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## Reproduction and Fertility Parameters of Dairy Cows Supplemented with Omega-3 Fatty Acid-rich Canola Oil

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author JRO researched and wrote the first draft of the manuscript as part of his PhD thesis literature review. Authors MJF, BSMA, PAL, PDN and AEOMA contributed in the design, reading and making needed changes to the final manuscript as a review article. All authors read and approved the final manuscript.*

**Review Article**

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### **ABSTRACT**

Dietary supplementation of lactating dairy cows with fat has long been used as a management tool to increase the energy density of feeds, improving milk production, reproduction and alleviating the menace of postpartum negative energy balance. In this paper, we show that while attempts have been made to investigate the effects of omega-3 (n-3) polyunsaturated fatty acid (PUFA) on reproduction and fertility parameters the results have been diverse and inconsistent. The effect of n-3 fatty acids on prostaglandin F2 $\alpha$

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(PGF<sub>2α</sub>) synthesis are well established, however, little is known about the effect of n-3 PUFA on progesterone, oestrogen, luteinising hormone, follicle stimulating hormone, insulin-like growth factor-1(IGF-1) and fat related genes linked to reproductive performance. There is contrasting evidence linking n-3 PUFA with ovulation, oestrous cycle, PGF<sub>2α</sub> and progesterone production. In addition, the mechanisms behind the influences of n-3 PUFA on reproductive traits such as, number of services per conception and embryo survival are not well understood. Therefore, this paper reviews the effect of n-3 PUFA on cow reproduction and the causal factors of fertility problems in the dairy industry. It also substantiates the need to establish a better understanding of the interactions between n-3 PUFA and cow fertility, with the aim of ameliorating the progressive subfertility issues emerging in the dairy industry. This review also summarizes the identified knowledge gaps and highlights fruitful directions for future research on high producing dairy cows in pasture-based systems.

*Keywords: Polyunsaturated fatty acid; prostaglandin F<sub>2α</sub>; progesterone; reproductive traits; fat supplementation.*

## ACRONYMS

*AA: Arachidonic acid; ALA: alpha-linolenic acid; BCS: Body condition score; bIFN-τ: Bovine interferone-tau; BLAD: Bovine leukocyte adhesion deficiency; Ca: Calcium; CCK: Cholecystokinin; CL: Corpus luteum; CLA: Conjugated linoleic acid; DHA: Docosahexaenoic acid; DM: Dry matter; DMI: Dry matter intake; DNA: Deoxyribonucleic acid; E<sub>2</sub>: Oestrogen; E-FLAX: Extracted flaxseed; EPA: Eicosapentaenoic acid; E-SUN: Extracted Sunflower; FS: Flax Seed; FSH: Follicle stimulating hormone; GH: Growth hormone; GHR: Growth hormone receptor; GnRH: Gonadotrophin releasing hormone; HF: Holstein-Friesian; IGF-1: Insulin like growth factor-1; LA: Linoleic acid; LC-PUFA: Long-chain polyunsaturated fatty acid; LH: Luteinising hormone; MFD: Milk fat depression; MUFA: monounsaturated fatty acid; n-3: Omega-3 PUFA; n-6: Omega-6 NADPH: Nicotinamide adenine dinucleotide phosphate; NEBAL: Negative energy balance; NEFA: non-esterified fatty acid; P<sub>4</sub>: Progesterone; PGF<sub>2α</sub>: Prostaglandin F<sub>2α</sub>; PGFM: 13,14-dihydro-15 keto PGF<sub>2α</sub>; PPAL: postpartum anovulatory interval; PPARs: Peroxisome proliferator-activated receptor; PUFA: Polyunsaturated fatty acid; Δ<sup>12</sup> and Δ<sup>15</sup>: (delta) desaturase enzymes; TAG: Triacylglycerol; SCC: Somatic cell count; SREBP-1: Steroyl response element binding protein 1; TMR: Total mixed ration.*

## 1. INTRODUCTION

The high-yielding, modern dairy cow is a product of many decades of genetic selection that continually placed emphasis on milk yield through the utilization of progeny tested bulls, sexed semen technology, improved management technologies and better nutrition [1]. However, the genetic progress resulting in increased milk production has concurrently led to a gradual but progressive decline in reproductive performance in diverse dairy production systems throughout the world [2,3,4]. The lengthening of calving intervals in dairy cows has already been observed in the USA, United Kingdom and Portugal; where an annual increase of 1.7 days in inter-calving interval was reported by Rocha et al. [5]. Studies by Royal et al. [2] and Butler et al. [4] also confirmed a decline in pregnancy rates from 55.6% to 39.7% at a potential rate nearing 1% annually.

Dairy farmers located in the southern region of Australia are largely dependent on pasture as the main feed source, using limited supplementation with grains due to the associated high price [6]. Stockdale [7] outlined the fact that most Australian dairy farmers are now heavily reliant on the Holstein-Friesian (HF) breed from North America. The contribution of the HF to the Australian dairy industry is very high. However, to continuously achieve high milk production, it is essential that HF cows are fed energy-dense feeds [7,8,9]. Generally, pasture alone is insufficient to meet the energy requirements of high merit dairy cows particularly during the postpartum period. During the postpartum phase, the lactating cow experiences an increase in both physiological and metabolic processes resulting in escalated nutritional demands to maintain continuous milk flow [10]. In the USA, most dairy farmers use total mixed rations (TMR) to provide adequate nutrition for high yielding cows [11], whereas Australian dairy farmers are still mostly reliant on pastures [7]. The pasture-based dairy system practiced in Australia, attempts to obtain uniform annual calving patterns within which parturition is rigorous during early to late spring to allow thorough consumption of the lush spring pasture growth [7]. However, to achieve high milk yields over the lactation period and maintain a yearly calving interval, it is paramount that dairy cows resume cyclicity, ovulate, conceive early postpartum and carry the pregnancy to term [12].

