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ORIGINAL RESEARCH

Effect of feeding calcium salts on performance of nursing Awassi ewes

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Abstract Twenty nursing Awassi ewes (BW=50±2.35 kg, age=4.5±1.2 years) with their lambs were used to evaluate the effects of feeding calcium salts in lactation diets on performance and pre-weaning growth of their lambs. Treatments were 0% calcium salts (CON) or 5% calcium salts (FAT). At the end of the study, a digestibility experiment was performed. Milk yield was greater ($P<0.05$) for ewes fed the FAT diet than the CON diet. Milk composition was similar ($P>0.05$) between diets. However, milk energy value (kcal/day) tended to be greater ($P=0.07$) for the FAT diet than the CON diet. Concentrations of milk C18:1c9 and C20:0 were greater ($P<0.05$) in ewes fed the FAT diet than the CON diet. However, concentration of *trans*-10, *cis*-12 CLA was lower ($P=0.05$) in the FAT diet than in the CON diet. No differences in feed intake and body weight change were detected between diets. Digestibility of dry matter, organic matter, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber were similar ($P>0.05$) for diets. For lambs, weaning weight was not affected by treatments. However, average daily gain and total gain were greater ($P=0.053$) for the FAT diet than the CON diet. Results suggest that supplementing lactating ewes with calcium salts at the beginning of lactation phase improves daily milk yield of ewes and pre-weaning growth of their lambs with no major negative impact on feed intake and digestibility.

Keywords Calcium salts · Ewes · Milk yield · Digestibility

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Introduction

The use of fat, either as rumen-protected or unprotected, has been a common feed additive worldwide to improve animal performance by optimizing energy intake. Such practice has been implemented both in dairy ewes and cattle to increase the energy density in lactation diets (Gargouri et al. 2006). Great emphasis on the use of fat supplementation in rations of dairy ewes has been given due to the high energy density in fat compared to carbohydrates or protein supplementation (Kitessa et al. 2001). Most of the research conducted to investigate the effect of fat supplementation has been carried out on lactating animals due to their high energy requirements (Goulas et al. 2003; NRC 2007).

Supplementation of diets with calcium salts has been shown to improve milk fat content with no effects on milk production or lamb gain in Polypay ewes (Appeddu et al. 2004). In contrast, milk yield and composition (protein and total solids) increased in ewes when diets were supplemented with calcium salts (Alba et al. 1997). These contradictions could be due to differences in dietary composition, type, and level of supplemented fat and/or phase of lactation.

On the other hand, wheat and/or barley straw represent the main roughage source for sheep in the Middle East region due to its availability and reasonable cost. The use of straw is always complicated by its low energy content especially when used during high energy demand like those of early lactating ewes (NRC 2007). Limited data are available on supplementing calcium salts in diets of lactating Awassi ewes. Therefore, this experiment was conducted to evaluate the effects of adding calcium salts to Awassi ewes fed wheat straw on milk production, milk composition (i.e., total solids, crude protein, crude fat, and fatty acids profile), body weight change, pre-weaning growth of their lambs, and feed digestibility.

Materials and methods

Animals, diets, and design

The experiment was conducted at the Agriculture Center for Research and Production at Jordan University of Science and Technology. Twenty Awassi ewes (BW=50± 2.35 kg, age=4.5±1.2 years) and their lambs (nursing single lambs) were randomly assigned to two treatment diets (ten ewes/treatment diet). Treatment diets were either control diet with 0% fat (CON) or supplemented with 5% fat (FAT) (MAGNAPAC, calcium salts of palm oil fatty acids, NOREL SA, Madrid, Spain). Analysis of the fat supplement showed that it contained 3.5% moisture, 84% crude fat, 12.5% ash, and 9% calcium. Calcium salts contained 1.5% mirisitic acid (C14:0), 44% palmitic acid (C16:0), 5% stearic acid (C18:0), 40% oleic acid (C18:1c9), and 9.5% linoleic acid (C18:2). Diets were mixed every 2 to 3 weeks, and diets were sampled for laboratory analysis to ensure consistency in their chemical composition (three samples, Table 1). The concentrate mixtures were offered to all ewes at 1.2 kg/head/day in addition to 1.0 kg/head/day of wheat straw. Wheat straw contained 95.7%, 80.9%, 4.2%, 74.2%, 44.4%, and 0.21% DM, OM, CP, NDF, ADF, and EE, respectively. All ewes had free access to fresh water for the duration of the experiment. Diets were formulated to be isonitrogenous and to meet the NRC (2007) requirements of lactating ewes. No attempts were made to prevent the lambs from having access to their dam's diet. Therefore, lambs performance was not solely dependent on the milk produced by their mothers.

