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Influence of dietary calcium salts of long chain fatty acids supplementation on growth and onset of puberty in Rahmani ewe- lambs

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Abstract

The present investigation was to study the effect of dietary calcium salts of long chain fatty acids (CSFA) on puberty attainment of Rahmani ewes -lamb. Twenty Rahmani ewes –lamb of 6-7 months of age and average body eight 24.75 ± 0.16 kg. Animals were randomly allotted into two equal groups. Animals were fed a basal diet of hay (64.2%) and barley grain (35.0%) plus minerals and vitamins (0.8%). The first group was kept as a control (n = 10) were fed a basal diet. Ewes-lambs on the second group (n = 10) received the same basal diet supplemented with calcium salts of long chain fatty acids (CSFA) at 3% of the basal diet dry matter intake (1.2 kg/ewe/d). The ewe-lambs were weighed at the start and at the end of the experiment. In addition, the body condition score, withers height and heart girth were determined at the end of the study. Blood samples were collected weekly till the end of experiment. Sera samples were assayed for progesterone, insulin-like growth factor-1 (IGF-1) glucose, total cholesterol, total lipids, and urea. Results indicated that the dietary supplementation with CSFA to Rahmani ewes – lamb attained puberty 5 weeks earlier than control one. CSFA supplementation of Rahmani ewe lambs has higher body weight, and body condition score than those of the control one (34.67 ± 0.30 kg 3.25 ± 0.09 vs. 30.67 ± 0.95 kg 2.0 ± 0.0; p< 0.05 respectively). A similar tendency was observed in dry matter intake and average daily gain. CSFA has a beneficial effect on blood born metabolites as indicated by increased serum glucose, total lipids, cholesterol, IGF-1 and decreased urea of Rahmani ewes- lamb as compared to control. The highest concentrations of insulin-like growth factor-1 (IGF-1) were significantly (P < 0.05) detected at 2nd 4th and 10th weeks (258.42±6.0, 228.76 ±14.44 and 337.32 ±24.57 ng/ml, respectively) than those of control one (163.3 ±11.94, 135.5 ± 3.07 and 218.47 ± 9.18 ng/ ml, respectively). In conclusion, CSFA enhanced puberty in Rahmani ewe lambs. This is due to increased provision of trophic signals (represented by increased Serum IGF-1 secretions) and/or blood-borne metabolites (glucose, cholesterol and lipid).

Keywords: Rahmani ewe-lambs; blood metabolites, puberty.

INTRODUCTION

One way of enhancing reproductive performance of female sheep is through extending lifetime productivity. Age of puberty and age at first lambing are important traits concerning overall reproductive performance. While the end point used to define puberty is a discrete point in time, the process of sexual maturation occurs gradually during the pre, peri and post pubertal periods [3] .The mechanism timing puberty is sensitive to a critical level of metabolic signals that are implicated in activation of the hypothalamo-hypophyseal gonadal axis [5].Moreover, oxidizable metabolic fuels, regardless of their source (Fat, carbohydrates or proteins) have been implicated as a regulators of gonadotropin releasing hormone[23] and ovarian hormones[7]. The energy intake appears to be the primary determinant for ruminant's reproductive performance as ruminants are good nitrogen preservers. Calcium soap represents a rich source of metabolic fuel due to its fatty acids content (84% fatty acids); so it was recently implicated in ruminant ration to increase its caloric density [14] So the present investigation was conducted on this Egyptian native breed of ewe lambs to study the effects of prepubertal administration of calcium soap on puberty attainment (based on serum progesterone level), some of blood-borne metabolites (glucose, total cholesterol, lipid and urea).

MATERIALS AND METHODS

Animals and ration

This study was conducted at Faculty of veterinary medicine, Cairo university, Egypt located in the south part of Nile Delta (latitude 30' 01" N; longitude 31'21" E) during May to July. Twenty Rahmani ewe- lambs aged between 6 to 7 months and average body weight 24.25±0.38 Kg. Animals were randomly allocated into two groups of equal treatments (n=10) and kept in separate groups according to the type of supplementation. Animals were fed a basal diet of hay (64.2%) and barley grain (35.0%) plus minerals and vitamins (0.8%). The first group was kept as a control (n = 10) were fed only

a basal diet. Ewes-lambs on the second group (n = 10) received the same basal diet supplemented with calcium salts of long chain fatty acids (CSFA) at 3% of the basal diet dry matter intake (1.2 kg/ewe/d). Clean water was available free choice at all times. Additionally, the ewe-lambs were given ad libitum access to feed. Diet was formulated to meet the nutrient requirements[12] for sheep. All ewes were weighed at the start and the end of the study after a fast of 24 hours from feed and 16 hours from water .Moreover, body condition score withers height and heart girth were assessed at the end of study .The supplementation period was 12 weeks.

