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Effect of Different Fat Sources on some Blood Metabolites, Hormones, and Enzyme Activities of Lambs with Different Residual Feed Intake in Heat-Stressed Condition

Research Article

G. Halakoo[1], A. Teimouri Yansari[1*], M. Mohajer[2] and Y. Chashnidel[1]

[1] Department of Animal Science, Faculty of Agricultural Science, Sari Agricultural Science and Natural Resources University, Sari, Iran
[2] Department of Animal Science, Golestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Gorgan, Iran

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*Correspondence E-mail: astymori@yahoo.com

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ABSTRACT

Most efficient animals in heat-stressed condition, intelligently regulate their metabolism for maximizing their productivity and fats play an important role in reducing heat stress in these animals but the underlying mechanisms remain elusive. The effects of different fat sources on some blood metabolites and hormones and enzyme activities of heat-stressed lambs with different residual feed intake (RFI) were studied. In the preliminary trial, 48 male lambs of four different breeds (Zel, Dalagh, and their hybrids with Romanov; BW=16.87±0.55 kg) in a block randomized complete design used to assay RFI for 67 days. After that, 32 lambs of the same breeds as a block (BW=30.74±1.21 kg) with two different RFIs in heat stressed condition were used in a 2 × 4 factorial trail over 84 days. The treatments included four rations: 1) basal ration (control), 2, 3, and 4) rations supplemented with calcium soap fatty acids (FA), beef tallow, and canola oil, respectively. Dry matter intake (DMI) was high in control and high RFI groups (low efficient) ($P<0.05$). Lipid sources had significant effect on serum glucose, cholesterol, triiodothyronine (T_3), and Insulin, pulse and respiration rate ($P<0.05$). No differences found between treatments for triglyceride, thyroxine (T_4), low-density lipoprotein (LDL), and Very-low-density lipoprotein (VLDL). Glucose concentration had strong correlation with the RFI ($P<0.01$). The Low RFI lambs (LRFI) had high T_3 and low LDH levels. These lambs had high respiration and pulse rate ($P<0.05$). Lambs fed with fat supplemented rations had higher concentration of serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactic dehydrogenase (LDH) enzymes than control ($P<0.01$). Lipid supplementation in heat stressed lambs markedly alters glucose, cholesterol, T_3 and respiration and pulse rate that independently of reduced dry matter intake (DMI) through coordinated changes in fuel supply and utilization by multiple tissues. Even more challenging the most efficient lambs (LRFI) had high physiologic rate and high activity for removing heat from tissues.

KEY WORDS fat, heat stress, lamb, residual feed intake.

INTRODUCTION

Improving feed efficiency and productivity in heat-stressed animals is the main target of an intensive production system. The residual feed intake (RFI) is a measure of the net feed efficiency that describes the difference between an animal's predicted DMI and the actual DMI required for its maintenance and production (Flay *et al.* 2019). As feed accounts for 60 to 75 percent of the animal production costs, improving feed efficiency, and an increase in average

daily gain would increases farm income. The low RFI animals have improved digestive and metabolic efficiencies, or have lower maintenance requirements than high RFI animals in a given BW (Flay *et al.* 2019). Heat stress is defined as the animal's inability to dissipate heat to maintain homeothermy. Fats as a dense source of energy for heat stress reduction, generate lower metabolic heat, convert more efficiently to energy than other nutrients, and improve the metabolic efficiency of energy for milk production or weight gain. Animals adapt their physiological condition through changes in the endocrine system to maintain animal hemostasis regardless of environmental challenges (Bernabucci *et al.* 2010). Triiodothyronine (T_3), thyroxin (T_4), leptin, and insulin have a main role in regulating energy metabolism (Min *et al.* 2016). Concentrations of these key hormones altered with heat stress (Haraki *et al.* 2018; Gonzalez-Rivas *et al.* 2019) and it probably reflects the animal's attempt to regulate metabolic heat production and metabolic modifications to physiological state and animal efficiency. Further, T_3 and T_4 concentration decline with heat and restricted intake. Insulin regulates glucose and energy metabolism in different tissues, and heat increases stressed animals (O'Brien *et al.* 2010). Leptin reflects the nutritional status of the animals and its levels vary in heat-stressed animals. The Dalagh and Zel breeds are two domestic sheep breeds in northern Iran. Crossbreeds with Romanov have been welcomed to increase productivity, especially on industrial farms. The comparison of these native and hybrid breeds is an important issue in this area with high humidity and temperature. Feeding a fat-rich diet induces extensive metabolic adaptations to partitioning fats on different tissues without metabolic disturbance. Increasing feed efficiency using fat supplementation is one of the main strategies for lowering the effects of heat stress (Saeed *et al.* 2019). Some studies reported that in comparison to low RFI animals, the extra feed energy consumed by high RFI animals could be attributed to the energy retained as fat, and concluded that efficient animal has an efficient metabolic response to maintain their body steady state.

In tropical and subtropical areas, sheep subject to high ambient temperatures and high relative humidity and they experience heat stress. Heat stress leads to reduced productivity, livestock production, reproductive performance, and health hazard. Some animals have a good performance and regulate their metabolism to tolerated better this condition. Understanding the mechanism of hormonal regulation and efficient sheep breeds response to different sources of fat under heat stress conditions is important to adopt an empirical and practical strategy to counteract or reduce its negative effects on livestock production.

It is one of the basic requirements to develop commercial sheep breeding. Therefore, this experiment was designed to investigate the nutritional of different fat effects in the Zel and Dalagh and their hybrids with the Romanov breed under heat stress.

