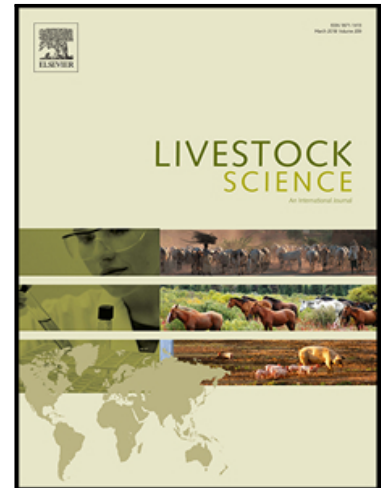


Journal Pre-proof

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Highlights:

- The transition of dairy cows is characterized by metabolic and health disorders.
- A state of insulin resistance and pro-inflammatory is highlighted.
- Beneficial effects of PUFAs on health have been evidenced in several species.
- PUFAs can contribute to the cows' adaptation during the transition period.

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Relevant aspects of dietary n-3 polyunsaturated fatty acids in the adaptation of dairy cattle to the transition period

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Short title: n-3 PUFA effects on the transition period of dairy cows

Abstract

During the transition period, cows undergo a series of metabolic and immunological changes, which represent a physiological challenge that is often a risk for their production and health. During this period, dairy cows have a pro-inflammatory state associated with frequent infectious diseases and lipomobilization, and present an insulin resistance state related to fat mobilization and a high body condition score. In humans, the polyunsaturated fatty acids (PUFAs) from the omega-3 (n-3) family, which are involved in many biological systems and processes, have been shown to exert beneficial effects on health, particularly on the metabolic syndrome. Therefore, the incorporation of n-3 PUFAs in the diets of dairy cows could represent an interesting supplement for them to afford the challenge of the peripartum. This review describes the novel information and advances in the understanding of relevant aspects concerning the effects of dietary PUFAs on dairy cows, focusing on metabolic and inflammatory aspects with possible effects on the cows' physiology and adaptation to the peripartum period.

Keywords: PUFAs; PRO-INFLAMMATORY STATE; INSULIN RESISTANCE; DAIRY CATTLE; TRANSITION PERIOD.

1. Introduction

In recent decades, genetic selection of dairy cows has allowed a significant increase in milk production per cow (Oltenucu and Broom, 2010). However, for this increase, cows have had to adapt to the higher energy and nutrient requirements, particularly in the first postpartum weeks, a period during which the dry matter intake (DMI) has not been restored and the animals are in negative energy balance (NEB) (Sundrum, 2015). In accordance with the increase in milk production and the adaptation problems, the prevalence of metabolic diseases has increased (van Saun and Sniffen, 2014), adversely affecting reproductive performance, a fact that has become one of the main factors that influences the profitability of a dairy farm (Giordano et al., 2011; Cattaneo et al., 2015). In this sense, the current genetic selection aims not only to breed dairy cattle that are productive but also fit for use, providing good health and fertility with good animal welfare conditions in dairy farms (De Vries, 2020).

The challenge associated with the increased energy and nutrient demand of dairy cows is particularly relevant during the so-called transition or peripartum period (three weeks before and three weeks after calving). This period represents the most critical moment of the productive cycle of dairy cows and the time during which the greatest metabolic and endocrine changes (Grummer, 1995) and most of the nutritional, infectious and productive problems occur (Roche et al., 2013). Drackley (1999) described the transition period as the period of main limitation in milk production and, in recent years, numerous researchers have focused on this period, trying to develop different technologies to improve cow health and milk production. In this context, polyunsaturated fatty acids (PUFAs) have emerged as nutritional supplements that could help dairy cows to face the challenge of milk production during the transition period.

The omega-3 PUFAs family, usually found in some seed and vegetable oils, like flaxseed and rapeseed, fish, seafood and also algae, have shown benefits on human health and prevention of several diseases, such as cardiovascular disease, atherosclerosis, inflammation, cancer, type II diabetes, immune function, dementia, cognitive function, and arthritis (Ruxton et al., 2004; Doughman et al., 2007). Thus, several studies have been carried out with the aim to increase the content of PUFAs in animal products such as meat and milk (Dewhurst et al., 2006; Kouba and Mourot, 2011). Furthermore, researchers have also studied whether the beneficial effects of PUFAs on human health could be transferred to dairy cows, particularly during the transition period (Lessard et al., 2004; Carriquiry et al., 2009; Gandra et al., 2016).

The peripartum period of dairy cows has been described as a state of insulin resistance similar to that observed in type II diabetes in humans (De Koster and Opsomer, 2013). In addition, during this period, cows, especially overweight ones, may present some elements similar to those of the human metabolic syndrome (Bertoni et al., 2009; Sordillo and Mavangira, 2014), a disorder that includes abdominal obesity, insulin resistance, dyslipidemia, and increased blood pressure, and is associated with other comorbidities such as pro-thrombotic state, pro-inflammatory state, nonalcoholic fatty liver disease, and reproductive disorders (Cornier et al., 2008). In this context, since n-3 PUFAs have shown to be relevant in the prevention of type II diabetes mellitus, especially in overweight persons (Nettleton and Katz, 2005), these PUFAs could represent an important nutritional alternative to be incorporated in the diets of dairy cattle to help them cope with the challenge posed by the transition period. Thus, in the last years, some studies have evaluated the supplementation of the cows' diet with different sources of n-3 PUFAs (Greco et al., 2015; Elis et al., 2016). This review highlights some of the recent advances in the understanding of relevant aspects

concerning the effects of dietary PUFAs on dairy cows during the transition period, focusing on the metabolic and inflammatory aspects that could have effects on the physiology and adaptation to this period.

