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Effect of a Calcium Soap of Fatty Acids on Reproductive Characteristics and Lactation Performance of Fat-Tailed Sheep

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Abstract: Experiments were carried out to study the effect of feeding Megalac, calcium soaps of fatty acids (protected fat), on reproduction and lactation of sheep. In the first experiment, 20 Ghezel and 20 Mehraban cyclic fertile ewes (4-5 years old) were randomly allotted to 4 groups. The control group was fed with a balanced ration and the other groups received the same diet as well as a daily allowance of 40 g non-protected fat (NP), 40 g protected fat (LP), or 80 g (HP) protected fat. The ewes were fed with their respective rations for one cycle length. Blood samples were collected and analyzed for progesterone (P_4), cholesterol (CHOL), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and triacylglycerols (TG). The ewes were slaughtered on their next estrous period and the size and number of follicles in ovaries were recorded. There were no significant effects of feeding fat on ovarian weights, cycle length and follicular numbers in each class, or on the size of the largest follicle. Serum concentrations of P_4, CHOL, TG and HDL were greater for HP ewes as compared with the control ewes ($p<0.05$). In the second experiment, effects on lactation and lamb performance of feeding protected fat during mating, late gestation and early lactation were studied in Mehraban ewes. Milk and fat yields on day 25 of lactation were significantly increased by feeding protected fat. Protected fat resulted in lower weight loss in ewes and a higher lamb birth weight. Average daily weight gain of lambs from birth to day 60 and the weaning weight of lambs were increased by feeding protected fat ($p<0.05$). In conclusion, calcium soaps of fatty acids increased serum P_4 between days 10 to 14 of the cycle which may be beneficial to early pregnancy maintenance. Protected fat seemed to have a beneficial effect on milk yield, fat yield, lamb daily gain, lamb birth weight and ewe weight loss.

Key words: Protected fat, ewe, reproduction, lactation, blood parameters

INTRODUCTION

Nutrition has a considerable effect on reproduction and lactation. Increased consumption of fats by lactating cows resulted in increases in the number of ovarian follicles, increase in the number and size of corpora lutea, stimulation of post-partum ovarian activity and improvement in pregnancy rate (Thomas *et al*., 1997; De Fries *et al*., 1998; Staples *et al*., 1998). Increased dietary lipid also increases plasma cholesterol and progesterone and the supply of lipoproteins which play significant roles in regulating ovarian steroidogenesis (Williams, 1996). In contrast to cattle, there are few observations on the effects of dietary lipid on reproductive function in sheep. Intravascular infusion of lipid into ewes stimulated progesterone and prostaglandin synthesis (Burke *et al*., 1996) and dietary supplementation of calcium soaps of fatty acids (CSFA) increased the progesterone concentration in the serum (Kuran *et al*., 1999) and follicular fluid (Espinoza *et al*., 1998). Sadeghipanah *et al*. (2006) have recently studied the effect of fat on several reproductive parameters of a fat-tailed sheep.

Supplemental fats have increased milk production of dairy cows (Fatahnia *et al*., 2007), but the responses to protected lipids have been variable. Supplementation of cow diets with Ca salts of long chain fatty acids increased milk or FCM yield in most (Brumby *et al*., 1978; Sklan *et al*., 1989, 1991; Klusmeyer *et al*., 1991; Moallem *et al*., 1997, 2000) but not in all experiments (Schauff and Clark, 1989).

The response of ewes to CSFA has also been variable. Perez Hernandez *et al*. (1986) reported that CSFA had no effect on milk yield but increased milk fat concentration. Sklan (1992) showed that CSFA increased yield of milk, milk fat, milk protein and milk lactose in Asaf ewes, but Horton *et al*. (1992) reported that supplementing Dorset ewes with rumen-protected lipid had no effect on milk production or body weight change of ewes or nursing lambs. A recent review (Gargouri *et al*.,

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2006) of the literature published on sheep response to CSFA supplementation indicated that CSFA had no significant effect on milk yield, but significantly increased milk fat content and yield and reduced milk protein content. Milk fat and protein responses were dependent on the dose and lactation phase. Supplementation of ewe diets in late pregnancy with 20 g fish oil per day improved the lamb survival to weaning (Dawson and Edgar, 2006). Inert fat at 3% of the total diet increased milk fat percentage of high producing dairy goats in early lactation (The *et al*., 1994). Supplementing dairy goats with 9% polyunsaturated fatty acid-rich protected fat increased milk production and protein and fat yield (Sanz Sampelayo *et al*., 2004).