MATERIALS AND METHODS

Two experiments were conducted from April to September 2018 in a Gonbad, Golestan province, north of Iran (37˚ 23'07.0"N 55˚ 07'15.6"E). The entire experiment was conducted following guidelines of the Iranian Council of Animal Care (Iranian Council of Animal Care, 1995).

Preliminary trail

Forty-eighth male lambs (BW=17.1±3 kg; body condition score=2.9) of 4 breeds (Zel, Dalagh, Zel×Romanov, and Dalagh×Romanov) the same number of animals each breed were housed in individual pens and had *ad libitum* access to the feed, water, and mineral block. Lambs were initially drenched against parasites, and fed with a total mixed ration (control; Table 1) twice daily at 7:00 and 19:00 according to their requirements.

Refusals of the daily feed of each lamb were collected before 07:00h. After a 14-d acclimation period, individual daily DMI was measured over 67 days. Lambs were weighed individually every 14 days, and average daily gain and DMI were determined.

Residual feed intake calculation

The body weight (BW) on the first day (initial weight) and on the last day (final weight), were used to calculation metabolic mid-test as: $BW \times ((\text{final BW}+\text{initial BW}))/2)^{0.75}$ and average daily gain as: ([final BW – initial BW] / 67].

The model that fitted for each period was:

$$Y_i = \beta_0 + \beta_1 ADG + \beta_2 MBW + e_i$$

Where:
Y_i: expected daily feed intake.
β_0: regression intercept.
β_1: partial regression coefficient of the average daily DMI on the metabolic mid-test BW.
β_2: partial regression coefficient of the average DMI to the average daily gain.
e_i: residual error of animal I.

The RFI was computed for each lamb for each period by subtracting actual DMI from expected DMI (Koch *et al.* 1963).

Subsequently, lambs were categorized into two general high and low RFI groups.

Table 1 The experimental rations that supplemented with

Item	No added lipid (Control)	Calcium soap fatty acids	Beef tallow	Canola oil
Feed ingredients (% of DM)				
Barley grain	38	34	34	34
Beet pulp	21	20	20	20
Corn silage	20	20	20	20
Wheat bran	12	11.5	10	10
Soybean meal	2	3.5	4	4
Wheat straw	5	5	5	5
Calcium soap fatty acids	-	5	-	-
Beef tallow	-	-	5	-
Canola oil	-	-	-	5
Limestone	1	-	1	1
Vitamin and mineral premix[1]	1	1	1	1
Chemical composition				
Dry matter (%)	77.54	77.87	78.12	78.09
Organic matter (%)	92.08	92.46	92.28	92.29
Crude protein (%)	12.29	12.23	12.29	12.29
Neutral detergent fiber (%)	38.61	37.11	36.75	36.75
Non-fiber carbohydrate[2] (%)	41.55	39.15	38.66	38.66
Acid detergent fiber (%)	19.46	18.97	18.87	18.87
Ether extract (%)	2.01	6.11	6.81	6.81
Crude Ash (%)	7.92	7.54	7.72	7.71
Sugar[3] (%)	4.20	4.16	4.14	4.14
Metabolizable energy[3] (Mcal/kg)	2.81	2.99	2.97	3.01

[1] Vitamin and mineral premix per kg of diet: vitamin A: 500000 IU; vitamin D_3: 100000 IU; vitamin E: 1000 IU; Ca: 150g; Na: 60 g; Mg: 40 g; Mn: 3500 mg; Zn: 4500 mg; Fe: 3500 mg; Cu: 1000 mg; Se: 25 mg; I: 40 mg; Co: 40 mg and Antioxidant: 400 mg.
[2] Non-fiber carbohydrate calculates= 100 - (% NDF + % CP + % Fat + % Ash) (NRC, 2001).
[3] Values estimated from the library tables of SRNS 2016 and CNCPS sheep (2007).

Experiment

Animals, experimental design, and diets

Once the RFI was calculated and lambs were categorized. Thirty-two lambs were ranked on RFI and assigned to two groups (high and low RFI and each group had 16 lambs). In each RFI group 4 breeds including Zel, Dalagh, and their hybrid with Romanov (BW=30.74±1.21 kg) allotted as a block. This experiment was conducted in a complete randomized block design with a 2 × 4 factorial arrangement in an 84-day feeding period. The treatments were four rations including 1) control (no lipid supplemented), 2, 3, and 4) supplemented with calcium soap FAs, beef tallow, and canola oil, respectively. The ingredients and chemical composition of experimental rations and FA composition of rations lipid are presented in Tables 1 and 2, respectively. The diets were adjusted according to lambs requirements (CNCPS Sheep, 2007; Table 1). The lambs were fed with TMR in two equal meals at 0700 and 1900 h with at least 200 g/kg of refusals remained for each lamb. Animals had free access to water and to a commercial mineral supplement offered separately from concentrate. Lambs were weighed weekly and DMI was measured daily. Feed and orts samples were analyzed for DM, Kjeldahl N, ether extract (EE), organic matter (OM) and ash at 605 ˚C for 3h (AOAC, 1995), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al.* (1991); with α-amylase being added and without sodium sulfite).

The NDF was expressed without residual ash. The non-fiber carbohydrate (NFC) was calculated from 100 - (% CP + % NDF + % Ash + % EE) (NRC, 2001). The total FA were extracted from feeds based on the method of Rajion *et al.* (1985) using a gas-liquid chromatography on an Agilent 7890A GS system (Agilent, Palo Alto, CA, USA) equipped with a 100 m × 0.25 mm internal diameter (0.20 µm film thickness) Supelco sp-2560 capillary column (Supelco, Inc., Bellefonte, PA, USA).