Table 1 Ingredient and chemical composition of the concentrate mixtures fed to ewes during the experiment

Item	Diets[a]		
	CON	FAT	SE
Ingredient (% of DM)[b]			
Barley	58.0	51.0	
Corn, ground	13.0	13.0	
Soybean meal, 44% CP	11.5	13.5	
Wheat bran	15.0	15.0	
Calcium salts	0.0	5.0	
Salt	1.0	1.0	
Calcium carbonate	1.0	1.0	
Mineral and vitamins[c]	0.5	0.5	
Nutrient (% of DM)			
Dry matter (DM)	90.2	90.2	0.14
Organic matter (OM)	89.0	86.8	0.25
Crude fat (EE)	2.7	6.8	0.05
Crude protein (CP)	19.0	18.9	0.08
Neutral detergent fiber (NDF)	20.9	20.2	0.65
Acid detergent fiber (ADF)	5.7	6.0	0.50
ME (Mcal/kg)	2.95	3.2	0.03

ME metabolizable energy calculated using NRC (2007)

[a] Treatment diets were a control diet with 0% (CON) or 5% calcium salts (FAT)

[b] All results are expressed on dry matter basis

[c] Composition per 1,000 g contained vitamin A, 1,100,000 IU; vitamin D3, 450,000 IU; vitamin E, 3.18 g, Mn, 10.9 g; I, 1.09 g; Zn, 22.73 g; Fe, 22.73 g; Cu, 2.73 g; Co, 0.635 g; Mg, 100 g; Se, 0.1 g; plus antioxidant

All ewes gave birth within 5 days of each other (day 0= parturition). The experiment started within 1 week after lambing and lasted for 8 weeks (until weaning). Ewes with their lambs were housed and fed individually in shaded pens (1.5×0.75 m) once daily at 0900 hours. Animals were adapted to the pens for 1 week before receiving the experimental diets. After adaptation, ewes and their lambs were fed their assigned diets for the following 7 weeks. Animals were weighed weekly before the morning feeding throughout the study. One ewe receiving the FAT treatment diet was removed from the experiment due to problems in the udder unrelated to treatment diets.

At the end of the experiment, eight ewes were chosen (four from each group) at random for the digestibility study. Ewes were housed individually in metabolism crates (105× 80 cm) equipped with collection pan designed for separate collection of urine and feces. The experiment lasted for 15 days with 10-day adaptation for the crates followed by another 5 days for samples collection. The concentrate and wheat straw was offered as total-mixed ration once daily at 0900 hours, and refused feed was collected after 24 h, weighed, sampled, and kept at −20°C for future analysis.

Daily fecal output was collected, weighed, mixed, sampled, and kept at −20°C (10% *w*/*w*) for subsequent analyses. Samples of the concentrate and wheat straw mix, refusals, and feces were dried at 55°C in a forced-air oven to reach a constant weight, air equilibrated, and then ground to pass 1-mm screen (BRABENDER Ohg, Kulturstrase 51–55, type 880845, Nr 958084, DUISDURG, Germany). According to procedures of AOAC (1997), offered feed, refusals, and feces were analyzed for DM (100°C for 24 h), OM (500°C for 8 h), EE (Soxtec procedure), and CP (Kjeldahl procedure). Neutral detergent fiber (NDF) and ADF were analyzed according to procedures described by Van Soest et al. (1991) with modifications for use in the ANKOM^{200} fiber analyzer apparatus (ANKOM Technology Cooperation, Fairport, NY, USA). Neutral detergent fiber analyses were conducted with using sodium sulfite and alpha amylase (heat stable) and expressed with residual ash content.