Calcium soaps of fatty acids are manufactured in Iran and sold under the trade name of Megalac. Two experiments were carried out to study the effects of these fats on ovarian follicular number, progesterone levels, blood parameters and lactation performance of fat-tailed sheep.

MATERIALS AND METHODS

The experiments were carried out in the Animal Research Station of the College of Agriculture, Shiraz University, Shiraz, Iran in September 2001. Calcium soap of fatty acids (CSFA), Iran Megalac, was supplied by Behparvaran Co., Esfahan. The sheep employed in this study were from two Iranian fat-tailed breeds known as Ghezel and Mehraban.

Experiment 1

Effect of CSFA on follicular characteristics and blood parameters: The estrous cycles of cyclic fertile Ghezel (62.5±1.0 kg) and Mehraban (56.5±0.9 kg) ewes (4-5 years old) were synchronized by two intra-muscular injections, 11 days apart, of cloprostenol (Nasr Pharmaceutical Co., Iran). Within each breed (n = 20), the ewes were randomly allotted to 4 equal groups. The control group (no supplemental fat; NF) was fed with a maintenance diet, balanced for metabolizable energy and crude protein according to the National Research Council (1985) recommendations. The other groups received the same diet as well as a daily allowance of 40 g non-saponified fat (NS40; the same fats used in manufacturing Megalac), 40 g Megalac (SF40), or 80 g Megalac (SF80). The ewes were gradually adapted over one week to the diets and were then fed with their complete rations for one cycle length, starting on the day of induced estrus. Blood samples were taken one day before feeding the experimental diets and again on days 10, 12 and 14 of the cycle. Blood sera were prepared after centrifugation at 3000 g for five minutes and kept frozen at -20°C until analysis. Blood sera from days 10, 12 and 14 were pooled and progesterone (P_4), cholesterol (CHOL), high density lipoproteins (HDL), low density lipoproteins (LDL) and triacylglycerols (TG) were analyzed on duplicate sub-samples. Progesterone concentration was determined by using radioimmunoassay kits (Spectria Orion Diagnostica). The inter-assay (n = 30) and intra-assay (n = 33) coefficients of variations were 4.30 and 4.97, respectively. Other blood parameters were analyzed by using enzymatic methods (Stein, 1986).

The ewes were slaughtered on their next estrous period and their ovaries were removed and transferred to the laboratory on ice. The ovaries were weighed (±0.01 g) by using a digital balance. The number and size (determined by using a caliper) of follicles in ovaries were recorded. The follicles were classified into small (<4 mm), medium (4-8 mm) and large (>8 mm) groups.

Experiment 2

Effect of CSFA on lactation and lamb performance of Mehraban sheep: The effect of CSFA on milk production and composition and lamb and ewe weights after lambing was studied in a completely randomized design. Forty mature cyclic Mehraban ewes were randomly divided into two groups (n = 20) and were fed daily (at 8.00 am and 15.00 pm) with a basal ration consisting of 1000 g lucerne hay and 500 g ground barley supplemented with either 50 g CSFA or vegetable oil per ewe per day. After four weeks, the estrous cycles of the ewes were synchronized as described in experiment 1 and were joined with rams for the duration of four estrous cycles during which they were observed for return to estrus. The ewes were fed with this ration for up to six weeks after estrous synchronization, when they were run with the station flock receiving the routine ration. On day 100th of gestation, the ewes were divided into two groups, each comprising of 10 ewes on CSFA diet and 10 ewes on vegetable oil diet. They were fed with two isoenergetic diets, one consisting of 1300 g lucerne hay and 600 g barley (control group) and the other one consisting of 1150 g lucerne hay, 500 g barley and 100 g CSFA (CSFA group). One week before lambing the rations were gradually increased so that at parturition the ewes were receiving on a daily basis, either 1000 g lucerne hay and 800 g barley (control group) or 1000 g lucerne hay, 800 g barley and 150 g CSFA (CSFA group). This feeding strategy was continued until weaning the lamb at 60 days of age. The lambs received the ewe milk only until weaned.