A typical dairy cow experiences negative energy balance (NEBAL) at the beginning of lactation. This is because the energy requirements for both metabolic processes and milk synthesis outweigh the amount of energy being replenished through dry matter intake (DMI) [13,14,15]. Some authors have further proposed that the nadir DMI at early lactation may be related to a linear increase of plasma cholecystokinin (CCK) [16,17,18]. Plasma CCK is a pancreatic polypeptide hormone that regulates satiety and feed intake in animals [16]. Faverdin [19], Grovum [20] and McLaughlin and Baile [21] found that intravenous injection of CCK in cow, sheep and mice resulted in reduced appetites. Bauman and Bruce-Currie [22], found that NEBAL of lactating cows usually continued until 6-8 weeks postpartum. In an attempt to sustain a continuous flow of energy and maintain copious milk synthesis postpartum, the liver elevates the rate of gluconeogenesis in the body. When plasma glucose levels decrease, body fat remobilization is instigated from nutrient accrual to provide sufficient energy that can maintain continuous milk production until the animal returns to a positive energy balance. Adewuyi et al. [23], Grummer [24] and Bauman and Griinari [25] found that cows suffering from NEBAL have increased concentrations of serum glucagon and growth hormone (GH), whereas the concentrations of insulin and IGF-I are decreased, indicating that NEBAL is heavily influenced by high milk production. Therefore, it is important that cows receive adequate nutrition prepartum and postpartum to accentuate their energy needs, in order to minimize the production of non-esterified fatty acid (NEFA), which could potentially compromise the reproductive performance of dairy cows. The onset of parturition is usually accompanied by high energy demand to support high milk production, however in the period leading up to the transition period (2-3 weeks prior to parturition), it is usual for the DMI of high merit cows to be at nadir and therefore the energy requirement for lactating cows is compensated through remobilization of adipose tissue by lipolytic process. Lipolysis of adipose tissues leads to a surge of fatty acids that are reversibly bound to albumin in the plasma [23]. Plasma NEFA have been implicated with poor follicle and granulosa cell development [26,27].

One suggested nutritional method for improving the energy and fertility status of lactating cows both prepartum and postpartum, is the addition of fat supplements to the cow's diet [28,29]. De Veth et al. [30] conducted five controlled meta-analysis studies and found that dairy cows supplemented with conjugated linoleic acid (CLA) decreased the median time to first ovulation. A study in Wisconsin showed that cows fed with supplements containing long-

chain polyunsaturated fatty acid (LC-PUFA:  $\geq 20$ ) exhibited stronger oestrus, had more active ovaries and less exogenous PGF $2\alpha$  was required for oestrous induction [31]. Other studies have also demonstrated that supplementation of dairy cows with fat was consistent with an increased concentration of cholesterol [32,33] and arachidonic acid (AA) in the follicular fluids [34]. Cholesterol is reported to be the precursor for the synthesis of steroid hormones, progesterone (P4) and oestrogen (E2), while AA is the precursor for PGF $2\alpha$  [29,32,35]. PGF $2\alpha$ , P4 and E2 are key hormones involved in ovulation, oestrous cycle and maintenance of pregnancy in dairy cows [32,36,37].

To our knowledge, the response of pasture-based dairy cows to supplementation with canola oil containing n-3 PUFA and the subsequent impact on reproduction and fertility parameters is not well known. Examination of literature reveals that fat supplementation trials in dairy cows have been mainly nutritional in focus and inconsistent, without deliberate evaluation of the impact on reproductive and fertility parameters. The mechanisms by which LC-PUFA affects fertility in dairy cows are also largely unknown. However, as suggested by Staples et al. [35] and Lucy et al. [38], some mechanisms by which fat could influence reproductive performance include: follicular growth through insulin manipulation, inhibition of PGF $2\alpha$  affecting longevity of the corpus luteum (CL) and overall improvement in the energy status of cows. The over-arching objective of this paper is to review the studies conducted on effects of fat supplementation on reproductive parameters in bovines. This paper also aims to explore the proposed mechanisms of fat metabolism and impacts on vital reproductive hormones and plasma metabolites. It also reviews the causes of reproductive problems in the dairy industry, summarizes the identified knowledge gaps and highlights fruitful directions for research aimed at unraveling the specific effect of n-3 PUFA on reproduction and fertility parameters of high-producing dairy cows.

## 2. FINDINGS

### 2.1 Canola Oil

Canola (*Brassica napus L.*) is a product of many decades of genetic engineering with emphasis on the reduction of erucic acid [39] and glucosinolates [40,41,42]. Conventional canola was bred to contain high concentrations of PUFA in comparison to the parentage line of rapeseed [42]. Although superior in production of more PUFA, canola oil when subjected to frying heat becomes rancid and produces more *trans*-fatty acids, a major cause of cardiovascular diseases [43]. As a consequence, the modern canola plant is capable of producing oil with a greater concentration of n-6 and adequate n-3 PUFA [44]. The different proportions and relative abundance of shorter and LC-PUFA in canola oil have been implicated with alteration of fatty acid profiles in the plasma and milk fat of dairy cows [45].

### 2.2 Fatty Acid Metabolism

Plant and animal materials contain organic complexes that can readily be dissolved in organic solvents. These complexes are referred to as lipids [46,47], as depicted in Table 1. Fat is by far the most significant lipid that is of nutritional, metabolic and physiological importance to animals [46]. The physical compartments of most animal cells rely heavily on fat-generated energy to enable the cells to work and function adequately [29,48]. Fat is simply defined as an ester of fatty acid with glycerol [49]. Esterification of the trihydric alcohol glycerol by fatty acid results in compounds known as triacylglycerols [50]. Structurally, they

generally have unbranched carbon chains and a single carboxyl group [34]. Fatty acids occur either as saturated or unsaturated [46].

