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Dietary saturated and unsaturated fatty acids on hepatic and subcutaneous tissues intermodulation, gene expression in transition dairy goats, and influence of maternal diets on kids' immune response

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CHAPTER 1

Foreword

1.1 Worldwide goat population

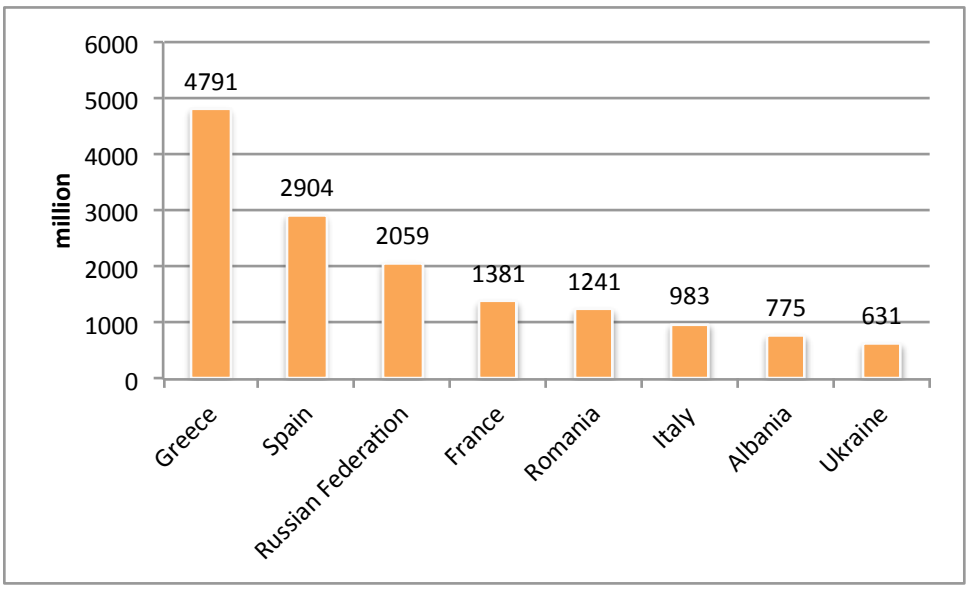
In the recent years, the importance of small dairy ruminants has increased significantly. First of all because they are an interesting and important alternative for the supply of dairy products for human consumption, whereas they are considered as a healthier alternative to cows' milk with significant organoleptic properties, either for direct consumption (e.g. Northern Europe) or cheese-making (e.g. Mediterranean basin)(Lerias et al. 2014). Even if the interest in small ruminants it's not yet well understood, goats are disseminated all around the world. This was possible due to their ability to adapt to varying environmental conditions and to different nutritional regimes. Their productivity, small size, independence and tolerance to many diseases and parasites (that have made themselves much better able to adapt than other livestock species) were the main features for which they have been revalued.
The importance of the valuable genetic resource is underestimated; they are often neglected in comparison with cow.
Nevertheless, goats are going to be more important source of livelihood for many more people in coming years and for an ever-growing number of consumers demanding healthy food, thus, they deserve greater attention at both the micro and macro levels. Therefore is the important to consider and pay attention to the value and capacity of goats for producing food.
According to FAO statistics, in 2011 there were over 875.529 million heads of goats

Continent	2000	2002	2004	2006	2008	2010	2011	2011 compared to 2000 (%)
Europa	18.941	18.214	18.635	17.886	17.819	17.115	17.072	-9.87
America	34.935	36.187	37.088	38.067	37.507	37.595	37.678	7.85
Africa	236.624	254.741	272.066	284.98	301.222	312.448	276.684	16.93
Asia	458.521	463.395	484.398	499.138	516.925	537.766	539.178	17.6
Oceania	2.396	3.219	3.372	4.197	3.618	4.923	4.917	105.22
Total	**751.417**	**775.756**	**815.559**	**844.268**	**877.091**	**909.847**	**875.529**	**16.52**

Table 1: The evolution of the worldwide goat population, by continents (thousand heads).

An increase of 16.52% of the goat population in the world was registered in 2011, while a fell to 9.87% in Europe in the same period. According to FAO, in 2011, China, Pakistan and Nigeria were the top countries in the world with the biggest goat population (18.9%, 8.2% and 7.6% of total respectively). In Europe the largest goat populations were in Greece (4.791 million head), followed by

Spain (2.904 million head), Russian Federation (2.059 million head) and France (1.381 million heads), as shown in graphic 1:



Graphic 1: European countries top regarding the goat population in 2011 (FAOSTAT, 2011).

1.2 Transition period

The transition period, also know as peripartum, has been defined as the 3 weeks before to 3 weeks after parturition (Grummer 1995; Drackley 1999).
The term transition is to underscore the important physiological, metabolic and nutritional changes occurring in this time frame (Block 2010). The manner in which these changes occur and how they are managed are of great importance as they are closely linked to lactation performance, clinical and subclinical postpartum diseases, and reproductive performance that can significantly affect profitability.
Peripartum is a period marked by changes in endocrine status to accommodate parturition and lactogenesis. The most characteristic event during the transition period is the reduction in feed intake during a moment of very high nutrients demand for the fetal growth and lactogenesis (Drackley 1999). These circumstances can lead ruminants to experience a negative energy balance (NEB) particularly before parturition and at the beginning of the lactation period. A number of profound physiologic changes occur in the transition that modifies the metabolism drastically. The decrease in prepartum DMI (attributed to the rapid growth of the fetus taking up abdominal space and displacing rumen volume) (Block 2010); the rapidly increasing demands of the fetus and the development of the mammary glands (including the initiation of synthesis of milk components); etc. The hormonal changes not only contribute to the decline in DMI, but also coordinate the metabolic changes that favor the mobilization of body fat reserves from adipocytes (Grummer 1995) (Figure 1). The result of this mobilization of lipids is an increase in concentration of plasma non-esterified fatty acids (NEFA), that is obligatory and under hormonal control (Grummer 1995; Bertics and Grummer 1999), this is a normal and required process to help the cow meet her energy demands for lactation. However, when the quantity and/or the speed of mobilization are exaggerated, the incidence of metabolic problems increases significantly. Fat, or lipid, stored in adipose is in the form of triglycerides (TG) that are mobilized as NEFA plus the glycerol backbone of the triglyceride. NEFA follow one of 3 desirable fates: 1) to be utilized by the mammary gland for milk fat synthesis; 2) to be used by peripheral tissues as a source of energy; 3) to be reesterified by the liver into triglycerides and exported as triglycerides incorporated into very low density lipoproteins (VLDL). Ruminant liver possesses a low capacity for TG export VLDL (Kleppe et al. 1988), but they large increases secretion would be needed to greatly affect liver TG accumulation during the periparturient period. Once deposited, the fat accumulated in the liver will remain there until the end of the NEB (Grummer 1993).

Depending on its severity, NEB, leads in ketosis and strongly influences reproductive system and immunological status (Drackley 1999; Duffield 2000).
An improved understanding of the inflammatory pathways at the molecular level which play an important role in normal immune function, metabolism and reproduction may improve our ability to predict and prevent transition disorders (Esposito et al. 2014) (Figure 2)..
The magnitude of the NEB prepartum, therefore, appears to be a variable that can be mitigated through nutritional management.

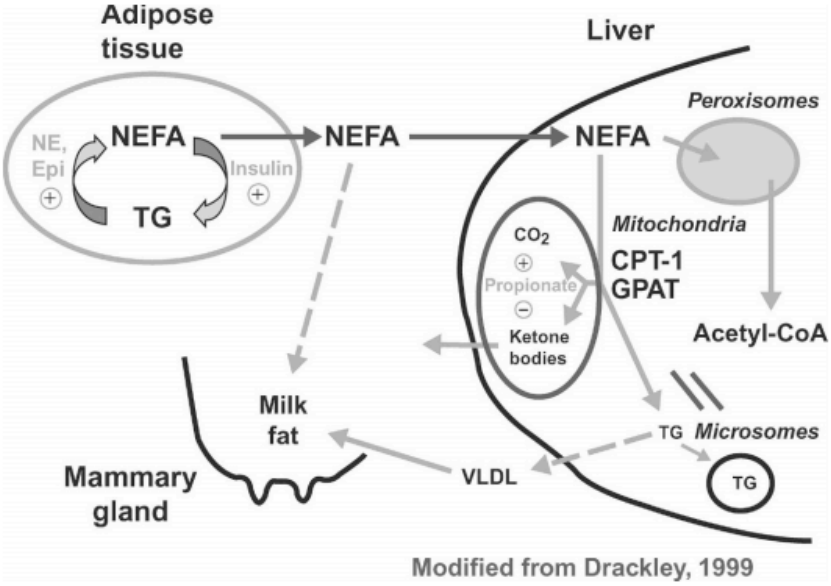


Figure 1: Schematic of metabolic relationships among adipose tissue, liver, and mammary gland during the transition period; NE = norepinephrine, Epi = epinephrine, CPT-1 = carnitine palmitoyltransferase-1, GPAT = glycerol-3 phosphate acyltransferase, TG = triglyceride, CoA = coenzyme A, VLDL = very low density lipoprotein (Drackley et al. 2006).

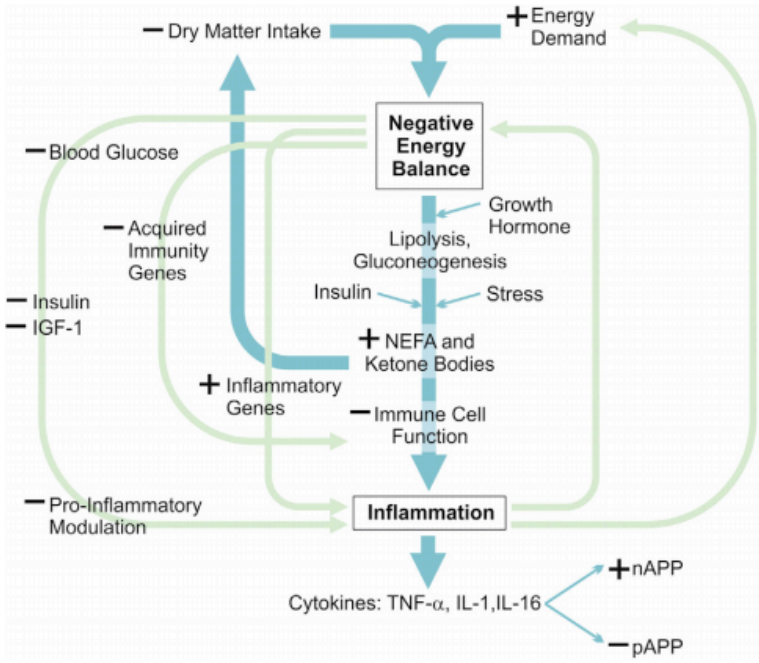


Figure 2: Major interactions between the immune, endocrine and metabolic systems in ruminants during the transition period. Inflammation postpartum, high cellular metabolism and upregulated immune gene expression increase energy requirements when there is a reduced dry matter intake. The symbols + and - indicate an increase (+) or a decrease (-) in biomolecule levels. NEFA, non esterified fatty acids; IGF-1, insulin-like growth factor 1; TNF-α, tumor necrosis factor α; IL-1 and IL-6, interleukin 1 and 6; nAPP and pAPP, negative and positive acute phase proteins (Esposito et al. 2014).

1.3 Definition, nomenclature and properties of fatty acids

The term fatty acid (FA) is used to indicate any one of the aliphatic monocarboxylic acids that can be liberated by hydrolysis from naturally occurring fats and oils (Fahy et al. 2009). FAs play multiple and essential cellular roles:

- Energy production,
- Membrane structures,
- Immune cell regulation,
- Cell signaling,
- Gene expression and regulation (Davidson and Cantrill 1985; Lai et al. 2005).

The FA is called "saturated" (SFA) (e.g. palmitic acid 16:0, arachidic acid 20:0) if there is an absence of bouble bond, or "unsaturated" (UFA) if there is a presence of double bond; "monounsaturated" (MUFA) if there is just one double bond or, if there are seveveral bonds it is called "polyunsaturated" (PUFA) (Davidson and Cantrill 1985; Ratnayake and Galli 2009).
The MUFA group, represented by the oleic acid (18:1), can be obtained by biotransformation of SFAs (Menaa et al. 2013).
The PUFA group is divided into two major families:

- Omega-3 such as linolenic acid 18:3, EPA 20:5 or DHA 22:6;
- Omega-6 such as linoleic acid 18:2 or arachidonic acid 20:4 (Davidson and Cantrill 1985; Ratnayake and Galli 2009).

PUFAs can be converted into longer unsaturated carbonated FAs chain by elongations and desaturations (e.g. linolenic acid 18:3 can be converted into EPA 20:5 which, in turns, can be converted into DHA 22:6) (Fig. 3).

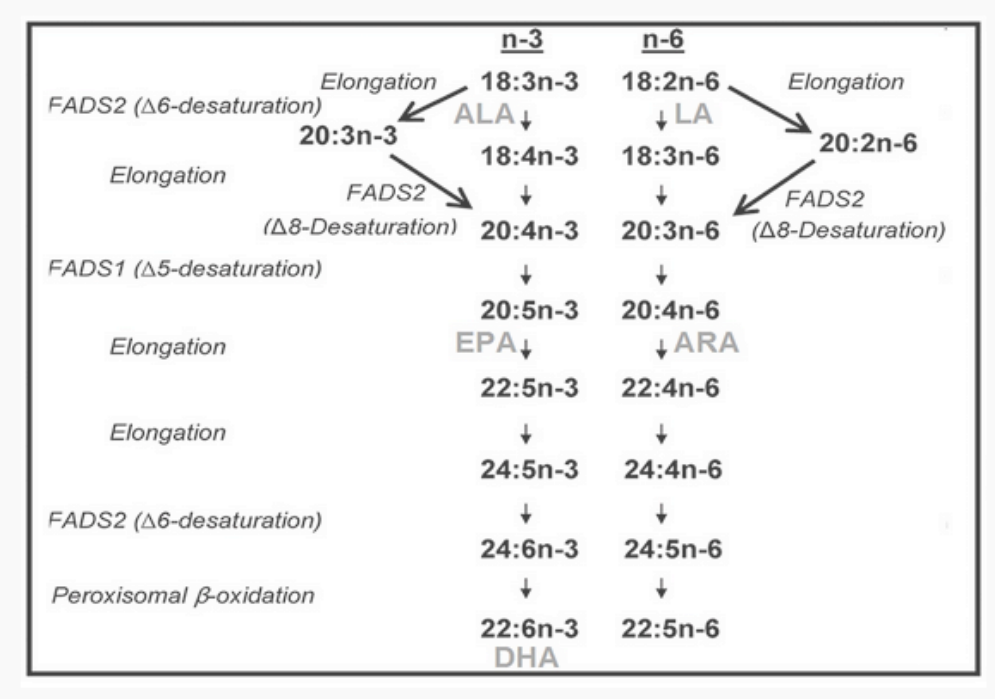


Figure 3: Endogenous pathway for LC-PUFA synthesis DHA, EPA and ARA by enzymatic desaturation and chain elongation steps (Rizzi et al. 2013).

The FAs have a *–cis* configuration when the two hydrogen atoms are on the same side of the carbon chain with respect to the double bond, whereas in the configuration *-trans,* the two atoms of hydrogen are diagonally opposed to each other, straightening the carbon chain.

Naturally occurring FAs usually have the *cis*-configuration. Nevertheless, under certain conditions (e.g. partial catalytic hydrogenation or enzymatic hydrogenation), a double bond in FAs may change from a *-cis* to a *-trans* configuration and/or move to other positions in the carbon chain (Sebedio 2007; Ratnayake and Galli 2009) (Fig. 4).

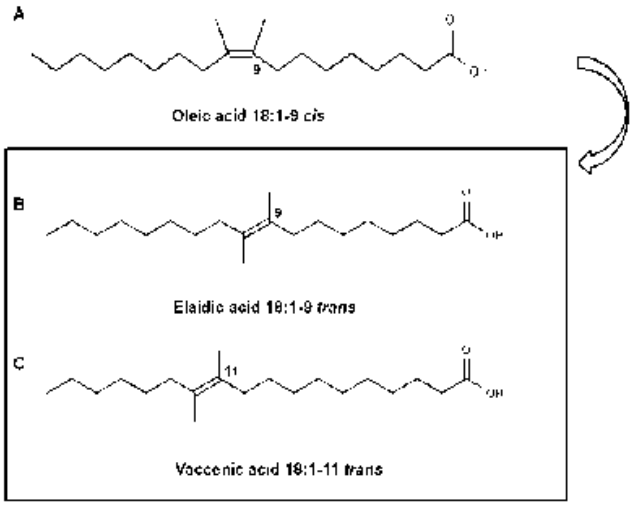


Figure 4: Isomers of the oleic acid (Block 2010)

The double bonds of PUFAs can be found in configuration trans or cis/trans, eg linoleic acid (18:2 9c , 12c) (Fig. 5).

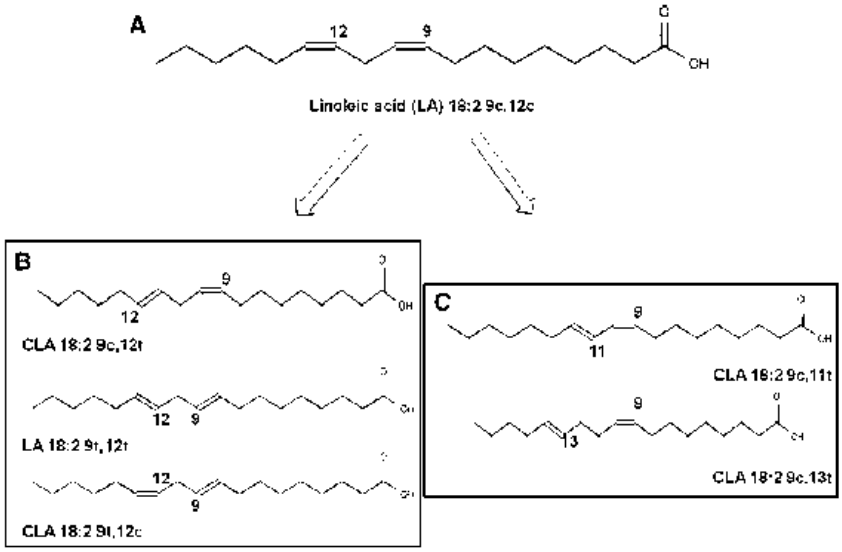


Figure 5: Isomers of the linoleic acid (Block 2010).

In the group of natural conjugated linoleic acid (CLA), the isomers, 18:2 9c, 11t, is always the major isomer formed during microbial biohydrogenation of linoleic acid (LA). Interestingly, CLA is a fatty acid that is currently receiving considerable attention because of a range of properties that may make a positive contribution to health at moderate doses.

The biohydrogenation mainly results from the enzymatic transformation of *cis*-

FAs into TFAs by the bacterial flora (microbiome) present in the rumen of animals (e.g. cows, cheeps, goats) (Sommerfeld 1983). They also can be produced by mammalian breasts through the action of the D 9-desaturase (Griinari et al. 2000; Mosley et al. 2005; Kraft et al. 2006).

1.3.1 Biohydrogenation of dietary lipids

In the rumen, dietary unsaturated lipids undergo hydrogenation, which is characterized in two steps: hydrolysis of ester bonds by microbial lipases resulting in the release of FA; and conversion of UFA through biochemical progress into SFA by microbial activity (Jenkisn 1994; Lourenco et al. 2010).
In the rumen, dietary lipids are subject to hydrolysis by microbial lipases followed by biohydrogenation of the unsaturated free fatty acids by rumen bacteria (Or-Rashid et al. 2007). The result is not only the production of 18:0 but also a wide range of isomers of PUFA and MUFA, especially trans and conjugated FA (Harfoot and Hazlewood 1997; Palmquist et al. 2005; Chilliard et al. 2007). Microorganisms convert oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) into stearic acid (C18:0) (Kellens et al. 1986; Lock and Bauman 2004) (Fig.6). The biohydrogenation progresses with isomerization of UFA, including conversion of the isomer *cis*- to a *trans* stereotype.
In the rumen, dietary linoleic acid (9c12c-18:2) is converted to 9c11t- CLA and 10t12c-CLA by isomerization (Harfoot and Hazlewood 1997; Kim et al. 2002; Harvatine et al. 2009). After forming the trans-11 isomer further hydrogenation of the cis-9 bond in C18:2 takes place by the enzyme reductase and a result trans-11 C18:1 is formed. Further hydrogenation of trans-11 C18:1 into C18:0 can also occur (Jenkins 1993). However, the rate of this step depends on the rumen environment and type of microorganisms (Kellens et al. 1986).
Ruminant animals harbor large populations of microorganisms in the rumen, such as bacteria, protozoa, and fungi, and are dependent upon fermentation of feed constituents by these microorganisms.
The 10t12c-CLA isomer derived from the rumen is usually found in small quantities in dairy products (Odongo et al. 2007; Or-Rashid et al. 2008b). Minor isomers of CLA, such as 11c13t, 12c14t, 11t13c, 7t9t, 8t10t, 9t11t, 10t12t, 11t13t, and 12t14t have also been reported in rumen contents (Shingfield et al. 2003; Loor et al. 2005b). However, there are no reports on how these minor CLA isomers are formed. Some other isomers of 18:1 monoenes (e.g., 4t, 5t, 6-8t, 9t, 12t, 13-14t, 15t, 16t, 12c, 13c, and 15c) also accumulate in rumen contents (Shingfield et al. 2003; Loor et al. 2005b). These intermediates of ruminal biohydrogenation vary largely with changes in diet composition, as demonstrated

by their appearance in milk fat under various conditions (Kraft et al. 2003; Shingfield et al. 2006).
Other factors affecting the biohydrogenation are: feed composition, feeding level and frequency, dietary FA level and composition (Jenkins 1993; Harfoot and Hazlewood 1997).

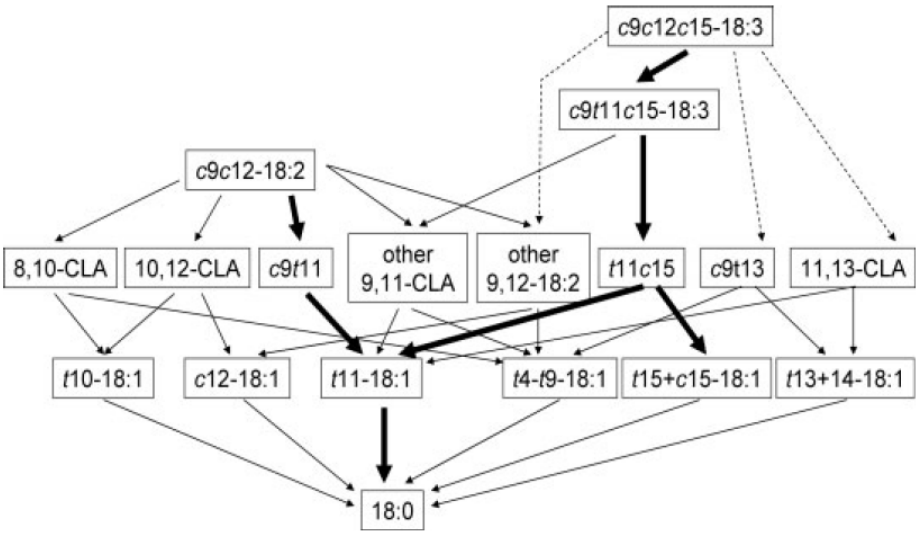


Figure 6: Main putative rumen biohydrogenation pathways. Thick arrows represent the major pathways (Harfoot and Hazlewood 1997) thin arrows represent other putative pathways, dotted arrows represent RBH pathways including unknown 18:3 isomer intermediates. Not all putative FA are mentioned, and the numerous interconversions among 18:1 isomers (Mosley et al. 2002; Proell et al. 2002).

Some intermediates that are not absorbed in the gut and directly secreted into milk, are transformed by body tissues, especially by the mammary gland where the D-9 desaturase acts by adding a cis9-double bond on different FA (Harfoot and Hazlewood 1997; Bauman et al. 1999; Griinari and Bauman 2006), which partly reverses the effect of rumen biohydrogenation and decreases the saturation level and the melting point of milk fat (Chilliard et al. 2000) (Fig. 7).
Furthermore, ruminal biohydrogenation intermediates act as regulators or disruptors of mammary lipogenesis, which results in changes in the amount of secreted milk fat but also in milk FA composition (Bauman and Griinari 2003), including short- and medium chain *de novo* synthesized FA.
In the end, not absorbed PUFA and 18:0 produced in the rumen are the residual precursors and the endproduct of rumen biohydrogenation. Thus, rumen biohydrogenation modifies all milk FA and plays a pivotal role in the interaction

between ruminant diet and mammary FA synthesis and secretion.

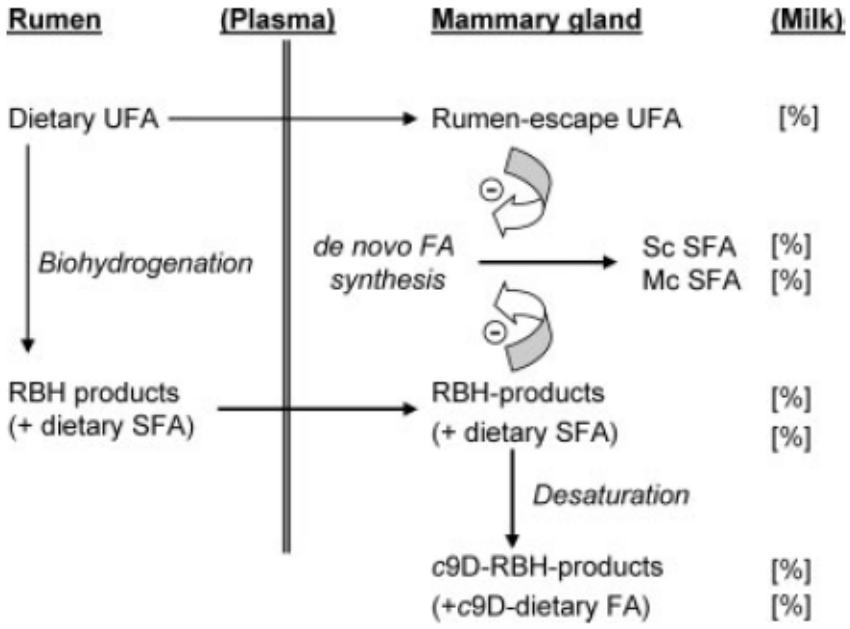


Figure 7: Schematic relationships between rumen biohydrogenation and milk FA composition. Sc, Mc = short-, medium-chain; c9D = cis9-desaturated; [%] = changes in milk FA concentrations (g/100 g total FA), as a result of changes in the flows of the different FA (Chilliard et al. 2007).

1.3.2 Saturated and/or unsaturated fatty acids in ruminants diet

The principal energy source in a diet is carbohydrates and fats. Including fat to the ruminants' diet is a common method to increase the energy density of the diet (Grummer 1995), improve energy balance, feed efficiency, yields milk and milk fat percentage (Rabiee et al. 2012). It has been speculated that feeding supplemental fat would reduce fatty acid mobilization from body stores and reduce liver fat accumulation (Kronfeld 1982). Fats in the diet are incorporated into lipoproteins in the intestine and absorbed into the circulation. As they are already in the form of lipoproteins they can be used directly by the tissues and not contribute to fat accumulation in the liver. Fatty acids, in fact, have been recognized to be biologically active molecules that can regulate gene expression, enzyme activities, binding proteins, and other cellular processes (Sessler and Ntambi 1998). Fats are classified as lipids, which are biological compounds that are soluble in organic solvents. Lipids include cholesterol and fats such as

triacylglycerols and phospholipids. Phospholipids are the major components of cellular membranes, and are a source of fatty acids for the synthesis of a variety of effector molecules such as the eicosanoids, a group of compounds that includes prostaglandins, thromboxanes and leukotrienes. Cholesterol is another component of the cellular membrane and is the precursor for the synthesis of steroid hormones (Mattos et al. 2000).

The effects of individual FA have not yet been adequately studied to successfully allow for the identification of specific FA that maximize the yield of milk and milk components (Rico et al. 2014). The concentration and yield of milk fat can be increased by feeding SFA supplements to dairy cows, compared with both control (non-added-fat diets) and unsaturated FA supplements (Relling and Reynolds 2007). Even though the effects of SFA supplements have been reported in numerous studies, it is still unclear what effects individual SFA have, with only a limited number of studies available that used pure sources of individual SFA, such as palmitic (C16:0) and stearic (C18:0) acids (Steele and Moore 1968; Enjalbert et al. 1998). There is a lot of research focused on studying the effects of C 16:0 (Mosley et al. 2007; Agazzi et al. 2010; Lock et al. 2013; Piantoni et al. 2013). Information on the effects of feeding C18:0 is much more limited, with only a few studies available (Steele and Moore 1968; Steele 1969; Enjalbert et al. 1998).

Palmitic (C16:0) and stearic (C18:0) acids have unique and specific functions in lactating dairy cows beyond a ubiquitous energy source.

Stearic acid is an 18-carbon SFA denoted as n-octadecanoic acid with a chemical formula of CH3(CH2)16CO2H. Michel Eugene Chevreul first described C18:0 in the early 1800s (Lemay 1948). Stearic acid is a prevalent FA in nature, found in many animal and vegetable fats, but is usually higher in animal fat than vegetable fat. It has a melting point of 69.4°C and a pKa of 4.50 (Sukhija and Palmquist 1990).

Stearic acid is the most abundant FA available to the dairy cow and is used to a greater extent for milk production and energy balance than C16:0.

Stearic acid was the only FA where the amount flowing from the rumen was greater than amount fed. In all diets, the amount of C18:0 flowing into the duodenum was higher than for any other FA (Glasser et al. 2008; Loften et al. 2014). Loor et al. (2004) also reported an amount 25 times higher into duodenum compared with the amount fed when the animal fed a high or low concentrate diet where C18:0 was only 2.5% of the total fatty acids.

Studies by Doreau and Ferlay (1994), Sauvant and Bas (2001) and Schmidely et al. (2008) estimated that ± 10g of FA/kg of DM intake flowing into the duodenum was of microbial origin, and two-thirds of the microbial FA was from the synthesis of C16:0 and C18:0.

Other studies (Doreau and Ferlay 1994; Lock et al. 2006; Glasser et al. 2008)

concluded that mean digestibilities of C16:0 and C18:0 were 77.1 and 76.3%, respectively. Schmidely et al. (2008) also found that intestinal digestibility of FA tended to increase with increasing chain length.
C18:0 does not accumulate in tissues of cows in negative energy balance and cows metabolize C18:0 for energy (e.g., β-oxidation) in the liver and muscle or secrete large proportions of C18:0 through milk as both C18:0 and C18:1 (Loften et al. 2014).
Feeding supplemental C16:0 can increase body tissue reserves of cows when fed during positive energy balance, but the energy stored will be in the form of C18:0 rather than C16:0. Most of the C18:0 in adipose tissue likely comes from elongation of C16:0 and not from direct uptake and esterification of C18:0 into TAG. The preferred substrate for synthesis of TAG is C16:0, whereas C18:0 itself is a poor substrate for TAG synthesis in adipocytes (Sampath and Ntambi 2005).
During years, attention has been paid to the digestibility of different fat sources. Fat sources that are high in stearic acid (i. e., hydrogenated fats) have a lower digestibility than the unsaturated C:18 fatty acids, but Harvatine and Allen (2005) reported a greater increase in milk protein yield in high-producing cows relative to low-producing cows in response to a saturated fat supplement compared with an unsaturated fat supplement. A reduced digestibility implies that the energetic value of different fats will differ based on their degree of hydrogenation and their ability to be biohydrogenated in the rumen (i. e., free fats vs. calcium salts of long chain fatty acids). Obviously, the lower the digestibility of the fatty acids, the less chance there is to improve energy balance or reduce lipolysis (Block 2010). There are different indications that saturated and unsaturated fatty acids will cause different blood and liver profiles in fat metabolism.
Dairy cow's milk products generally contain 0.5–0.9% total CLA of total fatty acids, with the 9c11t-CLA isomer accounting for at least 80.0% of the total CLA (Collomb et al. 2001; Luna et al. 2005; Odongo et al. 2007). This concentration can be increased several fold with supplementation of CLA-enhancing feeds, but total milk fat content drops significantly in most cases (Bauman et al. 2000; Donovan et al. 2000; Wright et al. 2007). The c9-t11 18:2 isomer CLA has been demonstrated to have potentially healthy properties. In fact, in a study by Marin et al. (2012) the supplementation of linseed or sunflower oil confirmed to have more vaccenic and rumenic acids in milk.
In addition, the inclusion of fish co-products in the diet of ruminants also can increase the concentration of 9c11t- CLA and 11t-18:1 in duodenal or omasal contents compared with control diets (Shingfield et al. 2003; Loor et al. 2005b), which would subsequently increase the concentration of these healthful fatty acids in ruminant origin food products. The possible mechanism for this may be that DHA from fish co-products and/or their derivatives inhibit the reductase

enzyme within certain rumen microorganisms (e.g., Butyrivibrio fibriosolvens and Megasphaera elsdenii), causing the increased accumulation of trans-18:1 and CLA within the rumen (AbuGhazaleh and Jenkins 2004). Several nutritional and technological strategies for animals have been designed and implemented to enhance n-3 VLCPUFA delivery post ruminal over the years, with varying degrees of success. Some of the methodologies and reasoning for nutritional manipulation have been reviewed (Demeyer and Doreau 1999; Jensen 2002; Lock and Bauman 2004; Wright et al. 2007; Kęsek et al. 2014), and therefore, will not be discussed in details here. Research of n-3 PUFA enrichment have been designed for diary cow but there are also similar investigations of n-3 VLCPUFA enrichment with sheep (Kitessa et al. 2001; Bichi et al. 2013; Clayton et al. 2014; Ferreira et al. 2014; Gallardo et al. 2014) and goats (Chilliard et al. ; Cattaneo et al. 2006; Marin et al. 2011; Marin et al. 2012; Ghazal et al. 2014a; Toral et al. 2014).