2. PUFAs: synthesis and source

PUFAs participate in multiple physiological processes, playing, among others, a structural role in the phospholipids of cell membranes and being substrates for the synthesis of different physiological mediators. These molecules have double carbon-carbon bonds and are named according to the total length of the chain and the position of these bonds, which allows them to be classified into two main families: omega-3 (n-3) and omega-6 (n-6), according to the position of the first double bond from the methyl end group of the fatty acid.

Alpha-linolenic acid (ALA, 18:3) and linoleic acid (LA, 18:2) are precursors of other n-3 and n-6 PUFAs, respectively (Figure 1). Their metabolism generates the long chain PUFAs (LC-PUFAs) with 20 or more carbon atoms. Particularly, the most important bioactive lipids of the n-3 family are docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5), and, as mentioned above, numerous studies and clinical investigations have provided evidence of their beneficial effects for health and prevention of several chronic diseases (Lombardo and Chicco, 2006; Lombardo et al., 2007; Zárata et al., 2017).

LC-PUFAs are synthesized by desaturation and elongation processes of fatty acids where reactions involving Δ -6 and Δ -5 desaturases are limiting (Tosi et al., 2014) (Figure 1). Moreover, the Δ -6 desaturase activity is influenced by a large number of hormones and while some are depressants of its activity (glucagon, adrenaline, and thyroxine), insulin can activate it (Brenner, 2003). In humans, these enzymes have a preference for ALA, but the presence

of high levels of plasma LA (caused by high n-6 PUFAs intakes) can shift their actions towards the n-6 pathway, resulting in an inhibition of the pathway that converts ALA to EPA and DHA (Ruxton et al., 2004). Mammalian cells have no desaturases with the ability to introduce a double bond in the n-3 and n-6 position, so this group of fatty acids cannot be synthesized and the n-3/n-6 families cannot be interconverted, so they must be incorporated into the food and are consequently considered essential. In this context, the dietary n-6/n-3 ratio is nowadays considered an important nutritional aspect in the human diet. For example, in infant nutrition, the optimal n-6/n-3 ratio must be lower than 10 (Abedi and Sahari, 2014).

In humans, ALA supplementation does not significantly change the plasma concentration of EPA and DHA, suggesting that the conversion of ALA to DHA is inefficient (Plourde and Cunnane, 2007). Therefore, for many decades, there has been a growing interest in foods that directly contribute EPA and DHA, especially fishes, shrimps, prawns, crabs, shellfishes, and algae (Abedi and Sahari, 2014). Based on the results observed with these foods in human nutrition, in the last decades, there has been a growing interest in the use of these PUFAs in the diets of dairy cattle, and thus studies supplementing diets with n-3 PUFAs from fish oil, algae and flax seeds or derivatives thereof have been performed (Moallem, 2018).

The information regarding the elongation and desaturation processes of ALA in ruminant tissues is scarce. However, some researchers have shown that the incorporation of sources of ALA in the diet increases the content of n-3 LC-PUFAs in plasma, muscle and milk, confirming a limited, but significant elongation and desaturation of ALA in ruminant tissue (Herdmann et al., 2010; Moallem and Zachut, 2012; Shingfield et al., 2013). Also, some authors have described a low rate of elongation and desaturation of ALA in the bovine liver (Moallem, 2018). In this sense, Cherfaoui et al. (2012) have identified gene and protein

expression of several enzymes involved in n-3 LC-PUFAs biosynthesis in the liver, adipose tissue and muscle of bulls.

However, other studies in bovines fed diets containing flaxseed derivatives have shown increases in the concentrations of ALA, EPA and docosapentaenoic acid (DPA; Figure 1), but not necessarily of DHA (Nassu et al., 2011; Moallem and Zachut, 2012), suggesting that the desaturation/elongation chain of n-3 fatty acids seems to be blocked at the level of DPA. Similar results have been observed in non-ruminants, suggesting that DHA biosynthesis in ruminants and non-ruminants is strictly metabolically regulated, so that its concentration cannot be substantially influenced by the diet, unless it is supplied directly through the diet (Raes et al., 2004).