**Table 1. Types and classification of lipids**

Lipids		
	Glycerol based	Non-glycerol based
<b>Simple</b>	<b>Complex</b>	<b>Waxes Steroids</b>
Fats	Glycolipids Phospholipids	Terpenes Eicosanoids

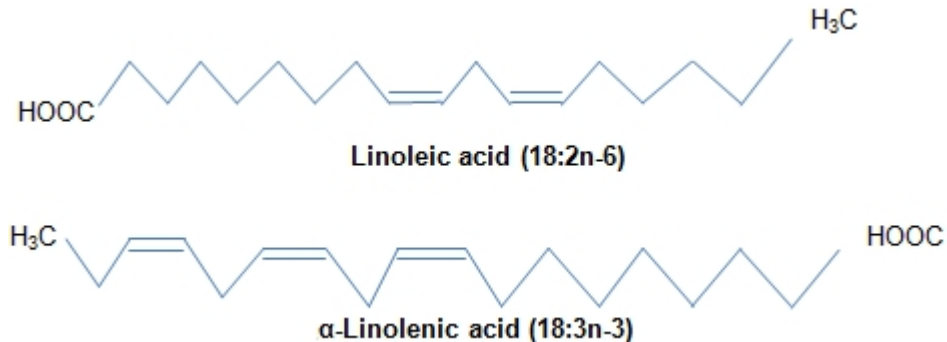
Source: Maynard et al. [46] and McDonald et al. [47]

### **2.2.1 Sources of n-3 and n-6 PUFA**

The n-3 and n-6 LC-PUFA are primarily the fatty acids of interest in dairy reproduction studies [51]. The major sources of n-3 and n-6 PUFA are fish, vegetables and plant oil [29,48]. The LC-PUFA eicosapentaenoic acid (EPA, 20: 5n-3) and docosahexaenoic acid (DHA, 22: 6n-3), are mainly found in fish. However, microalgae is also a major source, while the shorter chain PUFA such as  $\alpha$ -linolenic acid (ALA) and linoleic acid (LA), largely originate from plants [51,52,53]. Most of the n-3s found in dairy cow diets are obtained from grazing pasture [54]. Plants and vegetables with prominent levels of LA, oleic acid and ALA include sunflower, rapeseed, flaxseed, soybean, corn, safflower, linseed, soya bean, echium, peanut and canola [45,55,56]. Previous studies have reported improved reproduction performance of dairy cows with fat/oil supplementation.

### **2.2.2 Structure of PUFA**

The primary characteristics of the n-3 structure are the numbers and positions of the double bonds, chain length and the types of isomers formed [48], as shown in Fig. 1. The position of the first double bond relative to the terminal methyl group of the fatty acid is essential for grouping PUFA, e.g. EPA contains 20 carbon atoms, with five double bonds, with the first double bond at the third carbon from the methyl end, thus making it an n-3 LC-PUFA EPA, 20:5n-3 [57]. Enzymes such as desaturase and elongase are responsible for changing the structure of fatty acids [58]. Consequently, the changes in the carbon chain length of the fatty acids and the position of the double bond influence the biochemical properties and functions of unsaturated fatty acids [34]. These structural and chemical changes affect cattle reproduction [35,48].



**Fig. 1. Structures of linoleic and alpha-linolenic fatty acids**

Source: Gulliver et al. [51]

### 2.2.3 Fat metabolism in the rumen

Metabolism is the term used to illustrate the sequence of chemical processes taking place within living organisms. Metabolism is divided into two parts; catabolism and anabolism [49]. Metabolism of lipids in the rumen is facilitated by the rumen indwelling microorganisms, in particular, bacteria and protozoa [59]. Bacterial lipase hydrolyses triacylglycerols (TAG) and phospholipids consumed in food [60]. Once the fatty acids are liberated from their ester linkages, the end products (glycerol and NEFA) are utilised in the biohydrogenation process [50].

### 2.2.4 Rumen biohydrogenation and fatty acid synthesis

Biohydrogenation is an extensive microbial process that involves the addition of hydrogen molecules to unsaturated free fatty acids concentrated in the rumen [61]. During biohydrogenation, unsaturated fatty acids (LA and ALA) are extensively hydrogenated to form saturated fatty acids (stearic acid 18:0 and palmitic acid 16:0; 50). The process of biohydrogenating LA to stearic acid is demonstrated in Fig. 2.

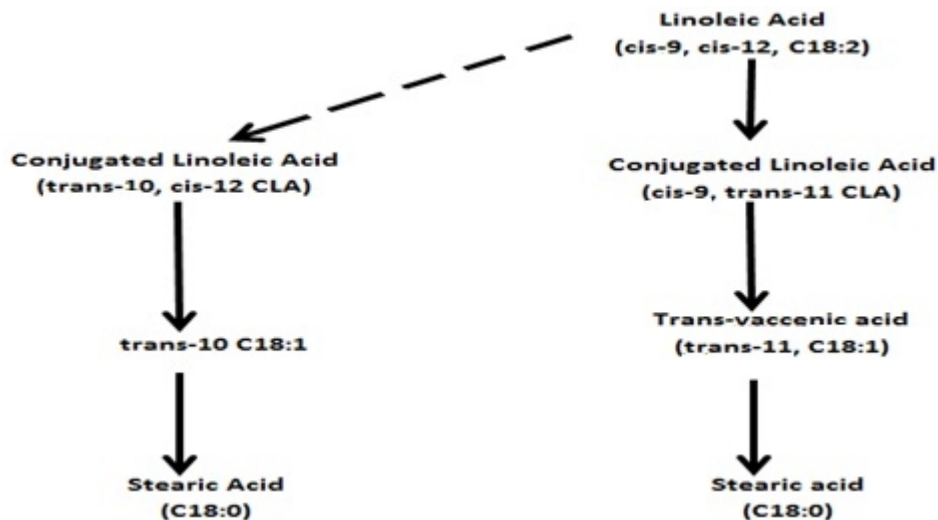


Fig. 2. Pathway for rumen biohydrogenation of linoleic to stearic acid by microbes

Source: Bauman & Griinari [25] and D'Mello [50]

Following biohydrogenation, the saturated and unsaturated fatty acids that escape this process are subsequently absorbed in the small intestine. As a result of rumen biohydrogenation, approximately 85% and 15% saturated and free fatty acids respectively, are transported into the small intestine and this process illustrates the efficiency of rumen microbes [50,61]. Rumen biohydrogenation is the major factor affecting the delivery of LC-PUFA in the small intestine and subsequent transportation in the blood of ruminants [29,62]. Attempts have been made to produce rumen protected lipids [63]. Currently, the addition of calcium (Ca) salts is the main method used to protect fat from rumen biohydrogenation [63,64].

## 2.3 Feeding System and Reproductive Performance

Australian southern states are known for their dairy systems that are largely reliant upon pasture as the main feed source and where calving is managed to coincide with spring pasture growth peaks. The objective of calving in spring is to allow dairy cows to take full advantage of the abundant lush pasture available during this period to increase milk production [6], while at the same time, bolstering early return to cyclicity [65]. Although the pasture system is cost effective, it cannot maintain the required body condition score (BCS) postpartum [7,66].

