UNIVERSITÀ DEGLI STUDI DI MILANO

Scuola di Dottorato in Scienze Veterinarie e dell'Allevamento

Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare (VESPA)

XXX ciclo

USE OF FUNCTIONAL FATTY ACIDS IN ANIMAL NUTRITION TO IMPROVE ANIMAL HEALTH STATUS AND DERIVED PRODUCTS

AGR18

Greta Farina
Matr. R10943

TUTOR
Prof. Alessandro Agazzi

COORDINATORE DEL DOTTORATO
Prof. Fulvio Gandolfi

Anno accademico
2017-2018

1. GENERAL INTRODUCTION 6

1.1. DEFINITIONS, NOMENCLATURE AND PROPERTIES OF FATTY ACIDS 6

1.2. FATS IN ANIMAL NUTRITION 9

1.2.1. Ruminants 10

1.2.1.1. Fat digestion in ruminants 10

1.2.1.2. Dietary fat during periparturient period 13

1.2.1.3. Lipid sources for ruminants 16

1.2.2. Non-ruminants 17

1.2.2.1. Fat digestion in non-ruminants 17

1.2.2.2. Lipid sources for non-ruminants 18

1.3. ANIMAL HEALTH – THE ROLE OF PUFA 18

1.3.1. Physiological adaptations during transition period and dietary fat supplementation 18

1.3.2. Nutrigenomic approach 23

1.3.2.1. Tissue - level mechanisms investigation – mRNA expression in dairy cattle 25

1.3.2.2. The emerging approach of miRNA in ruminants 27

1.4. HUMAN HEALTH AND PUFA 30

1.4.1. Dietary manipulation of milk fatty acid composition 35

1.4.2. Dietary manipulation of egg quality 41

1.4.3. Dietary manipulation of meat quality 46

2. OBJECTIVES 50

3. LIVER AND ADIPOSE TISSUE METABOLISM- AND INFLAMMATION-RELATED MRNA AND MICRORNA EXPRESSION IN PERIPARTURIENT DAIRY GOATS FED POLYUNSATURATED VS. SATURATED FATTY ACIDS 51

3.1. ABSTRACT 51

3.2. INTRODUCTION 52

3.3. MATERIALS AND METHODS 54

3.3.1. Animal management and diets 54

3.3.2. RNA Extraction and Quantitative PCR *58*
3.3.2.1. Liver biopsies 58
3.3.2.2. Adipose biopsies 58
3.3.3. Total RNA Extraction, Target Genes, Primers Design, and Quantitative PCR *59*
3.4. STATISTICAL ANALYSIS 66
3.5. RESULTS 66
3.5.1. mRNA expression of metabolism-related genes in liver *66*
3.5.2. mRNA expression of metabolism-related genes in adipose tissue *68*
3.5.3. mRNA and microRNA for adipose tissue, inflammatory-related genes *73*
3.6. DISCUSSION 77
3.6.1. Liver and adipose tissue metabolism gene expression *78*
3.6.1.1. Fat sources and gene expression in liver and adipose tissue 79
3.6.2. Adaptations to kidding *80*
3.7. CONCLUSIONS 82
4. DIETARY *CAMELINA SATIVA* IMPROVES CONVERSION RATE IN LAYING HENS AND LEADS TO HEALTHIER EGGS 83
4.1. ABSTRACT 83
4.2. INTRODUCTION 84
4.2.1. Camelina sativa meal: chemical composition and nutritional value *86*
4.2.2. Camelina sativa in poultry diet *87*
4.3. MATERIALS AND METHODS 90
4.3.1. Experimental design, animals and housing conditions *90*
4.3.2. Chemical composition of the diets, sample collection and recorded parameters *91*
4.3.3. Egg quality and shelf-life assays *95*
4.3.4. Egg yolk fatty acid analysis *96*
4.4. STATISTICAL ANALYSIS 97
4.5. RESULTS 98

4.5.1. Hen performance, egg quality and shelf-life *98*

4.6. DISCUSSION 105

4.6.1. Fatty acid profile of egg yolk *105*

4.7. CONCLUSIONS 108

5. GENERAL DISCUSSION **109**

6. REFERENCES **111**

7. SUMMARY **138**

8. RIASSUNTO IN ITALIANO **141**

9. PUBLISHED WORKS **145**

9.1. SCIENTIFIC PAPERS 145

9.2. MEETING ABSTRACTS 145

9.3. ORAL PRESENTATIONS 146

10. ACKNOWLEDGEMENTS **147**

11. TABLE OF CONTENT **148**

11.1. LIST OF FIGURES 148

10.2. LIST OF TABLES 149

1. GENERAL INTRODUCTION

1.1. Definitions, nomenclature and properties of fatty acids

Fatty acids (FA) are substantial part of lipids, one of the three major components of biological matter, along with proteins and carbohydrates (Lehninger et al., 2005). Hwang and Rhee (1999) stated they play a significant role in survival and well-being of mammalian life, being progenitors of a wide variety of biological lipids and participating to many signalling pathways. Therefore, fat are considered an essential dietary component (Spector and Kim, 2015).

In biochemistry, a fatty acid is a carboxylic acid with a long aliphatic chain and it can be liberated by hydrolysis from naturally occurring fats and oils (Fahy et al., 2009). Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28 (McNaught and Wilkinson, 1997).

They have important biological functions, structural and functional roles, such as immune cell regulation, cell signalling, energy production, membrane structures (Nagy and Tiuca, 2017), and gene expression and regulation (Bionaz et al., 2015).

Fatty acids are synthesized in the cytoplasm from two carbon precursors, thanks to acyl carrier protein (NADPH) and acetyl-CoA-carboxylase and their degradation is through β-oxidation in mitochondria, with the subsequent release of energy (Tvrzicka et al., 2011).

According to their structure, fatty acids are either saturated or unsaturated carboxylic acids with carbon chains varying between 2 and 36 carbon atoms.

Saturated FA (SFA) do not contain any double bonds. They can be divided into subgroups according to their chain length:

- ✓ Short chain (saturated) fatty acids (SCFA), such as acetic (2:0), propionic (3:0), and butyric (4:0) acids;

- ✓ Medium chain (saturated) fatty acids (MCFA), like caproic (6:0), caprylic (8:0), and capric (10:0) acids;
- ✓ Long chain (saturated) fatty acids (LCFA), which includes lauric (12:0), myristic (14:0), palmitic (16:0), and stearic (18:0) acids
- ✓ Very long chain (saturated) fatty acids (VLCFA), like include arachidic (20:0), behenic (22:0) acids.

On the other hand, unsaturated FA (UFA) includes the presence of double bonds.

They can have two different configurations depending on the three-dimensional orientations those results in different physical characteristics: *cis* or *trans*. The former makes fatty acids more fluid than the *trans* configuration. Plant lipids have only *cis* configuration and the *trans* is only the result either of microbial modification or chemical hydrogenation (Drackley, 2007).

There is a further subdivision, depending on the number of double bonds: MUFA, as monounsaturated fatty acids, with just one double bond and PUFA (polyunsaturated fatty acids) with more than one double bound (Tvrzicka et al., 2011). In general, the more double bonds there are in the fatty acid, the more prone they are to lipoperoxidation (Kaasgaard et al., 1992).

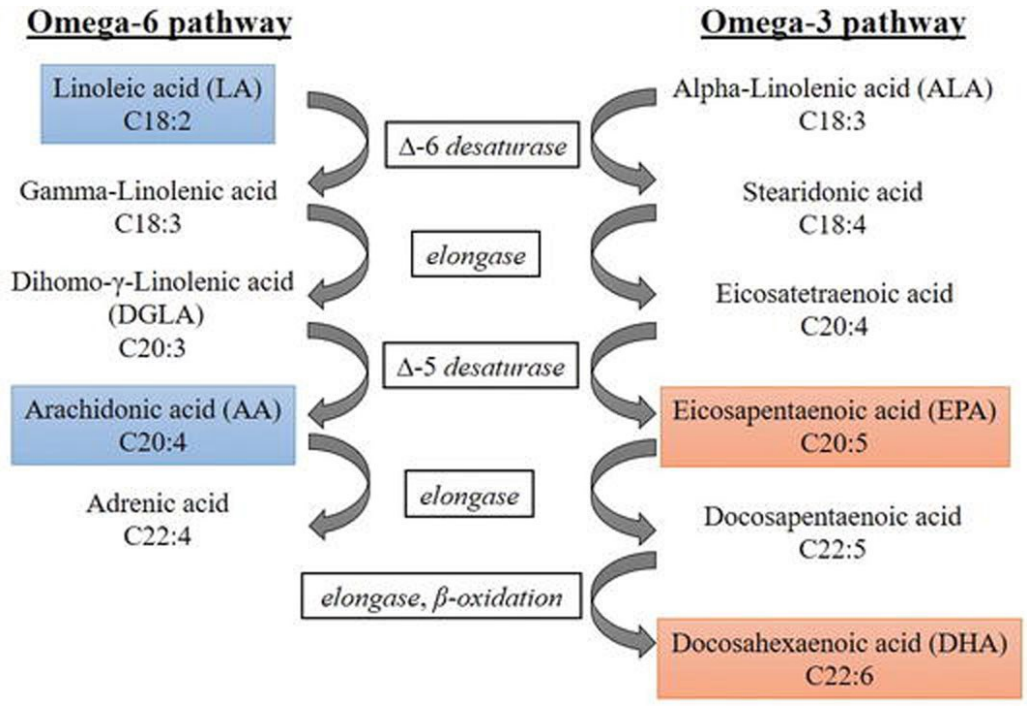
Within the PUFA group, we can find two major families, omega 3 and omega-6.

In the ω-3 PUFA family, the term n-3 identifies the position of the double bond. The parent fatty acid is α-linolenic acid (ALA; 18:3n-3), which is synthesized from linoleic acid (18:2n-6) (LA) through desaturation, catalysed by delta-15-desaturase. Since the lack of certain enzymes in mammals, the endogenous desaturation of these compounds can take place not beyond Δ9 position, so some fatty acids, like α-linolenic acid, are considered essential and must be supplied in the diet. Although this lack of enzymes in humans and animals, they can metabolize α-linolenic acid through additional desaturation and elongation to obtain long-chain

PUFA (Tvrzicka et al., 2011). As shown in Figure 1, α-linolenic fatty acid can be firstly converted by elongation, desaturation to Eicosapentaenoic acid (EPA; 20:5n-3) and then, further catalysed reactions lead to yield Docosahexaenoic acid (DHA; 22:6n-3). Although it is possible for the body to convert ALA to EPA and DHA, researches have demonstrated that only a small amount can be synthesized from this process (Swanson et al., 2012).

Both the conversions of ALA to EPA and linoleic acid to arachidonic acid (20:4n-6) (AA) need the same enzymes to take place, so they are in competition, especially for Δ6 desaturase (Calder, 2013). The activity of Δ6 and Δ5 desaturases are regulated, among the different factors, also by nutritional status (Calder, 2013).

Figure 1. Pathways of biosynthesis ω−6 and ω−3 fatty acids. (Adapted from Nagy and Tiuca (2017)).



Dietary sources of ALA are oils from both plant, like linseed and soybeans (Gunstone et al., 1994) and animal sources , like eggs. Whereas, its metabolites EPA and DHA, are available from marine food sources, such as fish, fish oils and algae (Ganesan et al., 2014).

On the other side, the parent fatty acid of ω-6 is linoleic acid and one of its metabolic products such as arachidonic acid. They can be found in some vegetable oils, for instance sunflower seed oil and soybean oil.

The PUFAs health benefits have been widely demonstrated (Ganesan et al., 2014). Indeed, these essential fatty acids play an important role at different levels, not only for normal growth and for development, but also for the evolution of chronic diseases, such as cancer and diabetes (Simopoulos, 2010). Indeed, twenty-carbon PUFAs have been shown to be promoters of eicosanoids that regulate inflammatory and immune responses through pro- and anti-inflammatory activities (Calder, 2013, Calder, 2015).

1.2. Fats in animal nutrition

In the last decades, supplementation of fats in animal nutrition has become a standard practice. That's because lipid are not only passive energy-providing molecules but also metabolic regulators able to act in an hormone-like manner in the control of transcription factors involved in lipid metabolism (Jump, 2004, Jump et al., 2005, Agazzi et al., 2010) , immune response (Agazzi et al., 2004) and milk fatty acid secretion (Cattaneo et al., 2006, Bernard et al., 2008).

As an example, studies on ruminants have demonstrated that increasing the supply of energy with fat from late pregnancy to the first weeks of lactation lead to improved health status and to ameliorate some aspects of immune response. This can occur especially when polyunsaturated n-3 and n-6 omega fatty acids, rather than saturated fatty acids, were added in

the diet (Thering et al., 2009, Agazzi et al., 2010, Sordillo, 2016). Conversely, increasing energy density during the close-up dry period was recently reported to increase the incidence of metabolic disorders in dairy cows after calving (Vailati-Riboni et al., 2016).

Newly, consumer demand in developed countries looks towards the search of food with positive effects on health. As a consequence, there is an increasing interest in functional foods (Bhat and Bhat, 2011). In this context, essential is the transfer of dietary fatty acids into the animal products, which depends on the nature of lipid digestion of the animal, different between ruminants and non-ruminants.

1.2.1. Ruminants

1.2.1.1. Fat digestion in ruminants

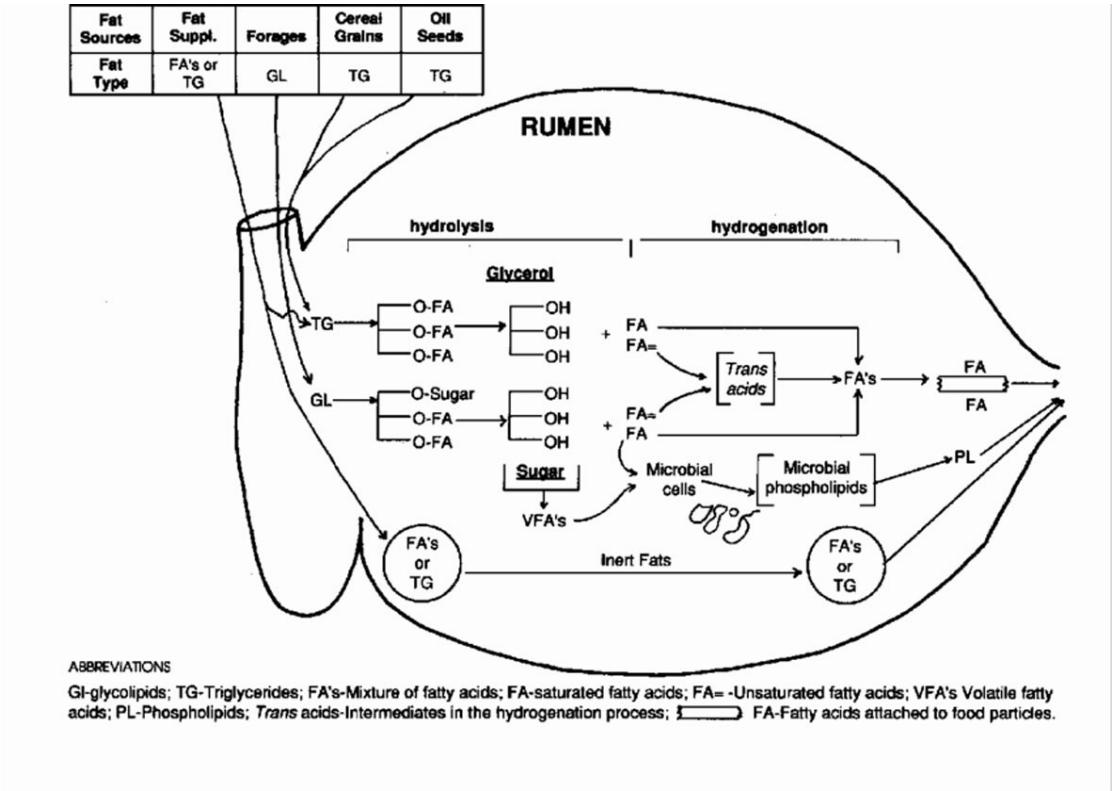
The presence of rumen and its microbial population determine the composition of fatty acids that leave the rumen for the following absorption in the small intestine. Although the profile of the fatty acids can be different, the outflow of total fatty acids is very similar to the dietary intake of them (Lock et al., 2005).

Diets for lactating dairy cows are usually around 4-5% of lipid content. The PUFA contained in the diet are part of plant triglycerides and glycolipids (Drackley, 2007). Among these polyunsaturated fatty acids, the main are linolenic acid from forage crops and linoleic acid from oil seeds and concentrates (Lock and Bauman, 2004). The disappearance of these two fatty acids in the rumen is approximately around 93% for the former and 85% for the latter (Chilliard et al., 2007).

Lipid metabolism in ruminants consists of two major processes. The first step is the hydrolysis of the ester linkages found in triglycerides, phospholipids and glycolipids by enzymes of rumen bacteria and the second one is the ruminal hydrogenation of long unsaturated fatty acids into

partially and fully saturated derivates. This process provides that the major fatty acid leaving the rumen is stearic acid (C18:0) (Zymon et al., 2014) and, thanks to the ruminal microbes, the configuration of fatty acids is changed from *cis*- to *trans* (Drackley, 2007).

Figure 2. Lipid metabolism in the rumen. The prevalent fat types in feedstuff are triglycerides and glycolipids (adapted from Bauman and Lock (2006)).



Many factors can affect the rate and the extent of these two processes. For instance, the extent of the hydrolysis is reduced by the increasing of dietary fat level and by the reduction of ruminal pH (Bauman and Lock, 2006). Likewise, the rate of biohydrogenation is faster along

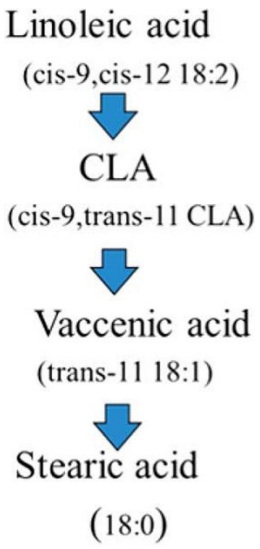
with the increasing of unsaturation level (Bauman and Griinari, 2003) and it is more intense with lower amount of concentrates (Chilliard et al., 2007).

During hydrolysis, rumen bacteria separate the fatty acids from the glycerol backbone and this process ensures that no monoglycerides or diglycerides pass the lower digestive tract (Drackley, 2007). The only exception to this process would be for saturated fatty acids for their low solubility due to their high melting point.

Afterward, the unsaturated fatty acids are hydrogenated to saturated fatty acids because toxic to many rumen bacteria (Bauman and Lock, 2006).

During the biohydrogenation, intermediate compounds with trans-double bonds are produced. One of these is conjugated linoleic acid (CLA), which recently has been investigated for its beneficial effects on human health (Kim et al., 2016).

Figure 3. Rumen biohydrogenation of fatty acids (adapted from Savoini et al., (2016)).



As a result through the first chamber of the alimentary canal, the lipid material consists mainly of free fatty acids (80-90% of lipids) attached to feed particles. The very small part not included in this component (20-10%) is composed by microbial phospholipids and traces of triglycerides and glycolipids from residual feed material, which are hydrolysed by intestinal and pancreatic lipases (Doreau and Ferlay, 1994, Lock and de Souza, 2016).

Thanks to the greater capacity of the bile salt and the micellar system to solubilize fatty acids, as well as the opportune acidic pH of the duodenum and jejunum (pH 3.0 – 6.0), the intestinal absorption coefficient of singular fatty acid is higher in ruminants than non-ruminants (for the former it is around 80% for SFA and 92% for PUFA in low fat diets) (Nieto and Ros, 2012). Once absorbed, they are re-esterified into triglycerides (with glycerol produced from metabolism of blood glucose) and wrapped into chylomicrons or very low-density lipoproteins (VLDL) to be transported to the lymphatic system. Then, through the bloodstream, they can be delivered to various organs of the body (like mammary gland and heart) that can use the triglycerides. Thanks to lipoprotein lipase, they are broken down to free fatty acids in the capillaries of these tissues. Once inside the cells, they can either be built back into triglycerides or burned to release energy (Drackley, 2007).

Differently form absorbed nutrients like amino acids, dietary fats do not reach the liver directly. Therefore, they can not play a role in the onset of fatty liver.

The small amount of PUFA which escapes biohydrogenation is absorbed from the intestine and incorporated into cell membrane phospholipids (Woods and Fearon, 2009).

1.2.1.2. Dietary fat during periparturient period

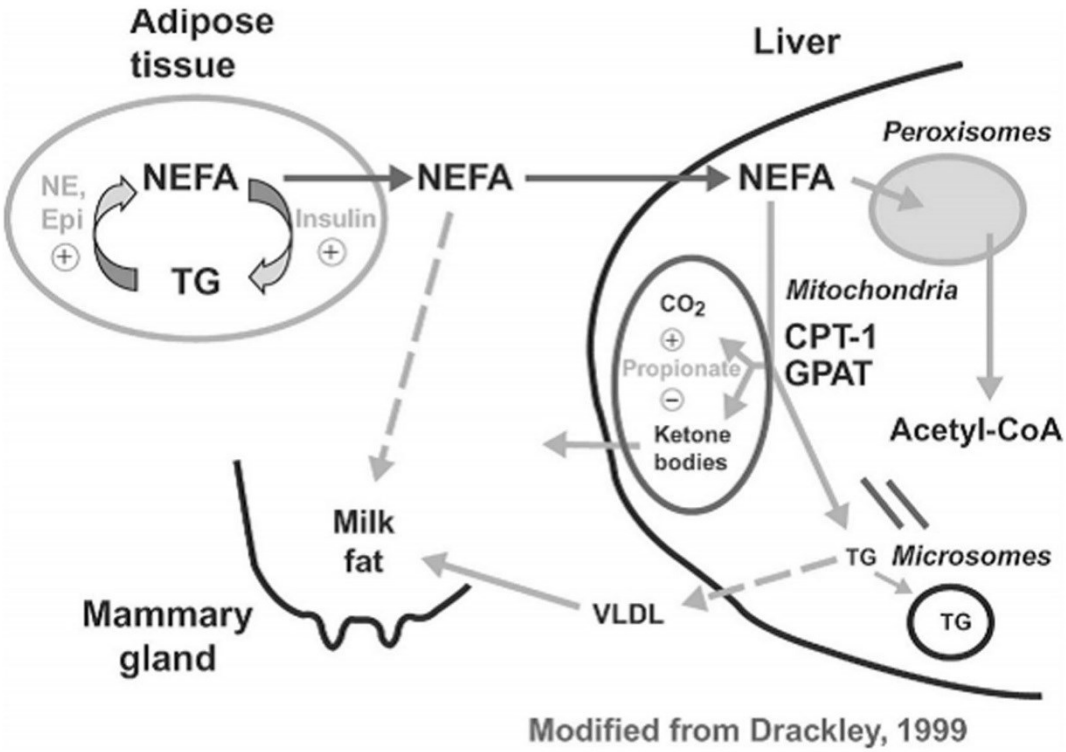
Fat are widely used in dairy animals being a strategy to modulate the answer to stressing events that are common during peripartum period. Because of that, the transition period, namely

from 3 weeks before to 3 weeks after parturition (Drackley, 1999), is considered the most appropriate phase to increase productive performance of dairy animals (Bronzo et al., 2010). A number of changes in endocrine status can take place to accommodate parturition and lactogenesis, which lead to modify the metabolism drastically (Vailati Riboni et al., 2015). Indeed, this timeframe is marked by enhanced energy requirements (due to the onset of lactation), diminution in dry matter intake (DMI) as well as negative energy balance (NEB) (Sordillo et al., 2009). In particular, some degree of negative energy balance is beneficial during early lactation because it can raise the metabolic efficiency, conveying the nutrients towards the mammary gland (Vernon, 2005). On the other hand, if it is severe, the hepatic function can be impaired, leading in ketosis, fatty liver (Eşki et al., 2015), and strongly influencing reproductive system and immunological status (Drackley, 1999, Duffield, 2000).

Since the liver can "sense the fuel needs of all of the other tissues in the body and respond by adjusting its metabolism accordingly" (Arias et al., 2009), it plays a key role in coordinating nutrient fluxes across the periparturient period (Drackley et al., 2001).

In order to adapt to NEB, mobilization of fat from body stores (in the form of non-esterified fatty acids - NEFA) into blood stream can occur (Sordillo et al., 2009), with their subsequent increment in plasma. After being carried to the liver, NEFA are oxidized to generate energy, ketone bodies, or esterified to triacylglycerol (TAG) followed by synthesis of very low-density lipoproteins (Invernizzi et al., 2016). When NEFA become excessive, fatty liver or hepatic lipidosis will occur in early lactating cows (Kuhla and Ingvartsen, 2018). Instead, in small ruminants this frequently happens during the last phase of pregnancy (Pinotti et al., 2008, Savoini et al., 2010).

Figure 4. Schematic metabolic relationships among lipid metabolism in adipose tissue, liver, and mammary gland during the transition period. (+) indicates stimulatory effects, (-) indicates inhibitory effects. Dashed lines indicate processes that happen at low rates or only during certain physiological states. (NE = norepinephrine, Epi = epinephrine, CPT-1 = carnitine palmitoyltransferase-1, GPAT = glycerol-3 phosphate acyltransferase, TG = triglyceride, CoA = coenzyme A, VLDL = very low density lipoprotein. Adapted from Drackley (1999).



Evidence from dairy cow studies underscores the usefulness of supplemental fat administration during this productive phase (Staples et al., 1998, Ballou et al., 2009). Specifically, dietary fats can provide dense energy sources or act as modulators of metabolic and immune pathways (Tsiplakou and Zervas, 2013b, a) that have been proved to be linked to each other, enhancing the incidence of disorders around calving (Sordillo and Mavangira, 2014).

1.2.1.3. Lipid sources for ruminants

Since has been proven that diet can influence fatty acid composition of animal products (Woods and Fearon, 2009), object of interest is the kind of lipid compounds provided by the different fat sources.

With the general term "lipid", we consider a wide category of substances insoluble in water or other aqueous solvents and soluble in organic solvents (Kates, 1986).

Changing the composition of the diet adding fatty acid-rich sources, the basal diet itself can influence the lipid composition of animal products.

Considering the different raw materials fed to ruminants, we can account for four types of lipids. We can find triglycerides (a molecule of glycerol combined with three fatty acids) in cereal grains, oilseeds and by-product feeds. Then, in forages, the main components are glycolipids (like the previous compounds but instead of the third fatty acid, with more sugars linked to the glycerol). The very small role in feedstuff for dairy cows is played by phospholipids (where the third position of glycerol is linked to a phosphate group, in turn attached to an organic base) and free fatty acids (few in feed, but the main component in fat supplements) (Drackley, 2007).

In terms of free fatty acids, depending on the raw materials (and their specific composition) taken into consideration, they can differently affect the fatty acid composition of animal products.

Forages are rich of n-3 PUFA (level can depend on the maturity and variety of the grass), in particular it has been proven how they can transfer linolenic acid (C18:3) into animal products (French et al., 2000, Chilliard et al., 2007). Even though the benefits obtained in milk and meat, feeding forages is less effective than the ones obtained with protected fat supplements, they are

a cheaper solution and have a more positive feedback of the consumers (Woods and Fearon, 2009).

Whereas, oilseeds can enrich milk and meat with the linolenic acid (C18:3) (Ebrahimi et al., 2014, Doreau and Ferlay, 2015) , while marine algae or fish oil have impact on increasing EPA and DHA (Greco et al., 2015).

1.2.2. Non-ruminants

1.2.2.1. Fat digestion in non-ruminants

In contrast to ruminants, in non-ruminants dietary fats are absorbed unchanged before incorporation into the tissue lipids. Therefore, dietary lipid sources have a direct effect on fatty acid composition of these animal products, and for this reason, the protection of feed during digestion is not required (Woods and Fearon, 2009).

Lipid digestion takes place for a small part (10-30% of the lipid hydrolysis) in the stomach, under gastric lipase and for the most in the small intestine by pancreatic lipase. Thereby, the principal site of digestion of these dietary compounds in monogastrics is the small intestine (Mun et al., 2007). Pancreatic lipase breaks the TAG down to 2-monoacylglycerols and free fatty acids. Afterward, these two latter compounds are associated with bile salts and phospholipids forming micelles, which allow these poor soluble substances to be carried to the surface of the enterocyte, where they can be absorbed. Once inside the enterocyte, monoglycerides and fatty acids are re-synthesized into TAG. The TAG is packaged, along with cholesterol and fat-soluble vitamins, into chylomicrons. Chylomicrons are lipoproteins, special particles that are designed for the transport of lipids in the blood circulation (Harrison and Leat, 1975).

In general, the ability of non-ruminants to digest fatty acids decreases when chain length increases and it enhances along with the number of double bonds (Lessire et al., 1992).

1.2.2.2. Lipid sources for non-ruminants

The cereal-based diet offered to monogastric provides mostly n-6 PUFA and only a small amount of n-3 PUFA. In order to obtain omega 3-riched products, dietary strategies are focused on evaluating the effects of the supplementation of both terrestrial and marine n-3 FA sources.

Researches using vegetable oil addition (linseed, sunflower, soybean, and olive oil) in non-ruminants have showed how plant oils have different impacts on the deposit fat and how the site of fatty acid deposition depends on fatty acid content of the oil (Crespo and Esteve-Garcia, 2002).

Moreover, testing marine oils (like fish oil) have shown that the transfer of n-3 long chain polyunsaturated fatty acids, of which they are rich in, is influenced by time and duration of supplementation (Nieto and Ros, 2012).

Marine algae, as the DHA source par excellence, have been fed to laying hens and chickens to look into the possible effects of this lipid supplementation on fatty acid composition of eggs (Meluzzi et al., 2001) and meat (Sirri et al., 2003).

1.3. Animal health – the role of PUFA

1.3.1. Physiological adaptations during transition period and dietary fat supplementation

The efficiency of the immune system, as a complex biological process aimed to protect the animal, determines the answer of the animal to withstand the outset of a disease. Its role deals

with the prevention of microbial invasion or, if already existing, with the elimination of those infections or injuries restoring the normal and physiological functions of tissues (Sordillo, 2016). Both the innate and adaptive immune systems cooperate to provide the most effective protection from external risks. The former is the fastest response after a tissue insult and with a nonspecific nature. Whereas the latter is delayed and more specific, able to improve its response to repeated exposures of the same pathogen (Sordillo, 2016).

Cytokines (such as *TNFa*, *IL1* and *IL6*), as an innate defence, are a common feature of many inflammatory conditions (Calder, 2009, Calder, 2011, Calder, 2013). Their activity, resultant as the interaction with their receptors located on host cells (Sordillo and Streicher, 2002, Bannerman, 2009), facilitates the migration of leukocytes from blood into tissues, followed by the cell recognition of bacteria (Ryman et al., 2015).

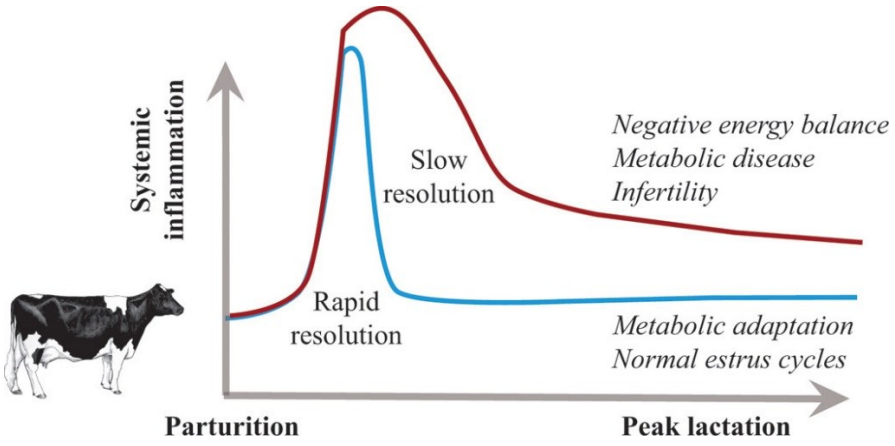
If this immune response fails, will be the role of the adaptive immune response, which recruits lymphocytes and memory cells to recognize specific antigenic determinants of the pathogen (immunological memory) (Sordillo, 2016).

Inflammation occurs in the initial phase of the immune response (innate defence) and determines if the tissue insult can be eliminated and functions can return to normality (Raphael and Sordillo, 2013, Sordillo and Mavangira, 2014). The fundamental signs of inflammation are redness, swelling, heat, pain and loss of function (Calder, 2015). If local defences are not effective, the acute phase reaction will take place, so from a local inflammation it moves to a systemic reaction that can lead to both acute and chronic forms (Ceciliani et al., 2012, Bradford et al., 2015, Calder, 2015). Therefore, the lack of an appropriate balance between the inception and the resolution of the inflammatory state converts into chronic (Aitken et al., 2011).

Under inflammation, all nutrients are directed to sustain the immune system at the expense of storage and growth (Gifford et al., 2012).

An evident inflammatory response is manifested, for example, in transition cows as well in small ruminants, due to their proximity to pregnancy and lactation (Humblet et al., 2006, Sordillo et al., 2009, Trevisi et al., 2012, Bradford et al., 2015, Caroprese et al., 2015). A typical signature is the release of pro-inflammatory cytokines, with metabolic changes (Kushibiki et al., 2003, Bertoni et al., 2004). Dairy goats, similarly to what reported in cows (Bertoni et al., 2008, Trevisi et al., 2012), could experience some degrees of inflammation around kidding (Invernizzi et al., 2016) just as a homeorhetic role of physiological adaptation to lactation (Farney et al., 2013, Bradford et al., 2015, Vailati Riboni et al., 2015).

Figure 5. Hypothesized responses to resolved and unresolved inflammation in early-lactation dairy cows (adapted from Bradford et al. (2015)).

