



Feed restriction around parturition does not affect colostrum immunoglobulin G concentration in dairy fat-tailed sheep but does affect performance and blood metabolites in newborn lambs

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ABSTRACT

This study aimed to investigate the effect of prepartum and postpartum feed restriction of fat-tailed dairy sheep on colostrum IgG concentration, and performance and blood metabolites of newborn fat-tailed lambs. Twenty fat-tailed dairy sheep were randomly allocated into control (Ctrl; $n = 10$) and feed restriction (FR; $n = 10$) groups. The Ctrl group received a diet that met 100% of energy requirements, both prepartum (from wk -5 to parturition) and postpartum (from parturition to wk 5). The FR group received a diet equivalent to 100, 50, 65, 80, and 100% of the energy requirements in wk -5 , -4 , -3 , -2 , and -1 relative to parturition, respectively. After parturition, the FR group received a diet equivalent to the 100, 50, 65, 80, and 100% of the energy requirements in wk 1, 2, 3, 4, and 5, respectively. At birth, lambs were assigned to their dam's experimental group. Both the Ctrl lambs ($n = 10$) and the FR lambs ($n = 10$) were allowed to suck colostrum and milk from the dams. Colostrum samples (50 mL) were collected at parturition (0 h) and then at 1, 12, 24, 36, 48, and 72 h postpartum. Blood samples were collected from all lambs before suckling colostrum (0 h) and then at 1, 12, 24, 36, 48, and 72 h after birth and weekly until the end of the experimental period (i.e., wk 5 relative to birth). The data were evaluated using the MIXED procedure of SAS (SAS Institute Inc.). The model included feed restriction, time, and the interaction feed restriction \times time as fixed effects. The individual lamb was set as a repeated subject. Variables measured in colostrum and plasma were considered dependent variables, and significance was set at $P < 0.05$. Prepartum and postpartum feed restriction in

fat-tailed dairy sheep did not affect colostrum IgG concentration. Consequently, no differences in blood IgG concentrations were observed in the lambs. In addition, the prepartum and postpartum feed restriction experienced by fat-tailed dairy sheep caused decreased body weight and milk intake in lambs from the FR group compared with the Ctrl group. Feed restriction also promoted increased concentration of blood metabolites such as triglycerides and urea in FR lambs compared with control lambs. In conclusion, prepartum and postpartum feed restriction in fat-tailed dairy sheep did not affect either colostrum IgG concentration or blood IgG concentration of the lambs. However, prepartum and postpartum feed restriction decreased lamb milk intake and, therefore, lamb body weight gain during the first 5 wk after birth.

Key words: ewe, metabolism, milk, offspring

INTRODUCTION

The sudden demand of energy for fetal growth prepartum and milk production postpartum and the reduced feed intake capacity represent an important metabolic challenge for dairy ruminants (Zachut et al., 2020). In this situation, energy intake does not meet energy requirements for body maintenance, fetal growth, and milk production, which results in negative energy balance (Bell and Bauman, 1997; Drackley, 1999) and high adipose tissue mobilization. If the animal fails to adapt to negative energy balance, the risk of metabolic disorders increases considerably, affecting not only animal performance, but also animal health and welfare (Hernández-Castellano et al., 2020). In addition, insufficient energy intake may affect fetal growth prepartum and has strong consequences for milk yield and quality, affecting lamb development during the first days of life (Zarrin et al., 2021; Silva et al., 2022). Livestock systems in developing countries located in tropical and subtropical regions are heavily dependent on natural

Received July 11, 2022.

Accepted November 12, 2022.

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resources (i.e., pastures). In these countries, the dry season decreases the quality and availability of local forage resources, which has important consequences for performance and health of dairy ruminants (Palma et al., 2017; Hernández-Castellano et al., 2019).

Fat-tailed sheep are highly resilient to harsh environmental conditions such as water scarcity and low-quality pastures. The main characteristic of all fat-tailed sheep is the deposition of a substantial amount of fat in the tail. According to the literature, fat depots are differently regulated in fat-tailed sheep compared with other sheep breeds during periods of feed scarcity (Alves et al., 2013; van Harten et al., 2015; Almeida et al., 2016). Most studies using fat-tailed sheep breeds under feed restriction have been done on breeds used for meat production, and the literature on dairy breeds is very limited (Ahmadzadeh et al., 2020; Zarrin et al., 2021). However, none of these studies have described the effect of prepartum and postpartum feed restriction on blood metabolites in newborn fat-tailed dairy lambs raised together with dams.

Therefore, in this study, we hypothesize that colostrum IgG concentration, and performance and blood metabolites in newborn fat-tailed lambs will be affected by prepartum and postpartum feed restriction of fat-tailed dairy sheep. The objective of this experiment was to study the effect of prepartum and postpartum feed restriction of fat-tailed dairy sheep on colostrum IgG concentration, and on performance and blood metabolites of newborn fat-tailed lambs.

