# The effect of n- 3 polyunsaturated fatty acid supplementation on immune and reproductive parameters in dairy cows: a review

## *O efeito da suplementação de ácidos graxos poliinsaturados n-3 sobre os parâmetros imunológicos e reprodutivos em vacas leiteiras: revisão de literatura*

Luisa Cunha Carneiro[1]; Erin Jane Williams[2]; João Paulo Elsen Saut[3]; Ricarda Maria dos Santos[3]; Eneiva Carla Carvalho Celeghini[1]

[1] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Reprodução Animal, Pirassununga – SP, Brazil
[2] University of Edinburgh, The Royal (Dick) School of Veterinary Studies, Edinburgh, Scotland
[3] Universidade Federal de Uberlândia, Uberlândia – MG, Brazil

**ABSTRACT**

In dairy cattle, supplementation with polyunsaturated fatty acids (PUFAs) is considered to be an important tool to decrease the negative energy balance of periparturient dairy cows and improve the reproductive and immune systems. The most common PUFAs added to ruminant diets are omega 3 (n-3 PUFA) as linolenic acid and omega 6 (n-6 PUFA) as linoleic acid. This paper aims to review the potential effects of n-3 PUFA. We consider the effects of n-3 PUFA on the bovine immune system, especially on immune cells, and on *in vivo* and *in vitro* reproductive parameters, emphasizing how n-3 PUFAs act as modulators through one or more molecular mechanisms. The incorporation of n-3 PUFA in the dairy cow diet has positive effects on animal fertility and immunity. Future research on n-3 PUFA should be more explored concerning reproduction and immune function, starting from the investigation of basic biology to their potential for application in the clinical and preventive medicine fields.

**Keywords:** Bovine. Immune response. Linolenic acid. Reproductive efficiency.

**RESUMO**

Em rebanhos leiteiros, a suplementação com ácidos graxos poliinsaturados (PUFAs) é considerada uma ferramenta importante para diminuir o balanço energético negativo de vacas leiteiras durante o periparto e contribuir para a reprodução e sistema imunológico. Os efeitos da suplementação com PUFA sobre estes sistemas têm sido pouco explorados na literatura. Os PUFAs mais comuns na dieta dos ruminantes são Ômega-3 (n-3 PUFA) como ácido linolênico e Ômega-6 (n-6 PUFA) como ácido linoleico. Esta revisão abordará os aspectos gerais do n-3 PUFA, seus efeitos mais relevantes no sistema imune, principalmente seus efeitos nas células imunes, bem como seus efeitos na parte reprodutiva, tanto *in vivo* como *in vitro*, enfatizando a ação do n-3 PUFA através de mecanismos moleculares. A incorporação de n-3 PUFAs na dieta de vacas leiteiras exerce efeitos positivos na fertilidade e imunidade. Mais estudos a fim de explorar a função do n-3 PUFA na modulação do sistema imune e parâmetros reprodutivos, desde a investigação da biologia básica até a aplicação a campo de modo clínico e preventivo, devem ser requeridos.

**Palavras-chave:** Ácido linolênico. Bovinos. Eficiência reprodutiva. Resposta imune.

**Correspondence to:**
Luisa Cunha Carneiro
Universidade de São Paulo, Faculdade de Medicina Veterinária
e Zootecnia, Campus Fernando Costa
Av. Duque de Caxias, 225, Jardim Elite
CEP: 13635-900, Pirassununga – SP, Brazil
e-mail: luisacunhacarneiro@hotmail.com

## Introduction

The dairy industry has a significant impact on the world economy. However, it faces many challenges. A key issue is a detrimental effect of puerperal disease on reproduction and production outcomes in postpartum dairy cattle, exacerbated by the lack of accurate and early diagnosis or efficient treatments for such disease.

Puerperal diseases are among the main health problems in dairy herds, especially in those animals that suffer an intense negative energy balance (NEB) during the transition period and early lactation. In this scenario, polyunsaturated fatty acids (PUFAs) are considered to be an important option to minimize NEB and support the immune and reproductive systems via their effect in modulating cellular functions. Still, they can influence the physical and functional properties of membranes and may influence molecular signaling and gene expression (Calder, 2020).

