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Blood Biochemical Parameters and Physiological Indices in Fattened Heat Stressed Lambs Fed with Higher Protein Level and Glutamine Supplementation

Research Article

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ABSTRACT

The research aims to determine the effect of protein levels and glutamine supplementation on the blood biochemical parameters and physiological indices of fattened lambs during heat stress. Sixteen male lambs of the Afshari breed (average BW=31.5±0.22 kg; aged 3-4 months) were randomly selected and assigned into four groups for forty-five days. The experimental treatments included animals fed with a basal diet (C), basal diet supplemented with glutamine at the rate of 0.2 g/kg of body weight (BW) (G), basal diet with 10% higher protein (CP), and basal diet with 10% higher protein and glutamine (GP). The mean temperature-humidity index was 82.26, which indicated heat stress condition during the experimental periods. Changes in body weight, dry matter intake, and feed conversion ratio were similar in all the groups. Rectal temperature and respiratory rate significantly decreased with glutamine supplementation (P=0.018, P=0.004 and P=0.051, P=0.004 on the 30th and 45th days respectively). Glutamine supplementation in G and GP groups decreased concentrations of creatinine (P=0.013 on the 30th), lactate (P<0.0001 on the 15th day) and conversely increased glucose concentration (P=0.027, P=0.012 and P=0.001 on the 15th, 30th and 45th days), but the effect of glutamine supplementation on blood urea nitrogen, cholesterol, and triglycerides was not significant. The significantly increased blood urea nitrogen, cholesterol (P<0.05) and triglycerides (P=0.001, P=0.019 on the 15th and 30th) when the higher level of protein was used; however, glucose, creatinine, and lactate were found non significant. The interaction of glutamine and protein level significantly increased glucose concentration (P=0.010 and P=0.005 on the 15th and 45th days) and decreased cholesterol and triglyceride concentrations in fattening lambs (P<0.05). The results showed that glutamine supplementation improves the health state of heat-stressed lambs during the fattening period.

KEY WORDS blood urea nitrogen, glutamine, heat stress, lactate, respiratory rate, temperature humidity index.

INTRODUCTION

Small ruminants are exposed to a variety of stressors, including physical, nutritional, chemical, physiological, and heat stress (HS). Heat stress is currently the most concern due to climate change among the stressors. High ambient temperatures, combined with high humidity, enhance the stress level which in turn resulted in depression of the physiological and metabolic activities of these animals (Rathwa *et al.* 2017), disrupts enzymatic reactions, hormonal secretions, and blood metabolites (Marai *et al.* 2007). In addition, common homeostatic responses to HS in sheep and goats include increased respiration rate, body temperature, and water intake (Gupta *et al.* 2013) and decreased dry matter intake (DMI) (Caulfield *et al.* 2014).

matter intake (DMI) (Caulfield *et al*. 2014). Vasodilation with increased blood flow to the skin surface and increased rectal temperature but reduced metabolic rate, DMI, feed consumption efficiency, and altered metabolism are the physiological responses associated with negative impacts of HS, on small ruminants (Caulfield *et al*. 2014).