MATERIALS AND METHODS

Experimental Design

The present study was carried out at the experimental farm of the Yasouj University (Naregah, Yasouj, Iran). All animal procedures followed the ethical law on Animal Protection and were approved by the Committee of Animal Experiments of Yasouj University (Yasouj, Iran) under experimental procedure 950441011–15950. All sheep and lambs used in this study were visually healthy.

In this study, 20 multiparous fat-tailed sheep (Lori-Bakhtiari, $n = 10$; and Turkey-Qashqai, $n = 10$) with average age of 3.4 ± 0.5 yr and BW of 56 ± 1.8 kg were used. From wk -7 to wk -5 relative to expected parturition date, all animals were fed a TMR diet formulated to meet 100% of the energy requirements recommended by the NRC (2007) for dry sheep (dry diet). In wk -5 relative to parturition, animals were randomly allotted into 1 of 2 experimental groups: control (**Ctrl**; $n = 10$) and feed restriction (**FR**; $n = 10$).

From wk -5 relative to parturition, sheep in the Ctrl group received the dry diet. Sheep in the FR group were fed different energy levels from wk -5 to parturition. Thus, the FR group was fed a diet equivalent to 100, 50, 65, 80, and 100% of the energy content of the dry diet in wk -5 , -4 , -3 , -2 , and -1 relative to parturition, respectively. The sudden reduction in energy level aimed to simulate conditions of reduced energy intake prepartum (i.e., from wk -5 to -4). Energy intake increased progressively prepartum (i.e., from wk -4 to -1) to prevent metabolic problems such as rumen acidosis. After parturition, sheep from the Ctrl group were fed a TMR diet formulated to meet 100% of the energy requirements recommended by the NRC (2007) for early-lactation sheep (early-lactation diet). Similarly, the FR group received a diet equivalent to 100, 50, 65, 80, and 100% of the energy content of the early-lactation diet in wk 1, 2, 3, 4, and 5, respectively. This sudden reduction in energy level aimed to simulate conditions with reduced energy intake postpartum (i.e., from wk 1 to 2). Energy intake increased progressively postpartum (i.e., from wk 2 to 5) to prevent metabolic problems such as rumen acidosis. Animals had free access to drinking water and mineral blocks throughout the experimental period. During the trial, all animals were kept in individual pens (1.2×1.0 m) located in a closed barn. Each pen was equipped with individual drink and feed containers. The chemical composition and ration ingredients of the dry and early-lactation diets were described in Zarrin et al. (2021).

Lamb Management, Feeding, and Weight

At birth, all lambs ($n = 20$; 8 females and 12 males) were immediately separated from their dams. Within the first 15 min after birth, lambs were dried, ear tagged, and weighed (MDS9800, Mahak; ± 10 g accuracy). After that, newborn lambs were allowed to suck colostrum from their dams and remained together from birth to wk 1. From wk 1 to wk 5 relative to birth, lambs were allocated in pens designed for lambs (3.0×3.0 m), with up to 5 lambs allocated per pen. Lambs had free access to water and were allowed to suck milk from their dams for 30 min 3 times a day (0800, 1400, and 1800 h). Colostrum and milk intake were estimated using the weigh–suckle–weigh method described by Benson et al. (1999) with slight modifications. Briefly, all lambs were weighed before and after suckling, and the weight difference was registered as colostrum or milk intake. During the experimental period, lambs had no access to any solid feed. Lambs were allocated to either the FR ($n = 10$) or the Ctrl group ($n = 10$) groups, based on the experimental group of each lamb's dam.

Colostrum Sampling

Colostrum samples (50 mL) were collected at parturition (0 h) and then at 1, 12, 24, 36, 48, and 72 h postpartum. Two aliquots (1.5 mL) were then stored at -20°C .

Blood Sampling

One blood sample was collected from each lamb immediately after birth before suckling colostrum (within the first 15 min relative to birth; labeled as 0 h). Additional blood samples were collected at 1, 12, 24, 36, 48, and 72 h relative to birth and then weekly until the end of the experimental period (i.e., wk 5 relative to birth). All blood samples were collected from the jugular vein using heparinized vacuum tubes (6 mL) before morning feeding. After collection, the tubes were placed in wet ice and centrifuged at $3,000 \times g$ for 20 min at 4°C (Hemle Labortechnik GmbH). The plasma was then aliquoted (1.5 mL) and stored at -20°C .

IgG Concentration in Colostrum

Concentrations of IgG in colostrum were measured using a commercial sheep IgG ELISA kit (#ab190546; Abcam) following the manufacturer's instructions. The inter- and intraassay coefficients of variation (CV) were 7.2 and 8.7%, respectively.