### 2.3.1 Body condition score (BCS)

In his review, Stockdale [7] defines BCS as the subjective measure of subcutaneous fat tissues, body fat and inter-muscular fat taken at the 12<sup>th</sup> rib or the rump of a bovine. Different countries use different scales for assessing BCS [7,67]. For instance, Australia has adopted the 8 point scale, while USA uses the 5 point scale [68,69].

The drastic prepartum and postpartum physiological changes in dairy cows utilise large amounts of metabolised energy [66]. Energy is utilised for milk synthesis and maintaining the body in good condition [70]. Unfortunately, the energy required to sustain milk production postpartum is far greater than that obtainable from the potential feed intake of the cow [10,71]. As a consequence, there is continuous remobilisation of fat from peripheral tissues, aided by lipolysis, to enhance the provision of adequate energy for milk production [72]. As a result, BCS of the dairy cow drops to nadir postpartum [7,14,73]. Research evidence suggests that remobilisation of fat from the adipose tissue can support the synthesis of approximately 7kg of milk per day [74]. In their review, Chagas et al. [14] outlined that fatty cows were more at risk of fat remobilisation than lean cows during early lactation. This supports the findings of Garnsworthy [75], who established a strong association ( $r^2=0.82$ ) between BCS at calving and during early lactation.

The relationship between BCS and reproductive performance is well documented [3,76,77], however, the results are conflicting. Studies by Veerkamp et al. [76], and Royal et al. [3] found a negative correlation between BCS and dairy fertility, while Berry et al. [78] found a positive relationship, and further indicated that cows with greater genetic excellence for BCS require less services per-conception and can also maintain more pregnancies. However, the studies by Veerkamp et al. [76], and Royal et al. [3] contained only small datasets with few observations. Dechow et al. [79] observed longer calving intervals to first service in cows rapidly losing BCS during early lactation as a result of genetic selection for milk production. Conception rate decreases in dairy cows with low BCS at the start of mating [7]. However, there are inconsistencies in previous reports. For instance, Grainger et al. [80] found that anoestrous was reduced by 5.7 days in each cow gaining an additional condition score postpartum, whereas Garnsworthy and Jones [81] observed no effect of differing BCS on days to resumption of oestrus cycle and number of services per conception in cows at parturition. Therefore, managing body reserves prepartum and postpartum through consistent and accurate measurement of BCS could be essential for enhancing reproductive performance in dairy cows.

Supplementation of dairy cows with fat has been shown by some authors to reduce fat remobilisation during early lactation [82,83,84,85]. Fat supplements containing large proportions of n-3 PUFA have been shown to decrease adipose tissue remobilisation through inhibition of *de novo* mammary milk fat production [48]. Decrease in BCS (on

average 2.6) during early lactation is a reflection of NEBAL in a dairy cow [86]. Cows in NEBAL state are prone to greater levels of NEFA (greater than 0.7mM) in the plasma [87,88], which promote increased production of glucagon [89] and growth hormones [90], and in turn, exacerbate plasma glucose an essential metabolite necessary for sustaining dairy cows in positive energy status postpartum [91,92].

Research has shown that concentration of plasma growth hormone is greater in high milk yielders compared to low yielders [93]. Lucy and Crooker [15] detected surges in the concentration of growth hormones prior to calving in high merit cows, signifying that they played a vital role in the initiation of adipose tissue remobilisation. The physiological role of growth hormones is only realised when they bind to growth hormone receptor (GHR) on target cells, primarily found in the liver and adipose tissue [10]. The production of insulin like growth factor-1 (IGF-1) by the liver regulates the production of GH [86]. The production of IGF-1 is triggered when a cow is in a positive energy status [94]. Growth hormone binds to the growth hormone receptor-1A (GHR-1A) in the liver when the cow is in a positive energy status [86]. Consequently, the production of IGF-1 increases, resulting in a reduction of growth hormone production via a negative feedback signal to the pituitary gland [86].

### **2.3.2 Non-esterified fatty acid (NEFA)**

During NEBAL, various hormones modify peripheral tissue reactions to diminish the rate of hepatic lipogenesis and increase the rate of hepatic lipolysis. This in turn optimises the plasma concentration of NEFA [95,96]. This is a homeostatic process which results in the provision of energy to the mammary glands for milk synthesis [23]. The energy is provided by palmitic and stearic acids which are components of NEFA [97]. However, a high concentration of NEFA in the blood results in high concentration of ketones in the plasma due to the inability of the liver to keep up with the oxidation of concentrated TAG in the blood stream. High concentrations of NEFA in the blood can also trigger the release of CCK that causes the cow to experience false satiety consequently leading to reduced DMI and rumen digestion [98,99]. Excessive NEFA are involuntarily stored in the liver and utilised in the  $\beta$ -oxidation pathway to form acetyl CoAs and NADH [100]. During NEBAL, gluconeogenesis takes precedence over oxidation [100] and this results in the accumulation of acetyl CoAs in the liver [89]. Unfortunately, removal of acetyl CoAs is through conversion to ketone bodies or beta-hydroxy-butyrate [10] which results in the development of fatty liver [101,102].

Elevated NEFA is heavily associated with poor health and reproduction [10, 86,87,88,97,103]. In these studies, Vanholder et al. [27] observed that increased concentrations of NEFA in the serum can cause cell apoptosis resulting in depressed granulosa cell proliferation and steroidogenesis. Supplementation of dairy cows with *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA during early lactation can decrease the concentration of plasma NEFA [83]. However, information regarding the mechanism by which dietary supplements rich in PUFA affect blood NEFA concentration is still limited. Some proposed theories postulate that dietary fat supplement favours lower blood NEFA concentration by providing extra energy postpartum [48]. Other research findings indicate that feeding dairy cows with fat supplements could promote increased insulin and IGF-1 production [15,85,104]. However, studies investigating the response of plasma insulin to fat supplementation are inconsistent. For instance, some studies reported decreased plasma concentrations of insulin, while others reported steady insulin increases postpartum in cows fed six different diets containing fats [38]. Addition of insulin to follicles and granulosa cells *in vitro* is known to increase cell proliferation and production of P4 [105,106]. Hence, a



nutritional strategy is required for the improvement of fertility parameters at the cost of greater milk production in dairy animals.

