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THE EFFECT OF DIETARY SUPPLEMENTATION WITH CALCIUM SOAPS OF POLY UNSATURATED FATTY ACIDS ON ADAPTIVE TRAITS IN SHEEP

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ABSTRACT

New born lambs are facing many environmental challenges that are totally different from the uterus one. Adaptation to extra-uterine life involves functional changes with almost each organ and system in the body undergoing a series of metabolic and anatomical modifications. Thirty healthy Barki ewes (3-4 years old) with average live body weight of 40±0.2 kg were used in the present study. This study aimed to investigate the effect of dietary supplementation with calcium soaps of poly unsaturated fatty acids (CSFA) on adaptive traits for ewes and their new lambs during pre and after parturition. Total protein (TP), albumin (Al), total lipids (TL) high density lipoprotein (HDL), triglycerides (TG), and cholesterol (CHOL) were investigated.

Lamb's sex ratio of 32 lambs born, control dams had 7 females vs. 7 males compared to 13 females vs. 5 males for CSFA supplemented ewes. Total protein of ewes in late of pregnancy was decline however increased after parturition (6.7 and 6.8 vs. 6.88 and 7.6) for control and treated group, respectively. The decrease in serum TP recorded from the mid (3^{rd} month) to the end of pregnancy could be attributed to the increased required of fetus from nutrient for purpose of growth and development.
Results revealed that serum CHOL concentration were significantly ($P<0.05$) higher in treated group than control (85.6 and 84.2 Vs 141.2 and 80.6), respectively. Lipid metabolites including TG, LDL and HDL were significant increased and take the same trend of cholesterol during treatment period. Serum glucose was elevated but not significant whereas, serum TL, and Gl concentration were significantly ($P<0.05$) higher in treated group compared with control group. It is therefore essential that supplementation CSFA improve performance of ewes and the physiological response, which may have beneficial effects on lambs survival. Further evidence was required to study their influence on sex ratio and twining rate.

Keywords: Barki sheep, new born lamb, CSFA, biochemical parameters, blood electrolytes

INTRODUCTION

Lipids are a major source of energy, fat-soluble vitamins, hormones and essential fatty acids for neonates. The essential fatty acids are important for cell membrane integrity as phospholipids and are precursors of prostaglandins (kennaugh and Hay, 1987).

The energy intake appears to be the primary determinant for ruminant’s reproductive performance as ruminants are good nitrogen preservers. Furthermore, ruminant’s performance and responses are dependent on the source of energy fed rather than variations of rumen degradable and undegradable protein contents of the diets (Abdelgadir *et al*., 1996). Bypass fat sources such as Calcium salts of fatty acids (CSFA) represent a source of metabolic fuel due to its fatty acids contents (84% fatty acids) so it was implicated in ruminants ration to increase its energy density (Palmquist, 1994).

Feeding of energy rich additives similar to calcium soap may modulate the recrudescence of hypothalamic and pituitary function and therefore ovarian activity through effects on the overall energy status of the female. Furthermore, greater Dietary fat ingestion has direct effects on ovarian structures (Rhodes *et al*., 1978). Therefore, fat may result in higher progesterone production (Hawkins *et al*., 1995).

Newborn lambs are usually threatened by multiple risk factors particularly to perinatal diseases, disorders of noninfectious etiology or by pathological conditions of microbial or parasitic, which may lead to high rate of mortality due to weak or less developed immune system at this critical stage of life span (Fragkou *et al*., 2010). About 10% of the alive born lambs can die on the day of birth because of different factors and this indicates death of almost half of all pre-weaning lambs (Dawyer, 2008).

Modification and adjustment of extra-uterine environment according to the needs of newborn in the first week of life can greatly reduce the death rate (Nowak *et al*., 2000; Sawalha *et al*., 2007). However, because of differences in breed and species the adaptation response to the new environment by lambs may differ and evidence does exist that lambs may fail to cope with the modifications associated with independent life because of their inability to maintain homeothermy and establish breathing (Sawalha *et al*., 2007).