The ewes and lambs were weighed (±50 g) on the day of lambing and again on days 15, 25, 40 and 60 of parturition. The ewes were weighed before the morning feeding and after complete milking of the udders. The lambs were weighed in the morning before being allowed to suckle their dams.

Milk production was measured (±10 g) on days 25, 40 and 60 after lambing. The lambs were separated from their dams for an overnight period of about 12 h. On the following morning, the ewes were first hand-milked and then the lambs were allowed to suckle their dams so that the udders became devoid of milk as much as possible. After 6 h, the ewe was intramuscularly injected with 2 IU of oxytocin (Scanpharm, Denmark) and hand-milked. The amount of milk produced during this 6 h period was multiplied by a factor of 4 as an estimation of 24 h milk yield. Milk samples were taken for determination of pH (digital pH meter), dry matter (oven drying), fat (Milkoscan) and ash (burning in furnace at 520°C).

Statistical analysis: The data were analyzed by using the GLM procedure and the means were compared by using the SNK test (SAS, 1996). Serum levels of blood parameters before feeding the experimental diets were used as covariates in analysis. Appropriate covariates were included in the statistical models for the analysis of other data. The percentage data were arcsine transformed before analysis, but the actual percentages are reported in the study.

RESULTS

Experiment 1

Effect of CSFA on follicular characteristics and blood parameters: There were no significant effects of feeding CSFA on ovarian weights, cycle length and the number of small and medium-sized follicles, however the number of large follicles tended to be greater ($p = 0.09$) in the ewes supplemented with 40 g CSFA (Table 1). On the other hand all fat supplements tended to decrease the total number of follicles in the ovaries ($p = 0.08$). Ovarian weights ($p = 0.02$), number of small follicles ($p = 0.09$) and total number of follicles in the ovaries ($p = 0.08$) were greater in Mehraban ewes than in Ghezel (Table 2). The size of the largest follicle was not significantly affected by the diet (Table 1) or breed (Table 2). Diet by breed interaction was not significant for any of these measurements.

Table 1: Effect of calcium soap of fatty acids on ovarian follicles and blood parameters of fat-tailed sheep (mean±SE)

	Ration†				
Parameters	Basal	NS40	SF40	SF80	p-value
Ovarian weight (g)	3.2±0.2	3.1±0.2	3.2±0.1	3.1±0.2	>0.10
Size of largest follicle (mm)	6.7±0.5	7.5±0.4	7.1±0.4	7.1±0.4	>0.10
No. of follicles					
Small (<4 mm)	38.0±5.0	32.0±4.0	32.0±3.0	30.0±4.0	>0.10
Medium (>4 and <8 mm)	2.5±0.8	1.9±0.5	2.3±0.4	2.3±0.5	>0.10
Large (≥8 mm)	1.3±0.2	1.3±0.2	1.9±0.2	1.2±0.2	0.09
Total	42.0±5.0	36.0±4.0	36.0±4.0	34.0±3.0	0.08
Estrous cycle length (day)	17.3±0.4	17.5±0.2	17.7±0.2	17.6±0.3	>0.10
Progesterone (nmol per L)	8.4±0.9b	10.9±0.8b	11.8±0.6b	15.6±1.7a	0.001
Cholesterol (mg per 100 mL)	66.5±3.9b	87.5±8.4ab	82.8±7.2ab	104.2±10.2a	0.05
Triacylglycerols (mg per 100 mL)	18.5±0.9b	19.8±0.9b	24.3±2.1a	24.6±1.6a	0.02
High density lipoproteins (mg per 100 mL)	31.1±1.9c	46.9±2.4a	40.5±2.7b	49.2±3.4a	0.0001
Low density lipoproteins (mg per 100 mL)	33.2±3.5	37.6±7.2	38.0±6.3	50.4±10.2	>0.10

†Basal: basal ration (no supplemental fat); NS40: Basal ration plus 40 g non-saponifiable fat used for making CSFA (Megalac); SF40: Basal ration plus 40 g CSFA (Megalac); SF80: Basal ration plus 80 g CSFA (Megalac); a,b: Within rows indicated, means with similar superscripts are not significantly different (SNK test; $p>0.05$)

Table 2: Ovarian characteristics and blood parameters of Mehraban and Ghezel sheep (mean±SE)