The most important nutrients used for incorporation of DHA into animal diet are fishmeal, fish oil (Najafi et al. 2012; Ferreira et al. 2014; Toral et al. 2014), or algae (Franklin et al. 1999; Papadopoulos et al. 2002; Or-Rashid et al. 2008a; Or-Rashid et al. 2008b; Moreno-Indias et al. 2012; Bichi et al. 2013).

Every single study have been used to evaluate as the administration of n-3 FAs can change milk composition and quality, oxidative stability and so on.

Among different dietary sources of n3-PUFAs, fish oil seems to be the most interesting for livestock nutrition. The long-chain n-3 PUFAs contained in marine oils, mainly eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), have been shown to be essential in mammals species for normal growth and development, leading to nutritional and health beneficial actions. In particular, EPA has revealed anti inflammatory properties as therapeutic agent for inflammatory and auto immune diseases (Calder 2001). On the other side, a specific functional role for DHA in fetal and early infant neuronal development has been recognized (Hornstra 2000). Maternal requirements for DHA increase during pregnancy and lactation, and DHA status of the mother and the newborn may be sub-optimal if maternal intake is insufficient. Studies by (Simopoulos 2002) recommend a total daily intake of about 650 mg of DHA plus EPA in adults, while pregnant and lactating woman should ingest 300 mg/day of DHA. Unfortunately, ruminant tissues cannot synthesize n-3 PUFAs. The presence of these compounds in milk fat depends from the dietary amounts that escaped rumen biohydrogenation and have been absorbed in the small intestine, but still the extent to which long chain n-3 PUFAs are transferred from dietary fish oil to ruminants' milk fat is still debated. A study by Cattaneo et al. (2006), demonstrated that the administration of fish oil to periparturient dairy goats resulted in an apparent transfer efficiency from dietary fish oil to mature milk of 14% for EPA and 7% for DHA. In a study by

Agazzi et al. (2010) apparent transfer rates of n-3 PUFAs from dietary rumen protected fish oil into milk for EPA (7.01%) and DHA (8.14%) were similar to values reported by Gulati et al. (1999) in dairy goats fed protected fish oil (7% EPA, 8.16% DHA); but lower than results observed in previous study (Cattaneo et al. 2006). The transfer efficiency values evidenced in this trial (Cattaneo et al. 2006) for EPA were alternatively higher than those reported in some studies (Donovan et al. 2000; Kitessa et al. 2001; Chilliard et al. 2007), and lower than those evidence by others authors (Keady et al. 2000). These discrepancies have been explained by the authors with the different dietary sources of n-3 PUFAs, dosages, duration of fish oil administration, and/or level of ruminal protection. Feeding fish oils to goats has been reported to affect milk production and composition of the main constituents in a way different than that observed in lactating cows (Chilliard et al. ; Sanz Sampelayo et al. 2000). Casals and Caja (1993) reported that different types of dietary fats, tested in lactating goats, can increase the production of milk, together with a higher concentration of fat and even of protein. These results were to be closely related with many factors as the level of fat in the diet, the productive capability of the animal and the state of lactation (Sanz Sampelayo et al. 2000). Under normal rumen conditions, dietary 18:2n6 is mainly converted to 11t-18:1 via 9c11t-CLA (Harfoot and Hazlewood 1997). If the cows' diets are high in oil and/or fermentable carbohydrates, the level of 10t-18:1 in milk fat may increase in comparison to 11t-18:1, because these diets can induce a shift in the ruminal microbial biohydrogenation process, resulting in production of more 10t-18:1 from 18:2n6 via 10t12c-CLA in the rumen (Pottier et al. 2006). Milk fat depression is associated with an increase in the content of the 10t-18:1 isomer (2.9% of total fatty acids), but milk fat percent is not depressed when the range of 10t- 18:1 is much lower (0.33-0.70% of total fatty acids) (Griinari et al. 1998; Odongo et al. 2007).

The 10t12c- CLA is also responsible for reduced milk fat synthesis, whereas 9c11t-CLA has no effect (Harvatine et al. 2009). Reports by Chilliard et al. (2003), Chilliard et al. (2006) underlined the fact that, contrary to dairy cows (Dallaire et al. 2014), in goats there is no decrease in the milk-fat content when the diet is supplemented with polyunsaturated fatty acid-rich vegetable oils (Tudisco et al. 2014). The goat is much less sensitive that the cow to alteration in ruminal biohydrogenation patweys (Chilliard et al. 2007; Bernard et al. 2010; Schmidely and Andrade 2011; Serment et al. 2011; Bernard et al. 2012).

In the study by Cattaneo et al. (2006), dietary treatment with fish oil in periparturient dairy goats had no significant effects on the quantity of milk secreted, but the supplementation significantly reduced milk fat and lactose concentrations. A study by Ghazal et al. (2014a) reported that CLA administration in the diet, decreased milk fat content (17%) and milk yield (19%). On the contrary, Kitessa et al. (2004) evidenced a significant milk yield

decrease without changes in milk fat content in response to unprotected tuna oil supplementation to dairy cow and this result markedly differs from what observed in other dairy ruminants. Longer term feeding of fat supplements that were rumen protected, had a content of long chain mono- and poly- unsaturated fatty acids.
Many papers report that when fish oil provided is in protected form, there is no change in milk production or fat content (Kitessa et al. 2001; Cattaneo et al. 2006; Agazzi et al. 2010).
In a recent study by Toral et al. (2014) the administration of fish oil to high- or low- diets changes the levels of bioactive fatty acids in milk without decreasing milk fat content and milk yield in dairy goats.
Gallardo et al. (2014) reported that the addition of fish oil decreased the milk fat content and increased healthy FAs, such as n-3 PUFAs and rumenic acid in ewes. Najafi et al. (2012) evaluated as the effects of fish oil in goat kids increased EPA and DHA concentration and decreased the n6:n3 ratio in the muscle without changing the sensory properties of colour of meat.
Ferreira et al. (2014) reported an increase of vaccenic acid, EPA and DHA in meat of lambs fed fish oil plus soybean.
Clayton et al. (2014) evaluated the effects of algae administration in diet of dams and the transfer in lambs. This study reported an increase of EPA and DHA in plasma, DHA increased in lambs born from dams that fed a diet higher in n-3 PUFAs compared with dams fed n-6 PUFAs.
Generally, variations in the response of different ruminant species to dietary fat supplementation concerning quantity of milk produced and fat and protein content may derive, according to Chilliard et al. (2003), from the interactions between the animal's digestive and metabolic activity and the nature of the diets used in each case.
Sampelayo et al. (2007) indicated that the digesta passage rate may be higher in goats than in cows, reducing the negative effects that fatty acids would have in the rumen.

1.4 Dietary n-3 PUFAs and immune response

The immune system is integrated by a diversity of cells and molecules that are capable of recognizing and eliminating invading foreign microorganisms in a specific manner (Baumann and Gauldie 1994). There are two mechanisms of defense: innate and specific (Calder 2007). In both cases the central role is recognize and discriminate between foreign substances and the host's own molecules (Kehrli Jr and Harp 2001).

Immune response is a interesting system that coordinates interactions amongst many different cell types in the body. The main important actions that caractherising immune response are: phagocytosis of bacteria, processing of antigens derived from intracellular and extracellular pathogens, activation of T cells with clonal expansion (proliferation) and production of cytokines that elicit antibody production and killing cell activity.
An important role for modulation of immune system is characterized by dietary fatty acids (Calder 2001; Calder 2002), the most powerful is the n-3 PUFAs (Simopoulos 2002) and those derived from fish oil, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Calder 2006; Lecchi et al. 2011).
Fatty acids have a lot of function as fuels for generation of energy; components of cell membrane phospholipids or covalent modifiers of protein structure influencing the cellular location and function of proteins; regulators of gene expression either through effects on receptor activity, on intracellular signaling processes, or on transcription factor activation; precursors for synthesis of bioactive lipid mediators like prostaglandins (PGs), leukotrienes (LTs), lipoxins and resolvins (Fig.8).
Thus, changes in membrane phospholipid fatty acid composition might be expected to influence immune cell function in a variety of ways:
- Alterations in the physical properties of the membrane such as membrane order and raft structure;
- Effects on cell signaling pathways, either through modifying the expression, activity or avidity of membrane receptors or modifying intracellular signal transduction mechanisms;
- Alterations in the pattern of lipid mediators produced, that have different biological activities and potencies.
The mechanism of action of these n-3 PUFA is still under investigation, and not fully understood, but they have been shown to be not merely energy-providing molecules (Desvergne and Wahli 1999). Polyunsaturated fatty acids (PUFAs) have been postulated as modifiers of the immune response in humans and some livestock species, although the mechanisms involved are only partially characterized (Lecchi et al. 2011).
Long chains n-3 PUFA may act by replacing arachidonic acid as eicosanoids substrate and inhibiting arachidonic acid metabolism, but also by giving rise to a family of anti-inflammatory mediators, named resolvins and protectins, whose physiological role is that of dampening the excessive effects of inflammation (Serhan and Savill 2005). Their inclusion in plasma and intracellular membranes may also modify both the fluidity and the composition of lipid rafts, as well as second messenger production and delivery (Calder et al. 1990; Madani et al. 2001).
Changing the fatty acid composition of immune cells also affects phagocytosis, T

cell signaling and antigen presentation capability. These effects appear to be mediated at the membrane level suggesting important roles of fatty acids in membrane order, lipid raft structure/function and membrane trafficking (Calder 2007)(Fig.9).

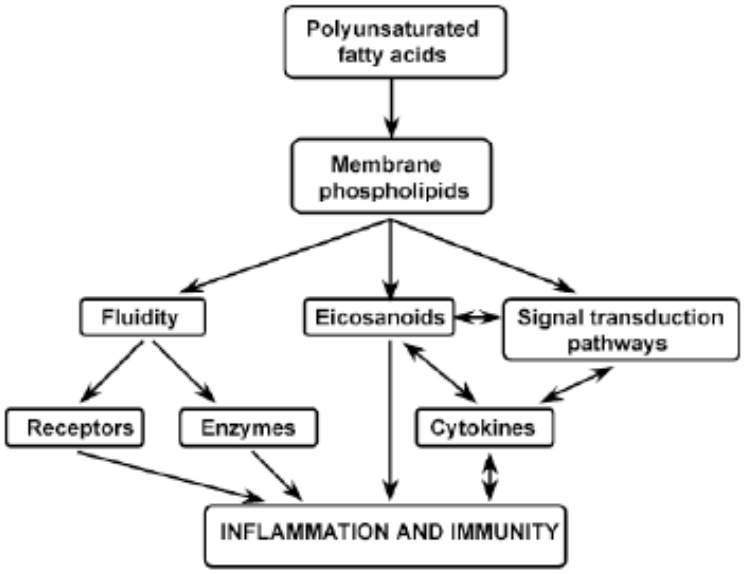


Figure 8: Mechanisms whereby polyunsaturated fatty acids might exert effects on inflammation and immunity (Chilliard et al. 2007).

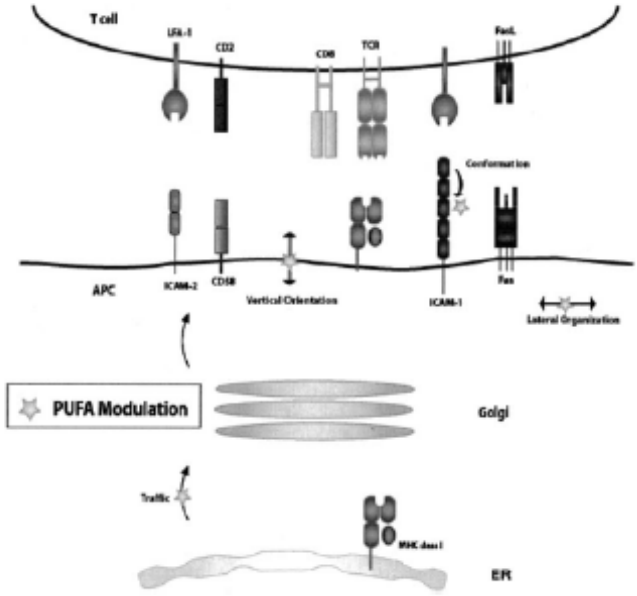


Figure 9: Polyunsaturated fatty acids (PUFAs) exert their immunomodulatory effects, indicated by stars, by modulating surface protein expression. On incorporation into the plasma membrane, PUFAs may alter the conformation, lateral organization, and vertical displacement or orientation of molecules involved in interactions with cognate T cells. In addition, uptake of PUFAs into endomembranes may alter the rate of trafficking of molecules such as major histocompatibility complex (MHC) class I to the plasma membrane and may explain the reduction in surface molecule expression often observed with n3 PUFAs. Lipid modulation of antigen presenting cell (APC) membranes may result in changes in recognition by the T cell. The membrane and proteins are not drawn to scale relative to one another. TCR, T cell receptor; ICAM, intracellular adhesion molecule; ER, endoplasmic reticulum; LFA-1, lymphocyte function associated antigen-1 (Shaikh and Edidin 2006).

As neuroendocrine–immunomodulatory effects have been demonstrated in humans supplemented with n-3 FA (Calder 2006; Michaeli et al. 2007), there is reason to believe that similar effects might also be seen in livestock that are being supplemented with fish meal (Stryker et al. 2013).

As is reported in literature, the use of administration of n-3 PUFAs in ruminants' diet is due because the high level of saturated fatty acid and the low level of PUFAs in milk of ruminants (Mattos et al. 2004; Cattaneo et al. 2006; Moghadasian 2008; Marin et al. 2012; Clayton et al. 2014; Ghazal et al. 2014b; Toral et al. 2014). Extensive studies have been performed on the role of lipids on cytokine responses, inflammatory reactions, and both specific and nonspecific immune responses in experimental animals. An highlights of a review of the literature with the influence of n-3, n-6 and n-9 on immune status are shown in table 2 because is too extensive to be cited in detail.

	ω-3	ω-6	ω-9
Effects on cytokine production			
IL-1	↓↑	↑	↓−↑
IL-2	↓	↑	↑
IL-4	↓	↑	↑
IL-6	↓		
IL-8	↓		
IL-10	↓		
TGF-β	↓		
IFN-γ	↓−	↑	↑
TNF-α	↓	↓↑	↓
Effects on Intracellular Signaling			
Adenyl cyclase	↑↓	↑	↑
PKA	↑↓	↓	↑↓
PKC	↓	↑	↑
NFκB		↑	
Effects on Inflammation			
CD-2 expression	↓		
ICAM-1 expression	↓		
VCAM-1 expression	↓		
ELAM-1 expression	↓		
L selectin expression	↓		
LFA-1 expression	↓		
Lymphocyte adhesion	↓		
Monocyte adhesion	↓		
Neutrophil chemotaxis	↓		
C reactive protein	↓		
NO production	↑↓	↑	↑
Superoxide production	↑↓	↑	↑
Effects on Immune Responses			
GVH response	↓		↓
Allograft survival	↑	↑	↑
TcR expression	↓		
Phytomitogen response	↓	↓	↓
Antigen stim. lymph. resp.	↓	↓	
Accessory cell function	↓		
DTH response	↓		
Ia expression	↓		
Effects on Infection (Direction of Arrow Relates to Mortality)			
Myocoplasma hypopneumonia	↓	↑	
Gut-derived sepsis	↓		
Peritonitis	↓	↑	↓

Table 2 : Effects of long chain unsaturated fatty acids on cytokine production and cell function in the immune system (Alexander 1998).

Calder (2002)reported that the fatty acid composition of lymphocytes and other immune cells was influenced by the fatty acid composition of the diet, which affects the ability of lymphocytes to produce eicosanoids. Among fatty acids, the administration of n-3 and n-6 polyunsaturated fatty acids to goats have been shown to play a critical role as modulators of immune reactions (Calder 2002; Simopoulos 2002; Agazzi et al. 2004; Bronzo et al. 2010), and the most effective have been shown to be those largely contained in fish oil, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)(Calder 2006). Since n-3 PUFAs reduce tissue levels of the immunosuppressive agents arachidonic acid and prostaglandin E2 (PGE2), increased intake of n-3 PUFAs should enhance immune response.

The effect of administration of fish oil on cell-mediated immune response has been investigated in periparturient dairy goats (Agazzi et al. 2004), where has been shown to modify the ratio between mononuclear and polymorphonuclear cells.

Calder (2007) suggests that EPA, but not DHA, can compete with arachidonic acid as substrate for cyclooxygenases and lipoxygenases being converted to eicosanoids as reported in a previous study (Desvergne and Wahli 1999).

The effect of PUFAs on oxidative burst is uncertain, since some studies have reported an increase in reactive oxygen species (ROS), whereas others have reported a decrease (Schonfeld and Wojtczak 2008). In an in vitro study by Pisani et al. (2009) phagocytosis and extracellular ROS production in dairy goats were determined after cells incubation with increasing concentration of EPA and DHA (25, 50, 100, 200 mM). Phagocytic activity was significantly increased by EPA and DHA, while treating PMN with EPA did not affect extracellular ROS production which was instead down-regulated by DHA. The finding that DHA has greater effects in both phagocytosis up-regulating and oxidative burst down regulating activities when compared to EPA is remarkable.

In a second *in vitro* study by Lecchi et al. (2011) blood monocytes incubated with increasing concentrations of EPA or DHA (25–200 μM) demonstrated increased phagocytosis compared to unexposed monocytes. Generation of ROS was not markedly affected in the presence of EPA and DHA, except at 200 μM, at which concentrations monocyte viability was also reduced.

1.5 Nutrigenomics

In the recent years the interest on the modification of liver metabolism in livestock animals with a nutrigenomic approach is increased. Nutrigenomic is defined as the study of the genomewide influences of nutritional altering the expression end/or structure of an individual's genetic makeup. In the short term, nutrigenomics describes the use of functional genomics tools to probe a biological system following a nutritional stimulus that will permit an increased understanding of how nutritional molecules affect metabolic pathway and homeostatic control (Müller and Kersten 2003). More practically from a nutrigenomics perspective, nutrients are dietary signal that are detected by the cellular sensor systems that influence gene, protein expression and metabolic production (Müller and Kersten 2003).

Studies about nutrigenomics in ruminants are relatively scant compeared with the literature in monogastics (Bionaz et al. 2013), therefore nutrigenomics can be considerated a relatively new area in ruminants. In this class of animals, peripartum is the most important period because the animal is particular sensitive to nutritional imbalance. In this situation, nutrigenomics can provide new tools that can be applied to address disease, performance and productivity in animals (Figure 10). In other words, the objective of nutrigenomics in ruminants is to study the effects of diet on changes in gene expression or regulatory process that may be associated with various biological process related with animal health and production (Bionaz et al. 2008).

In small ruminant fatty liver syndrome frequently develops during the last phase of pregnancy instead of the beginning of lactation leading to metabolic disorders such as ketosis that can impair the goat health status and, consequently, productive performance (D'Ambrosio et al. 2007; Pinotti et al. 2008).

Previous researches demonstrated that ruminant liver seems to possess a relatively greater capacity to initiate fatty acid oxidation in peroxisome than liver from rodents (Grum et al. 1994). Increase in peroxisomal FA oxidation may help the liver to cope with the large influx of mobilized non-esterified fatty acids (NEFA) during transition, preventing excessive accumulation of fat (Drackley et al. 2005; Janovick-Guretzky et al. 2007) and fatty liver occurrence.

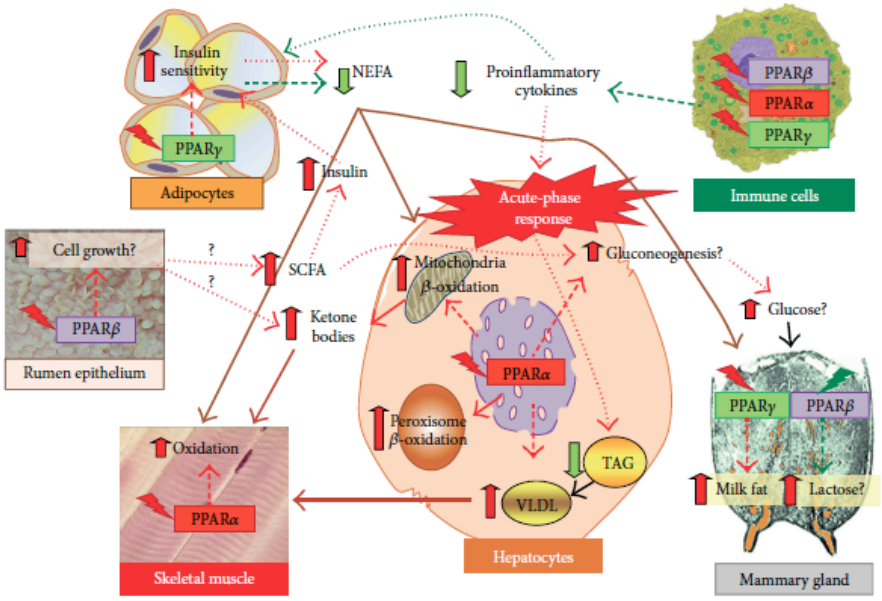


Figure 10: Transition, from pregnancy into lactation, in high producing dairy cows by nutrigenomics approach through PPAR isotypes (Bionaz et al. 2013).

1.5.1 PPARs

The peroxisome proliferator activated receptors (PPARs) were identified first in mouse liver tissue (Schoonjans et al. 1996), actually they are members of the steroid and thyroid nuclear receptor superfamily, being ligand-activated transcription factor (Fekete and Brown 2007) that control a variety of genes in several pathways of lipid metabolism.

PPARs are ligand dependent transcription factors that regulate target gene expression by binding to specific peroxisome proliferator response element (PPREs) in enhance sites of regulated genes. Each receptor binds to its PPREs as a heteodimet with a retinoid X-receptor (RXR). Once the ligand binds to the ligand binding domain, it produces a modification of PPAR structure (Waku et al. 2009). The activated PPAR/RXR binds to a specific DNA sequence in the promoter region of specific target genes including or repressing their expression (Bionaz et al. 2013).

Three isothype of PPAR have been identified: α, β/δ and γ; every single isoform is encoded by separate gene.

As reported in a review (Bionaz et al. 2013), the three isoforms were tested in different ruminants' tissues and cells, to evaluate the expression of the single

PPAR isoforms (Figure 11).

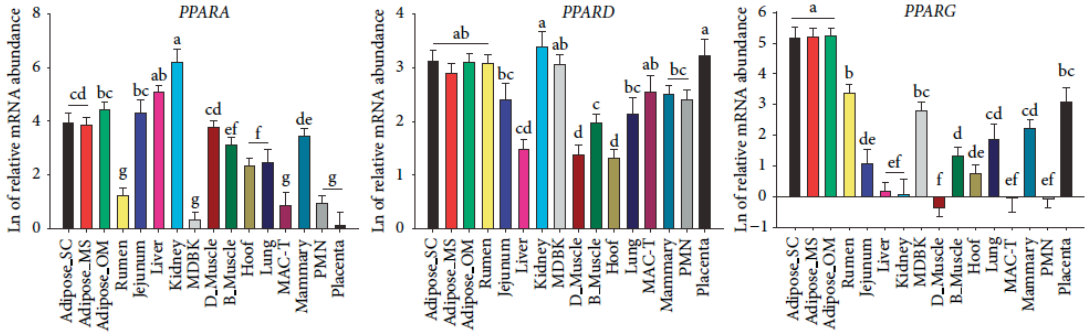


Figure 11: Relative transcript abundance of each PPAR isotype in 14 bovine tissues and 2 cell line [Madin- Darby BovineKidney(MDBK) and bovine mammary alveolar cells(MAC-T)] (Bionaz et al. 2013).

PPARA, the less studied isoform compared with PPARG, is largely expressed in kidney, liver, adipose tissues and jejunum. Recent studies also confirmed expression of this isoform in liver of ruminants (Loor et al. 2005a; Selberg et al. 2005; Loor et al. 2006; Bionaz et al. 2007a; Bionaz et al. 2007b; Loor et al. 2007); adipose and muscle tissues in bulls (Cherfaoui et al. 2012); endometrium tissue in ewes (Cammas et al. 2006) and heart samples in sheep (Buroker et al. 2008).
There are few available publications on PPARB/D isoform, this PPAR in ruminant is expressed in kidney, placenta, followed by adipose tissue and rumen.
PPARG, the most studied isoform in ruminants, is high in all adipose tissues, followed by rumen Madin- Darby BovineKidney (MDBK) cells and placenta. The lower expression is registered in liver, kidney, Mac-T cells and blood polymorphonuclear leucocytes (PMN). The highest expression in adipose tissue is also confirmed in mice (Kliewer et al. 1994) and humans (Vidal-Puig et al. 1996). Expression of PPARG was also detected in goat mammary, although at a significant lower level compared to bovine (Bernard et al. 2013).
In conclusion, it's evident that PPARA is more abundant in tissues where LCFA oxidation is generally higher and, otherwise, PPARG in lipogenic tissues.

1.5.2 Transcriptomic in lipid metabolism

During years, have been performed a lot of *in vitro* studies on ruminant PPAR response to synthetic agonist. The results testified a valid approach to uncover

the existence of an active PPAR isotype in cells or tissues and PPARs target genes. Using synthetic agonist is not feasible because of the high costs.
Among all nutritional factors able to affect cell biology by changing gene expression, dietary energy, fatty acids and amino acids have the strongest potential. The most powerful nutrigenomics dietary components are fatty acids. The effects of fatty acids vary depending on their level of saturation.
Numerous fatty acids and ecosanoics, including a variety of CLA (Desvergne and Wahli 1999; Weldon et al. 2004) serve as ligand of PPARs.
In particular Jump (2002) and Anderle et al. (2004) reported that preferred ligands for PPAR activation are thought to be 18C and 20C fatty acids, because shorter than 14C or longer than 20C will not it correctly into the PPAR binding pocket and stabize the AF-2 helix.
Göttlicher et al. (1992) have shown that PPARA can be activated by a wide variety of saturated and unsaturated fatty acids (palmitic, oleic, linoleic and arachidonic acids).
Xu et al. (1999) reported that PPARG clearly prefers PUFA, including the essential fatty acids, arachidonic acid and EPA.
Selberg et al. (2005) have been shown that hepatic PPARA gene expression is upregulated by dietary trans C18:1 in transition dairy cow. The same authors did not find alteration of hepatic PPARA mRNA in response to supplemental CLA.
A *in vitro* study confirmed that the expression of PPARA target genes is faster and more pronounced if cells are treated with free palmitate instead of palmitate bound to albumin (Thering et al. 2009a).
In a study by Ruby et al. (2010) the activation of PPARA by free LCFA was demonstrated. In this study only the free fatty acids relased by LPL are the ones able to activate PPARA.
EPA and arachidonic acid had low micromolar affinities for PPARG (Forman et al. 1997).
It was deserved that palmitate and stearate induced a very strong actraction of transcription of PPARG and PPARA target genes (Bionaz et al. 2008; Kadegowda et al. 2009; Bionaz et al. 2012).
The saturated LCFA are the most abundant in the circulation of ruminants (Or-Rashid et al. 2009; Zachut et al. 2010) due to extensive ruminal hydrogenation of unsaturated LCFA.
As is reported in literature the changes in PPARs is followed by a different expression of multiple enzymes involved in fatty acyl-CoA formation and hydrolysis, fatty acids elongation and desaturation and fatty acids oxidation (Hunt et al. 2002; Tong et al. 2006). Genes encoding proteins required for fatty acid uptake and activation (ACSS2, LPL); intracellular fatty acid transport (FABP3, FABP4), *de novo* fatty acid synthesis (ACACA, FASN), esterification (DGAT1, DGAT2, LPIN1), desaturation (SCD), elongation (ELOVL6),

transcriptional regulation (INSIG1, MED1, PPARG, RXRA, SCAP; SREBF1, THRSP), lipid droplet formation (ADFF, BTN1AA, XDH), β-oxidation and lipoprotein-related genes (ACOX1, APOB, CPT1A, PPARA, RXRA).
PPARG in ruminants plays a pivotal role in adipogenesis and the expression is high in adipose tissue and also plays a role in LCFA oxidation (Sharma et al. 2012). In this study is also reported that PPARG control the expression of carnitine palmitoyltransferase 2 (CPT2) and carnitine O-acetyltransferase (CRAT) genes involved in the entry of LCFA into mitochondria.
The activation of PPARA seems to controls catabolism of fatty acid. The catabolism in mitochondria or peroxisome increase during transition period in ruminants (Drackley et al. 2001) and the expression of PPARA in liver of ruminants increase from pregnancy to early post partum (Loor et al. 2005a; Schlegel et al. 2012). In the same time, also ACOX1 and Acyl-CoA dehydrogenase medium chain (ACADM) increased, as all the genes involved in lipid catabolism (Thering et al. 2009a; Bionaz et al. 2012) as carnitine palmitoyltransferase 1 (CPT1) that catalyzes the rate-limiting step in the translocation of activated fatty acids into the inner membrane of the mitochondria (Brady et al. 1989).
Correlated with PPARA gene expression and metabolism are acetyl-CoA carboxylase (ACC1), enzyme for fatty acid biosynthesisi; and malonil-CoA decarboxylase , enzyme able to convert malonil-CoA into acetyl-CoA.
As reported in literature, the activation of PPARD increased activity of GADPH in ruminants (Vidal-Puig et al. 1996) and in monogastrics (Desvergne et al. 2006). In the latter is reported that PPARD controls fatty acid oxidation in skeletral muscle, heart and adipose tissue ; probably the same is for ruminants.
To date, few available data suggest a role of PPARD in lipid catabolism in ruminant.
PPARG have another role in mammary gland during lactation. As reported by (Bionaz and Loor 2008) PPARG controls expression of key genes involved in milk fat synthesis as SREBF1 (Kadegowda et al. 2009), insulin induced gene 1 and 2 (INSIG1 and INSIG2) (Bionaz et al. 2008).
Recently has been confirmed the pivotel role of PPARS on the anti-inflammatory status. In a study by Hauner (2002) the effects of PPARG is not only by counteracting the effect of TNFα, but also by reducing the production of this cytokine (Perdomo et al. 2011).
In an *in vitro* study the activation of PPARG caused downregulation of several proinflammatory cytokines and increased the expression of CCL2 and TNFα (Lutzow et al. 2008).
The involvement of PPARB/D in the process of inflammation was recently underscored, an intrammamary infusion of LPS led to an upregulation of PPARD and several proinflammmatory genes (TNF, NFKB1) (Graugnard et al.

2013).

1.5.3 Gene expression and marine lipid administration in ruminants' diet

Starting from the beginning of 2000, the interest of marine inclusion on diets and the nutrigenomics approach has been increased.
In literature has been reported that PPARA can be activated by n-3 PUFAs fish oil (Thering et al. 2009b), although the mechanism of action of PUFAs is still debated.
In a study by Jump et al. (2005) has been outlier that the effectiveness of EPA and DHA as PPARA natural ligand and activator could be attributed not to the fatty acids themselves, but to their chain shortened to C20:5-CoA with the subsequent retroconversion to C20:5 n-3 as a preferred PPARA ligand.
Agazzi et al. (2010) reported a higher value in PPARA mRNA in a group of goat fed palm oil compared with control group. In the same study has been registrated an increase from day 7 before to 21 day after kidding for PPARA, CPT1A, ACADVL and ACACA in palm oil group. Palm oil (containing C16 and C18) was more effective in enancing mRNA expression of PPARA and target genes in liver during transition period.
The analysis of mammary mRNA expression patterns associated with saturated or marine lipid feeding in dairy cow has been reported by Invernizzi et al. (2010). In this study the milk fat depression was not characterized by lower mRNA abundance of lipogenic enzymes (FASN, ACACA), but upregulation of INSIG1, decreased SREBF1 and increased of SCAP.
In the study by Bichi et al. (2013) the influence of marine lipid administration has been recorded in ewes. In this case, the administration of marine algae not had effects on mammary and adipose tissue expression of the most important genes involved in protein and lipids synthesis.
Always in a situation of marine lipid administration, Vahmani et al. (2014) reported that cow on pasture, that registered a reduced secretion of *de novo* synthesized fatty acids in milk compared with confined ones, had a downregulated hepatic expression of FASN, SCD1, FADS2 and THRSP.
In a study by Karcher et al. (2014) the influence of n-3 on immune status in calves has been evaluated. Milk replacer with different sources of fat were administered. The results reported that flax oil had a greater growth rate and feed efficiency, furthermore flax oil decreased the expression of IL-4 and IL-8 compared with fish oil group.
A lot of studies has been performed in dairy cow or calves and, because there a re subtle differences between PPARs across species, it cannot be excluded that there are species-specific differences in the affinity of a receptor for given ligand

and a given species (Bragt and Popeijus 2008).
Thus all this studies are a testimonial that nutrigenomics approach can play a critical role in future strategies to feed ruminants, and this point of view it's very important to improve efficiency of highproducing animals and modify quality for an ever growing number of consumers demanding "healthy" food.