Variables Measured in Plasma

The plasma concentration of glucose (#1500017), calcium (#108100), total protein (TP; #1500028), urea (#1400029), albumin (#101500), creatinine (#1400009), triglycerides (TG; #1500032), cholesterol (#1500010), and the plasma activity of lactate dehydrogenase (LDH; #122400) and glutamic oxaloacetic transaminase (GOT; #1400018), were determined using commercial kits (Pars Azmoun), and an automated analyzer (B850, Mindray), following the manufacturers' instructions. The intraassay CV were 1.5, 1.3, 0.9, 3.3, 1.3, 3.2, 1.6, 0.9, 2.5, and 2.4%, respectively. The interassay CV were 0.9, 1.6, 1.3, 4.1, 1.5, 1.8, 1.2, 1.1, 1.7, and 2.2%, respectively. Concentrations of free fatty acids (FFA; #FA115) in blood plasma were measured using a commercial kit (Randox Laboratories Ltd.). The inter- and intraassay CV were 4.7 and 4.4%, respectively. Concentrations of IgG in blood plasma were measured using a sheep IgG ELISA kit (#ab190546; Abcam) following the manufacturer's instructions. The inter- and intraassay CV were 7.2 and 8.7%, respectively.

Statistical Analysis

The minimum number of lambs per group was calculated based on the colostrum yield reported by Zarrin et al. (2021) for fat-tailed dairy sheep (4.45 ± 0.45 kg) using the POWER procedure of SAS (version 9.4, SAS Institute Inc.) to comply with the assumption of power = 0.80 and $\alpha = 0.05$ and 32% variance caused by feed restriction. The data were tested for normal distribution using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc.). The data were evaluated using the MIXED procedure of SAS. The model included feed restriction (FR) and time (T; from birth to wk 5 relative to birth) and the interaction (feed restriction \times time) as fixed effects. The individual lamb was set as a repeated subject, and pen location was used as co-variable. Variables measured in colostrum and plasma were considered dependent variables. Significant effects were assumed when $P < 0.05$. Results are presented as least square means (LSM) \pm standard error of the mean (SEM).

RESULTS

Colostrum IgG concentration (Figure 1) was not affected by prepartum or postpartum feed restriction ($P_{\text{FR}} = 0.168$) but decreased from 0 h (31.2 ± 1.95 mg/mL) to 72 h (17.4 ± 1.88 mg/mL) ($P_{\text{T}} < 0.001$). A 2-way interaction between feed restriction and time was observed for lamb BW (Figure 2A; $P_{\text{FR} \times \text{T}} = 0.038$). Feed restriction did not affect lamb BW at birth (4.4 ± 0.62 and 4.9 ± 0.74 kg in the FR and Ctrl group, respectively) or in wk 1 (5.5 ± 0.62 and 6.5 ± 0.74 kg in the FR and Ctrl groups, respectively). However, Ctrl lambs were heavier than FR lambs during the rest of the experimental period (11.8 ± 0.83 and 8.6 ± 0.64 kg in wk 5, respectively).

Similarly, a 2-way interaction between feed restriction and time was observed for milk intake (Figure 2B; $P_{\text{FR} \times \text{T}} = 0.004$). Milk intake was lower in the FR group than in the Ctrl group during the entire experimental period. No differences in milk intake were observed in the Ctrl group from birth to wk 5 (1.1 ± 0.07 kg/d). However, milk intake decreased in the FR group from wk 1 (0.8 ± 0.08 kg/d) to wk 2 (0.5 ± 0.08 kg/d), and then increased constantly until wk 5 (0.7 ± 0.08 kg/d) relative to birth.

Table 1 shows the concentrations of plasma metabolites in lambs from the Ctrl and FR groups. A 2-way interaction between feed restriction and time was observed for TG ($P_{\text{FR} \times \text{T}} = 0.047$). Thus, TG concentrations (Figure 3D) were higher in the FR group than in the Ctrl group at birth (104.1 ± 6.04 and $80.8 \pm$

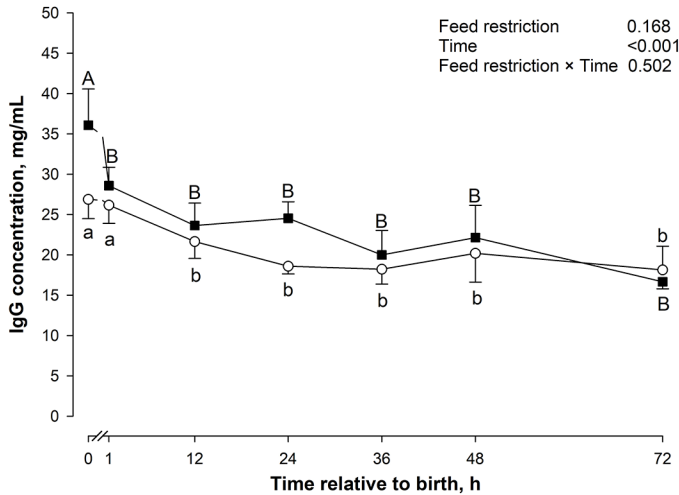


Figure 1. Colostrum IgG concentrations in control (Ctrl, $n = 10$; ○) and feed-restricted (FR, $n = 10$; ■) fat-tailed dairy sheep. Different lowercase letters (a, b) indicate significant differences ($P < 0.05$) between time points within Ctrl group. Different uppercase letters (A, B) indicate significant differences ($P < 0.05$) between time points within FR group. Data are expressed as LSM \pm SEM.

