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Survey of different responses of non-pregnant fat-tailed ewes to the intravenous glucose tolerance test during the feed restriction period

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Abstract

Introduction: Blood glucose concentration varies according to the physiological stages and daily milk yield. In ruminants, the gluconeogenesis process supplies most of the glucose requirements, which plays a prominent role in the metabolism and maintaining energy homeostasis. Due to the pivotal role of glucose and its metabolic processes in ruminants, particularly in milk production and optimizing reproductive efficiency, comprehensive studies have been conducted on dairy cows. The glucose tolerance test (ivGTT) is one of the techniques to assess glucose metabolism and the capacity of various tissues to react over insulin secretion (Kaneko *et al.* 2008). Although ivGTT studies have achieved significant success in glucose and insulin metabolism, the effect of some factors, such as feed restriction, physiological status, and dose of glucose infusion, need to be sufficiently clarified. The published results demonstrated that feed restriction at 65% of diet energy density increases the plasma glucose concentration in non-pregnant ewes (Zarrin *et al.* 2021b). This study evaluated the effect of intravenous glucose infusion on blood metabolite and insulin concentration in non-pregnant fat-tailed ewes during feed restriction.

Materials and Methods: Ten multiparous non-pregnant fat-tailed Turki-Qashqai ewes (3-4 years old and 49.2 ± 3.60 kg body weight), after two weeks of adaptation, were randomly allocated into two treatment groups: the control group (Control; n =5) and the feed restriction group (Restriction; n =5). The Control ewes had free access to feed throughout the experiment (weeks 1 to 5). The restricted ewes received a diet equivalent to 100, 50, 65, 80, and 100% of the energy content of the diet at weeks 1, 2, 3, 4, and 5 relative to the start of the experiment (Ding *et al.* 2016). Intravenous glucose infusion (1 ml/kg BW) was administered in the third week of the experiment. Blood samples were taken 10 and 5 minutes before and 2, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, and 180 minutes after the infusion. The area under the curve (AUC) was calculated for each parameter during the ivGTT (0-180 min) using the trapezoidal roll (sum of the rectangular and triangular areas under the curve). The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was calculated using the proposed

equation (Holtenius and Holtenius, 2007). For the calculation of the Glucose clearance rate (the rate at which the concentration of glucose is removed from the blood, k (%/min)) and the half-life of glucose (T1/2; the time required to reduce the concentration of glucose by half), were calculated by Kaneko et al.(2008). Data was analyzed by the MIXED procedure of SAS. Treatments (Control and Restriction), time points (0-180 min), and the interactions (treatment × time) were considered as fixed effects. The animal breed was considered as random effect. Measured data were considered as dependent variables. The data obtained for ivGTT parameters were evaluated using the SAS GLM Procedure. The results from reference samples were included in the model as covariates for individual differences between ewes. Data are presented as Means ± SEM and differences were considered significant if $P \leq 0.05$.

Results and discussion: Glucose infusion increased glucose concentration in both groups ($P<0.01$). No significant changes were observed for glucose turnover, half-life, and the area under the glucose curve between the groups. Insulin concentration increased in both groups a few minutes after glucose infusion, but the increase was slighter in Restriction ($P<0.05$). Free fatty acids declined due to intravenous glucose infusion in Control ($P<0.05$). Glucose infusion did not affect beta-hydroxybutyrate concentration. The current study was designed and conducted to study the glucose clearance pattern during feed restriction regardless of the many potentially confounding effects of the pregnancy presented in other studies. The animals were not matted during the experiment to avoid the influences of the particular endocrine and metabolic changes during pregnancy and the transition period. Researchers indicated that glucose and insulin increased in response to dextrose administration in pregnant and non-pregnant sheep (Lunesu *et al.* 2020; Morgante *et al.* 2012). An instantaneous increase in insulin concentration after the glucose infusion is expected because of its glucose-regulatory role (Zarrin *et al.* 2015; Francis and Bickerstaffe, 1996). In agreement with the current study, increases in insulin levels were reported in Ghezel ewes from zero to 1st hour after dextrose administration (Chalmeh *et al.* 2020). Previous studies documented the high insulin concentration level in response to intravenous glucose infusion (Wolfe *et al.* 1986). Glucose infusion, increases glucose, insulin, cortisol, and prolactin concentration, while it decreases the concentration of free fatty acid, beta-hydroxybutyrate, and insulin-like growth factor-1 during pre- and post-parturition in sheep (Chalmeh *et al.* 2020). These researchers suggested that induced hyperglycemia through intravenous infusion causes metabolic and hormonal changes in fat-tailed sheep that may be due to providing energy sources and glucose as a metabolic regulator (Chalmeh *et al.* 2020). However feed restriction-induced insulin resistance (Petterson *et al.* 1993) and the low insulin concentration during the ivGTT in the Restriction group compared to Control showed a different glucose homeostasis in the fat-tailed sheep. The low insulin concentration in Restriction compared to Control might be a comeback to the decreased energy during the whole experiment in this group (Zarrin et al. 2021b, 2021c). Circulatory levels of beta-hydroxybutyrate and free fatty acids significantly decreased following intravenous hypertonic dextrose administration in Ghezel ewes (Chalmeh et al. 2020). Low insulin concentration and reduced sensitivity of the tissues around parturition increase lipid mobilization and induce further rise in plasma-free fatty acid concentrations (Hayirli, 2006). The rapid drop in free fatty acid concentrations after the glucose infusion demonstrated low lipid mobilization (Chalmeh et al. 2020). Previous studies have demonstrated the negative correlation between free fatty acid concentrations, area under the insulin curve, and insulin concentration at its peak (Bossaert et al. 2008). Adipose tissue and its derivatives are crucial in determining and modulating insulin sensitivity during glucose metabolism in dairy cows (De Koster and Opsomer, 2013). Direct manipulative studies have also demonstrated that providing excess free fatty acid by abomasal fat infusion produced peripheral insulin resistance in nonlactating cows (Pires et al. 2007; Pires and Grummer, 2007). The current results indicate the ability of fat-tailed sheep to preserve blood glucose levels in periods of feed restriction, which is accomplished through hormonal mechanisms, especially hormones involved in energy metabolism, such as insulin. The reduction of insulin in

Restriction compared to Control, even when a rich source of glucose is provided through intravenous infusion, might be due to increased insulin sensitivity in the restricted group ewes compared to Control.

