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Milk production, milk composition, liver lipid contents and C18 fatty acid composition of milk and liver lipids in Awassi ewes fed a diet supplemented with protected *cis-9*, *trans-11* and *trans-10*, *cis-12* conjugated linoleic acid (CLA) isomers

F. Husvéth[a,*], E. Galamb[a], T. Gaál[b], K. Dublecz[a], L. Wágner[a], L. Pál[a]

[a] Department of Animal Science and Animal Production, Georgikon Faculty, University of Pannonia, H-8360 Keszthely, Hungary
[b] Department of Internal Medicine, Faculty of Veterinary Science, Szent István University, H-1078 Budapest, Hungary

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ABSTRACT

The effect of a rumen protected form of a conjugated linoleic acid (pCLA) supplement containing approximately equal proportions of *cis-9*, *trans-11* and *trans-10*, *cis-12* isomers (11.8% and 12.1%, wt/wt as free fatty acid equivalents) on milk production, milk composition and liver lipid content; as well as the fatty acid profile of milk and liver in lactating sheep was studied. Fifty multiparous Awassi ewes were randomly divided into two equal groups after parturition and were fed a total mixed diet of corn silage, grass hay and concentrate from d 2 to 42 postpartum. Diet of ewes in the pCLA group was supplemented with 25 g/(d × ewe) of pCLA providing 2.2 g/(d × ewe) of both CLA isomers while sheep in the control group were fed an isocaloric diet supplemented with 21 g of a hydrogenated triglyceride of palm oil (HTG group). Milk yield was measured daily and milk composition was analysed on a weekly basis. Liver total lipid, triglyceride and fatty acid composition was determined from liver samples collected by percutaneous needle biopsy on d 2, d 21 and d 42 postpartum. Higher milk production (1.89 ± 0.13 vs. 1.73 ± 0.25 kg/d) and milk protein yield (96.2 ± 8.7 vs. 84.9 ± 10.2 g/d) and lower milk fat content (56.8 ± 6.2 vs. 62.7 ± 7.1 g/kg milk) was detected in the pCLA sheep than in the HTG group. No differences were observed in the dry matter intake, milk fat yield or milk protein concentration between the two groups. Supplementation with pCLA resulted in lower total lipid (72.6 ± 5.6 vs. 82.0 ± 6.0) and triglyceride (49.1 ± 5.9 vs.60.9 ± 5.7) concentrations in the liver samples collected d 21 postpartum than in the HTG group. The pCLA supplementation increased *c9*, *t11* C18:2 and *t10*, *c12* C18:2, and decreased *c9* C18:1 fatty acid contents in the milk lipids. In the liver lipids pCLA supplementation resulted in higher proportions of C18:0, *c9*, *t11* C18:2 and *t10*, *c12* C18:2 fatty acids and lower *c9* C18:1 comparing to HTG. We concluded that pCLA supplementation, containing *t10*, *c12* isomer could decrease the risk of lipid accumulation in the liver of high-lactating dairy ewes in the postpartum period. The other isomer of pCLA increased the *c9*, *t11* CLA content of milk lipids, which may have favourable health effects for humans consuming sheep milk or milk products.

1. Introduction

Conjugated linoleic acids (CLA) occur in the milk and meat of ruminant animals and have numerous favourable physiological effects. It has been demonstrated that *cis-9*,

* Corresponding author. Tel.: +36 83 545 314; fax: +36 83 545 143.
E-mail address: husveth@georgikon.hu (F. Husvéth).

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trans-11 CLA prevents the development of several tumour types induced by specific environmental effects, such as certain skin tumours (Ha et al., 1987; Belury et al., 1996), mammary tumours (Ip et al., 1991; Thompson et al., 1997) and gastrointestinal carcinomas (Ha et al., 1990). Some experimental results prove that CLA isomers have substantial antioxidant effect (Ha et al., 1990; Ip et al., 1991; Flintoff-Dye and Omaye, 2005). Anti-inflammatory effect of *cis-9*, *trans-11* CLA was also shown in human cells (Jaudszus et al., 2005). Experiments conducted with pigs (Dugan et al., 1997) and mice (Park et al., 1997) have proved that supplementation of the feed with CLA markedly reduces the deposition of fat in the body. The *trans-10*, *cis-12* CLA is associated with diet-induced milk fat depression (Bauman et al., 2008). In lactating ruminants the administration of a supplement containing this isomer can be used as a tool to manipulate milk fat synthesis for reducing the harmful effects of negative energy balance in the critical peripartal period and improving reproductive performance (Moore et al., 2004; Lock et al., 2006; De Veth et al., 2009).

As CLA isomers are intermediate metabolites of hydrogenation processes of polyunsaturated fatty acids (PUFA) in the rumen, feeding diets containing ingredients reach in PUFA, such as oilseeds, vegetable oils (Zhang et al., 2006; Castro et al., 2009) or fresh grass (Atti et al., 2006) increase CLA content of ruminant products. In the recent years chemically synthesized and rumen protected CLA supplements are also available which can be used for the manipulation of milk fat content and fatty acid profile of milk lipids in lactating ewes or goats (Sinclair et al., 2007; Lock et al., 2008). The results of these studies provides support that dairy sheep and dairy cows respond similarly to *trans-10*, *cis-12* CLA supplementation when doses are compared on a metabolic BW basis. To date, there have been no investigations, however, studying the effects of CLA supplementations on liver lipids and liver fatty acid compositions in lactating sheep.