5.29 mg/dL, respectively) and at 1 h relative to birth (92.1 ± 5.68 and 77.5 ± 5.29 mg/dL, respectively). However, those differences disappeared during the rest of the experimental period, except in wk 5 when TG concentrations were higher in the Ctrl group than in the FR group (97.7 ± 6.00 and 86.0 ± 6.04 mg/dL, respectively). In general, lambs from the FR group showed higher urea concentrations (Figure 3A; $P_{FR} = 0.038$) than those from the Ctrl group (23.7 ± 1.73 and 18.5 ± 1.73 mg/dL, respectively). Urea concentrations (Figure 3B) increased from 0 h (16.2 ± 3.61 mg/mL) to wk 1 (26.7 ± 3.73 mg/mL) and remained constant until wk 5 ($P_T = 0.037$).

Glucose, calcium, and TP concentrations (Table 1) were not affected by either feed restriction ($P \geq 0.147$) or time relative to birth ($P \geq 0.076$). Albumin (Figure 3B), creatinine (Figure 3C), cholesterol (Figure 4A), GOT (Figure 4C), FFA (Figure 4D), and IgG (Figure 5) concentrations as well as LDH activity (Figure 4B) were not affected by feed restriction ($P \geq 0.099$) but were affected by time relative to birth ($P_T = 0.038$, $P_T < 0.001$, $P_T < 0.001$, $P_T = 0.046$, $P_T < 0.001$, $P_T < 0.001$, respectively). Albumin concentrations increased from 0 h (3.1 ± 0.20 g/dL) to wk 1 (3.9 ± 0.20 g/dL) and decreased progressively until the end of the experimental period (3.4 ± 0.21 g/dL). Similarly, creatinine concentrations increased from 0 h (0.9 ± 0.12 mg/dL) to 36 h (1.5 ± 0.10 mg/dL) and remained constant until the end of the experimental period. Cholesterol concentrations increased from 0 h (27.8 ± 3.58 mg/dL) to wk 2 (61.4 ± 3.58 mg/dL) relative to birth when it

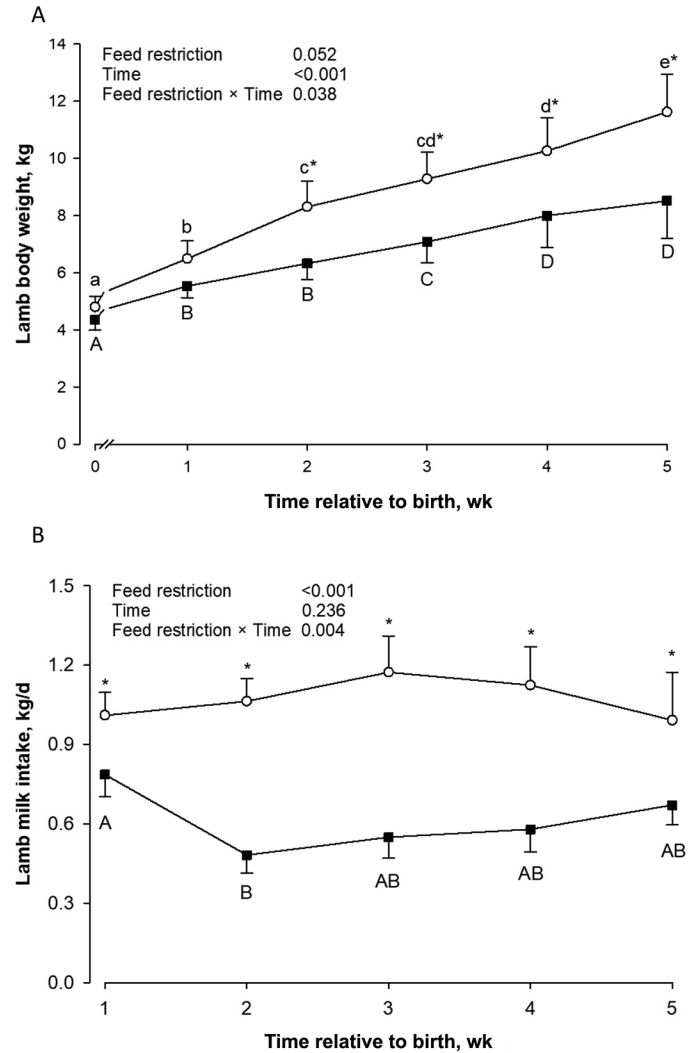


Figure 2. Lamb BW (A) and milk intake (B) in control (Ctrl, $n = 10$; ○) and feed-restricted (FR, $n = 10$; ■) lambs from birth (0) to wk 5 relative to birth. Different lowercase letters (a–e) indicate significant differences ($P < 0.05$) between time points within Ctrl group. Different uppercase letters (A–D) indicate significant differences ($P < 0.05$) between time points within FR group. *Indicates a significant difference ($P < 0.05$) between control and FR groups within each time point. Data are expressed as LSM \pm SEM.