Ewes supplemented with fish oil during pregnancy and lactation period had a physiologically more mature lamb at parturition, thus altering neonatal behavior (Capper *et al*., 2006). Bines *et al*, (1978) reported that plasma level of calcium and magnesium decreased on fat feeding in dairy cows. Fat feeding in general and a high intake of unsaturated fatty acids in particular, inhibit microbial activity and depresses rumen fermentation both in vivo as well as in vitro (Chalupa *et al*., 1984; Jenkins 1987).

Therefore, the current study was carried out to investigate the effect of the dietary supplementation of CSFA on serum biochemical parameters and some blood electrolytes for Barki ewes and their new lambs.

MATERIALS AND METHODS

Study area and animal characteristics

The current work was carried out at Maryout research station, Desert Research Center (DRC), located 35 km southwest of Alexandria, Egypt. This experiment aimed at scrutinizing the effect of some supplemented fatty acids to ewes on adaptability of their lamb's under semi arid conditions.

Thirty healthy Barki ewes (3-4 years old) with average live body weight of 40±0.2 kg were used in the present study. The animals were housed in closed pens throughout experimental period from July, 2008 to May, 2009. Ewes were fed a concentrate mixture according to their body weight requirement (NRC, 1985). All animals were given Egyptian clover (*Trifolium alexandrinum*) hay as a roughage ration adlib. Each animal also received 1 Kg/day of palliated concentrate mixture that contained 65% total digestible nutrients (TDN) and 14% crude protein.

The ewes were divided into two groups the first as control and the second received (50g/day) of calcium soaps of poly unsaturated fatty acids (CSFA, Alpha Chemical Co; Alexandria, Egypt). The chemical composition of CSFA is presented in Table (1). The treatment started at one month before mating season and continued for a second month during which synchronization and mating occurred. Repeat treatment of CSFA at one month before the expected date of parturition and continued until weaning of their lambs. Freshwater was presented twice daily. Live body weight was recorded monthly for each ewe throughout the experiment. Birth weights of new lamb born were recorded biweekly until them weaning.

Table (1): Chemical composition (g/kg) of calcium soaps of fatty acids (CSFA)

Item	g/kg
Total fat	840.0
Myristic acid	12.6
Palmatic acid	369.6
Stearic acid	42.0
Oleic acid	336.0
Linoleic acid	79.8
Ash	110.0
Ca++	9.9
Moisture	50.0

Blood sampling

Blood samples were collected via jugular vein puncture at fortnight from the beginning of the study, till the end of the experimental period. Blood samples were allowed to clot and sera were separated. Sera were divided into aliquots and frozen at −20°C until assayed for serum components. Lambs blood samples were collected biweekly directly after parturition till weaning.

Serum biochemical analysis

Ewes serum samples throughout the experimental period (before and after parturition) were colorimetric analyzed. Total protein (TP), albumin (Al), were determined according to Dumas *et al*., (1971), while globulin (Gl) and albumin/globulin (A/G) ratio were calculated. Total lipids (TL), and cholesterol (CHO), were determined according to Roeschlau *et al*., (1974). High density lipoprotein (HDL), and triglycerides (TG) were determined according to Finley *et al*., (1978), Warnick, (1983) and Scheletter and Nussel, (1975), respectively. Alanine amino taransferase (ALT) and aspartate amino taransferase (AST) were determined using according to Reitman and Frankel, (1957). Glucose (GLU) according to Trinder, (1969), Low Density Lipoprotein (LDL) was calculated according to the equation (LDL= TCH-HDL-TG/5) proposed by Friedewald *et al*., (1972).

The serum electrolytes in terms of, Sodium (Na), potassium (K), and Calcium (Ca) concentrations were analyzed according to Tietz, (1976).