Conclusion: The finding results proved the physiological ability of fat-tailed sheep to spare glucose concentration through hormones and metabolites involved in energy metabolism. This ability is important for these breeds in harsh environmental conditions or husbandry systems.

Keywords: Beta-hydroxybutyrate, Feed restriction, Free fatty acids, Energy homeostasis, Insulin

Introduction

Demand for glucose or its precursors for milk biosynthesis, maintenance, and reproduction in ruminants is high. Blood glucose concentration varies according to physiological stages and daily milk yield (Firat and Ozpinar, 1996). In ruminants, the gluconeogenesis process supplies most of the glucose requirements, which plays a prominent role in metabolism and maintaining energy homeostasis (Herdt, 2000). Due to the pivotal role of glucose and its metabolic processes in ruminants, particularly regarding milk production and optimizing reproductive efficiency, comprehensive studies have been conducted on dairy cows. Previous studies showed that feed restriction during the transition period and in non-pregnant ewes changed metabolites and hormones related to energy metabolism such as FFA, BHB, insulin, and growth hormone (Zarrin *et al.* 2021b, c). Contrastingly, several studies concerning energy supply during the transition period in fat-tailed ewes and dromedary camels provided that induced feed restriction ameliorates the circulatory glucose level in pre- and post-partum (Ahmadpour *et al.* 2019; Zarrin *et al.* 2017, 2021a, b, c).

Intravenous glucose tolerance test (ivGTT) through an orally administration or intravenous infusion over time is one of the primary techniques to assess glucose metabolism and the capacity of various tissues to react over insulin secretion (Kaneko *et al.* 2008). Intravenous glucose tolerance test affects glucose half-life and turnover (Freyer *et al.* 2006), insulin resistance or insulin sensitivity(De Koster and Opsomer, 2013), FFA concentration (Boston *et al.* 2008), and energy metabolism (Kneeskern *et al.* 2016).

Among ruminants, sheep is one of the most suitable animal models to study glucose metabolism in response to pre-pregnancy (Long *et al.* 2010; Todd *et al.* 2009), early and mid-pregnancy (Ford *et al.* 2007) and late pregnancy nutrition (Hosted *et al.* 2008; Ahmadzadeh-Gavahan et al. 2021). Various studies have shown that an imbalance diet (feed restriction, high-fat feeds, and overfeeding) during the transition period increases physiological risks such as insulin resistance, diminished insulin sensitivity, reduced glucose tolerance, and changes the glucose and fat metabolism (Kenyon and Blair, 2014; Khanal and Nielsen, 2017; Parlee and MacDougald, 2014).

Moreover, several trials have investigated the response of organs to glucose infusion (Freyer *et al.* 2006). Intravenous glucose infusion is influential in calculating glucose recovery rate due to its remarkable properties (Kaneko *et al.* 2008). Many studies have used intravenous glucose infusion using hypertonic dextrose to determine ruminant insulin resistance (Chalmeh *et al.* 2020; De Koster and Opsomer, 2013).

Despite the advantage of ivGTT in investigation of glucose and insulin metabolism, there is lack information related to the effects of feed restriction, physiological status, and dose of glucose infusion during the ivGTT in ewes. The published results demonstrated that feed restriction at 65% of energy content of diet increases the plasma glucose concentration in non-pregnant ewes (Zarrin et al. 2021b). We hypothesized that intravenous glucose infusion in non-pregnant fat-tailed ewes under the feed restriction affects glucose, FFA, BHB, and insulin concentration. The objective of the present

study was to determine the effect of ivGTTon metabolites and hormone related to energy metabolism in non-pregnant fat-tailed ewes during the feed restriction.

Table 1. Diet composition and calculated nutrient composition of diets fed ewes during pre- and postpartum (dry matter basis).

	Diet
Ingredients, DM %	
Alfalfa hay	26.7
Barley (grain)	34.9
Wheat straw	35.5
Chemical Composition [1]	
DM [2], %	89.0
Calculated ME [2], Mcal/kg	2.13
CP [2], DM %	11.0
NDF [2], DM %	49.0
ADF [2], DM %	32.9
EE [2], DM %	2.10
Calcium, DM %	0.51
Phosphorous, DM %	0.24

[1] Estimated using values obtained from the NRC (2007).
[2] DM = Dry matter; ME = Metabolizable energy; CP = Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; EE = Ether extract.

Materials and Methods

Animals and management: Ten multiparous non-pregnant fat-tailed Turki-Qashqai ewes (3 to 4 years old and 49.2 ± 3.60 kg body weight (mean±SD)) were selected from pastoral herds. In order to investigate the effects of intravenous glucose infusion in ewes, without influences of the particular endocrine and metabolic status of pregnancyor transition period and lactation, this study was conducted in non-pregnant ewes. The ewes were inspected for health by routine veterinary examinations before and during the experiment. Two weeks before the start of the trial, the animals were kept in individual biological pens (1.0 × 1.2 m, with separate feed and water troughs) and fed to adapt to housing and feeding conditions. Water and mineral blocks were available ad libitum, and the feeding frequency was twice daily (0800 and 1600 h). Total mixed ratio (TMR) diets were formulated to fulfill 100% of the energy requirements recommended by the NRC 2007 for non-pregnant ewes. The diet and its ingredients are detailed in Table 1.