Modifications of ration, feeding schedules, such as fiber adjustment, using high-quality forage, increasing energy density and the use of feed additives can greatly help in reducing the negative effect of HS. In HS conditions, both the quantity and quality of the dietary protein are important because protein consumption at higher and lower levels will increase heat production. Nutritional strategies such as adding ruminally protected amino acids such as glutamine (Gln) may be considered to reduce the negative effects of HS (Lima *et al*. 2005). During catabolic/hyper catabolic situations, Gln can become essential for metabolic function but its availability may be compromised due to the impairment of homeostasis in the inter-tissue metabolism of amino acids. For this reason, Gln is currently part of clinical nutrition supplementation protocols and / or recommended for immune-suppressed individuals. Glutamine, as the most abundant amino acid in the blood, has various anabolic and stimulatory roles such as stimulating protein synthesis, enhancing nitrogen balance, stimulating the immune system, anabolic and anti-catabolic effects on muscles, regulating and modifying glucose from gluconeogenesis pathway, branched-chain amino acid production (BCAA), initiation of trans-amination, and deamination pathways (Kul *et al*. 2009). Glutamine is involved in nucleic acid biosynthesis that is essential to support cell proliferation. At least in sheep, Gln may exert a protective effect against the oxidation of hepatic amino acids, particularly for methionine. *In vitro* studies as well as experiments in animals have shown Gln to be one of the most effective substrates for gluconeogenesis. Glutamine also improved the sensitivity of adipose tissue to insulin, decreased lipolysis, and subsequently improved glucose metabolism (Roth, 2008). However, the direct or even indirect effects of Gln on *in vivo* anabolism are controversial and may be limited to improving catabolic conditions. Cereal-based diets are relatively deficient in Gln (Li *et al*. 2011), and supplements can correct this deficiency without the need to increase total protein. However, it is important to decide on the results used to determine the need. Weight gain, DMI, or more variables such as blood biochemistry, or even a detailed study of muscle growth and quality can be investigated. To date, little attention has been paid to such variables, and the mechanisms involved and their physiological significance has not been adequately addressed.

The role of Gln in reducing the negative effects of HS on pig and bird species has been extensively studied, but there is a lack of information about its effects on goat and sheep species. Therefore, in this study, the effect of protein level and Gln supplementation on DMI and blood biochemical indices of Afshari sheep in Mazandaran were investigated.

MATERIALS AND METHODS

Design of experiment and treatments

The research was carried out in the second half of July to the end of August in the north part of Iran (Latitude: 36˚ 33' 47.95" N and Longitude: 53˚ 03' 36.32" E) during HS (temperature-humidity index (THI): 82.26). Sixteen apparently healthy male lambs of the Afshari breed (aged 3-4 months) with average BW= 31.5 ± 0.22 kg were randomly selected. Prior to the experiments, all animals were examined for their physiological parameters and then treated against internal and external parasites. Water was offered *ad libitum* and lambs were fed with TMR ration for 45 days. The experimental treatments included animals fed with a basal diet (C), basal diet supplemented with Gln at the rate of 0.2 g/kg of BW (G), basal diet with 10% higher protein (CP), and basal diet with 10% higher protein and Gln at the rate of 0.2 g/kg of BW (GP) (Table 1).

The glutamine, for protection, was sprayed with 2% formaldehyde for 72 h, dried at room temperature, and finally, formaldehyde residues were evaporated. Physiological parameters, rectal temperature (RT, ˚C) was recorded by clinical thermometer at each weighing time and respiratory rate (RR) recorded by counting lateral movements per minute (low: 40 to 60, medium: 60 to 80, high: 80 to 120, and severe: >200) from a distance of 4 to 5 meters without disturbing the animal (Shilja *et al*. 2015).

Over the study, meteorological data were obtained from the Sari Weather Station. Daily ambient temperature and relative humidity were recorded to calculate the THI (Table 2). The degree of HS experienced by animals is estimated by the THI (Mader, 2006):

$$THI = (0.8 \times temperature) + [(\% RH/100) \times (temperature - 14.4)] + 46.4$$

Where:
THI: highest daily temperature in Celsius degrees.
RH: maximum relative humidity (%) for each day.

Blood analysis

Blood sampling was carried out between 7 and 8 am, to avoid diurnal influences, on days 15, 30, and 45 of fattening. Blood samples were collected in serum clot activator vacutainers from the jugular vein. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at −20 ˚C till analyzed.