1.6 The importance of colostrogenesis on kids' immune response

In ruminants the characteristics of the placenta, epitheliochorial in cows and water buffaloes (Wildman et al. 2006; Padua et al. 2010), synepitheliochorial in small ruminants (Wooding et al. 1986), do not admit an appropriate transfer of immunoglobulins (Ig's) from the dam to the fetus.
Newborn ruminants are agammaglobulinemic (calves) or hypogammaglobulinemic (lambs and kids) at birth (Arguello et al. 2004; Castro et al. 2005; Castro et al. 2009; Moreno-Indias et al. 2012).
In a study by Constant et al. (1994) has been asserted that goat kids are agammaglobulimnemic at birth, otherwise Guerrault and Ouin (1990), Rabbani et al. (1990) and Sherman et al. (1990) reported low concentration of Ig's serum in ruminants. Chen et al. (1999) confirmed a peak blood serum concentration 24 h after birth, while O'brien and Sherman (1993) have defined failure of passive transfer in the kid. Consequently, it's evident that the consumption of colostrum is fundamental for ruminants acquisition of immunity (Lascelles 1979).
Colostrogenesis is a complex process extremely important for the pre-partum transfer of components, mainly immunoglobulins, from maternal bloodstream into mammary secretions during the first 3 days of lactation (Barrington and Parish 2001). Colostrum is the first source of nutrition in neonate ruminants, having a fundamental biological function in these animals, promoting immunoglobulin transfer from the dam to the newborn, providing protection against infections in the newborn (Kramer et al. 2001; Bendixen et al. 2011) and in the survival rate of newborns (Hernández-Castellano et al. ; Lascelles 1979; Stelwagen et al. 2009).
Several factors can affect the final colostrum composition: species, breed, age, nutrition, litter size, length of dry period and health status (Csapó et al. 1994; Awadeh et al. 1998; Maunsell et al. 1998), although colostrogenesis is still not fully understood (Barrington and Parish 2001; Castro et al. 2011b).
In general, colostrum is formed and stored in the mammary gland during late pregnancy (Linzell and Peaker 1974) and the input of colostrum secretion components is regulated by several mechanisms, controlled by local and systemic factors (Castro et al. 2011b)
It has been described that colostrum is a mixture of diverse components, such as fat, lactose, vitamins and minerals that have a high nutritional importance (Ontsouka et al. 2003; Hernandez-Castellano et al. 2014a; Hernandez-Castellano et al. 2014b), but colostrum also contains hormones, growth factors, cytokines, enzymes (Blum and Hammon 2000; Vetter et al. 2013), metabolites derived from alveolar epithelial cells (Linzell and Peaker 1974) and immunocompetent cells (Lee et al. 1980).

The immunoglobulins are the most deeply studied proteins because they play a crucial role in the protection of newborn ruminants against infections (Moreno-Indias et al. 2012). The most important immunoglogulins present in colostrum are IgG, IgM and IgA.
There are some factors that can modulate the colostrum composition and, consequently, the passive immune transfer.

- Birth body weight: Castro et al. (2007) reported no significant effects on IgG blood serum concentrations in kids, but there was just a tendency for IgG concentrations to increase when the birth BW was between 2.5 and 3.2 kg.
- Litter size: studies by Nowak and Poindron (2006), Csapó et al. (1994), Chen et al. (1999) confirmed that this parameter is related with the amount of colostrum accumulated in the udder before partum. Obviously the twin-bearing small ruminants generally yielded more colostrum and IgG concentration than in single bearing animals. Nevertheless, Banchero et al. (2002), Argüello et al. (2006) and Romero et al. (2013) reported no effect of the litter size on the colostrum composition and IgG concentration.
- Sex: Rabbani et al. (1990), Bekele et al. (1992), Chen et al. (1999) and O'brien and Sherman (1993) observed no significant relationship between the sex of kids and serum IgG concentrations after the ingestion of colostrums.
- Time, suckling duration and colostrum composition: considering ruminants' ability to absorb molecules decreases along the first 48 hours after birth, the first colostrum ingestion is very important to acquire a correct passive immune transfer, although IgG concentration or volume of ingested colostrum also take high relevance in passive immune transfer. As reported by Chigerwe et al. (2009), Holstein calves fed with 3 litres of colostrum between the first 4 hours after birth acquire an adequate passive immune transfer. Otherwise the calves that took their first colostrum later (4 and 12 hours after birth) also had an adequate passive immune transfer, but the latter animals were fed with a high quality colostrum (IgG concentration > 100mg/mL).
- Number of lactations: Oyeniyi and Hunter (1978) and Ha et al. (1986) observed an increase on IgG concentration in multiparous bovine and caprine colostrum compared to primiparous, otherwise Dossantos et al. (1994) and Argüello et al. (2006) did not observe differences in the colostrum IgG concentration due to the number of lactations in Saanen and Majorera goats, respectively.
  Other differences has been observed by Swanson et al. (2008) and Castro et al. (2011a) regarding pregnancy periods and the effects on IgG

concentration in animal that have had and induction of parturition.

Nutrition during pregnancy also affect both colostrum composition and yield (Castro et al. 2011b). The supplementation of selenium during pregnancy increased colostrum yield, fat, lactose and protein percentage (Meyer et al. 2011). Ramírez-Vera et al. (2012) showed that corn supplemented goats had higher colostrum yield than non-supplemented goats. Awadeh et al. (1998) reported that the inclusion in late pregnancy increased IgG content in cow. On contrast, Swanson et al. (2008) did not observe any effect in sheep.

In a recent study by Garcia et al. (2014) the influence of nutrition on the immune status of newborn calves was evaluated. Primiparous cattle were fed with a saturated or unsaturated (plus essential fatty acids) diets during the last 8 weeks of gestation. The newborn calves were fed with milk replacer plus a low linoleic acid (LLA) or high linoleic acid (HLA) on diet until 30 days of life; and a mixture with minimal linoleic acid from 31 to 60 days of life. As reported in this study, unsaturated acid had a positive effects in calves fed a HLL diet on performances, increased plasma glucose concentration, increased Insulin-like growth factor I and influenced the function and composition of some blood cell population toward a more active proinflammatory response.

Haptoglobin (Hp) is deemed a major positive acute phase protein in ruminants (Eckersall and Bell 2010). As reported in bibliography Hp, whose concentration changes in the response to the damaging factors and being a part of the inflammatory reaction, increased in the presence of subclinical inflammation (Gånheim et al. 2007), indeed only calves with gastroenteritis (Deignan et al. 2000) or viral respiratory infection (Heegaard et al. 2000) showed an increase of Hp in plasma. Hp is proven to rise considerably in acute conditions and remain e elevated for up to 2 weeks (Panndorf et al. 1976; Petersen et al. 2004; Rahman et al. 2010). In the study Garcia et al. (2014), feeding fat prepartum appeared to slightly increase inflammation of calves during the pre weaning period.

Although it's established the influence of fatty acids, in the specific case of the n-3 PUFAs, on immune response and the importance of colostrogenesis in ruminants; few available study have been reported on the influence of maternal lipid supplementation in diet on kids, calves and lambs immune response.

The trials present in literature on the importance of colostrogenensis, Igs' transmissions and so on, not rated this potential way to strengthen the newborn immune response.

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CHAPTER 2

Objectives

2 Objectives

The objective of this thesis was to study the variations of lipid metabolism in periparturient dairy goats supplemented with polyunsaturated (Fish oil) or saturated (Stearate) fatty acid and their kids' immune-response.
To achieve this objective, three experiments were conducted. In the first study the immune-metabolic adaptations in 12 periparturient dairy goats were evaluated using a biochemical and histological approach (Chapter 3), the second one studied the effects on hepatic and adipose mRNA levels of important genes involved in fatty acids metabolism in 23 transition dairy goats (Chapter 4) and the last examined the effects of inclusion of the different sources of fatty acids in maternal diets on 48 newborn kids immune status (Chapter 5).

CHAPTER 3

Hepatic and subcutaneous adipose tissues intermodulation in transition dairy goats fed saturated or unsaturated fat supplemented diets

3 Hepatic and subcutaneous adipose tissues intermodulation in transition dairy goats fed saturated or unsaturated fat supplement diets

3.7 Abstract

The aim of this study was to evaluate immune-metabolic adaptations in periparturient dairy goats fed saturated or unsaturated fatty acids supplements throughout a biochemical and histological approach. 12 spring kidding goats were divided in a randomized complete block design into three homogenous groups for parity, age and milk production in the previous lactation and assigned to three experimental treatments. Goats fed a pre- or post-kidding basal diet (C) or pre-/pos-kidding basal diet supplemented with fish oil (FO) or pre-/pos-kidding basal diet supplemented with stearic fatty acid (ST). At 14, 7 and 2 days before the expected kidding date as well as at 0, 2, 7, 14 and 21 DIM individual blood samples were taken. Liver and adipose tissue biopsies were harvested on days -7, +7 and +21 relative to parturition for each experimental subject.
Significantly higher ALAT serum content in FO than C was observed on day -7 (16.17 vs. 13.57 IU/L; $P = 0.08$) and +21 (16.20 vs. 11.29 IU/L; $P < 0.01$) from kidding. Significant higher BOHB serum content for ST than FO (0.54 mmol/L ST and 0.33 mmol/L FO $P < 0.05$). ST had lower NEFA levels compared with C during pre-kidding week, FO in the first week of lactation had highest levels (0.28 mmol/L C and 0.82 mmol/L FO $P < 0.01$). At 21 days in milk, both FO had lower NEFA levels than C (0.27 mmol/L FO vs. 0.44 mmol/L C $P < 0.05$). Morphometric evaluation showed a significant decrease of adipocytes surface between -7 and 21d in ST and C, whereas in FO the adipocyte surface reduction was related to the -7 to 7 d interval reaching a plateau until day 21. Morphological evaluations showed a significant increase of fatty infiltrated cells percentage between -7 and + 21 d, in FO. In ST and C no differences were observed between -7 and + 21, although fatty infiltrated cells percentage decreased at 7 days. Comparing the three diets at day 21, percentage of this type of cells was significantly higher in ST than in C and FO. Distribution of vacuolated cells percentage, within times and treatments reflected distribution of total fatty infiltrated cells previously described. Distribution of cloudy cells percentage in ST was comparable to that described previously for total fatty infiltrated cells; on the contrary, a significant decrease of cloudy cells percentage between 7 and 21 d were observed, both in C and FO. Comparing the three

diets, at day 21, the highest percentage of vacuolated cells was in FO, while the highest percentage of cloudy cells was in ST.
Data on subcutaneous adipose tissue are well coupled with NEFA (and BOHB) serum levels: in fact, the highest adipocyte area reduction in C was observed between days -7 and 7, that matches with spiking levels of NEFA, both clear signs of strong lipomobilization. From 7 to 21 days adipose tissue seemed to be still mobilized considering the negative energy balance of not supplemented goats. ST had a similar pattern but its energy balance got positive already between the 7th and 14th day post parturition; most likely its lipolysis would be reduced earlier than C, however after 21 days. Percentage of fatty infiltration in ST was the highest, only in FO the increase of fatty infiltrated cells was significant and it occurred more gradually than in C and ST. Change in percentage of cells categorized as normal, cloudy and vacuolated during the time reflects this trend on the basis of the severity of the cytological lesion. Comparing the treatments, percentage of normal, vacuolated and cloudy cells was quite similar at day -7, although the percentages of cloudy cells were the highest, while the percentage of vacuolated cells were the lowest both in control and treated animals. A day 21, the highest percentage of cloudy cells was observed in ST, while the highest percentage of vacuolated cells was observed in FO indicating that FO might induce slight more detrimental effects on liver.
In conclusion, data suggest dietary saturated and unsaturated lipid supplements are able to modulate the lipomobilizing machinery; in particular fish oil could reduce lipomobilization. On the other side it appeared to have slight more detrimental effects on liver.

Keywords: dairy goats, fatty liver, adipose tissue, stearate, fish oil

3.8 3.2 Introduction

The regulation and coordination of lipid metabolism among adipose tissue, liver and mammary gland are key components of the adaptions to lactation in dairy species (Drackley 1999). Transition period, critically important for health and production of dairy ruminants, is characterized by marked changes in the endocrine status of the animal that is much more considerable than at any other time in the lactation-gestation cycle (Drackley 1999). Increased incidence of metabolic and production-related diseases during the period around calving is not unexpected because of inadequate homeorhetic adaption of metabolism

during this period (Ingvartsen and Andersen 2000; Ingvartsen et al. 2003; Friggens et al. 2004).

The most characteristic event during transition period is the reduction in feed intake just when there is a very high nutrients demand for the developing conceptus and lactogenesis (Drackley 1999). The conjunction of these factors can lead the goat to experience a negative energy balance (NEB) particularly before parturition and at the beginning of the lactation period, when extra energy requirements for fetal growth before and milk production after are not met by low feed energy intake caused by a general low feed intake.

In such conditions goats mobilize fatty acids from adipose tissue reserves to compensate lack of glucose and fatty acids, this mechanism leads to increased circulating concentration of NEFA during late pregnancy and postpartum (Magistrelli and Rosi 2014). Liver metabolic pathways of NEFA are related to energy and ketone bodies production, or to the secretion through very low-density lipoproteins via triacylglycerols (TAG) conversion (Herdt 1988). If formation of TAG overcomes the liver secretion capacity, the accumulation of these compounds results in a so-called fatty liver syndrome (Herdt 1988). TAG are organized in lipid droplets of various dimensions, as demonstrated by histological analysis of liver biopsy of periparturient dairy cows and sheep (Komatsu et al. 2002; Kalaitzakis et al. 2007; Cal et al. 2009; Novais et al. 2009; Kalaitzakis et al. 2010a; Kalaitzakis et al. 2010b). Fatty liver has been evaluated either by chemical and histological analysis; from a histological point of view, fatty-infiltrated hepatocytes display different features that, in ascending order of severity range from a cloudy-swelling aspect to a vacuolated aspect due to the presence of lipid droplets of increasing dimension. Therefore, liver fatty change (FCL) has been categorized into normal, mild, moderate and severe (Bobe et al. 2004; Kalaitzakis et al. 2007). In dairy cows the majority of FCL cases appeared within the first 21 days in milk (DIM), whereas the majority of moderate-to-severe and severe FCL cases arose in the first 7 DIM.

Some classical efforts to enhance the energy status during transition are relative to the optimization of pre-partum nutritional level by increasing energy density of the diets and dry matter intake (DMI), while decreasing early post-partum energy output (Son et al. 1996; Dann et al. 2006; Grummer 2007). The partial replacement of grains or forages in the ration with fats sources, such as n-3 polyunsaturated fatty acids (PUFA), can considerably increase the energy level of the diet, and may enhance energy intake if DMI is not depressed (Staples et al. 1998): as a results energy balance might be improved in early lactating dairy goats (Ballou et al. 2009). Anyway 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 et al. 2005; van Knegsel et al. 2007). Serum calcium, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BOHB) content, were frequently used together with body condition score (BCS) to evaluate adaption to NEB during peripartum (McNamara et al. 1995; Rukkwamsuk et al. 1998; Kokkonen et al. 2005). As a result of NEB adaptation, significant lipid mobilization from subcutaneous adipose tissue leads to progressive loss of body mass that decrease BCS (Chilliard et al. 1999).
Fish oil has been supplemented in dairy animals often with the aim of enrich animal products with essential fatty acids considered healthy in particular for the human cardiovascular system (Breslow 2006). Dietary fat has been not anymore classified just as energy source but specific fatty acids have peculiar roles in lipid metabolism and organismal defense system. In fact, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been able to modulate immune and inflammatory response as observed either in vivo (Bronzo et al. 2010) and in vitro (Pisani et al. 2009; Lecchi et al. 2011; Lecchi et al. 2013).
Furthermore, also 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)
Total fat tissue mass and adipocyte mean cell size are fairly well related and this relationship could be used to indirectly assess total fat mass in living animals (Hirsch and Gallian 1968; Hood and Allen 1973; Alzón et al. 2007). The increase in adipocyte diameter can be used as an indicator of lipogenic activity under different metabolic challenging situations as undernourishment or breeding (Alzón et al. 2007; Faulconnier et al. 2007). Therefore, adipocyte diameter was used to estimate the amount of body fat deposition during goat lactation (Pike and Roberts 1980; Waltner et al. 1994).
However, data about the effects of highly unsaturated or saturated fats on liver and adipose tissue histology in dairy goats are nowadays limited.
The main aim of this study was to evaluate immune-metabolic adaptations in periparturient dairy goats fed saturated or unsaturated fat supplements throughout a biochemical and histological approach.

3.9 Materials and methods

3.9.1 Animals and diets

The present study was performed at the Animal Production Research and Teaching Center of the Veterinary School of the University of Milan (Lodi, Italy) and the protocol was approved by the Ethics Committee of the University of

Milan (attachment n. 5 January 26th 2011). Twelve spring kidding goats were divided in a randomized complete block design into three homogenous groups for parity, age and milk production in the previous lactation and assigned to three experimental treatments.
Goats were housed in individual boxes with free access to water and individually fed ad libitum. After kidding each goat shared the box with the relative suckling kids (on average 1.75 weighing 4.14 ± 0.68), the feeder being settled out of reach of kids. A pre-kidding or a post-kidding basal diet was administered to all the experimental animals in the three groups. Diets ingredients and chemical composition of the six experimental diets are detailed in Table 1. Pre kidding basal diet consisted of ad libitum mixed hay (refusal weight of at least 10%), 600g/head/d of concentrate and 100g/head/d of corn meal, while post kidding basal diet was composed by ad libitum alfalfa hay and mix hay (refusal weight of at least 10%), 1500g/head/d of concentrate and 200g/head/d of corn meal. Concentrates were separately fed from forage during the whole trial. The three experimental groups consisted of: a) Control (C), fed the basal pre or post kidding diet plus calcium carbonate (9 g/d during pre-kidding period, 12 g/d after kidding); b) Fish oil (FO), fed the pre or post kidding basal diet plus calcium carbonate (9 g/d during pre-kidding period, and 15 g/d after kidding) and 30 g/d of fatty acid (81 g/d of supplement) before kidding or 50g/d of fatty acid (135g/d of supplement) from a rumen-inert fish oil (10.4% EPA and 7.8% DHA) during lactation (Ufac Ltd., Stretton, UK); c) Calcium Stearate (ST), fed the pre- or post-kidding basal diet plus 30 g/d of fatty acid (34 g/d of supplement) before kidding or 50 g/d of fatty acid (56 g/d of supplement) from stearic acid (C16:0 26% and 69.4% C18:0) during lactation (Brenntag S.p.a., Milan, Italy). All the diets were vitamin E supplemented in order to supply 72 mg/head/d during pre-kidding period and 80 mg/head/d after kidding. Pre and post kidding dietary treatments in the three groups were designed to provide similar crude protein (CP) and calcium content, while fat enriched treatments (FO and ST) had similar ether extract (EE). All goats were fed concentrates and corn meal twice a day, fat supplementation was provided in the morning meal mixed into 50g or 100g of corn meal during pre-kidding or post-kidding periods.

Table 1: Ingredients and chemical composition of experimental diets.

	Experimental Diets					
	Pre-kidding			Post-kidding		
	C	FO	ST	C	FO	ST
Ingredient %of DM						
Alfalfa hay	0.0	0.0	0.0	31.2	29.8	30.7
Mixture hay	62.3	59.6	61.4	15.3	14.6	15.1
Concentrate mixture	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
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
DM, %	88.4	88.7	88.6	89.3	89.5	89.4
CP	12.3	11.9	12.2	17.8	17.2	17.5
EE	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
Nel (Mcal/d)	1.61	1.66	1.67	1.67	1.72	1.72

Table 2: Fatty acids profile of the three experimental diets (g/100g of FAMEs).

	Pre-kidding			Post-kidding		
	C	**FO**	**ST**	**C**	**FO**	**ST**
Ingredient %of DM						
Alfalfa hay	0.00	0.00	0.00	31.20	29.80	30.70
Mixture hay	62.30	59.60	61.40	15.30	14.60	15.10
Concentrate mixture	31.90	30.50	31.40	46.80	44.80	46.20
Corn meal	5.30	5.00	5.20	6.20	5.90	6.20
Fish oil	0.00	4.40	0.00	0.00	4.30	0.00
Stearate	0.00	0.00	2.00	0.00	0.00	1.90
$CaCO_3$	0.50	0.50	0.00	0.50	0.50	0.00
DM, %	88.40	88.70	88.60	89.30	89.50	89.40
CP	12.30	11.90	12.20	17.80	17.20	17.50
EE	2.90	4.90	4.50	3.20	5.20	4.80
NDF	43.90	43.80	43.30	33.70	34.00	33.20
Ashes	6.30	6.50	6.00	7.20	7.30	6.80
Ca	0.80	0.80	0.90	1.10	1.10	1.20
P	0.40	0.40	0.40	0.80	0.80	0.80
Nel (Mcal/d)	1.61	1.66	1.67	1.67	1.72	1.72

3.9.2 Live Body Weight, Body Condition Score, Energy Balance, Milk Yield and Composition

Individual DMI was assessed weekly until 21 days after kidding as the difference between feed dry matter (DM) offered and feed DM refused. 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 the same days as for LBW, body condition score (BCS) was determined using a five points scale according to Santucci et al. (1991). Goats were milked once daily at 8:00 am. Milk yield and milk samples were recorded and collected by dividing the suckling kids from the mothers for two consecutive milkings, starting from the evening milking on the day before to the end of the evening milking on the subsequent day. Separated kids were fed the relative mother's milk after milk sample collection.
For each goat, milk yield was recorded once a week. Individual milk samples were taken on day 0, 7, 14 and 21 of lactation and an aliquot subsequently analyzed for fat, protein, lactose and urea content by infrared analyzer (MilkoScan™, FOSS, Hillerød, Denmark) while somatic cells count (SCC) were assessed using fluoro-opto-electronic assay (Fossomatic FC™; FOSS, Hillerød, Denmark). A second aliquot was stored at -20 °C and subsequently analyzed by gas chromatography to determine FA composition of milk. Energy balance was weekly calculated by Small Ruminant Nutrition System (Cannas et al. 2007).

3.9.3 Blood samples and analysis

In order to evaluate serum metabolites, white blood cell count (WBC) and haemochromocytometric parameters (HCM), individual blood samples were taken at 14, 7 and 2 days before the expected kidding date as well as at 0, 2, 7, 14 and 21 DIM. Blood samples were collected from the jugular vein of the goats before the morning feeding in 2 vacuum sterile tubes containing either EDTA (Terumo Venoject® 10-mL VF-109SDK) or a clot activator (VF-109SP).
WBC and HCM were assessed in whole blood samples with a Hemat 8 (SEAC, Calenzano, Florence, Italy). Blood samples were subsequently centrifuged and plasma was obtained via centrifugation for 10 min at 1000 g. Serum for the determination of concentration of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), non-esterified fatty acid (NEFA), glucose, beta-hydroxybutyrate (BOHB), urea, triglycerides and cholesterol was stored at -20 °C until analyzed. Serum concentrations of ALAT, ASAT, urea, cholesterol, glucose and triglycerides were measured by a clinical chemistry analyzer (ILab 300 plus, Instrumentation Laboratory s.p.a., Milan) using reagents provided by

the same company; NEFA and BOHB were tested using Randox reagents (Randox, Crumlin, UK).

3.9.4 Adipose tissue and liver collection

Liver and adipose tissue biopsies were harvested on days -7, +7 and +21 relative to parturition for each experimental subject, via puncture biopsy under local anesthesia. The biopsy area was shaved and cleaned with disinfectant. For liver biopsy a 14G biopsy needle was introduced in a small incision made at the right 11th intercostal space at about 15 cm below the spine. Incision were sutured and treated with topical antibiotic agents.
Subcutaneous adipose tissue biopsy was taken from alternate sides of the tail-head region. The biopsy area was shaved and cleaned with disinfectant, an incision of 2-3 cm length was made between the tail head and the ischiatic bone, and a sample of approximately 1 cm3 of subcutaneous white adipose tissue was removed. Incisions were sutured and treated with topical antibiotics agents.
Biopsied tissue was fixed in B5 fixative (Bio-Optica, Milan, Italy) for 5 to 7 h, dehydrated by a graded series of ethanol, cleared with xylene, paraffin embedded and sectioned at 8 □ m.
Serial sections were placed on glass microscope slides, previously treated with Vectabond (Vector Laboratories, Burlingame, CA, USA) to enhance the adherence of tissues and stained with Haematoxylin and Eosin (H.E; Bio-Optica, Milan, Italy).
For each biopsy, 5 randomly chosen fields were photographed at 200X magnification on a light microscope (Nikon Diaphot TMD- Nikon, Japan).
To assess the effects of the diets on adipose tissue, variations in adipocytes area were evaluated (Fig.2). For each biopsy, 5 randomly chosen fields of 40000 μm2 in surface (1 randomly chosen field/image) were scored and the unit of mean adipocyte area, expressed as square micrometers (μm2), was calculated.
To evaluate the effect of the diets on hepatic tissue, variations in adipocytes fat infiltration were measured. Experiments were divided in two steps.
In the first step, the level of infiltration was classified histologically according to 6 different degrees (Grades der Leberverfettung or GdL), as previously described (Kalaitzakis et al. 2007). Range of severity of fatty infiltration (GdL) scores was from 0 (no fat droplets visible) to 5 (pan-lobular fatty infiltration), following the point score scale of Mertens (Mertens 1992): according to this scale the area from the central vein to the portal triad of the hepatic lobule was divided into 3 equal concentric regions. In each of these regions, fatty infiltration was evaluated and a score was obtained using 5 points in ascending order of severity of cellular lesion: no lesion with all the cells showing the typical aspect of hepatocytes, a fine granular cytoplasm and a centrally placed nucleus (0

points), "cloudy" swelling, cells with a foaming aspect of the cytoplasm (0.5 points); "cloudy" swelling with/and some small vacuoles (1.0 points); many small vacuoles (2.0 points); moderately sized vacuoles (3.0 points); large vacuoles (4.0 points); and stamp cells, with the cytoplasm completely occupied by a single vacuole (5.0 points).
Regions were scored according to the presence of the most severe lesion. For every sample, 5 randomly chosen lobules were scored (1 randomly chosen lobule/image) and the median calculated. From that median, each biopsy was classified according to a 6-point scale of severity of fatty infiltration, using a semi-quantitative scoring system (Kalaitzakis et al. 2007). When a sample did not contain 5 entire lobules to evaluate, the assessment was performed on 10 partial lobules.
In the second step, starting from the cellular lesion formerly described, hepatocytes were classified as normal cells, cloudy cells with a foaming aspect due to cloudy-swelling cytoplasm and or to the presence of small or moderately sized vacuoles and vacuolated cells, with large vacuoles or one single vacuole inside the cytoplasm. For each lobule, a triangular shaped area representative of one fourth of a single lobule was selected and the percentages of normal, cloudy and vacuolated cells were calculated as single type of cell or as the sum of these cells, classified as fatty infiltrated cells. All sections were analyzed by one technical expert using an image analysis system (ImageJ 1.41g, NIH) (Abràmoff et al. 2004) to avoid individual variation. Samples were blind analyzed to prevent any bias. For each biopsy, on additional sections, to evaluate the presence of inflammatory the presence of fibrosis features was evaluated with the Masson's trichrome staining, that specifically dyes collagen (Brunt et al. 1999).

3.9.5 Statistical analysis

Data relative to DMI, LBW, BCS, energy balance, milk yield and milk composition were analyzed by a repeated measures model using a MIXED procedure in SAS 9.2 (SAS Inst., Inc., NC, USA). The statistical model considered as fixed effects time, treatment and time x treatment interaction as well as goat as the random effect. A general linear model (GLM) followed by a Bonferroni post –hoc test to compare the differences within diet supplementations and within lactation time points were used on histological study. Hematological and histological data statistics were computed by using IBM SPSS 21.0 for Windows (IBM SPSS, Armonk, New York, USA). Because

of the repeated measurement in the data, in order to determine the effects of different diets and time of sampling on the blood differential leukocyte count (dependent variables), a Generalized Estimating Equation (GEE) was used. The dependent variables had an inverse gaussian distribution for blood leukocytes differential cell count and a negative binomial distribution for haemochromocytometric, blood metabolites and histological parameters, so an identity link function was used. Goodness of fit was assessed using a quasi-likelihood under independence model criterion (QICC). The threshold for statistical significance was considered to be $P<0.05$. All data in tables are presented as marginal means ± SEM or SD where stated.

3.10 Results

3.10.1 LBW, BCS, DMI, EB and milk yield and composition

In the present trial no significant differences were found for LBW, BCS and forage or concentrate DMI from the week before kidding to the third week of lactation between the three experimental groups. In the same way milk yield, fat-corrected milk, and milk composition were not affected by the dietary treatments except for a higher overall milk protein content in ST compared to C and FO and milk urea content in FO than ST (41.50 vs. 32.20 mg/100ml) on day 14 of lactation (Table 2). Energy balance was not different in the three experimental groups although a tendency can be observed in the third week with positive balances in both fat-supplemented groups and negative for C (1.18 vs. -0.48 Mcal/d respectively, $P = 0.07$). At the same time the two treatment groups in the first week postpartum had energy balance much more negative than the control group (-1.11 Mcal/d FO group and -1.13 Mcal / d ST group).

Table 2: Performance of dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST).

	Treatment				P		
					Trt	Time	Trt*Time
Time	**C**	**FO**	**ST**	**SE**			
Milk production (kg)							
7	3.52	4.15	3.45	0.33	0.27	<0.01	0.63
14	4.20	4.59	3.76				
21	4.23	4.32	3.64				
7-21	3.98	4.35	3.62				
Fat-corrected milk (kg)							
7	4.98	4.40	4.06	0.49	0.10	0.62	0.13
14	4.34	5.24	3.85				
21	5.21	3.88	3.55				
7-21	4.84	4.51	3.82				
BCS							
-7	2.56	3.19	2.89	0.19	0.35	<0.01	0.17
7	3.00	3.38	3.38				
14	3.19	3.50	3.31				
21	3.31	3.44	3.31				
DMI (kg)							
-7	3.08	2.44	2.48	0.44	0.47	0.01	0.48
7	2.46	2.77	2.29				
14	2.99	2.80	2.84				
21	3.27	3.62	3.01				
Energy Balance (Mcal)							
7	-0.09	-1.11	-1.13	0.71	0.35	0.02	0.07
14	-0.96	-0.59	0.76				
21	-0.48	1.18	1.18				

3.10.2 Blood components and serum metabolites

ALAT content was constantly higher in FO fed animals than ST in the two weeks after kidding (Table 3), while significantly higher ALAT serum content in FO than C was observed on day -7 (16.17 vs. 13.57 IU/L; $P = 0.08$) and +21 (16.20 vs. 11.29 IU/L; $P < 0.01$) from kidding. ASAT serum content did not vary in the three experimental groups. Cholesterol content was lower in both

fat-supplemented animals than C in the first week of lactation (57.68 mg/dL ST, 53.74 mg/dL FO and 90.55 mg/dL C $P < 0.01$). Glucose serum content was higher in both FO and ST goats than C in the week before kidding (56.45 mg/dL ST, 52.43 mg/dL FO and 44.73 mg/dL C $P < 0.05$), while non-significant differences were measured during the first three weeks of lactation. No significant interaction of dietary treatment per time effects was observed for BOHB serum content either before or after kidding, but means values during the whole trial period revealed significant higher BOHB serum content for ST than FO (0.54 mmol/L ST and 0.33 mmol/L FO $P < 0.05$). No differences were detected on triglycerides serum content in the three experimental groups during the trial; in the same way serum urea content was not influenced by the treatments in the single sampling days, but overall there was a tendency to higher levels (treatment effect $P = 0.07$) in ST than FO and C. ST had lower NEFA levels compared with C during pre-kidding week, FO in the first week of lactation had highest levels (0.28 mmol/L C and 0.82 mmol/L FO $P < 0.01$). At 21 days in milk, both FO had lower NEFA levels than C (0.27 mmol/L FO vs. 0.44 mmol/L C $P < 0.05$).

Table 3: Serum metabolites of dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST).