Temperature humidity index

Diurnal variations in ambient temperature (T; ˚C), relative humidity (RH; %), and temperature humidity index (THI) during 90 days (from 5 Jun to 3 September) were taken from the Golestan Meteorological Administration. The THI was calculated from the following equation (NRC, 1971):

$$THI = (1.8 \times T + 32) - ((0.55 - 0.0055 \times HR) \times (1.8 \times T - 26)).$$

Where:
T: hourly temperature (˚C); RH: hourly relative humidity (%).

Blood sampling and biochemical assessment

Blood samples (10 mL) were taken from the jugular vein on days 0 and 84, just before the morning feeding and at 6 h after feeding.

Table 2 Dry matter intake, body weight, and condition of lambs (n=48) evaluated for residual feed intake during the growth period

Item	RFI groups		Breeds				SEM	P-value	
	Low RFI	High RFI	Dalagh	Dalagh × Romanov	Zel × Romanov	Zel		Breed	RFI
DMI (g/d) day 84	1489.38	1546.25	1603.75[a]	1516.875[b]	1500[b]	1401.25[c]	0.007	0.165	0.007
Initial body weight (kg)	16.98	16.77	16.8625	16.8734	16.9375	16.7688	0.933	0.327	0.933
Initial BCS	2.25	2.24	2.250	2.1875	2.125	2.1250	0.565	0.161	0.565
Final BW (kg)	30.64	30.84	31.6763[a]	30.7429[b]	30.4238[b]	29.9617[c]	0.027	0.609	0.027
Final BCS	2.77	2.78	3.0[a]	2.7656[b]	2.6250[c]	2.6875[bc]	0.012	0.694	0.012
Metabolic BW (kg)	10.81	10.742	10.9342	10.7771	10.7333	10.6270	0.112	0.437	0.112
Average daily gain (g)	216.67	223.73	238.9329[a]	223.7007[b]	217.5216	212.7895	0.033	0.126	0.033
RFI (g/d)	-278.57[b]	217.16[a]	35.2735	17.801	4.4335	19.5975	0.236	< 0.001	0.236

DMI: dry matter intake; BCS: body condition score; BW: body weight and RFI: residual feed intake.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effect of different lipid sources on physiological indices of heat stressed lambs

Lipid sources	The experimental rations that supplemented with								SEM	P-values			
	No lipid (control)		Calcium soap fatty acids		Beef tallow		Canola oil			Diet	RFI	Breed	Diet × RFI
Residual feed intake	Low RFI	High RFI	Low RFI	High RFI	Low RFI	High RFI	Low RFI	High RFI					
Rectal temperature (˚C)	39.93	39.88	39.65	39.55	39.58	39.45	39.94	39.88	0.06	0.018	0.143	0.044	0.551
Respiration rate (breath/min)	55.08	54.75	51.42	49.00	48.83	47.08	51.67	49.33	0.04	0.048	0.026	0.000	0.972
Pulse rate (beats/min)	96.08	93.42	82.92	79.92	85.08	83.92	89.08	86.08	1.32	0.032	0.021	< 0.0001	0.690

SEM: standard error of the means.

The serum was stored at 20 ˚C after separation by centrifuging at 1500 × *g* for 15 min. Serum biochemical parameters including glucose (GLU), triglyceride (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL) concentration, aspartate-amino-transferase (AST), gamma-glutamyl-transferase (GGT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities were assessed with a biochemistry autoanalyzer (BT-1500, Biotechnica, Italy), using Pars Azmoon kits (Iran). The ELISA kit including STAT-FAX, Awareness Technology Inc., and Webster, Texas USA, DSL Insulin ELISA, was used to measure serum leptin and insulin. In addition, the T_3 and T_4 hormones were assayed by the gamma counter (Orion Diagnostica, Finland kit, Finland).

Statistical analysis

Data were analyzed by general linear models procedures of SAS (SAS, 2013), based on the following statistical model:

$$Y_{ijkl} = \mu + T_{j(j)} + R_j + RFI_k + T \times RFI_{j(j)k} + e_{ijkl}$$

Where:
Y_{ijkl}: dependent variable.
μ: overall mean.
T_i: effect of treatment (1, 2, 3, and 4).
R_j: effect of the breed j as a block (1, 2, 3, and 4).
RFI_k: effects of RFI (low and high).
$T \times RFI_{j(j)k}$: iteration of treatment and RFI.
e_{ijkl}: experimental error.

Means were separated using Duncan's multiple range tests with an alpha level of 0.05.

RESULTS AND DISCUSSION

Preliminary trail

Initial and final BW, metabolic BW, and average daily gain of animals are shown in Table 4. The values for RFI ranged from –64.02 to 56.05 g DM day^{-1}, which represents a difference of about 120 g DM day^{-1} between the highest and lowest RFI sheep. Lambs consumed 1,164 g day^{-1} DMI on average.

However, maximum and minimum DMI were 1.263 and 1.059 g DM day^{-1}, respectively. Besides, high efficient lambs (low RFI lambs) consumed 1156 g DM $day^{-1}$and low efficient lambs (high RFI lambs) eat 1172 g DM day^{-1} at end of the preliminary trial (day 67). According to the results, the lambs were classified into two groups for RFI. Overall, the high efficient lambs consumed on average 110 g DM day^{-1} less DM than the low efficient lambs. However, the initial and final BW and body condition scores (BCS), metabolic BW, and average daily gain were similar between breeds and were not significantly influenced by the RFI (Table 4).