Blood Sampling

Individual blood samples were collected prior to the beginning of the study (pretreatment or 0 blood samples); thereafter, blood samples were twice weekly obtained till the end of the study period. Blood samples were allowed to clot and sera were separated by centrifugation at 3000 rpm for 15 minutes. Sera samples were divided into aliquots and frozen at -20 0C until assayed for progesterone, glucose, total cholesterol, total lipids and urea.

Hormonal assay

Progesterone level was determined in weekly collected sera samples by competitive ELISA according to [27] using kits obtained from Dima, Germany. Age of puberty is defined as defined as the first day on which serum progesterone reached ≥ one ng/ml for two consecutive weeks [3, 21] Insulin-like growth factor-1 was assayed, in the pretreatment sample and then every two weeks till the end of the investigation, by two-sites immunoradiometric assay (IRMA) according to the method adopted by [11] using kit purchased from DSL, Webster, Texas, USA.

Blood metabolites assay

Serum glucose, cholesterol, lipids and urea were determined spectrophotometrically in the collected serum samples. Serum total cholesterol was assayed according to [17].Glucose was determined according to [26]. Total lipid were measured according to [28] and urea was analyzed according to [24[.All serum metabolites were determined using diagnostic kit brought from Biodiagnostics, Egypt.

Statistical analysis

Data were expressed as mean ± SEM .The data were analyzed statistically by ANOVA method and Duncan's test was used to detect differences among means using SPSS® Statistical Software [20].Additionally, chi square was applied to compare control group's cumulative numbers of ewe-lambs attaining puberty with those of supplemented one. Significant differences were set at $P < 5\%$.

RESULTS

Control animals had significantly decreased body weight and body condition score (30.67 ± 0.95 kg 2.0 ± 0.0; $p< 0.05$ respectively) than those on the CSFA treatments (34.67 ± 0.30 kg 3.25 ± 0.09, respectively). Furthermore, dry matter intake and average daily gain were significantly improved in CSFA treatments than those in control (1.55± 0.03; 115 ± 3.5 gm vs. 1.2 ± 0.02; 73.17 ± 11.27 gm, respectively) as shown in Table 1. In addition, withers height and heart girth were significantly ($p< 0.05$) increased in CSFA supplemented ewe-lambs than those of control one (71.5 ±1.65; 89.80 ±3.52 vs. 60.5 ±1.58;76;75 ±2.7cm, respectively).

It is evident from Table 2 that puberty was first detected 5 weeks after the start of the experiment, when 4/10 of the CSFA supplemented Rahmani ewe-lambs started to attain puberty. In the control group, puberty was first detected 6 weeks after the beginning of the investigation, when 2/10 of control ewe-lambs began to attain puberty. All ewe-lambs in the calcium soap supplemented and control treatments attained puberty at 7^{th} and 10^{th} weeks, respectively after the beginning of the study.

Regarding serum progesterone concentration during dietary supplementation is shown in Table 3. At the beginning of dietary supplementation, there were no significant differences between the treatments in the concentration of serum progesterone. It was observed that the effect of CSFA on serum progesterone concentration was time-dependent; as the level of progesterone in CSFA was significantly higher ($p< 0.05$) at the 5^{th} and 6^{th} week of the study(1.3 ± 0.3; 1.7 ± 0.2 ng/ml, respectively) than those of the control (0.09 ± 0.06; 0.77 ± 0.11 ng/ml, respectively). Moreover, the level of progesterone showed time-dependent effect which is evident statistically at the 10^{th} and 11^{th} week of the study, where serum progesterone concentration in the control was significantly ($p< 0.05$) higher than that of CSFA treatment (1.5 ± 0.31; 1.45 ± 0.19 vs. 0.80 ± 0.09; 0.45 ± 0.08 ng/ml, respectively).

It is clear from table (4) that the overall concentration of insulin-like growth factor-1 (IGF-1) in CSFA group was higher than that of the control one. Moreover, the increasing effect of CSFA was interacting with the weeks of the study, since the level of (IGF-1) in the CSFA group was significantly ($p< 0.05$) elevated than that of the control during the 2^{nd}, 4^{th} and 10^{th} week of the investigation (258.42±6.0, 228.76 ±14.44 , 337.32 ±24.57 vs. 163.3 ±11.94, 135.5 ± 3.07 ,218.47 ± 9.18 ng/ml, respectively).

Data presented in Table 5 showed that the effect of calcium soap supplementation has an overall significantly decreasing ($p< 0.05$) effect on serum urea concentration at 6th and 12th weeks of study (8.3 ± 0.19; 12.1 ± 0.24 mg/dl, respectively) as compared to control (13.1 ± 0.67; 14.0 ± 0.14 mg/dl, respectively).There were

overall increment effects of CSFA supplementation on serum concentration of glucose, cholesterol and lipids as compared to control. Moreover, CSFA supplementation was time- dependent. Additionally, the elevating effect of 12th week supplementation was higher than that of the 6th week supplementation (Table 6).

