.

Table 2. Milk yield and composition in Sanjabi and Mehraban ewes at different stages of lactation

ab, within each productive trait at each day of lactation, least-squares means without a common letter are significantly different ($P < 0.05$) between breeds. s.e., standard error of the mean

Productive traits	Breed	Day of lactation			s.e.
		28	56	77	
Daily milk yield (g/day)	Sanjabi	448	338b	245b	66.6
	Mehraban	499	503a	414a	
Fat (%)	Sanjabi	3.91b	5.33	8.21a	0.446
	Mehraban	5.08a	5.41	6.13b	
Protein (%)	Sanjabi	6.47b	6.98	6.54b	0.152

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	Mehraban	6.86a	7.02	7.13a	
	Sanjabi	4.61	4.62	4.52b	
Lactose (%)	Mehraban	4.62	4.61	4.63a	0.016
	Sanjabi	12.2	12.6	12.0b	
Solids not fat (%)	Mehraban	12.5	12.7	12.6a	0.16
	Sanjabi	17.7	18.1	20.0	
Fat yield (g/day)	Mehraban	27.2	27.6	25.9	4.93
	Sanjabi	29.0	23.6b	16.1b	
Protein yield (g/day)	Mehraban	34.3	35.1a	29.3a	4.74
	Sanjabi	20.5	15.6b	11.1b	
Lactose yield (g/day)	Mehraban	23.0	23.2a	19.1a	3.17
	Sanjabi	3.56b	4.09	5.09a	
Energy value of milk (Mj/kg milk) ^A	Mehraban	3.93a	4.10	4.39b	0.146

^APredicted with the equation of [Brett *et al.* \(1972\)](#). $E = 0.0328 F + 0.0025 D + 2.203$; where F is fat concentration (g/kg milk) and D is day of lactation.

Table 3. Blood plasma metabolite contents in Sanjabi and Mehraban ewes just before morning feeding (BF) and 6 h after morning feeding (AF) at different days of sampling (DIM) during the lactation period

abc, within each breed at each sampling time, least-squares means without common letters are significantly different ($P < 0.05$) among days of lactation. s.e., standard error of the mean

Blood metabolites	Time of sampling	Sanjabi			Mehraban			s.e.	Breed (B)	Time (T)	<i>P</i> -value			
		DIM			DIM						Week (W)	B ×T	B ×W	B ×T×W
		28	56	77	28	56	77							
Glucose (mg/dL)	BF	62.9b	68.0a	59.3b	57.6b	62.4a	57.5b	1.37	0.26	0.26	0.001	0.001	0.33	0.68
	AF	60.5ab	64.3a	57.4b	60.6	61.9	58.8							
Urea (mg/dL)	BF	18.7c	36.9a	28.5b	28.2b	33.7a	34.5a	1.32	0.13	0.78	0.001	0.19	0.001	0.03
	AF	25.4b	33.8a	27.4b	27.7b	30.5ab	34.2a							
Creatinine (mg/dL)	BF	0.63b	0.77a	0.79a	0.76	0.78	0.73	0.03	0.54	0.21	0.01	0.90	0.001	0.03
	AF	0.56b	0.80a	0.77a	0.79	0.72	0.70							
Uric acid (mg/dL)	BF	0.16ab	0.12a	0.16b	0.21	0.17	0.20	0.01	0.04	0.82	0.04	0.13	0.27	0.37
	AF	0.14	0.16	0.18	0.21a	0.15b	0.19a							
Cholesterol (mg/dL)	BF	55.1b	63.0a	63.6a	57.5c	71.8a	64.6b	3.44	0.65	0.001	0.001	0.001	0.17	0.23
	AF	55.3b	62.3a	64.5a	55.4b	65.6a	62.5a							
HDL ^A (mg/dL)	BF	34.7b	38.7a	36.8ab	36.5b	41.0a	37.5b	1.98	0.79	0.001	0.001	0.01	0.93	0.18
	AF	34.7b	38.5a	37.0ab	35.8	37.8	36.4							
LDL ^B (mg/dL)	BF	14.0b	20.6a	20.3a	16.2b	23.7a	22.0a	1.16	0.36	0.001	0.001	0.001	0.73	0.20
	AF	14.0b	20.0a	20.7a	15.5b	20.7a	20.6a							
Triglycerides (mg/dL)	BF	26.9a	24.8a	17.3b	26.0a	26.3a	15.5b	0.69	0.35	0.08	0.001	0.15	0.55	0.08
	AF	24.5b	28.3a	15.8c	22.8b	26.3a	15.0c							

^AHigh density lipoprotein cholesterol.

^BLow density lipoprotein cholesterol.

Table 4. Blood plasma enzyme activities in Sanjabi and Mehraban ewes just before morning feeding (BF) and 6 h after morning feeding (AF) at different days of sampling (DIM) during the lactation period

abc, within each breed at each sampling time, least-squares means without common letters are significantly different ($P < 0.05$) among days of lactation. s.e., standard error of the mean

Blood enzymes	Time of sampling	Sanjabi			Mehraban			s.e.	Breed (B)	Time (T)	P-value			
		DIM			DIM						B ×T	B ×W	B ×T ×W	
		28	56	77	28	56	77							
AST ^A (U/L)	BF	113.2a	107.5ab	96.8b	121.9ab	125.8a	108.9b	5.4	0.20	0.26	0.01	0.04	0.22	0.43
	AF	121.9a	108.0b	99.9b	120.3ab	125.5a	107.1b							
ALT ^B (U/L)	BF	12.3b	15.5a	12.0b	12.6b	18.8a	20.6a	0.98	0.03	0.04	0.001	0.03	0.001	0.01
	AF	14.5	16.1	13.8	12.4c	18.0b	21.5a							
ALP ^C (U/L)	BF	200.8b	211.4ab	229.1a	197.4b	228.5a	227.5a	15.4	0.87	0.001	0.04	0.03	0.30	0.95
	AF	184.6b	190.0b	214.9a	163.3b	193.8a	198.3a							
LDH ^D (U/L)	BF	487.1ab	513.3a	474.9b	456.3b	520.4a	458.7ab	26.3	0.46	0.001	0.02	0.01	0.59	0.77
	AF	550.4	537.5	517.5	491.9	502.7	479.9							

^AAspartate transaminase.

^BAlanine amino transferase.

^CAlkaline phosphatase.

^DLactate dehydrogenase.