The average daily ambient temperature, RH, and THI over 90 days from 5 Jun to 3 September 2018 were presented in Figure 1 (NRC, 1971). Traditionally, the THI is employed as the index to evaluate the degree of heat stress.

Table 4 Fatty acid composition (% of ether extracts) of the experimental diet

Item	The experimental rations that supplemented with				SEM	P-value
	No lipid (control)	Calcium soap fatty acids	Beef tallow	Canola oil		
Lauric acid (C_{12})	0.12	0.13	0.13	0.13	0.001	0.122
Myristic acid (C_{14})	0.26	0.34	0.42	0.27	0.01	0.071
Palmitic acid (C_{16})	21.10^{b}	22.63^{a}	21.15^{b}	20.14^{c}	0.16	0.034
Palmitoleic acid ($C_{16:1}$)	0.20^{b}	0.19^{c}	0.38^{a}	0.21^{b}	0.01	0.012
Stearic acid (C_{18})	1.54^{d}	1.73^{b}	2.43^{a}	1.64^{c}	0.06	0.012
Vaccenic acid ($C_{18:1\text{- Trans}}$)	0.04^{b}	0.05^{b}	0.25^{a}	0.23^{a}	0.02	0.001
Oleic acid ($C_{18:1\text{- sis}}$)	13.95	15.18	15.28	16.06	0.14	0.795
Linoleic acid ($C_{18:2}$)	50.61^{a}	48.74^{b}	47.83^{c}	48.74^{b}	0.18	0.032
Linolenic acid ($C_{18:3}$)	7.86	7.84	7.73	8.08	0.03	0.069
Other lipids	2.32	2.26	2.42	2.53	0.02	0.465
SFA[2]	23.03^{c}	24.84^{a}	24.12^{b}	22.18^{d}	0.18	0.054
MUFA[3]	14.19^{d}	15.42^{c}	15.91^{b}	16.49^{a}	0.15	0.047
PUFA[4]	58.47^{a}	56.48^{c}	55.56^{d}	56.81^{b}	0.19	0.050
n-6:n-3[5]	6.44	6.30	6.19	6.03	0.03	0.067

[1] Saturated fatty acids (SFA)= (C12:0+C14:0+C16:0+C18:0).
[2] Total monounsaturated FA (Monounsaturated fatty acid (MUFA), C16:1+C18:1n-9).
[3] Polyunsaturated fatty acids (PUFA)= (C18:2n-6+C18:3n-3).
[4] n-6:n-3 fatty acid ratio= (C18:2n) / (C18:3n).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

During the experiment, the THI was greater than the 70 and confirmed that animals were under heat stress conditions through the duration of the trial (Table 3).

The effect of feeding different lipid sources on physiological indexes in heat-stressed lambs is presented in Table 5. Lambs rectal temperature was different between treatments. Rectal temperature had a tendency to be lower in lipid supplemented diets (P=0.02). Ca-fat and beef tallow had the lowest pulse rate (P=0.03) and respiration rate (*P*=0.05), respectively. Overall Lambs in control treatment had the highest rectal temperature, respiration rate, and pulse rate (Table 5). It seems that lipid source decreases heat load in lambs.

Animals with low RFI had a high respiration rate (P=0.02) and high residual rectal temperature against DMI (P=0.04, R^2_{adjust}= 0.2). Rectal temperature correlated with metabolic BW (P<0.0001), DMI (P=0.02), and RFI (P=0.04). Animals when challenged to high temperature dissipated heat via an increase in respiration rate. Heat dissipation is the key response to heat adaptation and in many cases, heat generated in core body tissues exchange to the environment with respiration rate increases. Some animals had a lower heat load they did not need to acclimation response for climate changes. The heart rate increases in response to high ambient temperatures, to increase blood circulation from the depths of the body to eliminate excessive heat through conduction and convection. In this way, more water reaches the surface of the body and sweat glands.

In this study, THI was above 70 and more than 78 in some hour of days (Figure 1). In this situation, the animal experienced moderate and severe heat stress. As mentioned Belhadj Slimen *et al.* (2016) "Indeed, heat stress reorganizes the use of the body resources including fat, protein and energy" and as well as Herd *et al.* (2019) report that animal with low RFI had more heat production, it seems that efficient lambs generate more heat, that in bioenergetics view they had a high metabolism.

Dalagh lambs had a lower rectal temperature, lower heart pulse, and lower breathing rate vs. other breeds. Zel and their hybrid with Romanov had a more rectal temperature and heart pulse, and respiration rate (P<0.05). The Dalagh sheep had a lower metabolic rate and it seems that they generate low heat in comparison to other breeds. Although the body heat load is affected by animal factors (like breed, sex, BW, DMI, level of production) (Baumgard and Rhoads Jr, 2013; Belhadj Slimen *et al.* 2016; Morera *et al.* 2012; Wheelock *et al.* 2010), and environmental factors (diet, humidity, temperature, exposer to hyperthermia and …) (Carabano *et al.* 2016). The Dalagh breed seems to have adapted to the subtropical climatic conditions.

Experimental rations and RFI significantly affected DMI. The high RFI lambs had significantly greater DMI in comparison to low RFI lambs. Similar to Bhatt *et al.* (2013), the supplementation with calcium soap FA decreased DMI, but supplementation with beef tallow and canola oil did not affect DMI as compared to control.