	Treatment						P		
							Trt	Time	Trt*Time
Time	**C**	SD	**FO**	SD	**ST**	SD			
ALAT (IU/L)									
-7	13.57^{a,g}	3.05	16.17^{f}	0.69	15.58^{fg}	2.35	0.007	0.004	0.003
7	15.03^{a,fg}	3.56	16.47^{f}	0.86	14.27^{g}	2.33			
21	11.29^{b,e}	1.91	16.20^{d}	2.64	13.27^{de}	2.96			
Cholesterol (mg/dl)									
-7	55.72^{c}	5.75	54.77^{b}	4.90	57.24^{b}	3.19	0.001	<0.001	0.001
7	90.55^{a,d}	17.69	53.74^{b,e}	4.43	57.68^{b,e}	8.25			
21	69.86^{b,g}	9.25	84.49^{a,f}	16.40	71.65^{a,fg}	14.57			
Glucose (mg/dl)									
-7	44.73^{b,e}	4.63	52.43^{b,d}	7.31	56.45^{b,d}	4.74	0.012	<0.001	<0.001
7	59.70^{a}	12.36	49.29^{ab}	14.98	62.80^{ab}	6.66			
21	51.10^{ab,e}	8.34	63.08^{a,d}	10.53	67.88^{a,d}	13.48			
BOHB (mmol/L)									
-7	0.31	0.09	0.29	0.22	0.32	0.09	0.029	0.003	0.207
7	0.56	0.21	0.40	0.04	0.61	0.26			
21	0.42	0.12	0.32	0.04	0.64	0.27			
NEFA (mmol/L)									
-7	0.59^{a,d}	0.31	0.38^{ab,de}	0.44	0.27^{e}	0.02	0.166	0.081	0.006
7	0.28^{b,e}	0.13	0.82^{a,d}	0.69	0.37	0.26			
21	0.44^{ab,d}	0.17	0.27^{b,e}	0.07	0.27^{de}	0.16			

^{a,b,c}Means within each column with different superscripts are significantly different ($P < 0.05$).
^{d,e}Means within each row with different superscripts are significantly different ($P < 0.05$).
^{f,g}Means within each row with different superscripts are significantly different ($P < 0.09$)

3.10.3 Haemocromocytometric parameters

Neutrophils count was higher at day 21 in C compared with FO, in the non-supplemented group neutrophils were higher during postpartum compared with day -7. In FO, day 7 had the highest count of neutrophils (Table 4).
There was a tendency (P=0.09) in lymphocytes concentration at day 21; FO tended to have higher values than C. C had the highest values during prepartum.
Eosinophils were higher at day -7 in C and FO compared with day 7, while ST had the highest count at day 21 compared with day 7.

Table 4: Haemochromocytometric parameters of dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST).

	Treatment						P		
							Trt	Time	Trt*Time
Time	**C**	SE	**FO**	SE	**ST**	SE			
HGB									
-7	88.00	2.98	99.00	6.85	90.25^{ab}	1.52	0.147	0.002	0.037
7	83.75	4.56	98.28	6.30	93.00^{a}	3.37			
21	83.65	3.93	92.75	4.22	86.50^{b}	2.49			
Neutrophils									
-7	45.55^{b}	5.34	49.10^{b}	1.37	46.45	2.73	0.253	0.001	0.014
7	63.90^{a}	3.23	56.85^{a}	0.49	50.35	3.26			
21	60.40^{a,d}	1.80	50.02^{b,e}	0.70	49.27^{de}	7.49			
Monocytes									
-7	4.15	0.60	4.65	2.16	4.97	1.36	0.253	0.158	<0.001
7	3.85	1.66	3.87	0.95	6.72	0.93			
21	2.65	1.41	4.20	0.83	5.50	0.90			
Lymphocytes *									
-7	49.37^{a}	4.73	44.65^{ab}	2.53	47.77	3.99	0.381	<0.001	0.089
7	31.62^{b}	2.53	38.50^{b}	0.71	42.25	3.86			
21	35.92^{a,e}	2.03	43.90^{a,d}	0.65	43.90^{de}	7.57			
Eosinophil									
-7	1.24^{a}	0.26	0.97^{a}	0.23	2.08^{ab}	0.98	0.515	<0.001	0.050
7	0.23^{b}	0.02	0.30^{b}	0.06	0.28^{b}	0.07			
21	0.45^{ab}	0.07	1.03^{ab}	0.30	0.63^{a}	0.05			

^{a,b,c}Means within each column with different superscripts are significantly different ($P < 0.05$).
^{d,e}Means within each row with different superscripts are significantly different ($P < 0.05$)

3.10.4 Adipose Tissue Histology

In all the samples, histological examination showed that adipocytes were individually held in place by delicate reticular fibers clustering in lobules bounded by fibrous septa within the adipose tissue. Typical, uni-locular signet-ring shaped adipocytes were found in all the biopsies examined (Fig. 3). Morphometric evaluation showed a significant decrease of adipocytes surface between -7 and 21d in ST and C, whereas in FO the adipocyte surface reduction was related to the -7 to 7 d interval reaching a plateau until day 21 (Table 5).

Table 5: Mean Adipocyte Area (μm^2) of subcutaneous adipose tissue of dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST).

	Treatment						***P***		
							Trt	**Time**	**Trt*Time**
Time	**C**	SD	**FO**	SD	**ST**	SD			
-7	3200.00[a]	1001.86	2877.69[a]	875.51	3088.80[a]	1010.39	0.598	<0.001	<0.001
7	1970.44[b]	523.28	1801.80[b]	547.69	2156.33[b]	718.04			
21	1157.74[c,e]	216.20	1851.85[b,d]	638.18	1066.66[c,e]	269.13			

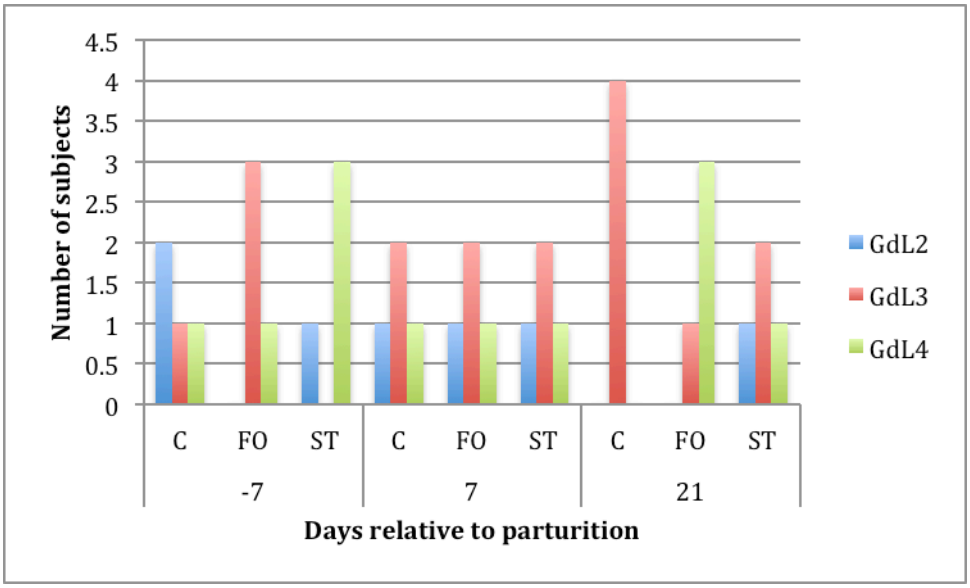
[a,b,c]Means within each column with different superscripts are significantly different ($P < 0.05$).
[d,e]Means within each row with different superscripts are significantly different ($P < 0.05$).

3.10.5 Hepatic Tissue Histology

In all the samples, histological examination revealed that goats experienced fatty liver before and after parturition. Cell morphology was comparable to that described in the dairy cow. No foci of inflammatory cells were observed and Masson's trichrome staining showed no signs of fibrosis in all specimens examined.

Semi-quantitative evaluation performed in first step of analysis demonstrated that liver tissue ranged from a mild grade of infiltration (Gdl 2), moderate (GdL 3) to a severe grade of infiltration (Gdl 4) (Fig. 1). No goats were classified as Gdl 0, 1 or 5 (Fig.1) within time and treatments

Figure 4: Histological classification of fatty infiltration change of the liver (FCL or hepatic lipidosis). Classification made according to six different degrees (Grade der Lebervettung or GdL) ranging from 0 (no fat droplets, totally normal hepatocytes) to 5 (pan lobular infiltration) of FCL (Mertens 1992; Kalaitzakis et al. 2007; Kurosaki et al. 2008).



Morphological evaluations of the second step showed a significant increase of fatty infiltrated cells percentage between -7 and + 21 d, in FO (Fig.6). In ST and C no differences were observed between -7 and + 21, although fatty infiltrated cells percentage decreased at 7 days. Comparing the three diets at day 21, percentage of this type of cells was significantly higher in ST than in C and FO. Distribution of vacuolated cells percentage, within times and treatments reflected distribution of total fatty infiltrated cells previously described. Distribution of cloudy cells percentage in ST was comparable to that described previously for total fatty infiltrated cells; on the contrary, a significant decrease of cloudy cells percentage between 7 and 21 d were observed, both in C and FO. Comparing the three diets, at day 21, the highest percentage of vacuolated cells was in FO, while the highest percentage of cloudy cells was in ST (Fig.5 A&B).

Figure 6: Percentage of fatty infiltrated liver cells of dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST) (** differs from * P<0.05).

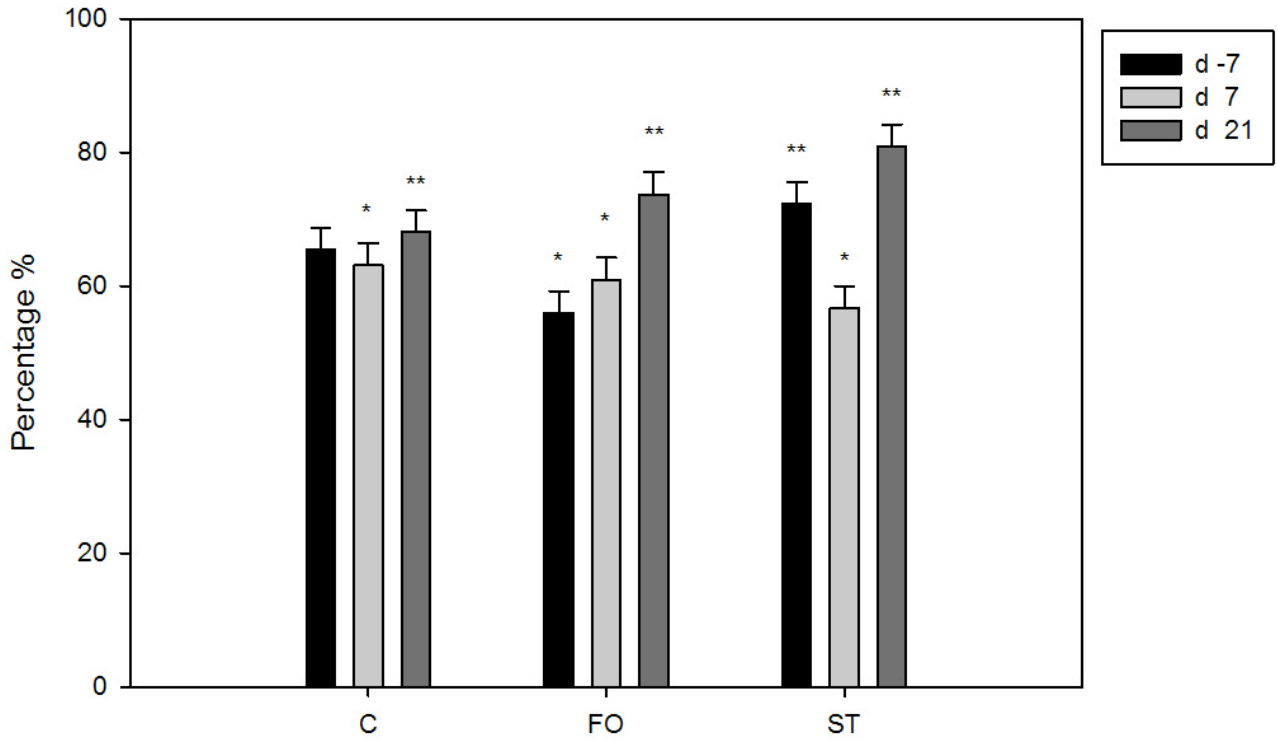
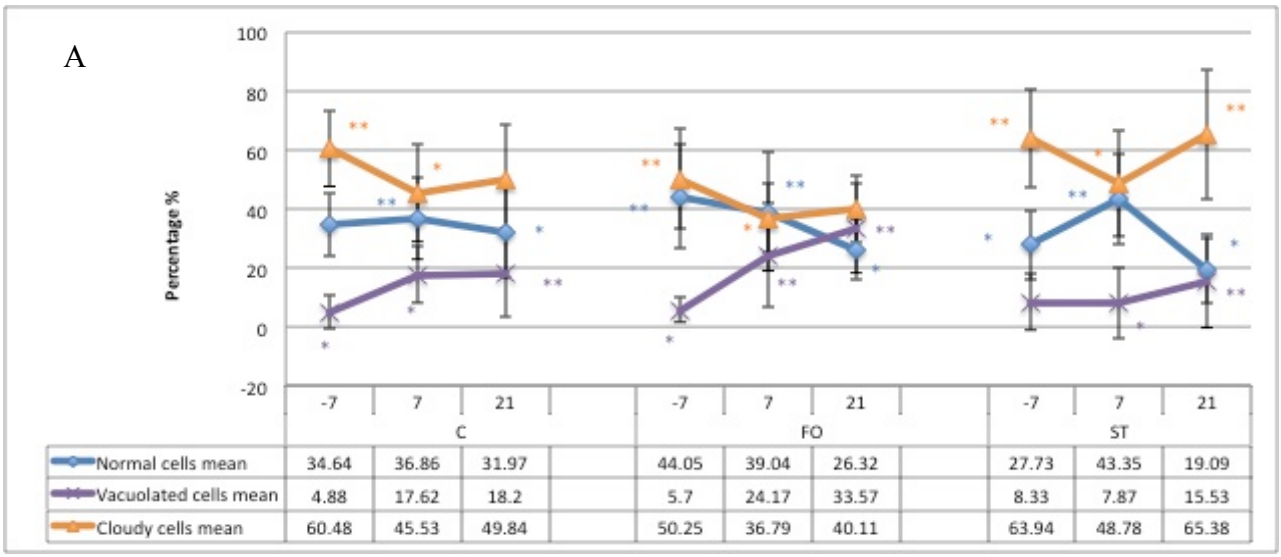
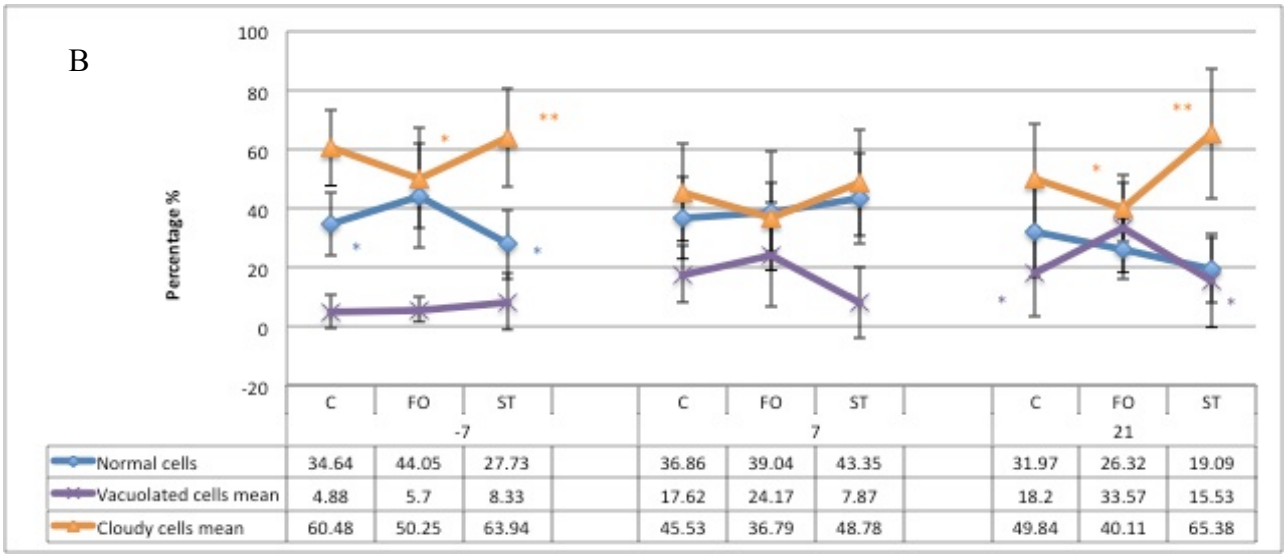


Figure 5A&B Percentage of normal cells, vacuolated and cloudy cells in liver tissue from dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST) (** differs from * P<0.05)



	-7	7	21	-7	7	21	-7	7	21
	C			FO			ST		
Normal cells mean	34.64	36.86	31.97	44.05	39.04	26.32	27.73	43.35	19.09
Vacuolated cells mean	4.88	17.62	18.2	5.7	24.17	33.57	8.33	7.87	15.53
Cloudy cells mean	60.48	45.53	49.84	50.25	36.79	40.11	63.94	48.78	65.38

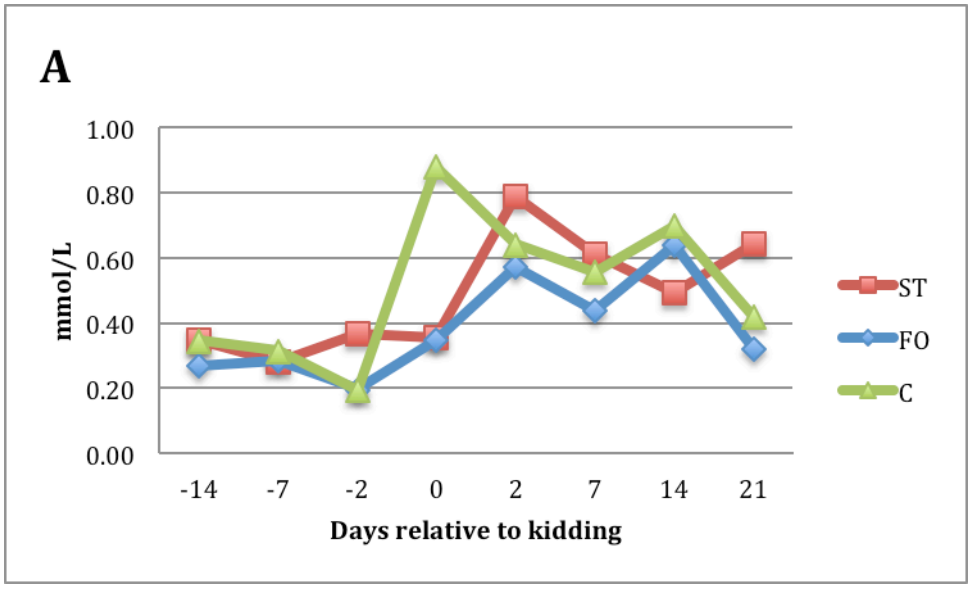


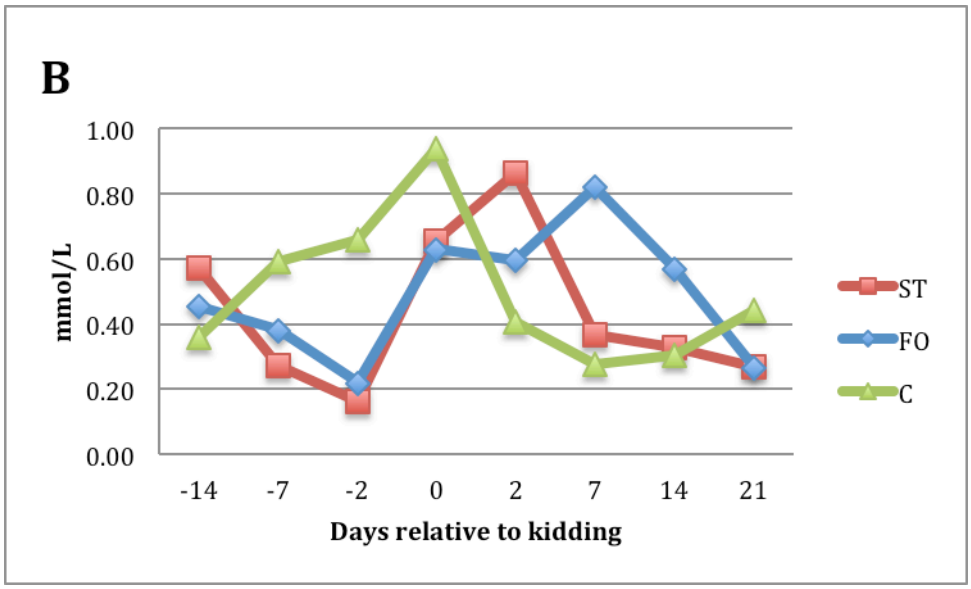
	C	FO	ST	C	FO	ST	C	FO	ST
	-7			7			21		
Normal cells	34.64	44.05	27.73	36.86	39.04	43.35	31.97	26.32	19.09
Vacuolated cells mean	4.88	5.7	8.33	17.62	24.17	7.87	18.2	33.57	15.53
Cloudy cells mean	60.48	50.25	63.94	45.53	36.79	48.78	49.84	40.11	65.38

3.11 Discussion

Goats fed C diets and supplemented with saturated and unsaturated fat had similar milk production and composition. Although in the trial a limited number of goats per treatment was used and milk production has been measured once a week by separating suckling kids, as reported in literature (Chilliard et al. 2014) and observed in a previous trial performed by our research group using a rumen-inert supplement (Agazzi et al. 2010), goats fed fish oil did not face milk fat depression (MFD) like cows (Invernizzi et al. 2010). In the previous trial, palm oil increased milk production; in contrast, ST did not affect any performance. NEFA blood levels are tightly linked to energy balance (Eknæs et al. 2006). NEFA suggested values for does with neutral EB are 0.200–0.217 mmol/L (Chilliard et al. 1977); concentrations observed were almost constantly higher with FO peaking at day 7 and passing the concentration of 0.7 mmol/L proposed as borderline for toxemia (D'Angelo et al. 2005). NEFA lowest concentrations were recorded for ST; data were, however, congruous with results obtained by Pinotti et al. (2008) and Magistrelli and Rosi (2014). NEFA and BOHB (Fig.6) had a similar pattern in C and ST spiking at day 0 and 2 respectively, in agreement with González et al. (2011), while NEFA in FO spiked at day 7 and BOHB had a couple of lower spikes at 2 and 14 days. EB values showed a continuous NEB in C for three weeks after kidding, while ST was the fastest to get back to positive values at 14 and 21 days.
BOHB and ALAT results were consistent with those obtained in a previous experiment (Agazzi et al. 2010) and, about the latter, FO had the highest values ($P<0.05$).

Figure 6: Figure 1 A&B Serum BOHB (A) and NEFA (B) content of dairy goats fed either a basal diet (C) or supplemented with fish oil (FO) or stearate (ST)





Data on subcutaneous adipose tissue are well coupled with NEFA (and BOHB) serum levels: in fact, the highest adipocyte area reduction in C was observed between days -7 and 7, that matches with spiking levels of NEFA, both clear signs of strong lipomobilization. From 7 to 21 days adipose tissue seemed to be still mobilized considering the negative energy balance of not supplemented goats. ST had a similar pattern but its energy balance got positive already between the 7th and 14th day post parturition; most likely its lipolysis would be reduced earlier than C, however after 21 days. Histological data on adipose tissue support the idea that FO could limit lipolysis even if energy balance was still negative (at least until day 7). The initial decrease of adipose cells surface, common to all treatments, stopped between days 7 and 21. This behavior is in agreement with studies on cows (Thering et al. 2009; Schmitt et al. 2011). Since important interspecies differences have been outlined in MFD, very interesting would be exploring the fine mechanisms that regulate this response.

Morphology of hepatocytes affected by fatty infiltration was comparable to that previously described in periparturient dairy cows (Kalaitzakis et al. 2007).

Our research demonstrated FLC in healthy peri-parturient dairy goats, although severe level of FCL were described, feature of perisinusal fibrosis indicating a progression to inflammatory processes were not observed (Brunt and Tiniakos 2010), in agreement with haemocromocytometric parameters.

Evaluation of level of severity did not show any differences between treatments and during the time. FLC is characterized by dynamic changes inside the hepatocyte, ranging from a cytoplasm with a foamy appearance due to the abundance of tiny fat droplets to a vacuolated appearance due to the presence of micro and macro vesicles, where large vesicles often arise from the joining of many smaller vesicles. Accumulations of fat usually start in zone 3 and with the increase of severity may progress to the whole acinus. Due to this histological and cytological complexity of FLC, it is possible that to support the evaluation of level of severity with a semi-quantitative approach, it would be needed a greater number of subjects. When FLC was assessed by considering the percentages of normal and infiltrated cells, the differences arose. We observed that FLC was finely modulated by metabolic and biochemical changes that occurred before and after partum. The highest level of FLC severity appeared at day 21, independently from the diet. Interestingly, although at this time percentage of fatty infiltration in ST was the highest, only in FO the increase of fatty infiltrated cells was significant and it occurred more gradually than in C and ST. Change in percentage of cells categorized as normal, cloudy and vacuolated during the time reflects this trend on the basis of the severity of the cytological lesion. Comparing the treatments, percentage of normal, vacuolated and cloudy cells was quite similar at day -7, although the percentages of cloudy cells were the highest, while the percentage of vacuolated cells were the lowest both in control

and treated animals. A day 21, the highest percentage of cloudy cells was observed in ST, while the highest percentage of vacuolated cells was observed in FO indicating that FO might induce slight more detrimental effects on liver. This might be in contrast with biochemical results, however we can hypothesize that the gradual onset of FLC observed in FO might have better preserved the hepatocyte functions since cells had time to adapt to cytoplasmic change induced by fatty infiltration. In human, macro vesicular steatosis is a common histological finding in human liver biopsy from patient with non alcoholic fatty liver (NAFL). It is characterized by both large and small droplets and, in absence of other cytological lesions and histological lesions, is considered to have a good long-term prognosis (Tiniakos 2009).
In conclusion, data suggest dietary saturated and unsaturated lipid supplements are able to modulate the lipomobilizing machinery; in particular fish oil could reduce lipomobilization. On the other side it appeared to have slight more detrimental effects on liver.

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CHAPTER 4

How dietary Fish oil and stearate regulates hepatic and adipose Lipid Metabolism Transcriptomics in periparturient dairy goats

4 How dietary fish oil and stearate regulates hepatic and adipose lipid metabolism transcriptomics in periparturient dairy goats

4.1 Abstract

The aim of this study was to evaluate the effects of saturated or unsaturated fatty acids on hepatic and adipose mRNA level of genes involved in the major metabolic pathways of lipogenesis. For this study 23 spring kidding goats were divided in a randomized complete block design into three homogenous groups for parity, age and milk production in the previous lactation and assigned to three experimental treatments. Goats fed a pre- or post-kidding basal diet (C) or pre-/pos-kidding basal diet supplemented with fish oil (FO) or pre-/post-kidding basal diet supplemented with stearic fatty acid (ST). At 14, 7 and 2 days before the expected kidding date as well as at 0, 2, 7, 14 and 21 DIM individual blood samples were taken. Liver and adipose tissue biopsies were harvested on days -7, +7 and +21 relative to parturition for each experimental subject. No differences were found on BW, BCS, milk yield and milk composition; in particular, no differences were found for milk fat percentage in animals fed FO. This result confirmed that goats are much less sensitive then cows in ruminal biohydrogenation pathway. Saturated fatty acid or PUFA supplementation during peripartum had no effect on abundance of mRNA encoding for SREBF2, SOD, CAT and PPARA in liver. Moreover, SCD mRNA expression was significantly differentially expressed in FO compared to ST ($P<0.03$): at day 7 was up regulated in ST (1.8-fold; $P<0.01$) and at day 21 (1.68-fold; $P<0.01$) compared with FO. With an opposite trend SCD expression in FO was down regulated (2.57 and 3.14-fold) respectively at days 7 and 21. Differences were found in EB between C compared to ST and FO at d 7 (0.34 vs. -1.12 and 0.91 respectively; $P\leq0.05$). Differences were observed in serum cholesterol at different time points ($P<0.01$), in particular at day 21 its amount was significantly higher in FO compared to C ($P<0.01$) and ST ($P<0.05$). ACOX1 mRNA expression at d 7 was significantly higher in ST compared to C (1.64 vs. 0.87 fold; $P<0.03$); no statistical differences were observed between ST and FO (Fig.3). Both FA-supplemented groups had higher ACOX1 mRNA levels ($P<0.01$) at day 7 compared to their expression before kidding, furthermore, expression in ST at day 21 was almost completely restored at pre-kidding levels. A very similar expression pattern to ACOX1 at least for ST and FO although at overall lower expression levels, was observed for ACAA1. At day 21 significantly

higher mRNA levels were observed in FO than ST. Different fat supplementation significantly affected subcutaneous adipose genes involved in TAG synthesis (LPIN1), in the regulation of lipogenesis (THRSP) and nuclear receptor (SREBF1). No effects were observed on the expression of the adipokine (ADIPOQ). LPIN1 expression was significantly higher in FO group compared to ST (1.46-fold vs. 0.59-fold; $P<0.04$). In conclusion this study point out the important role of saturated or unsaturated fat administration in dairy goat diets to modulate mRNA expression of central genes involved in lipogenesis.

Keywords: dairy goats, transcriptomic, lipid metabolism, stearate, fish oil

4.2 Introduction

In literature several studies reported the nutrition influences on gene expression in mammary, adipose or liver tissues in ruminant species (Chilliard et al. 2007; Bernard et al. 2008; Shingfield et al. 2010), but there are differences among ruminants as reported by Chilliard et al. (2014) and Tsiplakou et al. (2011). Different fat sources have been shown to differentially impact adipose and liver tissue in small ruminants (Agazzi et al. 2010; Tsiplakou et al. 2011; Bernard et al. 2012; Bichi et al. 2013a; Ebrahimi et al. 2013; Toral et al. 2013). Dietary marine oil or algae have been associated with milk fat depression in cow (Invernizzi et al. 2010) and in dairy ewes (Toral et al. 2010), but lipid supplements increase milk fat content in goats (Chilliard et al. 2003; Bouattour et al. 2008; Marin et al. 2011; Nudda et al. 2013). Chilliard et al. (2014) confirmed a not altered fat content and yield by dietary fish oil supplementation in dairy goats probably because they are much less sensitive than the cow in ruminal biohydrogenation pathways (Chilliard et al. 2007; Bernard et al. 2012). As reported by Toral et al. (2013), up to now, is not clear the physiological basis for the particular response of dairy goats to lipid supplements. Studies in dairy goats characterizing lipid metabolism in body tissues and the influence of different fat sources are still scant, especially in fresh dairy goats when they are in NEB. Diet has effects on the transcription of the major genes involved in fatty acid uptake and de novo synthesis. Stearoyl-CoA desaturase (delta-9-desaturase) 1 (SCD1) expression depends on the type of lipid supplement fed (Shingfield et al. 2010; Bernard et al. 2013). Toral et al. (2013) demonstrated in late lactating dairy goats in positive energy balance that fish oil administration reduced the mRNA abundance of SCD1. In a previous experiment we showed the effectiveness of hydrogenated palm oil, mainly containing C16:0 and C18:0, in enhancing expression of PPARA and selected target genes in liver of transition dairy goats in comparison with fish

oil (Agazzi et al. 2010). A study by Tsiplakou et al. (2011) demonstrated the pivotal role of subcutaneous adipose tissue during lactation and confirmed that gene expression depends on genetic, nutritional and environmental factors.
The aim of the present study is to evaluate the effects of saturated or unsaturated fatty acids on hepatic and adipose mRNA levels of a substantial number of genes involved in the major metabolic pathways of lipogenesis.

4.3 Materials and methods

4.3.1 Animals, experimental diet and management

The in vivo trial was performed at the "Centro Zootecnico Didattico Sperimentale" of the University of Milan, Lodi, Italy using 23 spring dairy goats homogenous for parity, age and milk production, from 21 days before the expected kidding date to 21 days of lactation. Goats were housed in individual boxes and individually fed ad libitum. All experimental procedures were approved by the Ethics Committee of the University of Milan (attachment n. 5 January 26th 2011). Details of the experimental pre- and post- kidding diets have been described previously (Invernizzi et al. 2015). Briefly, during pre-kidding, animals fed ad libitum mixed hay, 600g/head/d of concentrate and 100g/head/d of corn meal pus 9 g/d of calcium carbonate (C), or 81 g/d of fish oil (FO) or 34 g/d of calcium stearate (ST). During post- kidding, animals fed ad libitum alfalfa hay and mix hay, 1500g/head/d of concentrate and 200g/head/d of corn meal plus 12 g/d of calcium carbonate (C), or 135 g/d of fish oil (FO), or 56 g/d of calcium stearate (ST).
Diets ingredients and chemical composition of the six experimental diets are detailed in table 1 and the fatty acids profile in table 2. All the diets were vitamin E supplemented in order to supply 72 mg/head/d during pre kidding period and 80 mg/head/d after kidding. Pre and post kidding dietary treatments in the three groups were designed to provide similar crude protein (CP) and calcium content, while fat enriched treatments (FO and ST) had similar ether extract (EE). All goats were fed concentrates and corn meal twice a day, fat supplementation was provided in the morning meal mixed into 50g or 100g of corn meal during pre kidding or post kidding periods.

Table 1: Diets ingredients and chemical composition of the six experimental diets.