Milk production and chemical composition

Milk production measurement was initiated during the first week of the experiment (approximately during the second week of lactation). Milk production over a period of 12 h was estimated weekly throughout the study at 0800 hours using hand milking. Lambs were separated from their dams 12 h before milking, and milk yield was then calculated over the 24-h period (Awawdeh et al. 2009). To ensure consistency, all ewes were milked by the same trained person throughout the study. At the same time of measuring the milk yield, five ewes from each treatment group were randomly chosen, and a 125-mL sample was collected and analyzed immediately for total solids, fat, and protein. Similar ewes were chosen every week for sampling purposes and their milk yield used to calculate the amount total solids, fat, and protein. Total solids were evaluated using an air-force oven (50°C) to reach a constant weight. Crude protein was evaluated using Kjeldahl procedure ($N\% \times 6.38$). Fat content was analyzed according to Gerber procedure. Milk energy value (kilocalories per kilogram) was calculated with the following equation: $203.8+(8.36\times \text{fat}\%)+(6.29\times \text{protein}\%)$ as described by Baldi et al. (1992). Milk samples of each week were composited and kept at (−20°C) for determination of fatty acid composition. Fatty acids composition was analyzed as described by Titi and Obeidat (2008).

Statistical methods

Linear mixed model analysis was used to examine milk production data (during the whole period of experiment including the adaptation week) using the mixed model procedure of SAS (2000). Treatment, week, and their interaction were considered as fixed effects, and animal within treatment was incorporated in the model as a random factor and week as a repeated measure. Because no week× treatment interaction was detected, the treatment effect was discussed.

The rest of the data were analyzed using Student's independent samples *t* test. Tests of equality of variance were conducted to assure the use of pooled sample variance in Student's *t* test. Whenever the test of equality of variance revealed significant difference between the variance of the two treatment groups, the Satterthwaite unequal variance *t* test method was used to examine differences in means between the two treatments.

Results and discussion

The chemical composition of the experimental diets was similar for all nutrients except for the EE content which was higher in the FAT group (Table 1) due to the inclusion of the calcium salts.

The average milk yield for ewes in this study was lower than expected, mostly as a result of incomplete milking, and may not represent the actual milk yield for the ewes (Table 2). Nevertheless, ewes on the FAT diet had greater average milk yield ($P<0.05$) than ewes on the CON diet. Previous work with the same breed showed that milk yield was increased with the addition of different fat sources (Titi and Obeidat 2008). In contrast, Dobarganes Garcia et al. (2004) and Casals et al. (2006) also reported no differences in milk production in ewes fed diets with or without fat supplement. The differences among studies in the effect of fat supplementation on milk yield could be related to differences in genetic potential of ewes, which affects the efficiency of utilization of supplemented fats (Mele et al. 2005) and/or to the source of supplemented fat.

No differences in milk components percentages and yields were observed during the course of the study (Table 2). Another study has reported that milk fat percentages increased when ewes or dairy cows were supplemented with protected fat (Appeddu et al. 2004). In the current study, level of milk fat was low for both treatment diets when compared to previous studies with Awassi ewes (Titi and Obeidat 2008) or other breeds (Chiofalo et al. 2004; Appeddu et al. 2004). However, results of fat content presented in the current study were relatively close to the low values reported by Awawdeh et al. (2009), who performed their study on group of ewes selected from the same herd used in the current study. The differences in milk fat content between our results and other research studies could be related to differences in the experimental diets, or source and level of supplemented fat. Moreover, the low level of fat content in the ewes used in

Table 2 Effect of feeding calcium salts on milk production and composition in nursing Awassi ewes

Item	Diets[a]			
	CON	FAT	SE	*P* value
Milk production (g/day)	480	674	25.3	<0.05
Fat				
%	3.72	3.69	0.222	0.93
g/day	19.78	20.66	3.014	0.85
Protein				
%	4.63	4.51	0.082	0.28
g/day	23.97	26.35	3.807	0.67
Total solids %	14.40	14.70	0.338	0.53
Milk energy value (kcal/kg)	806.0	795.7	31.135	0.83
Milk energy value (kcal/day)	389.8	531.8	47.813	0.07

[a] Treatment diets were a control diet with 0% (CON) or 5% calcium salts (FAT)

the current studies is likely in part due to inbreeding, which may cause a depression in milk fat synthesis. The decrease in milk fat content and/or yield due to inbreeding that was observed in the current study is in agreement with others (Kasarda and Kadlecik 2007; Parland et al. 2007) who reported similar reduction in milk fat production in cows.

No differences in milk protein were detected between the two treatment diets. These results agreed with Bernal-Santos et al. (2003) who reported that milk protein content was not affected in dairy cows supplemented by mixture of rumen protected CLA and palm fatty acids. However, these results are inconsistent with the previous study (Appeddu et al. 2004) that reported a significant reduction in milk protein content with consumption of rumen protected fat, changes that have often been attributed to increases in milk yield (dilution effect) rather than decreases in milk protein synthesis.