2.1. Source of n-3 PUFAs in ruminants

The supplementation with PUFAs in the diet of dairy cows presents two major challenges. The first one is to avoid underestimating the composition and quantity of PUFAs in the supplementation, since this would threaten the survival of the normal ruminal flora due to the toxic effect of certain fatty acids, limiting their incorporation into the diet (Maia et al., 2007; Carreño et al., 2019). The second one is to consider that PUFAs in the rumen have short half-lives due to the ruminal biohydrogenation (BH) by the action of the microbial flora (Jenkins, 1993; Carreño et al., 2019). Thus, in recent years, the discovery of other fatty acids with beneficial effects in ruminants, such as conjugated linoleic acid (CLA, cis-9, trans-11C18:2) and other n-3 fatty acids, has led to studies aimed to identify the intermediates formed during ruminal BH and evaluate the microbial ecology of the rumen involved in these processes (Jenkins et al., 2008; Buccioni et al., 2012).

In recent decades, numerous strategies have been developed to avoid the toxic effects of fatty acids on ruminal bacteria, as well as the changes that they cause in fatty acid

molecules, especially by analyzing the bioactive agents that are incorporated into the ruminant diet. In this regard, several sources of PUFAs as well as treatments or protection technologies have been evaluated. One strategy to avoid ruminal BH could be feeding cows with whole oilseeds, because the seed coat prevents access of the rumen microorganisms to the PUFAs (Mach et al., 2006). Flaxseeds (*Linum usitatissimum*), for example, have significant concentrations of LA, and contain approximately 40 % oil and approximately 55 % of the total fatty acids in the oil as LA (Petit, 2010). Regarding protection technologies, different seed treatments have been evaluated for supplementation: an example of this is the protection of flaxseeds with formaldehyde to increase the proportion of LA in plasma triacylglycerols and milk fat, while preventing the BH of LA but also decreasing the digestibility of the total tract of crude fat (Sterk et al., 2012).

A recent review has extensively described the advantages and disadvantages of the different ruminal fat protection technologies (Gadeyne et al., 2017). Two main types of lipid protection of the rumen have been described: blocking the free carboxyl end of the fatty acid (by using for example calcium salts) or encapsulating a covering to prevent the hydrogenating bacteria from accessing the fatty acid. Although calcium salts are one of the most widely used technologies, some studies evaluating this technology have observed low transfers of PUFAs to milk, suggesting that fatty acids in the rumen could be partially dissociated (Castañeda-Gutiérrez et al., 2007; Côrtes et al., 2010). Other protective coatings have been developed using different elements, such as formaldehyde, which exhibits high transfer of PUFAs to milk, but the high toxicity of the crosslinking agent has limited its use. Furthermore, the heating step necessary to produce the protective coatings could promote the oxidation of the PUFAs, increasing the concentration of hydroperoxides in the oil (Gadeyne et al., 2017).

Other sources of LC-PUFAs, such as fish oil or marine algae, which have high concentrations of n-3 fatty acids, particularly EPA and DHA, should also be considered for ruminant supplementation. Since fish oil contains approximately 1.34 % and 6.13 % of EPA and DHA, respectively (Woods and Fearon, 2009), this could solve the problem of the inefficient conversion of ALA to DHA. However, although calcium salts have been produced from fish oil, some authors have not found protection against ruminal BH of EPA and DHA (Castañeda-Gutiérrez et al., 2007).

3. Relationship between PUFAs, milk production and DMI in dairy cows

During the postpartum period, dairy cows with high body condition score (BCS) tend to exhibit more NEB and lipomobilization and are thus more predisposed to metabolic and infectious diseases (Roche et al., 2009). The temporary period of insulin resistance described in the postpartum of dairy cows aims to reduce the glucose uptake by peripheral tissues to prioritize milk production, but this situation can further increase the NEB, compromising the cow's health and postpartum reproduction (De Koster et al., 2015).

Supplemental fat could increase the energy density of diets and energy intake by dairy cattle. Nevertheless, a study in which fat was added with the aim to improve energy intake and milk yield showed inconsistent results (Allen, 2000). In this sense, Rabiee et al. (2012) described vast differences in milk production and composition in response to different commonly used fats. These authors also showed that fat supplements tend to reduce the DMI, a fact that could increase the NEB and is directly related to milk yield. This greater hypophagic effect of fat may be due to a reduction in ruminal fiber digestion, a fact that increases distension in the rumen, and may be related to a greater proportion of unsaturated fatty acids (Allen, 2000). However, depressed feed intake has also been observed after

feeding cattle with rumen protected fats or after performing abomasal infusions of fat, suggesting gut or metabolic regulation (Bremmer et al., 1998; Relling and Reynolds, 2008). Relling and Reynolds (2007) demonstrated that the intestinal secretion of cholecystokinin and glucagon-like peptide 1, activated by the presence of lipids, had an inhibitory effect on the ingestion and reticulum-rumen motility. The detrimental effects of fat supplementation on DMI depend on several factors such as physical and chemical characteristics of the fat supplement, palatability, chain length, saturation or unsaturation of fatty acids, level and duration of the supplemental fat in the diet and stage of lactation (Moallem, 2018). On the other hand, Rabiee et al. (2012) described a variability in milk components regarding the addition of different fat sources in the diet. Regarding this, the biosynthesis of some CLA isomers by the rumen BH of PUFAs has been related to milk fat depression (Jenkins and Harvatine, 2014).