Table 1 Ingredients and chemical composition of experimental rations

Ingredients (%)	Basal ration with protein at requirement levels	Ration with 10% protein higher than requirement levels
Barley grain	27.00	28.94
Corn grain	21.00	17.95
Alfalfa hay	22.90	22.46
Wheat straw	6.00	6.29
Beet pulp	5.00	5.49
Wheat bran	12.00	10.48
Soybean meal	4.00	5.99
Salt	0.40	0.40
Premix*	1.00	1.00
Calcium carbonate	0.70	0.80
Urea	0.00	0.20
Chemical composition (%)		
Dry matter	89.20	89.32
Crude protein	13.40	14.50
Neutral detergent fiber (NDF)	36.20	35.07
Acid detergent fiber (ADF)	16.17	15.90
Crude fat	3.07	3.40
Ash	10.11	9.83
Non fiber carbohydrate (NFC)	37.33	37.20
Metabolizable energy (Mcal/kg)	2.40	2.41

* 1 kg of control premix contained: vitamin A: 80 KIU/kg; vitamin D_3: 20 KIU/kg; vitamin E: 200 mg/kg; Fe: 640 mg/kg; Mn: 640 mg/kg; Cu: 120 mg/kg; Zn: 640 mg/kg; Co: 2.5 mg/kg; I: 10.5 mg/kg and Se: 2.5 mg/kg.

Table 2 Temperature, relative humidity and temperature humidity index (THI) of the environment during the experimental periods

Days of fatting	Average temperature (°c)	Average Relative humidity (%)	THI
1-15	31.26	72.66	83.61
15-30	31.53	72.73	84.03
30-45	28.06	76.46	79.16

Serum biochemical parameters such as glucose, blood urea nitrogen (BUN), creatinine (Cr), cholesterol, triglyceride, and lactate with using Pars Azmon kits (Pars Azmon Laboratory, Tehran, Iran) in biochemical analyzer (Mindray BS-120) and non-esterified fatty acids (NEFA) (using Randox Company's kits, England) by colorimetric methods (Perkin-Elmwr-35) were estimated.

Statistical analysis

The experiment was conducted as a factorial 2 × 2 in a completely randomized design. Research results were processed by the SAS (2001) software. The effects of treatment on the concentration of biochemical indicators in the blood of lambs, were analyzed by two way repeated measures ANOVA. Results are presented as least square means with SEM and *P*-value. Statistical differences were confirmed at $P < 0.05$.

$$Y_{(ij)k} = \mu + A_{(i)} + B_{(j)} + AB_{(ij)} + \varepsilon_{(ij)k}$$

Where:

$Y_{(ij)k}$: value of any measured data.

μ: mean of the statistical society.

$A_{(i)}$: protein level effect.

$B_{(i)}$: factor Gln effect.

$AB_{(ij)}$: interaction of factors protein level and glutamine. and $e_{(ij)k}$: experimental error.

RESULTS AND DISCUSSION

The effects of heat stress on the metabolism

Glutamine increased glucose concentration (P=0.027, P=0.012 and P=0.001 on the 15th, 30th and 45th days), and decreased the concentration of lactate (P<0.0001 on the 15th day) and creatinine (P=0.013 on the 30th day) at during of fattening (Table 3). The lowest concentration of glucose was determined in lambs in C group; higher glucose concentrations were found in G, CP, and GP groups. Higher protein levels significantly increased BUN (P=0.037, P=0.010 and P=0.001 on the 15th, 30th and 45th days), cholesterol (P<0.0001, P=0.002 and P=0.001 on the 15th, 30th and 45th days) and triglycerides (P=0.001 and P=0.019 on the 15thand 30th days) concentrations. However, the effect of protein levels on the glucose, creatinine and lactate values was not significant. The lowest concentration of urea was determined in lambs in C and G groups (lower protein treatment); significantly higher urea concentrations were found in CP and GP groups, respectively. Besides, the lowest concentration of triglycerides on the 30th day was determined in GP.