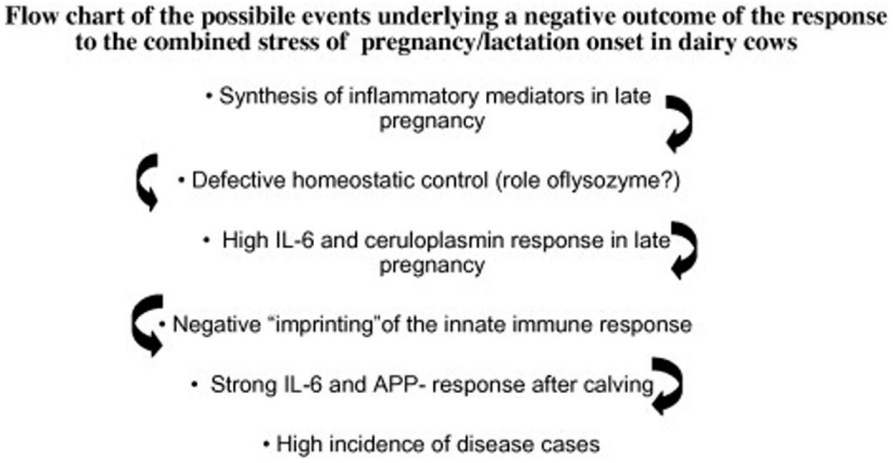


Actually, transition period can compromise the immune system (hypo responsiveness) because characterized by health disorders with consequences on the productive efficiency of cows (Pinedo et al., 2010, Sordillo, 2016). Indeed, it has been proved how the metabolic and immune pathways are linked to each other and how this correlation can enhance the incidence

of disorders around calving (Sordillo and Mavangira, 2014). Moreover, long since, it was established how metabolic and infectious diseases take place concurrently rather than as single events (Curtis et al., 1983) and how nutrient intake is their common nexus. As matter of fact, both over- and under-conditioned cows have higher incidences of disease (Heuer et al., 1999, Roche et al., 2009). In the first case, the normal prepartum falling in DMI is aggravated with a worsened NEB, a more intensive lipid mobilization from tissue stores to the liver (NEFA), and a greater sensitivity to infectious and/or inflammatory diseases (Janovick and Drackley, 2010, Bertoni et al., 2015). On the other hand, the insufficient intake in terms of energy and protein is not enough to cover cow needs for milk production and disease resistance (Hoedemaker et al., 2009, Roche et al., 2009).

Since the transition period is characterized by a high incidence of immunological changes associated with metabolic and physical stresses (where stress is an imbalance in the physiological homeostasis of an organism (Sordillo and Mavangira, 2014)), this physiological stage is the most appropriate to increase productive performance and improve the health of dairy animals, including goats (Bronzo et al., 2010).

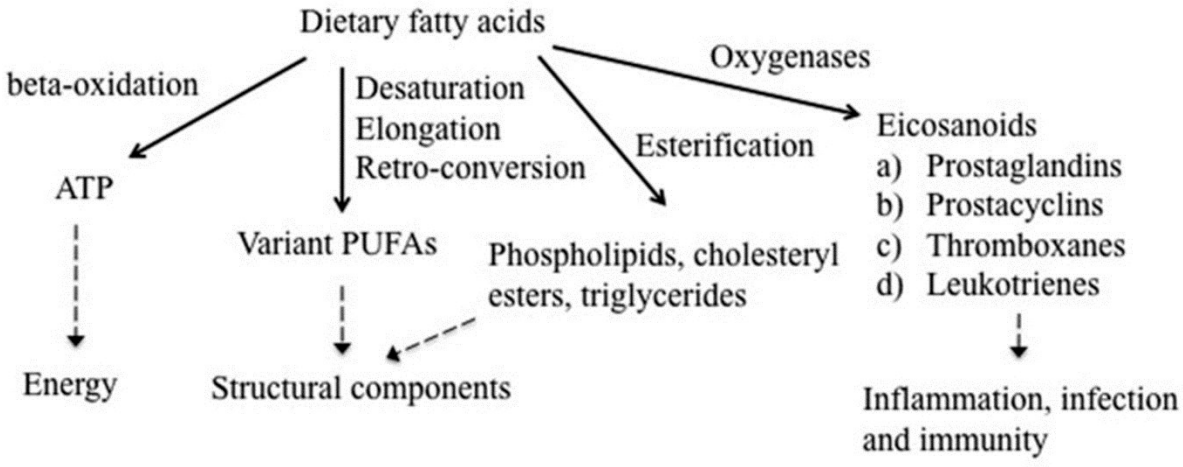
Figure 6. Flow chart of the possible events underlying a negative outcome of the response to the combined stress of pregnancy/lactation onset in dairy cows (adapted from Trevisi et al. (2012)).



Proved in human field and confirmed in animal medicine as well, the optimum energy level is crucial for a proper immune function (Calder, 2013). Indeed, cells from both innate and adaptive immune systems, when active, require high-energy. Although NEFA (produced through β-oxidation) can be used as an energy source, there is evidence that changes in fatty acid composition of immune cells can affect the immune response (Raphael and Sordillo, 2013). For instance, EPA and DHA can exert anti-inflammatory functions being able to interact with signalling pathways, including peroxisome proliferator-activated receptor (*PPAR*) and sterol response element binding protein (*SREBP*) family (Clarke, 2004).

As a result, lipid supplementation is widely used in dairy cows being a strategy to modulate the answer of animals to stressing events as during peripartum. In fact, dietary fats are more than just a source of energy, being also modulators of lipid metabolism and immune system (Tsiplakou and Zervas, 2013a, b).

Figure 7. Metabolic fates of dietary fatty acids (adapted from Ganesan et al. (2014)).

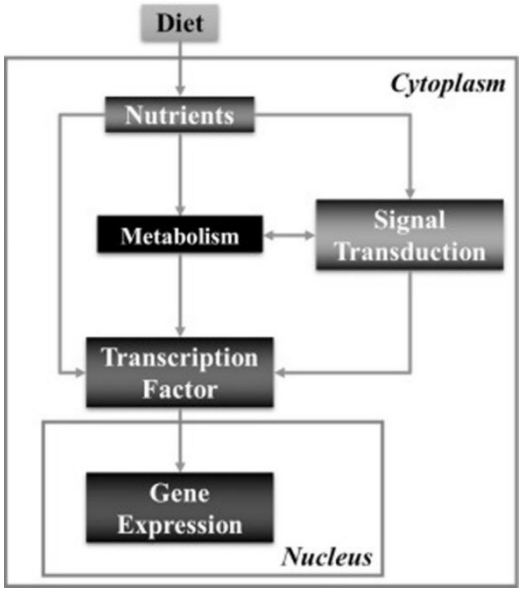


Similarly, increasing energy level during the close-up dry period has shown to enhance the occurrence of metabolic disorders in dairy cows after calving (Vailati-Riboni et al., 2016).

1.3.2. Nutrigenomic approach

Nutrigenomics, as a recent field of research in livestock species (Dawson, 2006, Fekete and Brown, 2007), is the study of the genome-wide influences of nutrition altering the expression of genes (Muller and Kersten, 2003, Kaput and Rodriguez, 2004). In other words, food nutrients (including fatty acids) are able to interact with the genome (not directly but through the intermediate action of transcription regulators) affecting biological functions and having an impact on the entire organism (Bionaz et al., 2015). In detail, transcriptional factors are proteins that bind short DNA sequences (response elements) located in the enhancer regions of genes (Shlyueva et al., 2014), inducing the transcription of genes. For this reason, transcriptional factors play a central key in nutrigenomics (Osorio et al., 2017).

Figure 8. Proposed model for transcription factor activation by nutrients in the cell (adapted from Osorio et al. (2017)).



Studies on lactating cows (Akbar et al., 2013, Hiller et al., 2013) have explicated a tissue-specific response in relation to different kind of FA, with positive nutrigenomic effects for saturated long-chain FA on liver and for unsaturated long-chain FA on adipose (Bionaz et al., 2015).

Several are the researches that have been performed to study how fat supplementation in ruminants could affect metabolism and/or immune response at level tissue, analysing mRNA and/or miRNA of genes involved in lipid and inflammatory pathways.

Schmitt et al. (2011), for instance, focused on the determination of adipose tissue level mRNA expression of some enzymes and transcription regulators, inflammation-related genes and adipokines after long chain FA supplementation, observing an increase adipogenic differentiation in response to SFA.

Few years ago, Bichi et al. (2013) investigated mRNA expression and abundance of key enzymes involved in lipid metabolism in different tissues (mammary, adipose, and hepatic) after feeding marine algae to sheep.

In regards to sheep, Conte et al. (2012) studied the effects of dietary substitution of sunflower oil with linseed oil (a dietary PUFA source) in regulating the SCD expression in skeletal muscle.

A more recent study to evaluate immunometabolic adaptations to lactation in subcutaneous adipose tissue was performed by Vailati-Riboni et al. (2017). They tested how different dietary energy levels during transition period can affect cows' health on metabolic level, measuring mRNA and miRNA expression of targets related to adipogenesis and inflammation.

1.3.2.1. Tissue - level mechanisms investigation – mRNA expression in dairy cattle

At the moment there is a well-established knowledge about dietary fat sources accounting for both metabolic and molecular changes during transition (Bionaz et al., 2015, Osorio et al., 2017), but tissue-level mechanisms are still poorly investigated. Moreover, despite belonging to the same ruminant suborder, some studies outlined as the way small and large ruminants face the transition period can differ (Sauvant et al., 1991, van den Top et al., 1995, D'Ambrosio and A. Agazzi, 2007, Pinotti et al., 2008).

At animal level, adipose tissue is not only a fat storage, but it is an active tissue. Indeed, it regulates animal reserves through the production of adipokines (McGown et al., 2014, Musi and Guardado-Mendoza, 2014) and controls inflammation locally through the recruitment and regulation of the innate immune system (Grant and Dixit, 2015).

The composition of dietary fatty acids, both in humans and animals, can regulate inflammation producing lipid mediators, whose substrates result from membrane phospholipids and depend on dietary fatty acid composition (Calder, 2011, Contreras and Sordillo, 2011, Raphael and Sordillo, 2013). Among FA, n-3 PUFA (EPA and DHA) are the most effective, and their influence on the cell types involved in inflammation and on the production of some chemical mediators has been studied for many years.

Contreras et al. (2012) showed that the exposition of Bovine Aortic Endothelial Cells to a mixture of FAs that reflects the composition of non-esterified fatty acids (NEFA) during the 1st week of lactation determined an increase of pro-inflammatory responses compared with cells exposed to a mixture of FAs enriched in EPA and DHA. Increasing the n-3 FA content of vascular phospholipids could mitigate the expression of cytokines (interleukin-6 and 8) (Fu et al., 2017, Mavangira and Sordillo, 2018), of adhesion molecules (intercellular and vascular

adhesion molecules) associated to an increase of inflammatory response, of reactive oxygen species (ROS), and of pro-inflammatory metabolites of linoleic acid.

Recently, Greco et al. (2015) have proved that reducing the n-6/n-3 FA ratio in the diet of early lactation dairy cows can attenuate inflammatory response to lipopolysaccharide (LPS) challenge. In particular, haptoglobin (*Hp*) was greatest in the mammary gland of cows fed the highest n-6/n-3 ratio (5.9). Moreover, interleukin-6 concentration in plasma increased as the ratio n-6/n-3 FA increased. In fact, it has previously demonstrated that n-6 PUFA can increase the proportions of arachidonic and linoleic acids in membrane phospholipids, escalating the pro-inflammatory response. On the opposite side, n-3 PUFA can increase the proportion of these FA in cell membranes, mitigating the inflammatory responses (Calder, 2012). Then, we can postulate that is the same at animal level (Gifford et al., 2012).

In a study by Agazzi et al. (2004), dietary fish oil fed to transition dairy goats was found to be effective on cell-mediated immune response, with modified mononuclear and polymorphonuclear (PMN) cells ratio as result. Treating cells with DHA (Pisani et al., 2009) exerted an increased PMN leukocytes phagocytic activity and lower Reactive Oxygen Species (ROS) production after in vitro challenged with EPA and DHA.

A subsequent validation in vivo of the obtained results demonstrated that both EPA and DHA have beneficial effects on goat health by improving the defensive performances of neutrophils (Bronzo et al., 2010), avoiding cellular and tissue damages by ROS. EPA and DHA also affected goat monocyte activities by up-regulating phagocytic activity and ROS production (Lecchi et al., 2011) and by interfering with the formation of lipid droplets and by up-regulating proteins belonging to PAT protein family, perilipin family, namely *PLIN1* (perilipin), *PLIN2* (adipophilin) and *PLIN3* (Lecchi et al., 2013).

Another study by Stryker et al. (2013) demonstrated that supplementation of fishmeal to pregnant and lactating ewes could alter both innate and acquired immune response.

Specifically, after a LPS challenge at 135 days of pregnancy ewes fed fishmeal showed an attenuated febrile response compared to soybean meal, and the basal *Hp* concentration was lower after a sensitization with hen egg white lysozyme during lactation.

The central role of mRNA expression in coordinating changes in different metabolic cellular pathways and endocrine functions has been highlighted in recent years, primarily in work with dairy cows (Arner and Kulyte, 2015, Vailati-Riboni et al., 2016).

Quite recently, Rezamand et al. (2016) determined the effects of ALA-enriched rations, adding camelina meal, on gene expression in Holstein dairy cattle. It was displayed both pro- and anti-inflammatory effects of this dietary source ALA-enriched on some of the local and systemic inflammatory markers at transcriptional level. They observed how feeding this dietary source (rich in n-3 FA) could synthesize eicosanoids with anti-inflammatory effects on the bovine immune system at local and/or systemic levels. On the other hand, eicosanoids originated from AA seem to have pro-inflammatory activities.

1.3.2.2. The emerging approach of miRNA in ruminants

In the early 21st century, microRNA (miRNA) has received particular interest from the research.

In molecular biology, miRNAs comprise a novel class of endogenous, small noncoding RNA, about 22 nucleotides in length, able to regulate gene expression, binding, directly or indirectly, to complementary sequences of target mRNA with a decreased mRNA levels (Guo et al., 2010).

It has been shown how they can control posttranscriptional regulation by preventing translation of mRNA (Osorio et al., 2017) and can get involved in diverse biological pathways dealing with development, physiology, and pathophysiology (Zhang, 2008).

One of the initial studies in small ruminants was performed on sheep in order to identify miRNA with a title role in hair growth (Wenguang et al., 2007), with results that highlighted the essential function of miRNA in follicle formation and subsequent future impact on the wool industry.

Among the different involvements, adipose miRNA have been suggested to be implicated in body controlling adipogenesis, regulation of metabolic and endocrine functions (Arner and Kulyte, 2015), adipocyte metabolism (Jin et al., 2009, Jin et al., 2010), and levels of the chemokine *CCL2* (Arner et al., 2012) (miR-92a, miR-126, and miR-193b) in response to administered diet (Romao et al., 2012).

Therefore, alterations of miRNAs affect gene expression and some biological processes, such as inflammation (Sayed and Abdellatif, 2011). Specifically on inflammation, Kloting et al. (2009) explained how miRNA expression patterns are associated with levels of inflammatory molecules such as cytokines and the degree of immune cell infiltration.

Another example is the study performed in 2014 by Wang et al. on goats. Focused on muscle maturation during two developmental stages of skeletal muscles (fetal stage and six month-old stage), this study was performed because limited information about goat muscle miRNAs has been reported. After extensive analysis of pathways and networks, they found two miRNA (miR-424-5p and miR-29a) with relevant regulatory effect on muscle development, elucidating the mosaic network between miRNA and mRNA in goats.

Nevertheless, under investigation was also the mammary gland on goats and sheep (Zidi et al., 2010, Galio et al., 2013).

In the latter study, 47 new ovine miRNA cloned from mammary gland in early pregnancy were object of study, in order to understand the role played by miRNA in the cellular remodeling processes of mammary gland. After the appropriate analysis, they observed that there were three miRNA (miR-21, miR-205 and miR-200) with an active role in the proliferation and maintenance of mammary epithelial cells.

More recently, Vailati-Riboni et al. (2017) used miRNA (in addition to an mRNA-based study) to further understand the adipose responses to the physiological changes induced by the high metabolic demands of early lactation of dairy cows. Data on miRNA function and correlation with immunity and inflammation result from human models but it is also common knowledge that they are conserved among species. For that reason, they took into consideration miRNA with a well-known role in human, speculating the same function in bovine. The obtained results (mRNA and miRNA expression-based) supported the idea of a homeorhetic mechanism of adipose tissue for the adaptation to lactation (because of a cross talk between inflammatory response and immune system), with a modulating role of peripartal nutrition in these mechanisms.

Interestingly, several proteomic studies in mammalian cells showed that miRNA can not only upregulate the translation of target mRNAs (Vasudevan et al., 2007) but also directly interfere with gene transcription (Kim et al., 2008), and their presence is closely linked to the production of several proteins levels of inflammatory molecules, like cytokines (Kloting et al., 2009).

On the whole, collected data on these small noncoding RNA are enhancing, underlining their importance in key physiological processes.

1.4. Human health and PUFA

Newly, the concept of "food" has switched from "source of nutrients for body's needs" to "health promoter" (Zymon et al., 2014), considering it's closely linked to the notion of health (Elsanhoty et al., 2009). This has therefore led to an increasing demand of "functional foods".

It is evident that foods contain biologically active components (Bhat and Bhat, 2011) able to affect some functions in the body, with subsequent positive effects on human health (Bellisle et al., 1998). First among all, fruits and vegetables were included in this category and only in recent times, animal-derived foods have been demonstrated to contain bioactive components (Bauman and Lock, 2006).

We speak of "nutritional safety", meaning the knowledge of how these nutrients can positively affect human health and how it is possible to increase their content in milk, meat and eggs (Cheli and Dell'Orto, 2015).

This new interest is also a consequence of the awareness that recently the westernized diet is characterized by too low a proportion of n-3 fatty acids and too high a content of n-6 fatty acids, with an associated change of n-6:n-3 FA ratio 1:1 to 15:1 (McDaniel et al., 2010).

Notably, particular interest is focused on two members of n-3 long chain polyunsaturated fatty acids, EPA and DHA, which could be provided by either food or could be synthesized in the organism from ALA (Markiewicz-Kęszycka et al., 2013).

Simopoulos (2008) showed in one of his papers that the optimal ratio between these two essential fatty acids is specific to different diseases. Since higher n-6:n-3 ratio is associated with health disorders (such as cardiovascular diseases, arthritis, psoriasis and colitis) (Kearns et al., 1999, McDaniel et al., 2010), the optimal n-6:n-3 ratio should be below 4 (according to the Food and Agriculture Organization – FAO / World Health Organization – WHO).

In particular, it has been determined that dietary saturated fatty acids (specifically C14:0 and C16:0) and trans-fatty acids can increase the incidence of these pathologies (especially when the latter are higher than the former) (Shingfield et al., 2008, Givens, 2010, Shingfield et al., 2013).

Nevertheless, a high amount of SFA is also associated with lowered insulin sensitivity, increasing the risk of metabolic syndrome and diabetes (Funaki, 2009, Kennedy et al., 2009).

On the other hand, an increased amount of omega 3 fatty acids in the diet are responsible for beneficial health effects, being capable of impacting the prevention and treatment of many diseases (Calon and Cole, 2007, Calder, 2012, Calder, 2013, Markiewicz-Kęszycka et al., 2013). For this reason, the European Food Safety Authority Journal (EFSA) recommends that the n-3 FA intake in the human diet has to be between 250 and 500 mg/day (EFSA Panel on Dietetic Products and Allergies, 2012).

These days, there is confusing information from the media about the effects of fat and FAs in dairy products on human health. Rather, we must consider biological and nutritional values of the individual FAs, and this is indeed true for saturated FAs.

Actually, there is an association between their presence and the risk of coronary diseases. Although the fat contained in milk is mostly saturated, some of this has no effect on plasma cholesterol (Bauman and Lock, 2006).

The estimated costs related to cardiovascular diseases in the European Union are estimated at €210 billion per year (Wilkins et al., 2017) and in the American economy more than $316 billion per year (Benjamin et al., 2017), with an even worse expectation in the near future due to longer life and increasing incidence of obesity (Givens, 2010).

The current strategies to improve animal product FA composition, to decrease SFA and increase MUFA and PUFA contents, can be applied in animal nutrition with the additional

positive effects on human diets as well (without any kind of change in consumer's eating habits) (Savoini et al., 2010, Shingfield et al., 2013).

Among all omega 3 PUFA, the essential EPA (C20:5) and DHA (C22:6) have been shown as health beneficial actions (Cattaneo et al., 2006), often with anti-inflammatory properties (Calder, 2001).

For instance, for humans DHA is important for supporting neuronal development in foetal and early infants (Hornstra, 2000) and with a marked role in the process of vision and prevention of inflammation (Markiewicz-Kęszycka et al., 2013). Unfortunately, the endogenous production of this fatty acid from ALA is very poor (Simopoulos, 2000) due to the competition with LA (18:2 n-6) for the same metabolizing enzymes, particularly at the first Δ6-desaturation step (Kuratko et al., 2013).

Long chain n-3 PUFAs are able to modulate immune functions in various ways by replacing, for example, arachidonic acid during the eicosanoid signalling cascade (Calder, 2006), thus decreasing the production of inflammatory eicosanoids such as of PGE2 (Rees et al., 2006), TXB2 (Caughey et al., 1996), LTB4 (Kelley et al., 1999), 5-hydroxyeicosatetraenoic acid (Endres et al., 1989) and LTE4 (von Schacky et al., 1993).

They can even directly interfere with the cytokine gene expression (Weldon et al., 2007) and the production of cytokines themselves (Korver and Klasing, 1997).

Additionally, they can regulate cell surface expression of adhesion molecules (De Caterina and Libby, 1996), membrane fluidity and apoptosis rates (Sweeney et al., 2001). They also have a crucial role as inducer of production of anti-inflammatory mediators, the resolvins (Serhan et al., 2002). Most of these activities directly target leukocyte function (Sijben and Calder, 2007).

Monocytes, as the major effector cells of the immune system, have the main role in the process of the immune response, from the initiation to its outcome. Their defensive activities, as well

as those of macrophages, can be affected by long chain ω-3 fatty acids in several ways. For instance, both EPA and DHA can increase phagocytosis (Halvorsen et al., 1997) and decrease chemotaxis of human monocytes (Schmidt et al., 1992, Schmidt et al., 1996). Cytokine expression can be modulated as well, since both EPA and DHA can down-regulate the in vitro production of IL-1β, IL-6 and TNF-α (Babcock et al., 2002, Zhao et al., 2005, Weldon et al., 2007).

Monocytes/macrophages, together with neutrophils, produce high amounts of reactive oxygen species (ROS) (involved in oxidative stress), commonly found in many disease processes and responsible for the killing of microorganisms. If this production is excessive, like in chronic inflammatory or immune diseases, ROS could damage cellular lipids, proteins and DNA, eventually impairing cell function. Despite the fact that it seems that long chain PUFA are able to modulate cellular production of ROS in macrophages (Yaqoob and Calder, 1995), neutrophils (Healy et al., 2003) and lymphocytes (Cury-Boaventura et al., 2005), their activity is still debated. Indeed, some authors found an increase while others, on the other hand, found a decrease in ROS production (Martins de Lima et al., 2006).

One more major homeostatic mechanism to control the activity of monocytes is the regulation of leukocyte apoptosis, decreasing or enhancing their lifespan during inflammation, eventually resolving the inflammatory process. There is increasing evidence that PUFAs can cause cell death in normal and cancer cells (Heimli et al., 2002, Sweeney et al., 2007). Even though it was evident that macrophages (Martins de Lima et al., 2006), neutrophils (Healy et al., 2003) and leukaemia cells (Cury-Boaventura et al., 2005) displayed morphological features of apoptosis and necrosis with PUFAs at high doses, the mechanisms by which these FA can cause it are still debated.

Unfortunately the content of EPA and DHA is minimal in ruminant diets and so milk has a very low amount of them (<0.1% of total fatty acids) (Lock and Bauman, 2004). Nevertheless, an improvement in their content in animal diets could lead to a higher available content of these fatty acids in milk for human consumption.

Fish oil, amongst the dietary sources of these essential fatty acids, seems to be the most promising one because of its high amount of EPA and DHA. The use of fish oil, however, is a costly supplementation of lipids compared to other more conventional fatty acid sources and there is increasing concern on the sustainability of the use of fish products in animal diet (Botsford et al., 1997), which leads to the consideration of alternative sources of long-chain n-3 PUFAs (Matthews et al., 2000).

One of these could be a source of ALA as a precursor for EPA and DHA, like linseed. Unfortunately, it presents two main disadvantages:

- a low digestibility for digestive enzymes due to the seed coat that can be usually solved with treatments like extrusion and expansion;
- the presence of anti-nutritional factors, which however can be destroyed by thermo-extrusion to some extent (Kouba and Mourot, 2011).

Alongside linseed, algae have high content of PUFAs and can be considered as an alternative to fish oil (Woods and Fearon, 2009). Among the numerous advantages there are the relatively low cost and the high growth rate. In addition, these compounds were found to have some interesting bioactive components such as antioxidants, sulfated polysaccharides, phlorotannins, diterpenes, minerals and vitamins, high protein content, valuable nutrients (Kouřimská et al., 2014, Maghin et al., 2014). Thus, the usage of algae as a dietary additive in livestock diets could be worthwhile (Rasoul-Amini et al., 2009), especially with the aim of decreasing SFAs content

through an increased content of conjugated linoleic acid (CLA) and other PUFAs in the ruminant's milk (Papadopoulos et al., 2002).

The content of PUFA in algae is higher than in terrestrial plants (Balboa et al., 2013) in spite of diversities in terms of fatty-acids composition among species and intra-species, depending on environmental and geographical factors (Li et al., 2002, Narayan et al., 2008), season (Terasaki et al., 2009), and algal parts (Khotimchenko and Kulikova, 2000).

The use of algal source as PUFA enrichment is a very new topic and not so common in dairy goat nutrition, but interest is continuously increasing because of several health benefits found in other species through their prebiotic functions, antimicrobial activities, improved digestibility, antioxidant role and anti-inflammatory and immunomodulatory properties (Maghin et al., 2014).

Another alternative could be *Camelina sativa*, also known as false flax. It is an oilseed crop of the Brassica (Cruciferae) family, whose oil is rich in omega-3 FA, specifically ALA and LA (Acamovic et al., 1999, Kakani et al., 2012). Native to Northern Europe and Central Asia, it has been used for over 2000 years for oil and livestock fodder. The interest as a biofuel source has enhanced its production, and consequently, its by-products (such as meal/cake) started to be considered as a possible feed source (Cherian, 2012, Meadus et al., 2014).

1.4.1. Dietary manipulation of milk fatty acid composition

Hippocrates stated that milk is 'nature's most perfect and complete food'. In actuality, it is a complete food, rich in bioactive components, able to prevent, and sometimes cure different diseases of modern civilization (Akalin et al., 2006, Lovegrove and Givens, 2016).

Lipids in milk are mainly triglycerides (98%) and the remaining part is composed by diacylglycerol, cholesterol, phospholipids, free fatty acids and traces of ether lipids.

Furthermore, milk, alongside dairy products, is the main source of C12:0 and C14:0 in the human diet, contributing also to the consumption of C16:0 and trans fatty acids in industrialized countries (Givens and Shingfield, 2006).

Notably, dairy milk fat contains between 60% and 70% SFA, 20% and 35% MUFA and trace amounts of PUFA depending on the animal species. In cow, goat and sheep the amount of PUFA is around 3-4% (Devle et al., 2009, Mayer and Fiechter, 2012), and the most representative are LA (C18:2 n-6) and ALA (C18:3 n-3). Milk of sheep and goat usually has a smaller n-6:n-3 ratio and greater concentration of ALA compared to cow's milk (Markiewicz-Kęszycka et al., 2013).

Since desaturation of FA does not occur at position Δ9 (Cook and McMaster, 2002), n-3 long chain PUFA cannot be synthesized, and are thus considered essential. Besides that, the conversion of ALA into EPA and DHA is restricted due to metabolic factors, owed to the excessive dietary intake of n-6 FA (in particular of C18:2 n-6) (Zymon et al., 2014).

Since fat content is the most sensitive and responsive component of milk to dietary changes (Jenkins and McGuire, 2006), the interest in manipulating milk fat content started more than two decades ago and the interest in reducing total fat content ha continued. Thus, applying different strategies is possible to enhance milk fat composition, (for instance with dietary addition of PUFA, like EPA and DHA). Making this, we should take into account the ruminal biohydrogenation, low intestinal digestibility and transportation to the mammary gland that mitigate the transfer rate of these FA into milk (Jenkins and McGuire, 2006, Zymon et al., 2014).

Milk fat composition is the outcome of both tissue fatty acid biosynthesis and fatty acid composition of the diet (Mansson, 2008). In monogastrics there is linearity between dietary fatty acid profile and milk fatty acid content, instead, in ruminants the ruminal

biohydrogenation process of dietary fatty acids determines fatty acid absorption (Jenkins, 1993, Doreau and Chilliard, 1997, Woods and Fearon, 2009).

In terms of FA synthesis and secretion of milk in ruminants, the mammary gland requires FA from two sources, uptake from peripheral circulation and synthesis in the mammary gland (*de novo* synthesis) (Shingfield et al., 2010).

De novo synthesis deals with the production of the 4:0-14:0 acids and about 50% of 16:0, starting with acetate and β-hidroxybutyrate produced during ruminal fermentation. Mammary desaturase enzymes can further modify FA with length between 14 and 18 carbons. The remaining C16:0 and almost all long-chain FA come from lipids in blood, after absorption in the small intestine or mobilization of adipose tissue (Nudda et al., 2014). They are derived from TAG fractions of VLDL and chylomicrons or albumin bound NEFA in plasma. Lipoprotein lipase (LPL) is responsible for importing fatty acids into mammary cell, anchoring VLDL and chilomicrons to mammary epithelium and subsequently acting on TAG (contained in the lipoprotein core) to release NEFA (Shingfield et al., 2010). In addition, the lipids from the membrane of bacteria are also a fat source (Vlaeminck et al., 2006).

Diverse studies about dietary fat supplementation in ruminants have been conducted, with the addition of different lipid sources, such as fish oil and marine algae to improve EPA and DHA content in milk (Loor et al., 2005, Cattaneo et al., 2006, Boeckaert et al., 2008, Mele et al., 2008, Toral et al., 2010, Shingfield et al., 2013, Toral et al., 2014, Bernard et al., 2015, Cappucci et al., 2018).

The supplementation of fish oil in dairy cows proves to be toxic on ruminal microorganisms, leading to a reduction in terms of milk fat content and conferring off-flavors due to FA oxidation (Lock and Bauman, 2004). This phenomenon is known as "Milk Fat Depression"

(MFD) and it can occur in case of low fiber content and high concentrations in the diet (Bauman and Griinari, 2003). In contrast to the bovine case, the occurrence of MFD is not common in small ruminants, even when they are fed diets rich in starch and containing vegetable or marine oils (Shingfield et al., 2010). One possible mechanism to explain what happens in cows could be the inhibition of lipid synthesis in the mammary gland in response to fatty acid intermediates (such as trans-C18:1) and related metabolites, formed during partial biohydrogenation of unsaturated fatty acids in the rumen (Bauman and Griinari, 2001, Toral et al., 2010).

Dealing with goat, data about milk yield and composition when adding n-3 PUFAs in the diet, often produced contradictory results. No effects on milk production when feeding fish oil but a decrease in terms of milk fat content were found in Cattaneo et al., (2006), El-Nor and Khattab (2012), and Toral et al. (2014). Differently, a study on sheep (Kitessa et al., 2001) showed a significant milk yield decrease without any change in milk fat content after tuna oil supplementation. To the author's knowledge scarce literature is available on the effect of dietary algae on production and quality of goat milk (Kouřimská et al., 2014), but typically no differences in milk yield and a significant decrease in milk fat content in cow (Franklin et al., 1999) and sheep (Papadopoulos et al., 2002, Toral et al., 2010) were observed.

Recently, Meignan et al. (2017) analysed milk fatty acid profiles of feeding dairy cows extruded with linseed. After administrating this source rich in ALA, they observed that SFA decreased linearly, whereas oleic acid, vaccenic acid, rumenic acid, α-linolenic acid, and the sums of mono- and polyunsaturated FA increased linearly when the daily intake of fat from EL was increased. Therefore, interestingly the addition of this vegetable source increased the proportion of human health-beneficial FA in milk.

Another example of enriching animal diets with fat is the dietary addition of both fish oil and algae in ruminants that is reported to strongly affect the fatty acid profile of milk, with a decrease in SFAs and with increased PUFAs content. In detail, unprotected fish oil was able to influence the milk fatty acid profile of goats, characterized by a lower average concentration of C18:0 and higher content of C16:1, C18:3, and very long-chain n-3 PUFAs EPA and DHA (Cattaneo et al., 2006).

The administration of algae in the goat diet are reported to decrease palmitic acid content in milk with increased levels of oleic, linoleic and linolenic acid. Furthermore, a positive change in SFA, MUFA, PUFA was observed, with a reduced proportion of SFA and a tendency to a positive shift in n-6:n-3 PUFA, with increasing proportion of n-3 (Kouřimská et al., 2014).

The observed augment in long chain PUFA content in milk (Papadopoulos et al., 2002, Toral et al., 2010) of dairy sheep when fed algae reflected a higher content of these fatty acids, in particular EPA and DHA (according with what previously observed in cow) (Franklin et al., 1999). The observed differences in transfer efficiencies could be explained not only by diversities of dietary sources, but also by dosage applied, duration of administration, and level/technological methods for rumen protection of the dietary fatty acid sources.

Particularly to this last point, the transfer efficiency of EPA and DHA from diet into milk is already generally limited without ruminal protection due to ruminal biohydrogenation together with the preferential incorporation into plasma phospholipids and cholesterol esters (Kitessa et al., 2001).

So, the possibility to introduce "inert" fats in the diet (rather than the native forms of supplementation) to escape or bypass fat rumen biohydrogenation is more advisable. As matter of fact, if protected, fatty acids can be preserved from oxidation, reducing the negative effects

of high levels of unprotected dietary fats on feed intake, ruminal fermentation, and milk fat content (Jenkins and Bridges, 2007).

Mentioning few examples of supplementation, first we can consider algae. Franklin et al. (1999) found a DHA transfer efficiency in dairy cow of 8.4% when using an unprotected form of algae, and 16.7% when a rumen-protected form was fed.

Similarly, the DHA transfer rate into sheep' milk from unprotected algae compound was between 5.0% and 8.0% (Toral et al., 2010), while 30% to 60% of dietary EPA was incorporated (Papadopoulos et al., 2002).

Instead, Cattaneo et al. (2010) tested a rumen-unprotected form of dietary fish oil in goats, observing that the apparent transfer efficiency of EPA and DHA from fish oil to goat milk ranged from 7% to 14% and 7% to 8%, respectively.