Nevertheless, in recent decades, there has been a growing interest in specific dietary fats with the potential to improve health, production and fertility (Lean et al., 2013). Regarding this sense, despite the relationship between fat supplementation and DMI described, some researchers have observed an increase in milk yield and postpartum DMI in cows supplemented with calcium salts of fish oil during postpartum (0.70 % DM and 2.4 % DM) (Moussavi et al., 2007; Greco et al., 2015). Also, Zachut et al. (2010) incorporated extruded flaxseed in the prepartum (7.9 % DM) and postpartum diet (9.2 % DM), and observed an increase in milk yield, DMI and energy balance. These studies may indicate that the metabolic advantages of incorporating n-3 PUFAs may represent a greater advantage compared to a possible effect on DMI. In this sense, long-chain fatty acids provided by protected fats could energetically replace glucose in the mammary gland, and thus contribute to the synthesis of lactose (Hammon et al., 2008). However, this statement is not conclusive

because other more recent studies with fish oil supplements during the peripartum (1 % to 1.4 % DM) have not found differences in milk yield (Badiei et al., 2014; Elis et al., 2016).

4. Effects of PUFAs on inflammation

Several authors have described the peripartum of dairy cows as a pro-inflammatory period characterized not only by frequent infectious diseases but also by high BCS and fat mobilization (Bertoni et al., 2008; Bradford et al., 2015). In human medicine, PUFAs have been shown to be beneficial in inflammatory diseases (Ruxton et al., 2004), whereas in dairy cows, Trevisi et al. (2011) observed that supplementation with n-3 PUFAs (protected algae-derived oil, 1.2 - 2 % DM) during the transition period affects the levels of cholesterol and bilirubin, which are inflammatory status indexes. These authors described these elements as negative acute-phase proteins because cholesterol is an index of the serum lipoprotein concentrations whereas the bilirubin concentration reflects changes in the enzymes responsible for its clearance (Bertoni and Trevisi, 2013).

PUFAs can influence immune responses by regulating the production of eicosanoids, which are signaling molecules that in turn regulate aspects of the initiation and resolution of inflammatory responses (Sordillo, 2016). Eicosanoids are a large number of compounds of 20 carbons, including prostaglandins (PGs), thromboxanes, leukotrienes (LTs) and hydroxy acids, with various biological functions (Figure 1). These compounds, which are derived from n-3 and n-6 LC-PUFAs, are stored as phospholipids in cell plasma membranes. A specific stimulus activates phospholipase A2 and then this enzyme hydrolyzes membrane phospholipids, releasing PUFAs. These fatty acids could be oxidized non-enzymatically by reactive oxygen species, or through different enzymatic pathways, including cyclooxygenases, lipoxygenases, and cytochrome P450. This leads to the production of a

variety of oxylipids, including PGs, thromboxanes, LTs, and lipoxins. The most important precursor of eicosanoids from which series 2 PGs are synthesized is arachidonic acid (AA, 20:4 n-6). Eicosanoids can also be derived from dihomo- γ -linolenic fatty acid (series 1) and EPA (series 3).

Considering the previously described competitive inhibition of $\Delta 6$ desaturase, the different series of eicosanoids could be regulated by the incorporation of precursors and competitors in the animal metabolism through the diet. According to this, an excessive intake of ALA inhibits the desaturation of LA and may decrease prostanoids of series 2 (Gulliver et al., 2012). On the other hand, increasing the levels of EPA and DHA in the diet could partially replace AA from the cell membranes of platelets, erythrocytes, neutrophils, monocytes, and hepatocytes where AA is usually found in high proportions (Patterson et al., 2012). Historically, the eicosanoids derived from n-6 PUFAs have been classified as pro-inflammatory, whereas those derived from n-3 PUFAs promote the resolution of inflammation. However, this description has been recognized as simplistic, because the functions of eicosanoids are complex, interactive, and cell-specific (Sordillo, 2016). However, some authors have described that prostanoids derived from n-3 PUFAs predominantly abrogate inflammation, while those derived from n-6 PUFAs have multiple effects, being able to stimulate or abrogate inflammation depending on the species and cell types (Raphael et al., 2014).

An *in vitro* study with bovine peripheral blood mononuclear cells also demonstrated the immunomodulatory effect of PUFAs, where the cells had lower production of leukotriene B₄ when cultured with ALA than when cultured with LA (Thanasak et al., 2005). Moreover, Mattos et al. (2003) showed a lower secretion of PGF₂ α by bovine endometrial cells after incubation with n-3 PUFAs than after incubation with n-6 PUFAs.

Several types of PUFAs are also able to regulate the inflammatory function by altering intracellular signaling pathways and transcription factor activation (Sordillo, 2016). EPA and DHA, for example, have been found to have anti-inflammatory functions by inhibiting the activation of Toll-like receptors (TLR), which can recognize pathogen-associated molecular patterns, like lipopolysaccharide (LPS) of gram-negative bacteria, mediating the innate immune response required for host defense. However, researchers have demonstrated that TLR can be activated by endogenous molecules derived from tissue injury, and by saturated fatty acids, mediating noninfectious inflammatory disorders. In this sense, increasing the concentration of n-3 PUFAs in the plasma membrane, especially in lipid rafts, can lead to disruption of recruitment and dimerization of TLR4 (Lee et al., 2010).