Table 3 Biochemical indicators in the blood of heat-stressed fattened lambs

Indicator	Experimental treatments				SEM	P-value			
	C	G	CP	GP		Treat	Protein level	Glutamine	Protein × glutamine
Glucose, mmol/L									
Day 15	73.66^{b}	82.66^{a}	77.66^{ab}	76.66^{ab}	0.741	0.0162	0.5187	0.0271	0.0097
Day 30	67.66^{b}	78.33^{a}	74.66^{ab}	78.00^{a}	1.080	0.0270	0.1614	0.0119	0.1281
Day 45	62.333^{b}	77.667^{a}	74.000^{a}	75.667^{a}	0.889	0.0012	0.0264	0.0014	0.0049
Blood urea nitrogen, mmol/L									
Day 15	33.00	33.66	36.33	35.33	0.499	0.1514	0.0369	0.8718	0.4288
Day 30	31.66^{b}	32.33^{ab}	34.00^{a}	33.00^{ab}	0.249	0.0279	0.0103	0.5237	0.0805
Day 45	30.00^{b}	31.33^{ab}	34.00^{a}	33.33^{a}	0.312	0.0068	0.0013	0.6075	0.1475
Cholesterol, mmol/L mmol/L									
Day 15	27.00^{c}	43.33^{b}	61.66^{a}	42.00^{b}	0.942	< 0.0001	< 0.0001	0.4025	< 0.0001
Day 30	47.667^{b}	55.000^{b}	64.667^{a}	54.667^{b}	0.913	0.0013	0.0018	0.4860	0.0015
Day 45	40.66^{c}	52.00^{bc}	66.00^{a}	58.00^{ab}	1.416	0.0014	0.0006	0.5726	0.0092
Triglycerides, mmol/L									
Day 15	14.00^{b}	20.33^{ab}	26.00^{a}	25.33^{a}	0.772	0.0020	0.0006	0.1041	0.0533
Day 30	20.00^{b}	31.33a	27.00^{a}	16.00^{b}	0.712	0.0003	0.0191	0.9097	< 0.0001
Day 45	20.00	28.00	25.33	20.33	1.037	0.0674	0.5893	0.4903	0.0140
Creatinine									
Day 15	1.12	1.08	1.12	1.04	0.029	0.7109	0.7406	0.3094	0.7406
Day 30	1.41^{a}	1.24^{ab}	1.33^{ab}	1.12^{b}	0.029	0.0415	0.1177	0.0133	0.7431
Day 45	1.20	1.08	1.29	1.24	0.025	0.0844	0.0390	0.1323	0.4351
Lactate									
Day 15	2.33^{a}	2.21^{a}	2.49^{a}	1.20^{b}	0.046	< 0.0001	0.0018	< 0.0001	0.0002
Day 30	1.52	1.49	1.42	1.23	0.058	0.3535	0.1613	0.3596	0.5294
Day 45	1.78	1.54	1.72	1.09	0.096	0.1247	0.2294	0.0541	0.3438

C: the experimental treatments were: basal diet; G: basal diet supplemented with glutamine at the rate of 0.2 g/kg of BW; CP: basal diet with 10% higher protein and GP: basal diet with 10% higher protein and glutamine.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Physiological responses in heat stressed fattened lambs

Indicator	Experimental treatments				SEM	P-value			
	C	G	CP	GP		Treat	Protein level	Glutamine	Protein × glutamine
RR									
Day 15	107.25^{ab}	77.50^{b}	113.50^{a}	86.00^{ab}	3.650	0.0133	0.3324	0.0020	0.8801
Day 30	74.75^{ab}	60.25^{b}	83.75^{a}	64.00^{ab}	2.400	0.0186	0.2089	0.0039	0.5945
Day 45	40.00^{b}	36.25^{b}	48.50^{a}	38.50^{b}	0.980	0.0045	0.0179	0.0043	0.1372
RT									
Day 15	39.75	39.55	39.65	39.50	0.052	0.3821	0.4890	0.1217	0.8160
Day 30	39.20	39.00	39.25	38.92	0.047	0.0950	0.8980	0.0176	0.5250
Day 45	39.02	38.82	39.07	38.70	0.666	0.2117	0.7820	0.0508	0.5214

C: the experimental treatments were: basal diet; G: basal diet supplemented with glutamine at the rate of 0.2 g/kg of BW; CP: basal diet with 10% higher protein and GP: basal diet with 10% higher protein and glutamine.
RR: respiratory rate and RT: rectal temperature.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The interaction of Gln and higher protein significantly increased glucose concentration and decreased cholesterol and triglyceride concentrations.