Polyunsaturated fatty acids are composed structurally of at least 18 carbon atoms and have a minimum of two unsaturated double bonds in their acyl chain. Their classification of either as n-3 PUFA or as n-6 PUFA is based on the position of the first double bond with the terminal methyl group (i.e., the third carbon atom in the case of n-3 and sixth in the case of n-6 PUFAs) (Roszkos et al., 2020).

Supplementation of the diet of dairy cattle with PUFAs is undertaken to increase the energy concentration of the diet. This is usually in the form of fat or oil, as the energy concentration of PUFAs is up to 2.5 times higher than other dietary components. Also, particular fat sources are advantageous not only because of the high energy content but also because of their positive effects on other physiological processes (Rosero et al., 2016).

The purpose of this paper is to give a brief overview of n-3 fatty acids and then review their most significant effects on immune and reproductive functions (both *in vivo* and *in vitro*) in dairy cows. It also discusses the potential applications of n-3 PUFA for improving dairy cow health and fertility.

## Omega 3 (n-3) PUFA

The importance of PUFA in the diet has been established as many of these fatty acids cannot be synthesized by mammals due to the lack of desaturase enzymes. In ruminants, the supply of PUFA for absorption by the small intestine is limited due to extensive hydrogenation (Santos et al., 2008).

The n-3 PUFAs are considered precursors of eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which is considered the most important fatty acid in neonatal development and one of the main components of brain fatty acids (Ramírez-Corría, 2001). N-3 fatty acids are found in large quantities in marine products and other subproducts (Haag, 2003). For a comprehensive review of the sources of n-3 fatty acids, we recommend Cholewski et al. (2018).

The biohydrogenation process, the conversion of unsaturated fatty acids to saturated fatty acids by rumen microbes, results in the conversion of n-3 to saturated fatty acids (Otto et al., 2014). While a high proportion of PUFA is biohydrogenated, a large amount of n−3 escapes this process and is transported to tissue (Ashes et al., 1992). Chilliard et al. (2007) reported that the main factor that affects the biohydrogenation process is the forage to concentrate ratio offered in the diet. Still, according to Jenkins et al. (2008), the rate of lipolysis and hydrogenation can vary concerning the surface area of the food particles in the rumen and can make modifications of the lipid molecule that inhibit microbial attack.

According to Church (1988), some rumen bacteria have lipolytic activity but not all of them. The extent of lipolysis is also dependent on the nature of the dietary lipid, and plant oils, such as flaxseed oil, are almost completely hydrolyzed (around 90%), whereas fish oils tend to be less hydrolyzed (around 50%) (Costa & Fontes, 2010).

In a study by Sinclair (2007) sheep diets were supplemented with n-3 from different dietary sources. The authors found that linseed oil enhanced duodenal flow but not plasma levels of n-3, suggesting that linseed oil did not reduce biohydrogenation rates or improve plasma levels of n-3 PUFA, despite higher microbial efficiency. When they assessed supplementation with fish oil, marine algae or fat encapsulated fish oil decreased the biohydrogenation rate of C20:5 and C22:6 n-3 PUFA, and increased the duodenal

flow and plasma concentration of these fatty acids. The study, therefore, demonstrated that the rate of biohydrogenation varies with the type of diet provided.

The inclusion of fatty acids in animal diets may be determined, at least partially, by the extent of post ruminal delivery of unsaturated fatty acids (Beam et al., 2000). Since dietary inclusion of fatty acids must be restricted to around 60 g/kg of dry matter consumed to prevent rumen damage, the manipulation of fatty acid components to be used by ruminal flora is limited (Dewhurst & Moloney, 2013).

According to Greco et al. (2015), reducing the ratio of n-6 to n-3 fatty acids in lactating dairy cow diets, whereas maintaining similar dietary concentrations of total fatty acids, enhanced productive performance in early lactation. Still, feeding more n-3 and less n-6 fatty acids decreased the acute phase response after the intramammary challenge with lipopolysaccharide (LPS). Progress in lactation performance by feeding diets that differ in fatty acids ratio might be related to altered nutrient partitioning by attenuating inflammatory responses.

## Immune system and n-3 PUFA

### The influence of n-3 PUFA on innate and adaptive immune function

Some challenges in animal production and reproduction can be minimized by the inclusion of PUFAs into the diet, although the specific mechanism by which these effects are mediated has not yet been elucidated. PUFAs may likely influence immune function as they have been shown to modulate the inflammatory response of different immune cells to various challenges (Greco et al., 2015).