TLR4 mediates inflammatory functions through the activation of nuclear factor kappa B (NF- κ B), which is a transcription factor that translocates to the nucleus and up-regulates target genes that encode inflammatory mediators such as tumor necrosis factor alpha (TNF- α), interleukin (IL)-1 β , and IL-6 (Chen et al., 2015). In dairy cows, Silvestre et al. (2011a) observed that the animals with greater ALA in the diet from 30 days prepartum until 35 days postpartum (1.5 % DM calcium salts of palm or safflower oils) showed greater phagocytosis and oxidative activities per neutrophil, and expressed more adhesion molecules, and produced more TNF- α and IL-1. The authors concluded that cows supplemented with a source of LA could improve their innate immunity to better cope with the bacterial challenges in the postpartum period. Similarly, other researchers observed that animals supplemented with microencapsulated fish oil (1.1 % DM) showed a lower lymphocyte response, determined by measurement site of skin-fold thickness, and lower humoral response following a subcutaneous injection with albumin (Caroprese et al., 2009).

In mice, Lee et al. (2010) observed a lower survival after bacterial infection but only in animals with a very high supplementation with fish oil. In this sense, some authors have suggested that the findings published to date are controversial because n-3 PUFA supplementation can both improve and impair host survival and pathogen clearance (Anderson and Fritsche, 2002). Husson et al. (2016) described that n-3 PUFA supplementation at a 2- to 4-fold higher dose and for a long time have worsened the response to infections, but the dose of n-3 corresponding to a daily dose of 0.5 g/day in healthy humans improved the results of experimental bacterial infections. These results could be due to the anti-inflammatory properties of n-3 PUFAs, which limit tissue damage associated with the pathogen and the inflammatory response.

In dairy cows, Greco et al. (2015) observed that reducing the n-6/n-3 ratio and increasing calcium salts of fish oil in the cow's ration (0.70 % DM vs 0.20 % DM) increased the DMI, with concurrent increases in milk yields. These authors also found that, after an intramammary LPS infusion, the cows with a lower n-6/n-3 ratio had lower IL-6 and haptoglobin plasma concentrations but that the dietary treatment did not affect phagocytosis or oxidative burst of neutrophils. In another study, the supplementation with flaxseed (n-3 PUFAs source, 6 % DM prepartum and 8 % DM postpartum) did not alter DMI or milk yield but increased the phagocytic capacity of monocytes and neutrophils and increased the percentage of T-helper cells and T-cytotoxic cells when compared with that observed with an n-6 PUFAs source (Gandra et al., 2016). Lessard et al. (2004) also observed a lower lymphocyte response in cows fed micronized soybeans (n-6 source, 9.4 % DM) than in those receiving flaxseed (5.9 % DM) or saturated fatty acid (calcium salt, 2.5 % DM), whereas Contreras et al. (2012) demonstrated that bovine aortic endothelial cells with higher concentrations of EPA and DHA reduced the mRNA expression of pro-inflammatory

cytokines, leukocyte adhesion molecules and cyclooxygenase, which could decrease eicosanoid biosynthesis.

5. Relationship between insulin resistance, high BCS and n-3 PUFAs

As previously described, a close relationship between pro-inflammatory elements and metabolic status, especially related to type II diabetes, insulin resistance and obesity, has been observed in humans (Dandona, 2004). The mechanisms linking these elements are yet not entirely understood, but some explanations have been proposed. In obese mice, adipocytes produce chemokines and cytokines such as IL-6, IL-1 β and TNF- α , which recruit monocytes into adipose tissue and activate their differentiation (Baker et al., 2011). These pro-inflammatory cytokines might interfere with insulin action by suppressing insulin signal transduction through NF- κ B activation. In this regard, NF- κ B has been proposed as an element that could connect the pro-inflammatory state, obesity and insulin resistance. NF- κ B activation is mediated by the activation of the I κ B kinase (IKK) complex, which degrades the inhibitor kappaB (I κ B), releasing and allowing the translocation to the nucleus and the upregulation of target genes that encode inflammatory mediators (Figure 2). Moreover, the activation of the IKK complex could affect the insulin signaling through phosphorylation of the serine residues from insulin receptor substrate 1 (IRS1) (Chen et al., 2015).

In dairy cattle, Pantelić et al. (2018) observed a lower phosphorylation of serine residues of IRS1 in animals supplemented with chromium during the transition period, and the consequent higher activation in the insulin signaling pathway. Also, Shi et al. (2015) found increases in the activation of NF- κ B in the liver of cows with higher levels of hepatic lipidosis than those found in a recent study from our group (data unpublished). In this respect, we found a relationship between a greater liver TNF- α expression and an alteration in the

liver insulin signaling pathway, but without changes in NF- κ B expression in the nucleus, noting that TNF- α can influence the insulin signaling pathway by means of mitogen-activated protein kinases also by the phosphorylation of IRS1.