Statistical analyses

The results of raw data statistically analyzed were conducted using SAS® (1998) software program. One way ANOVA of GLM procedure of SAS was used in each experiment.

RESULTS AND DISCUSSION

An increase in live weight was noted right from the start of trial (41.4±1.6 and 40.3±1.5 kg) to the end (52.4±2.2 and 53.7±2.1 kg) for ewes at control and treated groups, respectively (Table, 2). That increase due to the physiological stages of pregnancy development. Changes of ewe's weight were 26.57 and 33.25 % as a ratio of beginning weight.

Table (2) showed the variation in live body weight of lambs from birth to weaning and the effect of treatment on body weight and daily gain. Averages of birth weight were 3.7 and 4.0 kg in control and treated group, respectively, in which the differences were significant. The corresponding values of daily gain from birth to weaning were 118 and 102 g/day, respectively. Weaning weights were 15.3 and 14.0 kg for two groups, respectively. Supplementation of CSFA did not affect overall body weight of born lambs up to 14 weeks after parturition. Similar results were previously observed by Gargouri *et al*. (2006).

Table (2) Least square means ± SE of body weights (kg) of ewes and their lambs from birth to weaning throughout the experimental period

Item	Control group	Treated group
Ewes		
No.	15	15
1st month of pregnancy	41.4±1.6	40.3±1.5
Last month of pregnancy	52.4±2.2	53.7±2.1
Changes %	26.57	33.25
Lambs		
Lambing	92 (13/14)	100 (15/15)
Lambs sex ratio (F/M)	7/7	13/5
At birth	3.7±0.6^{a}	4.0±0.5^{b}
1st month	10.0±0.6	9.25±0.5
At weaning	15.3±0.6	14.0±0.5
Gain from birth to weaning (g/day)	118±4.2	102±3.1

Mean with different superscripts within the same row were significantly different at ($p < 0.05$).

It has been documented that the highest rate of lamb's growth in live weight occurs during the first month of life, lambs growth is associated with the level of milk consumption, so during these first month of complete depending on the mother ewes milk (Owen, 1976).

Ewes supplemented with CSFA had twining rate of 20% (3/15) that was not different from 7.14% (1/14) for the controls. Lambing rates take the same trend in both treated and control groups (Table, 2). Lamb's sex ratio of 32 lambs born, control dams had 7 females vs. 7 males compared to 13 females vs. 5 males for treated ewes. Despite the apparent different in sex ratio between control and treatment, the difference was not significant.

The theory explains that, skewed sex ratios could result from mechanisms that occur either prior to fertilization, through modulating the ability of either X or Y sperm to reach or penetrate the oocyte, or after fertilization via selective loss of one sex relative to the other (Rosenfeld and Roberts, 2004).

Results of blood biochemical parameters in late pregnancy and after parturition in Table (3) showed that the TP of ewes in late of pregnancy were decline and after parturition were increased (6.7 and 6.8 vs. 6.88 and 7.6 for control and treated groups, respectively. The decrease in serum TP recorded from the mid (3^{rd} month) to the end of pregnancy could be attributed to the increased required of fetus from nutrient for purpose of growth and development. The significant reduction in total protein at the mid and end of pregnancy observed in the present study agree with results of (Morsy *et al*., 2009). Globulin take the same trend of TP which decrease also which can be explained by decreased in serum globulin fraction that was transferred to colostrum at the end of pregnancy (Williams and Miller, 1979 and Rowlands *et al*., 1980).

The present results revealed that serum CHOL concentrations were significantly ($P<0.05$) higher in CSFA treated than control group (85.6 and 84.2 Vs 141.2 and 80.6 respectively). The lipid metabolites including TG, LDL and HDL were significant increased significantly ($P<0.05$) and take the same trend of cholesterol during treatment period. Similar finding were reported by Morsy *et al*. (2008) and Hegazy *et al*. (1999).