## 2.4 Milk Composition and Production Responses to Fat Supplementation

Bobe et al. [107] fed mid-lactating dairy cows with tallow at 4.2% of dry matter (DM) and found that both fat and protein contents of milk were increased. Chilliard et al. [108] obtained similar results when they fed ruminally inert tallow at 1.48kg/d to cows. Chichlowski et al. [45] fed ground canola seed to lactating Holstein cows and reported no change in milk yield, somatic cell count (SCC) and lactose percentage; however, protein and fat percentages were lowered. Feeding multiparous Holstein-Friesians with encapsulated fat containing 40.8% flaxseed oil (E-FLAX) had no effect on their milk production, but encapsulated fat containing 40.8% sunflower oil (E-SUN) decreased milk production [109]. In the same study, milk fat percentage was higher in the E-FLAX group than in the E-SUN group ( $P < 0.02$ ), although fat yield remained the same across treatment groups. The group of cows receiving E-FLAX produced less protein and had a lower protein percentage in their milk compared to the E-SUN and the control group. The E-FLAX and control groups produced milk with a higher lactose percentage but lower lactose yield than the E-SUN group. In another study by Caroprese et al. [110], feeding whole flaxseed (FS) at 2.2kg/d and microencapsulated fish oil at 200g/d (FO) to Italian Friesian cows did not affect milk yield, although higher numerical milk yield was obtained from the FS cows. In the same study, fat yield and fat percentage were significantly higher in the group receiving FS. However, no observable differences were seen between lactose, protein and SCC compositions across the different treatment groups. This result was also supported by the study of He and Armentano [111], who found no change in milk protein yield of multiparous Holstein cows supplemented with palm oil, corn oil, linseed oil, high oleic and linoleic safflower oil. However, the overall milk protein concentration was significantly lower for the cows on linseed, palm, high oleic safflower oil and the control group. Corn and high linoleic safflower oil significantly lowered fat concentration and yield, while palm oil increased milk yield, fat yield and fat concentration. High linoleic safflower oil greatly decreased milk yield compared with linseed oil.

In a study using CLA, Hutchinson et al. [28] reported significantly lower concentrations of milk fat and protein in both primiparous and multiparous lactating Holstein cows in Ireland, although milk yield increased significantly. In general, the differences in milk yield and milk composition between the different studies discussed above could be attributed to the different sources of lipid supplements and their fatty acid compositions. Observable differences in milk protein concentration have been reported by many authors to result from increasing or decreasing flow of rumen nitrogen to the duodenum, which upon diffusion to the blood, increases plasma concentration of amino acids [110,112]. An intravenous rumen infusion of sheep with linseed oil was reported to reduce protein digestibility but increased nitrogen flow to the duodenum [112].

Dairy cow supplementations with fat sources have been associated with milk fat depression (MFD) [110]. Plant oil that has been processed and containing *trans*-10, *cis*-12 CLA, has been shown to be a strong inhibitor of mammary milk fat synthesis [85,113,114]. Transcription factors responsible for the up-regulation of lipogenic enzymes are regulated by steroyl response element binding protein 1 (SREBP-1) [115,116]. The expression of these genes was found to be down-regulated following supplementation with *trans*-10, *cis*-12 CLA [117].

The fatty acid composition of milk containing C18:0, C18:1, C18:2 and C18:3 were reported to increase when a diet containing these fatty acids was fed as a supplement to dairy cows [45,109,110,118]. Hutchinson et al. [28] found that supplementation of dairy cows with CLA significantly reduced the proportions of short and medium chain fatty acids, although the proportions of C4:0, C14:0, C15:0 and C16:0 were not affected. Caroprese et al. [110] found that cows receiving whole flaxseed had significantly lower proportions of C14:0, C15:0, C16:0 and C17:0 in their milk, while the proportions of C18:0, C18:1 and *cis*-9 monounsaturated fatty acids (MUFA) were significantly higher ( $P < 0.01$ ). The decreased concentrations of short and medium chain fatty acids and the increased concentrations of LC-PUFA in both milk and plasma would be beneficial to the reproductive success of cows and the carryover effect may be beneficial for humans through the consumption of bovine milk.

## **2.5 Fat Supplementation and Reproductive Function**

Heifers are an important asset to the dairy farmer because they are the future replacement stock, and therefore are key determinants of the economic future of dairy farms. In modern dairy farms, calving interval is used as a management tool to determine the reproductive success of dairy cows. A fertile cow is one which delivers a healthy calf on a yearly basis following successful conception and parturition [32]. However, failure to successfully conceive may be due to cows not showing obvious signs of oestrus, no ovulation or delay in resumption of the ovarian cycle [119]. In order to identify the success and failure of the reproductive performance of dairy cows, it is essential to understand the reproductive traits responsible for enhancing successful fertility and try to unravel the links with the fat component of a dairy cow's diet.

### **2.5.1 Oestrous cycle**

The oestrus cycle occurs prior to ovulation and it is a rhythmic period in female bovines that is characterised by behavioural changes known as heat [32]. Dairy cattle are polyestrus, meaning they can ovulate many times during the year [120]. A typical female bovine oestrus cycle is characterised by an 18 to 24 day length period [37]. The oestrus cycle has four discrete phases namely: proestrus, oestrus, metestrus and diestrus [36]. Two organs namely the hypothalamus and anterior pituitary glands, which are located inside the cranium are essential for coordinating oestrous and other reproductive processes through the secretion of reproductive hormones [32]. The cascades of events of the oestrus cycle are controlled by reproductive hormones that are produced through the interaction of Graafian follicle, corpus lutea and hypothalamic-pituitary interrelationships [32]. The availability and concentration of these hormones at the required site at the right moment are essential for the success of the reproductive performance of dairy cows over their lifetime [37]. The oestrous cycle is initiated upon the release of gonadotropin releasing hormone (GnRH) from the hypothalamus [36,120]. GnRH stimulates the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary gland [32]. GnRH and FSH enhance the recruitment of primary follicles that are stimulated by LH into antral, and then into mature follicles, which through the presence of FSH in the ovary can be ovulated [121]. The presence of the dominant follicle is marked by the release of inhibin which causes the regression of the remaining antral follicles [122]. Inhibin also acts on the anterior pituitary gland to deploy a negative feedback mechanism that reduces the release of FSH [123].