Experimental Design and Treatments: Based on B.W, parity, and age, animals were randomly assigned to two experimental groups consisting of the control (Control; n=5) and feed restriction (Restriction; n=5). After the adaptation period, the Restriction group received a diet equivalent to 100, 50, 65, 80, and 100% of the energy content of the diet at weeks 1, 2, 3, 4, and 5 relative to the start of experiment (Ding et al. 2016). The Control group were fed with a TMR diet formulated to fulfill 100% of the energy requirements. This sudden reduction in energy level aimed to mimic energy restriction which accures mainly during dry seasone and feed and pasture insufficiency. Energy intake increased progressively to avoid metabolic problems such as rumen acidosis.

At the end of the third week of the experiment (65% of energy content), ewes were chosen to carry out the glucose tolerance test. One day before ivGTT, ewes were fixed in a jugular vein with intravenous catheters (I.V. Cannula, Bio-Med, Haryana, India) with a diameter of 1.3 mm and a length of 45 mm. On the day of the experiment, two blood samples were taken

as reference samples ten and five minutes before glucose infusion. A 50% dextrose solution (Glucojet, Dextrose 50%, Zoopha Parnian Pars, Tehran, Iran) was gently infused at 500 mg/kg, 1 mL/kg (De Koster and Opsomer, 2013) through the catheter for two minutes. The ewes were bleeding at 2, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, and 180 minutes after infusion from a jugular vein and preserved in EDTA tubes. The tubes were then kept on wet ice and centrifuged at 3000 g for 20 min in the lab. The plasma was harvested and frozen at -20 °C until the analytical procedures.

Laboratory Analysis: Plasma glucose (#1500017), free fatty acids (FFA; #FA115), and beta-hydroxybutyrate (BHB; #RB1007) were measured enzymatically with an automated analyzer (Autoanalyzer, Alcyon, 300, USA) using commercial kits (Glucose; Pars Azmon.; FFA and BHBA: Randox Laboratories Ltd. Crumlin, UK) based on manufacturer recommendation. According to the manufacturer's instructions, plasma insulin (#CSB-E17044Sh) was measured using commercial ELISA kits (Cusabio. USA) with a plate reader (SpectraMax® 190, Molecular Devices, USA). The intra-assay coefficients of variation for glucose, FFA, BHB, and insulin were 1.5, 4.4, 5.1, and 6.7%, respectively. The inter-assay coefficients of variation were 0.9, 4.7, 3.7, and 12.6%, respectively. All commercial kits used in the current study have been validated for sheep plasma, as confirmed by the parallelism test (Razavi et al. 2015) shown in Table 2.

Calculation of indicators related to the glucose tolerance test: The area under the curve (AUC) was calculated for each parameter during the ivGTT (0-180 min) using the trapezoidal roll (sum of the rectangular and triangular areas under the curve). The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was calculated using the proposed equation (Holtenius and Holtenius, 2007). For the calculation of the glucose clearance rate (k (%/min) the rate at which the concentration of glucose is removed from the blood) and the half-life of glucose (T1/2; the time required to reduce the concentration of glucose by half), the following formulas were used (Kaneko *et al.* 2008):

$$k\text{-value}\ (\%/\text{min}) = \{[\ln 1 - \ln 2]/[T2 - T1]\} \times 100$$

$$T1/2\ (\text{min}) = [0.693/k] \times 100$$

which [T1] and [T2] represent times 2 and 30 min after glucose infusion, respectively, and [ln 1] and [ln 2] represent the glucose concentration (mg/dL) at the same times.

Statistical Analysis: Normal distribution of data was tested using the SAS UNIVARIATE method (version 9.4, SAS Institute Inc. Cary, NC, USA). The results obtained were statistically evaluated using the SAS MIXED procedure. In the model, animals were considered as repeated factors. Treatments (Control and Restriction), time points (0-180 min), and the interactions (treatment × time) were considered as fixed effects. The animal breed was considered as random effect. Variables measured in plasma were considered as dependent variables. The data obtained for ivGTT parameters were evaluated using the SAS GLM method. The results from reference samples were included in the model as covariates for individual differences between ewes. Data were expressed as Mean ± SEM, and the significance level was a P-value below 5% ($P \leq 0.05$). Means comparison was performed using the Tukey-Kramer test. Figures were drawn using Sigma Plot software (version 14, Systat Software GmbH, Erkrath, Germany).

Table 2. Parallelism test results to check the validity of the results of different kits for sheep

Variables[1]	Sample	Dilution	Expected	Measured	Ratio%
Glucose, (mg/dL; n=12)	1	100	98.08	98.08	100.00
	2	50	49.04	48.41	98.71
	3	25	24.52	23.87	97.33
	4	12.5	12.26	12.69	103.53
	5	6.25	6.13	6.37	103.98
Insulin, (pmol/L; n=12)	1	100	10.50	10.50	100.00
	2	50	5.25	5.61	106.84
	3	25	2.62	2.94	112.20
	4	12.5	1.31	1.47	112.33
	5	6.25	0.66	0.74	112.29
BHB, (mmol/L; n=12)	1	100	0.32	0.32	100.00
	2	50	0.16	0.16	98.70
	3	25	0.08	0.08	98.65
	4	12.5	0.04	0.04	99.07
	5	6.25	0.02	0.02	97.41
FFA, (mmol/L; n=12)	1	100	0.11	0.11	100.00
	2	50	0.05	0.05	100.16
	3	25	0.03	0.03	104.19
	4	12.5	0.01	0.01	104.19
	5	6.25	0.01	0.01	101.71

[1]BHB = Beta-hydroxybutyrate; FFA = Free fatty acids

Results

No significant difference was detected between the experimental groups' baseline plasma glucose concentration (Table 4). However, the difference between the sampling times in both treatments was significant ($P<0.01$; Figure 1). Two minutes after the glucose infusion, circulatory glucose increased abruptly ($P<0.05$) in both groups (Table 4), and stayed high in both treatments up to 90 minutes after the infusion compared to the baseline ($P < 0.05$; Figure 1). Glucose levels then fell to the baseline in both groups by the end of the trial. No significant difference was observed between the groups for AUC, circulatory glucose peak, end-of-experiment level, glucose clearance rate, and glucose half-time (Table 4).