Table 1. Effect of calcium soap supplementation on body weight (Kg), dry matter intake (Kg/day) , body condition score , withers height (cm) and heart girth (cm) of Rahmani ewe lambs (mean± SEM).

Traits	Control	CSFA
Initial body weight	24.50±0.13 [a]	25.0 ± 0.77 [a]
Final body weight	30.67±0.95[a]	34.67±0.30 [b]
Body weight gain	73.17±11.27[b]	115 ± 3.55 [a]
Dry matter intake	1.20±0.02 [b]	1.55± 0.03 [a]
Body condition score	2.00 ± 0.0 [b]	3.25 ± 0.09 [a]
Withers height (cm)	60.5±1.58[b]	71.5 ±1.65[a]
Heart girth (cm)	76.75±2.7[b]	89.8 ±3.52[a]

Within the same row with different superscripts (a, b) are different (p<0.05).

Table 2. Cumulative numbers and percentages of calcium soap supplemented Rahmani ewe-lambs attaining puberty*

Weeks of supplementation	Control	Supplemented
4 weeks	0/10	0/10
5 weeks	0/10(0.0) [b]	4/10(40.0) [a]
6 weeks	2/10 (20.0) [b]	5/10 (50.0) [a]
7 weeks	2/10(10.0) [a]	10/10(100.0)[b]
8 weeks	4/10(40.0)	-
9 weeks	5/10(50.0)	-
10 weeks	10/10(100.0)	-

Within the same column with different superscripts (a, b) are different (p<0.05).
*Defined as the first day on which serum progesterone reached ≥ one ng/ml for two onsecutive weeks.

Table 3. Effect of calcium soap supplementation on Serum progesterone concentration (ng/ml) in Rahmani ewe lambs (mean± SEM).

Weeks of supplementation	Control	CSFA
0-week	0.05±0.00 [a]	0.05±0.00 [a]
1st-week	0.06±0.00 [a]	0.09±0.00 [a]
2nd-week	0.10 ±0.01 [a]	0.13±0.00[a]
3rd-weeek	0.09 ±0.00 [a]	0.13±0.00 [a]
4th-week	0.09±0.00 [a]	0.09±0.00 [a]
5th-week	0.85±0.06 [b]	1.30±0.30 [a]
6th-week	0. 77±0.11 [b]	1.70±0.21 [a]
7th-week	0.76 ±0.20 [a]	0.84±0.01 [a]
8th-week	0.80 ±0.09 [a]	0.54±0.11 [a]
9th-week	1.29 ±0.21 [a]	1.40±0.31 [a]
10th-week	1.55±0.31 [a]	0.80±0.09 [b]
11th-week	1.45±0.19 [a]	0.45±0.06 [b]
12th-week	1.94±0.28 [a]	0.48±0.08 [b]

Within the same row with different superscripts (a, b) are different (p<0.05).

Table 4: Effect of calcium soap supplementation on insulin-like growth factor 1 concentration (IGF-1, ng/ml) of Rahmani ewe - lambs (mean± SEM).

	Control	CSFA
0-week	130.4 ±18.93 [a]	124.87±18.5[a]
2nd-week	163.3±11.94 [a]	258.42±6.0 [b]
4th-week	135.5±3.07 [a]	228.76±14.4 [b]
6th-week	186.4 ±10.1 [a]	229.71±32.5 [a]
8th-week	188.6±11.1 [a]	223.39±25.2[a]
10th-week	218.4 ±9.1 [a]	337.32 ±24.5 [b]
12th-week	338.1 ±29.1 [a]	350.0 ± 9.0 [a]

Within the same row with different superscripts (a, b) are different (p<0.05).

DISCUSSION

The results of the current study identified that ewe lamb attained puberty by end of study about (270-300 days), as indicated by a rise of serum progesterone concentration to a level of ≥ one ng/ml for two consecutive weeks [3,21]. This puberty was associated with enhanced body growth as indicated by increased final body weight, body condition score and growth measures. . In agreement with the present study, the body weight gain tended to be higher for ewes fed the diet supplemented with calcium salts of olive fatty acids[15]. As well as, dietary fat increased energy intake and diminished body weight loss in summer but augmented body weight loss in winter [19]. These previously mentioned alterations may be due to the changes evoked by serum insulin-like growth factor-1 (IGF-1) and/or general metabolic pool levels (i.e., serum glucose, total cholesterol, and total lipid). This speculation is supported by the proposition of [22] that the blood-borne substances which may be hormones, metabolites or combination influence the reproductive system and initiate puberty. The current study recorded increased IGF-1 in Rahmani ewe-lambs during the onset of puberty. In this respect, it was observed that plasma IGF-1 concentration didn't markedly change until 5 weeks before puberty, they began to rise between weeks –4 and –1 and this increase continued throughout the onset of puberty in Shiba goat [18]. They concluded that, IGF-1 had a role in the initiation of puberty in ruminants. The recorded higher IGF-1 in the ewe-lamb might lead to an early activation of GnRH network, which is the rate limiting step of puberty onset; this would result in an increase in GnRH discharge and consequently LH secretions. This explanation is supported by [1] who reported that an increase in IGF-1 within the physiological range stimulated LH secretion in sheep. Moreover, the finding of the involvement of IGF-1 in GnRH [8] and LH secretions [9] further support the previously mentioned explanation. The results of the current study identify that calcium soap supplementation altered beneficially the general metabolic pool as indicated by increased serum glucose, lipid and cholesterol; metabolites critical to the