Increasing in serum concentration of lipid metabolites is due to increased intestinal secretion of lipoproteins with higher content of TG. Also addition of supplemented fat to sheep or cow diets probably stimulated lipoprotein cholesterol export by the intestine and increase circulating plasma CHOL, LDL, and HDL concentrations (Talavera *et al*., 1985). That might be attributed to the reduced activity of lipogenic enzymes in the liver and adipose tissues associated with feeding supplementary fat (Starry, 1981).

Serum alanine aminotransferase (ALT) is particularly useful in measuring hepatic necrosis and increases in serum when cellular degeneration or destruction occurs. On the other hand, AST is present in extrahepatic tissues including myocardium and kidney and can be used as a good indicator of hepatic injury (Lessard *et al*., 1986). The present results revealed that supplementation of CSFA did not has significant effect on the hepatic enzymes activity of both ALT and AST pre or after parturition this results were agree with (Westerbacka *et al*., 2005). Apparently no detrimental effects of higher contents of poly unsaturated fatty acids in CSFA containing diets on liver cells and functions.

Table (3) Least square means ± SE of serum biochemical parameters in late pregnancy and after parturition of ewes

Item	Control group	Treated group
Late pregnancy		
TP (g/dl)	7.68±0.16	7.48±0.10
Al (g/dl)	4.80±0.15	4.52±0.09
Gl (g/dl)	2.89±0.20	2.96±0.13
A/G	1.69±0.14	1.55±0.09
GLU (mg/dl)	57.28±8.78	62.49±5.55
TL (g/l)	262.31 ± 7.37^{b}	488.17 ± 6.13^{a}
TG (mg/dl)	16.2±1.9	16.2±1.9
HDL (mg/dl)	36.9±1.9	35.9±1.7
LDL (mg/dl)	44.0±3.6	46.0±3.3
CHOL (mg/dl)	84.2±3.7	85.6±3.4
AST (IU/L)	14.3±0.9	11.9±0.8
ALT (IU/L)	5.2±0.2	5.1±0.2
Urea (mg/dl)	59.33±4.21	48.09±2.66
After parturition (at 4th week)		
TP (g/dl)	6.88 ± 0.15^{b}	7.83 ± 0.32^{a}
Al (g/dl)	4.11±0.13	4.31±0.13
Gl (g/dl)	2.75 ± 0.18^{b}	3.61 ± 0.18^{a}
A/G	1.48±0.13	1.20±0.11
GLU (mg/dl)	62.6±2.2	62.2±2.0
TL (g/l)	302.46 ± 5.73^{b}	467.54 ± 6.87^{a}
TG (mg/dl)	24.2 ± 3.0^{b}	31.7 ± 2.8^{a}
HDL (mg/dl)	59.2±4.1	68.5±3.4
LDL (mg/dl)	19.3±6.8	68.6±5.8
CHOL (mg/dl)	80.6 ± 5.0^{b}	141.2 ± 4.6^{a}
AST (IU/L)	13.4±1.5	12.1±1.5
ALT (IU/L)	5.2±0.2	5.4±0.2
Urea (mg/dl)	45.84±3.77	41±0.37
After parturition (at 11th week)		
TP (g/dl)	6.57±0.17	6.99±0.25
Al (g/dl)	4.22±0.13	4.11±0.13
Gl (g/dl)	2.35 ± 0.18^{b}	2.89 ± 0.18^{a}
A/G	1.79±0.14	1.42±0.17
GLU (mg/dl)	65.5±1.6	64.3±1.4
TL (g/l)	222.43±6.12	341.34±5.46
TG (mg/dl)	9.4 ± 1.6^{b}	21.2 ± 15^{a}
HDL (mg/dl)	54.8±4.4	80.5±3.7
LDL (mg/dl)	24.3±6.8	51.6±5.8
CHOL (mg/dl)	79.5 ± 5.0^{b}	136.5 ± 4.6^{a}
AST (IU/L)	12.3±1.5	12.1±1.5
ALT (IU/L)	5.1±0.2	5.1±0.2
Urea (mg/dl)	38±0.35	39±0.47

Mean with different superscripts within the same row were significantly different at ($p < 0.05$).