Then, few years ago, Doreau and Ferlay (2015) showed the possibility to take advantage of the natural constitution of linseeds, thanks to partially protected lipids of whole seeds. Another way to protect feeds could be heating feeds at high temperature, using calcium salts of FAs or encapsulating the lipids in a matrix of rumen-inert protein (Jenkins and Bridges, 2007, Palmquist, 2009).

The selection of the best n-3 PUFA sources has to take account not only the transfer rate into animal products but also the economic aspect, environmental and ethical sustainability of the source. Zymon et al. (2014) discussed it in one of their papers, where they considered fish oil as a non-ethical PUFA source, even if fish oil can be obtained from farmed fish.

Thereby, it is critical to take into consideration alternative sources of n-3 PUFA, for example the natural and sustainable green pasture. Indeed, pasture has been proved to enrich milk fat in ALA, *cis*-9 *trans*-11 CLA and its precursor *trans*-11 C18:1 (vaccenic acid (VA) (Dewhurst et al., 2006, Coppa et al., 2011, Shingfield et al., 2013). Nonetheless, milk of pasture-fed dairy cows

contains higher levels of EPA and DHA (Hebeisen et al., 1993, Leiber et al., 2005, La Terra et al., 2010).

Increasing n-3 fatty acid content of milk may benefit human health but, at the same time, we should consider that it is more vulnerable to oxidation.

In this context, increasing milk vitamin E content may represent an advantageous tool to protect lipids from peroxidation, maintaining milk nutritional and organoleptic quality (Vagni et al., 2011). Other antioxidants that can be included in the diet for this aim are plant extracts rich in polyphenols (Gladine et al., 2007, Gobert et al., 2009), or superoxide dismutase, catalase and glutathione peroxidase (Marchi et al., 2015).

1.4.2. Dietary manipulation of egg quality

Characterized by digestible proteins, lipids, vitamins, minerals and molecules that promote health (Anton et al., 2006), as the other poultry products, eggs are the major source of animal protein around the world (Cherian, 2012). Therefore, particular interest is turned to ameliorate fatty acid profile of this animal product so widely consumed. Previous studies have demonstrated how their fatty acid composition can be modified by manipulating the poultry diet. Specifically, it has been shown how adding ω-3 rich meals can increase ω-3 fatty acid content of eggs (Kakani et al., 2012).

Although the positive effects of PUFA, their sources are not in sufficient quantity for the food industry and the research is looking for novel n-3 feedstuffs with nutritional and healthy effects (Taranu et al., 2014).

Numerous studies have been published on hen supplementation with either oilseeds and fish oil and recently researchers are exploring the possible addition of microalgae into the poultry diet (Fraeye et al., 2012).

Well known sources and widely used as hen dietary supplementations over the past 20 years are flaxseed, canola and other plants. These plants are indicated as an excellent source of ALA (Harris et al., 2009). Data on production parameters and egg characteristics of hens fed flaxseed are contradictory (Bean and Leeson, 2003, Hayat et al., 2009, Rizzi et al., 2009). Since many factors are involved in the determination of these parameters, this could explained the diversities in these findings (Fraeye et al., 2012).

Then, dealing with fatty acid composition, the general outcome shows an increase of ALA and EPA levels in yolk. DHA increases as well but with a lesser extent. This can indicate, as many authors report, the limit in the conversion of ALA in DHA and EPA, similar as in humans (Aymond and Van Elswyk, 1995).

Interestingly, alongside with enhancement of n-3 FA, they observed a parallel decrease of n-6 FA levels (Bean and Leeson, 2003, Hayat et al., 2009), especially arachidonic acid (Cherian and Quezada, 2016).

Since the conversion of ALA into EPA and DHA is inefficient, direct supplementation with a dietary source rich in these essential fatty acids, such as fish oil, could be worthwhile.

Similar to what found for studies about egg-enriched with plants, the supplementation of fish oil produced contradicting results about production parameters and egg quality characteristics (Gonzalez-Esquerra and Leeson, 2000, Cachaldora et al., 2008, Lawlor et al., 2010).

Referring then to the yolk composition after dietary supplementation, there is a recurrent component among the studies. Indeed, although fish oil is abundant in EPA and DHA, eggs have been found to be enriched mostly with DHA, while the increase of EPA content is limited (Bovet et al., 2007, Lawlor et al., 2010). Herber and Van Elswyk (1996) suggested the preference for DHA to be incorporated into membranes in comparison with EPA.

As we discussed about the use of plants, also the supplementation of this marine source has determined an increase of n-3 PUFA as well as a decrease in AA and total n-6 PUFA (Cachaldora et al., 2008, Poureslami et al., 2012).

Likewise to linseed and fish oil, discrepancies are found among the different performed trials feeding microalgae (Cachaldora et al., 2008, Rizzi et al., 2009). Similarly, to eggs from hens fed fish oil, DHA strongly increases and n-6 PUFA decrease (Rizzi et al., 2009, Baeza et al., 2015).

We should remember that feed represents the major cost for food animal production, and FA sources for animal products should not be in competition with human health-food uses. For this reason, the quest for sources of n-3 FA feeds is focused on looking for alternative feeding font that can reduce production costs and guarantee enriched food for human consumption (Cherian et al., 2009, Cherian, 2012, Cherian and Quezada, 2016).

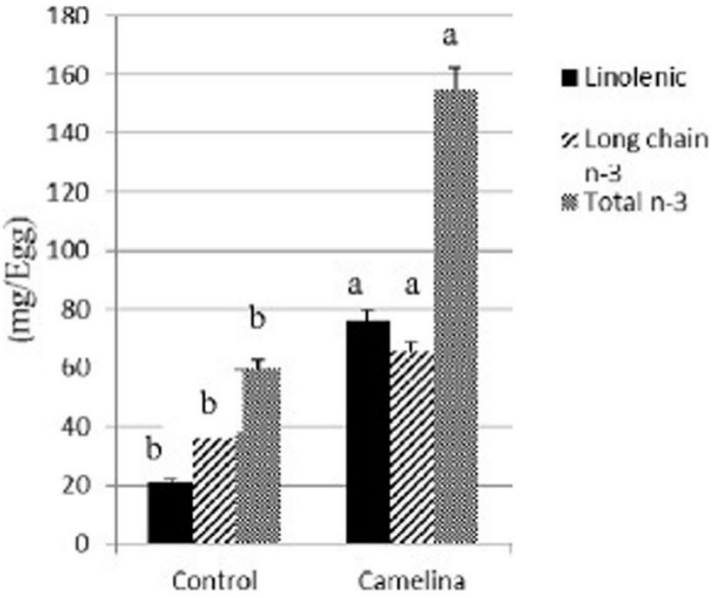
A possible option can be *Camelina sativa*, characterized by the same composition of flaxseed, high levels of ω3/ω-6 fatty acids protein, and ϒ-tocopherol (Zubr and Matthaus, 2002, Cherian, 2012).

Camelina is an oilseed crop of the Brassica (Cruciferae) family. The cultivation of the crop is environmentally friendly because it does not need application of pesticides/herbicides (Zubr, 2003). Cultivated in Europe for over 2000 years for oil and livestock fodder, there is a renew interest about its use as a biofuel source (Cherian, 2012). This increase production has led to exploit the meal produced from the oil extraction for poultry industry, in order not to compete with the human diet (Kakani et al., 2012). Camelina by-products (like meal/oil cake) are rich in protein, essential amino acids, fat and essential ω-3 and 6 fatty acids. For this reason, it could be fed in the diet of animals (Meadus et al., 2014).

As being monogastric, the quantity and the type of dietary lipids can affect the chemical characteristics of the egg yolk, modifying the content of fatty acids, and fat-soluble vitamins. As an outcome, egg lipids reflect dietary fat content in chickens (Cherian, 2012). Thanks to their high capacity for lipid biosynthesis, the alteration of fatty acid composition can be direct or indirect due to further acyl chain elongation and desaturation in the liver (Cherian et al., 1996).

Recent researches (Aziza et al., 2013, Cherian and Quezada, 2016) conducted on feeding camelina meal in egg layers have demonstrated that an inclusion up to 10% increases the omega-3 fatty acid content, not compromising bird performances. It has been proved that consumption of two eggs from hens fed with the inclusion of camelina meal could provide more than 300 mg/day of n-3 fatty acids to the human diet, which is the recommended daily intake of World Health Organization (WHO). Unfortunately, the information about administering camelina meal to laying hens and about egg lipid composition are scarce (Cherian et al., 2009).

Figure 9. a-Linolenic acid, long chain n-3 fatty acid and total n-3 fatty acid supplied through eggs from layer hens fed Control or Camelina. [a-b]Means between diets and within a fatty acid type without a common letter differ significantly (P < 0.05). Control and Camelina represent corn-soybean meal basal diet (Control); or basal diets containing camelina (Camelina) at 10 % (adapted from Cherian and Quezada (2016)).



Other interesting obtained results have shown how feeding *Camelina sativa* does not affect the functional properties and the sensory quality of eggs (Rokka et al., 2002). Rokka et al. (2002) fed camelina at a level of 5% in laying hens, observing that functional properties and quality of the eggs were not negatively affected by the concentration of seed oil. In this fashion, they have demonstrated that camelina did not impart the same off flavor and odor as flax meal and oil. Later, Kakani et al. (2012) tested extruded camelina meal in Lohmann White Leghorn hens with 3 different inclusions (0 -5 -10%), finding out that the addition of the meal up to 10% of concentration did not have effects on interior and sensory quality of eggs.

These outcomes suggest that *Camelina sativa* is a promising plant source for the enrichment of omega-3 fatty acids in hen eggs, but further investigations are needed.

Moreover, the inclusion of camelina has been proved to be beneficial because of the reduction of oxidative stress due to the presence of these bio-active compounds. This was observed, measuring the thiobarbituric reactive substances (TBARS) in eggs from hens fed <10% camelina meal (Cherian, 2012). Very few studies have reported sensory aspect of eggs from hens fed camelina.

1.4.3. Dietary manipulation of meat quality

Apart from supplying high biological value proteins, essential trace minerals, B-vitamins and micronutrients, meat is a valuable source of long-chain n-3 FA (Higgs, 2000).

The nutritional properties of meat are related to its fat content and the composition of FA contained. Indeed meat is the major source of fat in human diet, especially SFA that are related to the major incidence of chronic diseases (Wood et al., 2004). However, some MUFA and PUFA have positive effects on human health, so it is advisable the presence of these healthy fatty acids in meat (Nieto and Ros, 2012).

At standard conditions, the content of PUFA in the muscular tissue is different if we take into consideration different animals. Specifically, comparing their content among beef, lamb and pork shows some disparities. The most notable diversity is the higher quantity of linoleic acid in pork than in ruminants and it derives from the diet. Indeed, being monogastric, this fatty acid is unchanged by the stomach activity, it is absorbed in the small intestine, and, through blood stream, it reaches tissues unmodified (Harrison and Leat, 1975). Consequently, this incorporation into pig muscle fatty acids leads to a higher n-6:n-3 ratio, which is not desirable for human health (Nieto and Ros, 2012).

The situation is dissimilar for beef and lamb, containing a lower amount of C18:2 because, belonging to ruminant species, it is degraded into MUFA and SFA after ruminal microbial

biohydrogenation and, for a small amount, is incorporated into tissue lipids (Doreau and Ferlay, 1994). This fatty acid, along with linolenic acid, determines also the content of long chain PUFA (such as arachidonic acid and eicosapentaenoic acid) by the action of Δ5 and Δ6 desaturase and elongase enzymes (Nagy and Tiuca, 2017). Ruminal microorganisms also act on dietary PUFA, with the resulting conversion of these into SFA and their deposition in muscle tissue. This means that the PUFA:SFA ratio in lamb and beef is low, with higher risks of cardiovascular problems and other diseases (Kaić et al., 2016).

The rising interest of the consumers in health food and their opposition to the incorporation of synthetic additives lead to a growing application of dietary supplementation to modify fatty acid composition of meat, increasing the degree of unsaturation (Nieto and Ros, 2012). It is important to remember that fatty acid composition is more susceptible to manipulation than other components contained in meat, and that dietary fatty acid supply is the main factor determing the fatty acid composition of intramuscular fat and adipose tissue (De Smet and Vossen, 2016).

In order to do it, we should consider the digestion of nutrients in the animals. Specifically, in monogastrics, like in pigs and chickens, anaerobic microorganisms can hydrogenate UFA, forming as intermediates trans FA and conjugated fatty acids. The scenario is different in ruminants, where these FA are largely absorbed and the only way to avoid it is protecting them against hydrogenation in the rumen (Drackley, 2007).

Similar responses to the dietary supplementation were observed in four categories of animals (cattle, sheep, pig and poultry), with better results on the deposition of PUFA in poultry trials. The possible differences among the trials performed until now are imputable to diverse experimental design and kind of n-3 PUFA supply. Despite it, it is clear that is possible to

increase PUFA content in meat feeding alternative dietary fat sources (De Smet and Vossen, 2016).

Different are the dietary strategies applied over the last decades.

Diaz et al. (2011) used three diverse supplementations (fish oil, linseed and linseed plus microalgae) on lambs. Results showed how linseed favourably influenced the content of linolenic acid, meanwhile animals fed fish oil had the highest long-chain PUFA content. Interestingly, in both these two experimental groups they also found the highest PUFA/SFA ratio.

An example on chickens is performed by Nain et a. (2015). They supplied 4 different inclusions of *Camelina sativa* meal to 744 Ross 308 (0, 8, 16 or 24%) for 42 days to evaluate any effect on FA composition of some organs. Analysing the fatty acid content, they observed an enhanced of the overall n-3 PUFA concentration (especially ALA, DPA, and DHA) with greater α-linolenic acid content. In detail, increasing dietary camelina inclusion, the predominant n-3 PUFA were DPA and DHA in liver and brain tissue; then, in breast and thigh tissue DPA as well. These findings indicate that camelina can be potentially included in broilers rations in order to improve fatty acid profile of the meat.

Another group of research before them (Jaśkiewicz et al., 2014), tested the same FA source but in the form of oil. They fed 90 Ross 308 broiler chickens with camelina oil to compare its possible effects with two other supplementations, soybean and rapeseed oil. As in the trial before, they noticed a rise of α-linolenic content in the muscle and abdominal fat, in particular feeding camelina.

Recently Cherian's group of research (Moghadam and Cherian, 2017) remarked how feeding 10% flaxseed to broilers could lead to n-3 FA enrichment in both white and dark meat. With these results, they stated, as they did before in their previous published papers, that is possible

to incorporate n-3 FA in poultry food products and this may be an alternative strategy to increase n-3 FA consumption without changing existing dietary habits.

Lately in France (de Tonnac and Mourot, 2017) was performed 60 male pigs were assigned to five experimental groups with different n-3 PUFA sources to evaluate, among the diverse parameters, the fatty acid composition of pork. Dietary lipid supplements were composed of soybean and palm oil, de-hulled and extruded linseed, docosahexaenoic acid (DHA)-rich microalgae or a mixture of linseed and microalgae at 75%/25% and 50%/50%, respectively. They noticed that the n-3 PUFA content in the longissimus dorsi muscle, subcutaneous backfat and liver increased with a dietary supply of linseed and microalgae and corresponded to circulating fatty acids, indicating benefits of enriching animal feed with essential FA.

2. OBJECTIVES

Polyunsaturated fatty acids have been shown to work on different levels. On the one hand, they can modulate animal performance and health status, affecting the immune response. Furthermore, they have effects on the quality of animal products, enriching them with essential fatty acids and with consequent beneficial effects on human health.

The objective of this thesis was to evaluate the possible effects of fatty acid addition to animals on both health animal level and product quality level. To achieve this object, two different trials were designed. In the first one, the aim was to study the consequences of dietary fat supplementation (saturated and unsaturated fatty acids) around kidding in dairy goats on metabolism and immune response at hepatic and adipose tissue level through changes in the expression patters of both mRNA and miRNA of genes involved lipid metabolism and inflammation processes. In the second one, we evaluated the inclusion of 7.5% of camelina cake in the diet of laying hens on performance, egg quality characteristics, fatty acid composition and lipid oxidative stability.

3. LIVER AND ADIPOSE TISSUE METABOLISM- AND INFLAMMATION-RELATED mRNA AND microRNA EXPRESSION IN PERIPARTURIENT DAIRY GOATS FED POLYUNSATURATED vs. SATURATED FATTY ACIDS

3.1. Abstract

The transition into lactation is an important period to increase productive performance of dairy ruminants by supplementing fat in the diet. When compared to saturated fatty acids, polyunsaturated n-3 and n-6 fatty acids exert beneficial effects on immune response. The present study focused on the expression of selected genes and miRNA in the liver and adipose tissue of goats during transition. The major aim was to improve the knowledge of transcriptional responses in these tissues in the context of lipid metabolism and inflammation when goat are supplemented with saturated or polyunsaturated fatty acids around kidding. Twenty-three second-parity twin-diagnosed alpine dairy goats were either fed from one week before (30 g/head/d fatty acids) to three weeks after kidding (50 g/head/d fatty acids) calcium stearate (ST, n= 7) or fish oil (FO, n= 8), and were compared to a control group fed a basal diet (CON, n= 8). Liver and adipose biopsies were collected on day -7, 7 and 21 from kidding. Quantitative PCR was used to measure mRNA and microRNA expression of 38 targets. Dietary treatments up-regulated (ST) and down-regulated (FO) stearoyl-CoA desaturase (*SCD*) alone. Time around kidding strongly affected most of the target genes for lipid metabolism, being mostly up-regulated from -7 to 7 DIM and down-regulated in the third week of lactation in the liver, while in adipose tissue a gradual down-regulation was observed throughout the trial. Acute-phase proteins were increased in adipose tissue in the two weeks around kidding

and decreased from 7 to 21 DIM. Pro-inflammatory interleukins (*IL6* and *IL8*) peaked in the first week after parturition, while *IL10* was up-regulated in the last two weeks of the trial. With the exception of an up-regulation of miR-155 from -7 to 7 d from kidding, no effects of time were observed on miRNA expression in adipose tissue. Liver *ACOX1* was greater in ST than CON on day 7 while *SCD* showed a higher expression from 7 to 21 DIM in ST compared to FO. Then, dealing with adipose tissue, *LPIN1* had increase values in FO at 7 days after kidding (compared to ST) and *FASN* had the highest expression in stearate group 7 days before parturition, if compared to CON and FO. The present study revealed modest effects of dietary saturated or polyunsaturated supply around kidding on fatty acid metabolism- and inflammation-related genes in liver and adipose tissue of dairy goats. However, data indicated an increase in β-oxidation in the liver, and a decrease in fatty acid synthesis and inflammation in adipose tissue.

3.2. Introduction

The transition period is considered the most appropriate phase to increase productive performance of dairy animals (Bronzo et al., 2010), namely because of the marked decreased in dry matter intake (DMI) and negative energy balance (NEB) particularly after calving.

Changes in endocrine status to accommodate parturition and lactogenesis lead to modification of the animal's metabolism (Vailati Riboni et al., 2015). As a result, there is an increase in lipomobilization, which enhances the concentration of plasma non-esterified fatty acids (NEFA) and their transport to the liver where they can be oxidized to generate energy, ketone bodies, or esterified to triacylglycerol (TAG) followed by synthesis of very low-density lipoproteins (Invernizzi et al., 2016). Besides serving as an energy-storage organ, adipose tissue

can produce adipokines and is subject to localized inflammation through the recruitment and regulation of immune cells (Grant and Dixit, 2015).

Evidence from dairy cow studies underscores the usefulness of supplemental fat administration during transition (Staples et al., 1998, Ballou et al., 2009). Specifically, dietary fats can provide a source of energy or act as modulators of metabolic and immune pathways (Tsiplakou and Zervas, 2013b, a). A link may exist between metabolic and immune pathways during period with deficient metabolism, like during periparturient period, enhancing the incidence of disorders (Sordillo and Mavangira, 2014).

Increased supply of energy across the transition period leads to improved health status and ameliorates some aspects of the immune response especially when polyunsaturated n-3 and n-6 omega fatty acids are added in the diet (Thering et al., 2009, Agazzi et al., 2010, Sordillo, 2016). Conversely, increasing energy density by feeding readily-fermentable carbohydrate during the close-up dry period often increases the incidence of metabolic disorders in dairy cows after calving (Khan et al., 2014).

Recent work in dairy cows has highlighted a central role of mRNA and miRNA expression in coordinating changes in different metabolic cellular pathways (Arner and Kulyte, 2015, Vailati-Riboni et al., 2016). Adipose tissue microRNA (miRNA) have a biologic impact on both adipocyte metabolism (Jin et al., 2009, Jin et al., 2010) and inflammation, regulating gene expression through complex networks involving transcription factors (Arner and Kulyte, 2015). Expression patterns of miRNA are associated with the levels of cytokines (Arner et al., 2012) and the degree of immune cell infiltration (Kloting et al., 2009, Vailati-Riboni et al., 2017).

Although the potential of dietary fat sources to elicit changes in metabolic and molecular pathways in liver and adipose tissue of transition dairy cows has been recently highlighted

(Bionaz et al., 2015), a similar role in dairy goat or sheep around kidding is largely unknown (Osorio et al., 2017). Despite the well-established differences in which small and large ruminants navigate the transition period (Sauvant et al., 1991, van den Top et al., 1995, Pinotti et al., 2008), dairy goats (similar to cows (Farney et al., 2013, Vailati Riboni et al., 2015)) experience some degree of inflammation around kidding (Bronzo et al., 2010) as a homeorhetic adaptation to lactation.

Our hypothesis was that dietary fat supplementation around kidding in dairy goats could positively affect metabolism and immune response at the hepatic and adipose tissue level through changes in the expression patterns of both mRNA and miRNA of genes involved in lipid metabolism and inflammation. The aim was to study transcriptional responses in liver and adipose tissue in the context of lipid metabolism and inflammation when goats were supplemented with saturated or polyunsaturated fatty acids around kidding.

3.3. Materials and Methods

3.3.1. Animal management and diets

The protocol for care, handling, and sampling of animals was reviewed and approved by the Università degli Studi di Milano Animal Care and Use Committee (attachment n. 5 January 26th, 2011). The trial was performed at the Centro Clinico-Veterinario e Zootecnico-Sperimentale d'Ateneo di Lodi, Università degli Studi di Milano. Complete details of the experimental design have been published- Invernizzi et al. (2016). Briefly, twenty-three second-parity twin-diagnosed spring kidding Alpine dairy goats were divided in a randomized complete block design into three homogenous groups balanced for parity, age and milk production in the previous lactation (1.26 ± 0.45; 28.05 ± 6.15 months of age, 3.12 ± 0.33 kg/d, respectively). Goats were housed in individual boxes and fed ad libitum a pre-kidding or

a post-kidding basal diet (CON; n.8) with the inclusion of either fish oil (FO; n.8) or calcium stearate (ST; n.7) (Table 1). The pre-kidding basal diet consisted of ad libitum mixed grass hay, corn meal and concentrate, while post-kidding diet was composed of ad libitum alfalfa hay and mixed hay corn meal and concentrate. Forages were individually adjusted daily for a refusal weight of at least 10%, while concentrates were provided separately. Rumen-inert fish oil (EE 4.9% DM pre-kidding; 5.2% DM post kidding - 10.4% EPA and 7.8% DHA; Ufac Ltd., Stretton, UK) was included in the basal diet at a rate of 81 g/day of supplement (30 g/day of fatty acids) before kidding and 135 g/day of supplement (50 g/day of fatty acids) after kidding. Calcium stearate (EE 4.6% of DM pre-kidding; 4.8% of DM post kidding - C16:0 26% and 69.4% C18:0; Brenntag S.p.a., Milan, Italy) was included in the basal diet at a rate of 34 g/day of supplement before kidding and 56 g/day of supplement after kidding to ensure the same total amount of fatty acids supplemented in FO.

Calcium carbonate was included in experimental diets to balance calcium content both in the pre- (0.8% DM feed) and post kidding (1.1% DM feed) periods (Invernizzi et al., 2016). All diets were supplemented with vitamin E in order to supply 72 mg/head/d during the pre-kidding period and 80 mg/head/d after kidding. Pre and post kidding dietary treatments were designed to provide similar crude protein (CP) and calcium content, while fat enriched treatments (FO and ST) had similar ether extract (EE).

Table 1. Ingredients (g/100g feed as-fed) and nutrient composition (% of DM unless otherwise noted) of the experimental diets.

Item	Treatment[1]					
	CON		FO		ST	
	Pre-kidding	Post-kidding	Pre-kidding	Post-kidding	Pre-kidding	Post-kidding
Ingredient composition						
Alfalfa hay	0	31.2	0	29.8	0	30.7
Mixed hay[2]	62.3	15.3	59.6	14.6	61.4	15.1
Concentrate[3]	31.9	46.8	30.5	44.8	31.4	46.2
Steam-flaked Corn	5.3	6.2	5.0	5.9	5.2	6.2
Fish oil	0	0	4.4	4.3	0	0
Calcium Stearate	0	0	0	0	2.0	1.9
$CaCO_3$[4]	0.5	0.5	0.5	0.5	0	0
Chemical composition						
DM	88.4	89.3	88.7	89.5	88.6	89.4
CP	12.3	17.8	11.9	17.2	12.2	17.5
EE	2.9	3.2	4.9	5.2	4.6	4.8
NDF	43.9	33.7	43.8	34.0	43.3	33.2
Ash	6.3	7.2	6.5	7.3	6.0	6.8
Ca	0.8	1.09	0.8	1.1	0.9	1.2
P	0.4	0.8	0.4	0.8	0.4	0.8
NEL (Mcal/d)[5]	1.61	1.67	1.66	1.72	1.67	1.72

[1]CON = Basal diet; FO = basal diet with rumen-inert fish oil (10.4%EPA and 7.8% DHA; Ufac Ltd., Stretton, UK) included in the basal diet at a rate of 81 g/day of supplement (30 g/day of fatty acids) before kidding and 135 g/day of supplement (50 g/day of fatty acids) after kidding; ST = calcium stearate (C16:0 26% and 69.4% C18:0; Brenntag S.p.a.,Milan, Italy) included in the basal diet at a rate of 34 g/day of supplement (30 g/day of fatty acids) before kidding and 56 g/day (50 g/day of fatty acids) after kidding.

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

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

[4]Calcium carbonate was differently included to balance calcium content both in the pre- and post-kidding period within the groups.

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

3.3.2. RNA Extraction and Quantitative PCR

3.3.2.1. Liver biopsies

Tissue was harvested on days −7, +7and +21 relative to parturition for each experimental subject, via puncture biopsy under local anesthesia. The biopsy area was shaved and cleaned with disinfectants (ethyl alcohol and Betadine®, Povidone-iodine, 10%, Meda Pharma s.p.a., Monza, Italy) and a 14G biopsy needle was introduced through a small incision made at the right 11^{th} intercostal space at approximately 15 cm below the spine (Agazzi et al., 2010, Invernizzi et al., 2016) collecting 30 to 80 mg of liver tissue.

3.3.2.2. Adipose biopsies

On the same days as for liver biopsies, adipose tissue samples were collected from alternate sides of the tail-head region for each day of sampling. The biopsy area was shaved and cleaned with disinfectants (ethyl alcohol and Betadine®, Povidone-iodine, 10%, Meda Pharma s.p.a., Monza, Italy), and an incision of 2–3 cm length was performed between the tail head and the ischiatic bone. A sample of approximately 1 cm^3 of subcutaneous white adipose tissue was excised. The incisions for the liver and subcutaneous adipose tissue biopsies were sutured and treated with topical antibiotic reagents as described previously (Thering et al., 2009). Liver and adipose tissue samples were immediately placed in screw-capped, microcentrifuge tubes, snap frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

3.3.3. Total RNA Extraction, Target Genes, Primers Design, and Quantitative PCR

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 2-3. Primers were designed and evaluated as previously described (Bionaz and Loor, 2008). Primers were aligned against publicly available databases using BLASTN at NCBI. Prior to qPCR, primers were tested in a 20μL 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 adipose tissues) to ensure identification of desired genes. 5 μL of the PCR product were run in a 2% agarose gel stained with Sybr® Safe DNA Stain (EDVOTEK, Washington, DC, USA). The remaining 15 μL 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 hepatic and adipose tissues.

Immediately after biopsies the hepatic and adipose tissues were collected, stored in cryotubes and snap frozen in liquid nitrogen. Total RNA was isolated from the 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 was used to evaluate mRNA expression of genes involved in metabolic processes in adipose and liver, and genes taken part in the inflammatory response together with microRNA expression implicated in inflammatory response.

Genes selected for transcript profiling are associated with hepatic metabolism: methylsterol monooxygenase 1 (*MSMO1*), carnitine palmitoyltransferase 1 A (*CPT1*), and long-chain-fatty-acid-CoA ligase (*SLC27A2*), sterol regulatory element binding transcription factor 2 (*SREBF2*); superoxide dismutase 1 (*SOD1*), catalase (*CAT*), peroxisome proliferator-activated

receptor alpha (*PPARA*), stearoyl-CoA desaturase (*SCD*), acyl-CoA oxidase 1 (*ACOX1*), acetyl-CoA acyltransferase 1 (*ACAA1*); metabolism of adipose tissue: adiponectin (*ADIPOQ*), lipin 1 (*LPIN1*), lipoprotein lipase (*LPL*), sterol regulatory element binding factor 1 (*SREBF1*), thyroid hormone responsive protein (*THRSP*), peroxisome proliferator-activated receptor gamma (*PPARG*), acetyl-CoA carboxylase α (*ACACA*), fatty acid synthase (*FASN*), perilipin (*PLIN2*), leptin (*LEP*), and); adipose tissue inflammation: chemokine (C-C Motif) ligand 2 (*CCL2*), interleukin 6 (IL6), interleukin 10 (*IL10*), interleukin 18 (*IL18*), interleukin 1β (*IL1β*), interleukin 6 receptor (*IL6R*), serum amyloid A-3 (*SAA3*), haptoglobin (*HP*), interleukin 8 (*IL8*), retinol X receptor-α (*RXRA*), toll-like receptor 4 (*TLR4*).

The miRNA selected for expression profiling are associated with immune cell infiltration (miR-26b, miR-155), inflammation and lipolysis (miR-99a, miR-145, miR-221*)*, and positive regulation of adipogenesis (miR-143, mi-R378) (Vailati-Riboni et al., 2016, Vailati-Riboni et al., 2017).

Quantitative PCR for mRNA was performed using 4 µL diluted cDNA combined with 6 µL of a mixture composed of 5 µL SYBR Green master mix (cat#95073-05K, Quanta BioSciences), 0.4 µL each of 10 µM forward and reverse primers, and 0.2 µL DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems). Instead for miRNA PCR was performed using 1 µL diluted cDNA combined with 10 µL of a mixture composed of 5.25 µL SYBR Green master mix (cat#95073-05K, Quanta BioSciences), 0.45 µL each of 10 µM forward and reverse primers, and 3.85 µL DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems).

Each sample was run in duplicate and a 6-point relative standard curve plus the non-template control was used. The reactions were performed in an ABI Prism 7900 HT SDS instrument using the following conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C

(denaturation), and 1 min at 60 °C (annealing + extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95 °C for 15 s plus 65 °C for 15 s. Data were calculated with the 7900 HT Sequence Detection Systems Software.

For metabolic genes, the final data were normalized using the geometric mean of five internal control genes (ICG): Ribosomal Protein S15a (*RPS15A*), Ribosomal Protein S9 (*RPS9*), Ubiquitously-Expressed Prefoldin-Like Chaperone (*UXT*), Actin Beta (*ACTB*) and Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*). Instead for inflammatory genes, the three internal control genes are: Ribosomal Protein S15a (*RPS15A*), Ribosomal Protein S9 (*RPS9*) and Actin β (*ACTB*), while for microRNA were used microRNA-let7A (miR-Let-7A), microRNA-16-b (miR-16b), and microRNA-331 (miR-331).

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 2. Accession number, gene symbol, and forward and reverse primer sequences of genes analyzed in adipose and hepatic tissue, involved in lipid metabolism.