The period from three weeks before until around three weeks after calving is known as the transition period. During this time, the immune system is highly modulated as dairy cow ends their pregnancy, give birth, and adapt back to a non-pregnant state, although with the added physiological stress of lactation. Thus, dairy cows are at high risk of developing metabolic and infectious diseases (Grummer et al., 2004). Immune function can be compromised during the transition period (Mallard et al., 1998). Specifically, antibody responses (Mallard et al., 1997), neutrophil chemotaxis and adhesion (Weber et al., 2001), lymphocyte proliferation (Kimura et al., 2002), and the capacity of neutrophils to kill pathogens (Hammon et al., 2006) have all been reported to be compromised in some animals during this time.

The immune system is a multifactorial system comprising of innate (non-adaptive) and adaptive systems. The innate immune system is the first line of immune response to a challenge. Defenses include antimicrobial components, lysozyme bactericidal action, some complement proteins, and immune cells such as mononuclear cells (mainly macrophages), polymorphonuclear cells (mainly neutrophils), and natural killer cells (Harbige, 2003). The adaptive immune system is comprised of lymphocytes, both T and B. This next line of defense is usually activated around 4-7 days post-challenge and its effects are based on clonal activation and proliferation (Turvey & Broide, 2010) (Figure 1).

Mononuclear cells such as monocytes and macrophages play a major role in the immune system. Monocytes are derived from myeloid precursor cells in primary lymphoid organs, such as bone marrow and fetal liver, during embryonic and adult hematopoiesis (Ginhoux & Jung, 2014). They differentiate into macrophages or myeloid dendritic cells and migrate to peripheral tissues where they exercise their functions depending on growth factors, cytokines, and microbial molecules (Shi & Pamer, 2011).

According to Gandra et al. (2016), PUFA supplementation can modulate both innate and adaptive cellular immunity by increasing the phagocytic capacity and activity of monocytes. Some studies revealed that treating macrophages with n-3 PUFA as α-Linolenic acid (ALA), DHA and EPA decreased type 1 macrophage polarization, thereby increasing their phagocytic capacity. Conversely, in type 2 macrophages treated with n-3 PUFA, the polarization effect is decreased compared to type 1 macrophages (Titos et al., 2011).

In human peripheral blood mononuclear cells, some of the effects of fish oil supplementation have been explored, in particular, the suppressive effect of PUFA on the production of proinflammatory cytokines IL-1α, IL-1β, IL-2, IL-6, IL-8, and TNF-α (Calder, 1996). An anti-inflammatory
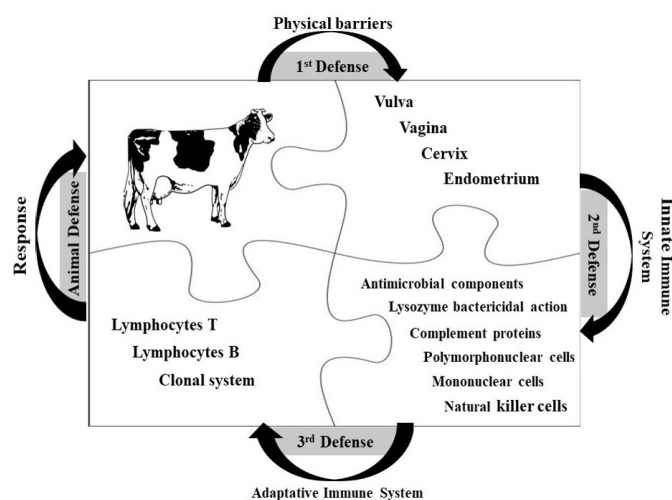


Figure 1 – Immune system composition and its characteristics. First defense: physical barriers; second defense: innate immune system and third defense: the adaptative immune system.

effect on human macrophages was observed by Lee et al. (2004), where C20:5 n-3 and C22:6 n-3 blocked the Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4), reducing the expression of nuclear factor kappa B (NF-κB).