reached a constant value until the end of the experimental period. Plasma LDH activity increased from 0 h (388.9 ± 58.96 U/L) to wk 1 (627.0 ± 87.02 U/L), thereafter remaining constant. Concentrations of GOT increased constantly from 0 h (56.1 ± 12.14 U/L) to wk 4 (117.2 ± 15.14 U/L). The circulating concentration of FFA increased from birth (0 h) to 24 h after birth (5.01 ± 0.21 and 6.2 ± 0.35 mg/mL, respectively) and remained constant until the end of the experimental period (wk 5).

Concentrations of IgG (Figure 5) increased rapidly from 0 to 1 h relative to birth and remained constant

Table 1. Plasma metabolite concentrations (LSM) of lambs in the control (Ctrl; n = 10) and feed-restricted (FR; n = 10) groups relative to birth (0–72 h and from wk 1–5)

Variable ¹	Group		SEM	Fixed effect ²		
	Ctrl	FR		F	T	F × T
Glucose, mg/dL	66.5	71.0	5.72	0.654	0.733	0.844
Calcium, mg/dL	10.3	10.8	0.49	0.483	0.267	0.457
TP, g/dL	3.9	4.5	0.24	0.147	0.076	0.924
Urea, mg/dL	18.5	23.7	1.73	0.038	0.037	0.723
Albumin, g/dL	3.2	3.6	0.15	0.099	0.038	0.564
Creatinine, mg/dL	1.4	1.3	0.07	0.679	<0.001	0.564
TG, mg/dL	88.0	92.0	3.73	0.434	0.135	0.047
Cholesterol, mg/dL	43.4	47.0	2.72	0.357	<0.001	0.135
LDH, U/L	453.8	503.8	57.49	0.530	0.023	0.904
GOT, U/L	67.8	85.2	8.16	0.239	0.046	0.301
FFA	5.57	5.64	0.15	0.735	<0.001	0.331
IgG, ³ mg/mL	6.3	8.4	0.89	0.112	<0.001	0.973

¹TP = total protein; TG = triglycerides; LDH = lactate dehydrogenase; GOT = glutamic oxaloacetic transaminase; FFA = free fatty acids.

²F = feed restriction; T = time; F × T = feed restriction × time interaction.

³LSM and SEM obtained from samples collected at 0, 1, 12, 24, 36, 48, and 72 h relative to birth.

for the next 72 h. Urea concentrations were affected by feed restriction ($P_{FR} = 0.038$) and time relative to birth ($P_T = 0.037$).

DISCUSSION

The current findings should be considered in the context of the performance results published in detail by Zarrin et al. (2021). Briefly, the decreased DMI observed in the FR group prepartum and postpartum did not cause differences in BW between the FR and the Ctrl groups. In dams, feed restriction increased FFA, BHB, and growth hormone concentrations and decreased insulin and prolactin concentrations during both the prepartum and postpartum periods. In addition, feed restriction caused a decreased colostrum yield and a relative increase of the main colostrum components. Similarly, milk yield decreased in the FR group compared with the Ctrl group, although milk components were not affected.

In the present study, the prepartum feed restriction in fat-tailed sheep did not affect lamb birth BW. However, feed restriction reduced both colostrum and milk yield of the dams (Zarrin et al., 2021), which reduced milk intake in lambs from the FR group compared with those from the Ctrl group. Consequently, lambs from the FR group did not show a rapid increase in lamb BW compared with Ctrl lambs, especially after wk 1 relative to birth. In agreement with these results, Santos et al. (2018) showed that the restriction of milk replacer intake impaired lamb BW compared with lambs fed ad libitum. In conditions such as feed restriction or specific physiological stages such as the onset of lactation, energy intake may not meet the requirements

for maintenance. In this situation, animals are under negative energy balance and they mobilize energy from different body compartments (i.e., fat, muscle) to meet energy requirements. During muscle catabolism, urea and creatinine concentrations are increased in the bloodstream (Haines et al., 2019). In the present study, urea concentrations were higher in the FR group than in the Ctrl group; however, creatinine concentrations were not affected. Therefore, it is unlikely that increased urea concentrations were caused by increased muscle catabolism in FR lambs. It is more likely that the reduced milk intake observed in the FR group could be caused by short-term dehydration, which is compatible with increased urea concentrations and unaffected creatinine concentrations (Mehta, 2008).