In this context, the beneficial effects of n-3 PUFAs by inhibiting the NF- κ B-driven inflammation can alleviate type II diabetes in patients and decrease hyperglycemia and insulin resistance (Baker et al., 2011). In non-lactating and non-gestating dairy cows, Pires et al. (2008) described that the abomasal infusion of linseed oil had an insulin-sensitizing effect during a glucose tolerance test compared to a tallow infusion in cow feeding at maintenance. However, these authors also observed that, during feed restriction, the abomasal infusion of linseed oil and its insulin-sensitizing effect were associated with a greater clearance of plasma non-esterified fatty acids (NEFAs) but no glucose clearance, concluding that, in ruminants, insulin has greater antilipolytic effects on adipose tissue than effects on glucose utilization in the whole body. Mashek et al. (2005) found similar results after intravenous infusion of linseed oil and fish oil emulsions. Similarly, Moussavi et al. (2007) described higher plasma glucose and insulin concentrations in postpartum cows supplemented with n-3 PUFAs (calcium salts of fish oil 2.4 % DM), with increased DMI, milk yield and without difference in energy balance. These authors suggested that fish oil increased ruminal propionate production, which is the single most important substrate for gluconeogenesis (Fievez et al., 2003). Also, Trevisi et al. (2011) described lower NEFA and higher glucose concentrations in dairy cows supplemented with protected algae-derived oil (1.2 - 2 % DM) during the transition period. In another study with a large number of postpartum cows, those fed with a diet containing extruded linseed (3.55 % DM) showed greater plasma glucose concentration (Jahani-Moghadam et al., 2015).

Other researchers have evaluated the supplementation with unprotected fish oil (1 % to 1.5 % DM) and found no effects on the concentration of glucose, NEFA or beta-hydroxybutyric acid (BHBA) comparing with a control diet or a diet supplemented with a source of saturated fatty acids, as well as no differences in the NEB (Ballou et al., 2009; Badiei et al., 2014). The absence of beneficial results with fish oil supplementation could be due to the unprotected source of this oil. However, Andersen et al. (2008) found no differences between a diet with saturated fat or linseed but only when supplementing cows during the prepartum period. Regarding these results, the beneficial effects of the addition of n-3 PUFAs in the diet of dairy cattle are not conclusive, but there are some evidences that indicate that n-3 PUFAs may decrease insulin resistance and lipolysis in adipose tissue.

This beneficial effect of n-3 PUFAs could occur by an agonist of peroxisome proliferator-activated receptors (PPAR), which are a family of transcriptional nuclear factors with three isoforms (alpha, beta and gamma) with key roles in the regulation of lipid metabolism (Hein et al., 2010). PPAR α has been shown to play a critical role in the regulation of β -oxidation mitochondrial and peroxisomal of fatty acids, inducing the expression of carnitine palmitoyltransferase I (CPT1) and acyl-CoA oxidase, respectively (Angeli et al., 2019b). In mice, PPAR α has been shown to be able to reduce hepatic fat accumulation in the liver and thus prevent steatosis (Stienstra et al., 2007).

In dairy cows, hepatic lipidosis is a major metabolic disorder in early lactation, which causes significant productive losses (Bobe et al., 2004). Thus, the role of PPAR α observed in mice (Stienstra et al., 2007) could represent an important element to prevent hepatic steatosis in dairy cattle. However, Litherland et al. (2010) described that the increase in the hepatic lipid metabolism observed in weaned dairy calves was lower than that observed in

rodents treated with a synthesized agonist (clofibrate) and that there was no response with fish oil.

Several authors have evaluated PPAR α expression during the transition period, but without clear conclusions. While some authors determined a postpartum increase in the mRNA of PPAR α and target genes involved in lipid metabolism (Loor et al., 2005), others found no changes or even evidenced a decrease in PPAR α gene and protein expression (Graber et al., 2010; Angeli et al., 2019b). In addition, some works have evaluated animals during the transition period with different BCS (Akbar et al., 2015) or reproductive performance (Angeli et al., 2019a), with differences in their liver triacylglycerol contents and in target genes like CPT1 but without differences in the PPAR α expression in the liver. Also, Petit et al. (2007) observed that cows supplemented with whole flaxseed (3.3 % DM during prepartum and 11.0 % DM during postpartum) showed lower hepatic lipid contents than cows supplemented with a saturated fatty acid source, but without differences with the control group. In contrast, Akbar et al. (2013) observed that supplementation of dairy cows with fish oil around partum had no effects on liver triacylglycerol contents or PPAR expression. Similarly, Carriquiry et al. (2009) observed that supplementation of cows with n-3 PUFAs (microcrystallized flaxseed and fish oil 1.5 % DM) had no effects on PPAR α mRNA abundance in the liver.