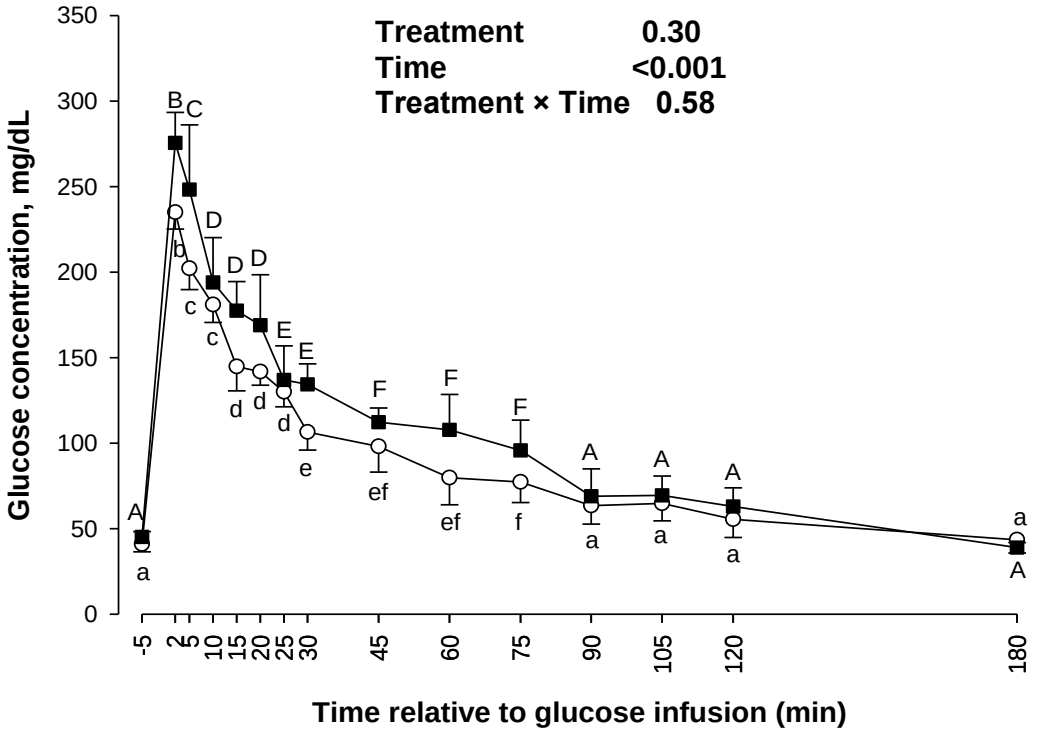

Figure 1. Blood glucose concentrations in Control (n= 10; o) and feed-restricted fat-tailed dairy sheep (Restriction, n= 10; ■). Different lowercase letters (a-f) indicate significant differences (P<0.05) between time points within Control. Different uppercase letters (A-F) indicate significant differences (P<0.05) between time points within Restriction. Data are expressed as least square means ± standard error of the mean.

Circulatory insulin levels before and after the infusion are revealed in Figure 2. Based on the results, there was no significant difference in the basal insulin level between the groups. After glucose infusion, insulin concentration was raised in both groups, so a significant difference between different time points was observed (P< 0.001). The effect of the glucose infusion on the plasma insulin was between the experimental groups; in Control, the insulin concentration increased from 5 min after the infusion and reached its peak 4o minutes later, the highest compared to the other time points (P<0.05). Afterward, the insulin concentration declined, matched the baseline level, and maintained at the same level until the end of the trial (180 min).

Nevertheless, the tendency of variation in insulin levels in Restriction was slightly different from Control, qua in this group, immediately after the infusion, the insulin significantly marked up (P<0.05) in the second minute compared to the pre-infusion levels. Furthermore, it yielded a basal level 10 minutes post-infusion. Insulin concentration in the restriction group was higher than baseline at 15, 20, 30, 50, and 60 minutes after glucose infusion (P<0.05). The results reveal that the feed restriction changed the blood insulin concentration at 10, 40, and 90 minutes post-infusion, taking it down compared to Control (P<0.05). AUC data indicated no significant differences between the groups (Table 3).