Heat stress causes changes in post-absorptive metabolism independent of reduced DMI and energy balance. The HS regulates protein synthesis at the transcription level, causing less protein deposition (Belhadj Slimen *et al*. 2016). Increased protein catabolism under chronic HS is likely to produce glucose through the gluconeogenesis pathway (Mahjoubi *et al*. 2015).

Reducing the oxidation of FA during chronic HS causes heat-stressed animals to depend on glucose to meet their energy needs for production, which is reduced and leads to negative energy balance.

Chronic HS reduces glucose circulating levels despite increased glucose uptake and increased hepatic glucose production. However, studies of blood glucose in response to HS are contradictory. In one study, glucose levels increased during HS in sheep (Al-Haidary *et al*. 2012), in the another, reduced in cows (Baumgard *et al*. 2011) or HS had no effe-

ct on blood glucose in goats (Hamzaoui *et al*. 2013). Decreased glucose levels may be due to decreased nutrient availability and decreased propionate production levels or due to increased glucose intake to provide energy required for high muscle activity with increased respiration rate. During HS liver glycogenolysis, which plays an important role in maintaining blood glucose levels, remains sensitive to adrenergic signals while no lipolysis is present (Baumgard *et al*. 2011). Reduced oxidation of fatty acids during HS causes animals to depend on glucose to meet their energy needs for production. McNeill *et al*. (1997) did not observe any significant difference in blood glucose levels by increasing the level of crude protein in the diet, which agreed with our results. On the other hand, HS stimulates proteolytic activity in muscle tissues to provide amino acids necessary for energy metabolism. Amino acids such as glutamine and arginine are deaminated by the liver to produce energy precursors and urea. However, Doepel *et al*. (2007) stated that Gln did not affect on glucose metabolism. Glutamine, through enhancement in hepatic gluconeogenesis, may increase post-splanchnic glucose (Doepel *et al*. 2007). Therefore, according to the results of the present experiment in HS, glutamine has an effective role in providing energy by increasing blood glucose levels. Animals under HS use new strategies to fuel and metabolic selection priorities independent of nutrient intake or energy balance. Protein metabolism is affected by HS because muscle damage may occur.

Tissue degradation increases the amount of blood urea nitrogen (BUN) and is often used as an indicator of muscle catabolism. In particular, HS increases plasma urea nitrogen in heifers, cows, and pigs (Pearce *et al*. 2013); they suggested that a higher level of blood BUN may be a consequence of deficient nutrient supply with increasing protein catabolism, causing N losses to occur. One percentage of Gln supplement diet corrected these negative effects and decreased serum BUN concentration to some extent because Gln provides precursors of several kinds of amino acids and promotes protein synthesis (Yu *et al*. 2002). Recently, research on molecular mechanisms has revealed that dietary Gln supplementation would increase the intestinal expression (120 to 124%) of genes that are necessary for cell growth and removal of oxidants, while reducing (34 to 75%) the expression of genes that promote oxidative stress (Hsu *et al*. 2012).

The results suggested that Gln supplementation may improve the intestinal oxidative-defense capacity and result in lower plasma stress-related hormones. Both Gln and glucose are major sources of energy for enterocytes (Hsu *et al*. 2012). It suggests that Gln can exert a "sparing effect" by reducing glucose contribution to the total ATP turnover (Hsu *et al*. 2012). In a study by Nemati *et al*. (2018), glutamine-fed cows had higher plasma glucose concentrations than controls, indicating a conversion of Gln to glucose, plasma concentrations of BUN were not affected by dietary Gln supplementation.