In addition to the reduced BW, lambs from the FR group showed increased TG concentrations immediately after birth, although those differences disappeared after 1 h relative to birth. No differences in circulating FFA were observed in these animals during the entire experimental period, which indicates that lambs were not mobilizing fat from their own body reserves. Under feed restriction, animals need to mobilize fat to meet energy requirements. This fact results in increased FFA concentration in the bloodstream. As shown in our companion paper (Zarrin et al., 2021), feed restriction increased FFA concentrations in the bloodstream of fat-tailed dairy sheep prepartum. As described by Toschi and Baratta (2021), the placenta of sheep under feed restriction is able to adapt the transfer of nutrients from the dam to the fetus. In case of FFA, these are transported through the placenta as triglycerides in lipoproteins. During feed restriction, both lipoprotein receptors and lipase activities are increased in the pla-

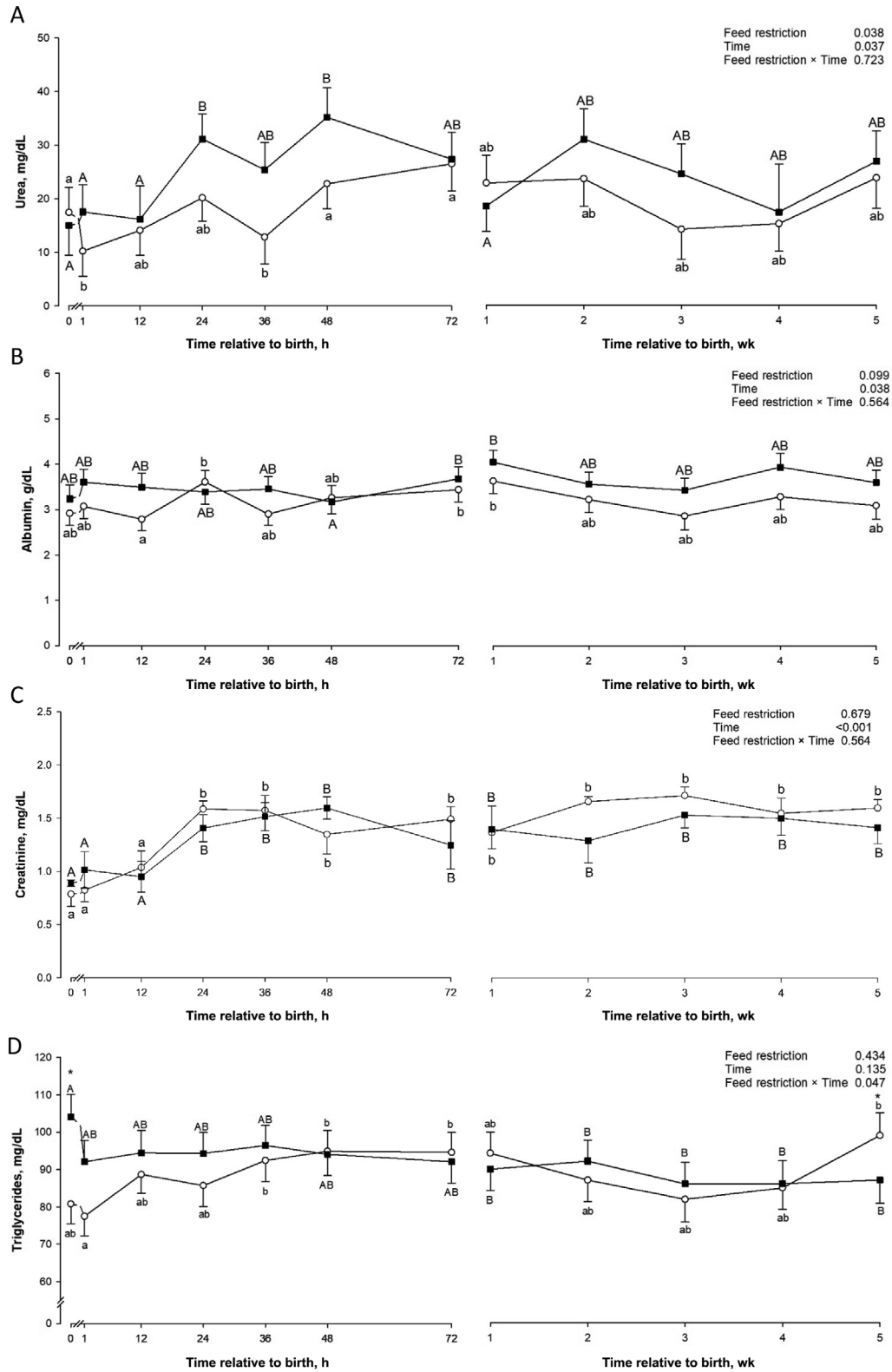