	Accession #	Symbol	Forward sequence	Reverse sequence
ICG	AF481159	ACTB	AAAGCCAACCGTGAGAAGATGA	ACAGCCTGGATGGCCACATA
	XM_005680968.1	GAPDH	TAATACTGGCAAAGTGGACATCGT	GAACTTGCCATGGGTGGAAT
	XM_005697526	RPS15A	TGCCGAGAAGAGAGGCAAAC	TTCGCCAATGTAACCATGCTT
	XM_005709411	RPS9	GGCCTGAAGATTGAGGATTTCTT	CGGGTATGGTGGATGGACTT
	XM_005700842	UXT	GAGAAAGTGCTGCGCTACGA	TGGCCAGCTGCTCATATACCT
Liver	AF422171.1	SCD	CCCAGGCTTTTGCTAGATGAA	CACATCATGCATGCTGACTCTCT
	JQ031288.1	FABP3	ATGGCCAAACCCACTGTGAT	GGCCCAATTTGAAGGACATCT
	XM_005694433.1	ACOX1	CAAACCCAGCAATATAAACTTTTTCC	CTTCATTAATCCGATGATAGGTCTCTT
	XM_005689606.1	ACAT1	AGGTCATACCATCCAGCTCATCA	CATAACTTCGCTCCGCATACC
	XM_005681212.1	PPARA	ATCATGGAGCCCAAGTTCGA	CTCCGCAGCAAATGATAGCA
	XM_005695550	ACAA1	GCCAGAGACTGCCTGATTCC	GTCCTGCTTCTCCCGTGAAA
	XM_005690077.1	CAT	ACACCCCCATTTTCTTCATCAG	ACCATGTCCGGATCCTTCAG
	NM_001285550.1	SOD1	AAAGTCGTCGTAACTGGATCCATT	CTGCACTGGTACAGCCTTGTG
	XM_005681190.1	SREBF2	CATCATCGAGAAGCGGTATCG	AACGCCAGACTTGTGCATCTT
	XM_005688487.1	GSS	ATCCTTTGTCCGGTCATTCCT	CCATGAAGACCCTGGAAATCTC
	XM_005700037.1	CPT1A	CATCAGATTCAAGAATGGCATCA	CTTGGCGTACATGGTCGACAT
	XM_005691142.1	MSMO1	GAGAGCAGCAGGAAGCACAGT	TCGCTTCTTGGGATTCACATG
	D86569	IL6	CTGGGTTCAATCAGGCGATT	TGATTTCCCTCAAACTCGTTCTG

	XM_005685790	SLC27A2	TGCTCTTGCCTCCGAGGTTA	AGAAGCGCAGCACTGTGGTA
Adipose	JQ266369	PPARG	GGAGATCACAGAGTACGCCAAGA	TCGTGCACGCCGTATTTTAG
	HM443643	SREBF1	TCTTCCATCAATGACAAGATCGTT	CGGATGTAGTCGATGGCTTTG
	JN684754	THRSP	ATTGGCCTAAAAGAGGGCTATGT	CCTCTGTTTCCGGGCTGTTA
	EU375363	ADIPOQ	CACCTTCACAGGCTTCCTTCTC	TGTGGACTGTTCTTTCACTTTGC
	AF422171.1	SCD	CCCAGGCTTTTGCTAGATGAA	CACATCATGCATGCTGACTCTCT
	DQ370053	LPL	TAAGGCGTACCGGTGCAATT	CTTGTTGATCTCGTAGCCCATGT
	XM_005686993.1	LPIN1	CTTACCTTGGGCTTGCTCATTAG	TACTCGGTTCAGAGCAGTTAAAATTC
	GU944974	LEP	CTGTCTGCAGAAGAGAGCCTATGT	GCAGTTTTCTGCCTTTGGAAGA
	XM_005682406.1	RETN	CCTGCAGGATGAAGGCTCTCT	TCCTGGATCTTCTTACTGACAGCTT
	DQ370054	ACACA	ATATGAGCTTCGCCACAACCA	CAAGATGAGTTTCTGCAGGTTCTC
	DQ223929	FASN	AGCCCCTCAAGCGAACAGT	GAATGACCACTTTGCCGATGT
	KC453970.1	PLIN2	CGGCCTGACTCCAAGATACCT	CATTAGAGACACCTTTTGAAGAAAAACA

Table 3. Accession number, gene symbol, and forward and reverse primer sequences of genes analyzed in adipose tissue with involvement in inflammatory and immune state

	Accession #	Symbol	Forward sequence	Reverse sequence
ICG	XM_005697526	RPS15A	TGCCGAGAAGAGAGGCAAAC	TTCGCCAATGTAACCATGCTT
	XM_005709411	RPS9	GGCCTGAAGATTGAGGATTTCTT	CGGGTATGGTGGATGGACTT
	AF481159	ACTB	AAAGCCAACCGTGAGAAGATGA	ACAGCCTGGATGGCCACATA
Adipose	NC_022311.1	CCL2	CTCGCTCAGCCAGATGCAAT	AGGTTGGGGTCTGCACAAAA
	NC_022308.1	IL10	CATGGGCCTGACATCAAGGA	ACCCCTCTCTTGGAGCATATTG
	NC_022308.1	IL18	AGAAGCTATTGAGCACAGGCAT	AGGTTCAAGCTTGCCAAAGTG
	NC_022303.1	IL1β	TCCACCTCCTCTCACAGGAAA	TACCCAAGGCCACAGGAATCT
	NC_022295.1	IL6R	GACCAGAGGACAATGCCACA	GCCTTCCCGGTAGCATGAAT
	NC_022308.1	SAA3	GGACATTCCTCAGGGAAGCTG	TGGCGTTACTGATCACTTTAGCA
	NC_022310.1	HP	CAAACTTCGCACCGATGGAG	CCGCACACTGCCTCACATT
	NC_022298.1	IL8	TGTGTGAAGCTGCAGTTCTGT	TGGGGTCTAAGCACACCTCT
	NC_022310.1	RXRA	GGTTGGTTGTGGGAGTGTCA	GGGGCAAAAGCTGGCATTAC
	NC_022310.1	TLR4	CTTGCGTCCAGGTTGTTCCT	TTGCAGTTCTGGGAACCTGG

3.4. Statistical analysis

The geometric mean of the internal control genes was used for normalization and qPCR data (mRNA and miRNA) were subsequently $\log_2$ transformed for a normal distribution of data before statistical analysis. Data were subjected to two-way-ANOVA and analyzed using repeated measures ANOVA with PROC MIXED (SAS, version 9.3, SAS Institute Inc., Cary, NC). The statistical model included time (T; −7, 7, and 21 days postpartum), dietary treatments (CON, FO and ST) and their interaction as fixed effects. Goat, nested within treatment, was the random effect. Data were considered significant at a $P \leq 0.05$ using the PDIFF statement in SAS. Data reported in Figures 10 through 13 are the $\log_2$ transformed least squares means that resulted from the statistical analysis. Standard errors were also adequately transformed.

3.5. Results

3.5.1. mRNA expression of metabolism-related genes in liver

Our results did not evidence significant up or down-regulation of metabolism-related genes at liver level from 7 d before to 21 d after kidding in the three experimental groups, with the exception of *ACOX1* and *SCD* (Figure 10). Specifically, the interaction between stearate supplementation and time increased *ACOX1* expression than CON (0.72 vs. -0.21; $P<0.001$) on day 7 post partum. Whereas, similarly, the interaction between fish oil and time decreased *SCD* expression on d 7 and d 21 with respect to ST (-1.37 vs. 0.85; $P<0.001$), and ST and CON (-1.65 vs. 0.33 and 0.75; $P<0.01$), respectively.

Then, the intra-group analysis revealed that *ACOX1* was up-regulated in ST group from -7 to 7 DIM (-0.3011, 0.7158 respectively, $P<0.01$) and in was instead down-regulated from the week post kidding to 21 d of lactation (-0.1543 at 21 DIM, $P<0.01$). In a similar way *ACOX1*

in FO group was up-regulated during the first two weeks of the trial (-0.2706, 0.304 respectively, *P*=0.02), but its expression was stable until 21 DIM (0.1773). *SCD* expression had the same trend during time in both CON and ST groups with an increase/up-regulation from -7 to 7 d from kidding (-1.9241, -0.4056 respectively for CON, *P*=0.02 and -0.8336, 0.8525 respectively for ST, *P*<0.01), but no differences were found from 7 to 21 d of lactation within the groups. Anyway, *SCD* expression at the end of the trial was higher in both CON and ST (0.3331 for CON, *P*<0.01 and 0.747 for ST, *P*=0.020, respectively) than at 7 d before kidding. Different trends were evidenced for ST and FO during time on *ACAA1* expression were ST showed a down-regulation after kidding (*P*=0.03), and FO an up-regulation from -7 to 7 DIM (-0.6566, 0.2601 respectively, *P*<0.01). *ACAA1* expression at three weeks of lactation resulted in a up-regulation with respect to -7 from kidding (*P*<0.01).

With the exception of hepatic *SCD* expression (*P*=0.03) which was higher in ST than FO (0.26 vs. -1.25; *P*<0.01) the administration of saturated or polyunsaturated fatty acids did not lead to significant variations of genes involved in oxidative status, β- oxidation or cholesterol biosynthesis when accounting for diet effect.

After that, hepatic genes related to metabolism strongly affected by time were involved in β-oxidation (*ACOX1, SLC27A2, CPT1)* and cholesterol biosynthesis (*SCD)*. In detail, *ACOX1, SLC27A2,* and *CPT1* were up-regulated from the week before kidding to 7 DIM and down-regulated at 3 weeks of lactation, while *SCD* expression was constantly increased throughout the experimental period.

3.5.2. mRNA expression of metabolism-related genes in adipose tissue

Evaluating the interactions that occur within a given treatment over time for adipose tissue, we found higher values for FO, compared to ST at 7 DIM for *LPIN1* instead *FASN* showed higher expression in ST than CON at the beginning of the trial (0.04 vs. -1.80; P=0.04) (Figure 11).

Moreover, the intra-group analysis showed a similar trend for ST and FO, with a down-regulation of *FASN* from -7 to 7 DIM (0.0413, -2.020 respectively for ST, P<0.01 and -0.2339, -3.062 respectively for FO, P<0.01) and a subsequent stable expression from the first week of lactation the end of the trial. On d 21 *FASN* was found to be down-regulated in ST and FO (-2.2728 for ST and -2.8934 for FO, P<0.01 for both groups) with respect to initial values (at day -7). *LPIN1* was up-regulated in FO from the week before kidding to 7 DIM (P<0.01). No effect of the three dietary treatments was evidenced in the expression of metabolism-related genes in adipose tissue.

As for mRNA liver metabolism, for mRNA adipose metabolism an effect of time was observed on most of the considered genes in adipose tissue (Table 4). Fatty acid synthesis-related genes, such as *PPARG* (P<0.01), *SREBF1* (P<0.05), *THRSP* (P<0.01), *ACACA* (P<0.01), and *FASN* (P<0.01) where down-regulated or decreased during lactation with respect to 7 days before kidding or between the first and the third week of lactation (Table 4). Adipose tissue maintenance-related genes showed the same trend as for fatty acid synthesis-related genes (*LEP*; P<0.01) or outlined an up-regulation from -7 to 21 DIM (*PLIN2*; P<0.01).

Table 4. Effect of time on mRNA and miRNA expression change-folds1 of liver and adipose tissue metabolism- and inflammation-related genes in transition dairy goats.

Item[2]	Days in milk					TRENDS
	-7	7	21	SEM[3]	*P*-value	
Metabolism						
Liver						
SOD1	-0.12	0.10	0.03	0.09	0.12	/-
CAT	-0.20	0.12	0.07	0.12	0.08	/-
ACOX1	-0.29^{B}	0.27^{Aa}	-0.09^{b}	0.11	<0.01	/\
ACAA1	-0.39	0.01	-0.18	0.14	0.06	/\
PPARA	0.12	0.01	0.01	0.10	0.63	_
SLC27A2	-0.23^{B}	0.24^{A}	-0.11^{B}	0.09	<0.01	/\
CPT1	-0.11^{b}	0.16^{Aa}	-0.32^{Ba}	0.08	<0.01	/\
SREBF2	-0.22	0.11	-0.04	0.12	0.06	/\
SCD	-1.16^{Bb}	-0.31^{a}	-0.19^{A}	0.31	0.01	/
MSMO1	0.04	0.15	0.12	0.14	0.82	/-
Adipose tissue						
PPARG	0.12^{A}	-0.02^{a}	-0.47^{Bb}	0.19	<0.01	\
SREBF1	0.13^{a}	-0.22	-0.29^{b}	0.15	0.04	\
THRSP	0.47^{Aa}	-0.46^{b}	-0.68^{B}	0.37	<0.01	\
ACACA	-0.18A^{a}	-0.93^{b}	-1.71^{Bc}	0.32	<0.01	\
FASN	-0.66^{A}	-2.24^{B}	-2.62^{B}	0.38	<0.01	\
LEP	0.11^{A}	-2.00^{Ba}	-3.23^{Bb}	0.51	<0.01	\
PLIN2	-0.50^{B}	0.58^{A}	0.20^{A}	0.17	<0.01	/\
LPIN1	-0.56	-0.04	-0.15	0.25	0.15	/\
ADIPOQ	-0.02^{a}	-0.23	-0.67^{b}	0.29	0.11	\
LPL	-0.18	-0.01	0.11	0.25	0.63	/
Inflamation						
Adipose tissue						
HP	-4.25^{Bb}	-0.32^{A}	-2.90^{Ba}	0.55	<0.01	/\
SAA3	-1.11^{B}	0.57^{A}	-0.52	0.41	0.01	/\
IL6R	0.18	-0.15	0.08	0.11	0.08	\/
RXRA	-0.25^{a}	-1.31^{Bb}	0.29^{A}	0.36	0.02	/-
TLR4	0.05	0.01	0.01	0.15	0.97	/-
CCL2	-0.36	0.01	0.05	0.26	0.39	/-
IL1β	0.06	-0.52	0.41	0.31	0.09	\/
IL6	-0.93^{b}	0.18^{a}	0.01^{a}	0.34	0.03	/\
IL8	-0.75^{B}	-0.43^{b}	0.44^{Aa}	0.27	0.01	/
IL18	-0.38	-0.08	0.20	0.21	0.08	/
IL10	-0.37^{B}	-0.40^{B}	0.47^{A}	0.21	0.01	-/
miR-26b	-0.35	-0.18	-0.41	0.20	0.70	/\
miR-155	-0.17^{B}	0.27^{A}	0.10	0.18	0.03	/\
miR-99°	0.41	0.13	0.15	0.17	0.43	\-
miR-145	-0.24	0.06	0.18	0.178	0.23	/
miR-221	-0.61	-0.32	-0.14	0.16	0.08	/
miR-143	0.30	0.29	0.37	0.26	0.96	--
miR-378	0.23	-0.15	0.11	0.32	0.68	\/

[A,B]*Different superscripts indicate a significant difference among days (P<0.01).*

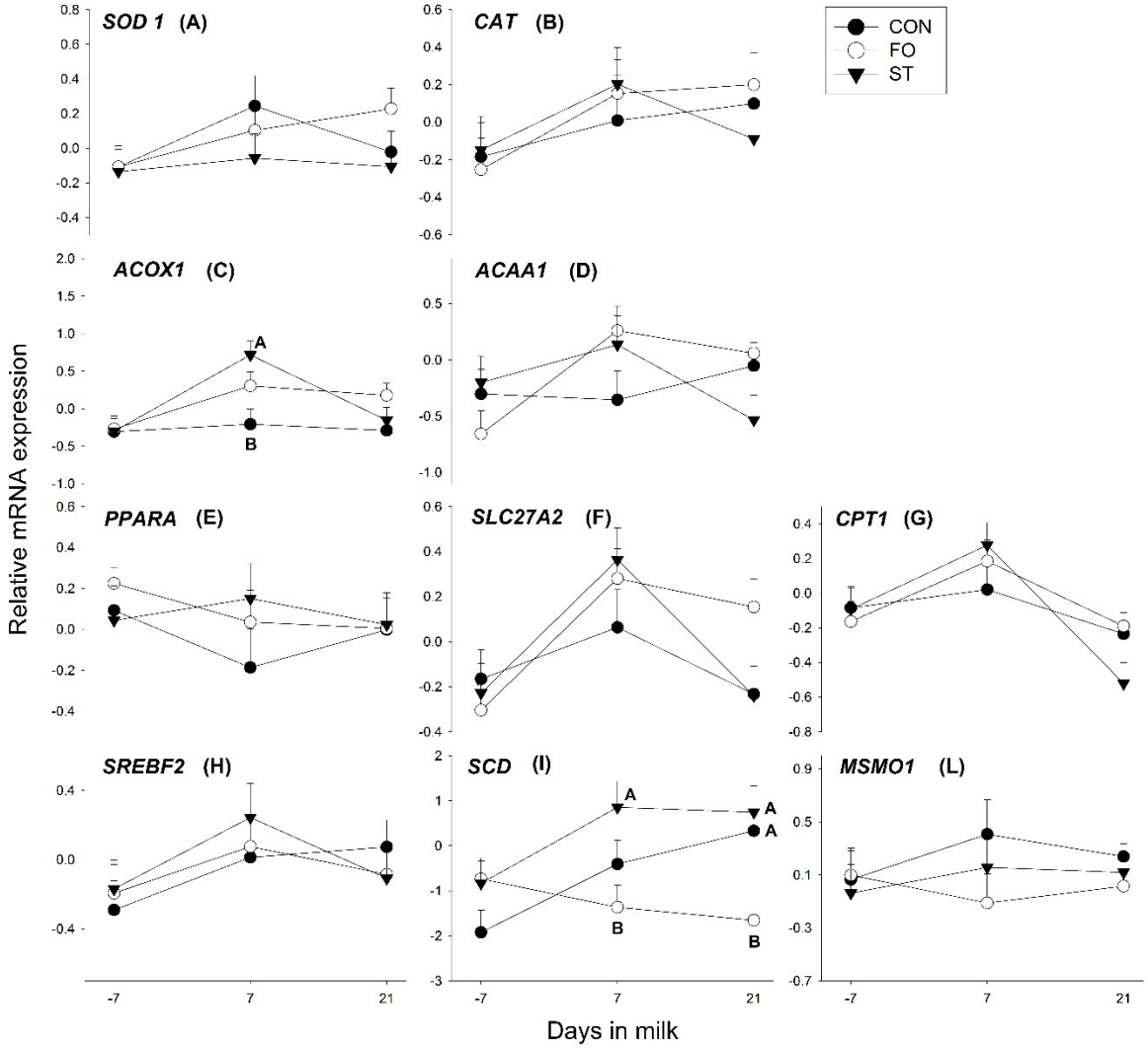
[a,b]*Different superscripts indicate a significant difference among days (P<0.05).*

[1]*log_2 transformed least squares means and SEM*

[2]*Involvement/class of gene: oxidative metabolism: Superoxide dismutase 1 (SOD1), catalase (CAT); β-oxidation: acyl-CoA oxidase 1 (ACOX1), acetyl-CoA acyltransferase 1 (ACAA1), peroxisome proliferator-activated receptor alpha (PPARA), long-chain-fatty-acid-CoA ligase (SLC27A2), carnitine palmitoyltransferase 1 A (CPT1); cholesterol biosynthesis: sterol regulatory element trascription factor 2 (SREBF2), stearoyl-CoA desaturase (SCD), methylsterol monooxygenase 1 (MSMO1); fatty acid synthesis: peroxisome proliferator-activated receptor gamma (PPARG), sterol regulatory element binding factor 1 (SREBF1), thyroid hormone responsive protein (THRSP), acetyl-CoA carboxylase a (ACACA), fatty acid synthase (FASN); maintenance of adipose tissue: leptin (LEP), perlipin (PLIN2); fatty acid oxidation: lipin 1 (LPIN1), adiponectin (ADIPOQ), lipoprotein lipase (LPL); acute phase proteins: haptoglobin (HP), serum amyloid A-3 (SAA3); transcription factors: interleukin 6R (IL6R), retinol X receptor-a (RXRA), toll-like receptor 4 (TLR4); chemokine: ligand 2 (CCL2); pro-inflammatory cytokines: interleukin 1β (IL1β), interleukin 6 (IL6); interleukin 8 (IL8), interleukin 18 (IL18); anti-inflammatory cytokine: interleukin 10 (IL10); immune cell infiltration: miR-26b, miR-155; inflammation and lipolysis: miR-99a, miR-145, miR-221; positive regulation of adipogenesis: miR-143, miR-378.*

[3]*Greatest standard error of the mean*

Figure 10. mRNA expression change-folds of liver metabolism-related genes[1,2] in transition dairy goats fed saturated or polyunsaturated fatty acids[3].



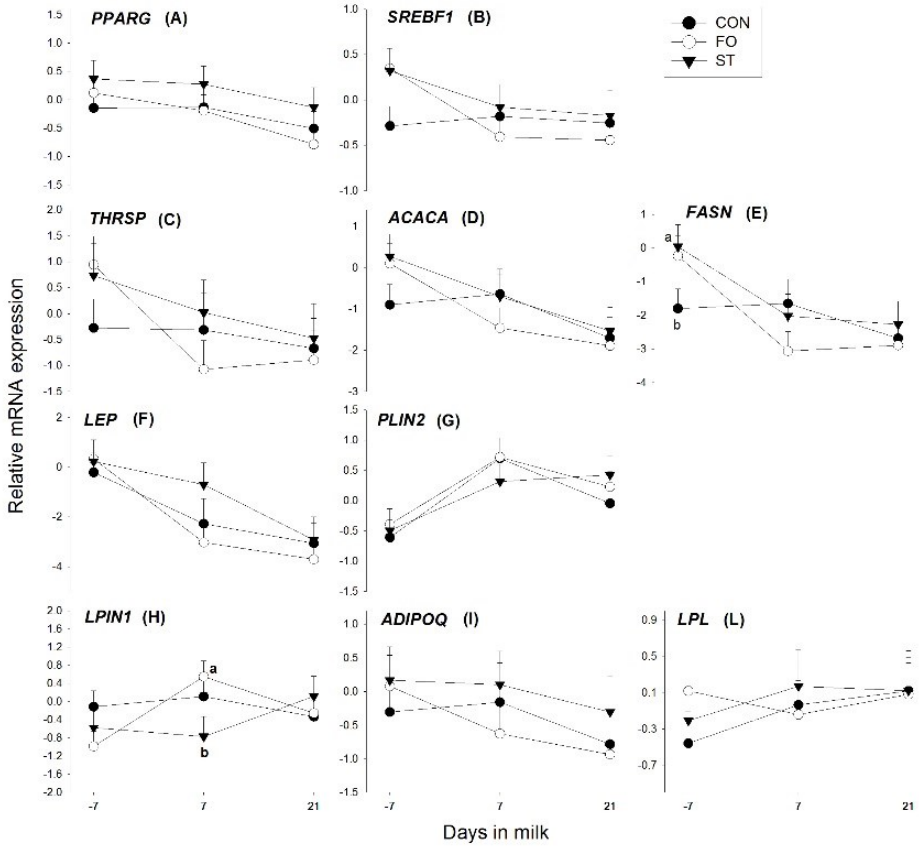
[A, B] Different superscripts indicate a significant difference among groups (P<0.01).

[1]log2 transformed least squares means and SEM

[2] Uppercase letters define the involvement of genes. A, B) oxidative metabolism: Superoxide dismutase 1 (SOD1), catalase (CAT); C, D, E, F, G) β-oxidation: acyl-CoA oxidase 1 (ACOX1), acetyl-CoA acyltransferase 1 (ACAA1), peroxisome proliferator-activated receptor alpha (PPARA), long-chain-fatty-acid-CoA ligase (SLC27A2), carnitine palmitoyltransferase 1 A (CPT1); H, I, L) cholesterol biosynthesis: sterol regulatory element trascription factor 2 (SREBF2), stearoyl-CoA desaturase (SCD), methylsterol monooxygenase 1 (MSMO1).

[3] *CON = Basal diet; FO = basal diet with rumen-inert fish oil (10.4%EPA and 7.8% DHA; Ufac Ltd., Stretton, UK) included in the basal diet at a rate of 81 g/day of supplement (30 g/day of fatty acids) before kidding and 135 g/day of supplement (50 g/day of fatty acids) after kidding; ST = calcium stearate (C16:0 26% and 69.4% C18:0; Brenntag S.p.a.,Milan, Italy) included in the basal diet at a rate of 34 g/day of supplement (30 g/day of fatty acids) before kidding and 56 g/day (50 g/day of fatty acids) after kidding.*

Figure 11. mRNA expression change-folds of adipose tissue metabolism-related genes[1,2] in transition dairy goats fed saturated or polyunsaturated fatty acids[3].



[a,b]*Different superscripts indicate a significant difference among groups (P<0.05).*

[1]*log_2 transformed least squares means and SEM*

[2] Uppercase letters define the involvement of genes. A, B, C, D, E) fatty acid synthesis: peroxisome proliferator-activated receptor gamma (PPARG), sterol regulatory element binding factor 1 (SREBF1), thyroid hormone responsive protein (THRSP), acetyl-CoA carboxylase a (ACACA), fatty acid synthase (FASN); F, G) maintenance of adipose tissue: leptin (LEP), perlipin (PLIN2); H, I, L) fatty acid oxidation: lipin 1 (LPIN1), adiponectin (ADIPOQ), lipoprotein lipase (LPL).

[3] CON = Basal diet; FO = basal diet with rumen-inert fish oil (10.4%EPA and 7.8% DHA; Ufac Ltd., Stretton, UK) included in the basal diet at a rate of 81 g/day of supplement (30 g/day of fatty acids) before kidding and 135 g/day of supplement (50 g/day of fatty acids) after kidding; ST = calcium stearate (C16:0 26% and 69.4% C18:0; Brenntag S.p.a.,Milan, Italy) included in the basal diet at a rate of 34 g/day of supplement (30 g/day of fatty acids) before kidding and 56 g/day (50 g/day of fatty acids) after kidding.

3.5.3. mRNA and microRNA for adipose tissue, inflammatory-related genes

Among the analyzed genes related to adipose inflammation, we found a significant interaction of diet*time only for *IL6*. A significant decrease of *IL6* expression in the first week of lactation in ST than CON (-1.14 vs. 1.64, respectively; $P<0.01$) (Figure 12).

There was a similar trend in CON and FO groups for *IL6* with an up-regulation from -7 to 7 DIM (-0.820, 1.604 respectively, for CON, $P<0.01$) (-1.537, -0.065 respectively, for FO, $P=0.02$), while it was up-regulated in ST from the first to the third week of lactation (-1.139, 0.365 respectively, $P=0.05$).

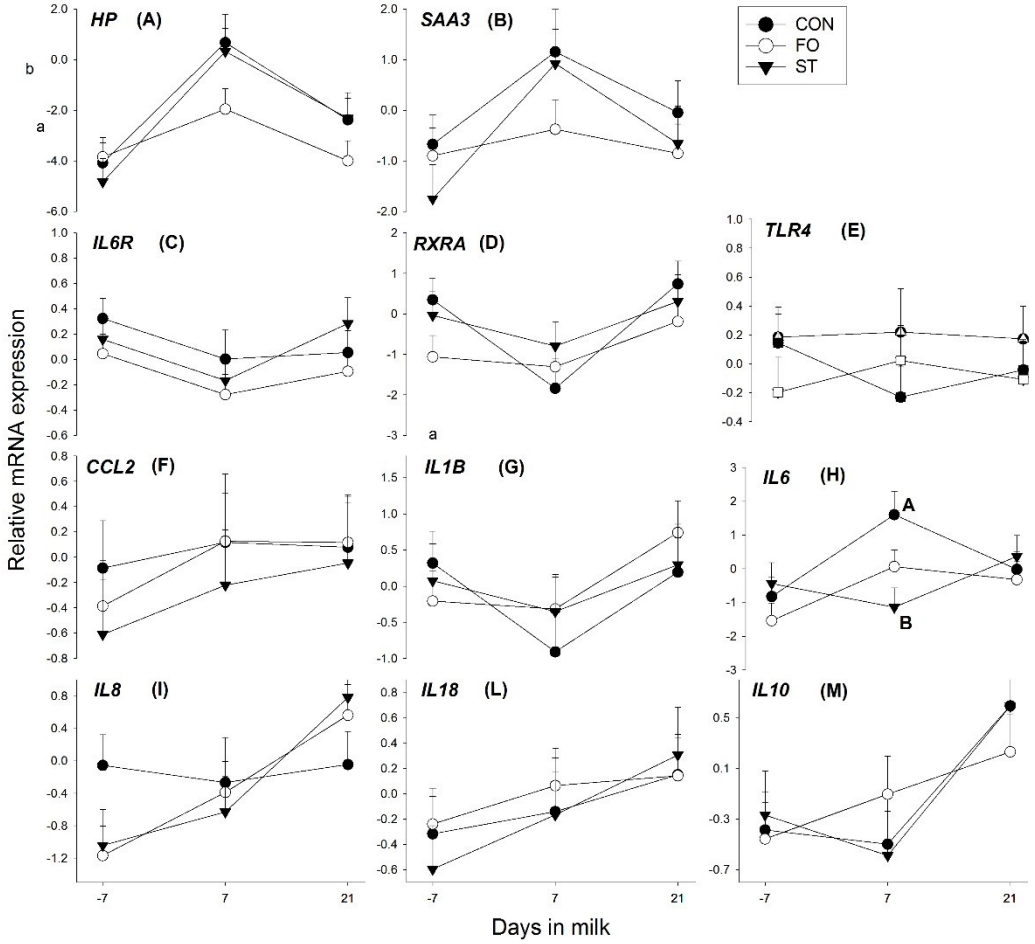
No effect of the three dietary treatments was evidenced in the expression of genes involved in inflammation processes. A significant effect of time was detected for acute phase proteins (*Hp*, $P<0.01$; *SAA3*, $P<0.05$), *RXRA* ($P<0.05$), pro-inflammatory (*IL6*, $P<0.05$; *IL8*, $P<0.01$), and anti-inflammatory cytokines (*IL10*, $P<0.01$) (Table 4).

Acute phase protein gene expression was increased during the trial (*Hp*; $P<0.01$) and up-regulated from -7 to 7 DIM (*SAA3*; $P<0.01$), while the transcription factor *RXRA* was decreased in the first week of lactation and up-regulated on 21 DIM ($P<0.05$). Interleukin 6 was up-regulated from -7 to 7 DIM ($P<0.05$), while a constant increase, and a consequent up-

regulation, was observed for *IL8* from the beginning of the trial to the first three weeks of lactation ($P<0.01$). Anti-inflammatory *IL10* did not show any variation in the two weeks around parturition, but it was up-regulated on 21 DIM ($P<0.01$) (Table 4).

No differences were evidenced for adipose infiltration of immune cells, inflammation and lypolisis, or proadipogenic-related miRNAs for diet and time effects or diet x time interaction (Figure 13), with the exception of an up-regulation of miRNA 155 from the week before kidding to the first 7 DIM ($P<0.01$) (Table 4).

Figure 12. mRNA expression change-folds of adipose tissue inflammation-related genes[1,2] in transition dairy goats fed saturated or polyunsaturated fatty acids[3].



[A, B] Different superscripts indicate a significant difference among groups (P<0.01).

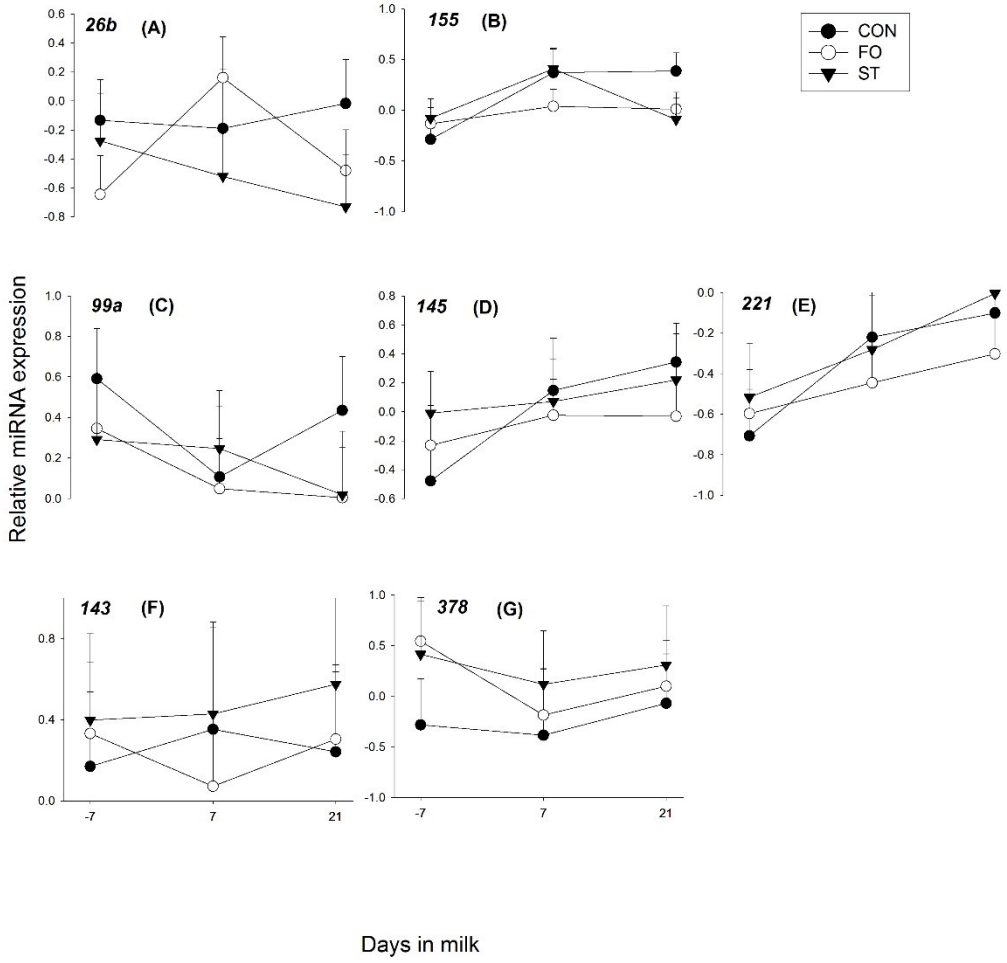
[1] log_2 transformed least squares means and SEM

[2] Uppercase letters define the classes of genes. A, B) acute phase proteins: haptoglobin (HP), serum amyloid A-3 (SAA3); C, D, E) transcription factors: inteleukin 6R (IL6R), retinol X receptor-a (RXRA), toll-like receptor 4 (TLR4); F) chemokine: ligand 2 (CCL2); G, H, I, L) pro-inflammatory cytokines: interleukin 1B (IL1B), interleukin 6 (IL6), interleukin 8 (IL8), interleukin 18 (IL18); M) anti-inflammatory cytokine: interleukin 10 (IL10);.

[3] CON = Basal diet; FO = basal diet with rumen-inert fish oil (10.4%EPA and 7.8% DHA; Ufac Ltd., Stretton, UK) included in the basal diet at a rate of 81 g/day of supplement (30 g/day of fatty acids) before kidding and 135 g/day of supplement (50 g/day of fatty acids) after kidding; ST = calcium stearate (C16:0 26% and 69.4% C18:0; Brenntag S.p.a.,Milan, Italy)

included in the basal diet at a rate of 34 g/day of supplement (30 g/day of fatty acids) before kidding and 56 g/day (50 g/day of fatty acids) after kidding.

Figure 13. Change-folds of microRNA expression in adipose tissue[1,2] of transition dairy goats fed saturated or polyunsaturated fatty acids[3].



[1] log_2 transformed least squares means and SEM

[2] Uppercase letters define the involvement of genes. A, B) immune cell infiltration; C, D, E) inflammation and lipolysis; F, G) positive regulation of adipogenesis.

[3] CON = Basal diet; FO = basal diet with rumen-inert fish oil (10.4%EPA and 7.8% DHA; Ufac Ltd., Stretton, UK) included in the basal diet at a rate of 81 g/day of supplement (30 g/day of fatty acids) before kidding and 135 g/day of supplement (50 g/day of fatty acids) after kidding; ST = calcium stearate (C16:0 26% and 69.4% C18:0; Brenntag S.p.a.,Milan, Italy) included in the basal diet at a rate of 34 g/day of supplement (30 g/day of fatty acids) before kidding and 56 g/day (50 g/day of fatty acids) after kidding.

3.6. Discussion

The combined analysis of mRNA and miRNA has helped improve knowledge of the molecular self-regulatory mechanisms in liver and adipose tissue depots during the transition of dairy cows. The aim of these studies has been to study metabolic changes and inflammation processes during transition or determine the effects of supplemental dietary energy intake around calving (Vailati-Riboni et al., 2016, Vailati-Riboni et al., 2017). Adipose tissue is recognized to play an important role in the adaptation to lactation in dairy cows, and its metabolism is closely linked to DMI (McNamara, 1991, McNamara, 1997). Furthermore, adipose tissue is characterized by self-regulation mechanism involving adipokines (Adamczak and Wiecek, 2013, McGown et al., 2014, Musi and Guardado-Mendoza, 2014) that can generate a local inflammatory response (Grant and Dixit, 2015).