Omega-3 fatty acids, and their metabolites, can alter the function of polymorphonuclear cells. Neutrophil function is modulated by PUFA in several ways. Cell migration, the ability to phagocytize, and the production of reactive oxygen species and cytokines are affected (Gutiérrez et al., 2019). According to Gorjão et al. (2009), the n-3 DHA increased neutrophil proliferation and monocyte phagocytosis, while the n-3 EPA did not have the same effect. Similarly, in an *in vitro* study conducted by Paschoal et al. (2013), the addition of DHA to rat peritoneal neutrophils increased their phagocytic capacity by 35% as well as inducing a two-fold increase in their fungicidal capacity.

The effect of n-3 fatty acids on NK cell function is less understood. Some studies in rodents observed that feeding linseed oil-rich in ALA and fish oil-rich diets suppressed NK cell activity (Calder, 1998), although Han et al. (2018) found that diets with DHA promoted the activation of splenic NK cells in mice.

Diets with high concentrations of long-chain n-3 fatty acids from fish oil were found to suppress the proliferation response of *ex vivo* lymphocytes in both animal and human studies (Calder, 1998). This finding is supported by some *in vitro* and *in vivo* animal studies following dietary supplementation with high concentrations of long-chain n-3 fatty acids (Yaqoob et al., 1994). Also, over expression of adhesion molecules in T-lymphocytes in response to n-3 PUFA supplementation suggests a proinflammatory effect of this PUFA in transition dairy cows (Gandra et al., 2016).

The addition of different sources of n-3 PUFA to lymphocyte cell cultures suggests that mitogen-induced proliferation is increased following supplementation at low concentrations. These *in vitro* functions are independent of the type of eicosanoids used (Calder, 1996, 1998). According to Gurzell et al. (2013), when fish oil was added to the diet of mice as a source of n-3 PUFA, different effects on lymphocytes B activation were observed, even if they induced a similar accumulation of n-3 PUFA on the cellular membrane.

High intakes of n-3 PUFA, principally in the form of fish oils, suppress a wide range of immune variables from the adaptive immune system, such as lymphoproliferation, CD4+ cells, antigen presentation, adhesion molecule expression, Th1, and Th2 responses, and antibody production (Harbige, 2003).

Despite some evidence that confirms the suppressive effect of n-3 PUFA on B cell activation, both EPA and DHA increase IgM production by B cells by enhancing the number of antibody-producing cells in mice and humans (Teague et al., 2013; Tomasdottir et al., 2014). Also, Prickett et al. (1982) showed an increase in IgG and IgE production in rats fed with a high n-3 PUFA diet, whereas a decrease in antigen-specific IgG1 (Th2-like), and to a lesser level of Ig2a (Th1-like), was observed in mice fed a high n-3 PUFA diet (Harbige et al., 1998).

Despite some questions over the importance of changes in the expression of factors related to immune cells, both immune and adaptive cell trafficking will likely be altered by feeding n-3 PUFA under both normal and inflammatory conditions (Harbige, 2003). A summary of the main effects of n-3 sources in both innate and adaptive immune cells is available in Table 1.

### Innate immune system and its mechanism after n-3 PUFA stimulation

The baseline of the innate immune system is the pattern recognition receptors (PRRs), receptors with the capacity to identify microbial components as pathogen-associated molecular patterns (PAMPs) (Albiger et al., 2007). Several classes of PRRs have been explored in innate immune system defense. The most studied and explored group of PRRs is the Toll-like receptors (TLRs) (Kumar et al., 2009). During the postpartum period, an elevated expression of genes encoding TLRs, such as TLR4, inflammatory mediators such as nuclear factor-kappa b (NF-κB), IL-8, IL-1, IL-6, IL-12, and effector molecules, such as acute phase proteins and activated proteins kinases, are characteristic of the local uterine innate immune response in dairy cows (Chapwanya et al., 2009).