Table 5 Effect of lipid sources on dry matter intake, plasma metabolite, some hormones and liver enzyme of heat-stressed lambs classified as high and low residual feed intake (RFI)

Lipid sources	The experimental rations that supplemented with								SEM	Diet	P-values		
	No lipid (control)		Calcium soap fatty acids		Beef tallow		Canola oil				RFI	Breed	Diet × RFI
Residual feed intake	Low	High	Low	High	Low	High	Low	High					
Dry matter intake (g/d)	1580^{b}	1675^{a}	1445^{d}	1473^{c}	1510^{c}	1528^{bc}	1405^{d}	1528^{bc}	35.76	0.024	< 0.001	0.051	0.217
Plasma metabolite (mg/dL)													
Glucose	60.25^{d}	62.75^{ab}	62.5^{cd}	65.25^{a}	62.25^{cd}	64.25^{a}	61.75^{d}	63.25^{ab}	1.35	0.062	0.006	0.593	0.553
Cholesterol	61.63^{bc}	69.25^{a}	45.88^{d}	66.73^{ab}	59.31^{c}	58.13^{c}	64.75^{b}	67.50^{a}	2.35	0.017	0.028	0.000	0.256
Triglycerides	31.50	30.88	33.25	35.25	35.00	31.25	34.75	41.00	2.95	0.073	0.579	0.271	0.239
High density lipoprotein	33.50^{ab}	35.75^{a}	28.75^{c}	34.75^{ab}	32.25^{b}	34.00^{ab}	35.00 ^{a}	36.50^{a}	1.57	0.028	0.005	0.501	0.288
Low density lipoprotein	18.29^{b}	23.46^{a}	7.17^{d}	22.10^{a}	17.40^{b}	14.55^{c}	19.98^{ab}	21.82^{a}	2.14	0.047	0.903	0.047	0.293
Very low density lipoprotein	3.24	3.44	3.35	3.28	3.07	2.97	3.18	3.32	0.06	0.151	0.683	0.186	0.666
Hormones													
T_3 (µg/dL)	1.25^{c}	1.05^{d}	1.49^{b}	1.33^{c}	1.40^{b}	1.39^{b}	1.60^{a}	1.39^{b}	0.03	< 0.0001	0.001	0.001	0.128
T_4 (µg/dL)	68.25^{bc}	65.50^{c}	75.25^{a}	72.75^{b}	65.25^{c}	60.75^{d}	67.00^{c}	71.50^{b}	2.17	0.251	0.733	0.011	0.843
T_3 toT_4	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.040	0.226	0.004	0.483
Insulin (µU/L)	6.15	6.08	7.11	5.93	7.31	6.94	8.60	7.43	0.42	0.211	0.290	0.001	0.901
Leptin (ngr/mL)	1.98^{e}	2.08^{d}	3.40^{b}	3.06^{c}	3.35^{b}	3.86^{a}	3.70^{b}	4.14^{a}	0.15	< 0.0001	0.023	0.716	0.161
Enzyme activities (U/L)													
Aspartate transaminase	69.25^{c}	71.25^{c}	83.25^{a}	83.25^{a}	77.75^{b}	76.50^{b}	75.50^{b}	76.00^{b}	1.03	< 0.0001	0.806	0.113	0.836
Alkaline phosphatase	79	69.75	97.25^{b}	98.75^{b}	105^{a}	98.75^{b}	90c	88.25	3.07	0.006	0.459	0.252	0.890
Alanine amino transferase	27.25	23.25	39	36.5	38.75	32.25	25.25	24.75	2.31	0.068	0.332	0.371	0.925
Lactate dehydrogenase	410^{e}	431^{d}	425^{d}	441^{c}	441^{c}	455^{b}	448^{a}	447^{a}	3.47	< 0.0001	< 0.0001	0.215	0.004

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

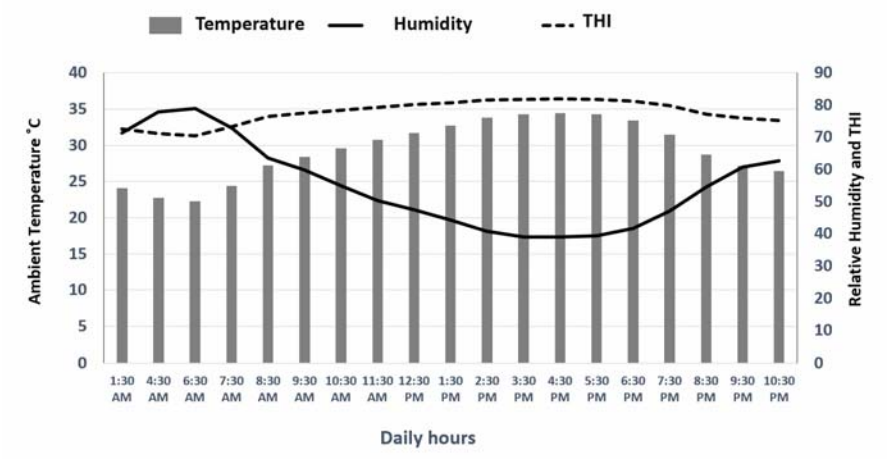

Figure 1 Diurnal variations in ambient temperature (˚C), relative humidity (%), and THI during 90 days (from 5 Jun to 3 September on trial)
Thanks to the Golestan Meteorological Administration, which provided us the raw data
THI calculated from THI= (1.8×T+32) - ((0.55-0.0055×HR) × (1.8×T-26)) equation (NRC, 1971)

The DMI decreased by 4.1%, 12% in treatment 2 in comparison to beef tallow, and control treatment respectively. The greatest DMI was observed in the control treatment (P=0.02). Adding fat sources often decreases DMI, especially when polyunsaturated fatty acids (PUFAs) can inhibit fiber digestion in rumen. Vegetable oils increased PUFA and decreased saturated fatty acids (SFA) (Tanaka, 2005). Canola oil presents a high content of PUFA (91.7%, mainly C18:1 and C18:2 in comparison to other vegetable sources).