In human models, adipocyte metabolic pathways have been demonstrated to be controlled both through the well-known changes in mRNA expression and miRNA signaling through complex networks, involving transcription factors and inflammatory molecules (Kloting et al., 2009). Considering that miRNA are highly conserved among species, this led to the hypothesis that inflammation in dairy cows plays a homeorethic role in the physiological adaptations to lactation, (Vailati-Riboni et al., 2017). However, the fact that dairy goats respond differently than dairy cows to lipid supplementation (Chilliard et al., 2007, Toral et al., 2013) suggests that physiologic adaptations during the transition period also may differ among species. At the

present time, studies on lipid metabolism or nutritional regulation in tissues of goats, especially when considering liver and adipose tissue, are scarce (Bernard et al., 2005, Bernard et al., 2009). Even if there are some papers about mammary gland mRNA and miRNA expression of gene involved in dairy goats (Osorio et al., 2017), very few have investigated the lipogenic gene mRNA abundance in late-lactation dairy goats in liver and adipose tissue when fed different fat supplementations (Toral et al., 2013).

3.6.1. Liver and adipose tissue metabolism gene expression

In our previous work (Invernizzi et al., 2016) from this same trial, no evident signs of serious metabolic disorders or milk fat depression, according to Chilliard et al. (2003) and Agazzi et al. (2010), and in contrast with Toral et al., (2015). Furthermore, data indicated that fish oil administration delayed fat mobilization.

All goats in the present trial exhibited fatty liver at 7 days before parturition independently from lipid supplementation. Although severe hepatocyte injury was detected, no features of perisinusal fibrosis or necrosis foci with poly-morphonuclear cells were evident suggesting the absence of inflammation (Brunt and Tiniakos, 2010) as it is often described in dairy cows with severe fatty livers (Kalaitzakis et al., 2010). Despite this, fish oil-fed goats had the highest percentage of vacuolated cells suggesting that the fish oil treatment induced slightly more detrimental effects on the liver over time. This effect was further confirmed by the Grades der Leberverfettungor (GdL) distribution and the higher alanine aminotransferase (ALAT) activity values.

An evaluation of the entire set of the obtained results led to the suggestion that the onset of fatty infiltration occurred more gradually in the fish oil group, indicating a sort of progressive adaptation to the lipid infiltration that allowed the cells to better preserve their functions.

With the exception of a lower liver *SCD* expression in FO than ST, mainly due to a decreased expression throughout 7 to 21 DIM, in the present trial we did not find variation on liver or adipose tissue metabolism-related genes due to feeding lipid alone. In contrast, ST led to higher liver expression of *ACOX1* at 7 DIM than CON.

3.6.1.1. Fat sources and gene expression in liver and adipose tissue

Changes in liver and adipose tissue metabolism-related gene expression within groups revealed different trends when periparturient goats were fed saturated or polyunsaturated fatty acids in the diet.

At hepatic level, both *ACOX1* (in ST) and *ACAA1* (in FO), showed that goats were subjected to fatty acid oxidation from -7 to 7 d from kidding, with an up-regulation of *SCD* in ST for cholesterol biosynthesis. Oxidation of fatty acids was further down regulated in ST at 21 DIM (*ACOX1* and *ACAA1*), but not in FO. The protein encoded by *ACOX1* is the first enzyme of the fatty acid beta-oxidation pathway (Vluggens et al., 2010) and its expression in our study was significantly higher in ST, with the highest value at 7 DIM. Akbar et al. (2013) observed an increase of its expression after kidding, purposely at 11 DIM, in periparturient cows fed SFA. They connected the increase of *ACOX1*, after SFA supplementation, to a potential enhancement in β-oxidation. Since these results, they stated that saturated LCFA are more potent in activating the espression of this gene, in agreement with in vitro data (Bionaz et al., 2012).

A very similar pattern to *ACOX1*, at least for ST and FO despite overall lower expression levels, was observed for *ACCA1* that encodes an enzyme operative in β-oxidation system of the peroxisomes. Considering the obtained results for genes related to β-oxidation seem to support a modulation of dietary FA over time, with a higher impact of FO and ST at days 21 and 7 respectively.

Although no differences were observed for miRNA-103 and miRNA 143, at the adipose tissue level, the observed down-regulation of the pro-adipogenic gene *FASN* in both ST and FO goats suggests a reduction in the differentiation and proliferation of adipocytes. The up-regulation of *LPIN1* from -7 to 7 DIM suggests to be involved in directing lipid to the appropriate storage site (i.e. adipose tissue) with the possible reduction in lipid accumulation to liver (Reue, 2007). Invernizzi et al. (2016), applying a histological approach to study the metabolic adaptations of dairy goats to the transition period, observed that the reduction of the adipocyte area was more intense in ST than FO group, leading to the idea that FO is able to limit lipolysis.

Thus, the decrease of *SCD* mRNA expression in liver of the FO group that we observed is in accordance with Akbar et al. (2013) in cows. Toral et al. (2013), in goats, reported that the effect of FO on the expression of *SCD* is also required for efficient cholesterol esterification and triglycerides synthesis and subsequent VLDL formation (Paton and Ntambi, 2010).

3.6.2. Adaptations to kidding

Studies with ruminants published to date indicate that there are species-specific differences, e.g. fatty liver in small ruminants develops frequently during the last phase of pregnancy rather than at the beginning of lactation (Sauvant et al., 1991, van den Top et al., 1995, Pinotti et al.,

2008). Furthermore, even if hepatic lipidosis is severe in small ruminants they do not show any serious metabolic disorders (Invernizzi et al., 2016) compared with cows. Moreover, also among the small ruminants, there are differences when dietary lipids are supplemented, showed by few publications for goats (Chilliard et al., 2003, Addis et al., 2005, Cabiddu et al., 2005).

Similar to the response in cows (Khan et al., 2013), the decrease in expression of genes involved in adipogenesis and lipogenesis (such as *SREBF1*, *THRSP*, *PPARG*, *ACACA*, *FASN*, and *LEP*) in adipose tissue, indicates that subcutaneous adipose tissue depot loses adipocytes and other cells with a subsequent regrowth of the tissue as lactation goes on. Then, the initial increase in expression of *PLIN2* from -7 to 7 DIM followed by a reduction contrasts the response found in cows, where expression of this gene is more stable across the transition period (Khan et al., 2013). This gene is linked with liver TAG accumulation and may serve to manage the turnover of lipid stores to facilitate the coordinated released of LCFA into lipoproteins in response to changes in metabolic state (Akbar et al., 2013). In accordance with us, Akbar et al. (2013) observed a down regulation of *PLIN2* in FO group and an upregulation in SFA and C group. It suggests a difference in potency of LCFA or alternatively, as in the case of the FO group, a protective mechanism of the cell to buffer from excessive LCFA influx.

Secondly, the upregulation postpartum of genes involved in inflammation let us to link it to the reduction of adipogenesis and lipogenesis (typical of this timeframe) and the increase of lipolysis. However, the expression of genes taken into consideration displays a lower intensity of lipomobilization, and its subsequent inflammation, than to what happens in cows (Bradford et al., 2015).

Compared to prepartum, the highest upregulation of *HP* and *SAA3* at 7 DIM (similar to cows (Vailati-Riboni et al., 2016, Vailati-Riboni et al., 2017)) supports the idea of an acute phase response in adipose tissue in the first week after kidding, which is likely related to a higher degree of peripartum inflammation. The reduction of their expression at 21 DIM in the current trial could be explained by a quick recovery from the inflammatory state. This led us to assume that dairy goats have to face a milder and less intense lipomobilization compared to cows. Furthermore, miR-155, which is involved in the infiltration of immune cells, had a similar expression pattern, showing the inflammation as a response to the physiological adaptations to lactation. In support of this hypothesis is also the expression that we found for *IL8*, *IL10* and miR-221, which was upregulated, showing the relationship of these genes with the acute phase reactants that we analyzed (*HP* and *SAA3*) and their role in inflammation and lipolysis.

3.7. Conclusions

In the present study, the analysis of the expression of miRNA and genes involved in lipid metabolism in liver and adipose tissue support the idea that FO can reduce or delay lipomobilization (even if with negative energy balance). Moreover, the increase of pro-inflammatory cytokines at 7 DIM showed how goats are different to cows and also to the other small ruminants, leading to the hypothesis of a postponed and mild inflammation in subcutaneous adipose tissue.

4. DIETARY *CAMELINA SATIVA* IMPROVES CONVERSION RATE IN LAYING HENS AND LEADS TO HEALTHIER EGGS

4.1. Abstract

There is growing interest in promoting human health and wellbeing through good nutrition and the provision of safe and healthy foods. In this respect, an important role can be played by food products of animal origin with improved beneficial fatty acids content achieved by animal diet modulation. The present study aimed to determine the effects of feeding *Camelina sativa* meal, a source of beneficial n-3 polyunsaturated fatty acids (PUFA) to laying hens on performance and egg quality, i.e. fatty acid composition and lipid oxidative stability. A total of 32 laying hens were randomly allocated to two experimental groups, with eight replicates per group. Animals were fed a common basal diet with (T) or without (C) inclusion of 7.5% camelina meal for a total period of 63 days. Performance was evaluated weekly, while egg quality was determined at days 0, 28, 56 and shelf-life/TBARS at days 0, 9, 21, 28. Camelina meal supplementation improved feed conversion rate in laying hens ($P<0.05$). Moreover, reduced saturated fatty acids (SFA) content was observed in yolk of camelina supplemented hens (33.18 C vs. 32.45 T; $P<0.01$), with a concomitantly enrichment in α-linolenic acid (1.05 C vs. 1.47 T; $P<0.01$). Animal products have been often criticized for their high content in SFA considered detrimental to human health. Results of the present study show that it is possible to improve yolk quality by feeding *Camelina sativa* meal to laying hens, reducing egg SFA content and providing increased amount of beneficial n-3 fatty acids.

4.2. Introduction

"Let food be thy medicine and medicine be thy food". 2500 years ago, Hippocrates espoused this philosophy about the role of food in human health, which is actual more than ever. Indeed, the awareness of the relation between nutrition and health started a long time ago. In this day and age, the concept of "functional food", born in Japan in 1980, has been spread with the meaning of food that is developed specifically to promote health or reduce the risk of disease (Serafini et al., 2012). Hence, the investigation of natural resources rich in active compounds with beneficial effects on both animal and human health. Amongst all the bioactive compounds, there is particular interest about polyunsaturated fatty acids (PUFAs), especially ω-3 and ω-6, and their positive role is widely studied in humans (Taranu et al., 2014).

Dietary n-3 fatty acids include α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA). The former occurs in terrestrial oils and the three latter are in marine oils (Cherian and Quezada, 2016). Whereas, among n-6 FA linoleic acid (LA) is the precursor to the n-6 series of fatty acids (Patterson et al., 2012).

In western countries, the increase use of LA-rich vegetable oils has led to a higher consumption of n-6 PUFA, if compared to n-3 PUFA (Anderson and Ma, 2009). In this fashion, dietary n-6:n-3 ratio moves away from the optimal value (1-4:1), being around 15-20:1 and exacerbating, in this way, the incidence of cardiovascular disease, rheumatoid arthritis, and cancer (Kakani et al., 2012).

Hence, there is an increased interest in modifying animal food products with beneficial fatty acid.

Particular interest is for eggs. Characterized by digestible proteins, lipids, vitamins, minerals and molecules that promote health (Anton et al., 2006), as the other poultry products, are the major source of animal protein around the world (Cherian, 2012). Previous studies have demonstrated how their fatty acid composition can be modified by manipulating the poultry diet. Specifically, it has been shown how adding ω-3 rich meals can increase ω-3 fatty acid content of eggs (Kakani et al., 2012).

Although the positive effects of PUFA, their sources are not in sufficient quantity for the food industry and the research is looking for novel n-3 feedstuffs with nutritional and healthy effects (Taranu et al., 2014). Flax is indicated as an excellent source of ALA and marine oils are rich of EPA and DHA but their demand for human health-food uses is high. The availability of the sources is not the only aspect to take into consideration. Indeed, we do not have to forget that feed represents the major cost for food animal production. So, the investigation for alternate sources of n-3 fatty acid-rich feeds could reduce production costs and guarantee enriched food for human consumption (Cherian et al., 2009, Cherian, 2012, Cherian and Quezada, 2016).

A possible option can be *Camelina sativa*, characterized by the same composition of flaxseed, high levels of ω3/ω-6 fatty acids, protein, and ϒ-tocopherol (Zubr and Matthaus, 2002, Cherian, 2012).

Camelina is an oilseed crop of the Brassica (Cruciferae) family. The cultivation of the crop is environmentally friendly because it does not need application of pesticides/herbicides (Zubr, 2003). Cultivated in Europe for over 2000 years for oil and livestock fodder, there is a renew interest about its use as a biofuel source (Cherian, 2012). This increase production has led to exploit the meal produced from the oil extraction for poultry industry, in order not to compete

with the human diet (Kakani et al., 2012). Camelina by-products (like meal/oil cake) are rich in protein, essential amino acids, fat and essential ω-3 and 6 fatty acids. For this reason, it could be fed to animals (Meadus et al., 2014).

4.2.1. *Camelina sativa* meal: chemical composition and nutritional value

The seed is rich in fat (>38.9%), α-linoleic acid (30%), 25.8% of crude protein and 42-45% of oil on dry matter basis (Cherian, 2012). The result of crushing and pressing the seeds is the oil, characterized by a high content of unsaturated fatty acids: the ALA is predominant (35% of the total fatty acids), LA and eicosanoid acid around 15% and then erucic acid about 3%. After the exploitation of the oil the result is camelina meal. The part not subjected to this mechanical process is used as bio-fuel (Zubr, 1997). The high protein, energy, omega-3 and omega-6 fatty acid and essential amino acid content of camelina meal makes it a potentially appropriate source of protein, essential fatty acids and amino acids for using in poultry ration, increasing the market value of the crop (Cherian, 2012).

However, camelina, as for most Brassica crops, can contain glucosilonates, with detrimental and antinutritional characteristics. For their toxicity, in humans they can be responsible of impairment of thyroid, growth, fertility, irritation of gastro-intestinal mucosa (Efsa Panel on Contaminants in the Food Chain, 2008, Russo et al., 2014).. On the other hand, feeding the animals can have negative effects, like reduced diet palatability, decreased growth and production (Pekel et al., 2009). For this reason, different inclusions of camelina have been testing in poultry diet, to assess the right level of integration to enrich food-derived animals without detrimental effects. However, recent experiments have demonstrated possible positive effects of those glucosinolates, such as being chemo-protective and anti-cancer (Meadus et al.,

2014).

4.2.2. *Camelina sativa* in poultry diet

As being monogastric, the quantity and the type of dietary lipids can affect the chemical characteristics of the egg yolk, modifying the content of fatty acids, and fat-soluble vitamins. As an outcome, egg lipids reflect dietary fat content in chickens (Cherian, 2012). Thanks to their high capacity for lipid biosynthesis, the alteration of fatty acid composition can be direct or indirect due to further acyl chain elongation and desaturation in the liver (Cherian and Sim, 1996).

Recent researches (Cherian, 2012, Aziza et al., 2013, Cherian and Quezada, 2016) conducted on feeding camelina meal in egg layers have demonstrated that an inclusion up to 10% increases the omega-3 fatty acid content, not compromising bird performances.

Newly, Cherian et al. (2016) have proved that consumption of eggs from hens fed with the inclusion of camelina meal could provide more than 300 mg/day of n-3 fatty acids to the human diet (when the recommended daily intake of EPA and DHA is 300-500 mg (Kris-Etherton et al., 2000)). Unfortunately, the information about administering camelina meal to laying hens and egg lipid composition are scant (Cherian et al., 2009).

The obtained results have shown that feeding *Camelina sativa* does not affect the functional properties and the sensory quality of eggs (Rokka et al., 2002). Rokka et al. (2002) fed camelina at a level of 5% in laying hens. They observed that functional properties and quality of the eggs were not negatively affected by the concentration of seed oil, demonstrating that camelina did not impart the same off flavor and odor as flax meal and oil. Later, Kakani et al. (2012) tested

extruded camelina meal in Lohmann White Leghorn hens with 3 different inclusions (0 -5 - 10%), finding out that the addition of the meal up to 10% of concentration did not have effects on interior and sensory quality of eggs.

Likewise, camelina has been supplemented to broiler chickens to assess the effects of this plant on their performance. Quite recently, Jaśkiewicz et al. (2014) tested camelina oil on 90 Ross 308 broiler chickens in comparison to other two supplementations, soybean and rapeseed oil. The addition of these oils affected the fatty acid profile of the meat, in particular camelina oil enhanced α-linolenic content in the muscle and abdominal fat. An example of supplementation of camelina cake has been made by Nain et al. (2015) on 744 Ross 308 for 42-d experiment, with 4 inclusions of the meal (0%- 8%- 16%- 24%). They evaluated the fatty acid content of different organs, observing an enhanced of the overall n-3 PUFA concentration (especially ALA, DPA, and DHA) with greater α-linolenic acid content. These outcomes suggest that *Camelina sativa* is a promising plant source for the enrichment of omega-3 fatty acids in hen eggs, but further investigations are needed. Moreover, the inclusion of camelina has been proved beneficial because of the reduction of oxidative stress due to the presence of these bioactive compounds. This was observed, measuring the thiobarbituric reactive substances (TBARS) in eggs from hens fed <10% camelina meal (Cherian, 2012). Very few studies have reported sensory aspect of eggs from hens fed camelina.

The demand for safer egg products by consumers leads to consider and evaluate deeply the quality of the eggs after deposition, starting from the distribution chain to the consumers' table.

Since the egg exit has the same of the passage of faeces, bacteria can be on the outside of a shell egg. The main element related to the conservation and the hygiene of fresh eggs that

determines also their commercial shelf life, is the presence of pathogenic microorganisms (like *Salmonella Enteritidis*). Legislation is different between USA and Europe. For the former, the U.S. Food and Drug Administration (FDA), responsible for protecting the American public health by providing science-based information to the public, announced a regulation expected to prevent each year approximately 79,000 cases of foodborne illness and 30 deaths caused by consumption of eggs contaminated with this bacterium (USDA, 2008). As it is recognized as a serious public health problem, the authority in charge of it are many and the regulation requires preventive measures dealing with egg production and supply. It means from poultry houses to consumers' table, including all the procedures to keep the product safety, such as washing and pasteurize the eggs.

European Union egg marketing laws, on the other hand, state that Class A eggs – those found on supermarkets shelves, must not be washed, or cleaned in any way. Indeed, water can promote bacterial growth and provide a vehicle for pathogens to pass through the pores on the surface of the egg shell. That is the reason why the European Union has excluded egg washing, declaring that this procedure can be more damaging than good (Hazards, 2015).

Conversely, a common and shared critical point between the two authorities is the control of temperature fluctuation to maintain the product safe. Indeed, it has been recognized that an effective way to minimize any increase in risk during extended storage is to keep the eggs refrigerated both at retail and at the household.

The aim of this trial was to evaluate the inclusion of 7,5% of camelina cake in the diet of laying hens on performance, egg quality characteristics, fatty acid composition and lipid oxidative stability.

4.3. Materials and Methods

4.3.1. Experimental design, animals and housing conditions

The trial was conducted at the Centro Clinico-Veterinario e Zootecnico-Sperimentale d'Ateneo di Lodi, Università degli Studi di Milano. The protocol for animal care, handling, and sampling was reviewed and approved by the Università degli Studi di Milano Animal Care and Use Committee (Protocol number No. 26/14). Thirty-two Hy-Line Brown laying hens from the same stock and homogenous for age (24 weeks) and initial live weight (1807 g ± 46) were randomly divided in two experimental groups on the basis of the adopted dietary treatments, in a 56-d trial. Experimental groups consisted of eight replicates per group with 2 hens per cage. Hens were all housed in the same room to avoid any room effect on performance. Animal welfare was ensured according to the existing regulation on laying hens rearing (D.L 267/2003) and cages were enriched with a nest, a roost and a scratch area. Lighting programme followed the Management guide for Hy-Line Brown laying hens (2015) and water and feeds were provided ad libitum. After a two-week acclimation period during which all animals were fed the same basal diet, the hens were assigned to one of the two dietary treatments: C, basal diet; T, diet containing 7.5% camelina meal (crude lipids 8% on dry matter basis) whose chemical and fatty acid composition is shown in Table 5). Camelina cake fatty acid composition is shown in Table 6. Experimental diets were designed to be isonitrogenous and isoenergetic, with no coccidiostatics inclusion for the whole duration of the trial.

4.3.2. Chemical composition of the diets, sample collection and recorded parameters

Before the start of the trial, samples of the two diets (200 g of each diet) were analysed for water content (Eu. Reg. 152/2009), Crude Protein (CP; AOAC 2001.11 ed18th 2005), Ether Extract (EE; DM 21/12/1998 - GU n°31 8/2/1999 suppl n°13), Crude Fibre (CF; AOCS Approved Procedure Ba 6a-05), Ash (AOAC 942.05 ed 18th 2005) and Neutral Detergent Fibre (NDF; AOAC 2002.04 ed 18th 2005) (Table 5). Moreover, fatty acid composition of camelina meal and diets were determined following the same procedure used for yolk fatty acid analysis as described below, and the n6/n3 fatty acids ratio was determined (Table 6). Individual body weight was recorded weekly and feed intake per cage determined. Egg production per cage was recorded daily and daily laying rate was calculated on weekly basis as the ratio between the number of eggs produced and an ideal number of eggs (2 eggs/cage/day). On days 0, 28, 56, two eggs per cage were collected for egg quality measurements. Egg weight was determined weekly and conversion rate was calculated during the laying period as weekly feed intake per cage over weekly produced egg mass per cage. All the parameters dealing with hen performance are shown in Table 7.

Skin and feather condition, footpad lesions and keel fractures were evaluated and recorded following the (Welfare Quality® Protocol, 2009) once a week. Daily incidence of mortality was registered.

Table 5. Ingredients, chemical composition and fatty acid composition of the two experimental diets (g/100 g total fatty acids) (C, control; T, treated).

Ingredients	C	T
Maize	56.55	57.22
Soybean meal (48% CP)	24.40	19.00
Oyster shell	4.65	4.65
Calcium carbonate	4.65	4.65
Soybean oil	4.55	4.00
Monobicalcium phosphate	1.90	1.90
NaCl	0.35	0.35
Methionine, DL	0.15	0.15
L-Lysine, HCL	0.00	0.08
Soybean hulls	2.30	0.00
Camelina cake	0.00	7.50
Vitamin mineral premix[1]	0.50	0.50
Chemical composition (%DM)		
DM	91.50	91.50
CP	18.66	18.65
EE	8.29	8.29
CF	3.58	3.60
NDF	10.96	10.72
Lys	0.97	0.97
Ca	4.62	4.60

P	0.81	0.84
Ash	15.33	15.24
EM (kcal/kg)	3192	3138
Fatty acid composition		
14:0	0.07	0.08
16:0	11.57	11.84
16:1 n-7	0.11	0.16
17:0	0.14	0.13
18:0	3.82	3.83
18:1 n-9	23.05	23.08
18:1 n-7	1.28	1.28
18:2 n-6	53.45	49.50
18:3 n-3	5.62	7.43
20:0	0.37	0.47
20:1 n-9	0.24	1.45
20:2 n-6	0.00	0.23
22:1 n-9	0.09	0.36
22:5 n-3	0.18	0.16
SFA	15.98	16.34
MUFA	24.78	26.34
PUFA	59.25	57.32
n-6 PUFA	53.45	49.73
n-3 PUFA	5.80	7.60
n-6/n-3	9.21	6.54

[1] *supplementation per kg:*

Vit A E672 (protected) U.I. 10000, Vit D3 E671 U.I. 2000, Vit E (protected) 20mg, Vit B1 2mg, Vit B2 5mg, D-calcium pantothenate 10mg, Vit B6 3mg, Vit B12 0.01mg, Nicotinic acid 25mg, Folic acid 0.50mg, Choline 100mg Iron E1 (ferrous

carbonate) 50mg, Iodine E2 (calcium iodate) 1mg, Copper E4 (copper oxide) 5mg, Manganese E5 (Manganic oxide) 60mg, Zinc E6 (zinc oxide) 94mg, Selenium E8 (sodium selenite) 0.15mg

Table 6. Chemical composition and fatty acid composition of camelina cake.

Chemical composition (%DM)	
DM	90.42
CP	38.41
EE	8.11
NDF	20.24
Ash	6.07
EM (kcal/kg)	2226
Fatty acids (g/100 g total fatty acids)	
16:0	6.83
18:0	2.40
18:1 n-9	13.85
18:1 n-7	1.09
18:2 n-6	22.29
18:3 n-3	31.16
20:0	1.32
20:1 n-9	12.91
20:1 n-7	0.92
20:2 n-6	2.07
20:3 n-3	1.31

22:1 n-9	3.14
24:1	0.73

4.3.3. Egg quality and shelf-life assays

Egg quality was determined overall the egg production on days 0, 28 and 56 of the trial, gathering eggs for two consecutive days to ensure the collection of 2 eggs per cage. Analysis were performed the day after the collection (+2, 30, 58). The analysis accounted for egg, yolk, shell and albumen weight, egg width and length, albumen and yolk height, albumen pH, Haugh unit, yolk colour (DSM Egg yolk fans®), air cell depth, and ω6:ω3 yolk ratio.

Specifically, Haugh unit (HU) was evaluated before all other parameters, measuring whole egg weight on a digital balance to nearest 0.01 mg, then carefully breaking the egg in a flat surface and measuring the albumen height with a specific micrometre (Albumen Haugh Tester Baxlo, Barcelona – Spain). HU was then calculated with the following formula:

$HU = 100 * \log (h - 1.7w^{0.37} + 7.6)$, where HU = Haugh unit, h = observed height of the albumen in millimetres and w = weight of egg in grams.

Yolk index was calculated by dividing the yolk height by the yolk diameter of the egg broken onto a flat surface.

The length and breadth (mm) of each egg were measured using a digital calliper to nearest 0.01 mm, and a shape index (SI) was calculated (SI=egg length:breadth). Shell weight (g) was measured after washing the shells and drying them overnight at 80°C. Egg surface area was calculated as described by Mabe et al. (2003).

Egg shelf life (Table 8) was assessed evaluating the antioxidant status on eggs harvested at days 56, 57 and 58. They were analysed at 0, 9, 21, and 28 days after storing at 9 °C and a 69% RH

environment. Oxidative stability of a homogenous-pooled sample of yolk per cage was evaluated by Thiobarbituric acid reactive substances (TBARS) as described by (Hayat et al., 2010). To avoid any contamination, the pooled samples were collected drawing off the yolk in the vitelline membrane with a syringe and moving the content in a tube kept in ice and then gently mixed. Briefly, egg yolks were weighed (2 g) and placed into a test tube with 18 ml of 3.86% perchloric acid. The mixture was homogenized with a Brinkman Polytron homogenizer (Type PT 10/35, Westbury, NY) for 15 sec at high speed. Then, butylated hydroxytoluene (50 μL, in 4.5% ethanol) was added to each sample during homogenization to control lipid oxidation.

The homogenate was filtered through Whatman no. 1 filter paper and the filtrate (2 ml) was mixed with 2 ml of 20 mM TBA in distilled water and incubated in the dark in water bath for 10 minutes (Irandoust and Ahn, 2015). After cooling, the absorbance of the filtrate was determined at 532 nm and thiobarbituric acid reactive substances (TBARS) were expressed as milligrams of malondialdehyde (MDA)/kg of yolk (Cherian et al., 1996). Moreover, on eggs collected at days 0, 28 and 56 of the trial fatty acid composition and n6/n3 fatty acid ratio were determined.

4.3.4. Egg yolk fatty acid analysis

The fatty acid profile of egg yolk was determined by GC-FID analysis. The extraction of total lipids was performed according to the Folch (1957) method with chloroform:methanol (2:1), using 1000 mg of omogenate yolk The preparation of fatty acid methyl esters was performed according to Christie (2003). Briefly, the lipid sample (20 mg) was dissolved 10% methanolic hydrogen chloride (2 mL). A 1 mL solution of tricosanoic acid (1 mg/ml) in toluene was added

as internal standard. The sample was sealed and heated at 50 °C overnight; then, 2 mL of a 1M potassium carbonate solution and 5 mL of 5% NaCl were added to each sample. The FAMEs were extracted with 2×2 mL of hexane and the mixture was evaporated under nitrogen. The sample was dissolved in 1 mL hexane and 1 μL sample was injected into the gas-chromatograph, in split mode (split ratio 1:100). Fatty acid analysis was carried out on an Agilent gas-chromatograph (Model 6890 Series GC) fitted with an automatic sampler (Model 7683) and FID detector. The carrier gas was helium with a flow rate of 1.0 mL min-1 and an inlet pressure of 16.9 psi. A HP-Innowax fused silica capillary column (30m×0.25mm I.D., 0.25 μm film thickness; Agilent Technologies) was used to separate fatty acid methyl esters. The oven temperature program for separation was from 100 to 180 °C at 3 °C min-1, then from 180 to 250 °C at 2.5 °C min-1 and held for 10 min. Carrier gas was helium at 1.0 mL min−1, inlet pressure 16.9 psi. Fatty acids were identified relative to known external standards (Fame 37 standard mix and Marine source fatty acid, Sigma-Aldrich, Milan, Italy) and were expressed as percentage of total fatty acids.

4.4. Statistical analysis

All the data were analysed by two-way ANOVA using the Proc MIXED for repeated measurements of SAS (SAS Inst. Inc., Cary, NC) with treatment and time as fixed effects and the animal or the cage as random effect. The cage was considered the experimental unit for feed intake, conversion rate and laying rate, while the hen represented the unit for all other investigated parameters. Differences were considered significant for $P<0.05$. A tendency toward a significant difference between treatment means was also considered at $P<0.10$.

Performance data of the first week were excluded from the analysis because of the influence associated to the change of the diet. Any difference was hardly attributable to the singular

treatment, but it was nested to the dietary change (from pre-trial to experimental diet). In particular, the control diet was soybean maize based, so, similar to the commercial pre-trial diet. At contrary, the treatment was quite different from the pre-trial diet because of camelina cake.

4.5. Results

4.5.1. Hen performance, egg quality and shelf-life

No differences were detected among the experimental groups for body weight during the trial (P=0.88) and at the end of the experimental period (1928 g ± 46, control group vs. 1842 g ± 46, camelina group; P>0.05). Laying rate and daily feed intake were not affected by dietary treatments, with the exception of a 7.6% decrement in feed consumption by T hens at 35-42 days of the trial (P<0.05) (Table 7). Feed conversion rate was significantly improved (P<0.05) in T hens from day 28 until the end of the experimental period, or at least it showed a trend for improvement (P<0.1).

Most parameters of egg quality were not affect by camelina meal supplementation, with the exception of lower yolk index in T group at day 56 (P<0.05)

Egg yolk total lipid content and fatty acid profile at 28 and 56 d of the trial are presented in Table 9 and Figure 14.

Egg yolk total lipid content was not modified by the dietary treatment. Dietary supplementation of camelina meal significantly reduced SFA content in egg yolk on day 28 and 56 of the trial by approximately 3.6% (P value<0.05), while total MUFA, PUFA, n-3, n-6 fatty acids were not modified. Egg yolk from T hens had lower content of myristic (C14:0) (P value= 0.02), palmitic (C16:0) (P value= 0.03), stearic (C18:0 (P value= 0.04), myristoleic

(C14:1) (*P* value= 0.05), ϒ-linolenic (C18:3 n-6) (*P* value= 0.05), arachidonic (C20:4 n-6) (*P* value<0.05), heneicosapentaenoic (C21:5 n-3) (*P* value< 0.05) and adrenic (C22:4 n-6) (*P* value< 0.05) fatty acids between 28 and 56 days of trial while with a concomitant increase of eicosanoic acid (C20:1 n-9) (+47%) (*P* value< 0.05) and α-linolenic acid (C18:3 n-3) (+49%) (*P* value< 0.05).

ω6/ω3 ratio was calculated for both the experimental diets at days 0, 28, 56. We observed a significant diet effect (8.21 C vs 6.88 T; *P* value = 0.02) and time effect (9.85, 6.61, 6.18 at days 0, 28 and 56 respectively; *P* value< 0.05) with a significant difference between days 0 – 28 and 0 – 56 (*P* value< 0.05). We did not observe a significant interaction between diet and time (*P* value= 0.81) (Table 9).

Egg shelf life was determined by the same parameters used for egg quality assessment, with no differences observed between C and T groups (Table 8).

Likewise, the spectrophotometric evaluation on the same eggs did not show diversities in lipid oxidation products in the eggs from both of the trial groups.

Table 7. Laying rate, daily feed intake and feed conversion rate of control (C) and treated (T) hens.

	Laying rate		Daily feed intake		Feed conversion rate	
Days	C	T	C	T	C	T
7-14	0.96	0.91	114.24	113.59	1.83	1.77
14-21	1.00	0.94	114.20	112.46	1.79	1.74
21-28	0.98	0.96	113.96	112.87	1.77	1.73
28-35	0.98	0.98	113.07	109.83	$1.77^{(a)}$	$1.70^{(b)}$
35-42	0.99	0.96	114.74^{a}	105.96^{b}	1.82^{a}	1.64^{b}
42-49	0.96	0.96	107.71	105.29	$1.70^{(a)}$	$1.63^{(b)}$
49-56	0.96	0.97	107.82	103.85	$1.69^{(a)}$	$1.62^{(b)}$
7-56	0.98	0.96	112.25	109.12	1.77^{a}	1.69^{b}
SEM	0.02		1.96		0.03	

^{a,b} $P<0.05$; $^{(a,b)}$ $0.1\geq P\leq 0.05$.

Table 8. Evaluation of egg shelf-life and TBARS (C, control; T, treated).