Table 5 Feed intake and feed conversion ratio of heat stressed fattened lambs

Indicator	Experimental treatments				SEM	P-value			
	C	P	CP	GP		Treat	Protein level	Glutamine	Proteinlev × glutamine
Body weight (kg)									
Day 1	31.47	31.45	31.27	31.77	0.576	0.9918	0.9577	0.8403	0.8238
Day 15	34.80	34.55	34.62	35.15	0.552	0.9805	0.8506	0.9030	0.7318
Day 30	37.95	37.72	37.77	38.70	0.550	0.9210	0.7395	0.7395	0.6267
Day 45	40.70	40.65	40.70	42.12	0.577	0.7621	0.5349	0.5625	0.5349
Average dry matter intake (g)									
1-15 days	1216.62	1201.10	1237.58	1237.36	8.108	0.3548	0.1031	0.6361	0.6457
15-30 days	1382.61	1339.65	1346.69	1365.17	9.586	0.4196	0.7905	0.5353	0.1350
30-45 days	1458.41	1427.15	1402.37	1409.81	13.931	0.5175	0.2125	0.6765	0.5006
1-45 days (total DMI)	1352.55	1322.63	1328.88	1337.45	10.104	0.7480	0.8302	0.6070	0.3598
Average daily gain (kg)									
1-15 days	0.221	0.206	0.223	0.225	0.008	0.8697	0.5758	0.7081	0.6404
15-30 days	0.210	0.215	0.210	0.236	0.010	0.7548	0.5995	0.4458	0.5995
30-45 days	0.183	0.191	0.195	0.228	0.008	0.3098	0.1839	0.2475	0.4798
1-45 days (total gain)	0.205	0.204	0.209	0.230	0.008	0.6914	0.3979	0.5698	0.5489
Feed conversion ratio									
1-15 days	5.59	6.01	5.64	5.50	0.224	0.8589	0.6127	0.7649	0.5439
15-30 days	6.69	6.38	6.61	5.86	0.258	0.6723	0.5700	0.3240	0.6780
30-45 days	8.88[a]	7.54[ab]	7.40[ab]	6.24[b]	0.268	0.0341	0.0240	0.0390	0.8706
1-45 days (total FCR)	6.85	6.61	6.49	5.85	0.218	0.4424	0.2239	0.3315	0.6508

C: the experimental treatments were: basal diet; G: basal diet supplemented with glutamine at the rate of 0.2 g/kg of BW; CP: basal diet with 10% higher protein and GP: basal diet with 10% higher protein and glutamine.
DMI: dry matter intake and FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The significant increase of BUN concentration of lambs determined in this study with an increase in protein levels is probably linked to the catabolism of amino acids or the stress. Other indicators of muscle catabolism include increased creatinine and plasma creatine. The increased level of BUN and creatinine may be due to reduced blood flow toward kidneys during HS condition. Similar findings of increased BUN and creatinine during the summer season have been reported in previous studies (Rathwa *et al*. 2017). Amino acids such as Gln and arginine are important transmitters of ammonia nitrogen mediators in its non-toxic form, which are deaminated by the liver to produce precursors of energy and urea. In HS conditions due to reduced gastrointestinal motility and passage rate, rumen microorganisms decompose more of the dietary protein, thus in higher protein diets, increased RDP significantly increases BUN. As a result, reducing the cost of urea by reducing dietary protein and using amino acids that can convert to other amino acids can be used as a nutritional strategy.

The effect of Gln supplementation on cholesterol and triglycerides was not significant. However, significant increases in cholesterol and triglycerides with higher protein level was observed. In the study of Rahmanifirozi *et al*. (2017), in late goat pregnancy, BUN concentration was significantly higher in high protein treatment, but there was no significant difference in triglyceride, cholesterol, and total blood protein concentration between treatments. The decrease in triglyceride and cholesterol concentrations during late pregnancy was similar to the results obtained in cows and contrast to sheep (Rahmanifirozi *et al*. 2017). A study showed that 30 g/d of Gln following each main meal had no effects on cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (Mansour *et al*. 2014). Another study showed that Gln supplementation increased cholesterol and increased adiposity, because Gln is lipogenic and could modulate lipid profile (Coqueiro *et al*. 2019). The lipid lipoprotein lipase gene expression is increased during HS compared to neutral heat conditions, indicating an increased capacity to store dietary and hepatic triglycerides (Sanders *et al*. 2009). In general, the lack of mobilization of adipose tissue is a paradox because HS not only reduces feed intake but also produces a good stress response by increasing released lipolytic signals (e.g. cortisol and circulating epinephrine).