The C-18 FAs and other UFA disrupt cell integrity of some bacteria and are toxic for rumen microorganisms, which reduce DMI, fermentation, passage rate, digestibility, and consequently feed efficiency. Otherwise, the heat stress decreases DMI that this issue considerable influence on sheep farming. Supply dense energy sources could help the animal to obtain their requirement and tolerated stress conditions. In most studies, calcium soap of palm oil (rumen-protected UFA) had no effect on DMI like other scientists

(Kellner *et al*. 2016; Palmquist and Jenkins, 2017; Rabiee *et al*. 2012). The DMI decreases with the increasing length of long-chain FA infusions into the abomasum in dairy cows. It was found that the hyperphagic effects of added fat, increased with the PUFA content. Bhatt *et al.* (2013) found that in Ca-soap supplemented lambs, metabolizable energy (ME) intake increased because of reduced heat energy because of lower microbial fermentation and less energy loss. In addition, Haddad and Younis (2004) found decrease DMI with the addition of 25 and 50 g/kg of SFA in the diets of Awassi lambs. The effects of dietary fat on the reduction of DMI is a complex mechanism and involves effects of FA on gut peptides and pancreatic hormones along with direct/indirect effects on hepatic energy oxidation (Allen *et al*. 2012). Moreover, the extent of DMI depression by dietary fat is related to PUFA concentration and this effect is greater for free FA compared to triglycerides (Palmquist and Jenkins, 2017). The EE content and subsequently the concentration of PUFA increased in supplemented rations in comparison to control. However, in the experimental diets the extent of DMI was affected by dietary fat and PUFA concentration.

Blood glucose concentrations differed significantly among RFI groups (P=0.006), but experimental diets, Diet × RFI, and breeds had no significant effect on the glucose concentration Results showed a tendency to increase in fat supplemented rations (P=0.06). However, the concentrations of blood glucose were within the normal range. Plasma glucose was higher in treatment 3 in comparison to other treatments. Heat stress exhibit increased basal insulin levels, greater insulin sensitivity, and glucose uptake, and lower plasma glucose. These results agree with the findings in dairy cows that chronic heat stress leads to hypoglycemia and increases basal insulin levels despite marked reductions in nutrient intake (Wheelock *et al*. 2010). These results do not agree with other studies that feeding oils did not affect plasma glucose concentration in sheep (Parvar *et al*. 2017). However, (Palmquist and Jenkins, 2017) found the concentration of glucose increased with fat supplementation in dairy cows and protected PUFA in pregnant ewes. The higher concentrations of glucose found in high-RFI lambs. Plasma glucose concentration was affected by RFI levels (P=0.006).

This finding was similar to other results showing that serum glucose decreased in the low RFI group of sheep (Rincon-Delgado *et al*. 2011), steers, and cows (Dechow *et al*. 2017). The RFI had a great relationship with glucose concentrations in ruminants (Dechow *et al*. 2017). Although the glucose increases in an animal under thermal stress, originates from both increased gluconeogenesis and increased glycogenolysis, it seems that more efficient or low RFI animals have lower plasma glucose.

The cholesterol concentration showed a tendency to increase in treatment 2 and 3 in comparison to treatments 1 and 4 (P=0.06) that was similar to Bianchi *et al*. (2014) and Bhatt *et al*. (2013). Bhatt *et al.* (2013) found higher cholesterol content in the serum of fat supplemented groups while no effect was recorded for other blood parameters. HDL increased by adding fat to rations (P=0.02) but Triglycerides did not differ among treatments (P=0.07). Long-chain FAs are synthesized from glucose by acetyl-CoA. Also, consequently, glycerol-3-phosphate is made in the liver and adipose tissue from glucose (Kaneko *et al.* 2008). Additionally, triglycerides elevated in sheep supplemented with protected fat due to the higher amounts of FAs released in the abomasum (Bianchi *et al*. 2014). Furthermore, triglycerides are stored in the liver and released in the form of LDL from the liver. In conclusion, rumen-protected (Ca-Fat) increases blood HDL concentrations, which may be derived from absorbing FAs in the small intestine. Triglyceride increased in heat-stressed dairy cows (Palmquist and Jenkins, 2017). The cholesterol and HDL were lower than other treatments in the low RFI group (P=0.03). The LDL and HDL contained cholesterol (HDL makes about 40 - 80% of lipoproteins; (Kaneko *et al*. 2008). Cholesterol biosynthesis is related to feed efficiency in sheep. Arthur *et al*. (2001) found the high RFI steers had a positive correlation with cholesterol levels compared to the low RFI group in intermuscular, intramuscular, subcutaneous, and average back fat of beef carcass. As well as, liver synthesis of cholesterol is liable for this increase (lack of cholesterol in the fat source) in dairy cows (Kellner *et al*. 2016; Palmquist and Jenkins, 2017; Rabiee *et al*. 2012). Moreover, the serum cholesterol appears to primarily be related to an increased HDL fraction in the high-fat-fed group (Koppe *et al*. 2009). So, the parallel changes of cholesterol and HDL in this study could be explained.