	Experimental Diets					
	Pre-kidding			Post-kidding		
	C	FO	ST	C	FO	ST
Ingredient %of DM						
Alfalfa hay	0.0	0.0	0.0	31.2	29.8	30.7
Mixture hay	62.3	59.6	61.4	15.3	14.6	15.1
Concentrate mixture	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
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
DM, %	88.4	88.7	88.6	89.3	89.5	89.4
CP	12.3	11.9	12.2	17.8	17.2	17.5
EE	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
Nel (Mcal/d)	1.61	1.66	1.67	1.67	1.72	1.72

Table 2: Fatty acids profile of the three experimental diets (g/100g of FAMEs).

	Pre-kidding			Post-kidding		
	C	FO	ST	C	FO	ST
Ingredient %of DM						
Alfalfa hay	0.00	0.00	0.00	31.20	29.80	30.70
Mixture hay	62.30	59.60	61.40	15.30	14.60	15.10
Concentrate mixture	31.90	30.50	31.40	46.80	44.80	46.20
Corn meal	5.30	5.00	5.20	6.20	5.90	6.20
Fish oil	0.00	4.40	0.00	0.00	4.30	0.00
Stearate	0.00	0.00	2.00	0.00	0.00	1.90
$CaCO_3$	0.50	0.50	0.00	0.50	0.50	0.00
DM, %	88.40	88.70	88.60	89.30	89.50	89.40
CP	12.30	11.90	12.20	17.80	17.20	17.50
EE	2.90	4.90	4.50	3.20	5.20	4.80
NDF	43.90	43.80	43.30	33.70	34.00	33.20
Ashes	6.30	6.50	6.00	7.20	7.30	6.80
Ca	0.80	0.80	0.90	1.10	1.10	1.20
P	0.40	0.40	0.40	0.80	0.80	0.80
Nel (Mcal/d)	1.61	1.66	1.67	1.67	1.72	1.72

4.3.1 Live Body Weight, Body Condition Score, Energy Balance, Milk Yield and Composition

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 (Flli Fascina snc, Castelvetro P.no, Italy). On the same days as for LBW, body condition score (BCS) was evaluated using a five points scale, where (1) indicates emaciated animals and (5) indicates fat animals (Santucci et al. 1991). Energy balance was weekly calculated by Small Ruminant Nutrition System (Cannas et al. 2007).
Milk yield and milk samples were recorded and collected by dividing the suckling kids from the mothers for two consecutive milking, starting from the evening milking on the day before to the end of the evening milking on the subsequent day. For each goat, milk yield was recorded weekly. Individual milk samples were taken on day 0, 7, 14 and 21 of lactation and an aliquot subsequently analyzed for fat, protein, lactose, urea and somatic cells count (SCC) content by infrared analyzer (Fosso-Matic, Fosso Electric, Denmark). A second aliquot was stored at -20 °C and subsequently analyzed by gas chromatography to determine FA composition of milk. Individual colostrum was sampled within the first 24 h postpartum. Individual mature milk yield records and samples were obtained at 7, 14 and 21 days in milk (DIM), at two consecutive milkings (0700 and 1500 h) and then bulked to make representative individual daily composites. Separate aliquots of each colostrum and milk sample were stored at □ 20 □ C for analysis of individual fatty acids by gas chromatography. Fatty acids profiling on milk and colostrum was performed using the same protocol described in Agazzi et al. (2010).

4.3.2 Blood samples and analysis

Individual blood samples were taken at 14, 7 and 2 days before the expected kidding date as well as at 0, 2, 7, 14 and 21 DIM. Blood samples were collected from the jugular vein of the goats before the morning feeding in 2 evacuated tubes containing either EDTA (Terumo Venoject® 10-mL VF-109SDK) or a clot activator (VF-109SP). Blood samples were subsequently centrifuged and plasma was obtained via centrifugation for 10 min at 1000 g. Serum was stored at -20 °C until analyzed in order to determine alanine transaminase (ALAT), cholesterol, betahydroxybutyrate (BHOB) and non-esterified fatty acids (NEFA) content. A second aliquot was stored at -20 °C and subsequently analyzed by gas chromatography to determine FA composition. A direct in situ transesterification method was used to extract serum fatty acids (Glaser et al. 2010), GC analysis was then performed as for milk samples.

4.3.3 Liver and Adipose tissues collection, primer design, RNA extraction and RT-qPCR

To evaluate genes mRNA levels, liver and adipose tissues biopsies were harvested on days -7, +7 and +21 relative to parturition for each experimental subject, via puncture biopsy under local anesthesia. All the procedures are described by Invernizzi et al. (2015). Briefly, for liver a 14G biopsy needle was introduced in a small incision made at the right 11th intercostal space at about 15 cm below the spine. Incision were sutured and treated with topical antibiotic agents. Adipose tissue biopsy was taken from alternate sides of the tail head. The biopsy area was shaved and cleaned with disinfectant, an incision of 2-3 cm length was made between the tail head and the ischiatic bone, and a sample of approximately 1 cm3 of subcutaneous white adipose tissue was removed. Incisions were sutured and treated with topical antibiotics agents. Biopsied tissue was stored in liquid nitrogen prior to RNA extraction.

All procedures, starting from primers design, were carried out at Dr. Loor Mammalian NutriPhysioGenomics Laboratory at the University of Illinois, Urbana-Champaign, IL.

Genes tested in the current study are listed in Table 3. Primers were designed and evaluated as previously described (Bionaz and Loor 2008b). Primer were designed using Primer Express 3.0 (Applied Biosystems, Carlsbad, CA) with minimum amplicon size of 80 bp (amplicons of 100 to 120 bp were used, if possible) and limited 3' G + C percentage. Primers were aligned against publicly available databases using BLASTN at NCBI. Prior to qPCR, primers were tested in a 20□ L PCR reaction using the same protocol described for qPCR except for the final dissociation protocol. For primer testing we used a universal reference cDNA (RNA mixture from all the samples for liver or adipose tissues) to ensure identification of desired genes. 5 □ L of the PCR product were run in a 2% agarose gel stained with Sybr® Safe DNA Stain (EDVOTEK, Washington, DC, USA). The remaining 15 □ L were cleaned using QIAquick® PCR Purification Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions and prepared for sequencing at the Core DNA Sequencing Facility of the Roy J. Carver Biotechnology Center at the University of Illinois, Urbana-Champaign. Only those primers that did not present primer-dimer, had a single band at the expected size in the gel, and had the right amplification product (verified by sequencing) were used for qPCR. The accuracy of a primer pair also was evaluated by the presence of a unique peak during the dissociation step at the end of qPCR.

The enzyme activities and/or mRNA abundance of enzymes involved in the major pathways of lipid metabolism were measured in liver and adipose tissues.

Immediately after biopsies the liver and adipose tissues were collected, stored in cryotubes and snap frozen in liquid nitrogen. Total RNA was isolated from the liver and adipose samples using QIAzol reagent (Qiagen) through tissues lysing operated by bead beater (BSP, Bartlesville, OK, USA) according to the manufacturer's instructions. Total RNA was purified using miRNeasy columns and on-column RNase-free DNase treatment (Qiagen), following the manufacturer's protocol. RNA quality was assessed for every sample with Bioanalyzer 2100 (Agilent, Santa Clara, USA): only samples with RIN equal to 5.0 or higher were used. Each cDNA was synthesized by RT using 100 ng RNA, 9 mL DNase/RNase free water and 1 mL Random Primers (Roche, Basel, Switzerland). The mixture was incubated at 65 °C for 5 min in an Eppendorf Mastercycler® Gradient and kept on ice for 3 min. A total of 9 μL of Master Mix composed of 4.0 μL 5X First-Strand Buffer (Fermentas, Thermofisher, USA), 1 mL Oligo dT18, 2 uL 10 mM dNTP mix (Invitrogen), 0.25 mL of Revert aid (Fermentas, Thermofisher, USA), 0.125 mL of Rnase inhibitor (Fermentas, Thermofisher, USA) and 1.625 mL DNase/RNase free water was added. The reaction was performed using the following temperature program: 25°C for 5 min, 42°C for 60 min and 70°C for 5 min followed by 4°C, forever (MyCycle™ Thermo Cycler, Bio Rad, Hercules, CA, USA). cDNA was then diluted 1:4 with DNase/RNase free water.

Real-time quantitative PCR using SYBR Green was used to evaluate stearoyl-CoA desaturase (SCD), peroxisome proliferator-activated receptor alpha (PPARA), acyl-CoA oxidase 1, palmitoyl (ACOX1), acetyl-CoA acyltransferase 1 (ACAA1), catalase (CAT), superoxide dismutase 1 (SOD1), sterol regulatory element binding factor 2 (SREBF2), gene expression in liver. Real-time quantitative PCR was used to evaluate sterol regulatory element binding transcription factor 1 (SREBF1), thyroid hormone responsive protein (THRSP), adiponectin C1Q and collagen domain containing (ADIPOQ) and lipin 1 (LPIN1) gene expression in adipose tissue. Ribosomal Protein S15a (RPS15A), Ribosomal Protein S9 (RPS9) and Ubiquitously-Expressed Prefoldin-Like Chaperone (UXT) were used as internal controls and the geometric mean calculated and used to normalize data. The primer sequences and references are shown in Table 3 .

qPCR was performed using 4 μL diluted cDNA, 5 μL of 1X SYBR Green master mix (Quanta BioSciences, Gaithersburg, MD), 0.4 μL of each of 10 μM forward and reverse primers, and 0.2 μL of DNase/RNase-free water in MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, USA). Each sample was run in triplicate, and a 6-point relative standard curve was generated using serial dilutions of cDNA prepared from liver or adipose samples to calculate efficiency of each primer pair. Each assay plate included a negative control in triplicate. The CV was below 10 % for all the primer pairs used. The

reactions were performed in an ABI Prism 7900 HT SDS instrument (Applied Biosystems, USA) using the following conditions: 5 min at 95 °C, 1 s at 95 °C, 40 cycles of 30 s at 60 °C, a final extension followed for a melting curve stage (15 min at 95 °C, 15 min at 60 °C, 15 min at 95 °C). Data were analyzed with the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, CA

Table 3: Primers sequence used for qRT-PCR and accession number at NCBI site

Gene	Accession no.	Forward primer (5'-3')	Reverse primer (5'-3')
ICG			
RPS15A	XM_005697526	TGCCGAGAAGAGAGGCAAAC	TTCGCCAATGTAACCATGCTT
RPS9	XM_005709411	GGCCTGAAGATTGAGGATTTCTT	CGGGTATGGTGGATGGACTT
UXT	XM_005700842	GAGAAAGTGCTGCGCTACGA	TGGCCAGCTGCTCATATACCT
Liver			
SCD	AF422171.1	CCCAGGCTTTTGCTAGATGAA	CACATCATGCATGCTGACTCTCT
ACOX1	XM_005694433.1	CAAACCCAGCAATATAAACTTTTTCC	CTTCATTAATCCGATGATAGGTCTCTT
PPARA	XM_005681212.1	ATCATGGAGCCCAAGTTCGA	CTCCGCAGCAAATGATAGCA
ACAA1	XM_005695550	GCCAGAGACTGCCTGATTCC	GTCCTGCTTCTCCCGTGAAA
CAT	XM_005690077.1	ACACCCCCATTTTCTTCATCAG	ACCATGTCCGGATCCTTCAG
SOD	NM_001285550.1	AAAGTCGTCGTAACTGGATCCATT	CTGCACTGGTACAGCCTTGTG
SREBF2	XM_005681190.1	CATCATCGAGAAGCGGTATCG	AACGCCAGACTTGTGCATCTT
Adipose			
SREBF1	HM443643	TCTTCCATCAATGACAAGATCGTT	CGGATGTAGTCGATGGCTTTG
THRSP	JN684754	ATTGGCCTAAAAGAGGGCTATGT	CCTCTGTTTCCGGGCTGTTA
ADIPOQ	EU375363	CACCTTCACAGGCTTCCTTCTC	TGTGGACTGTTCTTTCACTTTGC
LPIN1	XM_005686993.1	CTTACCTTGGGCTTGCTCATTAG	TACTCGGTTCAGAGCAGTTAAAATTC

4.3.4 Statistical analysis

Gene expression data with studentized residuals >2.5 were considered outliers and excluded from the analysis. After normalization, expression data were log-transformed (Bionaz and Loor 2008a; b). A repeated measures model was fitted to the adjusted ratios using Proc MIXED (SAS; SAS Inst., Cary, NC, USA). The model consisted of time, treatment, and time x treatment interaction as fixed effects, as well as goat as the random effect. Differences in body mass, dry matter intake (DMI), milk yield, milk composition were assessed using the same statistical model.

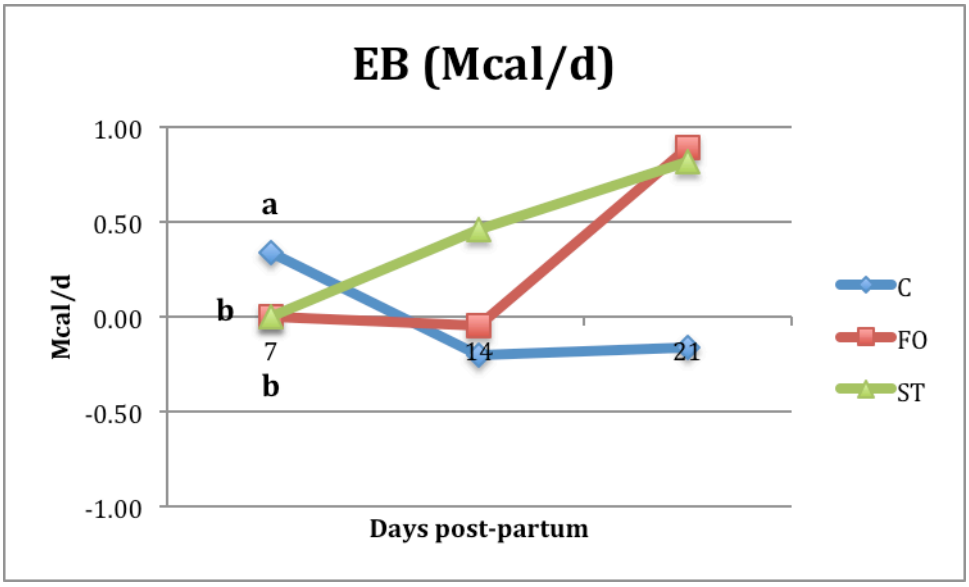
4.4 Results and discussion

The transition period is characterized by changes in the endocrine status of the animal based on the reduction in feed intake when there is a high nutrients demand (Drackley 1999). These conditions lead the goat to experience a NEB before parturition and at the beginning of lactation period.
Few available data concerning the effects of saturated or unsaturated fatty acid on performance, production and gene expression in liver and adipose goat tissues during the transition period are present in literature (Agazzi et al. 2010). The effects of fat enriched diets in small ruminants leads to different results from those reported in dairy cow. Fish oil administration do not have or have a limited effects on milk fat content as reported by Chilliard et al. (2014) in contrast with results observed in cows and ewes (Invernizzi et al. 2010; Bichi et al. 2013b). The similar results for the three experimental groups in our study in milk production and composition (Tab. 4) confirmed indeed, that goats are much less sensitive then cows in ruminal biohydrogenation pathway (Chilliard et al. 2007; Bernard et al. 2012). No differences were found in milk yield and composition (Tab. 4), in particular no differences in milk fat content in our study confirmed the literature that reported no change in milk fat percentage in dairy goats. Differences were found in EB between C compared to ST and FO at d 7 (0.34 vs. -1.12 and 0.91 respectively; $P \leq 0.05$) (Fig. 1).

Table 4: Milk production and composition

		Treatment				P		
	Time	C	FO	ST	SEM	Trt	Time	Trt*time
Milk production (kg/d)	7	2.85	3.87	3.36	0.3	0.13	<0.01	0.58
	14	3.52	4.24	3.79				
	21	3.66	4.21	3.88				
Milk fat (g/100 ml)	7	4.84	4.5	4.51	0.78	0.97	<0.01	0.56
	14	3.05	3.59	3.51				
	21	3.4	3.29	2.93				
Milk protein (g/100 ml)	7	4.17	4.19	4.36	0.18	0.62	<0.01	0.43
	14	3.59	3.68	3.66				
	21	3.41	3.31	3.61				
Milk SCC (log)	7	5.49	5.46	5.39	0.2	0.81	0.26	0.89
	14	5.66	5.52	5.62				
	21	5.74	5.57	5.48				

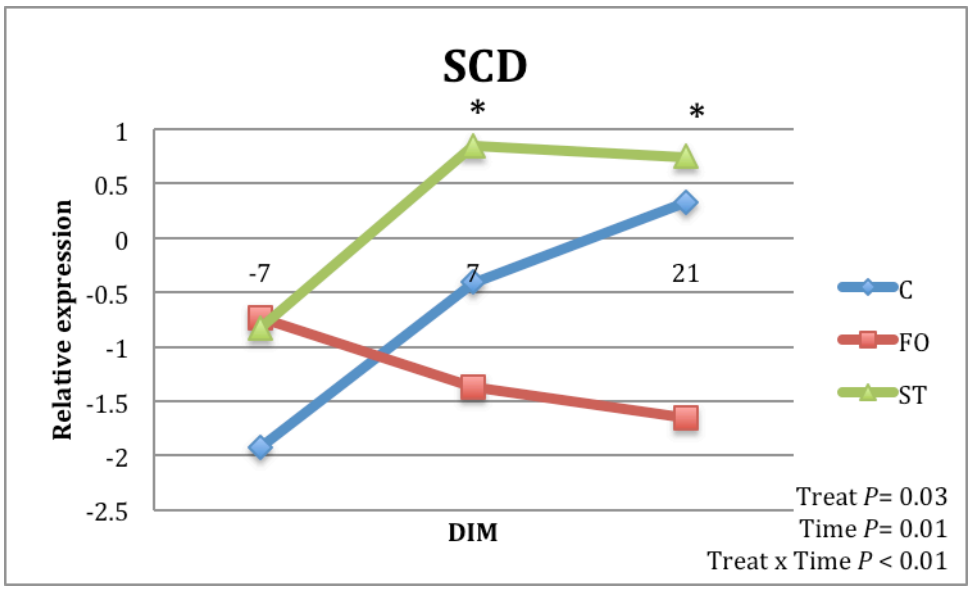
Figure 1: Energy Balance (Mcal/d)



Saturated fatty acid or PUFA supplementation during peripartum had no effect on abundance of mRNA encoding for SREBF2, SOD, CAT and PPARA in liver.

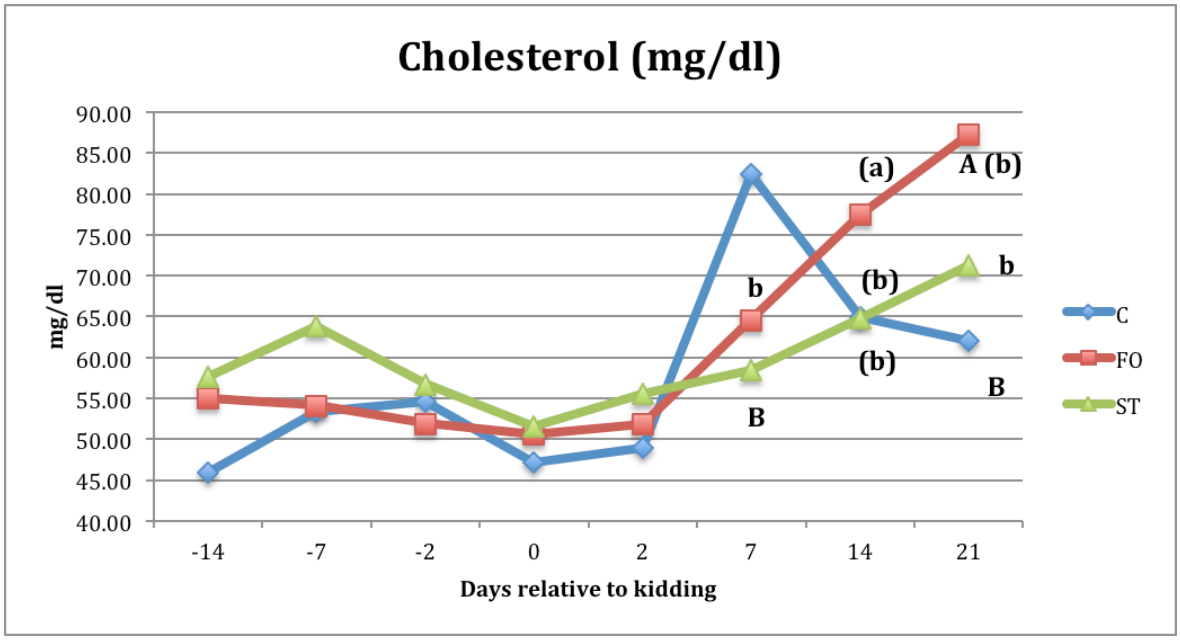
SCD mRNA expression was significantly differentially expressed in FO compared to ST ($P<0.03$): at day 7 was up regulated in ST (1.8-fold; $P<0.01$) and at day 21 (1.68-fold; $P<0.01$) compared with FO (Fig. 2). With an opposite trend SCD expression in FO was down regulated (2.57 and 3.14-fold) respectively at days 7 and 21. SCD gene encodes a lipogenic enzyme with a key role in the biosynthesis of monounsaturated fatty acids 18:1 and 16:1, from 16:0 and 18:0, important substrates for de novo synthesis of hepatic phospholipids, cholesterol esters and TAG. As is widely reported in vivo and in vitro studies, supplementing diets with saturated FA (C18:0 and C16:0) induced desaturase activity (Garg et al. 1986; Landau et al. 1997), otherwise, a diet supplemented with PUFA, especially linoleic (18:2n–6), arachidonic (20:4n–6) or linolenic (18:3n–3) acids down-regulates SCD expression in rat liver (Jeffcoat and James 1978; Ntambi 1999). These results are also confirmed in ruminants where SCD activity decreased in cow supplemented with rumen-protected fish oil (Ahnadi et al. 2002), in dairy goats fed formaldehyde-treated linseed (Bernard et al. 2005) and in positive energy balance late lactating dairy goats supplemented with fish oil (Toral et al. 2013). Bernard et al. (2009) and Toral et al. (2014) reported a relatively high expression of SCD1 gene in goat liver that supported a synergistic liver role in delta-9 desaturation of absorbed fatty acids. SCD activity is also required for efficient cholesterol esterification and triglycerides synthesis and subsequent VLDL formation but a reduced SCD-1 activity could lead also to higher free cholesterol in the cell that could have cytotoxic effects (Paton and Ntambi 2010). Differences were observed in serum cholesterol at different time points ($P<0.01$), in particular at day 21 its amount was significantly higher in FO compared to C ($P<0.01$) and ST ($P<0.05$) (Fig. 3). Protected fish oil most probably guaranteed a good level of unsaturated fatty acids (in particular MUFA) in blood stream and, on one side, induced a down regulation of SCD, on the other, assured enough substrate for cholesterol esterification and VLDL formation. Furthermore, cholesterol serum levels decreased at kidding and then increased constantly during the post-partum as previously observed (Agazzi et al. 2010).

Figure 2: mRNA relative expression of SCD in liver



* P≤ 0.05

Figure 3: Cholesterol plasma content (mg/dl)

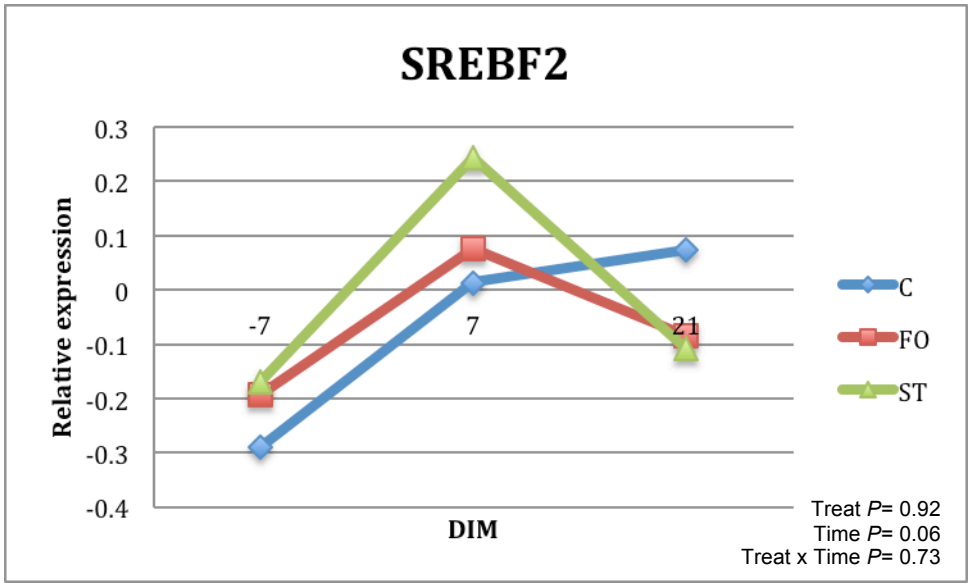


A,B P≤ 0.01

a, b P≤ 0.05

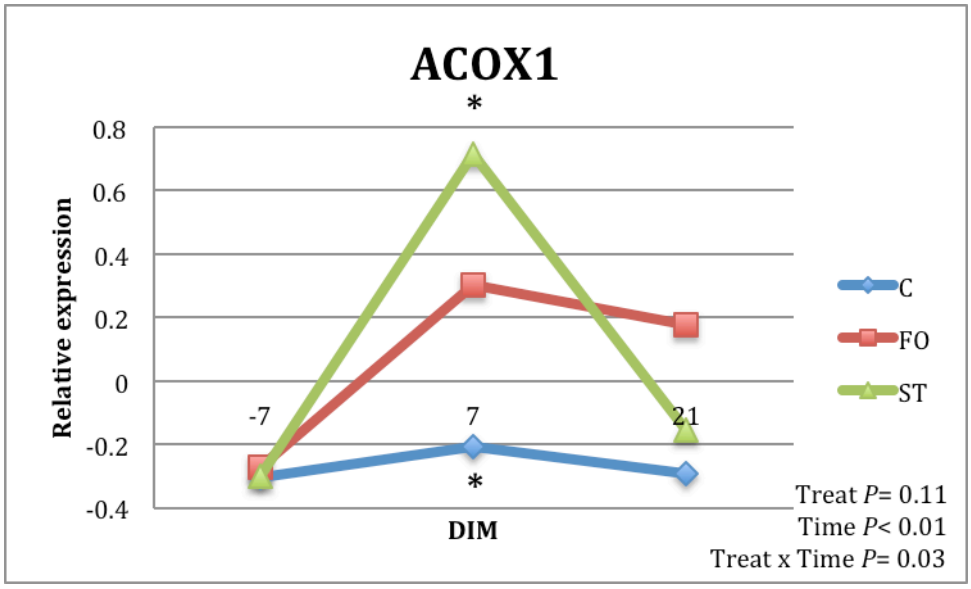
SREB proteins are indirectly required for cholesterol biosynthesis and for uptake and fatty acid biosynthesis. SREBFs are synthesized as endoplasmic reticulum membrane proteins. In the case of low cholesterol levels, they migrate to the Golgi apparatus where they are cleaved and then released as nuclear SREBF able to activate target genes (Desvergne et al. 2006; Daemen et al. 2013). SREBF2 gene, in particular, encodes a ubiquitously expressed transcription factor that controls cholesterol homeostasis by stimulating transcription of sterol-regulated genes. SREBF2 expression was not different between treatments but there was a tendency (P=0.06) for time effect; expression was up-regulated up to 1.18 fold at day 7 while was down regulated at -7 days preparturition (Fig. 4). Our results are in agreement with data obtained in periparturient cows (Kessler et al. 2014). Indeed, SREBF2 expression could be linked to the need of increasing cholesterol availability to export liver TG, even if the plasma level is decreased until kidding date. However, is hard to link SREBF2 expression to the observed cholesterol serum differences within dietary treatments.

Figure 4: mRNA relative expression of SREBF2 in liver



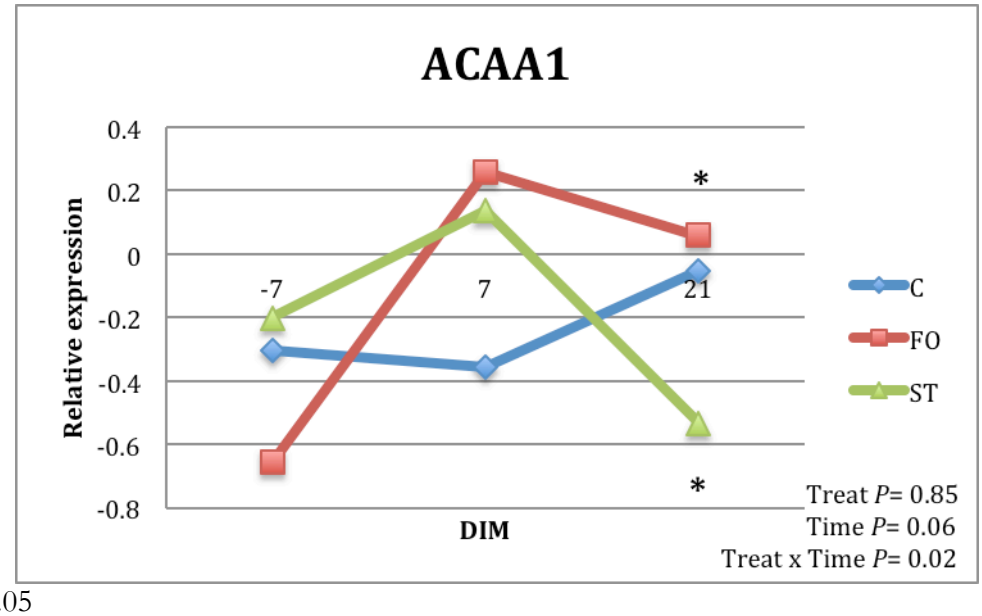
Peroxisomal acyl-coenzyme A oxidase 1 is an enzyme that is encoded by the ACOX1 gene. The protein encoded by this gene is the first enzyme of the fatty acid beta-oxidation pathway (Vluggens et al. 2010) which plays an important role in peripartal liver lipid metabolism (Grum et al. 1996). ACOX1 mRNA expression at d 7 was significantly higher in ST compared to C (1.64 vs. 0.87 fold; $P<0.03$); no statistical differences were observed between ST and FO (Fig.5). Both FA-supplemented groups had higher ACOX1 mRNA levels ($P<0.01$) at day 7 compared to their expression before kidding, furthermore, expression in ST at day 21 was almost completely restored at pre-kidding levels. Although the two experiments are quite different, our expression data are partially in agreement with Akbar et al. (2013), who also observed increased ACOX1 expression postpartum (at day 11 instead of day 7) in saturated fat supplemented cows during peripartum compared with -14 day (administration of supplement started at day -21 and pre-partum biopsy at day -14). FO ACOX1 expression was in contrast with results obtained in the same trial on cows in which postpartum expression was lower compared with pre-partum. A very similar expression pattern to ACOX1 at least for ST and FO although at overall lower expression levels, was observed for ACAA1 (Fig. 6) that encodes an enzyme operative in the β-oxidation system of the peroxisomes. At day 21 significantly higher mRNA levels were observed in FO than ST. Taken together, results obtained in genes related to β -oxidation seems to support a modulation of dietary FA over the time, with FO having a higher impact on liver at 21 days while ST at day 7.

Figure 5: mRNA relative expression of ACOX1 in liver



* P≤ 0.05

Figure 6: mRNA relative expression of ACAA1 in liver



* P≤ 0.05

SOD (Fig. 7), that catalyzes the dismutation of superoxide into oxygen and hydrogen, and CAT (Fig. 8), that catalyzes the decomposition of hydrogen peroxide to water and oxygen, are enzymes that have a pivotal role in oxidative stress which, as it is know, could occur during NEB with accumulation of TAGs, BOHB and NEFAs that can lead to fatty liver or lipidosis. We did not observe any effect of SFA or PUFA administration in the diet (P=0.33) on the mRNA abundance of the genes mentioned above that confirmed the results by Bellagamba et al. (2012) that reported not significant results of the oxidative stress biomarkers.