Items	0d		9d		21d		28d		SEM
	C	T	C	T	C	T	C	T	
Egg weight (g)	62.94	62.64	61.42	62.32	60.29	62.1	63.05	63.5	1.59
Shape index (%)	76.89	77.56	77.1	78.49	78.07	78.81	77.04	78.07	0.84
Albumen index (%)	9.83	10.11	7.85	8.25	7.32	7.3	6.71	7.06	0.48
Yolk index (%)	47.85	45.7	44.66	44.37	43.09	42.7	43.06	43.15	0.73
Haugh Unit (value)	87.29	88.06	78.57	81.1	77.16	75.97	73.72	76.04	1.95
Albumen pH (value)	8.48	8.44	8.99	9.04	9.08	9.07	9.12	9.08	0.44
Egg length (mm)	57.45	57.48	57.15	56.7	56.17	56.32	57.83	57.39	0.63
Egg width (mm)	44.11	44.56	44.05	44.49	43.82	44.34	44.44	44.75	0.37
Surface area (cm^2)	73.89	73.51	72.65	73.41	71.72	73.22	73.96	74.35	1.33
Area (cm^2)	74.96	74.58	73.78	74.51	72.9	74.33	75.02	75.4	1.27
Air chamber egg height (mm)	0.91	0.94	2.25	2.41	2.18	2.44	3.06	3.22	0.11

Albumen height (mm)	7.79	8.06	6.44	6.83	6.17	6.08	5.78	6.12	0.29
Albumen length (mm)	80.01	80.34	83.35	84.04	85.95	85.01	87.63	87.83	2.36
Albumen width (mm)	67.02	68.38	69.16	71.03	69.51	71.63	72.82	74.59	2.05
Yolk height (mm)	18.59	18.16	17.89	17.69	17.44	17.39	17.4	17.31	0.26
Yolk diameter (mm)	38.94	39.87	40.17	39.89	40.52	40.8	40.46	40.1	0.54
Yolk weight (g)	14.67	14.32	15.09	14.91	15.23	15.05	15.13	14.72	0.37
Yolk %	23.29	22.25	24.62	23.96	25.21	24.26	24.39	23.61	0.51
TBARS (mg MDA/kg yolk)	0.93	1.05	1.08	0.91	0.92	0.82	0.89	0.94	0.10

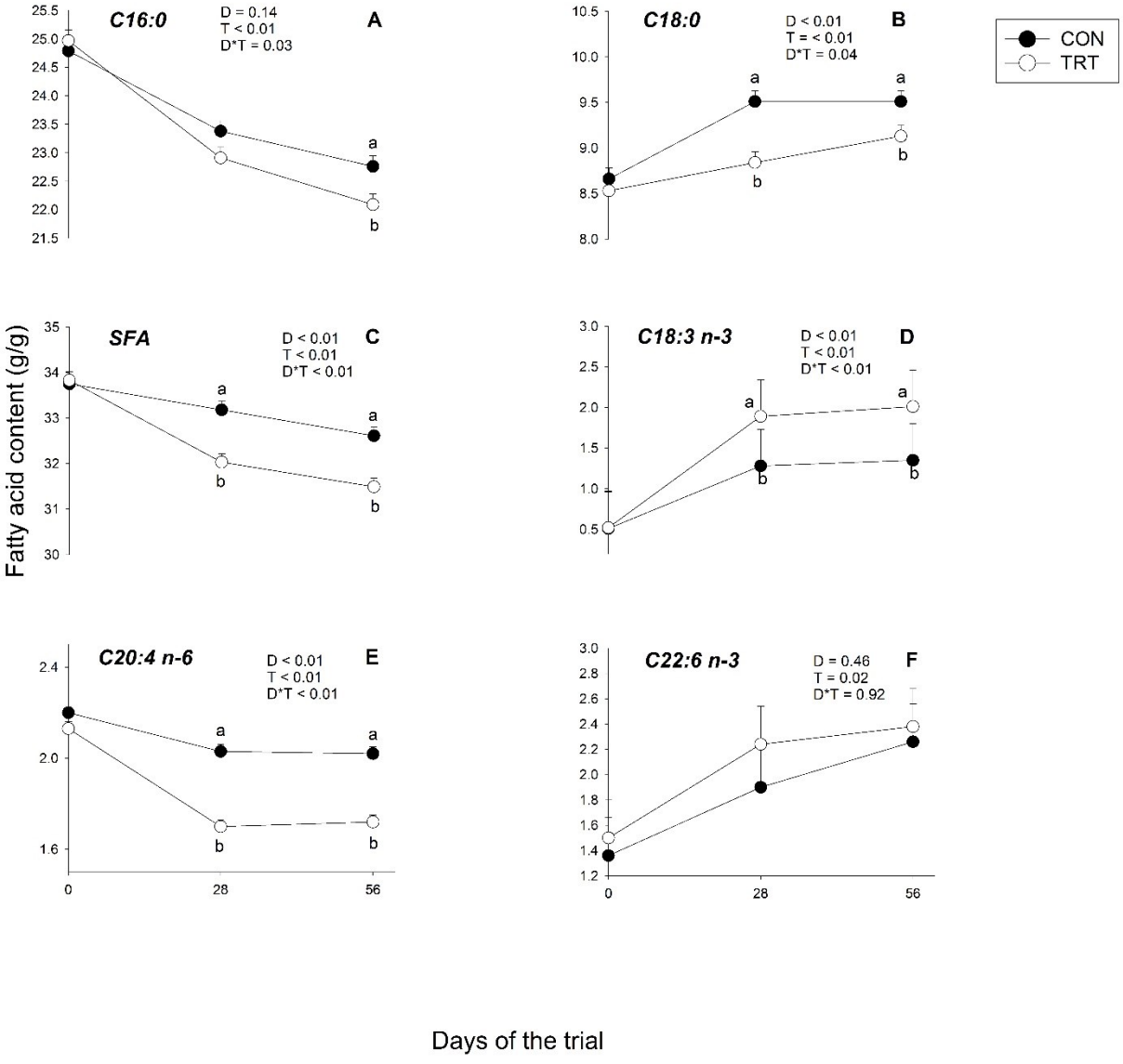
Table 9. Fatty acid composition of egg yolk assessed at days 28-56 of the trial (C, control; T, treated). (SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid). (g/100g total fatty acids).

Fatty acid	28C	28T	56C	56T	SEM
14:00	0.29	0.27	0.32[a]	0.26[b]	0.02
16:00	23.38	22.91	22.76[a]	22.09[b]	0.19
16:1 n-7	1.79	1.76	1.69	1.56	0.09
18:00	9.51[a]	8.84[b]	9.51[a]	9.13[b]	0.12
18:1 n-9	33.83	34.42	33.48	33.79	0.43
18:2 n-6	22.76	22.68	23.33	23.84	0.43
18:3 n-6	0.12[a]	0.10[b]	0.12[a]	0.09[b]	0.01
18:3 n-3	1.28[b]	1.89[a]	1.35[b]	2.01[a]	0.45
20:1 n-9	0.17[b]	0.28[a]	0.19[b]	0.28[a]	0.01
20:4 n-6	2.03[a]	1.70[b]	2.02[a]	1.72[b]	0.03
21:5 n-3	0.17[a]	0.14[b]	0.16[a]	0.14[b]	0.01
22:4 n-6	0.30[a]	0.20[b]	0.29[a]	0.18[b]	0.01
22:6 n-3	1.90	2.24	2.26	2.38	0.30
SFA	33.18[a]	32.03[b]	32.61[a]	31.49[b]	0.19
MUFA	37.28	37.98	36.85	37.12	0.49
PUFA	29.30	29.75	30.28	31.16	0.57
n-3	3.60	4.60	4.02	4.81	0.33
n-6	25.61	25.06	26.16	26.25	0.44
n-6:n-3	7.50[a]	5.72[b]	6.76	5.61	0.62

[a,b] $P<0.05$

*Figure 14. Trend of selected fatty acid content in egg yolk at different days of the trial (0, 28, 56). A Palmitic acid (C16:0); B Stearic acid (C18:0); C Saturated fatty acids (SFA); D α-linolenic acid (ALA) (C18:3 n-3); E Arachidonic acid (AA) (C20:4 n-6); F Docosahexaenoic acid (DHA) (C22:6 n-3). (CON, control; TRT, treated; D, diet; T, time; D*T, diet*time).* [a,b] *P<0.05.*

(g/100g total fatty acids).



4.6. Discussion

In the present trial, we observed a reduction in daily average intake between 35 and 42 days by camelina fed hens, with a concomitant improved feed conversion rate. Other studies (Cherian, 2012) reported that supplementation with camelina meal up to 10% of poultry diet did not compromise performance of broilers and egg laying hens. Furthermore, Kakani et al. (2012) did not observe any reduction in feed consumption when camelina meal was included at 5 and 10% of the diet.

On the other hand, Pekel et al. (2009) and Ryhanen et al. (2007) observed that inclusion of camelina (10% for Pekel et al. (2009); 5-10% for Ryhanen et al. (2007)) affected the ingestion in broiler chickens, reducing diet palatability, decreasing growth, and decreased production. This could be explained by the presence in *Camelina sativa* of glucosinolates, secondary plant metabolites with antinutritional activities (Schuster and Friedt, 1998, Russo and Galasso, 2014).

4.6.1. Fatty acid profile of egg yolk

Egg yolk total lipid content was not modified by the dietary treatment, in agreement with Cherian and Quezada (2016) who tested camelina oil seed at 10% of supplementation. In contrast, a previous study by Cherian (2009) with 10% inclusion of camelina meal in the diet, showed a lower fat content in egg yolk of treated compared to control (31.5, 31.9, 30.8, 29.5 % for control, CAM5, CAM10 and CAM15 respectively; $P<0.05$). The authors explained this starting from the point that triacylglycerol-rich very low density lipoprotein (VLDL) is the major lipoprotein in the dry yolk mass (Speake et al., 1998). In addition, its secretion by the liver is dependent on the activity of stearoyl-coenzyme A desaturase (able to convert saturated into monounsaturated fatty acids) (Cook, 2002). It seems that omega 3 fatty acids can reduce

the activity of this coenzyme, reducing the synthesis of VLDL and diminishing yolk mass and lipid content (Ntambi, 1999).

In the present trial we observed a decrement of total SFA content in egg yolks of camelina group (-3.6%) at days 28 (32.03 and 33.18 g/100 g total fatty acids for T and C, respectively) and 56 (31.49 and 32.61 g/100 g total fatty acids for T and C, respectively), in accordance to Cherian et al. (2009), Kakani et al. (2012) and Aziza et al. (2013). In particular, in our trial supplementation with camelina meal lowered levels of palmitic (C16:0) and myristic (C14:0) acids at day 56, and of stearic acid (C18:0) at days 28-56. Also Cherian et al. (2009) observed a reduction of palmitic acid in eggs of hens fed 10% camelina meal, but no differences for stearic content.

Total content of MUFA in eggs did not show any difference between the two experimental groups. Previous studies (Aziza et al., 2013, Cherian and Quezada, 2016) reported a reduction of MUFA following camelina supplementation. In our trial camelina enhanced eicosanoic acid (C20:1 n-9) in egg yolk, in accordance with Cherian and Quezada (2016) and this is not surprising because camelina seeds contained 15.7% of eicosanoic acid (Table 6).

Checking the overall amount of PUFA, no variations amongst the dietary treatments were observed over time. However, examining the abundance of the singular PUFA in egg yolk, we can point out some differences determined by camelina meal inclusion in the diet, such as a reduction of 17% ($P<0.05$) in arachidonic acid, in accordance with (Cherian et al., 2009, Aziza et al., 2013, Cherian and Quezada, 2016) and an important rise in α-linolenic acid ($P<0.05$), also reported in other studies (Cherian et al., 2009, Kakani et al., 2012, Aziza et al., 2013, Cherian and Quezada, 2016).

Arachidonic acid is formed from linoleic acid through desaturation and elongation (Cherian et al., 2009) and, in accordance with Cherian and Quezada (2016) , we did not find any significant

difference related either to the diets or the time for linoleic acid.

Cherian and Quezada (2016) also stated that high linolenic acid was found in yolk egg with a lower ratio of ω6:ω3 fatty acids in camelina treatment. It is well known that the enzyme Δ6-desaturase is the rate-limiting step in the synthesis of arachidonic acid and DHA form their precursors (Brenner, 1971). Moreover, in the competition between ω6 and ω3 FA the latter has priority as substrate (instead of n-6) in the desaturation-elongation pathway, resulting in a reduced n-6 and n-3 ratio in the egg (Cherian et al., 2009). Consequently, this occurrence may have decreased the competition of linoleic with α-linolenic acid for desaturase and elongase enzymes involved in bioconversion to AA and DHA, resulting in reduced egg content of these fatty acids. Furthermore, Goldberg et al. (2013) observed that increasing the dietary ALA, like in the case of camelina addition above certain amount (15%), did not affect yolk DHA content. This made them to make two hypotheses: the retro conversion of DHA back to DPA and EPA may have occurred or that ALA conversion was suppressed, or both.

Thus, our results related to DHA content, similar to Aziza et al. (2013), could be explained by these suppositions. On the contrary, in other studies DHA content in yolk was incremented by camelina treatment (Cherian et al., 2009, Kakani et al., 2012, Cherian and Quezada, 2016).

Concerning linoleic acid, our results are in agreement with Kakani et al. (2012), Cherian and Quezada (2016) but in contrast with the increment observed by Cherian et al. (2009) and Aziza et al. (2013).

No statistical differences for shelf life and lipid oxidative stability parameters were observed. Limited studies are available as regards oxidative stability and quality of meat and egg as influenced by camelina supplementation. Aziza et al. (2010) included camelina meal at either 5 or 10% in broilers diet and observed a drop in thiobarbituric reactive substances (TBARS) in 2 days (short-term) and 90 days (long-term) of storage of meat. This may be attributed to the

capacity of camelina meal to inhibit lipid oxidation and enhance antioxidant capacity thanks to its content of bioactive compounds. As matter of fact, camelina contains tocopherols and phenolic products (Matthäus, 2002), which can counteract the potential oxidation caused by fats in the diet (Quezada and Cherian, 2012).

More recently, Quezada and Cherian (2012) observed that camelina meal (10%) led to a significant reduction in lipid oxidation products in dark meat.

These results seem different looking at the ones obtained in other studies. For instance, observing data by Cherian et al. (2009), who fed camelina meal to 48 Brown Leghorn laying hens, we noticed higher TBARS values for animals fed with the 15% of inclusion of camelina meal, compared to the other two camelina groups (5-10% of addition) or control regimens. This could be related to a greater lipid oxidation due to the high content of n-3 fatty acids.

4.7. Conclusions

Camelina sativa, for its nutritional composition, could be incorporated into poultry diets as a source of functional fatty acids. Feeding *Camelina sativa* meal at the dose of 7.5% to laying hens has been able, not only to improve beneficial n-3 fatty acids (alfa-linoleic acid) and decrease SFA content, but also to reduce omega-6:omega-3 ratio in eggs. This strategy could be used to provide healthier food for human consumption (so called functional food), considering also the low competition for nutritional sources. Indeed, *Camelina sativa* is a non-food crop not competing for human food uses. Furthermore, we observed an improved feed conversion rate in laying hens, without any detrimental effect on performance or egg quality.

5. GENERAL DISCUSSION

Acting as not only a primary energy source, but also being progenitors of many biological lipids and participating at numerous signalling pathways, fatty acids play a central role for the health of aquatic and terrestrial organisms (Hwang and Rhee, 1999, Hixson et al., 2015).

It has been proved in human field and recently confirmed in animal medicine as well, that FA role is crucial for normal growth and a proper immune function (Calder, 2013, Tsiplakou and Zervas, 2013a, b). In fact, FA have a number of physiological roles as energy substrates, structural and functional components of cell membranes, precursors for lipid mediators, components affecting signal transduction pathways and gene transcription (Kremmyda et al., 2011).

As a result, at animal level, lipid supplementation is widely used being a strategy to modulate the animal answer to stressing events, as for cows during peripartum (Tsiplakou and Zervas, 2013a, b, Zapata et al., 2015). Indeed, most dairy cattle (perhaps most mammals) face an inflammatory state in the weeks across calving (Bradford et al., 2015). A moderate inflammation is a physiological adaptation to pregnancy and lactation (Sordillo et al., 2009). Bauman and Currie (1980) described "homeorhesis" as the orchestrated and coordinated control in metabolism of body tissues in order to support the physiological state during pregnancy and lactation. The situation, however, is exacerbated when the degree of inflammation reaches the point of impairing cow productivity (Bradford et al., 2015) and uncontrolled tissue damage, pathology and disease (Calder, 2018). These findings and the several researches on fat supplementation to cattle (Invernizzi et al., 2016, Meignan et al., 2017, Shin et al., 2017) let us to propose that the dietary fatty acid incorporation (in particular

polyunsaturated fatty acids) can affect both inflammatory state and lipid metabolism of dairy animals.

On the other side, at human level, the development of functional foods (dairy, meat and poultry products) enriched with larger amounts of long chain n-3 fatty acids is the new challenge of the industries (Nieto and Ros, 2012, Ganesan et al., 2014, Cherian and Quezada, 2016). This is possible applying feeding strategies in animal nutrition.

Observing the obtained results in the study on gene expression in goats let us to speculate that n-3 enriched fatty acids (FO) are able to reduce or delay lipomobilization typical of peripartum period. Moreover, the increase of pro-inflammatory cytokines after kidding showed how goats are different to cows and to the other small ruminants, leading to the hypothesis of a postponed and contained inflammation, if compared to cows.

Then, the supplementation of Camelina sativa cake on laying hens is an example of enrichment of foods with a n-3 fatty acid source. In fact, it increased n-3 fatty acids and decreased SFA content in eggs, without detrimental effects on both performance and egg quality.

6. REFERENCES

Acamovic, T., C. Gilbert, K. Lamb, and K. C. Walker. 1999. Nutritive value of Camelina sativa meal for poultry. Br. Poult. Sci. 40 Suppl:S27.

Adamczak, M. and A. Wiecek. 2013. The adipose tissue as an endocrine organ. Semin. Nephrol. 33(1):2-13.

Addis, M., A. Cabiddu, G. Pinna, M. Decandia, G. Piredda, A. Pirisi, and G. Molle. 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid cis-9,trans-11. J. Dairy Sci. 88(10):3443-3454.

Agazzi, A., D. Cattaneo, V. Dell'Orto, P. Moroni, L. Bonizzi, D. Pasotto, V. Bronzo, and G. Savoini. 2004. Effect of administration of fish oil on aspects of cell-mediated immune response in periparturient dairy goats. Small Rumin. Res. 55(1-3):77-83.

Agazzi, A., G. Invernizzi, A. Campagnoli, M. Ferroni, A. Fanelli, D. Cattaneo, A. Galmozzi, M. Crestani, V. Dell'Orto, and G. Savoini. 2010. Effect of different dietary fats on hepatic gene expression in transition dairy goats. Small Rumin. Res. 93(1):31-40.

Aitken, S. L., C. M. Corl, and L. M. Sordillo. 2011. Immunopathology of Mastitis: Insights into Disease Recognition and Resolution. J. Mammary Gland Biol. Neoplasia 16(4):291-304.

Akalin, S., S. Gonc, and G. Unal. 2006. Functional Properties of Bioactive Components of Milk Fat in Metabolism. Pakistan Journal of Nutrition 5(3):194-197.

Akbar, H., E. Schmitt, M. A. Ballou, M. N. Correa, E. J. Depeters, and J. J. Loor. 2013. Dietary Lipid During Late-Pregnancy and Early-Lactation to Manipulate Metabolic and Inflammatory Gene Network Expression in Dairy Cattle Liver with a Focus on PPARs. Gene Regul. Syst. Bio. 7:103-123.

Anderson, B. M. and D. W. Ma. 2009. Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis. 8:33.

Anton, M., F. Nau, and Y. Nys. 2006. Bioactive egg components and their potential uses. Worlds Poult. Sci. J. 62(03):429-438.

Arias, I. M., A. W. W. Wolkoff, J. L. Boyer, D. A. Shafritz, N. Fausto, H. J. Alter, and D. E. Cohen. 2009. The liver: biology and pathobiology. 5th ed.

Arner, E., N. Mejhert, A. Kulyte, P. J. Balwierz, M. Pachkov, M. Cormont, S. Lorente-Cebrian, A. Ehrlund, J. Laurencikiene, P. Heden, K. Dahlman-Wright, J. F. Tanti, Y. Hayashizaki, M.

Ryden, I. Dahlman, E. van Nimwegen, C. O. Daub, and P. Arner. 2012. Adipose tissue microRNAs as regulators of CCL2 production in human obesity. Diabetes 61(8):1986-1993.
Arner, P. and A. Kulyte. 2015. MicroRNA regulatory networks in human adipose tissue and obesity. Nat. Rev. Endocrinol. 11(5):276-288.
Aymond, W. M. and M. E. Van Elswyk. 1995. Yolk Thiobarbituric Acid Reactive Substances and n-3 Fatty Acids in Response to Whole and Ground Flaxseed. Poult. Sci. 74(8):1388-1394.
Aziza, A. E., A. K. Panda, N. Quezada, and G. Cherian. 2013. Nutrient digestibility, egg quality, and fatty acid composition of brown laying hens fed camelina or flaxseed meal. Journal of Applied Poultry Research 22(4):832-841.
Aziza, A. E., N. Quezada, and G. Cherian. 2010. Feeding Camelina sativa meal to meat-type chickens: Effect on production performance and tissue fatty acid composition. The Journal of Applied Poultry Research 19(2):157-168.
Babcock, T. A., W. S. Helton, D. Hong, and N. J. Espat. 2002. Omega-3 fatty acid lipid emulsion reduces LPS-stimulated macrophage TNF-alpha. Surg Infect 3(2):145-149.
Baeza, E., P. Chartrin, M. Lessire, K. Meteau, G. Chesneau, M. Guillevic, and J. Mourot. 2015. Is it possible to increase the n-3 fatty acid content of eggs without affecting their technological and/or sensorial quality and the laying performance of hens? Br. Poult. Sci. 56(6):748-754.
Balboa, E. M., E. Conde, A. Moure, E. Falqué, and H. Domínguez. 2013. I*n vitro* antioxidant properties of crude extracts and compounds from brown algae. Food Chem. 138:1764-1785.
Ballou, M. A., R. C. Gomes, S. O. Juchem, and E. J. DePeters. 2009. Effects of dietary supplemental fish oil during the peripartum period on blood metabolites and hepatic fatty acid compositions and total triacylglycerol concentrations of multiparous Holstein cows. J. Dairy Sci. 92(2):657-669.
Bannerman, D. D. 2009. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J. Anim. Sci. 87(13 Suppl):10-25.
Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms. J. Dairy Sci. 63(9):1514-1529.
Bauman, D. E. and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Production Science 70(1-2):15-29.
Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Bauman, D. E. and A. L. Lock. 2006. Concepts in lipid digestion and metabolism in dairy cows. Tri-State Dairy Nutrition Conference, Proceedings.

Bean, L. D. and S. Leeson. 2003. Long-term effects of feeding flaxseed on performance and egg fatty acid. Poult. Sci. 82(3):388-394.

Bellisle, F., J. E. Blundell, L. Dye, M. Fantino, E. Fern, R. J. Fletcher, J. Lambert, M. Roberfroid, S. Specter, J. Westenhofer, and M. S. Westerterp-Plantenga. 1998. Functional food science and behaviour and psychological functions. Br. J. Nutr. 80 Suppl 1:S173-193.

Benjamin, E. J., M. J. Blaha, S. E. Chiuve, M. Cushman, S. R. Das, R. Deo, S. D. de Ferranti, J. Floyd, M. Fornage, C. Gillespie, C. R. Isasi, M. C. Jimenez, L. C. Jordan, S. E. Judd, D. Lackland, J. H. Lichtman, L. Lisabeth, S. Liu, C. T. Longenecker, R. H. Mackey, K. Matsushita, D. Mozaffarian, M. E. Mussolino, K. Nasir, R. W. Neumar, L. Palaniappan, D. K. Pandey, R. R. Thiagarajan, M. J. Reeves, M. Ritchey, C. J. Rodriguez, G. A. Roth, W. D. Rosamond, C. Sasson, A. Towfighi, C. W. Tsao, M. B. Turner, S. S. Virani, J. H. Voeks, J. Z. Willey, J. T. Wilkins, J. H. Wu, H. M. Alger, S. S. Wong, and P. Muntner. 2017. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 135(10):e146-e603.

Bernard, L., C. Leroux, M. Bonnet, J. Rouel, P. Martin, and Y. Chilliard. 2005. Expression and nutritional regulation of lipogenic genes in mammary gland and adipose tissues of lactating goats. J. Dairy Res. 72(2):250-255.

Bernard, L., C. Leroux, and Y. Chilliard. 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. Adv. Exp. Med. Biol. 606:67-108.

Bernard, L., C. Leroux, Y. Faulconnier, D. Durand, K. J. Shingfield, and Y. Chilliard. 2009. Effect of sunflower-seed oil or linseed oil on milk fatty acid secretion and lipogenic gene expression in goats fed hay-based diets. J. Dairy Res. 76(2):241-248.

Bernard, L., C. Leroux, J. Rouel, C. Delavaud, K. J. Shingfield, and Y. Chilliard. 2015. Effect of extruded linseeds alone or in combination with fish oil on intake, milk production, plasma metabolite concentrations and milk fatty acid composition in lactating goats. Animal 9(5):810-821.

Bertoni, G., A. Minuti, and E. Trevisi. 2015. Immune system, inflammation and nutrition in dairy cattle. Animal Production Science 55(7):943-948.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and. J. Dairy Sci. 91(9):3300-3310.

Bertoni, G., E. Trevisi, and F. Piccioli-Cappelli. 2004. Effects of Acetyl-salicylate Used in Post-calving of Dairy Cows. Vet. Res. Commun. 28(suppl 1):217-219.

Bhat, Z. and H. Bhat. 2011. Milk and dairy products as functional foods: a review. International Journal of Dairy Science 6(1):1-12.

Bichi, E., G. Hervas, P. G. Toral, J. J. Loor, and P. Frutos. 2013. Milk fat depression induced by dietary marine algae in dairy ewes: persistency of milk fatty acid composition and animal performance responses. J. Dairy Sci. 96(1):524-532.

Bionaz, M. and J. J. Loor. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9(366):366.

Bionaz, M., J. Osorio, and J. J. Loor. 2015. TRIENNIAL LACTATION SYMPOSIUM: Nutrigenomics in dairy cows: Nutrients, transcription factors, and techniques. J. Anim. Sci. 93(12):5531-5553.

Bionaz, M., B. J. Thering, and J. J. Loor. 2012. Fine metabolic regulation in ruminants via nutrient–gene interactions: saturated long-chain fatty acids increase expression of genes involved in lipid metabolism and immune response partly through PPAR-α activation. Br. J. Nutr. 107(2):179-191.

Boeckaert, C., B. Vlaeminck, J. Dijkstra, A. Issa-Zacharia, T. Van Nespen, W. Van Straalen, and V. Fievez. 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91(12):4714-4727.

Botsford, L. W., J. C. Castilla, and C. H. Peterson. 1997. The management of fisheries and marine ecosystems. Science 277(5325):509-515.

Bovet, P., D. Faeh, G. Madeleine, B. Viswanathan, and F. Paccaud. 2007. Decrease in blood triglycerides associated with the consumption of eggs of hens fed with food supplemented with fish oil. Nutr. Metab. Cardiovasc. Dis. 17(4):280-287.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98(10):6631-6650.

Brenner, R. R. 1971. The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. Lipids 6(8):567-575.

Bronzo, V., M. Puricelli, A. Agazzi, G. Invernizzi, M. Ferroni, P. Moroni, and G. Savoini. 2010. Effects of protected fish oil in the diet of periparturient dairy goats on phenotypic variation in blood and milk leukocytes. Animal 4(9):1510-1517.

Brunt, E. M. and D. G. Tiniakos. 2010. Histopathology of nonalcoholic fatty liver disease. World J. Gastroenterol. 16(42):5286-5296.

Cabiddu, A., M. Decandia, M. Addis, G. Piredda, A. Pirisi, and G. Molle. 2005. Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. Small Rumin. Res. 59(2-3):169-180.

Cachaldora, P., P. Garcia-Rebollar, C. Alvarez, J. C. De Blas, and J. Mendez. 2008. Effect of type and level of basal fat and level of fish oil supplementation on yolk fat composition and n-3 fatty acids deposition efficiency in laying hens. Anim. Feed Sci. Technol. 141(1-2):104-114.

Calder, P. C. 2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36(9):1007-1024.

Calder, P. C. 2006. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot. Essent. Fatty Acids 75(3):197-202.

Calder, P. C. 2009. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie 91(6):791-795.

Calder, P. C. 2011. Fatty acids and inflammation: The cutting edge between food and pharma.

Calder, P. C. 2012. Long-chain fatty acids and inflammation. Proc. Nutr. Soc. 71(2):284-289.

Calder, P. C. 2013. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br. J. Clin. Pharmacol. 75(3):645-662.

Calder, P. C. 2015. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and. Biochim. Biophys. Acta 4:469-484.

Calder, P. C. 2018. Very long-chain n-3 fatty acids and human health: fact, fiction and the future. Proc. Nutr. Soc. 77(1):52-72.

Calon, F. and G. Cole. 2007. Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: evidence from animal studies. Prostaglandins Leukot. Essent. Fatty Acids 77(5-6):287-293.

Cappucci A., S.P. Alves, R.J.B. Bessa, A. Buccioni, F. Mannelli, M. Pauselli, C. Viti, R. Pastorelli, V. Roscini, A. Serra, G. Conte, M. Mele. 2018. Effect of increasing amounts of olive crude phenolic concentrate in the diet of dairy ewes on rumen liquor and milk fatty acid composition. J. Dairy Sci. 101:4992–5005.

Caroprese, M., I. Giannenas, and G. C. Fthenakis. 2015. Interactions between nutritional approaches and defences against microbial diseases in small ruminants. Vet. Microbiol. 181(1-2):8-14.

Cattaneo, D., V. Dell'Orto, G. Varisco, A. Agazzi, and G. Savoini. 2006. Enrichment in n-3 fatty acids of goat's colostrum and milk by maternal fish oil supplementation. Small Rumin. Res. 64(1-2):22-29.

Cattaneo, D., M. Ferroni, F. Caprino, V. Moretti, A. Agazzi, G. Invernizzi, and G. Savoini. 2010. Dietary fats in transition dairy goats: effects on milk FA composition. Pages 667-668 in Proc. Energy and protein metabolism and nutrition. 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition.

Caughey, G. E., E. Mantzioris, R. A. Gibson, L. G. Cleland, and M. J. James. 1996. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. The American Journal of Clinical Nutrition 63(1):116-122.

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. - Acute phase proteins in ruminants. J. Proteomics 75(14):4207-4231.

Cheli, F. and V. Dell'Orto. 2015. La sicurezza alimentare e nutrizionale. Biologi Italiani 45(2):24-29.

Cherian, G. 2012. Camelina sativa in poultry diets: opportunities and challenges. Biofuel Co-products as Livestock Feed: Opportunities and Challenges. Rome: FAO:303-310.

Cherian, G., A. Campbell, and T. Parker. 2009. Egg quality and lipid composition of eggs from hens fed Camelina sativa. Journal of Applied Poultry Research 18(2):143-150.

Cherian, G. and N. Quezada. 2016. Egg quality, fatty acid composition and immunoglobulin Y content in eggs from laying hens fed full fat camelina or flax seed. J Anim Sci Biotechnol 7:15.

Cherian, G. and J. S. Sim. 1996. Changes in the breast milk fatty acids and plasma lipids of nursing mothers following consumption of n-3 polyunsaturated fatty acid enriched eggs. Nutrition 12(1):8-12.

Cherian, G., F. W. Wolfe, and J. S. Sim. 1996. Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability. Poult. Sci. 75(3):423-431.

Chilliard, Y., A. Ferlay, J. Rouel, and G. Lamberet. 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86(5):1751-1770.

Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. European Journal of Lipid Science and Technology 109(8):828-855.

Christie, W. W. 2003. Lipid Analysis: Vol. 15, Isolation, Separation, Identification and Structural Analysis of Lipids (Oily Press Lipid Library Series). 3rd Edition. ed. Oily Press Ltd, UK.

Clarke, S. D. 2004. The multi-dimensional regulation of gene expression by fatty acids. Curr. Opin. Lipidol. 15(1):13-18.

Conte G., E. Jeronimo, A. Serra, R. J.B. Bessa, M. Mele. 2012. Effect of dietary polyunsaturated fatty acids on Stearoyl CoA-Desaturase gene expression in intramuscular lipids of lamb. Ital. J. Anim. Sci. vol.11:e79.

Contreras, G. A., W. Raphael, S. A. Mattmiller, J. Gandy, and L. M. Sordillo. 2012. Nonesterified fatty acids modify inflammatory response and eicosanoid. J. Dairy Sci. 95(9):5011-5023.

Contreras, G. A. and L. M. Sordillo. 2011. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comp. Immunol. Microbiol. Infect. Dis. 34(3):281-289.

Cook, H. W. and C. R. McMaster. 2002. Chapter 7 Fatty acid desaturation and chain elongation in eukaryotes. New Comprehensive Biochemistry 36:181-204.

Coppa, M., A. Ferlay, F. Monsallier, I. Verdier-Metz, P. Pradel, R. Didienne, A. Farruggia, M. C. Montel, and B. Martin. 2011. Milk fatty acid composition and cheese texture and appearance from cows fed hay or different grazing systems on upland pastures. J. Dairy Sci. 94(3):1132-1145.

Crespo, N. and E. Esteve-Garcia. 2002. Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. Poult. Sci. 81(10):1533-1542.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman, and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in. J. Am. Vet. Med. Assoc. 183(5):559-561.

Cury-Boaventura, M. F., C. Pompeia, and R. Curi. 2005. Comparative toxicity of oleic acid and linoleic acid on Raji cells. Nutrition 21(3):395-405.

Dawson, K. A. 2006. Nutrigenomics: feeding the genes for improved fertility. Anim. Reprod. Sci. 96(3-4):312-322.

De Caterina, R. and P. Libby. 1996. Control of endothelial leukocyte adhesion molecules by fatty acids. Lipids 31 Suppl(Journal Article):S57-63.