PPAR agonists have been found to promote insulin sensitization and to have anti-inflammatory effects (Stienstra et al., 2007; Hein et al., 2012). Nevertheless, the molecular mechanisms of the anti-inflammatory effects of PPAR antagonists are not clear (Dasu et al., 2012). PPAR agonists reduce the production of several molecules that are involved as markers or mediators of inflammation in adipose tissue such as IL-6 and TNF α , and induce the synthesis of adiponectin, an adipokine with a relevant role in the control of lipid

metabolism, glucose homeostasis and insulin sensitivity (Moller and Berger, 2003). Some *in vitro* results have shown similar effects, where PPAR agonists have anti-inflammatory effects in bovine peripheral blood mononuclear cells, mammary epithelial cells and endothelium (Bionaz et al., 2013). In this sense, a recent *in vitro* study has described that, in bovine mammary epithelial cells stimulated with LPS, pretreatment with DHA increased PPAR γ protein expression and decreased the phosphorylation of NF- κ B, leading to a lower gene expression of proinflammatory cytokines such as TNF α , IL-6 and IL-1 β (He et al., 2017). Also, an *in vivo* study during the postpartum period showed that treatment with 2,4-thiazolidinedione (TZD) linearly decreased the NEFA concentration, possibly in response to the action of PPAR, increasing insulin sensitivity in adipose tissue (Smith et al., 2009). This study also showed that the cows presented lower hepatic lipid accumulation, greater DMI and lower open day, associated with the treatment and the lower NEFA concentration.

6. Effects of n-3 PUFAs on reproduction performance in dairy cattle

Many of the studies regarding the supplementation with PUFAs in dairy cattle have evaluated their action on the reproductive function of animals. Although the results are not conclusive (Santos et al., 2008), some studies have shown that cows supplemented with n-3 PUFAs show a better reproductive performance (Ambrose et al., 2006; Silvestre et al., 2011b).

One of the main hypotheses is that n-3 PUFAs are precursors of PGF3 α and that n-6 PUFAs are precursors of PGF2 α . An *in vitro* study has shown that the secretion of PGF2 α by bovine endometrial cells is lower after incubation with n-3 PUFAs than after incubation with n-6 PUFAs, and that this effect was greater with EPA and DHA than with ALA (Mattos et al., 2003). Thus, diets with more n-3 PUFAs may reduce PGF2 α synthesis and may prevent

regression of the corpus luteum, allowing continued secretion of progesterone (P4), which may improve embryo survival (Gulliver et al., 2012). Ambrose et al. (2006) described that dairy cows fed a diet enriched in ALA showed better reproductive performance and lower pregnancy loss following artificial insemination. Regarding this issue, Robinson et al. (2002) described that dietary supplementation of dairy cows with n-3 PUFAs (heated whole flaxseed 1.3 kg/day) and n-6 PUFAs supplementation of dairy cows was associated with lower plasma concentrations of P4. In an *in vitro* study with bovine luteal cells, Hinckley et al. (1996) showed lower P4 production after incubation with n-3 PUFAs. These results suggest that n-3 PUFAs may alter the availability of cholesterol as a substrate for steroid synthesis. Nevertheless, the most significant effects of n-3 and n-6 PUFAs are most likely to be mediated through their effects on the synthesis of series-2 and series-3 PGs (Gulliver et al., 2012).

In dairy cows supplemented with rumen-protected encapsulated fish oil (1 % DM) during postpartum, Elis et al. (2016) found an increased number of follicles with a diameter ≥ 10 mm and a decrease in infertility and in the early embryo mortality rate 21 days after artificial insemination. Also, Ambrose et al. (2006) described larger ovulatory follicles in cows supplemented with n-3 PUFAs (rolled flaxseed 9 % DM), suggesting that the size of the follicles could determine the future success of the ovulation rate and oocyte viability. Regarding oocyte development, *in vitro* studies have demonstrated that oocytes cultured with ALA show better maturation than those cultured with LA, which showed worse maturation than the control (Fouladi-Nashta et al., 2007). In another *in vitro* study, Oseikria et al. (2016) described better oocyte maturation and embryo development with DHA supplementation. However, despite the higher concentrations of n-3 PUFAs observed in follicular fluid from

supplemented dairy cattle, these results could not be replicated in *in vivo* studies (Gulliver et al., 2012).

7. Conclusions

There is a lot of evidence about the metabolic benefits of supplementation with n-3 PUFAs in different species, particularly on the insulin resistance state in obese people. The main mechanism that determines these health benefits seems to be the anti-inflammatory action of n-3 PUFAs. However, in dairy cattle, the results regarding the supplementation with n-3 PUFAs have been controversial. Some researchers suggest that the action of n-3 PUFAs could be beneficial in a pro-inflammatory state with high lipomobilization, as that described in dairy cows during the transition period. However, further studies are necessary to determine the importance of these supplements to improve dairy cows' health, postpartum adaptation, reproductive efficiency, milk production and optimization of dairy systems.

Conflict of interest statement

The authors confirm that this work involves no conflict of interest.