Table (4) showed serum biochemical parameters in lambs. Glucose concentration was elevated but not significantly whereas, serum TL, and GI concentration were significantly ($P<0.05$) higher in treated compared with control group. A/G ratio take the oposit trend compared with Gl which showed higher ratio in control group compared with treated group were, 1.73 vi. 1.42 and 1.64 vi. 1.37 for lambs at 1st month and 3rd month, respectively. There was no change in albumin level among groups. Albumin showed a constant level from birth till the end of experimental period. This is probably due to the metabolic pathways typical during the postnatal age. In fact, protein metabolism plays an important role in the regulation of physiological functions during pregnancy and lactation (Diogenes *et al*., 2010 and Piccione, *et al*., 2011).

Also treatment by CSFA showed significant (P<0.05) effect on Ca and K level in serum, since the lowest values were recorded for ewes before parturition (5.32 and 5.27 nmol/l) in control group and their new lambs at 1st month (6.02 and 6.26 nmol/l) in the same group, respectively (Table, 5). Sodium/K ratio ranged between 22.72 to 25.81 and 20.22 to 23.06 for ewes and their new lambs, respectively. This might be due to equilibrium between Na and K levels in blood. Which due to aldosterone, it had been assured that it promoted the excretion of potassium and retention of sodium, thereby influencing extracellular volume homeostasis and blood pressure (Williams and Williams, 2003). The result of serum mineral composition showed difference among treatments, which is harmony with the observation of Bines *et al*, (1978) where they reported that plasma level of calcium and magnesium decreased on fat feeding.

Table (4) Least square means ± SE of serum biochemical parameters in newly lambs

Item	Control group	Treated group
lambs at 1st month		
TP (g/dl)	7.28±0.15	7.30±0.15
Al (g/dl)	4.60±0.13	4.25±0.13
Gl (g/dl)	2.67±0.18^{b}	3.05±0.18^{a}
A/G	1.73±0.13^{a}	1.42±0.13^{b}
GLU (mg/dl)	118.24±7.86	127.15±7.86
TL (g/l)	562.66±7.66^{b}	889.65±7.66^{a}
Urea (mg/dl)	33.96±3.77	27.58±3.77
lambs at 3rd month		
TP (g/dl)	7.18±0.21	7.65±0.21
Al (g/dl)	4.45±0.09	4.41±0.09
Gl (g/dl)	2.72±0.14^{b}	3.25±0.14^{a}
A/G	1.64±0.11^{a}	1.37±0.11^{b}
GLU (mg/dl)	118.24±7.86	127.15±7.86
TL (g/l)	562.66±7.66^{b}	889.65±7.66^{a}
Urea (mg/dl)	33.96±3.77	27.58±3.77

Mean with different superscripts within the same row were significantly different at ($p < 0.05$).

Table (5) Least square means ± SE of some blood electrolytes of ewes and their lambs

Item	Control group	Treated group
Ewes before parturition		
Na (nmol/l)	134.88±6.86	151.08±10.66
K (nmol/l)	5.27±0.39[b]	6.65±0.25[a]
Na/K ratio	25.59±1.03	22.72±1.05
Ca (nmol/l)	5.32±0.95[b]	6.68±0.60[a]
Ewes after parturition		
Na (nmol/l)	147.65±5.08	149.49±4.95
K (nmol/l)	5.72±0.35	5.94±0.35
Na/K ratio	25.81±1.12	25.17±1.12
Ca (nmol/l)	6.69±0.85	6.97±0.85
New lambs at 1st month		
Na (nmol/l)	144.34±5.08	141.91±5.08
K (nmol/l)	6.26±0.35[b]	7.02±0.35[a]
Na/K ratio	23.06±1.53	20.22±1.53
Ca (nmol/l)	6.02±0.85[b]	6.96±0.85[a]
New lambs at 3rd month		
Na (nmol/l)	139.34±4.78	144.91±5.57
K (nmol/l)	6.47±0.35[b]	7.16±0.35[a]
Na/K ratio	21.52±1.73	20.24±1.73
Ca (nmol/l)	6.45±0.65[b]	7.84±0.65[a]

Mean with different superscripts within the same row were significantly different at ($p < 0.05$).