De Smet, S. and E. Vossen. 2016. Meat: The balance between nutrition and health. A review. Meat Sci 120:145-156.
de Tonnac, A. and J. Mourot. 2017. - Effect of dietary sources of n-3 fatty acids on pig performance and. Animal 20:1-9.
Devle, H., E. O. Rukke, C. F. Naess-Andresen, and D. Ekeberg. 2009. A GC-magnetic sector MS method for identification and quantification of fatty. J Sep Sci 32(21):3738-3745.
Dewhurst, R. J., K. J. Shingfield, M. R. F. Lee, and N. D. Scollan. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim. Feed Sci. Technol. 131(3-4):168-206.
Doreau, M. and Y. Chilliard. 1997. Digestion and metabolism of dietary fat in farm animals. Br. J. Nutr. 78(1):S15-35.
Doreau, M. and A. Ferlay. 1994. Digestion and Utilization of Fatty-Acids by Ruminants. Anim. Feed Sci. Technol. 45(3-4):379-396.
Doreau, M. and A. Ferlay. 2015. Linseed: a valuable feedstuff for ruminants. OCL 22(6):D611.
Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82(11):2259-2273.
Drackley, J. K. 2007. Overview of Fat Digestion and Metabolism in Dairy Cows.
Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows during the Periparturient Period. J. Dairy Sci. 84:E100-E112.
Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 16(2):231-253
Díaz, M. T., V. Cañeque, C. I. Sánchez, S. Lauzurica, C. Pérez, C. Fernández, I. Álvarez, and J. De la Fuente. 2011. Nutritional and sensory aspects of light lamb meat enriched in n−3 fatty acids during refrigerated storage. Food Chem. 124(1):147-155.
D'Ambrosio, F., A. Campagnoli, F. Susca, E. Fusi, R. Rebucci, and L. P. A. Agazzi, and A. Baldi. 2007. Effects of rumen-protected choline supplementation in periparturient dairy goats. Vet. Res. Commun. 31(Suppl.1):393-396.
Ebrahimi, M., M. A. Rajion, and Y. M. Goh. 2014. - Effects of Oils Rich in Linoleic and α-Linolenic Acids on Fatty Acid Profile and Gene Expression in Goat Meat. Nutrients 6(9):3913-3928.

Efsa Panel on Contaminants in the Food Chain. 2008. Glucosinolates as undesirable substances in animal feed - Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA Journal 6(1):590-n/a.
EFSA Panel on Dietetic Products, N. and Allergies. 2012. Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal 10(7):2815.
El-Nor, A. S. A. and M. S. Khattab. 2012. Enrichment of milk with conjugated linoleic acid by supplementing diets with fish. Pak. J. Biol. Sci. 15(14):690-693.
Elsanhoty, R., A. Zaghlol, and A. Hassanein. 2009. The manufacture of low fat labneh containing barley β-glucan 1-chemical composition, microbiological evaluation and sensory properties. Current Research in Dairy Sciences 1(1):1-12.
Endres, S., R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonnemann, J. W. van der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempner, P. C. Weber, and et al. 1989. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N. Engl. J. Med. 320(5):265-271.
Eşki, F., İ. Taşal, M. A. Karsli, S. Şendağ, B. A. Uslu, H. Wagner, and A. Wehrend. 2015. Concentrations of NEFA, ß-HBA, triglycerides, and certain blood metabolitesin healthy colored Angora goats during the peripartum period. Turkish Journal of Veterinary and Animal Sciences 39(4):401-405.
Fahy, E., S. Subramaniam, R. C. Murphy, M. Nishijima, C. R. H. Raetz, T. Shimizu, F. Spener, G. van Meer, M. J. O. Wakelam, and E. A. Dennis. 2009. Update of the LIPID MAPS comprehensive classification system for lipids. J. Lipid Res. 50(Supplement):S9-S14.
Farney, J. K., L. K. Mamedova, J. F. Coetzee, B. KuKanich, L. M. Sordillo, S. K. Stoakes, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305(2):R110-117.
Fekete, S. G. and D. L. Brown. 2007. Veterinary aspects and perspectives of nutrigenomics: a critical review. Acta Vet. Hung. 55(2):229-239.
Folch, J., M. Lees, and G. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal. J. Biol. Chem. 226(1):497-509.
Fraeye, I., C. Bruneel, C. Lemahieu, J. Buyse, K. Muylaert, and I. Foubert. 2012. Dietary enrichment of eggs with omega-3 fatty acids: A review. Food Res. Int. 48(2):961-969.

Franklin, S. T., K. R. Martin, R. J. Baer, D. J. Schingoethe, and A. R. Hippen. 1999. Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J. Nutr. 129(11):2048-2054.
French, P., C. Stanton, F. Lawless, E. G. O'Riordan, F. J. Monahan, P. J. Caffrey, and A. P. Moloney. 2000. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. J. Anim. Sci. 78(11):2849-2855.
Fu, Z., M. Thorpe, R. Alemayehu, A. Roy, J. Kervinen, L. de Garavilla, M. Abrink, and L. Hellman. 2017. Highly Selective Cleavage of Cytokines and Chemokines by the Human Mast Cell Chymase and Neutrophil Cathepsin G. J. Immunol. 198(4):1474-1483.
Funaki, M. 2009. Saturated fatty acids and insulin resistance. J. Med. Invest. 56(3-4):88-92.
Galio, L., S. Droineau, P. Yeboah, H. Boudiaf, S. Bouet, S. Truchet, E. Devinoy. 2013. MicroRNA in the ovine mammary gland during early pregnancy: spatial and temporal expression of miR-21, miR-205, and miR-200. Physiol. Genomics 45, 151–161.
Ganesan, B., C. Brothersen, and D. J. McMahon. 2014. Fortification of foods with omega-3 polyunsaturated fatty acids. Crit. Rev. Food Sci. Nutr. 54(1):98-114.
Gifford, C. A., B. P. Holland, R. L. Mills, C. L. Maxwell, J. K. Farney, S. J. Terrill, D. L. Step, C. J. Richards, L. O. Burciaga Robles, and C. R. Krehbiel. 2012. Growth and Development Symposium: Impacts of inflammation on cattle growth and carcass merit. J. Anim. Sci. 90(5):1438-1451.
Givens, D. I. 2010. Milk and meat in our diet: good or bad for health? Animal 4(12):1941-1952.
Givens, D. I. and K. J. Shingfield. 2006. Optimising dairy milk fatty acid composition. Pages 252-280 in Improving the Fat Content of Foods. Woodhead Publishing.
Gladine, C., E. Rock, C. Morand, D. Bauchart, and D. Durand. 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols. Br. J. Nutr. 98(4):691-701.
Gobert, M., B. Martin, A. Ferlay, Y. Chilliard, B. Graulet, P. Pradel, D. Bauchart, and D. Durand. 2009. Plant polyphenols associated with vitamin E can reduce plasma lipoperoxidation in. J. Dairy Sci. 92(12):6095-6104.
Goldberg, E. M., D. Ryland, R. A. Gibson, M. Aliani, and J. D. House. 2013. Designer laying hen diets to improve egg fatty acid profile and maintain sensory quality. Food science & nutrition 1(4):324-335.

Gonzalez-Esquerra, R. and S. Leeson. 2000. Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. Poult. Sci. 79(11):1597-1602.

Grant, R. W. and V. D. Dixit. 2015. Adipose tissue as an immunological organ. Obesity (Silver Spring, Md.) 23(3):512-518.

Greco, L. F., J. T. Neves Neto, A. Pedrico, R. A. Ferrazza, F. S. Lima, R. S. Bisinotto, N. Martinez, M. Garcia, E. S. Ribeiro, G. C. Gomes, J. H. Shin, M. A. Ballou, W. W. Thatcher, C. R. Staples, and J. E. Santos. 2015. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on performance and inflammatory responses to a lipopolysaccharide challenge in lactating Holstein cows. J. Dairy Sci. 98(1):602-617.

Gunstone, F. D., J. L. Hardwood, and F. B. Padley. 1994. The lipid handbook. Second ed. Chapman and Hall, London.

Guo, H., N. T. Ingolia, J. S. Weissman, and D. P. Bartel. 2010. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466(7308):835.

Halvorsen, D. S., J. B. Hansen, S. Grimsgaard, K. H. Bonaa, P. Kierulf, and A. Nordoy. 1997. The effect of highly purified eicosapentaenoic and docosahexaenoic acids on monocyte phagocytosis in man. Lipids 32(9):935-942.

Harris, W. S., D. Mozaffarian, M. Lefevre, C. D. Toner, J. Colombo, S. C. Cunnane, J. M. Holden, D. M. Klurfeld, M. C. Morris, and J. Whelan. 2009. Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. J. Nutr. 139(4):804S-819S.

Harrison, F. A. and W. M. Leat. 1975. Digestion and absorption of lipids in non-ruminant and ruminant animals: a comparison. Proc. Nutr. Soc. 34(3):203-210.

Hayat, Z., G. Cherian, T. N. Pasha, F. M. Khattak, and M. A. Jabbar. 2009. Effect of feeding flax and two types of antioxidants on egg production, egg quality, and lipid composition of eggs. The Journal of Applied Poultry Research 18(3):541-551.

Hayat, Z., G. Cherian, T. N. Pasha, F. M. Khattak, and M. A. Jabbar. 2010. Oxidative stability and lipid components of eggs from flax-fed hens: Effect of dietary antioxidants and storage. Poult. Sci. 89(6):1285-1292.

Hazards 2015. Scientific Opinion on the public health risks related to the consumption of raw drinking milk. EFSA Journal 13(1):95.

Healy, D. A., R. W. Watson, and P. Newsholme. 2003. Polyunsaturated and monounsaturated fatty acids increase neutral lipid. Clin. Sci. 104(2):171-179.

Hebeisen, D. F., F. Hoeflin, H. P. Reusch, E. Junker, and B. H. Lauterburg. 1993. Increased concentrations of omega-3 fatty acids in milk and platelet rich plasma of grass-fed cows. Int. J. Vitam. Nutr. Res. 63(3):229-233.

Heimli, H., C. Giske, S. Naderi, C. A. Drevon, and K. Hollung. 2002. Eicosapentaenoic acid promotes apoptosis in Ramos cells via activation of caspase-3 and -9. Lipids 37(8):797-802.

Herber, S. M. and M. E. Van Elswyk. 1996. Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs. Poult. Sci. 75(12):1501-1507.

Heuer, C., Y. H. Schukken, and P. Dobbelaar. 1999. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J. Dairy Sci. 82(2):295-304.

Higgs, J. D. 2000. The changing nature of red meat: 20 years of improving nutritional quality. Trends Food Sci. Technol. 11(3):85-95.

Hiller, B., J. Angulo, M. Olivera, G. Nuernberg, and K. Nuernberg. 2013. How selected tissues of lactating holstein cows respond to dietary polyunsaturated fatty acid supplementation. Lipids 48(4):357-367.

Hixson, S. M., B. Sharma, M. J. Kainz, A. Wacker, and M. T. Arts. 2015. Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. Environ. Rev. 23(4):414-424.

Hoedemaker, M., D. Prange, and Y. Gundelach. 2009. Body condition change ante- and postpartum, health and reproductive performance in German Holstein cows. Reprod Domest Anim 44(2):167-173.

Hornstra, G. 2000. Essential fatty acids in mothers and their neonates. Am. J. Clin. Nutr. 71(5):1262s-1269s.

Humblet, M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a. Vet. Clin. Pathol. 35(2):188-193.

Hwang, D. and S. H. Rhee. 1999. Receptor-mediated signaling pathways: potential targets of modulation by dietary. Am. J. Clin. Nutr. 70(4):545-556.

Invernizzi, G., S. Modina, D. Corbani, V. Bronzo, L. F. Pisani, J. M. Caputo, A. Agazzi, V. Dell'Orto, and G. Savoini. 2016. Hepatic and subcutaneous adipose tissue variations in transition dairy goats fed saturated or unsaturated fat supplemented diets. Small Rumin. Res. 144:211-219.

Janovick, N. A. and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and. J. Dairy Sci. 93(7):3086-3102.
Jaśkiewicz, T., A. Sagan, and I. Puzio. 2014. Effect of the Camelina sativa oil on the performance, essential fatty acid level in tissues and fat-soluble vitamins content in the livers of broiler chickens. Livest. Sci. 165:74-79.
Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76(12):3851-3863.
Jenkins, T. C. and W. C. Bridges. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. European Journal of Lipid Science and Technology 109(8):778-789.
Jenkins, T. C. and M. A. McGuire. 2006. Major advances in nutrition: impact on milk composition. J. Dairy Sci. 89(4):1302-1310.
Jin, W., M. V. Dodson, S. S. Moore, J. A. Basarab, and L. L. Guan. 2010. Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development. BMC Mol. Biol. 11(29):29.
Jin, W., J. R. Grant, P. Stothard, S. S. Moore, and L. L. Guan. 2009. Characterization of bovine miRNAs by sequencing and bioinformatics analysis. BMC Mol. Biol. 10(90):90.
Jump, D. B. 2004. Fatty acid regulation of gene transcription. Crit. Rev. Clin. Lab. Sci. 41(1):41-78.
Jump, D. B., D. Botolin, Y. Wang, J. Xu, B. Christian, and O. Demeure. 2005. Fatty acid regulation of hepatic gene transcription. J. Nutr. 135(11):2503-2506.
Kaasgaard, S. G., G. Hølmer, C.-E. Høy, W. A. Behrens, and J. L. Beare-Rogers. 1992. Effects of dietary linseed oil and marine oil on lipid peroxidation in monkey liverin vivo andin vitro. Lipids 27(10):740-745.
Kaić, A., B. Mioč, A. Kasap, and K. Potočnik. 2016. Meta-analysis of intramuscular fatty acid composition of Mediterranean lambs. Archives Animal Breeding 59(1):1-8.
Kakani, R., J. Fowler, A. U. Haq, E. J. Murphy, T. A. Rosenberger, M. Berhow, and C. A. Bailey. 2012. Camelina meal increases egg n-3 fatty acid content without altering quality or production in laying hens. Lipids 47(5):519-526.
Kakani R. and A.-U. H. J. Fowler, E., J. Murphy, T. A. Rosenberger, M. Berhow, C. A. Bailey. 2012. Camelina meal increases egg n-3 fatty acid content without altering quality or production in laying hens. Lipids 47(5):519-526.

Kalaitzakis, E., N. Panousis, N. Roubies, N. Giadinis, E. Kaldrymidou, M. Georgiadis, and H. Karatzias. 2010. Clinicopathological evaluation of downer dairy cows with fatty liver. Can. Vet. J. 51(6):615-622.

Kaput, J. and R. L. Rodriguez. 2004. Nutritional genomics: the next frontier in the postgenomic era. Physiol. Genomics 16(2):166-177.

Kates, M. 1986. Influence of Salt Concentration on Membrane-Lipids of Halophilic Bacteria. FEMS Microbiol. Lett. 39(1-2):95-101.

Kearns, R. J., M. G. Hayek, J. J. Turek, M. Meydani, J. R. Burr, R. J. Greene, C. A. Marshall, S. M. Adams, R. C. Borgert, and G. A. Reinhart. 1999. Effect of age, breed and dietary omega-6 (n-6): omega-3 (n-3) fatty acid ratio on. Vet. Immunol. Immunopathol. 69(2-4):165-183.

Kelley, D. S., P. C. Taylor, G. J. Nelson, P. C. Schmidt, A. Ferretti, K. L. Erickson, R. Yu, R. K. Chandra, and B. E. Mackey. 1999. Docosahexaenoic acid ingestion inhibits natural killer cell activity and. Lipids 34(4):317-324.

Kennedy, A., K. Martinez, C. C. Chuang, K. LaPoint, and M. McIntosh. 2009. Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. J. Nutr. 139(1):1-4.

Kessler, E. C., J. J. Gross, R. M. Bruckmaier, and C. Albrecht. 2014. Cholesterol metabolism, transport, and hepatic regulation in dairy cows during transition and early lactation. J. Dairy Sci. 97(9):5481-5490.

Khan, M. J., A. Hosseini, S. Burrell, S. M. Rocco, J. P. McNamara, and J. J. Loor. 2013. Change in subcutaneous adipose tissue metabolism and gene network expression during the transition period in dairy cows, including differences due to sire genetic merit. J. Dairy Sci. 96(4):2171-2182.

Khan, M. J., C. B. Jacometo, D. E. Graugnard, M. N. Correa, E. Schmitt, F. Cardoso, and J. J. Loor. 2014. Overfeeding Dairy Cattle During Late-Pregnancy Alters Hepatic PPARalpha-Regulated Pathways Including Hepatokines: Impact on Metabolism and Peripheral Insulin Sensitivity. Gene Regul. Syst. Bio. 8:97-111.

Khotimchenko, S. V. and I. V. Kulikova. 2000. Lipids of different parts of the lamina of Laminaria japonica Aresch. Bot. Mar. 43(1):87-91.

Kim, D. H., P. Saetrom, O. Snove, Jr., and J. J. Rossi. 2008. MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc. Natl. Acad. Sci. U. S. A. 105(42):16230-16235.

Kim, J., A. Magnuson, L. Tao, M. Barcus, and X. G. Lei. 2016. Potential of combining flaxseed oil and microalgal biomass in producing eggs-enriched with n-3 fatty acids for meeting human needs. Algal Research-Biomass Biofuels and Bioproducts 17:31-37.

Kitessa, S. M., S. K. Gulati, J. R. Ashes, E. Fleck, T. W. Scott, and P. D. Nichols. 2001. Utilisation of fish oil in ruminants - I. Fish oil metabolism in sheep. Anim. Feed Sci. Technol. 89(3-4):189-199.

Kloting, N., S. Berthold, P. Kovacs, M. R. Schon, M. Fasshauer, K. Ruschke, M. Stumvoll, and M. Bluher. 2009. MicroRNA expression in human omental and subcutaneous adipose tissue. PLoS One 4(3):e4699.

Korver, D. R. and K. C. Klasing. 1997. Dietary fish oil alters specific and inflammatory immune responses in chicks. J. Nutr. 127(10):2039-2046.

Kouba, M. and J. Mourot. 2011. A review of nutritional effects on fat composition of animal products with. Biochimie 93(1):13-17.

Kouřimská, L., E. Vondráčková, M. Fantová, P. Nový, L. Nohejlová, and K. Michnová. 2014. Effect of Feeding with Algae on Fatty Acid Profile of Goat'S Milk. Scientia Agriculturae Bohemica 45(3).

Kremmyda, L. S., E. Tvrzicka, B. Stankova, and A. Zak. 2011. Fatty acids as biocompounds: their role in human metabolism, health and disease. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 155(3):195-218.

Kris-Etherton, P. M., D. S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R. L. Hargrove, G. Zhao, and T. D. Etherton. 2000. Polyunsaturated fatty acids in the food chain in the United States. Am. J. Clin. Nutr. 71(1 Suppl):179S-188S.

Kuhla, B. and K. L. Ingvartsen. 2018. Proteomics and the Characterization of Fatty Liver Metabolism in Early Lactation Dairy Cows. Pages 219-231 in Proteomics in Domestic Animals: from Farm to Systems Biology. Springer.

Kuratko, C. N., E. C. Barrett, E. B. Nelson, and N. J. Salem. 2013. The relationship of docosahexaenoic acid (DHA) with learning and behavior in. Nutrients 5(7):2777-2810.

Kushibiki, S., K. Hodate, H. Shingu, Y. Obara, E. Touno, M. Shinoda, and Y. Yokomizo. 2003. Metabolic and lactational responses during recombinant bovine tumor necrosis factor-alpha treatment in lactating cows. J. Dairy Sci. 86(3):819-827.

La Terra, S., V. M. Marino, M. Manenti, G. Licitra, and S. Carpino. 2010. Increasing pasture intakes enhances polyunsaturated fatty acids and lipophilic antioxidants in plasma and milk of dairy cows fed total mix ration. Dairy Science & Technology 90(6):687-698.

Lawlor, J. B., N. Gaudette, T. Dickson, and J. D. House. 2010. Fatty acid profile and sensory characteristics of table eggs from laying hens fed diets containing microencapsulated fish oil. Anim. Feed Sci. Technol. 156(3-4):97-103.

Lecchi, C., G. Invernizzi, A. Agazzi, M. Ferroni, L. F. Pisani, G. Savoini, and F. Ceciliani. 2011. In vitro modulation of caprine monocyte immune functions by omega-3 polyunsaturated fatty acids. Vet. J. 189(3):353-355.

Lecchi, C., G. Invernizzi, A. Agazzi, S. Modina, P. Sartorelli, G. Savoini, and F. Ceciliani. 2013. Effects of EPA and DHA on lipid droplet accumulation and mRNA abundance of PAT proteins in caprine monocytes. Res. Vet. Sci. 94(2):246-251.

Lehninger, A. L., D. L. Nelson, M. M. Cox, M. M. Cox, U. M. M. Cox, and S. W. Fund. 2005. Lehninger Principles of Biochemistry. W. H. Freeman.

Leiber, F., M. Kreuzer, D. Nigg, H. R. Wettstein, and M. R. Scheeder. 2005. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. Lipids 40(2):191-202.

Lessire, M., M. Doreau, and A. Aumaitre. 1992. Digestion and metabolism of fats in domestic animals. In: Manuel des Corps Gras. Pages 683-694. Lavoisier, Paris.

Li, X., X. Fan, L. Han, and Q. Lou. 2002. Fatty acids of some algae from the Bohai Sea. Phytochemistry 59(2):157-161.

Lock, A. L. and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to. Lipids 39(12):1197-1206.

Lock, A. L. and J. de Souza. 2016. Fatty Acid Digestibility and Impacts on Responses of Dairy Cows. Florida Ruminant Nutrition Symposium 27th Annual Meeting.

Lock, A. L., P. W. Parodi, and D. E. Bauman. 2005. The biology of trans fatty acids: implications for human health and the dairy industry. Australian Journal of Dairy Technology 60(2):134.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23(2):217-226.

Lovegrove, J. A. and D. I. Givens. 2016. Dairy food products: good or bad for cardiometabolic disease? Nutr. Res. Rev. 29(2):249-267.
Mabe, I., C. Rapp, M. M. Bain, and Y. Nys. 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from. Poult. Sci. 82(12):1903-1913.
Maghin, F., S. Ratti, and C. Corino. 2014. Biological functions and health promot-ing effects of brown seaweeds in swine nutrition. J Dairy Vet Anim Res 1(1):00005.
Mansson, H. L. 2008. Fatty acids in bovine milk fat. Food Nutr. Res. 52.
Marchi, L. B., A. R. G. Monteiro, J. M. G. Mikcha, A. R. Santos, M. M. Chinellato, D. R. Marques, A. S. Dacome, and S. C. Costa. 2015. Evaluation of antioxidant and antimicrobial capacity of pomegranate peel extract (Punica Granatuml.) under different drying temperatures. Chemical Engineering Transactions 44:121-126.
Markiewicz-Kęszycka, M., G. Czyżak-Runowska, P. Lipińska, and J. Wójtowski. 2013. Fatty acid profile of milk-a review. Bulletin of the Veterinary Institute in Pulawy 57(2):135-139.
Martins de Lima, T., M. F. Cury-Boaventura, G. Giannocco, M. T. Nunes, and R. Curi. 2006. Comparative toxicity of fatty acids on a macrophage cell line (J774). Clin. Sci. 111(5):307-317.
Matthews, K. R., D. B. Homer, F. Thies, and P. C. Calder. 2000. Effect of whole linseed (Linum usitatissimum) in the diet of finishing pigs on. Br. J. Nutr. 83(6):637-643.
Matthäus, B. 2002. Antioxidant activity of extracts obtained from residues of different oilseeds. J. Agric. Food Chem. 50(12):3444-3452.
Mavangira, V. and L. M. Sordillo. 2018. Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. Res. Vet. Sci. 116:4-14.
Mayer, H. K. and G. Fiechter. 2012. Physical and chemical characteristics of sheep and goat milk in Austria. Int. Dairy J. 24(2):57-63.
McDaniel, J. C., K. Ahijevych, and M. Belury. 2010. Effect of n-3 oral supplements on the n-6/n-3 ratio in young adults. West. J. Nurs. Res. 32(1):64-80.
McGown, C., A. Birerdinc, and Z. M. Younossi. 2014. Adipose tissue as an endocrine organ. Clin. Liver Dis. 18(1):41-58.
McNamara, J. P. 1991. Regulation of adipose tissue metabolism in support of lactation. J. Dairy Sci. 74(2):706-719.
McNamara, J. P. 1997. Adipose tissue metabolism during lactation: Where do we go from here? Proc. Nutr. Soc. 56(1A):149-167.

McNaught, A. D. and A. Wilkinson. 1997. IUPAC. Compendium of Chemical Terminology. 2nd ed. (the "Gold Book") ed. Blackwell Scientific Publications, Oxford.
Meadus, W. J., P. Duff, T. McDonald, and W. R. Caine. 2014. Pigs fed camelina meal increase hepatic gene expression of cytochrome 8b1, aldehyde dehydrogenase, and thiosulfate transferase. J Anim Sci Biotechnol 5(1):1.
Meignan, T., C. Lechartier, G. Chesneau, and N. Bareille. 2017. Effects of feeding extruded linseed on production performance and milk fatty acid profile in dairy cows: A meta-analysis. J. Dairy Sci. 100(6):4394-4408.
Mele M., A. Serra, A. Buccioni, G. Conte, A. Pollicardo, P. Secchiari. 2008. Effect of soybean oil supplementation on milk fatty acid composition from Saanen goats fed diets with different forage:concentrate ratios. Ital. J. Anim. Sci. vol. 7, 297-311.
Meluzzi, A., F. Sirri, N. Tallarico, and A. Franchini. 2001. Effect of different vegetable lipid sources on the fatty acid composition of egg yolk and on hen performance. Pages 207-213.
Moghadam, M. H. B. and G. Cherian. 2017. Use of flaxseed in poultry feeds to meet the human need for n-3 fatty acids. Worlds Poultry Science Journal 73(4):803-812.
Muller, M. and S. Kersten. 2003. Nutrigenomics: goals and strategies. Nat. Rev. Genet. 4(4):315-322.
Mun, S., E. A. Decker, and D. J. McClements. 2007. Influence of emulsifier type on i*n vitro* digestibility of lipid droplets by pancreatic lipase. Food Res. Int. 40:770-781.
Musi, N. and R. Guardado-Mendoza. 2014. Chapter 14 - Adipose Tissue as an Endocrine Organ A2 - Ulloa-Aguirre, Alfredo. Pages 229-237 in Cellular Endocrinology in Health and Disease. P. M. Conn, ed. Academic Press, Boston.
Nagy, K. and I.-D. Tiuca. 2017. Importance of Fatty Acids in Physiopathology of Human Body. in Fatty Acids. InTech.
Nain, S., M. A. Oryschak, M. Betti, and E. Beltranena. 2015. Camelina sativa cake for broilers: Effects of increasing dietary inclusion from 0 to 24% on tissue fatty acid proportions at 14, 28, and 42 d of age. Poult. Sci. 94(6):1247-1258.
Narayan, B., K. Miyashita, and M. Hosakawa. 2008. Comparative Evaluation of Fatty Acid Composition of DifferentSargassum(Fucales, Phaeophyta) Species Harvested from Temperate and Tropical Waters. J. Aquat. Food Prod. Technol. 13(4):53-70.

Nieto, G. and G. Ros. 2012. Modification of Fatty Acid Composition in Meat Through Diet: Effect on Lipid Peroxidation and Relationship to Nutritional Quality - A Review. Lipid Peroxidation:239-258.

Ntambi, J. M. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and. J. Lipid Res. 40(9):1549-1558.

Nudda, A., G. Battacone, O. Boaventura Neto, A. Cannas, A. H. D. Francesconi, A. S. Atzori, and G. Pulina. 2014. Feeding strategies to design the fatty acid profile of sheep milk and cheese. Revista Brasileira de Zootecnia 43:445-456.

Osorio, J. S., M. Vailati-Riboni, A. Palladino, J. Luo, and J. J. Loor. 2017. Application of nutrigenomics in small ruminants: Lactation, growth, and beyond. Small Rumin. Res. 154:29-44.

Palmquist, D. L. 2009. Omega-3 Fatty Acids in Metabolism, Health, and Nutrition and for Modified Animal Product Foods. The Professional Animal Scientist 25(3):207-249.

Papadopoulos, G., C. Goulas, E. Apostolaki, and R. Abril. 2002. Effects of dietary supplements of algae, containing polyunsaturated fatty acids, on milk yield and the composition of milk products in dairy ewes. J. Dairy Res. 69(3):357-365.

Paton, C. M. and J. M. Ntambi. 2010. Loss of stearoyl-CoA desaturase activity leads to free cholesterol synthesis through increased Xbp-1 splicing. Am. J. Physiol. Endocrinol. Metab. 299(6):E1066-1075.

Patterson, E., R. Wall, G. F. Fitzgerald, R. P. Ross, and C. Stanton. 2012. Health implications of high dietary omega-6 polyunsaturated Fatty acids. J. Nutr. Metab. 2012:539426.

Pekel, A. Y., P. H. Patterson, R. M. Hulet, N. Acar, T. L. Cravener, D. B. Dowler, and J. M. Hunter. 2009. Dietary camelina meal versus flaxseed with and without supplemental copper for broiler chickens: live performance and processing yield. Poult. Sci. 88(11):2392-2398.

Pinedo, P. J., A. De Vries, and D. W. Webb. 2010. Dynamics of culling risk with disposal codes reported by Dairy Herd Improvement dairy herds. J. Dairy Sci. 93(5):2250-2261.

Pinotti, L., A. Campagnoli, F. D'Ambrosio, F. Susca, M. Innocenti, R. Rebucci, E. Fusi, F. Cheli, G. Savoini, V. Dell'orto, and A. Baldi. 2008. Rumen-protected choline and vitamin E supplementation in periparturient dairy goats: effects on milk production and folate, vitamin B12 and vitamin E status. Animal 2(7):1019-1027.

Pisani, L. F., C. Lecchi, G. Invernizzi, P. Sartorelli, G. Savoini, and F. Ceciliani. 2009. In vitro modulatory effect of omega-3 polyunsaturated fatty acid (EPA and DHA) on phagocytosis and ROS production of goat neutrophils. Vet. Immunol. Immunopathol. 131(1-2):79-85.

Poureslami, R., K. Raes, G. Huyghebaert, A. B. Batal, and S. De Smet. 2012. Egg yolk fatty acid profile in relation to dietary fatty acid concentrations. J. Sci. Food Agric. 92(2):366-372.

Quezada, N. and G. Cherian. 2012. Lipid characterization and antioxidant status of the seeds and meals of Camelina sativa and flax. European Journal of Lipid Science and Technology 114(8):974-982.

Raphael, W. and L. M. Sordillo. 2013. Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid. Int. J. Mol. Sci. 14(10):21167-21188.

Rasoul-Amini, S., Y. Ghasemi, M. H. Morowvat, and A. Mohagheghzadeh. 2009. PCR amplification of 18S rRNA, single cell protein production and fatty acid evaluation of some naturally isolated microalgae. Food Chem. 116(1):129-136.

Rees, D., E. A. Miles, T. Banerjee, S. J. Wells, C. E. Roynette, K. W. Wahle, and P. C. Calder. 2006. Dose-related effects of eicosapentaenoic acid on innate immune function in. Am. J. Clin. Nutr. 83(2):331-342.

Reue, K. 2007. The role of lipin 1 in adipogenesis and lipid metabolism. Novartis Found. Symp. 286:58-68; discussion 68-71, 162-163, 196-203.

Rezamand, P., B. P. Hatch, K. G. Carnahan, and M. A. McGuire. 2016. Effects of α-linolenic acid-enriched diets on gene expression of key inflammatory mediators in immune and milk cells obtained from Holstein dairy cows. J. Dairy Res. 83(1):20-27.

Rizzi, L., D. Bochicchio, A. Bargellini, P. Parazza, and M. Simioli. 2009. Effects of dietary microalgae, other lipid sources, inorganic selenium and iodine on yolk n-3 fatty acid composition, selenium content and quality of eggs in laying hens. J. Sci. Food Agric. 89(10):1775-1781.

Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow. J. Dairy Sci. 92(12):5769-5801.

Rokka, T., K. Alén, J. Valaja, and E.-L. Ryhänen. 2002. The effect of a Camelina sativa enriched diet on the composition and sensory quality of hen eggs. Food Res. Int. 35(2):253-256.

Romao, J. M., W. Jin, M. He, T. McAllister, and L. L. Guan. 2012. Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet. PLoS One 7(7):e40605.

Russo, R., I. Galasso, and R. Reggiani. 2014. Variability in glucosinolate content among Camelina species. American Journal of Plant Sciences 5(3):294-298.

Russo R. and R. R. I. Galasso. 2014. Variability in glucosinolate content among Camelina species. American Journal of Plant Sciences 5(3):294-298.

Ryhänen, E. L., S. Perttilä, T. Tupasela, J. Valaja, C. Eriksson, and K. Larkka. 2007. Effect of Camelina sativa expeller cake on performance and meat quality of broilers. J. Sci. Food Agric. 87(8):1489-1494.

Ryman, V. E., N. Packiriswamy, and L. M. Sordillo. 2015. Role of endothelial cells in bovine mammary gland health and disease. Anim. Health Res. Rev. 16(2):135-149.

Sauvant, D., Y. Chilliard, and P. Morand-Fehr. 1991. Etiological aspects of nutritional and metabolic disorders of goats. Pages 124-142 in Goat nutrition. P. Morand-Fehr, ed. Wageningen Pudoc.

Savoini, G., A. Agazzi, G. Invernizzi, D. Cattaneo, L. Pinotti, and A. Baldi. 2010. Polyunsaturated fatty acids and choline in dairy goats nutrition: Production and health benefits. Small Rumin. Res. 88(2-3):135-144.

Savoini, G., G. Farina, V. Dell'Orto, and D. Cattaneo. 2016. Through ruminant nutrition to human health: role of fatty acids. Advances in Animal Biosciences 7(02):200-207.

Sayed, D. and M. Abdellatif. 2011. MicroRNAs in development and disease. Physiol. Rev. 91(3):827-887.

Schmidt, E. B., K. Varming, J. M. Moller, I. Bulow Pedersen, P. Madsen, and J. Dyerberg. 1996. No effect of a very low dose of n-3 fatty acids on monocyte function in healthy. Scand. J. Clin. Lab. Invest. 56(1):87-92.

Schmidt, E. B., K. Varming, J. O. Pedersen, H. H. Lervang, N. Grunnet, C. Jersild, and J. Dyerberg. 1992. Long-term supplementation with n-3 fatty acids, II: Effect on neutrophil and monocyte chemotaxis. Scand. J. Clin. Lab. Invest. 52(3):229-236.

Schmitt, E., M. A. Ballou, M. N. Correa, E. J. DePeters, J. K. Drackley, and J. J. Loor. 2011. Dietary lipid during the transition period to manipulate subcutaneous adipose tissue peroxisome proliferator-activated receptor-gamma co-regulator and target gene expression. J. Dairy Sci. 94(12):5913-5925.