The T_3 increased with fat supplementation (P<0.0001). The results of the current experiment indicated the significant effect of breed on T_3 and T_4 concentration that may be related to differences in BW, length, and body fat because larger frame cattle and sheep have higher circulating levels of T_4. Toufighi *et al*. (2014) found that in young steers, rumen-protected fat increases T_3 concentration. Thyroid hormones concentration in plasma is directly related to the level of DMI. In ruminants, decreased DMI reduces the release of plasma concentration of T_3 and T_4 (Sadeghi and Moghaddam, 2018). The increased thyroid hormones increase the cholesterol catabolism in the liver, which reduces plasma cholesterol. Therefore, plasma cholesterol has a negative correlation with T_3 and T_4 levels in animals (Sadeghi and Moghaddam, 2018). These hormones play a very important role in adapting the metabolism of animals to the environment and increasing the efficiency of live-

stock. However, T_3 concentration was 11% higher in the low RFI group (P=0.001) but T_3 was greater in the low RFI pigs (Lkhagvadorj, 2010). However, the T_3 level was lower in the low RFI chicken. Lkhagvadorj (2010) suggested that the greatest concentration of T_3 is low RFI group pigs were likely down-regulated for 3-iodothyronine deiodinases at the liver and this increase in T_3 concentrations may contribute to improving feed efficiency of the animal through enhanced growth and reduced DMI via nutrient-sensing mechanisms.

The Insulin level in serum was not affected by fat sources but its concentration in different breeds was significantly different (P=0.001). Plasma insulin concentration decreased for PUFA and SFA diet in human and lactating cow and lambs (Becú-Villalobos *et al*. 2007). A decrease and an increase in plasma glucose concentration were reported when 2% of fish oil and 2.3% Ca soap of fish oil were fed, respectively (Mattos *et al.* 2004). Increasing insulin prevents the mobilization of adipose tissue and prevents animals from entering glucose to produce milk or increase muscle (Bianchi *et al*. 2014). The effects of heat stress on blood glucose concentrations were less effective and decreased in some cases (Shwartz *et al*. 2009) and other studies were increased (Wheelock *et al*. 2010). Also, insulin-stimulated glucose uptake in high-fat diet, also long-term heat stress (more than 24 hours) increases basal and circulating insulin and reduces the mobilization of fatty tissues in different species (Baumgard and Rhoads Jr, 2013). Hyperinsulinemia or increased insulin sensitivity may be one of the strategies to reduce heat production because glucose oxidation is more effective in the production of ATP than lipid (Belhadj Slimen *et al*. 2016). On the other hand, animals that are raised in heat stress have higher adipose tissue than predicted, which seems to be explained by hyperinsulinemia.

Leptin concentration increased by lipid supplementation (P<0.0001). In addition, leptin concentration was not different among breeds. Leptin concentration significantly increased in high RFI lambs in comparison to low RFI lambs (P=0.02). Similar results were observed in young heifers and lactating cows that fed olive oil (Loor *et al*. 2005) or calcium salts of palm oil or conjugated linoleic acids (Block *et al*. 2003), sunflower seeds in high fat diet, corn oil, linoleic acid, and rumen-protected linoleic acid (Gillis *et al*. 2004). On the other hand, leptin in adipose tissue increases with fat supplementation, which indicates that the tissue has its fluctuations (Gillis *et al*. 2004). Leptin that is secreted from adipose tissue plays a vital role in feed intake, carbohydrate, and lipid metabolism balance, uptake of adipose tissue, energy production, and endocrine system activity. Serum leptin increased during thermal stress (Morera *et al*. 2012) and exposed to heat shock adipose tissue cells, may be considered as one of the adaptive mechanisms to the ambient temperature with a central action, which reduces DMI and limit body temperature. In some experiments, various sources of fat under certain physiological conditions reduced serum leptin levels (Becú-Villalobos *et al*. 2007). In other studies, supplementing fat to diet or replacing it with energy did not increase leptin levels in lactating cows (Becú-Villalobos *et al*. 2007). In addition, when a water-rich emulsion of 18:2 oil was injected into the jugular vein, no effect was observed at the level of leptin in early lactation cows, but leptin increased at the end of lactation, indicating that various hormonal fluctuations depending on the physiological state of the animals (Gillis *et al.* 2004). Leptin has a role in the regulation of acute triglyceride metabolism, HDL-cholesterol clearance, lipid oxidation, and the lipogenic effects of insulin at the cellular level. In addition, leptin is positively associated with cholesterol and HDL; has a weak positive correlation with triglyceride concentration (Gillis *et al*. 2004). Leptin induces satiety and might help maintain BW over the long term as its secretion by adipocytes is correlated with the size of the body fat deposits. Leptin concentration in blood was related to body condition score of lactating cows (r=0.61; P<0.001) while insulin, another putative adiposity signal, was only weakly related in a different experiment (r=0.33; P<0.10). Morera *et al.* (2012) found that heat stress improves leptin and adiponectin signaling and changes in the levels and sensitivity of these adipokines may be considered an adaptive response to heat stress conditions.

Lambs in the fat supplemented group had significantly more serum AST, ALP, and LDH enzymes than the control group (P<0.0001; P=0.006 and P<0.0001 respectively). This result is in agreement with other studies (Bianchi *et al*. 2014; Parvar *et al*. 2017). Parvar *et al*. (2017) found lambs that fed fish oil, soybean oil, and canola oil had the numerically the highest concentration of ALP, AST, and ALT in comparison to control treatment. High-fat diets induce oxidative stress (Pinho *et al*. 2017). Moreover, the average of enzymes in these groups was not sufficient to associate it to damage liver tissues (the tissue of the livers was seen to be healthy and without apparent lesion).