In conclusion, it can affirm that supplementing pregnant ewes with calcium soaps of fatty acids (CSFA) improve performance of ewes and the physiological response, which may have beneficial effects on lamb survival. The inclusion of CSFA in the prepartum di*et al*so severely reduced colostrum fat and protein yield, and dietary strategies investigating the effects of timing of CSFA supplementation in the pre-parturition period. It is likely that the calcium and magnesium included in the concentrate mix were sufficient to prevent symptoms of hypocalcemia and hypomagnesemia. Further evidence was required to study their influence on sex ratio and twining rate.

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تأثير إضافة الأحماض الدهنية المحمية بالكالسيوم على صفات التأقلم للأغنام

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تتعرض الحملان حديثة الميلاد لعديد من التحديات فى بيئتها الجديدة والتى تختلف كليتا عن البيئة التى داخل الرحم (خلال مرحلة الحمل) مما يتطلب تغيرات وظيفية أيضية وتشريحية فى أجهزة وأعضاء الجسم المختلفة لمجابهة هذه المتغيرات وتأتى التغذية على رأس العوامل البيئية التى قد تؤثر فى ذلك. أجريت هذه الدراسة بهدف معرفة تأثير إضافة الأحماض الدهنية المحمية بالكالسيوم (CSFA) بمعدل 50 جرام/رأس/يوم على بعض القياسات البيوكيميائية للنعاج وحملانها لمعرفة قدرتها على التأقلم لمثل هذه الظروف وذلك خلال مرحلة نهاية الحمل للأمهات ومرحلة الرضاعة للمواليد.

أجريت هذه الدراسة فى محطة بحوث مريوط التابعة لمركز بحوث الصحراء واستخدم فيها عدد (30) نعجة برقى. وتم تقدير البروتين الكلى والالبيومين والجلوبيولين وكذلك نسبة الالبيومين الى الجلوبيولين و الجلسريدات الثلاثية والكوليسترول الكلى والكوليسترول العالي والمنخفض الكثافة وإنزيمات الكبد (AST, ALT) وبعد العناصر المعدنية مثل الكالسيوم والماغنيسيوم والصوديوم.

أظهرت النتائج أن استخدام الأحماض الدهنية المحمية بالكالسيوم (CSFA) كان تأثيره معنوى على محتوى السيرم من البروتين الكلى والجلوبيولين فى المجموعة المعاملة مقارنة بالكنترول وذلك فى فترة نهاية الحمل بينما كان لايوجد اى اختلاف بين المجموعات التجريبية فى فترة ما بعد الحمل (الرضاعة). كذلك ارتفع محتوى السيرم من كل من الجلسريدات الثلاثية والكوليسترول الكلى والكوليسترول العالي والمخفض الكثافة فى المجموعة المعاملة مقارنة بالكنترول وكان ذلك الارتفاع معنويا ($P<0.05$). لم تتأثر إنزيمات الكبد AST, ALT بإضافة الأحماض الدهنية المحمية بالكالسيوم مقارنة بالمجموعة الكنترول خلال مرحلة التجربة.

ولهذا يجب الاهتمام بإضافة الأحماض الدهنية المحمية بالكالسيوم CSFA كمكملات والتى يمكن فى المساعدة فى تحسين الأداء من النعاج والاستجابة الفسيولوجية، والتي قد تكون لها آثار مفيدة على حيوية وبقاء حملانها والنسبة الجنسية للمواليد.

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