reproductive function. The attainment of puberty in ewe-lamb may be due to creation of favorable conditions that forward puberty; these conditions may be represented by increased trophic signals to the hypothalamus-pituitary ovary axis, higher levels of metabolites critical to the reproductive function (glucose and cholesterol) and improved energy and lipid status. CSFA treated Rahmani ewe lambs have greater glucose availability than their corresponding control; this increased glucose availability in the treated group may forward the onset of puberty[4]. Moreover, it has been identified that glucose availability is one of metabolic regulators of the GnRH pulse generator in ruminant species [13]. In addition to the central action of the increased glucose; glucose can affect positively the ovarian metabolism via acting as energy substrates and as a stimulator for the ovarian uptake of the precursors required for steroid hormones biosynthesis and suggested that glucose may promote cholesterol uptake into the ovine ovarian cells or vice versa [16]. Furthermore, the decreased serum urea concentration may be additional effect by which calcium soap supplementation increased serum through saving extra ATP for hepatic gluconeogenesis; this notion is supported by finding of [10] that hepatic detoxification of ammonia into urea has been reported to reduce plasma glucose level possibly by its direct inhibition effect on liver gluconeogenic activity of urea cycle. Moreover, the increased serum total lipids and cholesterol recorded in the present study, represents an additional beneficial effect of calcium soap feeding at general metabolic pool level of Rahmani ewe- lambs. The increased serum total lipids may be ascribed to the depressing effect of long chain fatty acids content of calcium soap on lipogenic enzymes activities in adipose tissue, since it has been found that feeding long chain fatty acids was reported to induce shifting in the balance from active protomeric to inactive polymeric forms of acetyl Co.A carboxylase in bovine adipose tissue [2].Moreover, the recorded increase in serum total cholesterol associated with calcium soap supplementation could be attributed to increased formation of chylomicrons required for cholesterol absorption from small intestine [6] . On other hand, the current study reported hyperlipidemic effect of calcium soap supplementation; this effect may lead to increased steroid hormones synthesis by the ovary; since the blood cholesterol is the primary source of ovarian steroid genesis among mammalian species [25]. In conclusion, calcium soap supplementation led to early puberty in Rahmani ewe lambs this may be due to their beneficial effect on rumen and general metabolic pools led to improvements in the energy and lipids status of the supplemented Rahmani ewe lambs and earlier attainment of puberty.

Table 5: Effect of dietary calcium soap supplementation on serum urea nitrogen levels (mg/dl) in Rahmani ewe lambs(mean± SEM).

Weeks of supplementation	Treatments	Urea
Day zero	Control	9.79±0.57^{a}
	CSFA	10.77±0.47^{a}
6 weeks	Control	13.12±0.67^{a}
	CSFA	8.36±0.19^{b}
12 weeks	Control	14.00±0.14^{a}
	CSFA	12.11±0.24^{b}

Within the same column with different superscripts (a, b) are different ($p<0.05$).

Table 6. Effect of calcium soap supplementation on blood metabolites related to energy metabolism (mean± SEM).

Weeks of supplementation	Treatments	Glucose mg/dl	Cholesterol mg/dl	Total lipid mg/dl
Day zero	Control	68.31±1.17^{a}	24.50±0.17^{a}	137.98±5.89^{a}
	CSFA	67.11±1.03^{a}	23.62±0.52^{a}	131.09±13.34^{a}
6 weeks	Control	68.31±1.17^{b}	30.79±1.28^{b}	254.89±16.27^{b}
	CSFA	81.16±2.72^{a}	41.72±3.50^{a}	312.85±10.80^{a}
12 weeks	Control	67.68±0.19^{b}	36.64±2.15^{b}	312.50±2.46^{b}
	CSFA	82.22±4.79^{a}	57.17±4.11^{a}	387.71±10.30^{a}

Within the same column with different superscripts (a, b) are different ($p<0.05$).

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