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Hepatic and subcutaneous adipose tissue variations in transition dairy goats fed saturated or unsaturated fat supplemented diets

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ABSTRACT

Biochemical and histological approaches were used to study the metabolic adaptations of transition dairy goats to dietary supplementation with saturated and unsaturated fatty acids. Twenty-three Alpine dairy goats were divided into three groups and fed a basal pre-kidding and lactation diet (C) or the same diet supplemented with fish oil (FO) or stearic acid (ST) starting 1 week before kidding until 21 days in milk (DIM). No differences were observed in milk production and composition. However, the serum non-esterified fatty acid (NEFA) and beta-hydroxybutyrate (BOHB) concentrations were changed over time by the treatments. The mean adipocyte area, measured on a subset of 12 goats, which included four subjects from each experimental group, decreased constantly in the C and ST groups from −7 to 21 days, while the FO group did not change between days 7 to 21. These results support the idea that FO is able to limit lipolysis, although the energy balance is still negative. No inflammatory processes were observed in the liver in accordance with the blood leukocytes trend, even if moderate to severe fatty changes in the liver were observed in the experimental goats. In the FO group, however, fatty infiltration appeared more severe and it occurred more gradually compared with the other diets. Overall, these results suggest an interesting ability of dietary lipid supplements to affect the fat mobilizing machinery; FO in particular seems able to reduce/delay fat mobilization and could improve hepatocyte adaptation to fatty infiltration, allowing the cells to better maintain their function.

1. Introduction

The regulation and coordination of lipid metabolism amongst adipose, liver and mammary gland tissues are key components of lactation adaption in dairy species (Chilliard, 1999). The most characteristic event during the transition period is the reduction in feed intake just when there is a very high nutrient demand for the developing conceptus and lactogenesis (Drackley, 1999). The conjunction of these factors can lead the ruminant to experience a negative energy balance (NEB) particularly before parturition and at the beginning of the lactation period, when it is almost impossible to meet the extra energy requirements needed for fetal growth before and milk production after.

In such conditions, goats mobilize fatty acids from adipose tissue reserves to compensate for the lack of glucose and fatty acids. This mechanism leads to an increase in circulating concentrations of NEFA during the late pregnancy and postpartum periods (Magistrelli and Rosi, 2014). The NEFA liver metabolic pathways are related to energy and ketone body production, or to the secretion of very low-density lipoproteins *via* triacylglycerol (TAG) conversion. If the TAG formation overcomes the liver secretion capacity, then their accumulation results in a so-called fatty liver syndrome (Herdt, 1988).

The partial replacement of grains or forages in rations with fat sources, such as n-3 polyunsaturated fatty acids (PUFAs), can considerably increase the energy level of the diet, and may enhance energy intake if the dry matter intake (DMI) is not depressed (Staples et al., 1998): as a result, the energy balance might be improved in early lactating dairy ruminants (Ballou et al., 2009). Moreover, some studies stated that higher supplemental levels of fat might increase the risk of peripartal lipid accumulation in the liver of dairy animals (Douglas et al., 2004). Some metabolites and metabolic hormones are well-recognized signals in the interaction between NEB and metabolic disorders in dairy goats (van Knegsel

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et al., 2007). Serum calcium, NEFA and BOHB contents are frequently used to evaluate the adaption to NEB during the peripartum period (McNamara et al., 1995; Kokkonen et al., 2005). As a result of NEB adaptation, significant lipid mobilization from subcutaneous adipose tissue leads to progressive body mass loss (Chilliard, 1999).

Fish oil has often been supplemented in dairy animals with the objective of enriching animal products with essential fatty acids considered healthy, particularly for the human cardiovascular system (Calder, 2013). Moreover, dietary fat is no longer just classified as an energy source because specific fatty acids have peculiar roles in lipid metabolism and organismal defense systems in food-producing animals (Tsiplakou and Zervas, 2013a,b). In fact, not only have eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) been demonstrated to be contained in dairy goats but their ability to influence immune and inflammatory responses has also been as observed *in vivo* (Bronzo et al., 2010) and *in vitro* (Pisani et al., 2009; Lecchi et al., 2011, 2013). Additionally, saturated fatty acids, such as palmitic and stearic acids, can affect lipid metabolism in dairy ruminants (Chilliard, 1993; Thering et al., 2009; Agazzi et al., 2010).

The increase in the adipocyte diameter can be used as an indicator of lipogenic activity under different metabolic challenging conditions, such as undernourishment or breeding (Alzon et al., 2007; Faulconnier et al., 2007). Currently, data regarding the effects of highly unsaturated or saturated fats on liver and adipose tissue histology in dairy goats are limited. The main aim of this study was to evaluate the effects of saturated or unsaturated fat supplements on metabolic adaptations in periparturient dairy goats with biochemical and histological approaches, and in particular, focusing on changes at the liver and subcutaneous adipose tissue levels.

Table 1
Ingredients and chemical composition of the experimental diets of the dairy goats fed either a basal diet (C) or a diet supplemented with fish oil (FO) or stearate (ST).