It is evident from Table 5 that the addition of Gln (G and GP) to the feed mixture of fattening lambs significantly affected the reduction of lactate compared with the group of lambs without the addition of Gln (C and CP). Superficial breathing under HS leads to respiratory alkalosis, thereby increasing the urinary excretion of bicarbonate. As a result, saliva bicarbonate, a source of rumen buffering, is reduced. At this time, by decreasing salivation and DMI and rumination due to the lack of physical stimulation of the food particles, all of them eventually lead to a decrease in rumen pH, which causes a subacute and mild acidosis, and the rumen pH falls below 6, This causes the death of fiber-digesting bacteria and protozoa and increases the lactate-producing bacteria. During HS and high catabolism conditions, the Gln utilization by the cells is higher or equal to glucose utilization (Shah *et al*. 2020). Glutamine in immune cells is converted into glutamate, alanine, and aspartate through partial oxidation of the carbon dioxide (glutaminolysis), while glucose is converted into lactate through glycolysis (Curi *et al*. 2016). Therefore, Gln can play a role in reducing lactate.

The effect of Gln supplementation on physiological responses of fattening lambs is shown in Table 4. Experimental groups had a significant effect on the respiration rate of lambs on the 15th, 35th and 45th days (P=0.013, P=0.019 and P=0.045). At the end of the fattening period, the respiration rate in all groups decreased with the declining trend in THI. Rectal temperature did not differ significantly between experimental groups, although the effect of Gln supplementation on mean rectal temperature on 30 and 45 days of fattening was significant (P=0.018 and P=0.051, respectively). Glutamine supplementation decreased respiration rate and rectal temperature during fattening. The interaction between Gln and higher protein levels did not differ significantly between groups.

Changes in respiration rate, heart rate, and rectal temperature are often used as indicators of physiological adaptation to HS in small ruminants (Gupta *et al*. 2013). In Rathwa *et al*. (2017) study, sheep rectum temperature and respiration rate in summer were 38.98 ± 0.04 ˚C and 40.02 ± 0.5 per minute, respectively. In the present experiment, the respiration rate on day 15 was between 80 and 120 respiration per minute, which indicates the high intensity of thermal stress, although this index decreased between 60 and 80 (medium intensity) on day 30 and around 40 to 50 breaths per minute (low HS) on day 45. The effect of protein levels on physiological parameters was not significant, but Gln supplementation was effective in reducing HS by reducing RR and RT.

The RT is controlled by a biological “clock”, located in the suprachiasmatic nucleus of the brain. This result agrees with the finding of Hsu *et al* (2012), who reported that L-glutamine lowered RT in lipopolysaccharide-challenged pigs and Ocheja *et al*. (2017) who reported that L-glutamine lowered RT in goats. The finding of the present study differs from that of Lindinger and Anderson (2014), who found no significant difference in RT of horses supplemented with L-glutamine-containing diet for nine weeks.

Although the mechanism by which L-glutamine reduced the RT and RR in the experimental lambs was not investi-

gated in the present study, Hsu *et al.* (2012) suggested that L-glutamine prevents the rise in RT by modulating the expression of plasma tumor necrotic factor-alpha, thus reducing inflammation. The mechanism by which L-glutamine exerted this effect has not been fully elucidated, but it could be through enhancement of glutathione production. L-glutamine is also involved in signaling pathways and may be involved in the regulation of heat-shock proteins, which facilitate the adaptation of animals to high environmental temperatures (Mohanarao *et al*. 2014). The difference in the present finding and that of Lindinger and Anderson (2014) may be due to different experimental subjects used, mode of L-glutamine administration, and the environmental conditions in which the horses were kept. It is hypothesized that L-glutamine exerts RT and RR lowering effect through an antioxidant mechanism by increasing glutathione production (Roth, 2008), thus, its administration may be beneficial in HS alleviation in sheep and goats. Further investigations into the molecular mechanism of the L-glutamine effect on RT are warranted.