There is evidence that saturated fatty acids and n-3 PUFA can modulate PRR responses. Thus, consequently, the activation of PRRs may be modified by the fatty acids consumed in the diet (Lee et al., 2010). Saturated fatty acids induce phosphorylation of protein kinase (AKT) and activation of NF-κB through TLR4, while PUFA inhibits TLR4-induced AKT phosphorylation and NF-κB activation (Lee et al., 2004). Similarly, saturated fatty acids stimulates, but n-3 PUFA (DHA) inhibits the dimerization and recruitment of TLR4 into lipid rafts (Wong et al., 2009). Lee et al. (2004) suggest that TLR's can be activated by non-microbial agonists including n-3 PUFA. On the other hand, n-3 PUFA (DHA) suppresses NF-κB activation and COX-2 expression induced by various TLR agonists, *i.e.* TLR2, 3, 4, 5, and 9. Strong evidence suggests that

Table 1 – Most relevant effects of n-3 fatty acids on immune cells from innate and adaptive immune systems

| Immune system | Immune cells | Actions | References |
|---|---|---|---|
| *Innate* | Monocytes | (+) Phagocytic; activity capacity; | Gandra et al. (2016) |
| | Macrophages | (-) Polarization effect type I; (+) polarization effect type 2; | Titos et al. (2011) |
| | | (-) Proinflammatory cytokines; | Calder (1996) |
| | | (+) An anti-inflammatory effect; | Lee et al. (2004) |
| | Neutrophils | (+) Migration; (+) phagocytosis; (+) production; of reactive oxygen species; (+) cytokines; | Gutiérrez et al. (2019) |
| | | (+) Proliferation by DHA acid; | Gorjão et al. (2009) |
| | | (-) Proliferation by EPA acid; | Paschoal et al. (2013) |
| | | (+) Phagocytic capacity; (+) Fungicidal activity; | |
| | Natural Killer | (+) Suppression effect; | Calder (1998) |
| | | (+) Activation of splenic NK cells; | Han et al. (2018) |
| *Adaptative* | Lymphocytes B | (+) Proliferation suppression effect; | Calder (1998), Yaqoob et al. (1994), Harbige (2003) |
| | | (+; -) Mitogen-induced proliferation; | Calder (1998, 1996) |
| | | (+) Activation; | Gurzell et al. (2013) |
| | | (+) IgM production; | Teague et al. (2013), Tomasdottir et al. (2014) |
| | Lymphocytes T | (+) Proliferation suppression effect; | Calder (1998), Yaqoob et al. (1994), Harbige (2003) |
| | | (+) Adhesion molecules; | Gandra et al. (2016) |
| | | (+; -) Mitogen-induced proliferation | Calder (1998,1996) |
| | | (+) CD4+ cells; (+) Th1 and Th2 responses; (+) antibody production; | Prickett et al. (1982) |
| | | (+) IgG and IgE production | Harbige et al. (1998) |
| | | (-) IgG1 and IgG1 production | |

Note: (+) means an increase effect; (-) means a decrease effect.

TLR-mediated target gene expression and cellular responses may be modulated by saturated and unsaturated fatty acids in different ways, over the reciprocal modulation of TLR signaling pathways (Lee et al., 2003).

As a response to immune system activation, cytokines regulate acute and/or chronic inflammation by activating neutrophils and macrophages and increasing the endothelial expression of cell adhesion molecules (Koh et al., 2007). Blok et al. (1996) reported that TNF-α and IL-1α cytokine expression was increased in mice fed a fish oil diet rich in n-3 PUFA. Yan et al. (2013), demonstrated that n-3 PUFA as DHA, EPA, and ALA decreased IL-1α secretion in bone narrow-derived macrophages stimulated with LPS. In a review where Gutiérrez et al. (2019) explored different studies with n-3 PUFAs, they concluded that the anti-inflammatory action of DHA was more potent than EPA. Still, the only cytokine whose secretion was increased by the treatment with n-3 PUFA was the anti-inflammatory cytokine IL-10. Studies developed by Calder (2012) revealed that n-3 PUFAS inhibit the activation of NFκB, which further blocks the synthesis of proinflammatory proteins.

The n-3 PUFA can resolve inflammation or reduce an inflammatory response (Karcher et al., 2014), but the reports on the effects of n-3 PUFA on cytokine production are conflicting (Calder, 2001) as the literature suggests both pro and anti-inflammatory roles for this particular PUFA (Fritsche, 2008). Evidence indicates that dietary supplementation with n-3 PUFA can modulate the immune system and data suggests that a key mechanism by which it causes these effects is via actions on TLR4-induced AKT phosphorylation and NF-κB activation. However, the precise mechanism of this effect remains to be elucidated.