	Experimental diets					
	Pre-kidding			Post-kidding		
	C	FO	ST	C	FO	ST
Ingredient (%)						
Alfalfa hay	0.0	0.0	0.0	31.2	29.8	30.7
Mixture hay[2]	62.3	59.6	61.4	15.3	14.6	15.1
Concentrate mixture[1]	31.9	30.5	31.4	46.8	44.8	46.2
Corn meal	5.3	5.0	5.2	6.2	5.9	6.2
Fish oil	0.0	4.4	0.0	0.0	4.3	0.0
Calcium Stearate	0.0	0.0	2.0	0.0	0.0	1.9
$CaCO_3$	0.5	0.5	0.0	0.5	0.5	0.0
Chemical Composition (% of dry matter)						
Dry Matter (%)	88.4	88.7	88.6	89.3	89.5	89.4
Crude Protein	12.3	11.9	12.2	17.8	17.2	17.5
Ether Extract	2.9	4.9	4.5	3.2	5.2	4.8
NDF	43.9	43.8	43.3	33.7	34.0	33.2
Ashes	6.3	6.5	6.0	7.2	7.3	6.8
Ca	0.8	0.8	0.9	1.1	1.1	1.2
P	0.4	0.4	0.4	0.8	0.8	0.8
NE_L (Mcal/kg DM)[3]	1.61	1.66	1.67	1.67	1.72	1.72

[1] The concentrate mixture was a commercial dairy goat mixed feed, chemical composition: 22.25% crude protein, 5.00% ether extract, 22.98% neutral detergent fiber, 6.51% ashes, 1.28% Ca and 0.76% P (on dry matter basis).

[2] The mixture hay was a grass hay, chemical composition: 7.6% crude protein, 1.8% ether extract, 57.5% neutral detergent fiber, 5.9% ashes, 0.6% Ca and 0.2% P (on dry matter basis).

[3] Net energy of lactation concentration of the diets were calculated using the Small Ruminant Nutrition System (SRNS) software (Tedeschi et al., 2010).

2. Materials and methods

2.1. Animals and diets

The present study was performed at the Animal Production Research and Teaching Centre of the Polo Veterinario of the Università degli Studi di Milano (Lodi, Italy), and the protocol was approved by the Ethics Committee of the Università degli Studi di Milano (attachment n. 5 January 26th, 2011). Twenty-three spring kidding Alpine goats (1.26 ± 0.45 kidding, 28.05 ± 6.15 months of age, 3.12 ± 0.33 kg of milk/d) were divided using a randomized complete block design in an attempt to achieve three homogenous groups for parity, age and milk production, per their previous lactations, and assigned to three experimental treatments. The goats were housed in individual 4.56 m^2 straw bedded boxes with free access to water and were individually fed. After kidding, each goat shared the box with their relative suckling kids (on average 1.83 kids/goat, weighing 4.18 ± 0.23 kg); however, the feeder was set out of reach of the kids. A conventional pre-kidding or a post-kidding basal diet was offered to all the experimental animals in the three groups. The diet ingredients and chemical compositions are detailed in Tables 1 and 1S. The pre-kidding daily basal diet consisted of *ad libitum* mixed grass hay (refusal weight of at least 10%), 600 g/head of concentrate and 100 g/head of corn meal. Post-kidding, the daily basal diet was composed of *ad libitum* alfalfa hay and mixed hay (refusal weight of at least 10%), 1500 g/head of concentrate and 200 g/head of corn meal. The concentrates were provided separately from the forage during the entire trial, and calcium carbonate was added to balance calcium content in all diets as follows: a) Control (C; n = 8 goats), animals were fed the basal pre- or post-kidding diet plus calcium carbonate (9 g/day during pre-kidding period, 12 g/day after kidding); b) Fish oil (FO; n = 8 goats), animals were fed the pre- or post-kidding basal diet plus calcium carbonate (9 g/day during pre-kidding period, and 15 g/day after kidding) and 30 g/day of fatty acids (81 g/day of supplement) before kidding or 50 g/day of fatty acids (135 g/day of supplement) during lactation from a rumen-inert fish oil (10.4% EPA and 7.8% DHA; Ufac Ltd., Stretton, UK); c) Calcium stearate (ST; n = 7 goats), animals were fed the pre- or post-kidding basal diet plus 30 g/day of fatty acids (34 g/day of supplement) before kidding or 50 g/day of fatty acids (56 g/day of supplement) during lactation from stearic acid (C16:0 26% and 69.4% C18:0; Brenntag S.p.a., Milan, Italy). All the daily diets were vitamin E supplemented to supply 72 mg/head during the pre-kidding period and 80 mg/head after kidding. The pre- and post-kidding dietary treatments in the three groups were designed to provide similar crude protein (CP) and calcium contents. The fat-enriched treatments (FO and ST) contained similar ether extracts (EE). The dietary supplements were stored in the dark at room temperature. All goats were fed concentrates and corn meal twice a day, and the fat supplementation was provided in the morning meal mixed into 50 g or 100 g of corn meal during the pre- or post-kidding periods, respectively. Stearic acid is preferred over palmitic acid as a positive control treatment because it is considered more neutral. Indeed, in a previous trial, palmitate showed a strong effect on lipid metabolism in the liver, increasing expression of peroxisome proliferator-activated receptor-α (*PPARA*), acyl-coenzyme A dehydrogenase very long chain (*ACADVL*) and carnitine palmitoyl-transferase 1A (*CPT1A*) at 21 days after kidding (Agazzi et al., 2010).

2.2. Dry matter intake, live body weight, energy balance, milk yield and composition

Individual DMI was assessed weekly until 21 days after kidding as the difference between the feed dry matter (DM) offered and the feed DM refused. The individual live body weight (LBW) was assessed at 7 days before kidding and at 7, 14 and 21 days of lactation by an electronic scale (F.lli Fascina snc, Castelvetro P.no, Italy). On a daily basis, goats were milked once a day at 8:00 a.m. To allow milk yield recording and milk samples collection, once a week, the suckling kids were separated from the mothers for two consecutive