Parameters	Mehraban	Ghezel	p-value
No. of ewes	19.0	19.0	-
Ovarian weight (g)	2.9±0.1	3.4±0.1	0.02
Diameter of largest follicle (mm)	6.8±0.3	7.4±0.3	>0.10
No. of follicles			
Small (<4 mm)	30.0±2.7	36.4±2.8	0.09
Medium (>4 and <8 mm)	2.1±0.3	2.4±0.4	>0.10
Large (≥8 mm)	1.4±0.1	1.5±0.2	>0.10
Total	33.4±2.7	40.3±2.7	0.08
Estrous cycle length (day)	17.5±0.1	17.6±0.2	>0.10
Progesterone (nmol per L)	11.4±1.1	12.0±0.7	>0.10
Cholesterol (mg per 100 mL)	90.2±7.5	80.3±4.2	0.08
Triacylglycerols (mg per 100 mL)	22.7±1.3	20.8±1.0	>0.10
High density lipoproteins (mg per 100 mL)	38.9±2.3	45.0±2.3	0.07
Low density lipoproteins (mg per 100 mL)	47.8±6.3	32.1±2.7	0.02

Table 3: Interaction effect of the ration and breed on concentration (mg per 100 mL) of blood serum high density lipoproteins (mean±SE; n = 5 per breed)

	Ration[†]			
Breed	Basal	NS40	SF40	SF80
Mehraban[‡]	27.2±1.3[c]	49.8±4.0[a]	37.8±3.6[b]	40.6±3.0[b]
Ghezel	35.0±2.5[b]	44.0±2.3[b]	43.2±3.9[b]	57.8±2.6[a]

[†]Basal: basal ration (no supplemental fat); NS40: Basal ration plus 40 g non-saponifiable fat used for making CSFA (Megalac); SF40: Basal ration plus 40 g CSFA (Megalac); SF80: Basal ration plus 80 g CSFA (Megalac); [‡]Within each row, means with similar superscripts are not significantly different (SNK test; p>0.05)

Table 4: Effect of calcium soap of fatty acids on ewe and lamb performance of Mehraban sheep from lambing to weaning (mean±SE)

Parameters	Control	CSFA	p-value
Ewe daily weight loss (g) from lambing to day			
15	192±39 (14)	164±23 (14)	>0.10
25	238±16 (17)	128±20 (17)	0.002
40	188±11 (16)	133±14 (17)	0.01
60	164±16 (14)	123±12 (14)	0.10
Milk yield (g) on day			
25	1275±103 (17)	1981±116 (17)	0.004
40	1017±86 (17)	1181±81 (17)	>0.10
60	694±52 (14)	722±86 (14)	>0.10
Milk fat yield on day 25 (g)	123±16 (17)	215±18 (17)	0.01
Mean lamb birth weight (g)	4329±190 (17)	4499±244 (17)	0.05
Total lamb birth weight (g)	6343±385 (17)	7023±298 (17)	>0.10
Lamb daily weight gain (g) to 60 days	224±13 (23)	252±11 (22)	0.04
Lamb weaning weight (g) at 60 days	17350±885 (23)	17440±842 (22)	0.04

Values in parenthesis show number of animals

Serum concentrations of P_4, CHOL, TG and HDL were significantly greater for SF80 ewes as compared with the control ewes, but LDL concentration was not significantly affected by fat supplementation (Table 3). Serum concentrations of CHOL (p = 0.08) and LDL (p = 0.02) were higher in Mehraban as compared with Ghezel ewes (Table 1), but the reverse was found for HDL (p = 0.07). A highly significant interaction (p = 0.0001) was found between the diet and breed for HDL concentration. In Ghezel ewes, the SF80 diet resulted in higher serum levels of HDL as compared with the other diets, whereas in Mehraban ewes all supplemental fats resulted in higher HDL as compared with the diet without supplemented diet (Table 3).

Experiment 2

Effect of CSFA on lactation and lamb performance of Mehraban sheep: Effect of feeding CSFA during the mating period was not significant, therefore this was omitted from the analysis of variance model. One ewe gave birth to triplets and its data was not included in the analysis.