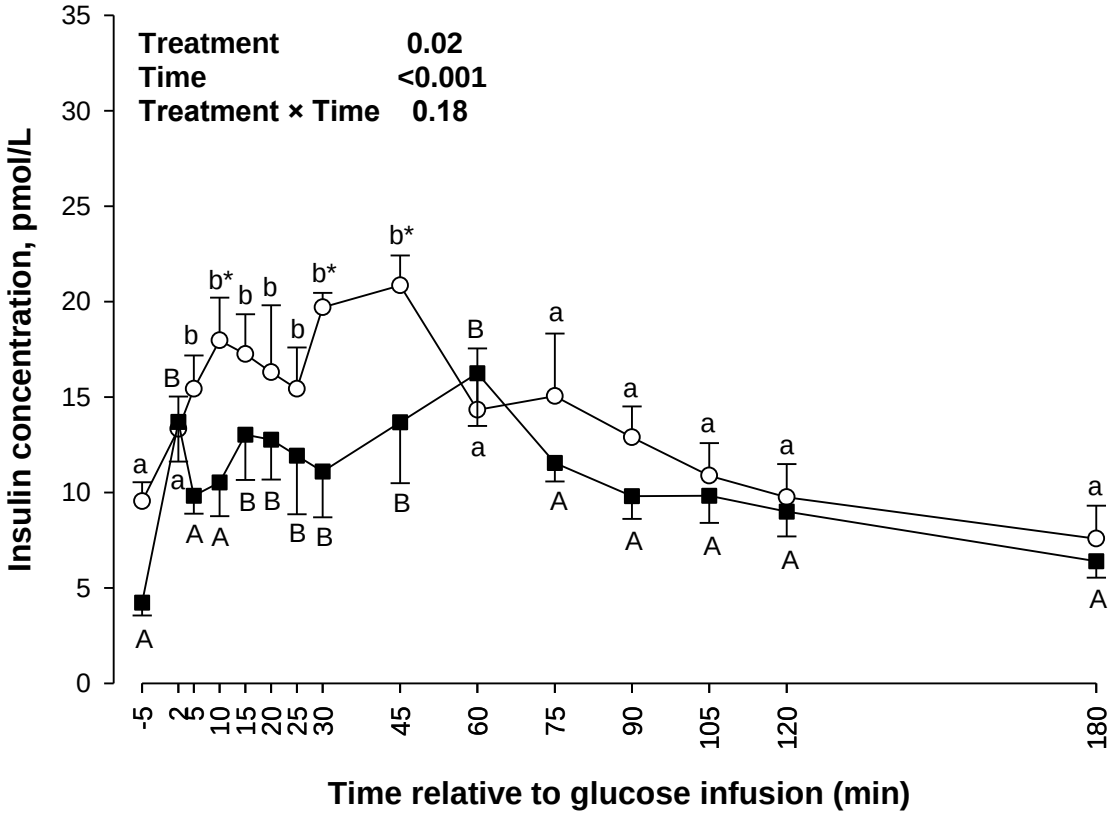

Figure 2. **Blood insulin concentrations in Control (n= 10; o) and feed-restricted fat-tailed dairy sheep (Restriction, n= 10; ■). Different lowercase letters (a-b) indicate significant differences (P<0.05) between time points within Control. Different uppercase letters (A-B) indicate significant differences (P<0.05) between time points within Restriction. Data are expressed as least square means ± standard error of the**

The differences in free fatty acid (FFA) concentration pre- and post-infusion in both groups are presented in Figure 3. The results showed no significant difference between the initial FFA concentration in both Control and Restriction (P>0.05). After the infusion, FFA concentration was unchanged by the treatments, and no difference was observed during the timepoint in Restriction. In Control, however, high blood glucose induced by the infusion reduced FFA concentration at 25, 30, 45, and 60 minutes compared to baseline (P<0.05; Figure 3). During the trial, no significant differences were observed between the groups for the AUC of the FFA concentration (Table 3).

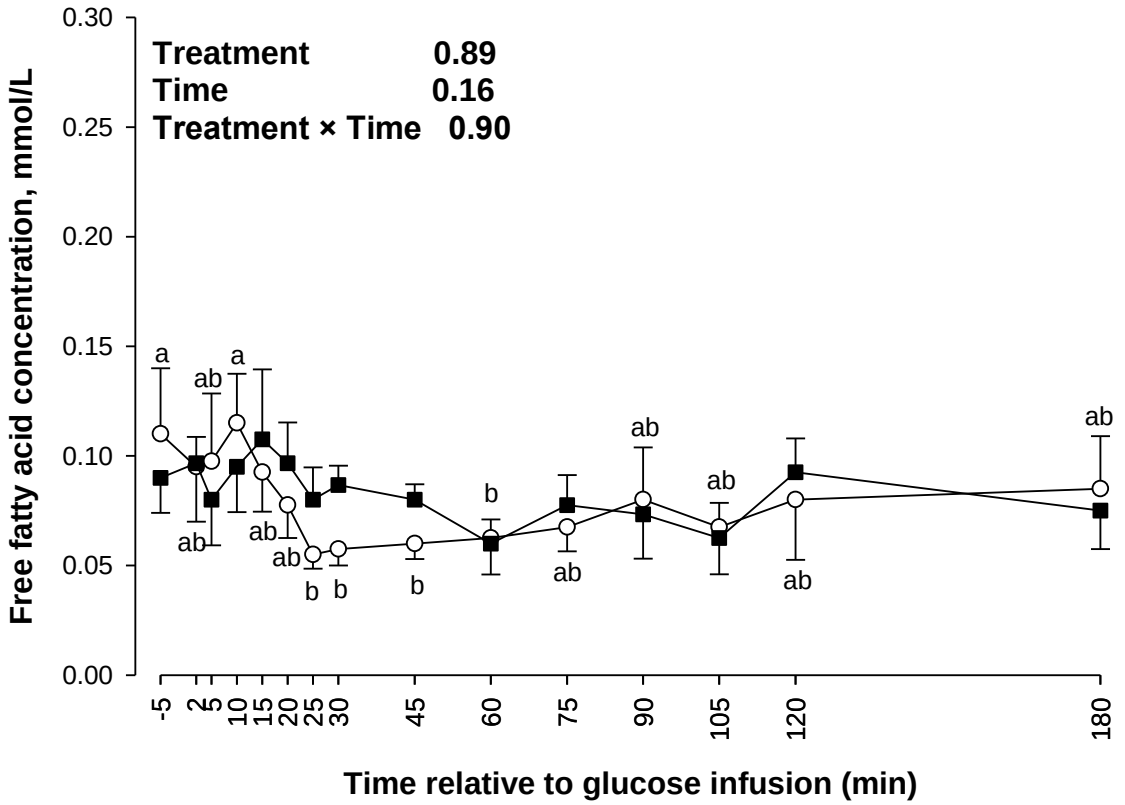

Figure 3. **Blood FFA concentrations in Control (n= 10; o) and feed restricted fat-tailed dairy sheep (Restriction, n= 10; ■). Different lowercase letters (a-b) indicate significant differences (P<0.05) between time points within Control. Data are expressed as least square means ± standard error of the mean.**

The mean concentration of BHB before the infusion in Control and Restriction was 0.32 and 0.39 mmol/l, respectively (Figure 4). After the glucose infusion, plasma BHB was not significantly altered in any groups. Its concentration unchanged within the pre-infusion concentration range throughout the trial period. The AUC results also showed no significant difference between the groups (Table 3).