## Reproductive system and n-3 PUFA

### *The importance of n-3 supplementation starts on prostaglandin 2-α (PGF2α) synthesis*

In this section, we highlight the importance of n-3, especially on prostaglandin 2-α (PGF2α) secretion. For a comprehensive review of the effects of n-3 fatty acids on reproductive hormones, we recommend Santos et al. (2008) and Soydan et al. (2017).

Essential steps to establish acceptable pregnancy rates in cows are the suppression of luteolytic PGF2α secretion and maintenance of the corpus luteum (CL) (Thatcher et al., 1994).

Supplementation with n-3 PUFAs blocks the synthesis of bovine endometrial PGF2α *in vitro* and *in vivo* (Mattos et al., 2002), resulting in improved fertility and embryonic survival (Soydan et al., 2017). The mechanism by which n-3 PUFA inhibits PGF2α is not known. What *is* established is that long-chain n-3 PUFA influences eicosanoid signaling in different ways. They decrease prostaglandin H synthase enzymes, which regulate prostanoid synthesis and increase eicosanoid catabolism producing less biologically active products, including the prostaglandins series 1 and 3 at the expense of series 2 prostaglandins (Gulliver et al., 2012).

Coyne et al. (2008) suggested that supplementation with PUFA not only decreased arachidonic acid concentrations but also attracted PUFA to PGE2 instead of PGF2α production, thus resulting in a switch in the uterine *milieu* from a luteolytic to a luteotrophic environment more favorable for embryo survival. Moreover, they affirmed that some positive effects of n-3 PUFA on fertility could be realized through peroxisome proliferator-activated receptors (PPAR), as they are ligands for PPARα and PPARγ.

The PPAR family are considered to be ligand-activated transcription factors and have important roles in endometrial cells in regulating multiple physiological processes (Michalik et al., 2002). The relative level of the expression of PPAR receptors in bovine endometrial cells is influenced by several factors, including the protein kinase C pathway and embryonic interferon-tau (IFNτ) production. Following ligand binding to the oxytocin receptor, the protein kinase C pathway is stimulated *in vivo* and causes secretion of PGF2α, thus inducing luteolysis. This pathway is suppressed in ruminants by embryo-derived IFNτ, which thereby prevents luteolysis in the process of maternal recognition of pregnancy (MacLaren et al., 2006).

Exogenous supplementation may therefore minimize the proportion of bovine embryos that are lost due to inadequate inhibition of PGF2α secretion by acting on these PG production pathways, thereby increasing embryo survival rates (Mattos et al., 2003).

### Bovine in vitro embryo production and n-3 PUFA supplementation

PUFA supplementation during in vitro embryo production (IVEP) does not alter the composition of fatty acids in oocytes (Sturmey et al., 2009). It is believed that the action of PUFA in IVEP systems occurs through mechanisms that regulate the expression of lipid genes during their metabolism (Ntambi & Bené, 2001), and indirectly via effects on oocyte maturation and *cumulus* cell expansion,

critical events for oocyte development and fertilization (Marei et al., 2009).

The main source of n-3 from the diet is linolenic acid (LNA), present in the chloroplasts of green vegetables, grass, and flaxseed (Dewhurst & Moloney, 2013). An appropriate concentration of LNA in bovine embryo maturation media can result in molecular variations associated with higher PGE2 synthesis, cAMPi concentrations, and mitogen-activated protein kinases (MAPK) phosphorylation in cumulus-oocyte complexes (COCs). Furthermore, the potential of an oocyte to develop into a quality embryo is enhanced (Marei et al., 2009). It is established that the presence of MAPK in cumulus cells is essential for gonadotropin-induced meiotic resumption and cumulus expansion (Su et al., 2003). Also, n-3 PUFA maintains COCs fatty acid composition by selective acceptance of saturated fatty acids related to the PUFAs supplemented in diet (Adamiak et al., 2005).

Marei et al. (2010) reported that the addition of LNA to IVEP maturation media improved the oocytes that reached the stage of metaphase II and had greater mitogenic action in the activated protein kinase signaling pathways. Embryos, grown in n-3 PUFA supplemented medium, were healthier, with better cleavage and blastocyst development.

The supplementation of IVEP media with LNA can inhibit the synthesis and uptake of triacylglycerols, reducing the accumulation of intracytoplasmic lipids, which contributes to increasing cryotolerance of bovine embryos (Pereira et al., 2008). The incorporation of PUFAs in the phospholipid double layer of the plasma membrane promotes the change in membrane fluidity thus improving embryonic cryotolerance (Seidel, 2006).