Figure 7: mRNA relative expression of SOD in liver

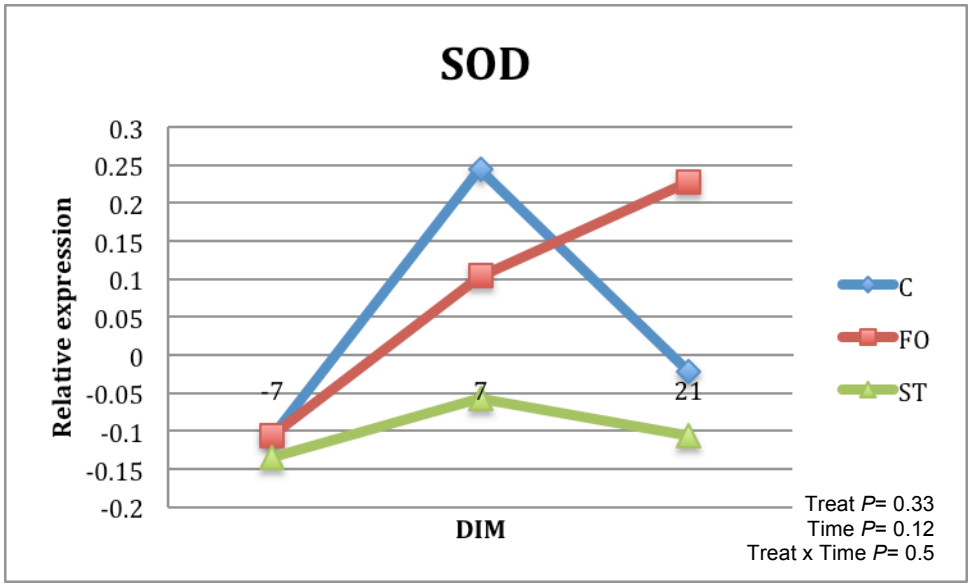
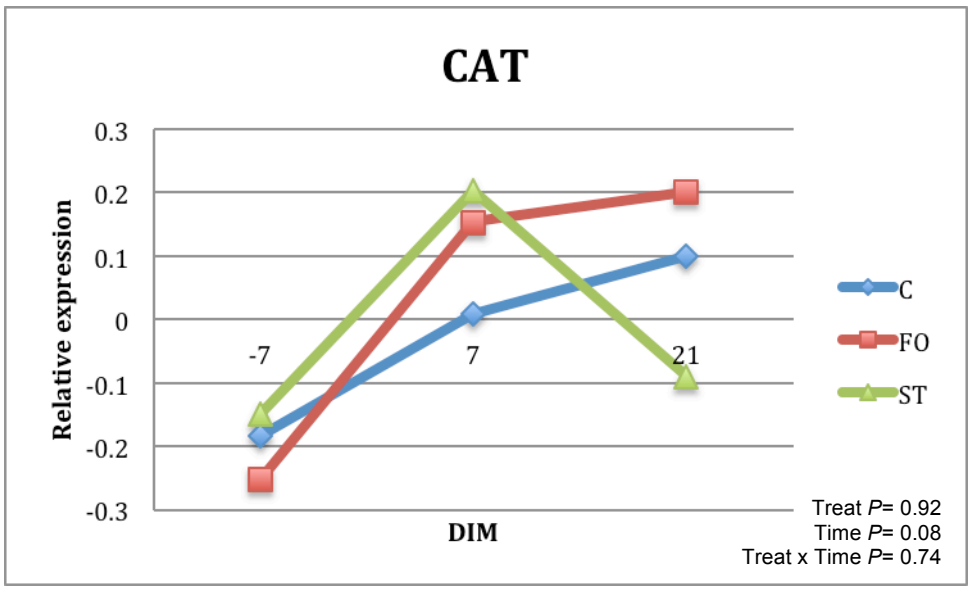


Figure 8: mRNA relative expression of SOD in liver



As it is well known PUFA, especially EPA and DHA, have the strongest action on PPARA, expression 10 times higher than SFA (Desvergne and Wahli 1999). The PPARA transcription factor, which is involved in β-oxidation of FA (Litherland et al. 2010), is relatively highly expressed in goat liver. In dairy cow PPARA expression is strongly up regulated by serum NEFA content and uptake during the three weeks prepartum (Loor et al. 2005). However, in our study the different fat source did not affect (P=0.77) (Fig. 9) the expression of PPARA as observed in a recent experiment (Toral et al. 2013). Moreover, in a previous experiment, our group observed the same pattern for PPARA expression for C and FO when early lactation dairy goats were fed fish oil and an up-regulation when fed hydrogenated palm oil (Agazzi et al. 2010).

Figure 9: mRNA relative expression of PPARA in liver

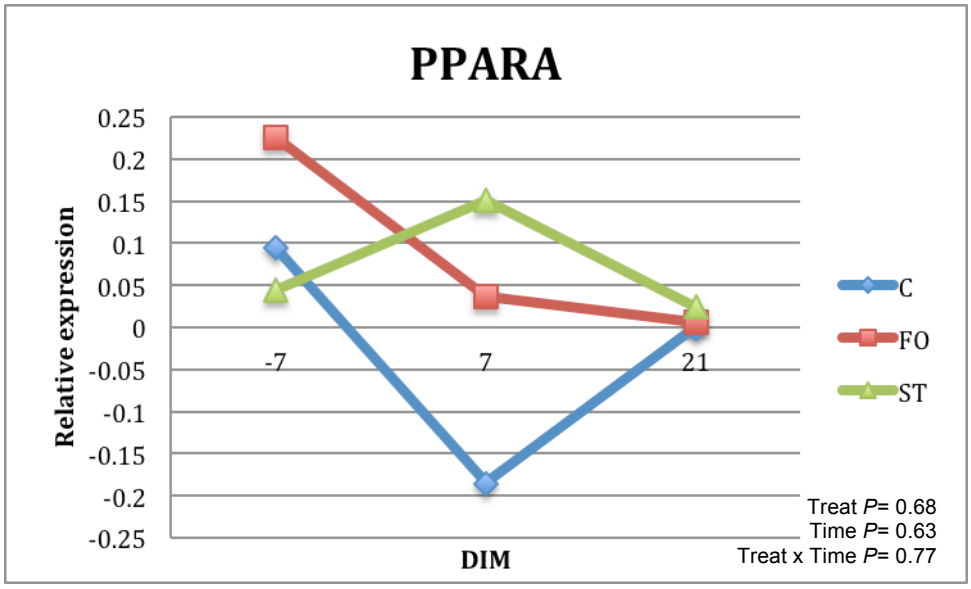
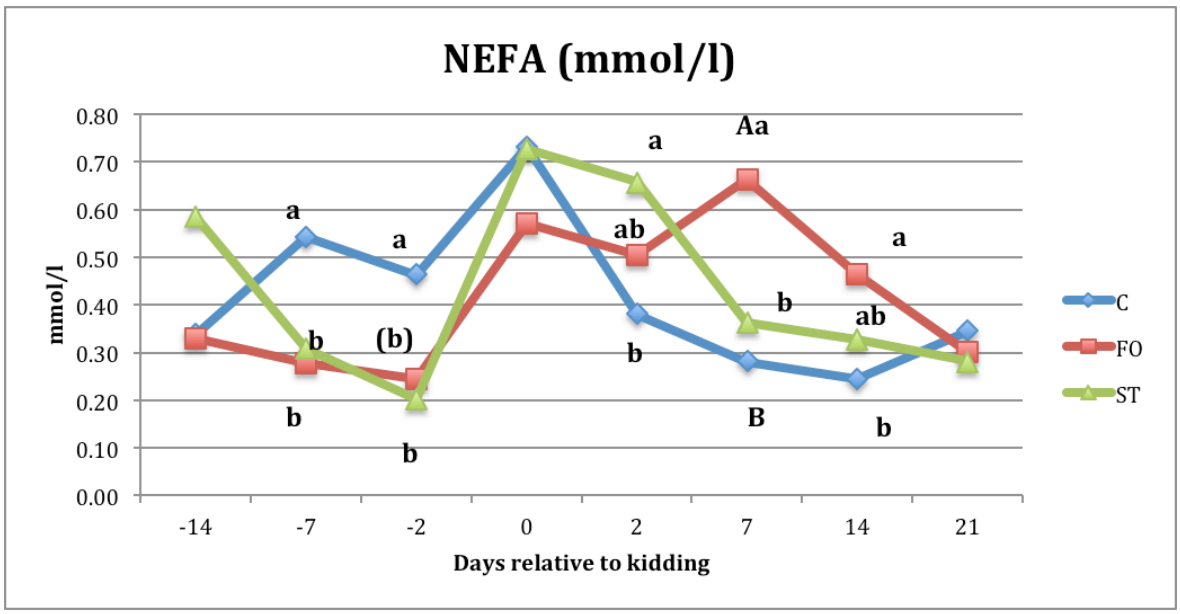


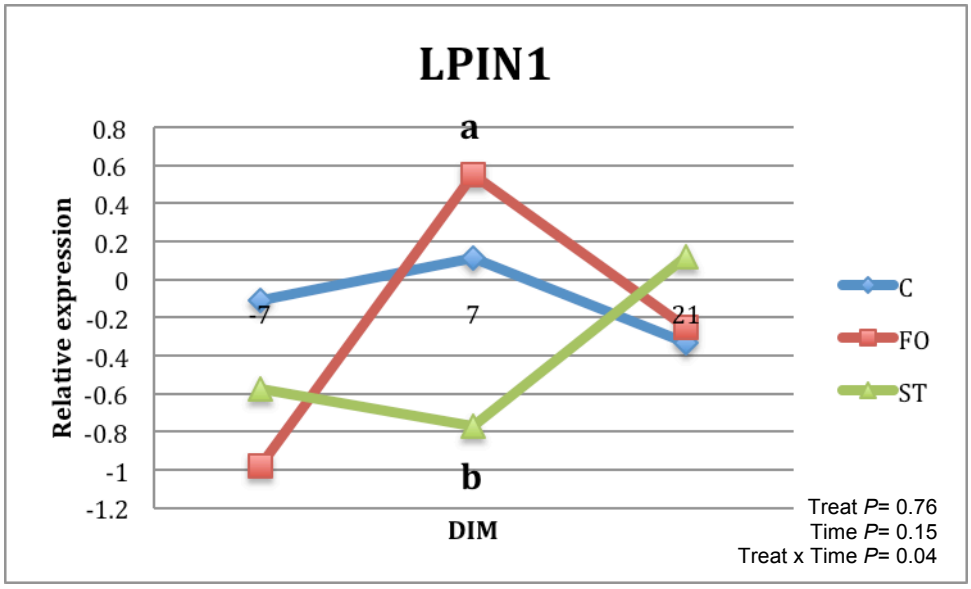
Figure 10: NEFA plasma content (mmol/L)



A,B P≤ 0.01
a, b P≤ 0.05

Different fat supplementation significantly affected subcutaneous adipose genes involved in TAG synthesis (LPIN1), in the regulation of lipogenesis (THRSP) and nuclear receptor (SREBF1). No effects were observed on the expression of the adipokine (ADIPOQ).
The expression of LPIN1 is required for adipocyte differentiation, but this gene have also a second function as nuclear transcriptional coactivator with some PPARs to modulate expression of other genes involved in lipid metabolism. This gene coding for enzymes of the TAG pathway are essential in adipose for TAG formation (Reue and Zhang 2008). In our study LPIN1 expression was significantly higher in FO group compared to ST (1.46-fold vs. 0.59-fold; P<0.04) (Fig. 11) at 7 DIM in agreement with Thering et al. (2009), which reported an up regulation of this gene in cows fed a diet supplemented with fish oil and soybean oil.

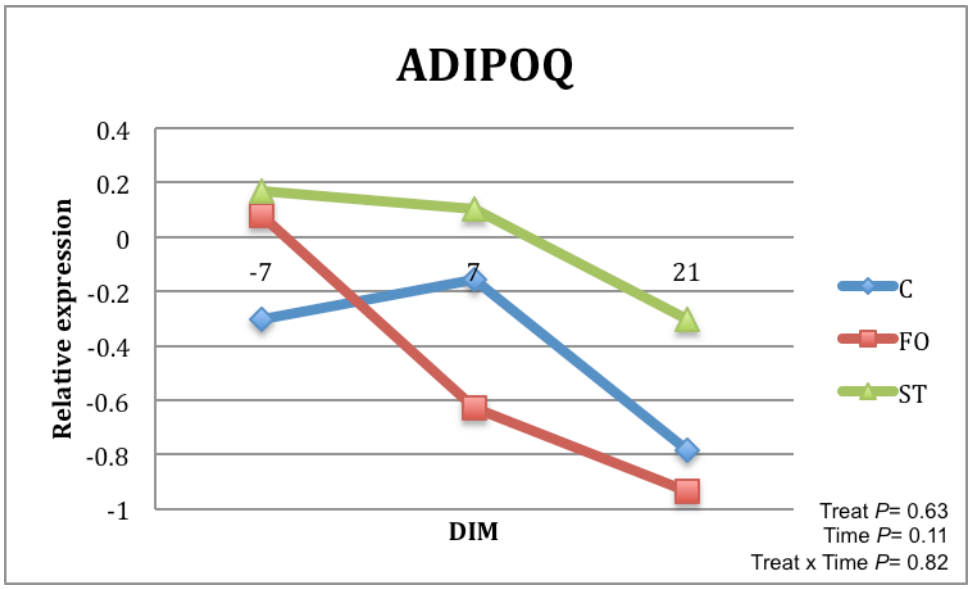
Figure 11: mRNA relative expression of LPIN1 in adipose



[a, b] P≤ 0.05

Schmitt et al. (2011) observed some proof that production of adipokines by adipose tissue could be influenced by lipid supplementation. In our study fat administrations did not modify ADIPOQ expression in adipose (P=0.63), but FO had a numerically lower expression post-partum (days 7 and 21) compared with ST or C groups (Fig. 12). Furthermore, there was a trend in all treatments (P=0.11) for lowering expression from prepartum to 21 DIM in agreement with Ji et al. (2012), who reported that ADIPOQ gradually decreased from -21 through 56 DIM in bovine subcutaneous adipose tissue. On the other hand, our results are not in agreement with observations by Schmitt et al. (2011) and (Akbar et al. 2013) in dairy cattle that detected a down regulation in ADIPOQ when fed FO compared with SFA administration or C diets (Akbar et al. 2013). Furthermore, the suggested link between ADIPOQ in adipose and ACOX1 in liver leading to an overall reduction in β-oxidation in FO is hard to be shared interpreting our results.

Figure 12: mRNA relative expression of ADIPOQ in adipose



SREBF1 (Fig. 13) and THRSP (Fig.14) expression pattern was very similar within each treatment; in ST and FO SREBF1 in particular, starting from a similar up regulated antepartum status (C values were already down regulated), postpartum mRNA level showed a sort of parallelism in all treatments with FO being more down regulated. A time effect (P=0.04 SREBF1; P=0.003 THRSP) was observed for both transcription factors showing a fast down regulation from prepartum to 7 DIM and to a lesser extent continued to d 21. Down regulation in THRSP was, however, more marked, going, in FO, from 1.92 to 0.47-fold change between -7 to 7 DIM and with a reduced values at day 21.

THRSP gene plays a role in the regulation of lipogenesis, especially in lactating mammary gland, fat and liver (Zhan et al. 2006). This transcription regulator is important for the biosynthesis of triglycerides with medium-length fatty acid chains. The expression of THRSP in liver and adipose of rodents was induced by lipogenic stimuli, thyroid hormone and dietary carbohydrates, which increased the rate of lipogenesis (Kinlaw et al. 1995; Obregon 2008). Jump et al. (1993) reported that feeding PUFA decreased the THRSP mRNA levels in rat liver; and in dairy cow both, saturated and PUFAs administration, decreased THRSP expression in mammary gland from 7 until 21 DIM (Invernizzi et al. 2010). Results obtained are in contrast with data observed by Harvatine et al. (2009) infusing CLA in dairy cow and for THRSP also with Thering et al. (2009) that supplemented FO or saturated fatty acids to midlactating cows, both studies observed an increase on expression. However, an in vitro study on bovine mammary cells found marked down regulation of SREBF1 with exogenous

20:5n3 (Kadegowda et al. 2009).

Figure 13: mRNA relative expression of SREBF1 in adipose

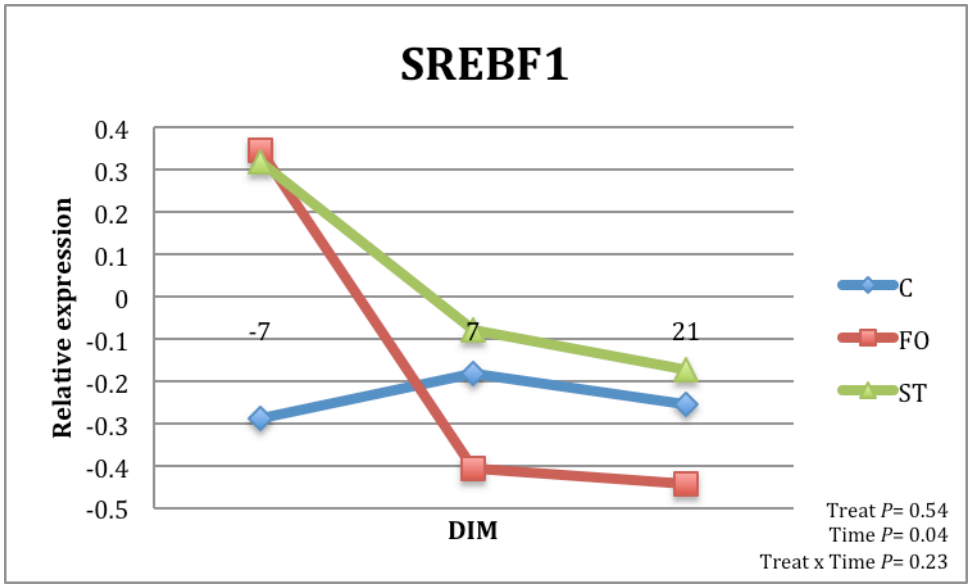
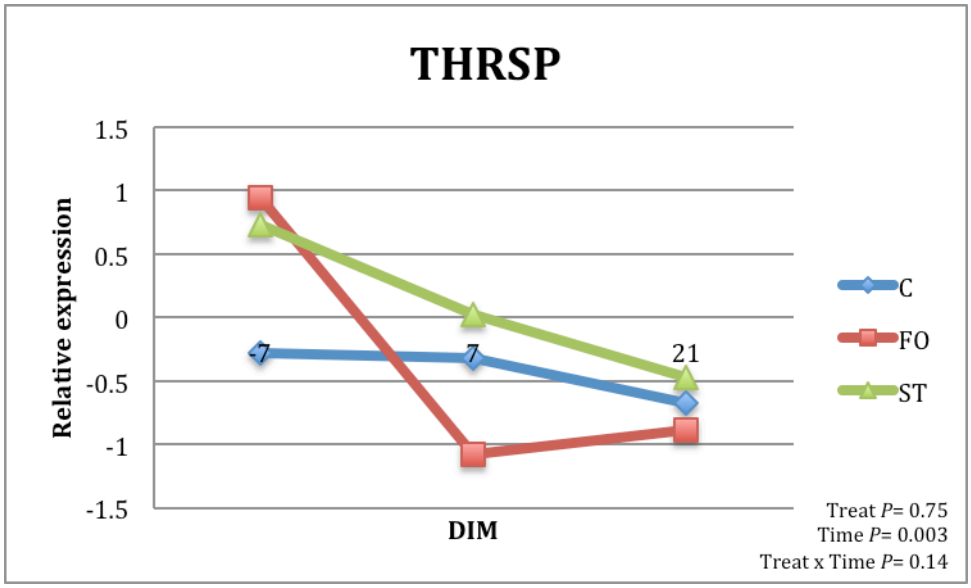


Figure 14: mRNA relative expression of THRSP in adipose



In conclusion this study point out the important role of saturated or unsaturated fat administration in dairy goat diets to modulate mRNA expression of central genes involved in lipogenesis.

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CHAPTER 5

Different dietary fat sources in dairy goat can affect metabolism and immune response in newborn kid

5 Different dietary fat sources in dairy goat can affect metabolism and immune response in newborn kid

5.1 Abstract

The aim of the study was to evaluate the influence of dietary saturated (C18:0) or polyunsaturated fatty acids (PUFA) on immune response of suckling kids. 42 newborn kids (18 female and 28 male) were divided in three groups based on the source of fat administered to mothers. The goats fed a basal diet (C) or a basal diet supplemented with fish oil (FO) or a basal diet supplemented with stearic acid (ST). Starting from the kidding day (d 0) until 21 d of life, individual body weight (BW) was weekly recorded and average daily gain (ADG) was computed. On day 21 skin test was performed. Blood samples were collected weekly from kidding until 21 days of life for haematological, haematochemical and immunological parameters evaluation. No differences were found in growth performances. Kids born from dams fed saturated fatty acids showed the most significant variations in both immune response and metabolism. At 2 days of life differences were found IgA plasma content was higher in ST compared to FO and C (0.54 vs. 0.20 and 0.14 mg/ml respectively; P≤0.01) and also the overall mean was higher in ST group compared to FO and C groups (0.18 vs. 0.07 and 0.07 mg/ml respectively; P≤0.01) and). Neutrophils percentage was higher in ST group compared to C at kidding (72.12 vs 57.79 %; P≤0.05), at 2 days of life between ST and C groups (60.85 vs 47.31 %; P≤0.05); at d 21 differences were found between FO and C (47.91 vs 34.82 %; P≤0.01). ALT plasma content was higher at d 14 in FO and C group compared to ST (38.34 and 29.06 vs 7.38 UI respectively; P≤0.01). AST content was higher at d 14 in FO and C groups compared to ST (186.85 and 161.00 vs 79.62 UI respectively; P≤0.05) and at d 21 in C and FO groups compared to ST (159.74 and 99.68 vs 75.82 UI respectively; P≤0.05).
The significantly higher IgA and the higher neutrophils plasma content in ST, support the idea that saturated fat dietary supplementation can have a positive effect on passive immunity transfer from goats to newborn kids, and can stimulate the immune system of offspring. Furthermore, ALT and AST results seem to underline a better hepatic condition.

Keywords: dairy goats, newborn kids, immune response, immunoglobulins, immunity, stearate, fish oil

5.2 Introduction

The immunoglobulins plays a crucial role in the protection of newborn ruminants against infections (Moreno-Indias et al. 2012). Anyway in small ruminants the synepitheliochorial placenta impedes the transfer of immunoglobulins from the dam to the fetus (Wooding et al. 1986) and, consequently, newborn ruminants are hypogammaglobulinemic at birth (Arguello et al. 2004; Castro et al. 2005; Castro et al. 2009; Moreno-Indias et al. 2012). In this case the acquisition of immunity from dams to kids is strongly dependent from the consumption colostrum during the first days of life (Lascelles 1979), where pre-partum colostrogenesis is responsible for the transfer of immune components, mainly immunoglobulins, from maternal bloodstream into mammary secretions (Barrington and Parish 2001).
Nowadays the usual rearing system of goat kids is performed by the use of colostrum replacers after an abrupt separation from their mothers at birth, but the absorption of IgG in kids using other compounds than the maternal colostrum is reported to be poor (Sherman et al. 1990; Constant et al. 1994; Zadoks et al. 2001). A natural rearing system where the kids are maintained with their dams should provide an adequate immunoglobulin transfer; anyway at the moment few data are available on this topic.
Improving the immune system of newborn animals beside immunoglobulin content of colostrum could be of value in all rearing systems. Since several years dietary fatty acids are evidenced not to be just energy-providing molecules, but nutrients that can positively act on immune response and metabolism of animals (Desvergne and Wahli 1999).
If stearic acid rapresents the most abundant and traditionally used saturated fatty acid in ruminat nutrition, this is not true for other fatty acids with quite longer and usaturated chains.
On the opposite, long-chain fatty acids (LCFA), are reported to be able to improve some immune response aspects in animals. If this is particularly true when LCFA are directly fed through the diet (Savoini et al. 2010), then these compounds could be a valuable tool to improve immunity in newborn kids by their administration through the relative mothers.
In particular long-chain n-3 polyunsaturated fatty acids of marine origin, mainly eicosapentaenoic acid (EPA C20:5 n-3) and docosahexaenoic acid (DHA C22:6 n-3), are known to explicate several health beneficial actions in mammals (Savoini et al 2010). DHA has been shown to play a specific role during fetal and neonatal development (Innis 2000). Dietary supply of LC n-3 PUFAs during late pregnancy and nursing period can improve EPA and DHA content in goat colostrum and milk (Agazzi et al. 2010; Cattaneo et al. 2006) and could be beneficial to the suckling kids.

Anyway placental passage of LCFA is debated since time. First Elphick et al. (1979) reported a placental impermeability to LCFA in sheep, while Bell et al. (1993) found a limited transportation of both short- and long-chain fatty acids. Otherwise, Campbell et al. (1994) reported a minimal passage of PUFA through the bovine placenta.
At the present moment, few studies are available on the influence of different dietary sources of lipids in the diet of the mothers on newborn calves, kids or lambs immune response. Even if there are differences between species (Johnson et al. 2007), Ballou and DePeters (2008) reported that fish oil (FO) supplementing in milk replacer attenuated many aspects of the acute phase response and health in pre-weaned calves. On the other side there are some evidence that saturated fatty acids (SFA) in the diet of the mothers could elicit positive results on immune status of very young kids as recently suggested by Santos et al. (2013) who found an improved transfer of passive immunity feeding newborn calves with colostrum from cows supplemented with SFA. Moreover, Garcia et al. (2014) reported that lipid supplement in pregnant Holstein cows diets during the last 3 wks of gestation changed some aspects of functional immunocompetence of calves in the first week of life. The objective of this study was to evaluate the influence of dietary saturated (C18:0) or polyunsaturated fatty acids (PUFAs) in periparturient dairy goats on immune response of kids.

5.3 Materials and methods

5.3.1 Goat diets

Animals were housed in the experimental farm "Centro Zootecnico Didattico Sperimentale" of the University of Milan. All procedures were performed under protocols approved by the Ethics Committee of the University of Milan (attachment n. 5 January 26th 2011).
Twenty-three pregnant Alpine goats were housed in individual boxes with free access to water and individually fed ad libitum. After kidding each goat shared the box with the relative suckling kids (on average 1.75 weighing 4.14 ± 0.68), the feeder being settled out of reach of kids. A pre-kidding or a post-kidding basal diet was administered to all the experimental animals in the three groups. Diets ingredients and chemical composition of the six experimental diets are detailed in Table 1. Pre kidding basal diet consisted of ad libitum mixed hay (refusal weight of at least 10%), 600g/head/d of concentrate and 100g/head/d of corn meal, while post kidding basal diet was composed by ad libitum alfalfa hay and mix hay (refusal weight of at least 10%), 1500g/head/d of concentrate and 200g/head/d of corn meal. Concentrates were separately fed from forage

during the whole trial. The three experimental groups consisted of: a) Control (C), fed the basal pre or post kidding diet plus calcium carbonate (9 g/d during pre-kidding period, 12 g/d after kidding); b) Fish oil (FO), fed the pre or post kidding basal diet plus calcium carbonate (9 g/d during pre-kidding period, and 15 g/d after kidding) and 30 g/d of fatty acid (81 g/d of supplement) before kidding or 50g/d of fatty acid (135g/d of supplement) from a rumen-inert fish oil (10.4% EPA and 7.8% DHA) during lactation (Ufac Ltd., Stretton, UK); c) Calcium Stearate (ST), fed the pre- or post-kidding basal diet plus 30 g/d of fatty acid (34 g/d of supplement) before kidding or 50 g/d of fatty acid (56 g/d of supplement) from stearic acid (C16:0 26% and 69.4% C18:0) during lactation (Brenntag S.p.a., Milan, Italy). All the diets were vitamin E supplemented in order to supply 72 mg/head/d during pre-kidding period and 80 mg/head/d after kidding. Pre and post kidding dietary treatments in the three groups were designed to provide similar crude protein (CP) and calcium content, while fat enriched treatments (FO and ST) had similar ether extract (EE). All goats were fed concentrates and corn meal twice a day, fat supplementation was provided in the morning meal mixed into 50g or 100g of corn meal during pre-kidding or post-kidding periods.

The proportion of C18:0 in ST diet was 69.4%, whereas the proportion of C16:0 was 26%. Otherwise FO supplement reported a 10.4% of EPA and 7.8% of DHA. All the proportions of the SFAs or PUFAs of the diets are reported in table 2.

Table 1: Ingredients and chemical composition of experimental diets.

	Pre-kidding			Post-kidding		
	C	**FO**	**ST**	**C**	**FO**	**ST**
Ingredient %of DM						
Alfalfa hay	0.00	0.00	0.00	31.20	29.80	30.70
Mixture hay	62.30	59.60	61.40	15.30	14.60	15.10
Concentrate mixture	31.90	30.50	31.40	46.80	44.80	46.20
Corn meal	5.30	5.00	5.20	6.20	5.90	6.20
Fish oil	0.00	4.40	0.00	0.00	4.30	0.00
Stearate	0.00	0.00	2.00	0.00	0.00	1.90
$CaCO_3$	0.50	0.50	0.00	0.50	0.50	0.00
DM, %	88.40	88.70	88.60	89.30	89.50	89.40
CP	12.30	11.90	12.20	17.80	17.20	17.50
EE	2.90	4.90	4.50	3.20	5.20	4.80
NDF	43.90	43.80	43.30	33.70	34.00	33.20
Ashes	6.30	6.50	6.00	7.20	7.30	6.80
Ca	0.80	0.80	0.90	1.10	1.10	1.20
P	0.40	0.40	0.40	0.80	0.80	0.80
Nel (Mcal/d)	1.61	1.66	1.67	1.67	1.72	1.72

Table 2: Fatty acids profile of the three experimental diets (g/100g of FAMEs).

Fatty Acid	Dry off diet			Lactation diet		
	C	**FO**	**ST**	**C**	**FO**	**ST**
C12:0	0.17	0.14	0.15	0.06	0.07	0.08
C14:0	0.48	2.98	1.08	0.28	2.69	0.98
C16:0	18.69	17.41	21.36	16.55	16.21	20.12
C16:1	0.42	3.85	0.27	0.27	3.54	0.17
C18:0	4.92	4.36	28.46	3.40	3.49	28.36
C18:1n9	17.86	17.23	11.34	24.41	21.19	15.18
C18:2n6	37.98	24.26	24.12	46.34	30.16	28.82
C18:3n3	15.14	9.36	9.62	5.52	3.98	3.43
C20:5n3	0.07	4.43	0.04	0.09	4.16	0.05
C22:6n3	0.00	3.29	0.00	0.00	3.07	0.00

5.3.2 Milk sampling and analyses

Individual colostrum was sampled within the first 24 h postpartum. Individual mature milk yield records and samples were obtained at 7, 14 and 21 days in milk (DIM), at two consecutive milkings (07:00 and 15:00 h) and then bulked to make representative individual daily composites.
Separate aliquots of each colostrum and milk sample were stored at □ 20 □ C for analysis of individual fatty acids by gas chromatography. Fatty acids profiling on colostrum and milk was performed using the same protocol described in Agazzi et al. (2010).

5.3.3 Kids management

For all the duration of the trial kids were housed in single boxes with their own dams. Based on the source of fat administered to mothers, 42 kids (18 female and 28 male) were assigned to the three groups, this scheme was chosen to ensuring a complete intake of colostrum.
Body weights (BW) were taken before colostrum feeding, and then at 2, 7, 14 and 21 days of life using a electronic scale.
The average daily gain (ADG) was calculated for each kid on day 2 and, subsequently, on a weekly base.
On day 21 after the initiation of treatments, skin test was performed for each kid. Each thickness at the site of injection was determined after 24 h post injection using a pressure-sensitive, digital caliper (Veterinaria Strumenti, Padova, Italy).
Kids blood samples were collected weekly from the kidding date (time 0) until 21 d of life for the determination of serum metabolite, blood differential leucocyte count, immunoglobulin A (IgA), immunoglobulin G (IgG) and acute phase protein (Hp) content for each kid. Kids plasma fatty acids (FAs) were evaluated at day 7 and 21. A direct in situ transesterification method was used to extract serum fatty acids (Glaser et al. 2010), analysis was then performed as for milk samples.

5.3.4 Blood samples and analysis

Blood samples were collected from the jugular vein of the kids using 2 evacuated tubes containing either EDTA (Terumo venoject ® 10-ml VF-109SDK) or a clot activator (VF-109SP).
Plasma and serum were separated by centrifugation for 10 minutes at 1000 g and stored at -20°C.

Concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, glucose, triglycerides and urea were analyzed by a clinical chemistry analyzer (ILab 300 plus, Instrumentation laboratory s.p.a. , Milan) using reagents provided by the same company. NEFA and betahydroxybutirate (BOHB) were tested using Randox reagents (Randox, Crumalin, UK).

5.3.5 Immune response and acute-phase protein (Hp)

Concentrations of IgA was determined using a commercial ELISA kit (Goat IgA ELISA Quantitation Set, Bethyl Laboratories, Inc., Montgomery, TX, USA) and IgG was determined using a commercial ELISA kit (Goat IgG ELISA Quantitation Set, Bethyl Laboratories, Inc., Montgomery, TX, USA) using reagents providing by the same company and following the manufacturer's protocol. Samples were analyzed in triplicate and an average calculated. IgG or IgA concentration were determined from a standard curve generate for caprine IgG or IgA.
Hp concentration was evaluated using a "PHASE" ™ Haptoglobin Assay (Tridelta Development Limited, Maynooth, County Kildare, Ireland) using reagents providing by the same company and following the manufacturer's protocol.

5.3.6 Cellular response to phytohemagglutinin

Cell-mediated immune response was evaluated to determine double skin thickness in response to phytohaemagglutinin injection (PHA, Sigma Chemicals, St. Louis, MO, USA) using the test procedure described by Agazzi et al. (2007) at 21 days after birth.
Intradermal injection of 250 μg PHA diluted in 0.1 ml of sterile PBS (Sigma Chemicals, St. Louis, MO, USA) was performed on a top-part shaved area of right shoulder using an automatic syringe (Veterinaria Strumenti, Padova, PD, Italy). Sterile PBS (0.1 ml) was injected into the corresponding area of left shoulder in order to check any skin response to PBS alone. Double skin thickness to both areas was measured with a constant tension calliper (Mitutoyo Italiana, Lainate, MI, Italy) before (time 0) and 24 hours after PHA injection. Values obtained at 24 h were considered the maximum PHA response (Lacetera et al. 1999).