Schuster, A. and W. Friedt. 1998. Glucosinolate content and composition as parameters of quality of Camelina seed. Ind Crop Prod 7(2):297-302.
Serafini, M., A. Stanzione, and S. Foddai. 2012. Functional foods: traditional use and European legislation. Int. J. Food Sci. Nutr. 63 Suppl 1:7-9.
Serhan, C. N., S. Hong, K. Gronert, S. P. Colgan, P. R. Devchand, G. Mirick, and R. L. Moussignac. 2002. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. The Journal of experimental medicine 196(8):1025-1037.
Shin, T. K., Y. J. Yi, J. C. Kim, J. R. Pluske, H. M. Cho, S. S. Wickramasuriya, E. Kim, S. M. Lee, and J. M. Heo. 2017. Reducing the dietary omega-6 to omega-3 polyunsaturated fatty acid ratio attenuated inflammatory indices and sustained epithelial tight junction integrity in weaner pigs housed in a poor sanitation condition. Anim. Feed Sci. Technol. 234:312-320.
Shingfield, K. J., L. Bernard, C. Leroux, and Y. Chilliard. 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4(7):1140-1166.
Shingfield, K. J., M. Bonnet, and N. D. Scollan. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7 Suppl 1:132-162.
Shingfield, K. J., Y. Chilliard, V. Toivonen, P. Kairenius, and D. I. Givens. 2008. Trans fatty acids and bioactive lipids in ruminant milk. Adv. Exp. Med. Biol. 606:3-65.
Shlyueva, D., G. Stampfel, and A. Stark. 2014. Transcriptional enhancers: from properties to genome-wide predictions. Nat. Rev. Genet. 15(4):272-286.
Sijben, J. W. and P. C. Calder. 2007. Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease. Proc. Nutr. Soc. 66(2):237-259.
Simopoulos, A. P. 2000. Human requirement for N-3 polyunsaturated fatty acids. Poult. Sci. 79(7):961-970.
Simopoulos, A. P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease. Exp. Biol. Med. 233(6):674-688.
Simopoulos, A. P. 2010. Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role. Exp. Biol. Med. 235(7):785-795.
Sirri, F., G. Minelli, N. Iaffaldano, N. Tallarico, and A. Franchini. 2003. Oxidative stability and quality traits of n-3 PUFA enriched chicken meat. Italian Journal of Animal Science 2(sup1):450-452.

Sordillo, L. M. 2016. Nutritional strategies to optimize dairy cattle immunity1. J. Dairy Sci. 99(6):4967-4982.
Sordillo, L. M., G. A. Contreras, and S. L. Aitken. 2009. Metabolic factors affecting the inflammatory response of periparturient dairy. Anim. Health Res. Rev. 10(1):53-63.
Sordillo, L. M. and V. Mavangira. 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Animal Production Science 54(9):1204-1214.
Sordillo, L. M. and K. L. Streicher. 2002. Mammary gland immunity and mastitis susceptibility. J. Mammary Gland Biol. Neoplasia 7(2):135-146.
Speake, B. K., A. M. Murray, and R. C. Noble. 1998. Transport and transformations of yolk lipids during development of the avian. Prog. Lipid Res. 37(1):1-32.
Spector, A. A. and H.-Y. Kim. 2015. Discovery of essential fatty acids. J. Lipid Res. 56(1):11-21.
Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998a. Influence of Supplemental Fats on Reproductive Tissues and Performance of Lactating Cows. J. Dairy Sci. 81(3):856–871.
Stryker, J. A., R. Fisher, Q. You, M. M. Or-Rashid, H. J. Boermans, M. Quinton, B. W. McBride, and N. A. Karrow. 2013. Effects of dietary fish meal and soybean meal on the ovine innate and acquired immune response during pregnancy and lactation. Animal 7(1):151-159.
Swanson, D., R. Block, and S. A. Mousa. 2012. - Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv. Nutr. 3(1):1-7.
Sweeney, B., P. Puri, and D. J. Reen. 2001. Polyunsaturated fatty acids influence neonatal monocyte survival. Pediatr. Surg. Int. 17(4):254-258.
Sweeney, B., P. Puri, and D. J. Reen. 2007. Induction and modulation of apoptosis in neonatal monocytes by polyunsaturated. J. Pediatr. Surg. 42(4):620-628.
Taranu, I., M. Gras, G. C. Pistol, M. Motiu, D. E. Marin, N. Lefter, M. Ropota, and M. Habeanu. 2014. omega-3 PUFA rich camelina oil by-products improve the systemic metabolism and spleen cell functions in fattening pigs. PLoS One 9(10):e110186.
Tedeschi, L. O., A. Cannas, and D. G. Fox. 2010. A nutrition mathematical model to account for dietary supply and requirements of energy and other nutrients for domesticated small ruminants: The development and evaluation of the Small Ruminant Nutrition System. Small Rumin. Res. 89(2-3):174-184.
Terasaki, M., A. Hirose, B. Narayan, Y. Baba, C. Kawagoe, H. Yasui, N. Saga, M. Hosokawa, and K. Miyashita. 2009. Evaluation of Recoverable Functional Lipid Components of Several

Brown Seaweeds (Phaeophyta) from Japan with Special Reference to Fucoxanthin and Fucosterol Contents(1). J. Phycol. 45(4):974-980.
Thering, B. J., D. E. Graugnard, P. Piantoni, and J. J. Loor. 2009. Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J. Dairy Sci. 92(9):4290-4300.
Toral P.G. and C. D. L. Bernard, D. Gruffat, C. Leroux, and Y. Chilliard. 2013. Effects of fish oil and additional starch on tissue fatty acid profile and lipogenic gene mRNA abundance in lactating goats fed a diet containing sunflower-seed oil. Animal 7(6):948-956.
Toral, P. G., Y. Chilliard, J. Rouel, H. Leskinen, K. J. Shingfield, and L. Bernard. 2015. Comparison of the nutritional regulation of milk fat secretion and composition in cows and goats. J. Dairy Sci. 98(10):7277-7297.
Toral, P. G., P. Frutos, G. Hervas, P. Gomez-Cortes, M. Juarez, and M. A. de la Fuente. 2010. Changes in milk fatty acid profile and animal performance in response to fish oil. J. Dairy Sci. 93(4):1604-1615.
Toral, P. G., J. Rouel, L. Bernard, and Y. Chilliard. 2014. Interaction between fish oil and plant oils or starchy concentrates in the diet: Effects on dairy performance and milk fatty acid composition in goats. Anim. Feed Sci. Technol. 198:67-82.
Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy. Res. Vet. Sci. 93(2):695-704.
Tsiplakou, E. and G. Zervas. 2013a. Changes in milk and plasma fatty acid profile in response to fish and soybean oil supplementation in dairy sheep. J. Dairy Res. 80(2):205-213.
Tsiplakou, E. and G. Zervas. 2013b. The effect of fish and soybean oil inclusion in goat diet on their milk and plasma fatty acid profile. Livest. Sci. 155(2-3):236-243.
Tvrzicka, E., L. S. Kremmyda, B. Stankova, and A. Zak. 2011. Fatty acids as biocompounds: their role in human metabolism, health and disease--a review. Part 1: classification, dietary sources and biological functions. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 155(2):117-130.
USDA. 2008. Foodborne illness: what consumers need to know.
Vagni, S., F. Saccone, L. Pinotti, and A. Baldi. 2011. Vitamin E bioavailability: Past and present insights. Food and nutrition sciences 2(10):1088-1096.
Vailati Riboni, M., S. Meier, N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, M. Crookenden, A. Heiser, J. R. Roche, and J. J. Loor. 2015. Adipose and liver

gene expression profiles in response to treatment with a nonsteroidal antiinflammatory drug after calving in grazing dairy cows. J. Dairy Sci. 98(5):3079-3085.
Vailati-Riboni, M., G. Farina, F. Batistel, A. Heiser, M. D. Mitchell, M. A. Crookenden, C. G. Walker, J. K. Kay, S. Meier, J. R. Roche, and J. J. Loor. 2017. Far-off and close-up dry matter intake modulate indicators of immunometabolic adaptations to lactation in subcutaneous adipose tissue of pasture-based transition dairy cows. J. Dairy Sci. 100(3):2334-2350.
Vailati-Riboni, M., M. Kanwal, O. Bulgari, S. Meier, N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, M. Crookenden, A. Heiser, J. R. Roche, and J. J. Loor. 2016. Body condition score and plane of nutrition prepartum affect adipose tissue transcriptome regulators of metabolism and inflammation in grazing dairy cows during the transition period. J. Dairy Sci. 99(1):758-770.
van den Top, A. M., A. T. van 't Klooster, T. Wensing, G. H. Wentink, and A. C. Beynen. 1995. Liver triacylglycerol concentrations around parturition in goats with either pre-partum restricted or free access to feed. Vet. Q. 17(2):54-59.
Vasudevan, S., Y. Tong, and J. A. Steitz. 2007. Switching from repression to activation: microRNAs can up-regulate translation. Science 318(5858):1931-1934.
Vernon, R. G. 2005. Lipid metabolism during lactation: a review of adipose tissue-liver interactions. J. Dairy Res. 72(4):460-469.
Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131(3-4):389-417.
Vluggens, A., P. Andreoletti, N. Viswakarma, Y. Jia, K. Matsumoto, W. Kulik, M. Khan, J. Huang, D. Guo, S. Yu, J. Sarkar, I. Singh, M. S. Rao, R. J. Wanders, J. K. Reddy, and M. Cherkaoui-Malki. 2010. Reversal of mouse Acyl-CoA oxidase 1 (ACOX1) null phenotype by human ACOX1b isoform [corrected]. Lab. Invest. 90(5):696-708.
von Schacky, C., R. Kiefl, E. Jendraschak, and W. E. Kaminski. 1993. n-3 fatty acids and cysteinyl-leukotriene formation in humans in vitro, ex vivo, and in vivo. J. Lab. Clin. Med. 121(2):302-309.
Wang, Y., C. Zhang, X. Fang, Y. Zhao, X. Chen, J. Sun, Y. Zhou, J. Wang, Y. Wang, X. Lan, H. Chen. 2014. Identification and profiling of microRNAs and their target genes from developing caprine skeletal Muscle. PLoS One 9, e96857.

Weldon, S. M., A. C. Mullen, C. E. Loscher, L. A. Hurley, and H. M. Roche. 2007. Docosahexaenoic acid induces an anti-inflammatory profile in. J. Nutr. Biochem. 18(4):250-258.

Welfare Quality® Protocol. 2009. Welfare Quality® Assessment Protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, The Netherlands.

Wenguang, Z., W. Jianghong, L. Jinquan, M. Yashizawa. 2007. A subset of skin-expressed microRNAs with possible roles in goat and sheep hair growth based on expression profiling of mammalian microRNAs. OMICS 11, 385–396.

Wilkins, E., L. Wilson, K. Wickramasinghe, P. Bhatnagar, J. Leal, R. Luengo-Fernandez, R. Burns, M. Rayner, and N. Townsend. 2017. European Cardiovascular Disease Statistics 2017. E. H. Network, ed, Brussels.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2004. Effects of fatty acids on meat quality: a review. Meat Sci 66(1):21-32.

Woods, V. B. and A. M. Fearon. 2009. Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. Livest. Sci. 126(1-3):1-20.

Yaqoob, P. and P. Calder. 1995. Effects of dietary lipid manipulation upon inflammatory mediator production by. Cell. Immunol. 163(1):120-128.

Zapata, R. C., R. Salehi, D. J. Ambrose, and P. K. Chelikani. 2015. Effects of prepartum fat supplementation on plasma concentrations of glucagon-like peptide-1, peptide YY, adropin, insulin, and leptin in periparturient dairy cows. J. Dairy Sci. 98(10):6876-6885.

Zhang, C. 2008. MicorRNomics: a newly emerging approach for disease biology. Physiol. Genomics 33:139-147.

Zhao, G., T. D. Etherton, K. R. Martin, J. P. Vanden Heuvel, P. J. Gillies, S. G. West, and P. M. Kris-Etherton. 2005. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochem. Biophys. Res. Commun. 336(3):909-917.

Zidi, A., M. Amills, A. Tomas, O. Vidal, O. Ramirez, J. Carrizosa, B. Urrutia, J.M. Serradilla, A. Clop. 2010. Short communication: genetic variability in the predicted microRNA target sites of caprine casein genes. J. Dairy Sci. 93, 1749–1753.

Zubr, J. 1997. Oil-seed crop: Camelina sativa. Ind Crop Prod 6(2):113-119.

Zubr, J. 2003. Qualitative variation of Camelina sativa seed from different locations. Ind Crop Prod 17(3):161-169.

Zubr, J. and B. Matthaus. 2002. Effects of growth conditions on fatty acids and tocopherols in Camelina sativa oil. Ind Crop Prod 15(2):155-162.
Zymon, M., J. Strzetelski, and G. Skrzynski. 2014. Aspects of appropriate feeding of cows for production of milk enriched in the fatty acids, EPA and DHA. A review. Journal of Animal and Feed Sciences 23(2):109-116.

7. SUMMARY

The aim of the present thesis is to evaluate the effects of different dietary fatty acid sources on animal health and product quality, in addition, the possible beneficial effects on human health from animal product consumption was also a central motivation for this evaluation. With this purpose, two different trials were performed in dairy goats and laying hens respectively. Accounted for, were: (i) The expression of selected lipid metabolism and inflammation-related genes, and miRNA in the liver and adipose tissue of small ruminants around kidding which were fed saturated (SFA) or polyunsaturated (PUFA) fatty acid supplements in their diets from stearic acid or fish oil, (ii) The performance and egg quality of laying hens fed PUFA from *Camelina sativa* dietary supply. In the last decades PUFA supply, especially EPA and DHA among others, was reported to be effective in both the improvement of animal health, through the modulation of the metabolism and immune systems, and the enrichment of animal products with essential fatty acids that can lead to positive effects on human health.

The first trial considered twenty-three second-parity twin-diagnosed alpine dairy goats that were either fed from one week before (30 g/head/d fatty acids) to three weeks after kidding (50 g/head/d fatty acids) calcium stearate (ST, n= 7) or fish oil (FO, n= 8), and were compared to a control group fed a basal diet (CON, n= 8). Liver and adipose biopsies were collected on day -7, 7 and 21 from kidding. Quantitative PCR was used to measure mRNA and microRNA expression of 38 targets. Dietary treatments up-regulated (ST) and down-regulated (FO) Stearoyl-CoA desaturase (SCD) alone. Time around kidding strongly affected most of the target genes for lipid metabolism in liver, being mostly up-regulated from -7 to 7 DIM and down-regulated in the third week of lactation, while in adipose tissue a gradual down-regulation was observed throughout the trial. Acute-phase proteins were increased in adipose tissue in the two weeks around kidding and decreased from 7 to 21 DIM. Pro-inflammatory

interleukins (*IL6* and *IL8*) peaked in the first week after parturition, while *IL10* was up-regulated in the last two weeks of the trial. With the exception of an up-regulation of miR-155 from -7 to 7 d from kidding, no effects of time were observed on miRNA expression in adipose tissue. Liver *ACOX1* was greater in ST than CON on day 7 while *SCD* showed a higher expression from 7 to 21 DIM in ST compared to FO. Then, dealing with adipose tissue, *LPIN1* had increase values in FO at 7 days after kidding (compared to ST) and *FASN* had the highest expression in stearate group 7 days before parturition, if compared to CON and FO. The expression of *IL6* in adipose tissue had a down regulation in ST compared to control group at 7 DIM. The present trial revealed limited effects of dietary saturated or polyunsaturated supply around kidding on fatty acid metabolism - and inflammation - related genes in the liver and adipose tissue of dairy goats. At the same time, a physiological trend was observed in the two weeks around kidding with increased β-oxidation in the liver, and decreased fatty acid synthesis together with the higher inflammation processes in adipose tissue.

The second trial involved a total number of 32 laying hens that were randomly allocated to two experimental groups, with eight replicates per group. Animals were fed a common basal diet with (T) or without (C) inclusion of 7.5% Camelina meal for a total period of 63 days. Performance was evaluated weekly, while egg quality was determined at days 0, 28, 56 and shelf-life/TBARS at days 0, 9, 21, 28. Camelina meal supplementation improved feed conversion rate in laying hens ($P<0.05$). Moreover, reduced saturated fatty acids (SFA) content was observed in the yolk of T hens (33.18 vs. 32.45 g/100g total fatty acids; $P< 0.01$), with a concomitant enrichment in α-linolenic acid (1.05 vs. 1.47 g/100g total fatty acids; $P< 0.01$). Results of the present study show that it is possible to improve yolk quality by feeding *Camelina*

sativa meal to laying hens, reducing egg SFA content and providing increased amount of beneficial n-3 fatty acids.

As a result, PUFA can exert a duplicate effect: on animal health and, indirectly, on human health.

Indeed, they can act not just as a source of energy but also as an integral part of animal feed during stressing events such as peripartum. As a consequence, these nutritional strategies can also improve FA composition of animal products, with the decrease of SFA and increase of monounsaturated FA (MUFA) and PUFA contents, and a fortification of human diet.

8. RIASSUNTO IN ITALIANO

Lo scopo della presente ricerca è stato quello di valutare degli effetti della somministrazione di differenti fonti di acidi grassi sulla salute animale e sulla qualità dei prodotti derivati; non di meno, sono stati oggetto di analisi i possibili effetti benefici sulla salute umana. A tale scopo, sono state effettuate due distinte prove sperimentali, una su capre da latte e l'altra su galline ovaiole.

La prima prova ha preso in considerazione lo studio dell'espressione di geni e miRNA coinvolti nel metabolismo lipidico e nel processo infiammatorio a livello di tessuto epatico e adiposo sottocutaneo di nella capra da latte in transizione a seguito della somministrazione di fonti grassate apportanti acidi grassi saturi (SFA) o polinsaturi (PUFA) della serie omega 3 e 6 quali acido stearico e olio di pesce.

La seconda sperimentazione ha preso in considerazione le performance e la qualità dell'uovo prodotto in galline ovaiole la cui dieta è stata supplementata con *Camelina sativa*.

Negli ultimi decenni l'integrazione della dieta con PUFA, in particolare con l'acido eicosapentaenoico (EPA) e docosaesaenoico (DHA), si è dimostrata efficace in termini di salute sia a livello animale, attraverso la modulazione del metabolismo e del sistema immunitario, sia a livello dell'uomo, con il consumo di alimenti animali arricchiti di acidi grassi essenziali.

Il primo caso di studio ha preso in esame 23 capre da latte di razza Alpina diagnosticate per un parto gemellare. La sperimentazione ha previsto l'integrazione della dieta da una settimana prima (30 g/head/d di acidi grassi) a tre settimane dopo il parto (50 g/head/d di acidi grassi) di calcio stearato (ST, n= 7) o olio di pesce (FO, n= 8), e il confronto di questi due gruppi sperimentali con un gruppo di controllo (CON, n= 8), alimentato con una dieta base. Sono state effettuate biopsie di fegato e tessuto adiposo sottocutaneo a -7, 7 e 21 giorni dal parto al

fine di determinare l'espressione mRNA e miRNA di 38 geni implicati nel metabolismo lipidico di fegato e tessuto adipose e nella risposta infiammatoria a livello adiposo, mediante Polymerase Chain Reaction (PCR). Ad eccezione di Stearoyl-CoA desaturasi (*SCD*) (fegato), non abbiamo riscontrato variazioni significative nell'espressione dei geni oggetto di studio a seguito della somministrazione dei trattamenti (FO e ST). Specificatamente, la supplementazione lipidica ha portato ad un aumento dell'espressione di *SCD* nel gruppo ST e una riduzione nel FO. L'effetto del tempo ha influenzato l'andamento della maggior parte dei geni target per il metabolismo lipidico del fegato, vedendone l'espressione aumentata da -7 a 7 giorni di lattazione e ridotta nella terza settimana di lattazione mentre, a livello di tessuto adiposo, è stata osservata una down regulation per tutta la durata della prova. Per quanto riguarda l'andamento dell'espressione dei geni implicati nei fenomeni di infiammazione nel tessuto adiposo, i risultati del presente studio hanno evidenziato una up-regulation delle proteine di fase acuta nelle due settimane in prossimità del parto, diminuita poi da 7 a 21 giorni di lattazione. Le interleuchine pro-infiammatorie (*IL6* and *IL8*) hanno raggiunto un picco nella prima settimana dopo il parto, mentre abbiamo osservato una up-regulation di *IL10* nelle ultime due settimane di prova. Considerando i microRNA, ad eccezione dell'aumento dell'espressione del miR-155 da -7 a 7 die dal parto, non è stato osservato nessun effetto del tempo. Analizzando l'effetto interazione dieta*tempo nel metabolismo del fegato, Acyl-CoA Ossidasi 1 (*ACOX1)* ha visto aumentata la sua espressione nel gruppo ST, rispetto al CON al giorno 7 mentre *SCD* ha evidenziato una maggiore espressione da 7 a 21 giorni nel gruppo ST, se comparato al gruppo FO. Passando poi al tessuto adiposo, Lipin1 (*LPIN1)* ha mostrato valori di espressione maggiori per il FO a 7 giorni di lattazione (rispetto a ST) e l'espressione di *FASN* ha raggiunto valori più elevati nel gruppo stearato a -7 DIM rispetto al controllo e al FO. L'espressione di *IL6* nel tessuto adiposo ha mostrato una down-regulation nel gruppo

stearato rispetto al gruppo controllo al giorno 7. Il presente studio ha evidenziato limitati effetti dell'espressione dei geni considerati, coinvolti nel metabolismo lipidico e nell'infiammazione in fegato e tessuto adiposo, a seguito dell'integrazione di acidi grassi saturi e polinsaturi in capre da latte vicine al parto. Nel contempo, è stato osservato un trend fisiologico nelle due settimane in prossimità del parto, caratterizzato da un aumento della β-ossidazione nel fegato e da una diminuzione nella sintesi di acidi grassi, accompagnata da più intensi processi infiammatori, a livello di tessuto adiposo.

La successiva prova sperimentale si svolta con un numero totale di 32 galline ovaiole, assegnate in modo casuale a due gruppi oggetto di studio, con otto replicati per gruppo. Gli animali sono stati alimentati con una dieta base con (T) o senza (C) l'inclusione del 7.5% di farina di camelina per un totale di 63 giorni. Le performance degli animali sono state valutate settimanalmente, mentre la qualità dell'uovo è stata determinata a 0, 28, 56 giorni e la shelf-life/TBARS a 0, 9, 21, 28 giorni. Si è visto come l'integrazione della farina di camelina abbia migliorato il tasso di conversione dell'alimento nelle galline ($P<0.05$). Inoltre, è stata osservata una riduzione del contenuto di acidi grassi saturi (SFA) nel tuorlo delle galline appartenenti al gruppo T (33.18 vs. 32.45 g/100g di acidi grassi totali; $P< 0.01$), concomitante ad un arricchimento in acido α-linolenico (1.05 vs. 1.47 g/100g di acidi grassi totali; $P< 0.01$). I risultati così ottenuti mostrano come sia possibile, integrando la dieta di galline ovaiole con farina di *Camelina sativa,* migliorare la qualità del tuorlo, agendo sulla riduzione del contenuto in SFA e apportando un aumento di acidi grassi essenziali n-3.

Ne consegue che i PUFA possano avere un duplice impatto: sulla salute animale e, indirettamente, sulla salute del consumatore.

Infatti, questi acidi grassi non solo si sono dimostrati essere una fonte energetica ma anche una valida integrazione alla dieta animale durante i periodi più stressanti, quale, ad esempio il

periparto. Di conseguenza, questo tipo di strategie alimentari possono agire su due fronti: da un lato migliorare la composizione acidica dei prodotti di origine animale, con un decremento nel contenuto di SFA e un aumento di acidi grassi monoinsaturi (MUFA) e PUFA, e, dall'altro, arricchire la dieta del consumatore.

9. PUBLISHED WORKS

9.1. Scientific papers

Farina G., Cattaneo D., Lecchi C., Invernizzi G., Savoini G., Agazzi A. 2015. A Review on the Role of EPA and DHA through goat nutrition to human health: could they be effective both to animals and humans? Journal of Dairy, Veterinary and Animal Research. DOI: 10.15406 /jdvar.2015.02.00027.

Savoini G., Farina G., Dell'Orto V., Cattaneo D. 2016. Through ruminant nutrition to human health: role of fatty acids. Advances in Animal Biosciences. 7(2): 200-207.

Savoini G., Dell'Orto, Farina G., Cattaneo D. 2016. Nutrients for animal health and for improvement of nutritional quality of animal products for human consumption. Atti dei Georgofili. *In press*

Vailati-Riboni M., Farina G., Batistel F., Heiser A., Mitchell M.D., Crookenden M.A., Walker C.G., Kay J.K., Meier S., Roche J.R., Loor J.J. 2017. Far-off and close-up dry matter intake modulate indicators of immunometabolic adaptations to lactation in subcutaneous adipose tissue of pasture-based transition dairy cows. Journal of Dairy Science. 100:2334-2350.

Ahasan A.S.M.L., Invernizzi G., Farina G., Pilotto A., Barbè F., Bontempo V., Rossi R., Bellagamba F., Lecchi C., Savoini G., Agazzi A. 2018. The effect of superoxide dismutase rich-melon pulp concentrate on antioxidant status, inflammation and growth performance of piglets exposed to chronic lipopolyssaccharide challenge. Animal. doi 10.1017/S1751731118001234.

9.2. Meeting abstracts

Farina G., Agazzi A., Caputo J.M., Campagnoli A., Ferroni M., Loor J.J., Savoini G., Invernizzi G. 2015. Hepatic and subcutaneous adipose lipid metabolism genes modulation by dietary fish oil and stearate in transition goats. Proceedings of Veterinary and Animal Science Days. 15th-17th July, Milan, Italy. International Journal of Health, Animal Science and Food Safety. 2(1s). DOI: 10.13130/2283-3927/5009.

Ahasan ASML, Agazzi A., Barbe F., Invernizzi G., Caputo J.M., Pilotto A., Farina G., Moretti V.M., Lecchi C.,Dell'Orto V., Savoini G. 2015. Effect of SOD-rich melon supplement in LPS challenged piglets on antioxidant status and growth performance. Book of abstract of the 66th of the Annual Meeting European Federation of Animal Science EAAP, 31st August - 4th September, Warsaw. 119

Farina G., Agazzi A., Invernizzi G., Campagnoli A., Loor J.J., Savoini G. 2016. Transcriptional regulation of lipid metabolism and inflammation in transition dairy goats by fish oil and stearate. Proceedings of Veterinary and Animal Science Days. 8th- 10th June, Milan, Italy.

International Journal of Health, Animal Science and Food Safety. 3(1s). DOI: 10.13130/2283-3927/7163.

Farina G., Mariani E., Vasconi M., Rebucci R., Moretti V.M., Agazzi A., Savoini G., Invernizzi G. 2017.Camelina cake in laying hens diet to enrich eggs with omega-3 fatty acids.. Book of abstract of the 22nd Congress of Animal Science and Production Association. 13th - 16th June, Perugia, Italy. Italian Journal of Animal Science 16(s1): 141.

Farina G., Agazzi A., Invernizzi G., Savoini G., Loor J.J. 2017. Can lipid supplementation modulate inflammatory state and immune response in periparturient goats? A case study on hepatic and adipose miRNA expression. Proceedings of Veterinary and Animal Science Days. 15th- 17th July, Milan, Itlay. International Journal of Health, Animal Science and Food Safety. 4(1s). DOI: 10.13130/2283-3927/8385.

Farina G., Invernizzi G., Caputo J.M., Agazzi A., Dell'Orto V., Loor J.J., Savoini G. 2016. Transcriptional regulation of lipid metabolism in transition dairy goats by fish oil and stearate. Book of Abstract of the 67th Annual Meeting of the European Federation of Animal Science. 29th August – 2nd September Belfast, United Kingdom. 359.

Agazzi A., Savoini G., Farina G., Mariani E., Doronzo M., Valentinelli C., Belladelli C. 2016. Changes in body condition and serum metabolites in endurance horses fed stabilized rice bran. Book of Abstract of the 67th Annual Meeting of the European Federation of Animal Science. 29th August – 2nd September Belfast, United Kingdom. 660

Agazzi A., Invernizzi G., Ahasan A.S.M.L., Farina G., Longo R., Barbe F., Crestani M., Dell'Orto V., Savoini G. 2017. Dietary SOD-rich melon on hepatic gene expression of antioxidant proteins in piglets and broilers. Book of abstracts of the 68th Annual Meeting of the European Federation of Animal Science. 28th August –1st September. Tallinn, Estonia. 395.

9.3. Oral presentations

Caputo J.M., Invernizzi G., Campagnoli A., Farina G., Ferroni F., Agazzi A., Loor J.J., Savoini G. 2015. Dietary fish oil and stearate action on adipose lipid metabolism transcriptomics in periparturient dairy goats. Book of abstract of the 21st Congress of Animal Science and Production Association. 9th - 12nd June, Milan, Italy. Italian Journal of Animal Science. 14(s1): 72.

Farina G., Invernizzi G., Perricone V., Agazzi A., Cattaneo D., Loor J.J., Savoini G. 2017. Fatty acid supplementation in transition goats: a transcriptional study related to inflammation. Book of the 68th Annual Meeting of the European Federation of Animal Science. 28th August - 1st September. Tallinn, Estonia. 444

10. ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Prof. Agazzi for his support of my PhD studies and related research, for his patience, motivation, and willingness. His guidance helped throughout my research and the writing of this thesis, and contributed to the achievement of this important goal.

In addition to my advisor, I would like to thank the Director of the Department of Health, Animal Science and Food Safety (VESPA), Professor Giovanni Savoini.

My sincere thanks also go to Prof. Juan J. Loor, who provided me with the opportunity to join his team at the Department of Animal Sciences & Division of Nutritional Sciences (University of Illinois – Urbana – IL) and who gave me access to the laboratory and research facilities (Animal Sciences Laboratory).

I thank my fellows (Elena, Vera, Marcello, Adriano, Fabio, Silvia, Guido, Cecilia, Elisabetta) for the time we worked together, and for all the fun we have had during the period of my studies.

I would like to thank my family: my parents and my sister for supporting me spiritually and in my day to day life throughout the writing of this thesis.

And finally, last but by no means least, a very special gratitude goes out to my boyfriend, Vishal, for bringing much color into my life over this time.

Thanks for all your encouragement!

11. TABLE OF CONTENT

11.1. List of Figures

Figure 1. Pathways of biosynthesis ω−6 and ω−3 fatty acids. (Adapted from Nagy and Tiuca (2017)). ..8

Figure 2. Lipid metabolism in the rumen. The prevalent fat types in feedstuff are triglycerides and glycolipids (adapted from Bauman and Lock (2006)). 11

Figure 3. Rumen biohydrogenation of fatty acids (adapted from Savoini et al., (2016)). 12

Figure 4. Schematic metabolic relationships among lipid metabolism in adipose tissue, liver, and mammary gland during the transition period. (+) indicates stimulatory effects, (-) indicates inhibitory effects. Dashed lines indicate processes that happen at low rates or only during certain physiological states. (NE = norepinephrine, Epi = epinephrine, CPT-1 = carnitine palmitoyltransferase-1, GPAT = glycerol-3 phosphate acyltransferase, TG = triglyceride, CoA = coenzyme A, VLDL = very low density lipoprotein. Adapted from Drackley (1999). 15

Figure 5. Hypothesized responses to resolved and unresolved inflammation in early-lactation dairy cows (adapted from Bradford et al. (2015))..... 20

Figure 6. Flow chart of the possible events underlying a negative outcome of the response to the combined stress of pregnancy/lactation onset in dairy cows (adapted from Trevisi et al. (2012)). 21

Figure 7. Metabolic fates of dietary fatty acids (adapted from Ganesan et al. (2014))..... 22

Figure 8. Proposed model for transcription factor activation by nutrients in the cell (adapted from Osorio et al. (2017))..... 23

Figure 9. α-Linolenic acid, long chain n-3 fatty acid and total n-3 fatty acid supplied through eggs from layer hens fed Control or Camelina. [a-b]Means between diets and within a fatty acid type without a common letter differ significantly ($P < 0.05$). Control and Camelina represent corn-soybean meal basal diet (Control); or basal diets containing camelina (Camelina) at 10 % (adapted from Cherian and Quezada (2016)). 45

Figure 10. mRNA expression change-folds of liver metabolism-related genes[1,2] in transition dairy goats fed saturated or polyunsaturated fatty acids[3]. 71

Figure 11. mRNA expression change-folds of adipose tissue metabolism-related genes[1,2] in transition dairy goats fed saturated or polyunsaturated fatty acids[3]. 72

Figure 12. mRNA expression change-folds of adipose tissue inflammation-related genes[1,2] in transition dairy goats fed saturated or polyunsaturated fatty acids[3]. 75

Figure 13. Change-folds of microRNA expression in adipose tissue[1,2] of transition dairy goats fed saturated or polyunsaturated fatty acids[3]. 76

Figure 14. Trend of selected fatty acid content in egg yolk at different days of the trial (0, 28, 56). A Palmitic acid (C16:0); B Stearic acid (C18:0); C Saturated fatty acids (SFA); D α-linolenic acid (ALA) (C18:3 n-3); E Arachidonic acid (AA) (C20:4 n-6); F Docosahexaenoic acid (DHA) (C22:6 n-3). (CON, control; TRT, treated; D, diet; T, time; D*T, diet*time). [a,b] $P<0.05$..... 104

10.2. List of Tables

Table 1. Ingredients (g/100g feed as-fed) and nutrient composition (% of DM unless otherwise noted) of the experimental diets. 56

Table 2. Accession number, gene symbol, and forward and reverse primer sequences of genes analyzed in adipose and hepatic tissue, involved in lipid metabolism. 63

Table 3. Accession number, gene symbol, and forward and reverse primer sequences of genes analyzed in adipose tissue with involvement in inflammatory and immune state 65

Table 4. Effect of time on mRNA and miRNA expression change-folds1 of liver and adipose tissue metabolism- and inflammation-related genes in transition dairy goats. 69

Table 5. Ingredients, chemical composition and fatty acid composition of the two experimental diets (g/100 g total fatty acids) 92

Table 6. Chemical composition and fatty acid composition of camelina cake. 94

Table 7. Laying rate, daily feed intake and feed conversion rate of control (C) and treated (T) hens.. 100

Table 8. Evaluation of egg shelf-life and TBARS (C, control; T, treated). 101

Table 9. Fatty acid composition of egg yolk assessed at days 28-56 of the trial (C, control; T, treated). (SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid). (g/100g total fatty acids)..... 103