Sources of n-3 PUFAs as EPA and DHA are little found in embryos (McEvoy et al., 2000). Hughes et al. (2011) reported that n-3 PUFA enriched serum and albumin increased the yield of ovine blastocysts, yet they were morphologically poor.

The balance between the production of reactive oxygen species (ROS) and the action of antioxidants is an important factor for oocyte maturation and fertilization (Tamura et al., 2009) and *in vitro* treatment with PUFA significantly reduced (ROS) species levels suggesting it may be able to modulate oocyte quality (Kim et al., 2001).

### Relevant effects of n-3 PUFA supplementation in reproductive parameters of dairy cows

Polyunsaturated fatty acid supplementation in cows has mainly focused on the nutritional aspects. In a review explored by Santos et al. (2008), the authors highlighted

how dietary supplementation with PUFA improved fertilization rate and embryonic development in dairy cows. The diameter and growth rate of the ovulatory follicle in cows fed with high-oil sunflower seeds rich in linoleic acid (18:2), or a mixture of Alifet-High Energy and Alifet-Repro (protected sources of linolenic (18:3), EPA, and DHA acids) (Carriquiry et al., 2009).

The conception rate to the first service was higher, and pregnancy loss between days 32 and 60 after artificial insemination (AI) was lower in cows that received linseed (rich in n-3 fatty acids) in their diets compared to control cows (Dirandeh & Ghaffari, 2018). Silvestre et al. (2011), reported that cows fed with fish oil had greater pregnancy rate at 60 days after AI and significantly lower embryo losses between 32- and 60-days post-AI than controls.

In a study conducted by Ambrose et al. (2006), cows were fed with 0.75 kg of fat from flaxseed (C18:3 n-3), or sunflower seed (18:2 n-6), and reported a non-significant increase inf pregnancy rate in the cows that received fat from flaxseed. Petit et al. (2001) fed cows with a flaxseed-based diet and observed an increase in conception rate compared with control cows fed with a calcium soap of palm oil. Others studies in lactating dairy cows fed with n-3 PUFA (C18:3 n-3) or EPA and DHA, observed a decrease in pregnancy loss (Ambrose et al., 2006; Petit & Twagiramungu, 2006).

In a study conducted by Colazo et al. (2004), they compared the effects of diets containing whole flaxseed and whole sunflower seed in a timed AI program in 983 beef heifers. The authors found no effects on conception rate between diets. The number of oilseeds included was considerably low (1 kg/d), suggesting that high levels of PUFA supplementation are necessary to increase conception rates.

Supplementation of ruminant diets with sources of fatty acids is mainly undertaken to increase the energy content in the food and improve animal performance. Adequate nutrition is essential to permit acceptable merit in dairy cows as well as to increase their production and achieve satisfactory calving rates. Specific PUFAs can contribute to the regulation of reproductive hormones, which are required for optimal fertility rates (Otto et al., 2014).

The observed effects of PUFA supplementation on reproductive function in dairy cattle may be partly mediated by altering the microenvironment of the follicular fluid around the oocytes since the fatty acid profile of the follicular fluid is related to the dietary fatty acid type and content (Childs et al., 2008). A summary of the main effects of n-3 sources in this review on reproductive function, both *in vitro* and *in vivo*, is available in Table 2.

### Future prospects for n-3 PUFA supplementation in dairy cows

In dairy cows, studies have shown that dietary supplementation with n-3 PUFA during the peripartum period increases the reproductive performance of these animals. In particular conception and pregnancy rates may be increased. Many studies have explored the mechanisms by which n-3 PUFA supplementation mediates effects on PGF2α synthesis and activation of the innate immune cascade. We hypothesize that dietary supplementation with n-3 PUFA during the peripartum period may result in minimization of postpartum inflammation, thereby increasing reproductive performance and reducing cow losses (Figure 2).