### **2.5.2 Ovulation**

Oestrogen (E2) production by the granulosa cells occurs pre-ovulation and is controlled by the action of insulin [123]. The presence of E2 pre-ovulation is necessary to prepare the reproductive system for fertilisation by increasing the LH concentration [32]. Inflammatory reaction stimulated by LH surge, thins and ruptures the follicle wall to release the mature dominant follicle through the ovulatory process. LH surge is also responsible for the formation and initiation of the function and production of P4 of the CL [124]. The ovum released during ovulation is dispensed into the oviduct and pushed downward through ciliary movement of the oviduct [32].

### **2.5.3 Conception**

The presence of P4 during the luteal phase inhibits the release of GnRH and LH through the negative feedback mechanism that it exerts on the hypothalamus and anterior pituitary gland [37]. P4 is also responsible for the preparation of the uterus for conception [125,126]. However, lack of fertilisation of released ova causes the CL to release a second hormone known as oxytocin [127]. Oxytocin stimulates the release of PGF<sub>2α</sub> from the endometrium [37]. When there is no fertilisation, PGF<sub>2α</sub> causes the regression of the CL through luteolysis [36]. If fertilisation occurs, the level of P4 remains high preventing PGF<sub>2α</sub> production and supports pregnancy [32]. The production of bovine interferone-tau (bINF-τ) increases in a pregnant cow and is used by the embryo to signal to the cow that conception has occurred [36].

### **2.5.4 Embryonic development**

The syngamy of the male and female pronuclei within the cytoplasm of oocyte results in zygote (embryo) formation [128]. Following fusion, continuous mitotic division of the embryo occurs and this creates a mass of unspecialised cells known as cell mass. Further division of the embryo results in a morula, which is a tight ball of cells formed between days 5-6 after fertilisation [120,128]. On approximately day six, the blastocyst is formed. The blastocyst consists of the trophoblast and inner cell mass. The latter is known to form the embryo while the former supplies the nutrients required by the cell mass [32,125]. The beginning of the embryo phase is marked by the development of three germ layers (ectoderm, mesoderm and endoderm) on day 14 [37]. The nervous system, hair, skin and hooves all arise from the ectoderm. The mesoderm is responsible for the formation of the heart, muscle and bones, while the endoderm ensures that the lining of the digestive tract and the respiratory tubes occurs [37]. Maternal recognition of pregnancy is possible at day 16 when the embryo has satisfactorily developed [32]. The embryo at this stage can rely on its own supply of nutrients from fluids within the uterine milieu. However, in the long run, the embryonic disc develops into a transparent membrane that impinges against the endometrium to allow uninterrupted flow of nutrients from the mother to the foetus and vice versa [120]. However, it should be noted that the reproductive processes discussed above are for a typical normal dairy cow. Conversely, there are many fertility issues that are currently faced by the modern dairy cow.

## **2.6 Factors Affecting Reproductive Performance**

The genetic progress in milk production has concurrently led to a gradual but progressive decline in reproductive performance in diverse dairy production systems around the world. Selection for high milk yield followed by inadequate nutritional management and large herd sizes have contributed tremendously to reduced fertility in the dairy industry for the past 20

years [4]. Reproductive problems in the dairy industry arise as a result of insufficient nutrition, deleterious inherited genes and infection from microbes [32]. Most developmental reproductive problems are associated with inheriting hazardous genes from either the sire or the dam. It is also possible that DNA damages during fertilisation or foetal development could result into reproductive failure in the resulting progeny. Two of the most commonly inherited diseases associated with cows are bovine leukocyte adhesion deficiency (BLAD) [129] and segmental aplasia of the Müllerian ducts (White heifer disease) [32]. The latter disease occurs when the gene for white coat colour hinders the development of the Müllerian ducts. Müllerian ducts also known as paramesonephric ducts are found in the developing embryo and subsequently develop into the uterus, cervix, oviduct and parts of the vagina [32]. When interference of the development of the Müllerian ducts occurs, it results in blockage of the reproductive tracts in the progeny [32]. Heterozygous individuals carrying the genes for BLAD tend to be infertile [130]. Reproductive failures can also occur as a result of infection of the reproductive system by invading pathogens such as bacteria, virus, fungus and protozoa [32]. A well-known example of an infection of the uterus is endometritis [131]. Endometritis is an inflammation of the uterus membrane specifically caused by *Campylobacter fetus* or non-specifically by *Escherichia coli* or *Campylobacter pyogenes* [131]. The condition arises usually following the processes of either artificial insemination (AI), dystocia and/or retained placenta [32].

### **2.6.1 Inactive ovaries**

Dairy cows can only reproduce if the waves of primary follicles develop to mature follicles (dominant) and go through ovulation. This is possible if the ovary is active postpartum. The ovaries of cows are usually latent just after parturition because nature requires that the newly born calf must first be weaned before a new conception can occur [132,133]. This sexual quiescence period can vary according to lactation, nutrition, farm management, environment, season, stress, suckling behaviour of the new born calf, the period taken to re-establish new ovarian activities and luteal activities [3]. The acyclicity of dairy cows following parturition can be divided into physiological and pathological periods depending on the length of the sexual quiescence [32]. The physiological period of acyclicity is usually between 30-40 days [134] while pathological acyclicity occurs at day 50 and over [32]. Physiological acyclicity also occurs to enable uterine involution [32]. Pathological acyclicity mainly occurs due to lack of ovulation, the occurrence of an ovarian cyst or the inability of a healthy CL to regress [135]. In a pasture-based system, acyclicity is the main factor causing reproductive problems in dairy cows and is correctly termed prolonged postpartum anovulatory interval (PPAI). Studies have shown that inadequate nutrition and NEBAL are to blame for PPAI [136]. Beam and Butler [137] and Canfield and Butler [138], reported a negative relationship between ovulation and NEBAL in cows, suggesting that to improve the ovulating status of dairy cows, energy-dense supplements are needed in the diets of lactating cows.