5.3.7 Statistical analysis

Data relative to BW, ADG, blood metabolites, IgA, IgG and Hp were analyzed by a repeated measures model using a MIXED procedure for repeated measurements in SAS 9.2 (SAS Inst., Inc., NC, USA). The statistical model considered as fixed effects time, treatment and time x treatment interaction as well as kids as the random effect.
Fatty acids content in colostrum was analyzed using a general linear model (GLM) of SAS ((SAS Inst., Inc., NC, USA). Fatty acids content in milk and kids plasma were analyzed a MIXED procedure for repeated measurements in SAS 9.2 (SAS Inst., Inc., NC, USA). The statistical model considered as fixed effects time, treatment and time x treatment interaction as well as kids as the random effect.
Hematological data statistics were computed by using IBM SPSS 19.0 for Windows (IBM SPSS, Armonk, New York, USA). Because of the repeated measurement in the data, in order to determine the effects of different diets and time of sampling on the blood differential leukocyte count (dependent variables), a Generalized Estimating Equation (GEE) was used. The dependent variables had an inverse gaussian distribution for blood leukocytes differential cell count and a negative binomial distribution for haemochromocytometric parameters, so an identity link function was used. Goodness of fit was assessed using a quasi likelihood under independence model criterion (QICC).
The threshold for statistical significance was considered to be $P<0.05$.

5.4 Results

5.4.1 BW, ADG and skin test

FO and ST administration did not affect initial body weight, while a trend of higher values were found in FO than C and ST groups at the end of the trial (Tab. 3). Average daily gain, was not influenced by the dietary treatment, and the differences were not statistically significant ($P=0.17$) (Tab.3). Skin thickness in PBS injection sites was not statistically different at 24 h, thus no correction was performed on PHA-induced thickness. At 21 days of life the skin thickness did not significantly ($P=0.41$) increase with time up to 24 hours post PHA intradermal injection in C compared with fat supplemented groups, and no differences were found between the experimental groups ($P=0.78$) (Tab. 3).

Table 3: BW, ADG and Skin test in kids of the three experimental groups.

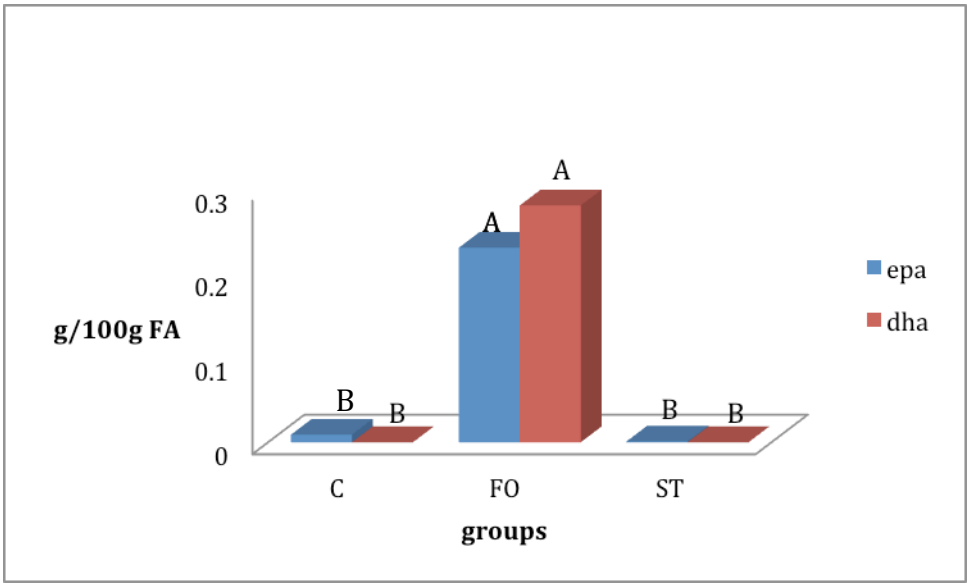
	Treatment				*P*		
	C	**FO**	**ST**	**SEM**	**Trt**	**Time**	**Trt*Time**
Weight (kg)							
Day							
0	3.94	4.40	4.19	0.23	0.04	<0.01	0.09
2	4.39	4.84	4.61				
7	5.31	6.07	5.39				
14	6.66	7.44	6.78				
21	7.92	8.80	7.99				
ADG (kg/d)							
Day 0-2	0.18	0.20	0.21	0.05	0.87	0.38	0.17
2-7	0.21	0.24	0.16				
7-14	0.18	0.16	0.20				
14-21	0.17	0.18	0.18				
0-21	0.20	0.20	0.18				
Skin thickness (mm)							
hrs 0	0.18	0.28	0.22	0.06	0.78	<0.01	0.41
24	1.26	1.04	1.53				

[A, B] P≤0.01
[a, b] P≤0.05

5.4.2 FAs profile in colostrum, milk and plasma of kids

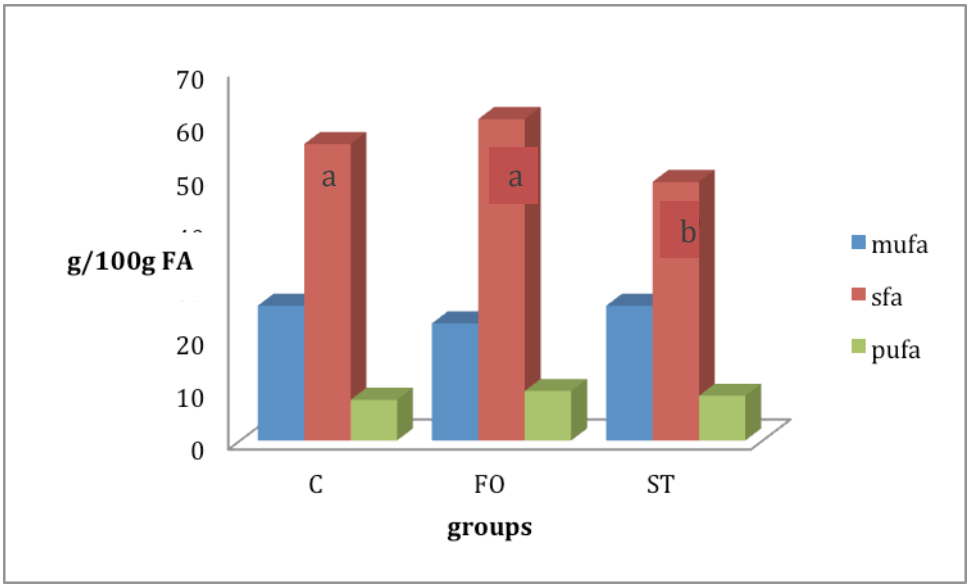
Colostrum FAs concentration was significantly higher for EPA and DHA content in FO compared to ST and C (EPA: 0.23 vs 0.001 and 0.009 g/100g FA respectively; P≤0.01(Fig. 1). Colostrum SFAs content was higher in FO and C groups compared to ST (60.5 and 55.79 vs 48.67 g/100g FA respectively; P≤0.05) (Fig.2). Colostrum n-3 content was higher in FO group compared to C and ST (1.78 vs 0.72 and 0.76 g/100g FA respectively; P≤0.05)

Figure 1: EPA and DHA content in colostrum (g/100g FA)



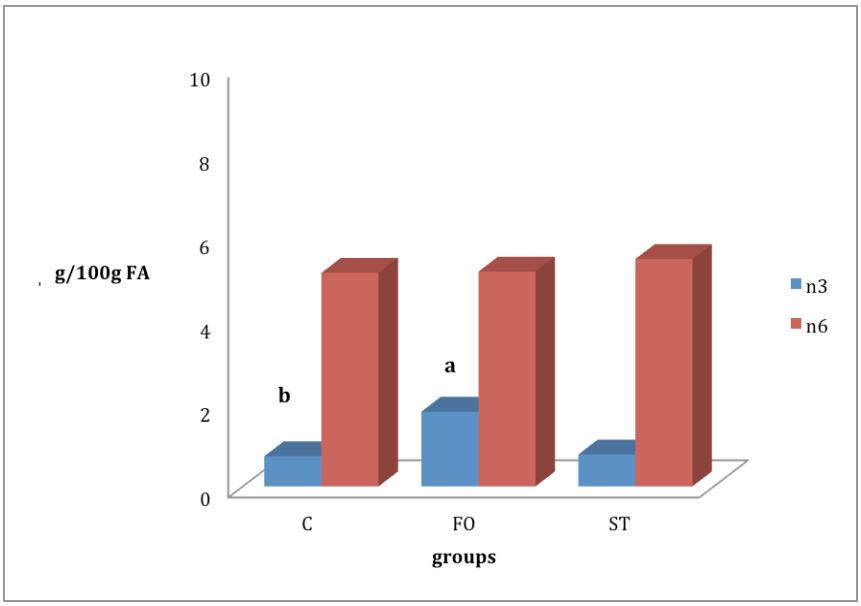
[A, B] P≤0.01
[a, b] P≤0.05

Figure 2: SFAs, MUFAs and PUFAs content in colostrum (g/100g FA)



[A, B] P≤0.01
[a, b] P≤0.05

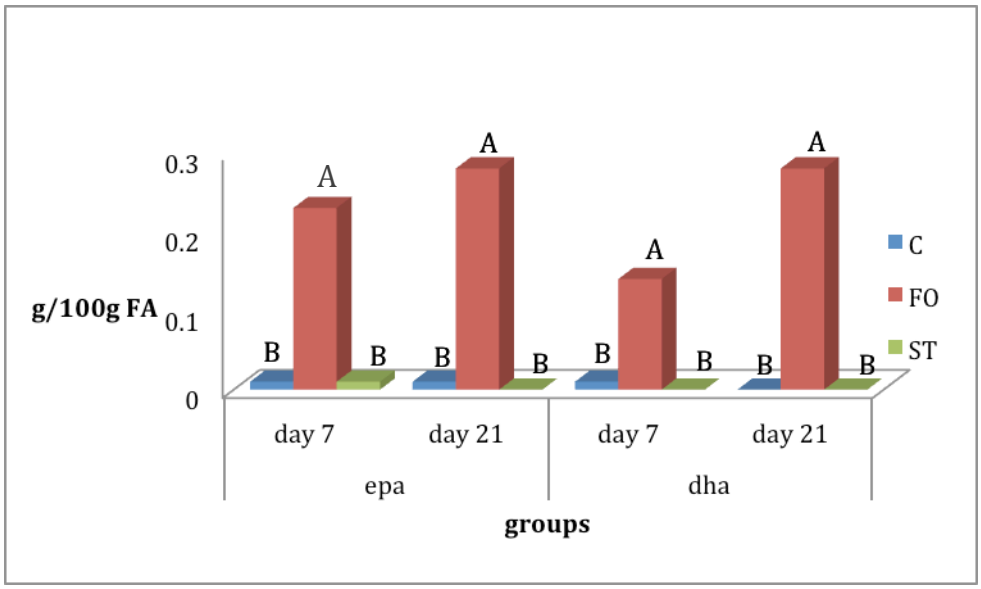
Figure 3: Colostrum n-3/n-6 content (g/100g FA)



[A, B] P≤0.01
[a, b] P≤0.05

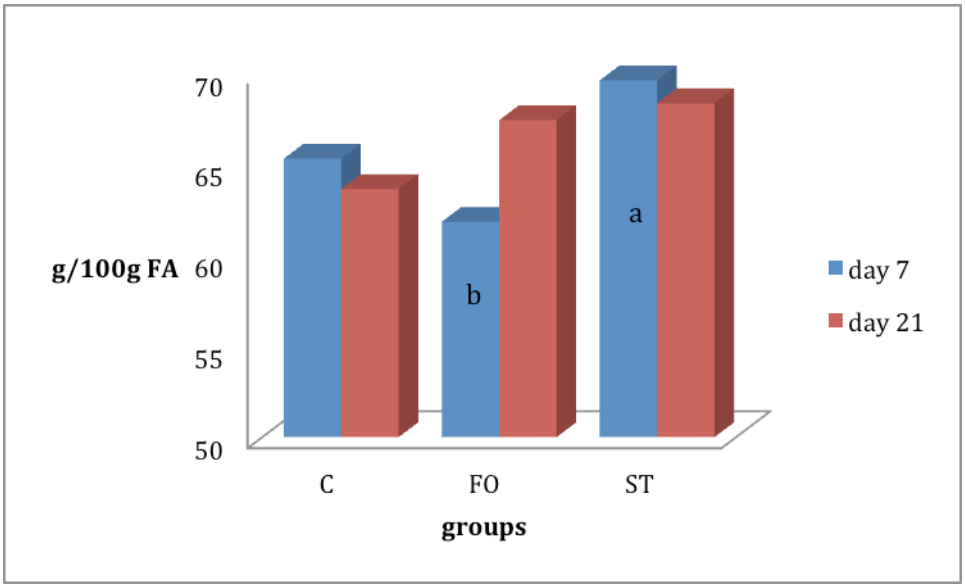
Milk EPA and DHA content was significantly higher at d 7 (P≤0.01) and at d 21 (P≤0.01) in FO compared to others two groups (Fig.4). Total SFAs content in milk was higher at d 7 in ST compared to FO (69.61 vs 61.84 g/100g FA respectively; P≤0.05) (Fig. 5). Milk MUFAs content was higher in C compared to ST at d 7 (33.56 vs 25.41 g/100g FA respectively; P≤0.05) (Fig. 6). Milk PUFAs concentration was significantly higher in FO group compared to ST (6.86 vs 4.76 g/100g FA respectively; P≤0.01); and in FO compared to C (6.86 vs 5.31 g/100g FA respectively; P≤0-05) (Fig. 7). Milk n-3 content was higher at d 7 in FO compared to C (1.46 vs 0.67 g/100g FA respectively; P≤0.05) and at d 21 in FO compared to ST and C groups (2.17 vs 0.85 and 0.60 g/100g FA respectively; P≤0.01) (Fig.8).

Figure 4: EPA and DHA content in milk (g/100g FA)



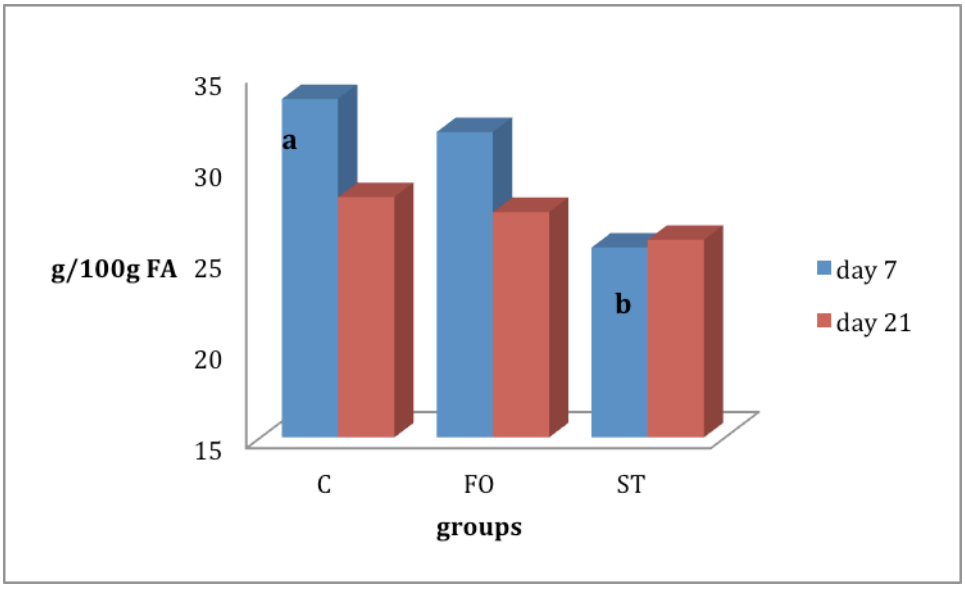
[A, B] P≤0.01
[a, b] P≤0.05

Figure 5: SFAs content in milk (g/100g FA)



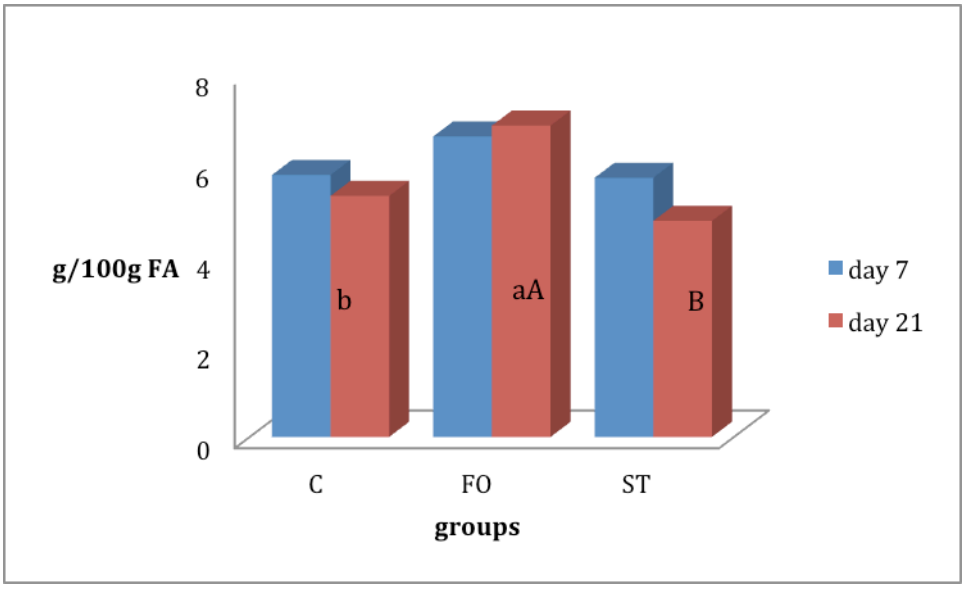
[A, B] P≤0.01
[a, b] P≤0.05

Figure 6: MUFAs content in milk (g/100g FA)



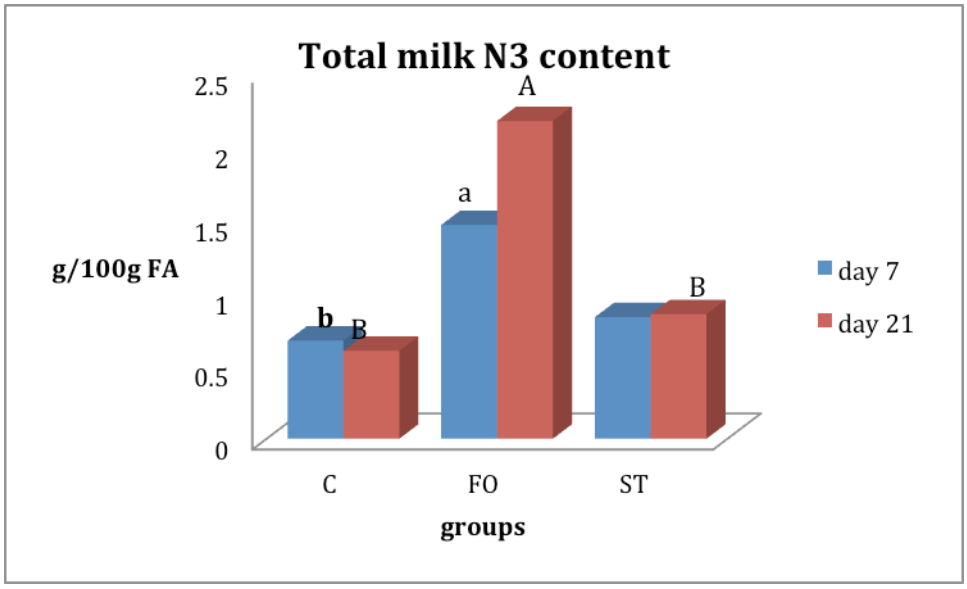
[A, B] P≤0.01
[a, b] P≤0.05

Figure 7: PUFAs content in milk (g/100g FA)



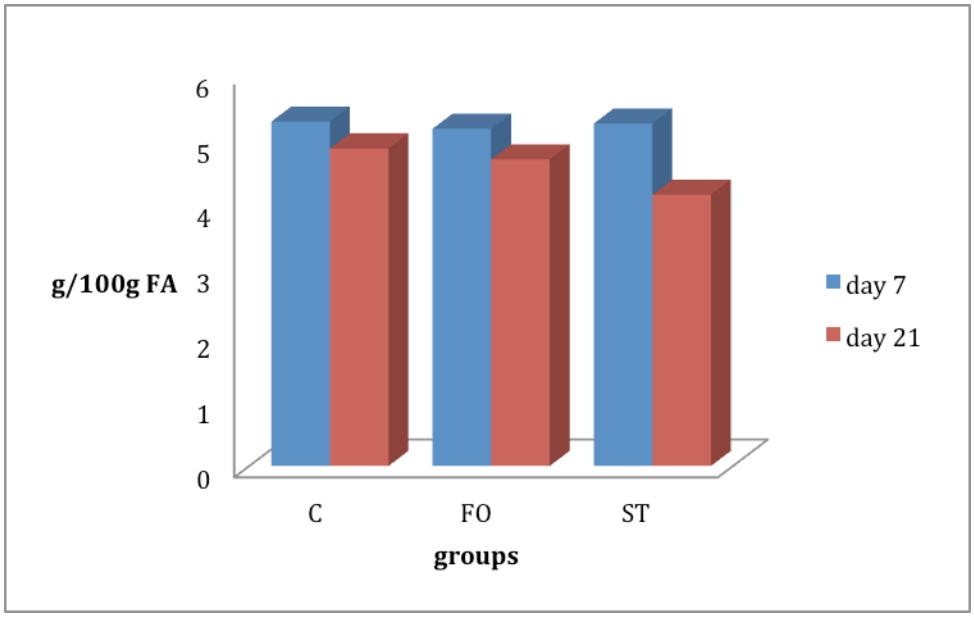
[A, B] P≤0.01
[a, b] P≤0.05

Figure 8: n-3 content in milk (g/100g FA)



[A, B] P≤0.01
[a, b] P≤0.05

Figure 9: n-6 content in milk (g/100g FA)



[A, B] P≤0.01
[a, b] P≤0.05

Fatty acids profile of kids plasma showed differences for C18:2 n-6 content at 7 days of life between FO and C (25.36 vs 15.98 g/100g FA; P≤0.01) and ST and C (22.96 vs 15.98 respectively; P≤0.05) (Tab. 4). The EPA, DPA and DHA content in plasma kids showed significantly variations for EPA content at day 7 between FO, ST and C (1.41 vs 0.16 and 0.14 g/100g FA respectively; P≤0.01); at d 21 between FO and ST (0.62 vs 0.20 g/100g FA; ≤0.05) (Fig.10). DPA plasma content was higher in FO compared to C (1.15 vs 0.35 g/100g FA; ≤0.01) and in FO group compared to ST and C (1.15 vs 0.88 and 0.35 g/100g FA respectively; ≤0.05) at d 7; and in FO group compared to ST and C (1.38 vs 0.89 and 0.22 g/100g FA respectively; ≤0.01) and between FO and C (1.38 vs 0.22 g/100g FA; ≤0.01) at d 21. DHA content was significantly higher in FO group compared to ST and C (2.19 vs 1.23 and 1.02 g/100g FA respectively; ≤0.01) at d 7; and in FO group compared to C (1.99 vs 1.31 g/100g FA; ≤0.01) at d 21.

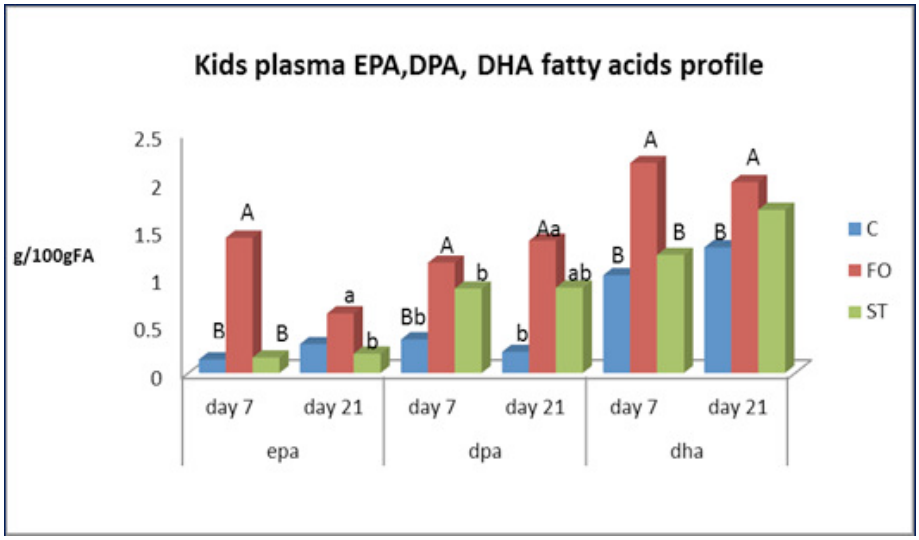
Table 4: Fatty acid profile of plasma kids (g/100g FA).

	Treatment				P		
	C	**FO**	**ST**	**SEM**	**Trt**	**Time**	**Trt*Time**
C14:0							
Day 7	1.00	1.60	0.99	0.05	0.01	<0.01	0.13
Day 21	2.34	2.30	1.50				
Mean	1.67	1.95	1.25				
C15:0							
Day 7	0.20	0.40	0.18	0.05	<0.01	<0.01	0.72
Day 21	0.39	0.58	0.41				
Mean	0.30	0.49	0.29				
C16:0							
Day 7	12.97	20.14	15.64	0.20	0.01	<0.01	0.08
Day 21	20.08	21.40	18.85				
Mean	16.52	20.77	17.24				
C16:1n-7							
Day 7	0.61	1.12	0.58	0.04	<0.01	<0.01	0.79
Day 21	0.86	1.40	0.94				
Mean	0.74	1.26	0.76				
C17:0							
Day 7	0.51	0.86	0.60	0.44	<0.01	0.03	0.10
Day 21	0.72	0.84	0.71				
Mean	0.61	0.85	0.66				
C18:0							
Day 7	11.51	14.49	15.10	1.10	0.30	0.12	0.64
Day 21	16.10	13.49	16.03				
Mean	13.81	13.99	15.60				
C18:1n-9							
Day 7	14.69	17.16	16.95	1.60	0.40	0.01	0.09
Day 21	21.34	16.78	21.71				
Mean	18.01	16.96	19.33				
C18:1n-7							
Day 7	1.30	5.12	1.49	0.30	<0.01	0.20	0.82
Day 21	1.93	5.44	1.69				
Mean	1.61	5.28	1.59				
C18:2n-6							
Day 7	15.98 ^{Bb}	25.36 ^{A}	22.96 ^{a}	1.98	0.10	0.07	0.02
Day 21	25.00	22.96	25.30				
Mean	20.49	24.16	24.16				
C18:3n-3							
Day 7	0.41	0.80	0.57	0.05	<0.01	<0.01	0.08
Day 21	0.88	1.01	0.90				
Mean	0.64	91.00	0.73				

$^{A, B}$ P≤0.01

$^{a, b}$ P≤0.05

Figure 10: Kids Plasma EPA, DPA and DHA fatty acids profile (g/100g FA).



[A, B] P≤0.01
[a, b] P≤0.05

Haematological parameters

No effects of different fatty acids administration were found on cholesterol content (Tab. 5).
ALT plasma content was significantly higher at d 14 in FO and C group compared to ST (38.34 and 29.06 vs 7.38 UI respectively; P≤0.01).
AST content was higher at d 14 in FO and C groups compared to ST (186.85 and 161.00 vs 79.62 UI respectively; P≤0.05) and at d 21 in C and FO groups compared to ST (159.74 and 99.68 vs 75.82 UI respectively; P≤0.05).
Both FO and ST groups decreased glucose content, C had highest amount compared with FO and at day 2 (157.55 vs 11.33 and 133.11 mg/dl respectively; P≤0.01), at d 7 (C 129.41 vs ST 88.88 mg/dl P≤0.01 respectively; C129.41 vs FO 99.54 mg/dl respectively; P≤0.05) and at d 14 (C 130.01 vs ST 75.10 mg/dl respectively; P≤0.01; C vs FO 103.24 mg/dl P≤0.05).
NEFA content was significantly higher at d 21 in ST group compared with FO group (0.60 vs 0.28 mmol/l; P≤0.01), and ST vs C groups (0.60 vs 0.41 mmol/l; P≤0.05) at the same day.
The administration of stearate (ST) significantly increased BHBO plasma content compared to C at d 7 (0.21 vs 0.12 mmol/l; P≤0.01); highest value in ST group compared to FO and C at d 14 (0.25 vs 0.12 and 0.10 respectively; P≤0.01) and 21 (0.23 vs 0.11 and 0.10 respectively; P≤0.01).

Triglycerides plasma content was higher in FO and C groups compared to ST at d 7 (63.33 and 55.12 vs 30.88 mg/dl respectively; P≤0.01), differences were found at d 14 between FO and ST (56.54 vs 27.47 mg/dl; P≤0.01) and between C and ST (49.98 vs 27.47 mg/dl; P≤0.05) at d 21 C had highest triglycerides amount compared to ST (62.04 vs 28.10 mg/dl; P≤0.01).
Urea plasma content was significantly (P≤0.01) higher in ST group compared with FO and C groups at d 7 (41.46 vs 20.13 and 16.60 mg/dl respectively; P≤0.01), at d 14 (34.05 vs 25.70 and 20.62 mg/dl respectively; P≤0.01) and at d 21 (33.02 vs 22.95 and 22.98 mg/dl respectively; P≤ 0.05).

Table 5: Serum metabolites of newborn kids

	Treatment				P		
Time	C	FO	ST	SEM	Trt	Time	Trt*Time
ALT (UI)							
0	7.71	5.15	6.72	8.51	0.10	<0.01	0.01
2	6.46	6.46	6.28				
7	12.98	10.91	10.84				
14	29.06^{A}	38.34^{A}	7.38^{B}				
21	35.84	22.89	11.66				
AST (UI)							
0	51.79	66.92	88.09	24.77	0.31	<0.01	0.05
2	66.96	69.32	66.61				
7	96.16	90.04	86.50				
14	161.00^{A}	186.85^{A}	79.62^{B}				
21	159.74^{A}	99.68^{A}	75.82^{B}				
Cholesterol (mg/dl)							
0	36.96	37.05	37.93	7.24	0.01	<0.01	0.12
2	87.87	74.68	79.01				
7	88.50	83.31	85.08				
14	100.40	75.54	93.82				
21	114.48	96.89	99.44				
Glucose (mg/dl)							
0	108.11	97.17	121.47	15.38	0.01	<0.01	0.01
2	157.55^{A}	111.33^{B}	133.11^{AB}				
7	129.41^{Aa}	99.54^{b}	88.8754^{B}				
14	130.01^{Aa}	103.24^{b}	75.10^{Bc}				
21	115.93	106.41	85.58				
NEFA (mmol/l)							
0	0.64	0.82	0.65	0.08	0.81	<0.01	0.01
2	0.51	0.62^{*}	0.44^{*}				
7	0.50	0.44	0.41				
14	0.26	0.30	0.35				
21	0.41^{b}	0.28^{B}	0.60^{Aa}				
BHBO (mmol/l)							
0	0.04	0.05	0.02	0.03	0.01	<0.01	<0.01
2	0.07	0.09	0.09				
7	0.12^{B}	0.14^{b}	0.21^{Aa}				
14	0.10^{B}	0.12^{B}	0.25^{A}				
21	0.10^{B}	0.11^{B}	0.23^{A}				
Triglycerides (mg/dl)							
0	42.15	44.24	43.04	10.72	<0.01	<0.01	0.02
2	57.39	63.80	63.81				
7	63.33^{A}	55.12^{A}	30.88^{B}				
14	49.98^{a}	56.54^{A}	27.47^{Bb}				
21	62.04^{A}	45.18	28.10^{B}				
Urea (mg/dl)							
0	36.22	32.01	35.79	5.55	<0.01	<0.01	0.01
2	41.65	35.58	41.06				
7	16.60^{B}	20.13^{B}	41.46^{A}				
14	20.62^{B}	25.70^{B}	34.05^{A}				
21	22.98^{b}	22.95^{b}	33.02^{a}				

$^{A, B}$ P≤0.01 $^{a, b}$ P≤0.05

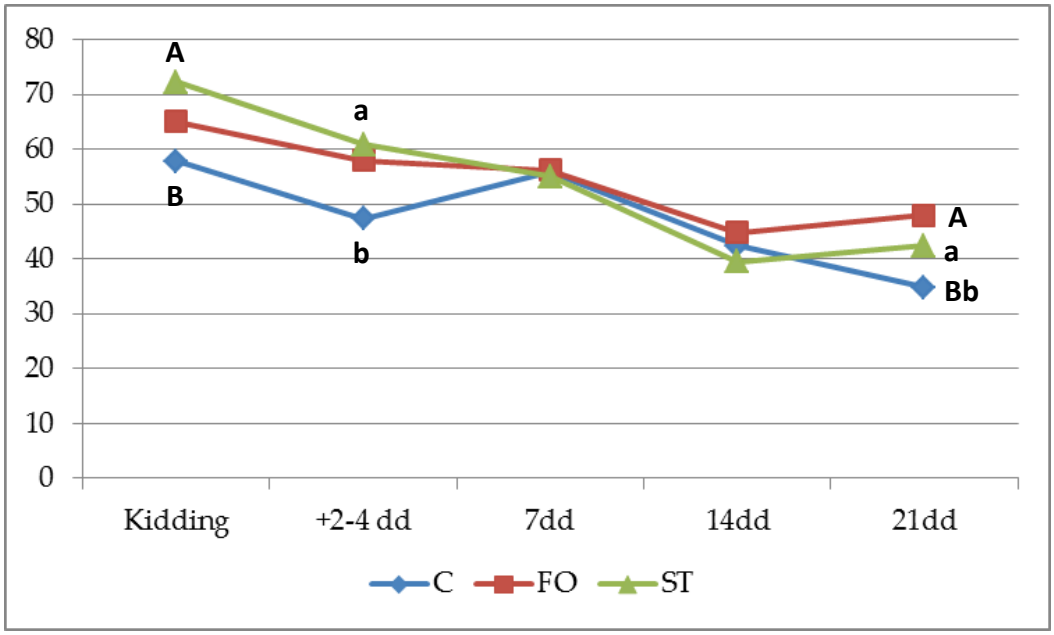
The blood differential leucocyte count was influenced by dietary fat administrations. Neutrophils percentage was statistically higher in ST group compared to C during kidding (72.12 vs 57.79 %; P≤0.05), at 2 days of life differences were found between ST and C groups (60.85 vs 47.31 %; P≤0.05), and at d 21 FO had the highest percentage compared to C (47.91 vs 34.82 %; P≤0.01) and differences were found between ST and C (42.38 vs 45.34 %; P≤0.05 (Tab. 11).