Table 2 – Most relevant effects of n-3 on the reproductive system in dairy cows

| Reproductive system | Actions | References |
|---|---|---|
| **In vitro effects** | (+) PGE synthesis; (+) cAMP concentration; (+) MAPK1 and MAPK3 phosphorylation; (+) oocyte maturation; (+) embryo quality; | Marei et al. (2009) |
| | (+) Oocytes maturation; (+) mitogenic action; | Marei et al. (2010) |
| | (+) embryos healthier; (+) cleavage and blastocyst rate; | Pereira et al. (2008), Seidel (2006) |
| | (-) Intracytoplasmatic lipids; (+) embryonic cryotolerance | McEvoy et al. (2000), Hughes et al. (2011) |
| | (+) Blastocyte rate; (+) embryo quality; (-) Reactive oxygen species | Kim et al. (2001) |
| **In vivo effects** | (+) Diameter and growth rate of the ovulatory follicle; (+) 1st service conception rate; (-) pregnancy loss; | Dirandeh & Ghaffari (2018) |
| | (+) Pregnancy rate; (-) embryo loss; | Silvestre et al. (2011), Ambrose et al. (2006), Petit & Twagiramungu (2006) |
| | (+) Conception rate | Petit et al. (2001) |
| | (n) Conception rate | Colazo et al. (2004) |

Note: (+) means an increase effect; (-) means a decrease effect; (n) means no effect.
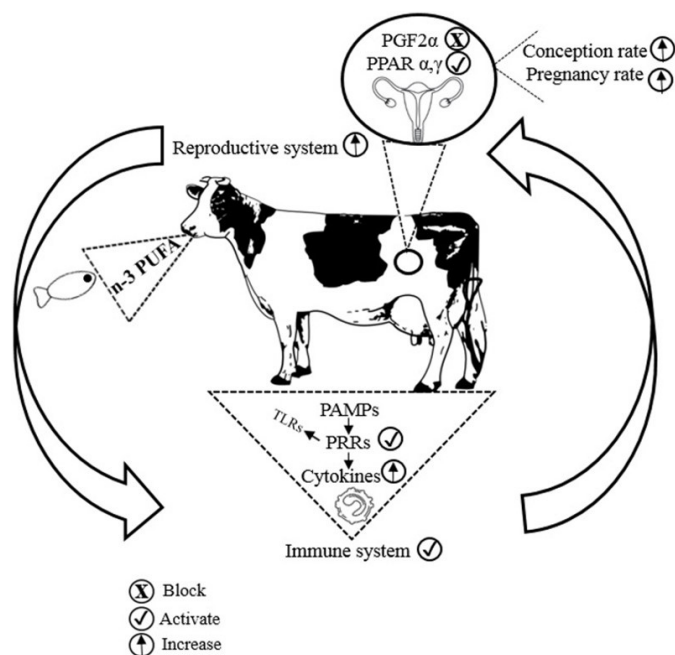
Figure 2 – Proposed effects of n-3 PUFA supplementation in dairy cow diets. A reduction in inflammation may increase cow health and thus improve reproductive performance. PGF2α = prostaglandin F2α; PPAR α, γ = peroxisome proliferator-activated receptors α, γ; PAMPs = pathogen-associated molecular patterns; TLRs = Toll-Like Receptors; PRRs = Pattern recognition receptors.

cellular membrane? How many of them act on the activation of transcription factors? Are these factors activated by n-3 fatty acids in all immune cells? (Gutiérrez et al., 2019).

The body of literature on n-3 PUFA suggests that it may be possible to improve dairy cow health and fertility via n-3 PUFA dietary supplementation across the peripartum period. This may include modulating peripartum immune responses, the development of preventive therapies and/or new treatments for puerperal infections, and the improvement of reproductive performance of the animal.

## Conclusion

Supplementation with n-3 PUFA in dairy cow diets has the potential to play an important role in achieving satisfactory reproductive efficiency and robust immune function in the peripartum period. Future studies are required to understand the contribution of n-3 PUFA supplementation in reproductive and immune responses, from the investigation of basic biological mechanisms through to the potential application of n-3 PUFA as prophylactic or clinical therapy.

## Conflict of Interest

We have no conflict of interest to declare.

## Acknowledgements

It is important to note that although a great progress has been made in our understanding of the effects of n-3 PUFAs acids on reproductive processes, we still do not fully comprehend the precise mechanisms by which n-3 PUFA exert their immunoregulatory effects. How many of the effects exerted by n-3 PUFA rely on their localization on the

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