Figure 3. Average plasma urea (A), albumin (B), creatinine (C), and triacylglyceride (D) concentrations in control (Ctrl, n = 10; ○) and feed-restricted (FR, n = 10; ■) lambs during sampling time (0–72 h and wk 1–5 relative to birth). Different lowercase letters (a, b) indicate significant differences ($P < 0.05$) between time points within Ctrl group. Different uppercase letters (A, B) indicate significant differences ($P < 0.05$) between time points within FR group. *Indicates a significant difference ($P < 0.05$) between control and FR groups within each time point. Data are expressed as LSM ± SEM.

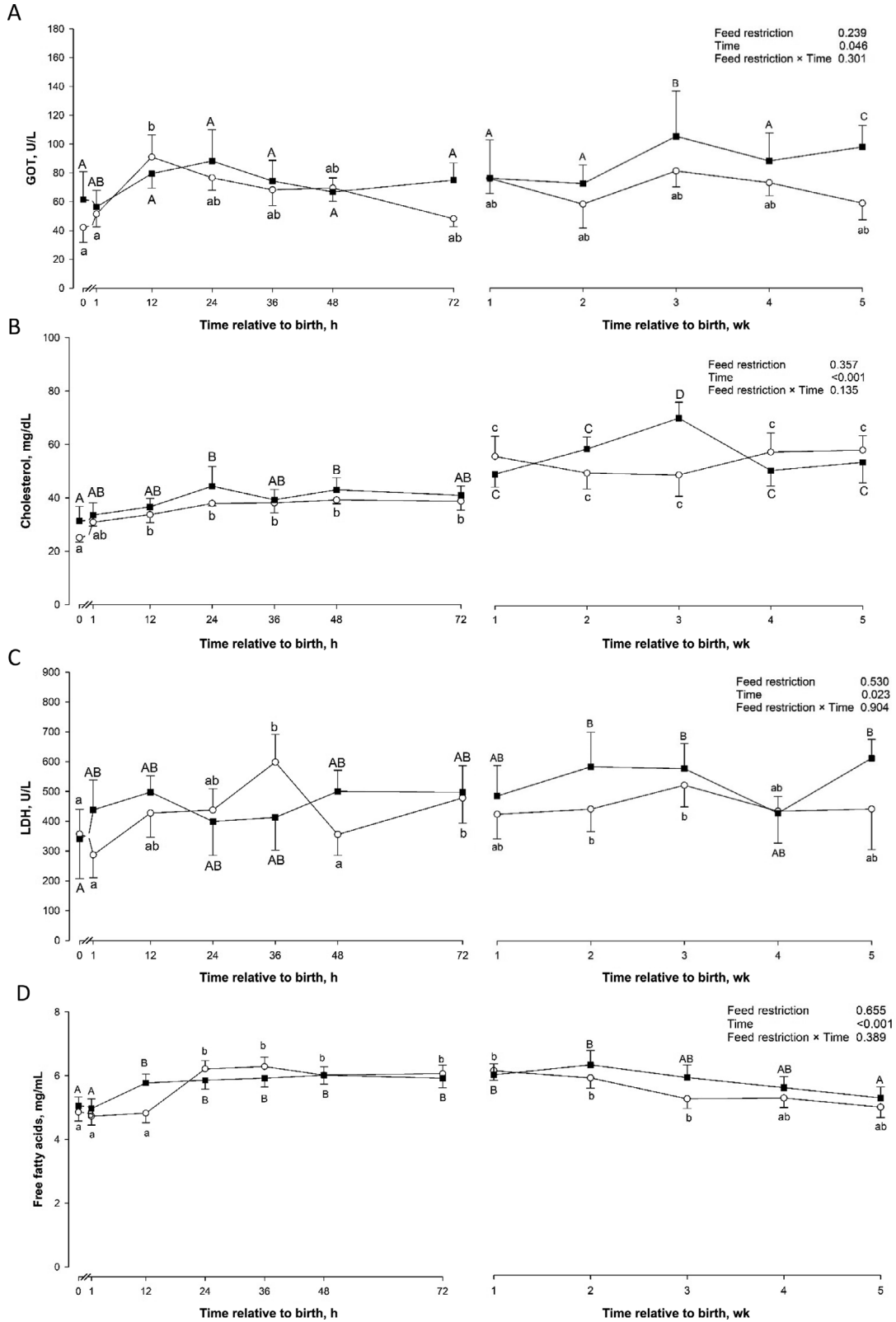


Figure 4. Average plasma glutamic oxaloacetic transferase (GOT; A), cholesterol (B), lactate dehydrogenase (LDH; C), and free fatty acids (D) concentrations in control (Ctrl, n = 10; ○) and feed-restricted (FR, n = 10; ■) lambs during sampling time (0–72 h and wk 1–5 relative to birth). Different lowercase letters (a–c) indicate significant differences ($P < 0.05$) between time points within Ctrl group. Different uppercase letters (A–D) indicate significant differences ($P < 0.05$) between time points within FR group. Data are expressed as LSM ± SEM.