Before the glucose infusion, the revised insulin sensitivity index (RQUICKI) was 0.66 and 0.82 in Control and Restriction, respectively (Figure 5). Forasmuch as RQUICKI is acquired based on an equation of the inverse ratio of other parameters, its value diminished post-infusion in both groups compared to pre-infusion, which resided significantly in Control up to the 60 minutes and up to 120 minutes in Restriction (P<0.05). After these time points, the RQUICKI index returned to the pre-infusion baseline value. The AUC results showed no significant difference between the groups for RQUICKI (Table 3).

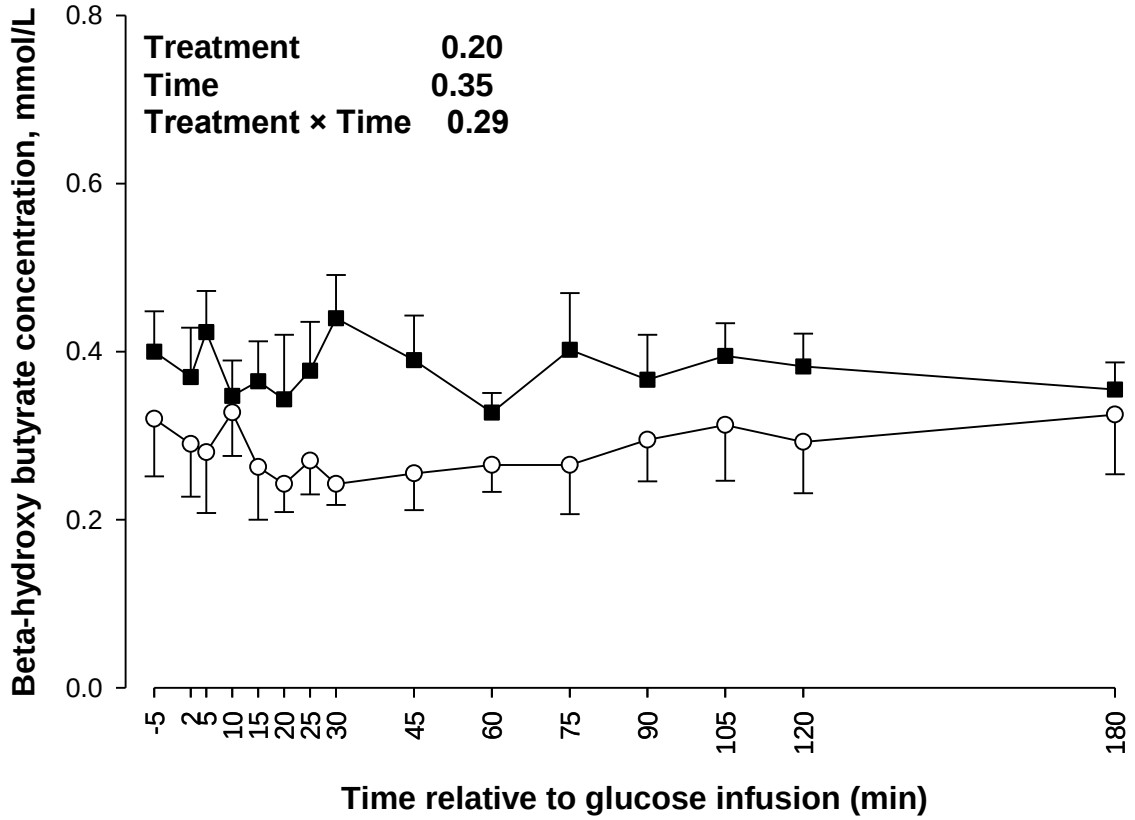

Figure 4. Blood BHB concentrations Control (n= 10; o) and feed restricted fat-tailed dairy sheep (Restriction, n= 10; ■). Data are expressed as least square means ± standard error of the mean.

Discussion

The current study was designed and conducted to study the glucose clearance pattern during feed restriction regardless of the many potentially confounding effects of the pregnancy presented in other studies. The animals were not matted during the experiment to avoid the adverse effects of pregnancy and the transition period. Researchers indicated that glucose and insulin increased in response to intravenous glucose infusion, in pregnant and non-pregnant ewes (Lunesu *et al.* 2020; Morgante *et al.* 2012). An instantaneous increase in insulin concentration after the glucose infusion is expected because of its glucose-regulatory role (Zarrin *et al.* 2015; Francis and Bickerstaffe, 1996). In agreement with the current study, increases in insulin levels were reported in Ghezel ewes from zero to 1st hour after dextrose administration (Chalmeh *et al.* 2020). Previous studies also confirmed the increase in insulin release from pancreatic beta cells due to intravenous glucose infusion (Wolfe *et al.* 1986).