Lympocytes content was statistically higher in C compared to ST at d 2 (46.05 vs 31.71 %; P≤0.05) and at d 21 in C groups compared to ST group (61.78 vs 51.55 %; P≤0.05), differences were found between C and FO (61.78 vs 47.86 %; P≤ 0.01) (Fig.12).

Monocytes blood content was significantly higher at kidding in C compared to ST (7.11 vs 1.98%; P≤0.05) and in FO compared to ST (4.30 vs 1.98 %; P≤0.01), at d 7 differences were found between ST and C (8.33 vs 4.75 %; P≤0.01) and between ST and FO (8.33 vs 5.045%; P≤0.01), at d 14 between C and FO (5.75 vs 3.88 %; P≤0.05) and between ST and FO (7.44 vs 3.88 %, P≤0.01) (Fig. 13).

Eosinophils percentage was significantly higher in C group compared to FO group at birth (1.29 vs 10.90 %; P≤0.01), and at d 21 in FO group compared to C (1.40 vs 0.90 %; P≤ 0.05 (Fig.14).

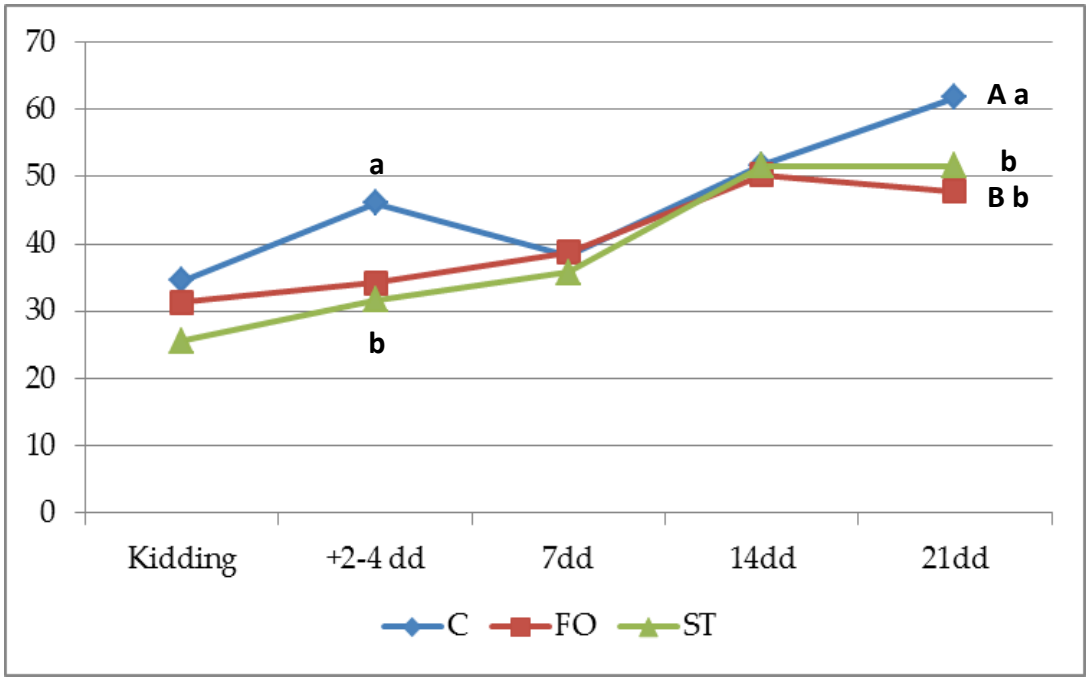
Figure 11: Neutrophils content in kids plasma (%).



[A, B] P≤0.01
[a, b] P≤0.05

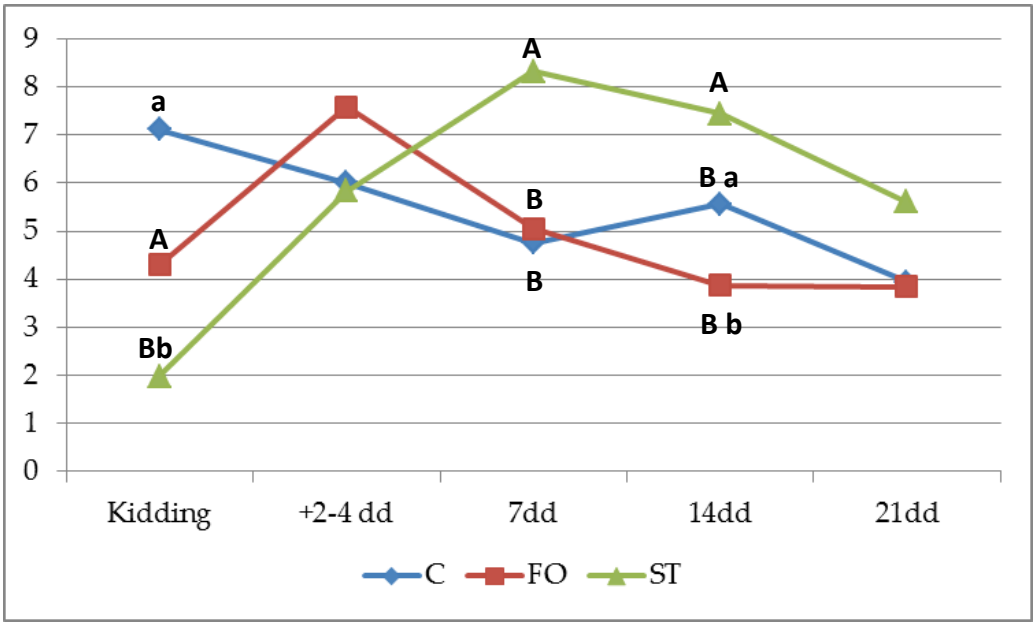
Figure 12: Lymphocytes content in kids plasma (%).

[A, B]



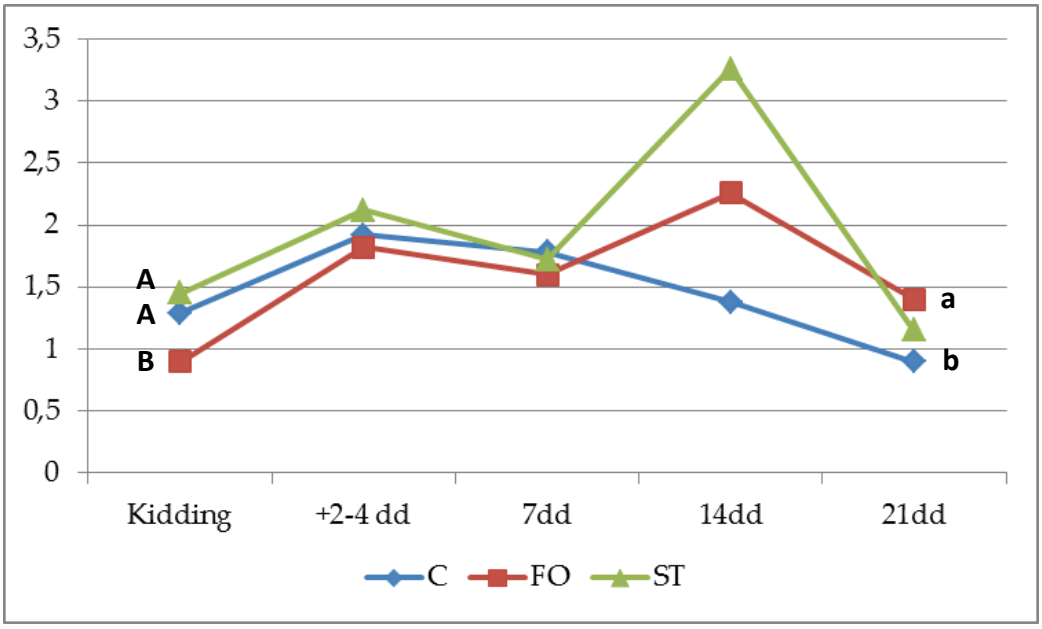
P≤0.01
[a, b] P≤0.05

Figure 13: Monocytes content in kids plasma (%).



[A, B] P≤0.01
[a, b] P≤0.05

Figure 14: Eosinophils content in kids plasma (%).

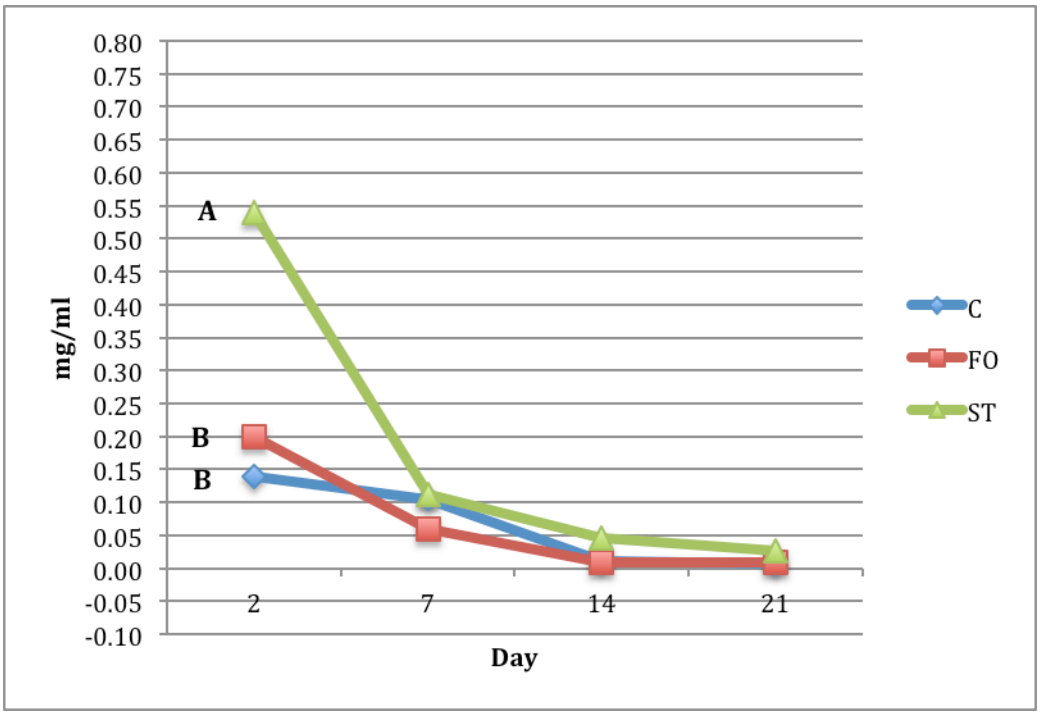


[A, B] P≤0.01
[a, b] P≤0.05

Immune response

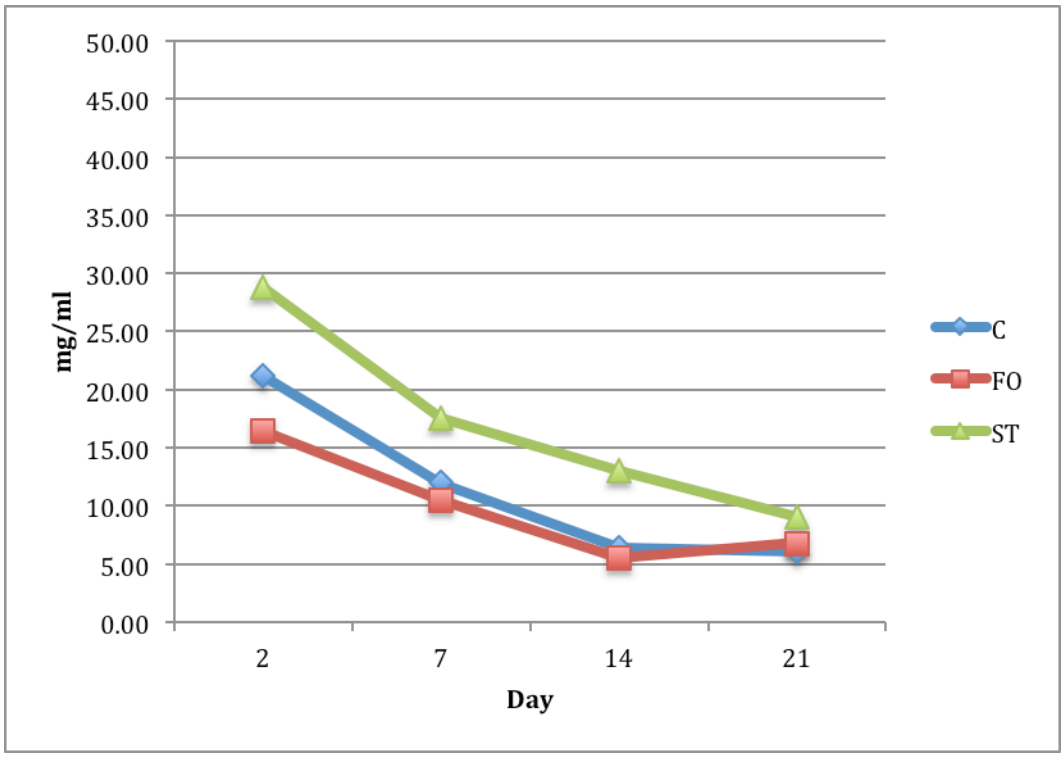
Peak serum IgA concentrations were observed at d 2 of life (Fig. 15). Differences were found at 2 day after birth between ST and FO snd C (0.54 vs 0.20 and 0.14 mg/ml respectively; P≤0.01), and mean IgA content during the trial was significantly higher ST group compared with FO and C groups (0.18 vs 0.07 and 0.07 mg/ml respectively; P≤0.01) and (Fig. 15).
IgG content was higher for whole the experimental period in ST group compared with FO (17.14 mg/ml vs 9.80 mg/ml; P≤0.01) and C (11.43 mg/ml; P≤0.05) (Fig.16).
Hp concentration was not statistically different in the three experimental groups even if was higher in C group (0.50 mg/ml) compared with FO (0.48 mg/ml) and ST (0.47 mg/ml) at d 2 (Fig. 17).

Figure 15: IgA content in plasma of kids (mg/ml)



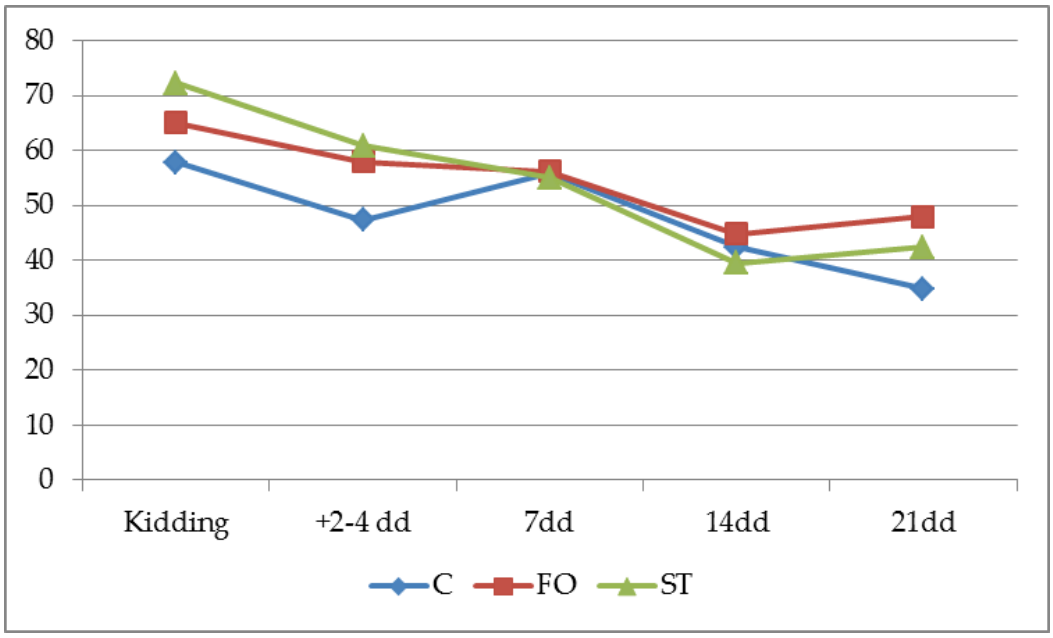
[A, B] P≤0.01
[a, b] P≤0.05

Figure 16: IgG content in plasma of kids (mg/ml)



[A, B] P≤0.01
[a, b] P≤0.05

Figure 17: Hp content in plasma of kids (mg/ml)



[A, B] P≤0.01
[a, b] P≤0.05

5.5 Discussion

In the present trial the effects of the administration of saturated or unsaturated dietary fatty acid sources in dairy goat were evaluated on immune status, metabolism and growth performance of newborn kids during the first three weeks of life.
Unfortunately, to our best knowledge, at the present moment very few data are available on kid performance, immune status and metabolism with a similar trial design. Moreover no information is available on the effect of the administration of stearic acid or fish oil to young kids on immune status or metabolism.
In the present trial the administration of fish oil in dairy goats increased EPA and DHA content in both colostrum and milk with and increased content also in the blood of relative kids according with Or-Rashid et al. (2010). Average milk secretion of EPA and DHA during the first 3 weeks of lactation was 0.40 g/d and 0.31g/d respectively, with an apparent transfer efficiency of 7.69% (EPA) and 8.00% (DHA), in accordance with previous studies (Cattaneo et al. 2006).
On the other hand, the administration of stearic acid increased saturated fatty acid content in milk on day 7 of lactation, but not in colostrum or on day 21 in milk. As a result higher stearic acid plasma content were found in ST kids only on day 7 of life. Main obtained outcomes of this trial showed different results then expected when adding stearic acid or fish oil to the diet of the mothers.
Kids from mothers fed high content of EPA and DHA were supposed to show increased immune response and a better metabolic status rather than control or ST group on the basis of reported general properties of n-3 and n-6 long-chain fatty acids on immune status and metabolism (Calder 2001; Simopoulos 2002; Calder 2009). Unexpectedly the kids from mother fed stearic acids showed the most significant variations in both immune response and metabolism respect to the other experimental groups.
Anyway the different dietary fatty acids sources in mothers' diets used in the present trial did not influence growth performance of relative kids.
Although a positive treatment effect was observed on BW for FO in comparison with ST and C kids, no differences were detected on body weights and average daily gain during the trial.
The lack of significant differences among the experimental groups could be probably attributed to general positive effect of the administration of mothers' colostrum and milk, and to the good hygienic rearing conditions of the experimental animals that can have in someway masked the dietary effect.
The growth rates obtained during our trial are comparable with data previously obtained by by Argüello et al. (2004a) and Rodríguez et al. (2008). These authors showed how kids and lambs raised with their dams in a natural rearing system, and fed the relative mothers' colostrum and milk, had higher performance that

kids fed artificial colostrum or milk replacer and kept away form the mothers. The benefiacial effect of mothers colostrum was also outlined later by Mellado et al. (2008) who found heavier kids than those fed a commercial colostral supplement containing immunoglobulins.
At the present time, IgG and IgA colostrum content in our trial is still to be determined, thus no information at the moment are available on the Ig's transfer from the mothers.
Very few and still debated literature on IgG or IgA blood content in new-born kids is available at the moment, but generally it is reported that blood IgG are undetectable (Constant et al. 1994; Castro et al. 2005) or, at least, poorly detectable at birth (Guerrault and Ouin 1990; Rabbani et al. 1990; Sherman et al. 1990; Castro et al. 2011) with a peak at 24 h of life (Chen et al. 1999) that can last until 48-60 hours of life (Argüello et al. (2004b).
The rearing system applied in our trial should have ensure the total transfer of Ig's from mothers to kids through colostrum and milk.
In the present trial the observed decreasing IgG plasma content in the three experimental groups from the second day of life showed the same trend as reported in other studies (Argüello et al. 2004b), and could be related to the closure of the intestine permeability to macromolecules (Staley and Bush 1985; Jochims et al. 1994; Argüello et al. 2004b).
During the present trial, a significant positive effect of stearic acid supplementation in the diet of the mothers on IgG kid plasma content was observed in early days of life. This finding is in agreement with a recent work by Santos et al. (2013) who evidenced higher IgG plasma contente in calves fed colostrum from cows administered with saturated fatty acids rather than fed essential fatty acids. According with Santos et al. (2013) it is possible to conclude that the dietary administration of saturated fatty acids to the mother can lead to an improvement of passive immune transfer to the newborn both in cow and goats.
To our best knowledge, no data are available at the present moment on IgA blood content in goat kids in the very early stages of life. Although some differences between species were evidenced (Johnson 2007), IgA plasma content in newborn kids show a similar trend as for what reported for IgG; low IgA content at birth are expected to peak at 24 hours of life and then decrease within five days after (Rodriguez et al. 2009).
The significant higher IgA kid plasma content observed in our trial on day two of life in ST group could provide evidence of the positive effect of stearic acid on the transfer of passive immunity from mother to newborn kids (as for IgG).
In the present trial the positive effect of stearic acid in the diet of dairy goat on immune status of kids is also supported by the higher neutrophil plasma content found in the first days of life.

Being neutrophils phagocytes that are normally found in the bloodstream during the beginning (acute) phase of inflammationthe progressively decreasing concentration of neutrophils detected in the three experimental groups were due to increased migration from the blood to the sites of inflammation. Higher initial levels of neutrophils in ST kids in the present trial could indicate an improved immune activity against infection and pathogenic agents although no significant differences were found by skin test.

Haptoglobin has an important function on immune cell function and differentiation, reduction of bacterial growth by reducing the bioavailability of iron and on prostaglandin production inhibition (Sadrzadeh and Bozorgmehr 2004). Recent studies reported a presence of acute –phase proteins (e.g. Hp) in colostrum (Reinhardt and Lippolis 2008), but the ability of colostrum Hp ingestion to induce hepatic Hp synthesis in ruminants is unknown, although it has been observed in swine (Hiss-Pesch et al. 2011). Petersen et al. (2004) reported that Hp is not present in normal bovine serum but only in case of acute inflammation. Hp is proven to rise considerably in acute conditions and remain elevated for up to 2 weeks (Panndorf et al. 1976; Petersen et al. 2004; Rahman et al. 2010). In the present trial no differences in Hp plasma content were observed in the three experimental groups. Unfortunately no reference values are available for such young kids at the moment, but similar Hp plasma content in the three groups show similar inflammatory conditions that can be linked with the hypothesis of a more reactive immune system of ST kids than C or FO.

Beside immune-related parameters, haematological profile showed values within the physiological ranges for glucose and triglycerides blood content (Celi et al. 2008) in the three experimental groups, although lower values were evidenced in ST than C and FO. Conversely, cholesterol was found to be higher than reference values (Celi et al. 2008) in all the experimental groups starting on day two of life, while the administration of stearic acid to the mother significantly increased urea blood content in kids.

Anyway most relevant results were obtained on ALT and AST blood content where ST group showed within-the ranges values (Magistrelli et al. 2013) respect to increased out-of ranges blood content of both these metabolites in C and FO kids, outlining a better hepatic condition.

Generally, NEFA and BHBO blood content in the three experimental groups were in the expected ranges (Sahlu et al. 1992; Doré et al. 2013), although higher values of BHBO were found in ST groups from the first week of life.

In conclusion this study reported saturated fat dietary supplementation can have a positive effect on passive immunity transfer from goats to newborn kids, and can stimulate the immune system of offspring.

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CHAPTER 6

General Discussion

6 General discussion

The fat administration in dairy ruminants during a critical period as peripartum, is a common strategy to prevent situation of NEB in productive animals. It has been speculated that feeding supplemental fat would reduce fatty acid mobilization from body stores and reduce liver fat accumulation.
The effects of individual FA have not yet been adequately studied to successfully allow for the identification of specific FA.
Fish oil has been supplemented in dairy animals often with the aim of enrich animal products with essential fatty acids considered healthy for human. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been able to modulate immune and inflammatory response. The extent to which long chain n-3 PUFAs are transferred from dietary fish oil to ruminants' milk fat is still debated. Also saturated fatty acids (C16:0 and C18:0) can affect lipid metabolism in dairy ruminants. Even though the effects of SFA supplements have been reported in numerous studies, it is still unclear what effects individual SFA have. Stearic acid is the most abundant FA available to the dairy cow and is used to a greater extent for milk production and energy balance.
Fatty acids have been recognized to be biologically active molecules that can regulate gene expression, enzyme activities, binding proteins, and other cellular processes. Among all nutritional factors able to affect cell biology by changing gene expression, dietary energy fatty acids and amino acids have the strongest potential. The most powerful nutrigenomics dietary components are fatty acids and the effects of fatty acids vary depending on their level of saturation. Numerous fatty acids and ecosanoics serve as ligand of PPARs. As is reported in literature the changes in PPARs is followed by a different expression of multiple enzymes involved in fatty acyl-CoA formation and hydrolysis, fatty acids elongation and desaturation and fatty acids oxidation. Different fat sources have been shown to differentially impact adipose and liver tissue in small ruminants. Dietary marine oil or algae have not been associated with milk fat in goats. As reported by Toral et al. (2013), up to now, is not clear the physiological basis for the particular response of dairy goats to lipid supplements. Studies in dairy goats characterizing lipid metabolism in body tissues and the influence of different fat sources are still scant, especially in fresh dairy goats when they are in NEB. Diet has effects on the transcription of the major genes involved in fatty acid uptake and de novo synthesis.
Our results in the first two experiments point out the importance of dietary fat supplementation to modulate lipid mobilization in transition dairy goats through a biochemical, histological and transcriptomic approach.
This thesis leads to some specific conclusions: dietary saturated and unsaturated lipid supplements are able to modulate the lipomobilizing machinery; in

particular fish oil could reduce lipomobilization. On the other side it appeared to have slight more detrimental effects on liver. Moreover, saturated or unsaturated fat administration in periparturient dairy goat diets are able to modulate mRNA expression of central genes involved in fatty acid uptake and de novo synthesis.
Furthermore, assuming that newborn ruminants are hypogammaglobulinemic (lambs and kids) at birth, the acquisition of immunity from dams to kids is strongly dependent from the consumption colostrum during the first days of life. Therefore, colostrogenesis is a complex process extremely important for the pre-partum transfer of components, mainly immunoglobulins, from maternal bloodstream into mammary secretions during the first 3 days of lactation. Nutrition during pregnancy was confirmed to affect both colostrum composition and yield. Long-chain n-3 polyunsaturated fatty acids of marine origin, mainly eicosapentaenoic acid (EPA C20:5 n-3) and docosahexaenoic acid (DHA C22:6 n-3), are known to explicate several health beneficial actions in mammals. DHA has been shown to play a specific role during fetal and neonatal development. Dietary supply of LC n-3 PUFAs during late pregnancy and nursing period can improve EPA and DHA content in goat colostrum and milk and could be beneficial to the suckling kids. On the opposite, long-chain fatty acids (LCFA), are reported to be able to improve some immune response aspects in animals. Anyway placental passage of LCFA is debated since time (placental impermeability to LCFA in sheep or a minimal passage of PUFA through the bovine placenta). At the present moment, few studies are available on the influence of different dietary sources of lipids in the diet of the mothers on newborn calves, kids or lambs immune response.
The results of the 3rd study point out the importance to administer dietary fat supplementation in periparturient dairy goats for the passive immunity transfer to newborn kids. Kids born from dams fed saturated fatty acids showed the most significant variations in both immune response and metabolism. The significantly higher IgA and the higher neutrophils plasma content in ST, support the idea that saturated fat dietary supplementation can have a positive effect on passive immunity transfer from goats to newborn kids, and can stimulate the immune system of offspring. Furthermore, ALT and AST results seem to underline a better hepatic condition.

CHAPTER 7

Summary

Dietary saturated and unsaturated fatty acids on hepatic and subcutaneous tissues intermodulation, gene expression in transition dairy goats, and influence of maternal diets on kids' immune response

Caputo Jessica Michela

7 Summary

Peripartum is a critically important period for health and production of dairy ruminants that is characterized by marked changes in the endocrine status that can lead to an increase incidence of metabolic and production-related diseases (e.g. NEB) to accommodate parturition and lactogenesis. Some classical efforts to enhance the energy status during transition are relative to the optimization of pre-partum nutritional level by increasing energy density of the diets. The most common practices are partial replacement of grains or forages in the ration with fats sources. On the other hand, dietary fat have peculiar roles in lipid metabolism and organismal defense system.

Fish oil has been supplemented in dairy animals often with the aim of enrich animal products with essential fatty acids considered healthy for human. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been able to modulate immune and inflammatory response as observed in vivo and in vitro studies. Also saturated fatty acids (C16:0 and C18:0) can affect lipid metabolism in dairy ruminants.

Studies in dairy goats characterizing lipid metabolism in body tissues and the influence of different fat sources are still scant, but diet has effects on the transcription of the major genes involved in fatty acid uptake and *de novo* synthesis.

About the influence of maternal lipid supplementation in diet on newborn animals, studies reported FO supplementing in milk replacer attenuated many aspects of the acute phase response and health in pre-weaned calves. Moreover, feeding calves colostrum from cows fed diets supplemented with saturated fatty acids (SFA) improved transfer of passive immunity.

The purpose of the present work is to study the variations of lipid metabolism in periparturient dairy goats supplemented with polyunsaturated (FO) or saturated (ST) fatty acid (1st and 2nd experiment) and their kids' immune-response (3rd experiment).

The first study evaluates immune-metabolic adaptation in periparturient dairy goats fed saturated or unsaturated fat supplement throughout a biochemical and histological approach. Results suggest dietary SFA and PUFA are able to modulate the lipomobilizing machinery. In particular, FO could reduce lipomobilization but, on the other hand, it appeared to have slight more detrimental effects on liver.
The second study shows that lipid inclusion in diet could modulate the expression of genes involved in fatty acid metabolism. Differences were found in liver tissue, for genes related to biosynthesis of monounsaturated fatty acids (SCD) and mRNA expression of genes encoding proteins associated with FA oxidation (ACOX1 and ACAA1). In adipose tissue LPIN1, gene involved in TAG synthesis was differentially expressed.
The last study shows that fatty acids supplements in goats diet can influence the immune response in newborn kids. The FAs profile of kids' plasma testifies a sure passage from diets into colostrum and milk. IgA and IgG content report a probably more reactive immune system in animals born from and fed by dams fed saturated fatty acids. Ig's, neutrophils and monocytes plasma content support the idea that saturated fat dietary supplementation can have a positive effect on passive immunity transfer from goats to newborn kids, and can stimulate the immune system of offspring.
Our results point out the importance of lipid supplementation in dairy goats diets on liver and adipose tissues intermodulation, mRNA expression of genes involved in FA oxidation in transition dairy goats and their role in the modulation of immune response in newborn kids.

CHAPTER 8

Published works

8 Published works

A. Agazzi, J.M. Caputo, M. Ferroni, M. Comi, V. Dell'Orto, V. Bontempo, G. Savoini. 2013. *Intestinal proinflammatory citokines could be modulated by dietary nucleotides in post weaning piglets.* Italian Journal of Animal Science . Vol. 12 (Suppl. 1)

X.R. Jiang, H.J. Zhang, G. Mantovani, G.L. Alborali, J.M. Caputo, G. Savoini,V. Dell'Orto, V. Bontempo. 2014. *The effect of plant polyphenols on the antioxidant defence system of weaned piglets subjected to an Escherichia coli challenge.* Journal of Animal and Feed Sciences. Vol. 23 (4) 324-330.

CHAPTER 9

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