Ewe weight loss was lower in CSFA supplemented ewes particularly up to day 25 when the difference in weight loss was more than 100 per day (Table 4). CSFA supplemented ewes produced significantly more milk and milk fat on day 25 lactation but not on days 40 and 60. Milk dry matter, fat percentage, pH and ash percentage were not significantly affected by CSFA feeding (data not tabulated). Mean lamb birth weight of CSFA supplemented ewes was significantly greater than non-supplemented ewes, although total lamb birth weight was not significantly affected by feeding CSFA. Average daily weight gain and weaning weight of lambs born to CSFA supplemented ewes were significantly higher than for non-supplemented ewes. Gestation length, number of lambs born per ewe lambing and sex ratio (data not shown) were not affected by the treatment.

DISCUSSION

Fat supplementation at the level and for the period used in the present experiment did not improve ovarian characteristics of the ewe but it tended (p = 0.08) to decrease the number of large follicles. Intravascular infusion of lipids (Burke *et al*., 1996) and feeding CSFA (Kuran *et al*., 1999) did not affect the number of follicles in the sheep although several studies have reported the beneficial effect of supplementary fat on ovarian follicular development in cows (Williams, 1996). The content of unsaturated fatty acids in the supplemental fat has been considered as a factor which affects the ovarian follicular characteristics in cattle (Thomas *et al*., 1997) where polyunsaturated fatty acids were more effective than saturated or highly unsaturated fatty acids. In the study of Kuran *et al.* (1999), the main fatty acid composition difference between the control and fat supplemented diets was in terms of the amount of saturated palmitic acid and to some extent stearic and oleic acids. Sadeghipanah *et al.* (2006) reported in the ewe that CSFA (4.5 g kg^{-1} diet) made of tallow significantly reduced the number of follicles ≥3 mm in diameter as compared with the non-supplemented ration and the rations supplemented with CSFA made from soybean oil fatty acids (4.5 g kg^{-1} diet) or a mixture of soybean fatty acids and tallow (2.25 g kg^{-1} diet each). The CSFA used in the present experiment was made from plant oil fatty acids as indicated by the manufacturer, but we did not analyze the actual composition of fatty acids in this product. Wehrman *et al.* (1991) were the first to note the beneficial effect of plant oil supplementation on follicular development in cattle. This effect seems to be, within limits, independent of metabolizable energy intake. The extent of increases in follicle number seems to be dependent on the duration of plant oil feeding; six weeks of supplementation was necessary in cattle (Williams, 1996). Maximum responses to plant oil supplementation occurred when cattle were fed at 4-6% of the diet dry matter and lesser increases noted with lower levels of added fats. The inclusion of

animal tallow, calcium salts of saturated fatty acids or fish oil were less effective in enhancing the follicular growth in cattle as compared with plant-derived oils (Williams, 1996). Based on extensive data in cattle, more refined experiments are needed before a final conclusion can be made about the effects of the type, amount and duration of fat supplementation for enhancing follicular development and reproductive performance in sheep.

Serum concentrations of cholesterol, progesterone, triacylglycerol and HDL, but not LDL, were significantly increased with of CSFA supplementation in the present experiment as compared with the non-supplemented ewes. Fat supplementation also increased the serum levels of cholesterol, progesterone, triacylglycerol and HDL in cows (Funston, 2004) and ewes (Burke *et al*., 1996; Espinoza *et al*., 1998; Kuran *et al*., 1999). Kuran *et al*. (1999) reported a significant increase in LDL levels of CSFA-supplemented ewes, but Espinoza *et al*. (1998) did not find a significant effect of CSFA on progesterone concentration. The mechanism of the effect of fat supplementation on enhanced progesterone level is not completely understood. Hawkins *et al*. (1995) suggested that the increase in plasma progesterone in cows on lipid-supplemented diets may not be due to increased progesterone synthesis but rather to its reduced clearance from the circulation. However, Kuran *et al*. (1999) showed that the luteal tissue from lipid-fed ewes secreted higher amounts of progesterone *in vitro* than that from the control. Therefore, increased plasma progesterone levels could be due to increased synthesis of progesterone following increased availability of cholesterol (Spicer *et al*., 1993) and (or) lipoproteins (Bao *et al*., 1995) and as well as reduced clearance from circulation. The correlation coefficient between HDL and cholesterol levels following CSFA feeding of ewes in our experiment was 0.36 ($p<0.05$). HDL represents the majority of total plasma lipoprotein cholesterol and addition of HDL and LDL into culture have resulted in increased progesterone secretion from bovine luteal cells *in vitro* (Carroll *et al*., 1992; Bao *et al*., 1995, 1997). Kuran *et al*. (1999) also found that lipid supplementation of ewes increased the total cholesterol and HDL-cholesterol levels of the follicular fluid but had no effect on the concentration of LDL-cholesterol and triacyglycerols. The significant interaction between breeds and fat supplements on HDL level in the present experiment is indicative of different responses to fat supplementation in different sheep breeds and requires further clarification. The fatty acid profile of the fat also affects plasma progesterone concentration (Staples *et al*., 1998). Exogenous lipids may also stimulate PGE1 and PGE2 synthesis, which are luteotropic in the ewe (Reynolds *et al*., 1981).