Milk energy value tended to be greater (P=0.07) for ewes fed the FAT treatment diet when compared to the CON treatment diet (Table 2). These results are consistent with Casals et al. (1999) who reported more milk energy value when ewes were supplemented with calcium salts.

Except for C18:1c9, C20:0, and *trans*-10, *cis*-12 CLA, dietary treatment had no effects (P>0.05) on profile of other fatty acids. The de novo fatty acids synthesis was not probably affected by calcium salts supplement because treatment did not affect even-number fatty acids. Chouinard et al. (1998) observed reductions in milk short- and medium-chain fatty acids with supplemental fat. This reduction was probably due to the negative effect of fat on ruminal fiber-fermenting bacteria (Jenkins 1993). We did not observe such depression in fiber digestibility.

Concentration of C18:1c9 in milk fat was greater (P< 0.05) in ewes fed the FAT treatment diet when compared to the CON treatment diet. Chouinard et al. (1998) also reported similar increases in dairy cows. Concentration of *trans*-10, *cis*-12 CLA was lower (P=0.05) in the FAT treatment diet than in the CON treatment diet. This might have resulted from higher grain content in the CON diet (Table 1) as suggested by others (Loor et al. 2004; AbuGhazaleh and Jacobson 2007; Table 3).

As indicated in intake parameters presented in Table 4, ewes on the FAT treatment diet consumed more (P<0.05) EE than ewes on the CON treatment diet due to greater EE content in the FAT treatment. Throughout the experiment, all ewes consumed the assigned diets (concentrate and wheat straw) without refusal. This indicates that calcium salts did not affect feed intake when included in the concentrate mixture at 5% (DM basis). Likewise, no differences were also detected in ME intake between the two treatment diets. Our earlier work found that inclusion of calcium salts in the diets of early lactating ewes had increased their ME intake (Titi and Obeidat 2008). However, in the current study, the level of concentrate intake assigned to ewes was lower than the amount provided in the above study, which might contribute to the lack in differences in ME intakes. Such differences in response could also be properly related to differences in fat sources utilized.

Initial weights ($BW = 50 \pm 2.35$ kg) were used as covariate to analysis for final weights which did not differ (P>0.05) between treatment diets (Table 4). As a result, body weight changes of ewes in both groups were not different during the trial. Intake data indicate that calcium salts have no negative effects on intakes when fed at a level of 5% of the concentrate mixture. On the other hand, Titi and Obeidat (2008) found that ewes gained more weight when supplemented with 5% calcium salts in the concentrate mixture possibly as a result of higher energy intake.

Data for lamb growth showed that inclusion of calcium salts in the ewes' diets had positive effects on performance (Table 4). Lamb ADG and total gain were greater (P= 0.053) for the FAT treatment diet than the CON treatment diet. Additionally, lamb weaning weight tended to be higher

Table 3 Effect of feeding calcium salts on fatty acid composition of milk fat in nursing Awassi ewes

Item (% milk fatty acid)	Diets[a]			
	CON	FAT	SE	*p* value
C4:0	2.04	2.31	0.420	0.67
C6:0	3.38	2.87	0.304	0.28
C8:0	4.18	3.76	0.612	0.67
C10:0	10.11	9.27	0.716	0.43
C11:0	0.95	0.49	0.299	0.31
C12:0	3.77	3.40	0.262	0.36
C14:0	9.46	8.14	0.662	0.22
C14:1	0.53	0.46	0.120	0.73
C15:0	0.78	0.99	0.181	0.48
C16:0	24.64	27.62	1.980	0.32
C16:1	0.74	0.86	0.238	0.74
C17:0	0.73	0.75	0.170	0.94
C17:1	0.73	0.66	0.267	0.87
C18:0	9.51	9.90	0.821	0.75
C18:1 *cis*-9	19.41	24.13	1.293	0.03
C18:2 n-6 *trans trans*	1.05	0.94	0.305	0.81
C18:2 n-6 *cis cis*	2.71	2.29	0.360	0.43
C18:3 n-6	0.73	0.47	0.278	0.53
C18:3 n-3	1.09	0.94	0.326	0.76
C20:0	0.45	1.05	0.174	0.04
Conjugated linoleic acid (CLA)				
Cis-9, *trans*-11	1.09	1.01	0.388	0.88
Trans-10, *cis*-12	1.02	0.27	0.220	0.05

[a] Treatment diets were a control diet with 0% (CON) or 5% calcium salts (FAT)