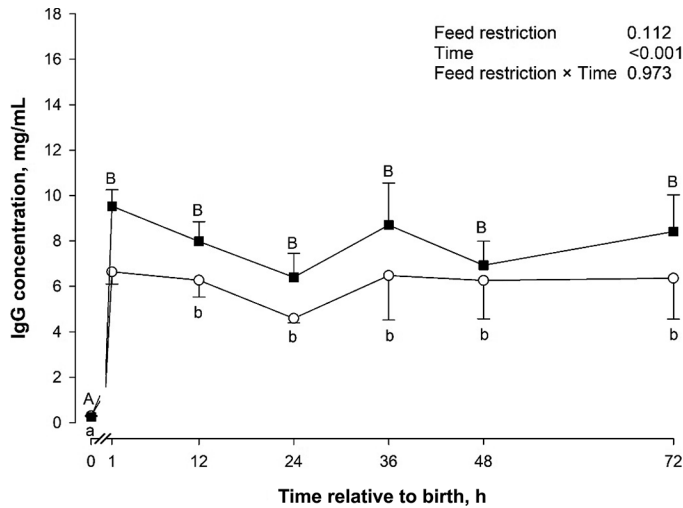


Figure 5. Plasma IgG concentrations in control (Ctrl, n = 10; ○) and feed-restricted (FR, n = 10; ■) lambs. Different lowercase letters (a, b) indicate significant differences ($P < 0.05$) between time points within Ctrl group. Different uppercase letters (A, B) indicate significant differences ($P < 0.05$) between time points within FR group. Data are expressed as LSM \pm SEM.

centa. Although changes in the placenta conformation caused by feed restriction are not immediate, Vonnahme et al. (2006) reported that sheep that used to be raised under harsh environments with limited food availability displayed faster placental adaptation than sheep facing undernutrition for the first time. As the Lori-Bakhtiari and Turkey-Qashqai fat-tailed dairy sheep are well known for being adapted to harsh environments and reduced food availability, it is possible that these breeds undergo rapid placental conformation changes caused by short-term feed restrictions. Therefore, feed restriction and increased FFA concentration prepartum in fat-tailed dairy sheep could have promoted placental changes that resulted in increased transfer of FFA through the placenta, and consequently increased TG concentration in FR lambs few hours after birth.

In the present study, feed restriction did not affect either TP or IgG concentration. Lambs are born with an immature immune system. In sheep, the insufficient transfer of immune components (i.e., immunoglobulins) through the placenta makes colostrum intake almost the only way to achieve optimal passive immune transfer (Hernández-Castellano et al., 2015b). Therefore, almost all IgG detected in the bloodstream of lambs during the first days after birth is colostrum derived (Hernández-Castellano et al., 2015a). Even though, in the companion paper (Zarrin et al., 2021), the FR group showed reduced colostrum yield, no differences in colostrum IgG concentration were detected between groups in the present study. This indicates that lambs from the FR group could have had less colostrum IgG

available for intake. However, it seems that colostrum synthesized by the FR group was enough to meet the intestinal absorption capacity of IgG by the newborn lambs because no differences in circulating IgG concentrations were detected between FR and Ctrl lambs. Massimini et al. (2006) described a robust correlation between TP and IgG during the first days after birth such that TP concentration can be used to assess passive immune transfer success. As no differences in IgG concentration were detected, the lack of difference in TP was also expected.

CONCLUSIONS

Prepartum and postpartum feed restriction in fat-tailed dairy sheep did not affect colostrum IgG concentration and therefore did not affect IgG concentrations in the bloodstream of the newborn lambs. However, prepartum and postpartum feed restriction in fat-tailed dairy sheep causes decreased milk intake and therefore decreased BW gain during the first 5 wk relative to birth, which promoted increased concentration of blood metabolites such as triglycerides and urea.

ACKNOWLEDGMENTS

The authors are grateful to SabzBavaran-e-NouAndish Co. (Shiraz, Iran) for providing expert technical and laboratory assistance and equipment for part of the study. Lorenzo E. Hernández-Castellano acknowledges funding from the Agencia Estatal de Investigación (Spain; RYC2019-027064-I/MCIN/AEI/10.13039/501100011033) and the European Social Fund (ESF) Investing in Your Future. M. González-Cabrera acknowledges financial support from the Formacion del Personal Investigador program (ACIISI, Gobierno de Canarias, Spain). The data presented in this study are available on request from the corresponding author. The authors have not stated any conflicts of interest.

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