Injection of progesterone during the early luteal phase accelerated the early embryo development in cattle (Garrett *et al*., 1988) and increased fetal size in sheep at day 74 of gestation (Kleemann *et al*., 1994). A considerable proportion of the high embryonic losses during early gestation of sheep and cattle are due to luteal insufficiency (Niswender and Nett, 1988), therefore, dietary supplementation with suitable fat sources could, by stimulating luteal progesterone production, improve embryo survival and fertility in these species. However, it is noteworthy that beef cattle research on the effect of fat supplementation on reproduction is inconclusive with regard to the amount and time of supplementation (Funston, 2004). It is not known exactly how to supplement fat to improve reproductive performance beyond the energy contribution; most studies have used isocaloric and isonitrogenous diets, however, this can be challenging. On the other hand, research on feeding supplemental fat to cattle has resulted in varied and inconsistent results as it relates to reproductive efficiency, including positive, negative and no apparent effects (Funston, 2004).

CSFA attenuated the ewe weight loss during the suckling period to some extent, particularly from lambing to day 25 of lactation. This was accompanied by higher milk and fat production in CSFA ewes. Lambs of CSFA-supplemented ewes were slightly heavier at birth and had a higher average daily gain although the difference ($p = 0.04$) in their weaning weights were not so large. Milk yield on days 40 and 60 were not significantly different between CSFA and control ewes. Sustained milk production in control ewes was accompanied by higher ewe weight loss. Similar trends were also reported by Casals *et al*. (1999). A survey of the published data on the effect of CSFA supplementation in sheep by Gargouri *et al*. (2006) indicated that CSFA had no significant effect on milk yield, but significantly increased milk fat content and yield and reduced milk protein content. Milk fat and protein responses were dependent on the dose and lactation phase. The optimum level was found to be 150-200 g per day per ewe.

Contradictory data have been reported for the effect of fat supplementation on energy balance in ewes (Horton *et al*., 1992; Ryan *et al*., 1992; Espinoza *et al*., 1998; Casals *et al*., 1999). Although fat supplementation increases the energy density of the ration, but daily energy intake may remain unchanged because of reduced dry matter intake (Staples *et al*., 1998). According to Komaragiri *et al*. (1998), lack of tissue response to fat supplementation in the cow could be due to lower sensitivity of tissues to dietary changes as compared to the effect of hormones during early lactation. Feeding of CSFA in isoenergetic diets during late pregnancy

improved the lamb birth weight in the present experiment. However, Lammoglia *et al.* (1996) found no effect of fat inclusion in isoenergetic diets on calf birth weight. Fat supplementation did not improve lamb birth weight in several studies (Horton *et al.*, 1992; Espinoza *et al.*, 1998; Casals *et al.*, 1999). Similarly, feeding of protected fat to lactating goats did not have a beneficial effect on milk production and kid performance (Martin *et al.*, 1999). Dawson and Edgar (2006) showed that source of fish meal had no significant effect on ewe and lamb performance, but feeding of 20 g fish oil per day per ewe improved lamb survival to weaning as compared with the control and 40 g fish oil per day.

CONCLUSIONS

Daily feeding of 80 g of a calcium salt of fatty acids for one cycle length did not change the number of follicles in different size groups, but significantly increased the serum concentration of P4 between days 10 to 14 of the cycle which may be beneficial to early pregnancy maintenance in sheep. The data also indicated that CSFA feeding during lactation can be beneficial in terms of milk production and lamb performance, but considering the degree of improvement in production performance further investigation with higher levels of CSFA seems appropriate.

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