### **2.6.2 Embryonic losses**

Embryonic death can be experienced as early as day 24 or 25 of pregnancy [32]. Cows and heifers raised and managed in a pasture-based system were found to have embryonic losses of 7.2% and 6.1% respectively [139]. Causes of embryonic loss are multifaceted and caused by nutritional, genetic, health, metabolic, hormonal and physiological factors [32]. To date, nutrition seems to be the most important factor affecting foetal survival and other reproductive parameters of high merit cows. The negative or positive energy status of some dairy cow postpartum depends on its pre-calving BCS, which relates back to the availability

and quality of available nutrition prepartum, although the amount of energy provided postpartum will have the biggest influence [66]. Postpartum negative energy status of the modern dairy cow has been implicated as one of the major problem affecting reproductive performance [4,13].

Inbreeding, lethal genes and abnormal chromosomes have been found to heighten the loss of embryos [140]. The ability of the embryo to signal to the dam its presence in the womb would prevent its loss. However, an under-developed embryo at around day 24 of pregnancy may not be able to produce bFN- $\tau$ , a signalling chemical indicating its presence in the uterus [141]. The correct hormonal interaction, particularly between PGF<sub>2 $\alpha$</sub>  and P4, is essential in maintaining and carrying the foetus to term without loss. López-Gatius et al. [142] found that the addition of P4 to high yielding cows at the early embryonic growth stage reduces pregnancy loss.

## **2.7 n-3 LC-PUFA and Dairy Fertility**

Infertility is considered a major problem in the dairy industry due to increasing number of services per conception, poor expression of oestrous signs, twinning and double ovulation [143]. n-3 PUFA in dietary supplements offered to dairy cows positively influence fertility traits [29,35]. However, most of the reproductive studies involving fat supplementation have utilised rumen protected CLA, particularly in America, Europe and Ireland [28]. It has been reported that improved dairy fertility resulting from fat supplementation stems from the significant effect of specific fatty acids and not from the provision of energy as previously thought [48]. Most of the reproductive hormones are steroids, and n-3 LC-PUFA is responsible for the synthesis of steroid hormones. Several studies have investigated reproductive traits such as oocyte quality and pre-ovulatory follicular growth in dairy cows using different sources of fat/oil containing n-3 PUFA, but the results have been inconsistent and warrant further research. Robinson et al. [103] observed an increase in medium sized follicle growth when experimental cows were supplemented with C18:2n-6 or C18:3n-3. Ponter et al. [144] utilised soybean (concentrated n-6 PUFA) and flaxseed (concentrated n-3 PUFA) and found that the number of small follicles were lower in cows fed flaxseed than soybeans.

### **2.7.1 n-3 PUFA effects on reproductive hormones**

P4, E2 and PGF<sub>2 $\alpha$</sub>  [32] are essential dairy reproductive hormones. E2 is involved with the preparation of the reproductive tracts for ova fertilisation and the initiation of pulsative surge of LH [32]. P4 is one of the most important fertility hormones responsible for carrying out pregnancy to term [32,145], whereas PGF<sub>2 $\alpha$</sub>  counteracts the functions of P4 on the CL after failed fertilisation [146]. The proposed mechanisms by which n-3 PUFA affect reproductive hormones rely on their ability to regulate the production of PGF<sub>2 $\alpha$</sub> , and increase the availability of ovarian cholesterol (the main precursor for steroid hormones) [33,35,147]. Some studies have reported a negative correlation between the consumption of rich dietary sources of n-3 PUFA and plasma cholesterol concentration, which could potentially lead to lowered concentrations of P4 and E2 *in vivo* [51,103]. Cows fed sources of n-6 PUFA are known to produce more cholesterol. The cholesterol can be utilised in the presence of steroidogenic acute regulatory hormone (StAR) to synthesise P4 [145,148,149]. n-3 PUFA enables P4 to be synthesised by negatively affecting PGF<sub>2 $\alpha$</sub>  synthesis [150]. Increased concentration of n-3 PUFA decreases the concentration of PGF<sub>2 $\alpha$</sub>  in the CL [32]. Robinson et al. [103] found that cows subjected to increasing concentration of n-3 PUFA in their mid-luteal stage had low P4. Hinckley et al. [151] found that low P4 in luteal cells was associated

with increasing n-3 PUFA. Steroidogenesis of reproductive hormones by n-3 PUFA is a known phenomenon; however, mechanisms through which n-3 PUFA modulate the synthesis of steroid hormones to affect the function of the ovary and the CL are largely unknown and warrant further elucidation. Conflicting findings amongst researchers investigating the effects of fat supplementation on dairy reproduction arise from use of different fat sources, numbers of animals and timing of the fat supplementation.

#### **2.7.1.1 LH and follicles development**

The availability of LH is paramount in the latter stages of development and maturation of ovarian follicles [32]. The future reproductive success of a dairy cow can only be determined by the availability and quality of the follicles produced [146]. The pulsative secretion of LH and the production of primary follicles require the availability of sufficient energy which can be provided by fat supplementation [146]. A review by Schillo [152] of the detrimental effects of under-nutrition on LH pulse frequency proposed a mechanism by which fat exerts its effect on LH secretion through the increased production of propionate. Propionate is a precursor for glucose which exerts its effect on the anterior pituitary gland to foster the release of LH [35]. However, the mechanism by which fat supplementation affects LH secretion is still poorly understood and warrants further investigation [48].

The size and number of pre-ovulatory follicles are essential in determining the overall size of the CL [51]. A large CL is known to produce more P4 which increases the rate of conception [146]. Ambrose et al. [153], Mendoza et al. [154] and Petit et al. [155] showed that a wider diameter of the CL and ovulatory follicles was possible to attain through supplementation of dairy cows with n-3 PUFA. Homa and Brown [156] reported a reduced follicle size following the consumption of n-6 PUFA by dairy cows. On the other hand, Zachut et al. [109] found that feeding multiparous Holstein-Friesian cows diets rich in ALA caused increased production of small sized ovarian follicles, but diets rich in LA assisted in producing larger follicles. Other studies have reported the effect of n-6 PUFA on follicle numbers, diameter and CL volume in dairy cows; however, there is a dearth of information on the effects of n-3 PUFA on these parameters [157,158].