Activities of LDH enzyme differed in lambs (P<0.01). High RFI lambs had higher levels of LDH than low RFI group as this corroborates earlier findings (Faure *et al*. 2013) who reported that the low RFI group exhibited different strategy than high RFI and showed low LDH. The liver plays a crucial role in metabolism, especially for lipid and carbohydrate especially in regulation of systemic glucose as well as insulin level. The ALP plays a multisystemic functions in FA absorption, in protecting gut barrier function, in determining the composition of the gut micro-

biota via its ability to dephosphorylate lipopolysaccharide, in bio-mineralization, facilitating the transport of long chain FAs, phosphate, Ca into cells, proactive role from bacterial endotoxin insult, neurotransmitter synthesis, regulation of growth, and apoptosis in the fetus (Buchet *et al*. 2013). The ALT enzyme catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, and the converted products are L-glutamate and pyruvate in the liver, which is a critical process of the tricarboxylic acid cycle. Liao *et al*. (2015) reported that trans-FA feeding results in higher serum ALT compared with a standard murine high-fat diet in rats. A high-fat diet had higher AST and ALT in Hamsters (Liao *et al*. 2015). Bianchi *et al*. (2014) report that in the group was treated with protected fat increased AST in comparison to the other groups, superior even to reference values (Kaneko *et al*. 2008).

CONCLUSION

Physiologic parameters are affected by many factors. The result showed that animals fed fat supplemented diet had the lowest rectal temperature, respiration rate, and pulse rate. In this study, efficient lambs (low RFI) showed numerically high rectal temperature and a significantly pulse and respiration rate. Blood transfer heat from tissue to lungs and skeletal muscles. These events showed efficient animals produce more heat for their metabolism and dissipated this heat through conduction and convection way. Efficient lambs, in bioenergetics view they had high metabolism. The comparison of 4 breeds showed Dalagh lambs had low activity in removing heat *vs*. other breeds. Zel and their hybrid with Romanov had the challenge to eliminate heat from body dept to the environment. The Dalagh sheep had a lower metabolic rate and it seems that they generate low heat in comparison to other breeds. The Dalagh lambs adapted easily to the subtropical climatic. Animal feed intake differs in innate and environmental condition. The low RFI lambs had lower DMI than high RFI lambs. The supplementation rations with calcium soap FA decreased DMI, but supplementation with beef tallow and canola oil did not affect DMI as compared to control. Fat supplementation decreased DMI *vs*. control treatment. Adding fat to rations often decreases DMI, especially when PUFAs can inhibit fiber digestion in the rumen. Canola oil has a high content of PUFA. The unsaturated FA disrupts some bacteria activity and is toxic for rumen microbes, which reduce DMI, flow rate, digestibility, and feed efficiency. The DMI decreases with the increasing length of long chain FA. Hypophagic effects of fat increased with the PUFA content. Ca-soap supplemented diet, increase ME intake, and reduce energy loss. Reduction DMI with fat is a complex mechanism with effects on hepatic energy oxidation. However, the DMI was affected by dietary fat and PUFA concentration. Plasma metabolites affect fat feeding and alter in different RFI groups. Blood glucose concentrations were high in high RFI groups. This study result showed the RFI had great relationship with glucose concentrations in lambs. More efficient lambs had less plasma glucose. Probably this result was presumed to be result of low feed intake. Plasma glucose was higher in the beef tallow treatment in comparison to other treatments. This treatment had lower PUFA than other treatments. Rumen-protected (Ca-Fat) increased HDL concentrations, which may be derived from absorbing FAs in the small intestine. The cholesterol and HDL were high in high RFI lambs. The LDL and HDL contained cholesterol. High RFI lambs had a positive correlation with plasma cholesterol levels compared to the low RFI group. Moreover, the serum cholesterol appears to primarily be related to an increased HDL fraction in the high-fat-fed group. So, glucose and HDL biosynthesis related to feeding efficiency in lambs. Secretion hormones change with multifactor. Fat supplementation increased T_3 concentration. The results of the current experiment indicated different breeds had distinct T_3 and T_4 concentration that may be related to differences in BW, length, and body fat because the larger frame of these sheep has higher circulating levels of T_4. The greatest concentration of T_3 is low RFI group lambs may contribute to improving the feed efficiency of the animal through enhanced growth and reduced DMI via nutrient-sensing mechanisms. The Insulin level in serum in different breeds were significantly different. Increased insulin may be one of the strategies to reduce heat production because glucose oxidation is more effective in the production of ATP than lipid. Leptin concentration increased by lipid supplementation and in high RFI. Leptin in adipose tissue increases with fat supplementation, which indicates that the tissue has its own effect. Leptin that is secreted from adipose tissue plays a vital role in feed intake, metabolism, energy production, and endocrine hormones. Leptin regulate triglyceride, HDL and lipid metabolism and the lipogenic effects. Leptin induces satiety and its concentration in blood was related to body condition score. Hormones that mentioned above play a very important role in adapting the metabolism of animals and affected diet, breed, and RFI. Liver enzymes have a major role in animal metabolism. Animals that fed fat supplemented diet had more serum AST, ALP, and LDH enzymes may reflect different protein metabolism rate. High-fat diets induce oxidative stress. The ALP plays multisystemic functions in gut absorption, FA and mineral transportation and regulation of growth. A high-fat diet had higher AST and ALT treated. Protected fat increased AST. Activities of LDH enzyme differed in RFI groups. Low RFI lambs had less LDH, maybe correspond with the stable condition in this animals. The ALT enzyme

catalyzes the transfer of amino groups which is a critical process of the tricarboxylic acid cycle. Given that the measurement of residual feed intake is time-consuming and costly, the measurement of some factors can partly indicate a prospect of livestock performance. The high correlation of some factors with the residual feed intake makes their performance more prominent.

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