Table 4 Least square means of ewe body weight changes and growth performance of their lambs during the pre-weaning period consumed diets containing calcium salts

Item	Diets[a]			
	CON	FAT	SE	*p* value
Ewes				
Intake				
Wheat straw (kg/head/day)	1.0	1.0	–	–
Concentrate (kg/head/day)	1.22	1.20	–	–
Ether extract (g/head/day)	32.95	81.61	2.20	<0.05
ME (Mcal/day)	3.60	3.79	0.15	0.38
Initial weight (kg)	49.5	50.4	2.28	0.78
Final weight (kg)	48.0	48.6	1.95	0.84
Body weight change (kg)	−1.5	−1.9	1.01	0.80
Lambs				
Initial weight (kg)	6.3	6.3	0.48	0.93
Weaning weight (kg)	18.3	20.9	1.08	0.11
Total gain (kg)	12.0	14.6	0.86	0.053
ADG (g/day)	214.8	259.9	15.36	0.053

ME metabolizable energy calculated using NRC (2007)

[a] Treatment diets were a control diet with 0% (CON) or 5% calcium salts (FAT)

(P=0.11) with the FAT treatment diet. These results are in agreement with Casals et al. (1999) who reported a slight improvement (not statistically observed) of pre-weaning growth of lambs when dairy ewes were supplemented with calcium soaps of palm oil fatty acids. In contrast, Alba et al. (1997) reported no improvement in growth rate for lambs when their dams consumed diets supplemented with calcium soaps of olive fatty acids. Our results and results

Table 5 Least square means of nutrient digestibility (percent) of nursing Awassi ewes consumed diets containing calcium salts

Item	Diets[a]			
	CON	FAT	SE	*p* value
DM	73.8	72.1	2.27	0.62
OM	75.9	76.0	2.15	0.98
CP	74.7	74.5	1.95	0.94
NDF	61.0	55.1	4.48	0.39
ADF	56.2	51.0	5.29	0.51
EE	74.6	78.8	2.02	0.20

[a] Treatment diets were a control diet with 0% (CON) or 5% calcium salts (FAT)

from previous studies (Alba et al. 1997; Casals et al. 1999) have demonstrated that growth rate of lambs during the pre-weaning period is greatly dependent on the mothering ability of ewes to produce milk, improvement in the milk energy value, and/or the type of dietary ingredients offered to ewes. However, it seems that level of milk production did not totally explain the observed growth rate of lambs. This could be due to underestimation of milk yield. Additionally, the part of growth rate of lambs that was not accounted for by the increase in milk yield was probably due to substantial consumption of dam's diet by their lambs.

Data of the digestibility trial are presented in Table 5. Inclusion of calcium salts did not influence (P>0.40) the digestibility of DM, OM, CP, EE, NDF, or ADF. Results of CP and NDF digestibilites are consistent with the findings of Manso et al. (2006) who fed growing lambs diets containing calcium salts (MAGNAPAC) similar to the fat source used in the present study. Similarly, Atkinson et al. (2006) observed no differences on OM, CP, and NDF digestibilities when crossbred whether lambs were fed high concentrate diets supplemented with high-linoleate safflower oil at levels of 0%, 3%, 6%, and 9%. However, our results for ADF digestibility disagreed with previous studies that reported that inclusion of fat decreased ADF digestibility (Reddy et al. 2003; Appeddu et al. 2004; Manso et al. 2006). Haddad and Younis (2004) evaluated the effects of protected fat inclusion at 0%, 2.5%, and 5% in high concentrate diets for growing Awassi lambs on nutrients digestibility. They reported that digestibility of DM, OM, CP, NDF, and ADF improved by inclusion of protected fat. These discrepancies between results could be related to the differences in concentrate to forage ratio, fiber content in basal diet, animals' age, and/or source or the level of supplemented fat. The inertness of the protected fat should not affect the digestibility of nutrients throughout the gastrointestinal tract (Manso et al. 2006); however, under some conditions where the fiber digestibility might be adversely affected, the poor digestibility of fiber rather than the fat supplement might have been the cause.

Conclusion

The current study indicates that supplementing lactating ewes with rumen-protected fat at the beginning of lactation phase improves daily milk yield of ewes and pre-weaning growth of their lambs with no major negative impacts on feed intake and nutrients digestibility under the conditions of the present study. More research is needed to evaluate the effects of using different levels of calcium salts in nursing ewes' performance and the use of calcium salts for the whole lactation period rather than the pre-weaning period only.

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