#### **2.7.2 Oocyte development**

Follicular fluids comprising high concentrations of n-3 PUFA have been shown by many authors to be essential for oocyte maturation [159,160]. Fouladi-Nashta et al. [159] and Zeron et al. [160] found that the addition of ALA and other n-3 PUFA such as soybean, linseed and fish oil in cow diets improved oocyte maturation. However, high concentration of n-6 PUFA in follicular fluids hinders meiosis, thereby reducing the maturation of oocytes [161]. A second experiment by Fouladi-Nashta et al. [162] did not find a significant impact of n-3 and n-6 PUFA on oocyte development. Assessment of the effect of n-3 PUFA on oocyte growth, development and maturation is challenging because of lack of effective equipment that can maintain the integrity of oocytes throughout the experiments. Therefore, inconsistencies and or lack of adequate information on the effect of n-3 PUFA on oocyte development are prominent and more studies are needed.

#### **2.7.3 Oestrous and ovulation**

Burke et al. [157] intravenously infused six mature Hampshire ewes with olive and soybean oil and found that ewes receiving olive oil had a shorter time to oestrus compared to the soybean group. The reason was because olive oil contains high proportions of

monounsaturated fatty acids (n-9) in comparison to soybean [51]. The interactions between n-3 and n-6 PUFA in the ovary have a huge impact on oestrus cycle and ovulation [51]. The availability of n-3 PUFA assists in the production of P4 which is responsible for CL formation and maintenance; while concentration of n-6 PUFA in the follicular fluids enable the synthesis of  $\text{PGF}_{2\alpha}$  hormone which initiates the luteolytic process that causes CL regression [48,163,164]. Supplementation of dairy cows with oils provides energy that is essential for stimulating ovulatory processes [51]. However, the effect of specific fatty acids on ovulation is not clear.

#### **2.7.4 Embryo survival**

A viable embryo implanted in the uterus of the cow should be able to send a signal to its dam to stop luteolysis [165]. Maternal recognition of pregnancy is achieved when the embryo releases a chemical compound b1FN-r that is recognised by the dam [32]. Increasing the concentration of b1FN-r in the cow's plasma prevents the expression of an oxytocin receptor which induces the release of  $\text{PGF}_{2\alpha}$  hormone [166]. Luteolytic processes occur with high concentrations of  $\text{PGF}_{2\alpha}$  hormone in the endometrium and this affects the survival of the embryo [167]. n-3 PUFA are known to inhibit the production of  $\text{PGF}_{2\alpha}$  and support the production of P4 essential for the survival of the embryo [168,169]. Results from Petit and Twagiramungu [170] suggest that n-3 PUFA formed from ALA may be responsible for embryo survival, although the result was not significant ( $p=0.07$ ). Many *in vitro* studies have been conducted to establish the impact of n-3 PUFA on embryo survival using BEND cells, however *in vivo* studies are needed to verify findings from the *in vitro* studies.

### **2.8 Knowledge gaps and Research Objectives**

- Progesterone and prostaglandin hormones have been identified as limiting factors in the reproduction and fertility successes of dairy cows. Contrasting reports on the effects of n-3 and n-6 PUFA from dietary oil/fat supplementation on progesterone and prostaglandin abound in published literature but there is limited information on the effect of canola oil containing n-3 PUFA on progesterone and prostaglandins in pasture-based dairy systems.
- A negative correlation exists between NEFA and reproductive traits in most dairy herds. This relationship is exacerbated by NEBAL and inadequate nutrition. There are inconsistent reports on the effect of fat supplementation on NEFA,  $\beta$ -hydroxybutyrate and ketone bodies.
- Many authors have reported the effect of supplementing dairy cows with differing sources of fat/oil on milk fatty acid composition, but the effect of canola oil supplementation on milk fatty acid profile of dairy cows in a pasture-based system has not yet been fully explored.
- Little attention has been given to investigating the relationships between fat supplementation and the immune response of dairy herds in pasture-based systems. Filling in this significant knowledge gap will assist dairy farmers operating under pasture-based settings to improve their health management techniques to enhance efficient reproduction and optimal profitability in their dairy system.
- A wide body of evidence exists that shows the effect of different sources of oil/fat supplementation on milk yield, milk composition, BCS and live weight in lactating dairy cattle but published empirical evidence of the impact of canola oil on these lactation parameters is lacking for dairy cows in pasture-based systems.

Therefore, the research objectives needed to fulfil the identified knowledge gaps are;

- To investigate the relationship between n-3 PUFA-containing canola oil supplementation of dairy cows and the associated BCS and live weight profiles of cows in pasture-based dairy systems.
- To evaluate the influence of n-3 PUFA derived from canola oil on reproductive hormones (progesterone, oestrogen, prostaglandin, insulin growth factor-1, luteinising and follicle stimulating hormone).
- To investigate the fatty acid profile of milk from dairy cows supplemented with canola oil.
- To examine the effect of canola oil supplementation on plasma metabolites.
- To investigate the influence of canola oil supplementation on milk composition.

### **3. CONCLUSION**

n-3 LC-PUFA has significant effect on reproductive success and general wellbeing of dairy cows mainly through PGF<sub>2</sub>, P<sub>4</sub>, oestrogen, LH and FSH. Measurable reproductive parameters such as oestrus cycle, ovulation, embryo survival, parturition and calving interval have been strongly linked with high concentrations of n-3 in the blood. However, there is a lack of information on the effect of n-3 on dairy reproduction and fertility traits in pasture-based systems. Filling this knowledge gap could have long term positive implications for pasture based dairy industries. This literature review has also shown that specific n-3 PUFA have a direct impact on reproduction and fertility traits in dairy cows. Fat supplementation may also provide extra energy postpartum capable of influencing lactation traits. The reproductive success of dairy cows in pasture-based systems will require early resumption of oestrus cycle postpartum, proliferation and ovulation of healthy oocytes, establishment of a healthy embryo and maintenance of pregnancy to term. Adequate and appropriate nutrition is required to allow high merit cows to continuously sustain increased milk production and maintain acceptable yearly calving patterns. Specific fatty acids found in supplemented fat can assist in the hormonal regulation essential for optimal reproduction and fertility.

### **4. COMPETING INTERESTS**

Authors have declared that there are no competing interests.

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