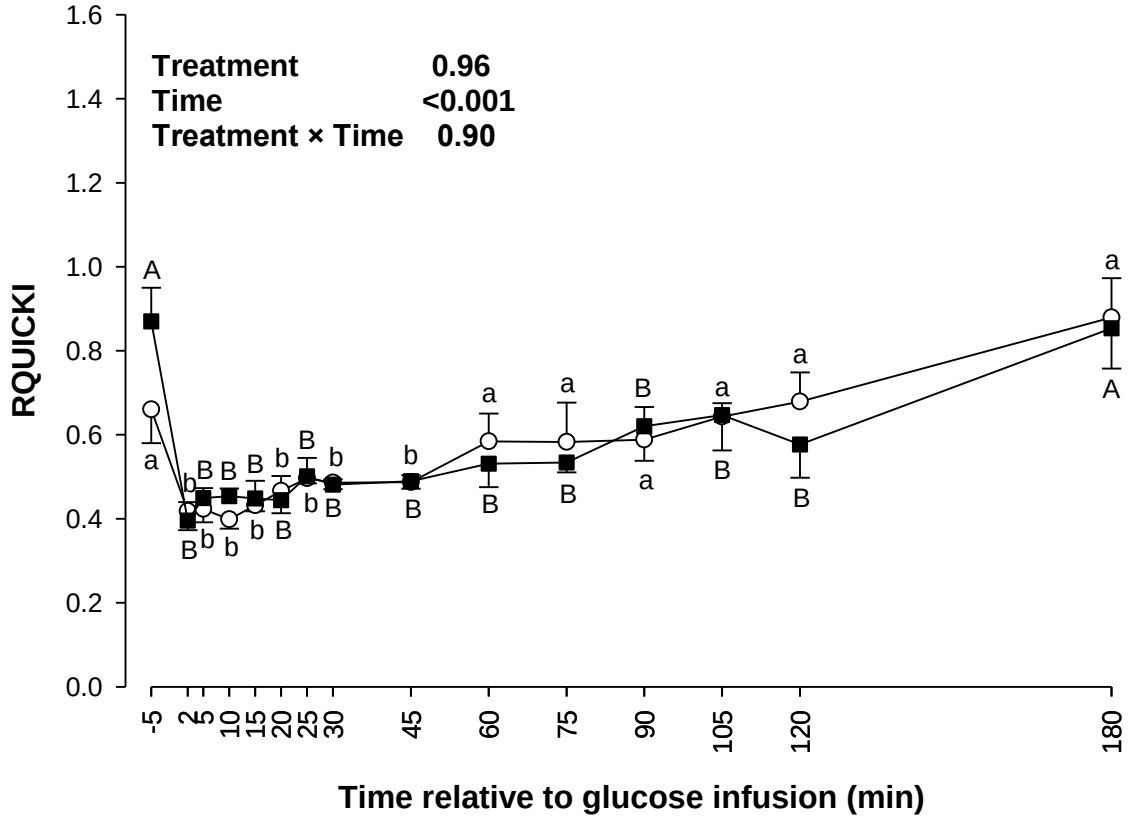

Figure 5. **RQUICKI index Control (n= 10; o) and feed restricted fat-tailed dairy sheep (Restriction, n= 10; ■). Different lowercase letters (a-b) indicate significant differences (P<0.05) between time points within Control. Different uppercase letters (A-B) indicate significant differences (P<0.05) between time points within Restriction. Data are expressed as least square means ± standard error of the mean.**

Glucose infusion in sheep, pre- and post-parturition, increases circulatory glucose, insulin, cortisol, and prolactin concentration; the infusion of 50% glucose decreases the concentration of FFA, BHB, and IGF-1 (Chalmeh *et al.* 2020). These researchers also suggested that induced hyperglycemia through intravenous infusion causes metabolic and hormonal responses in fat-tailed sheep due to providing energy sources and glucose as a metabolic regulator (Chalmeh *et al.* 2020). Although feed restriction-induced insulin resistance (Petterson *et al.* 1993), the low insulin concentration during the ivGTT in the restriction group compared to Control showed a different manner of mediating glucose homeostasis in the fat-tailed sheep. The low insulin concentration in Restriction compared to Control might be a response to the decreased energy during the entire trial in this group (Zarrin *et al.* 2021b, 2021c). Regardless of similar glucose clearance between the studied groups, Control had higher plasma insulin concentrations during an ivGTT, which could be deduced as a possible sign of decreased insulin sensitivity compared to Restriction. Based on the results obtained, the authors concluded that different insulin and glucose regulatory mechanisms might be promoted during feed restriction.

As observed, plasma insulin concentration increased in Control compared to Restriction, which may result from induced insulin resistance in Control and insulin sensitivity in Restriction. Also, Control performs an enhanced response to a glucose challenge at the beta cells level (increased insulin secretion to similar circulating glucose) and reduces insulin responsiveness in peripheral tissues regarding glucose uptake (greater circulating

insulin but similar glucose uptake). That might be demonstrable by the more significant insulin peak in Control than in the Restriction, without perceptible changes in glucose metabolism measures (glucose concentration curve, glucose peak, glucose clearance rate, T½, or AUC).

Table 3. Least square means ± SEM of the area under the curve (AUC) plasma variables and insulin concentration in ewes during glucose tolerance test in Control (n= 5) and restricted (Restriction; n= 5) groups. Data are shown as LSMeans ± SEM.

Variable [1]	Group[2]	LSMeans ± SEM	ANOVA (*P*-Value, group)
Insulin, pmol/L*h	Control	15.80 ± 1.27	0.13
	Restriction	10.97 ± 1.60	
BHB, mmol/L*h	Control	0.30 ± 0.01	0.09
	Restriction	0.35 ± 0.01	
FFA, mmol/L*h	Control	0.08 ± 0.01	0.78
	Restriction	0.08 ± 0.01	
RQUICKI*h	Control	0.54 ± 0.05	0.67
	Restriction	0.50 ± 0.06	

[1]BHB = Beta-hydroxybutyrate; FFA = Free fatty acids; RQUICKI = Revised Quantitative Insulin Sensitivity Check Index.