The effect of Gln supplementation on the growth performance of fattening lambs is shown in Table 5. There were no significant differences in initial BW between groups; however, the BW of GP group tended to be higher than the other groups. In general, the DMI in all stages did not differ between groups. The average daily gain (ADG) was compared based on different stages (0 to 15, 15 to 30 and 30 to 45 d) of growth. The GP group had trended to increase ADG compared to the control on days 1 to 45. However, the GP group had significantly lower feed conversion ratio (FCR) than the C group during the 30 to 45 days (P=0.034).

Heat stress regulates the secretion of leptin and adiponectin, which leptin stimulates the hypothalamic axis and reduces DMI, and adiponectin alters nutritional behavior using environmental and central mechanisms (Aleena *et al.* 2016). Heat-stressed animals decrease DMI in an attempt to create less metabolic heat because the heat increment of feeding is an important source of heat production. Also, the maintenance requirements increased by 30% because of HS and the energy intake would not be enough to cover the daily requirements which result in an apparent BW loss (Hamzaoui *et al.* 2013).

In many studies, BW, DMI, and gain decreased under HS conditions in sheep and goats (Pragna *et al*. 2018). In our study, daily weight gain in the whole period was higher in GP with higher protein levels and Gln addition than other treatments, but this difference was not statistically significant.

In a study by Nemati *et al*. (2018), consuming of Gln in the diet of fresh cows (250 and 350 grams per cow per day) increased DMI after calving, but DMI in the group with lower Gln (150 grams per cow per day) did not differ from the control diet. Doepel *et al.* (2007) in a study on Holstein cows receiving abomasal infusions of water or 300 g/d of Gln (unprotected-Gln), reported that Gln increased milk yield but DMI had the tendency to increase. In another study, the effect of 1% Gln and glutamic acid supplementation on the circulation of carbon in pig muscle and animal performance (DMI, ADG, and feed conversion ratio) was investigated; the supplements did not show a significant effect on performance variables, although the supplement accelerated carbon circulation in the muscles, indicating a faster recovery after weaning and the anabolic effect of these additives is proven, but the high cost of this additive limits its use (Borges Amorim *et al*. 2018).

The nature of diets in terms of level and carbohydrate sources fermentability and solubility of nitrogen sources affect metabolic changes as well as changes in the gastrointestinal tract and can affect the amount of DMI and nutrient digestibility (Mertens *et al*. 2009). There is a strong positive correlation between diet protein (amino acids) content and DMI. It seems that nitrogen resources which have lower rates of ruminal degradation tend to improve the forages digestion and increase the passage rate and DMI. One study found that increasing the level of dietary protein increased DMI in sheep (Cheema *et al*. 1991). In some studies, it has been reported that by increasing the level of degradable protein in the diet, the digestibility of neutral detergent fibers improved (Moradi *et al.* 2018). Some researchers have also reported that the amount of DMI is not affected by the level of diet raw protein and the degradability of crude protein in the rumen (Jabbar *et al*. 2013).

In our study, this significant effect on DMI was not observed with changes in protein levels and Gln supplementation in the diet. Although ADG was greater in GP who were fed Gln supplementation and higher protein levels, this difference statistically was not significant. The feed conversion ratio was also improved by increasing protein levels and adding Gln to the diet, so that GP group obtained the best-feed conversion ratio among the treatments.

CONCLUSION

From the results of the present study, it could be concluded that rumen protected-Gln at 0.2 g/kg BW/day to fattening lambs improved health status via increasing glucose and decreasing creatinine and lactate concentrations. Although parameters such as cholesterol and triglycerides increased significantly with increasing protein levels, Gln supplementation had no effect on cholesterol and triglyceride plasma concentrations. Changes in BW, DMI, and feed conversion ratio were not statistically significant between experimental groups. Physiological responses such as rectal temperature

(RT), respiration rate (RR) with Gln were significantly reduced. It can be concluded that Gln supplementation improves the performance variables and health condition of lambs during the fattening period under HS.

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