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Legends to figures

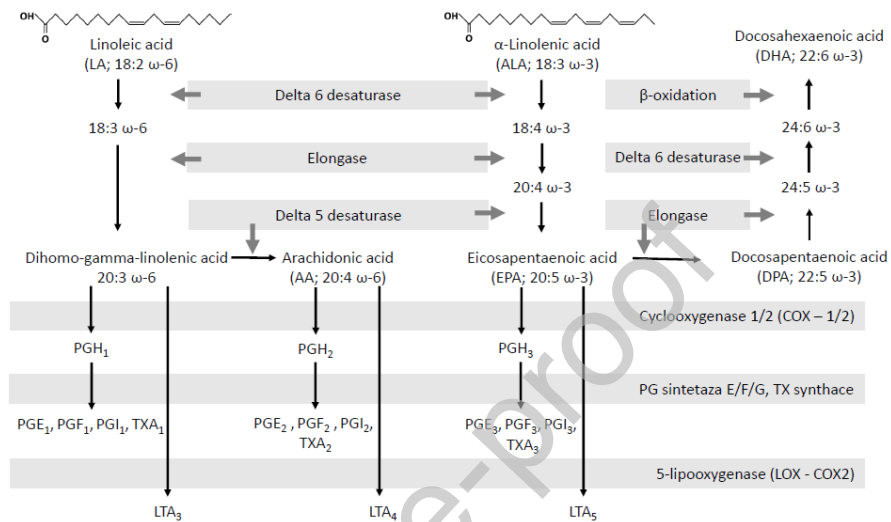


Figure 1. Scheme to illustrate the metabolism of n-3 and n-6 PUFAs and the generation of eicosanoids. PG: prostaglandin. TXA: thromboxane A. LTA = leukotriene A (based on: Wathes et al., 2007; Gulliver et al., 2012).

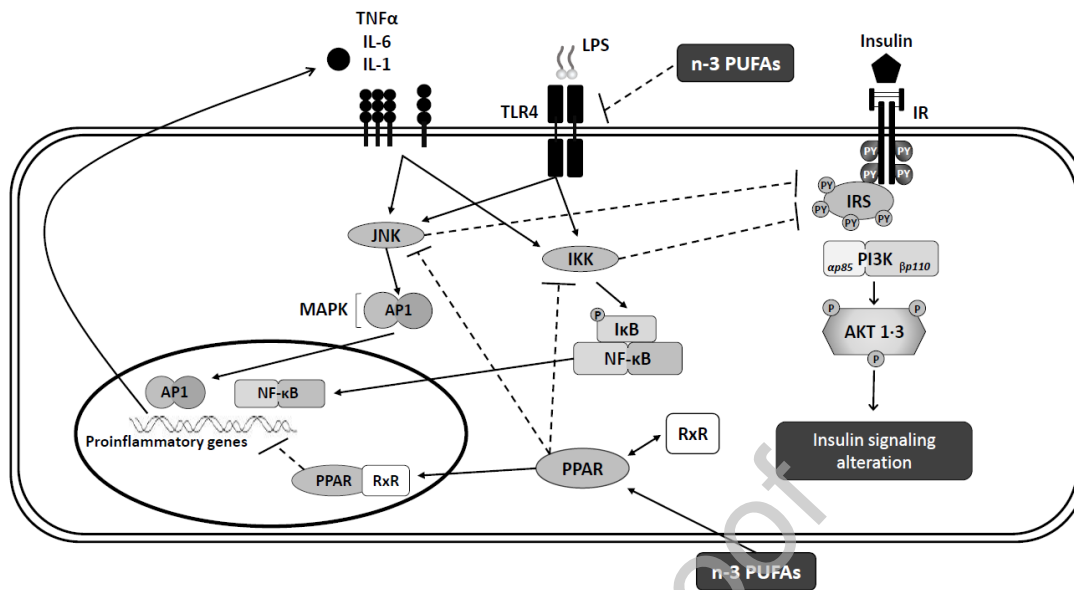


Figure 2. Schematic representation of n3-PUFA regulation of the inflammatory response and insulin signaling pathway. TLR can recognize LPS mediating the innate immune response and can be activated by endogenous molecules mediating non-infectious inflammatory disease. PPARs are nuclear receptors which regulate lipid metabolism and promote anti-inflammatory effects. TLR and PPAR can alter the NF- κ B and MAPK pathways with inflammatory and anti-inflammatory consequences, respectively. The stimulation of NF- κ B and MAPK pathways can alter the insulin signaling pathway through phosphorylation of serine residues. n-3 PUFAs have both inhibitory effects on TLR and agonist effects on the PPAR. Solid arrow: stimulating effects. Dashed arrow: inhibitory effects. n-3 PUFAs: n-3 polyunsaturated fatty acids. LPS: lipopolysaccharides. IL-6: interleukin 6. IL-1: interleukin 1. TNF α : tumor necrosis factor α . TLR: toll-like receptors. NF- κ B: nuclear factor kappa B. I κ B: inhibitor kappa B. IKK: I κ B kinase. JNK: jun N-terminal kinase. AP-1: activator protein 1. PPAR: peroxisome proliferator activated receptors. RXR: retinoid X receptor. IR: insulin receptor. IRS: insulin receptor substrate 1.

PI3K: phosphatidylinositol-3-kinase. AKT: protein kinase B. PY: phosphotyrosine. P: phosphate (based on the literature reviewed).

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