The objective of the present experiments was to study a rumen protected form of CLA supplement containing approximately equal proportion of *cis-9*, *trans-11* and *trans-10*, *cis-12* isomers on the milk production, milk composition and liver lipid content as well as fatty acid profile of milk and liver lipids especially C18 fatty acids in lactating Awassi ewes.

2. Materials and methods

2.1. Animals and their management

The experiment was carried out with 50 multiparous lactating Awassi ewes in the farm of Awassi Co., Bakonszeg, Hungary in compliance with the animal welfare regulations authorized by the County Veterinary Office (protocol #: DK157/2/2007). The experiment was conducted according to a complete randomized design from d 2 to 42 postpartum. The parturition occurred late February 2008. The lambs were weaned immediately after parturition and the ewes were randomly divided into two groups (25 sheep in both groups) on the basis of parity, weight and previous lactation performance. Parity, weight and lambs born per ewes, respectively were as follows; HTG: 4.8 ± 0.4, 75.2 ± 3.8, 1.6 ± 0.5 CLA: 4.6 ± 0.6, 74.9 ± 3.9, 1.5 ± 0.4. Animals were loose-housed in groups on straw bedding in partially covered pens in the same building in concordant environmental conditions. Experimental diets were formulated according to the allowances of NRC (2007) and were supplied as total mixed ration (TMR) twice daily at 7.00 and 19.00 h. Daily amounts of TMR were adjusted to ensure a 10% feed refusal. Ingredients and nutrient composition of TMR are shown in Table 1. Sheep had unlimited access to water and were milked twice a day (6.00 and 18.00 h) by an automated milking system (De Laval International AB, SE-147 21 Tumba, Sweden).

Table 1
Ingredients and nutrient composition of the experimental ration (TMR) fed to the ewes.

Composition	
Ingredients (g/kg DM)	
Corn silage	238
Grass hay	329
Corn grain	188
Wheat grain	91
Sunflower meal	40
Soybean meal	99
Premix[a]	15
Total	1000
Nutrients (% in DM)	
DM	63.1
Crude protein	14.6
NDF	37.5
NE_L (MJ/kg DM)	7.1

[a] Lactating ewe mineral and vitamine premix, Abomix Ltd., Nyíregyháza Hungary; Product No: α HU 033000021.

Before the experimental procedure with the 50 lactating ewes the rumen stability of the CLA supplement used in the experiment was measured by four adult and non lactating ewes surgically supplied with rumen cannula (#8C-Rumen; Bar Diamond, Parma, ID, USA). These animals were kept in free-ranging conditions. However, during the rumen stability test they were housed in individual cages in a shelter and were fed the same TMR described earlier. The length of adaptation period to the TMR was 1 week before sampling.

2.2. Experimental design and treatments

The TMR of ewes in one of the groups was supplemented with 25 g (0.5 MJ NE_L) protected CLA /(day × ewe). This product was manufactured by BASF SE (Ludwigshafen, Germany) and marketed as Lutrell® pure by Arravis Ltd. (Debrecen, Hungary). The supplement was a special complex of *cis-9*, *trans-11* and *trans-10*, *cis-12* CLA isomers and saturated fat of vegetable origin and silicic acid. The pCLA contained 788 g lipid and 212 g ash/kg dry matter. Of the lipid component (as free fatty acid equivalent) 11.8% was the *cis-9*, *trans-11* isomer of CLA, 12.1% was the *trans-10*, *cis-12* isomer of CLA, 16.5% was C16:0, 46.0% was C18:0, 9.8% was C18:1. Supplement provided 2.2 g of both isomers per ewes, respectively on a daily basis. This amount was very similar to that applied by Lock et al. (2008) in a study with lactating ewes. The TMR of the ewes in the other group was supplemented with 21 g of hydrogenated triglyceride of palm oil (HTG; Alifet® ERBO Agro AG, 4922 Bützberg, Switzerland) in the same energy content as pCLA. The HTG contained 951 g fat/kg dry matter (fatty acid composition, g/100 g: C12:0 3.2; C16:0 33.7; C18:0 56.6; C18:1 6.1). At first, both the daily amounts of pCLA and HTG supplements per group were blended with 2 kg of ground corn grain, respectively and added to the TMR of ewes in two equal proportions before feeding. Experimental diets were fed from d 2 to 42 postpartum.

2.3. Collection of samples

Rumen stability of pCLA was measured by the *in situ* method of Ørskov and McDonald (1979), originally developed for determining protein degradation in the rumen and modified in our laboratory to measure the rumen degradability of feed supplements stabilized by saturated vegetable fat encapsulation (Elek and Husvéth, 2007).

In the experiment with lactating ewes samples of TMR were collected weekly for analysis. The amount of feed refused (orts) was recorded daily per group and collected weekly, and sampled for analysis. The dry matter intake (DMI) of the groups was calculated daily by the difference between feed intake offered and feed DM refused. Data were grouped weekly and these weekly subsets were evaluated statistically.