Feed restriction increased FFA and BHB concentration in non-pregnant ewes (Zarrin *et al.* 2021b). Increased circulatory FFA indicates a higher need for body fat resources in cases of necessity like negative energy balance (LeBlanc, 2006) or reduced feed intake (Xue *et al.* 2019; Zarrin *et al.* 2021c). The FFA fall after the glucose infusion can be related to the homeostatic response caused by increased energy precursor. Also, according to the insulin results, it is supposed that the rise in the insulin concentration has controlled fat tissue breakdown, which has caused a lessened FFA concentration in Control (Karpe *et al.* 2011). Another possible reason for the decline in FFA is its hepatocyte absorption due to higher insulin secretion (Zachut *et al.* 2013). There is solid evidence on the effect of insulin on increasing oleic acid absorption by the hepatic cells in calves (Zammit, 1996). The results obtained in the present study are consistent with those of other researchers and show the reducing effect of the available energy source on fatty acid mobilization (Chalmeh *et al.* 2020; Karpe *et al.* 2011; Zachut *et al.* 2013). BHB and FFA circulatory levels significantly decreased following intravenous hypertonic dextrose administration in Ghezel ewes (Chalmeh *et al.* 2020). Low insulin concentration and reduced sensitivity of the tissues around parturition increase lipid mobilization and induce further rise in plasma FFA concentrations (Hayirli, 2006). The rapid drop in FFA values after the glucose infusion demonstrates that lipid mobilization is reduced regarding energetic balance conditions (Chalmeh *et al.* 2020). Previous studies have authenticated the negative correlation between FFA concentrations, insulin AUC, and insulin concentration at its peak (Bossaert *et al.* 2008). Adipose tissue and its derivatives are crucial in determining and modulating insulin sensitivity during glucose metabolism in dairy cows (De Koster and Opsomer, 2013). Direct manipulative studies have also demonstrated that providing excess FFA by abomasal fat infusion produced peripheral insulin resistance in nonlactating cows (Pires *et al.* 2007; Pires and Grummer, 2007).

Table 4. Least square means ± SEM of variables related to glucose tolerance test in control (Control: n= 5) and restricted (Restriction; n= 5) groups. Data are shown as LSMeans ± SEM.

	Intravenous glucose tolerance test variables					
	Glucose Baseline, mg/dL	Glucose Maximum, mg/dL	Glucose at the end of experiment, mg/dL	Glucose turnover rate, %/min	Glucose half-life time, min	Area under the curve, (mg/dL*180 min)
Control	42.57	235.00	43.50	2.13	32.89	119.90
Restriction	45.13	275.00	39.00	2.26	36.17	123.32
SEM	3.75	13.76	5.34	0.27	4.54	10.40
P-value	0.84	0.53	0.83	0.65	0.46	0.83

According to Grummer *et al.* (2004), increased plasma FFA concentration increases hepatocyte ketogenesis. After dextrose administration, the circulatory BHB followed the same pattern of FFA concentration. However, the current results were in contrast to studies showing a decline in BHB concentration (Chalmeh *et al.* 2020). The rapid decrease observed in BHB and FFA concentration after glucose administration has occurred in all time periods, which confirms that the production of ketone bodies was reduced (Chalmeh *et al.* 2020).

Conclusion

The current results indicate the ability of fat-tailed sheep to preserve blood glucose levels in conditions of feed restriction, which is accomplished through hormonal mechanisms, especially hormones involved in energy metabolism, such as insulin. The reduction of insulin in Restriction compared to Control, even when a rich source of glucose is provided through intravenous infusion, might be due to increased insulin sensitivity in the restricted ewes compared to Control. Therefore, the physiological ability of fat-tailed sheep to support body homeostasis and their high energy storage source in the tail shows the importance of these breeds in harsh environmental conditions or husbandry systems.

Conflicts of Interest: The authors declare no conflict of interest.

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بررسی پاسخ‌های متفاوت میش‌های دنبه‌دار غیرآبستن به تست تحمل گلوکز داخل وریدی در زمان ایجاد محدودیت خوراکی

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چکیده

به منظور بررسی پاسخ متابولیسمی به تزریق داخل وریدی گلوکز همراه با محدودیت خوراکی در میش‌های دنبه‌دار غیرآبستن، ۱۰ رأس میش غیرآبستن نژاد آمیخته لری بختیاری و ترکی قشقایی با میانگین سنی ۳ تا ٤ سال و وزن ۳/٦± ٤۹/۲ کیلوگرم انتخاب و به دو گروه آزمایشی شامل گروه شاهد (٥ رأس) و محدودیت خوراک (٥ رأس) تقسیم شدند. جیره پایه بر مبنای توصیه‌های NRC (۲۰۰۷) (شامل یونجه، جو و کاه تنظیم شد. میش‌های گروه شاهد به خوراک دسترسی آزاد داشتند، در حالی که گروه محدودیت پس از یک هفته دسترسی آزاد، به مدت سه هفته به ترتیب به ٥۰، ٦٥ و ۸۰ درصد از جیره توصیه شده دسترسی یافتند و در هفته پنجم دوباره به خوراک آزاد بازگشتند. تزریق داخل وریدی گلوکز (1 ml/kg BW) در هفته سوم آزمایش انجام شد و خون‌گیری از سیاهرگ وداجی در زمان‌های مشخص قبل و بعد از تزریق صورت گرفت. نتایج آماری با رویه Mixed بررسی شدند. تزریق گلوکز موجب افزایش ناگهانی گلوکز در هر دو گروه (P<۰/۰۰۱) و افزایش غلظت انسولین با تأخیر در هر دو گروه شد، اما این افزایش در گروه محدودیت کمتر بود (P<۰/۰٥). تزریق گلوکز بر غلظت اسیدهای چرب آزاد و بتاهیدروکسی‌بوتیرات در هر دو گروه اثر نداشت ولی در گروه کنترل با افزایش غلظت گلوکز خون کاهش غلظت اسیدهای چرب آزاد مشاهده شد. نتایج نشان داد گوسفندان دنبه‌دار در شرایط کاهش دسترسی به خوراک قادر خواهند بود از طریق هورمون‌های دخیل در متابولیسم انرژی نظیر انسولین هموستازی لازم برای حفظ انرژی و گلوکز بدن را انجام دهند.

واژه‌های کلیدی: بتاهیدروکسی‌بوتیرات، محدودیت خوراکی، اسیدهای چرب آزاد، هموستازی انرژی، انسولین