Fat supplementation: implication in dairy cattle

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Supplementation of fat or oil in the ration of high yielding dairy cattle increases the energy density of the ration without altering the composition of the other ingredients. A high proportion of free fatty acids in vegetable fat possibly improves fat digestibility in the total gastrointestinal tract and has been associated with variable effects on milk yield and milk fat composition. Vegetable fats are rich sources of essential fatty acids, viz. linoleic and linolenic acid, and varying amounts of monounsaturated oleic acid. Polyunsaturated fatty acids (PUFAs) in dietary fat are important substrates for the synthesis of reproductive hormones and prostaglandins and to augment the reproductive performance through stimulation of the growth of ovarian follicles, increase in follicle size and number, and increased function of corpus luteum (CL) in cattle. PUFA undergoes biohydrogenation in the rumen to produce conjugated linoleic acid (CLA). The milk CLA is identified to reduce body fat levels, improve glycaemic profile, resynthesize glycogen, modulate immune function, improve bone mineralization, prevent heart diseases, atherosclerosis, diabetes and cancer in humans. Significantly higher levels of fat/oil (>3%, generally) in the diet may adversely affect the fermentation pattern through adverse effects on fibrolytic bacteria and protozoa in the rumen, subsequently lowering the fibre and dry matter digestibility. To minimize the effect of higher levels of fat on rumen fermentation, fat may be supplied in a protected form that escapes rumen fermentation and is subsequently utilized in the lower digestive tract of high-yielding dairy cattle to produce beneficial effects.

Keywords: Dairy cattle, essential fatty acids, fat supplementation, milk production, reproduction.

IN high-yielding dairy cattle, negative energy balance is common during the early lactation phase, resulting in metabolic stress and subsequently a decrease in milk yield. One of the strategies adopted to combat this condition is increasing the energy density of the diet through the improvement of concentrate: roughage ratio of the ration. Generally, however, the inclusion of concentrate beyond the threshold level has been reported to cause ruminal acidosis. Also, alteration in the fermentation pattern due to reduced fibre availability is anticipated, which may lead to milk fat depression[1]. So, alternate approaches to increase the energy density of the diet of high-yielding cows through supplementation of oil have been studied extensively[2–4]. A high proportion of free fatty acids from fat was expected to improve the total tract and intestinal fatty acid digestibility. Several experimental findings reported improved energy balance, milk fatty acid profile and better reproduction status due to supplementation of additional oil/fat, though the effects were not always consistent[2–5].

Fatty acid profile of vegetable oils

Fat sources in the diet of ruminants are chiefly of plant origin, i.e. forages, oilseed cakes, vegetable oils, cereal grains and agro-industrial by-products. Forages are normally rich in glycolipids (chiefly galactolipids) and phospholipids, whereas other sources are rich in simple triglycerides. The lipid content in forage leaves is approximately 6–7% of their dry weight[6]. About half of the total fatty acids in forages consist of α-linolenic acid. The fatty acid profile of various oils of vegetable origin is given in Table 1. Vegetable oils are rich in fat predominated by polyunsaturated fatty acids (PUFAs), with a few exceptions, like coconut oil, which is rich in saturated fatty acids (SFAs). Among unsaturated fatty acids, oleic and linoleic acids are abundant in vegetable oils. Linoleic and linolenic acids are essential fatty acids for cattle as they cannot be synthesized in their body. Among the vegetable oils, canola oil has the highest content of unsaturated fatty acids (>90%) that constitute chiefly oleic acid[7]. Cottonseed, safflower, sunflower and soybean oils are rich in linoleic acid, while flaxseeds are rich in linolenic acid.

Digestion and metabolism of fat in cattle

Fat is hydrolysed to about 85% in the rumen by lipolytic bacteria (*Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens*). The extent of lipolysis is reported to reduce under high concentrations of dietary lipid or decrease in rumen bacteria count[8]. The rumen bacteria count may decrease due to the use of ionophore antibiotics, decrease in rumen

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Table 1. Fatty acid richness of vegetable oils

	Fatty acid profile (% FA)								
Oil	Palimitic	Stearic	Oleic	Linoleic	Linolenic	TSFA	MUFA	PUFA	Reference
Corn oil	12.94	2.12	31.97	48.97	0.76	16.6	33.67	49.74	67
Cotton seed oil	23.40	2.79	17.82	51.81	0.35	28.17	19.66	52.16	
Gingelly oil	9.58	5.76	40.72	41.92	0.41	16.45	41.21	42.34	
Groundnut oil	10.46	3.37	53.77	26.96	–	19.27	53.77	26.96	
Mustard oil	2.19	1.17	10.16	15.58	11.70	5.73	66.98	27.28	
Rice bran oil	19.34	2.0	43.42	32.04	0.59	23.63	43.71	32.66	
Safflower oil	6.24	2.35	13.8	76.58	0.13	9.19	14.04	76.78	
Soybean oil	11.00–20.32	3.46–3.87	18.68–24.77	42.56–54.5	5.16–8.26	15.90–27.91	21.27–24.77	50.82–59.33	4, 63, 67
Sunflower oil	6.43	3.69	25.92	62.69	–	11.39	25.92	62.69	67
Linseed oil	6.4–18.32	3.1–4.36	17.55–20.1	18.2–28.3	24.10	29.06	18.54	51.4–52.41	4, 63
Canola oil	4.8	1.9	51–58.5	23–25	7.7–14				63, 68

pH, etc.[9]. The free unsaturated fatty acids (USFAs) released due to hydrolysis are biohydrogenated, and approximately 1–2% of H_2 produced in the rumen is utilized in the biohydrogenation process[10]. Extracellular enzymes of bacteria are associated with more than 80% of biohydrogenation[6]. Though natural fat in the diet is rich in USFAs, through biohydrogenation a large part (80–90%) of the dietary PUFAs is converted to SFAs. For human health, a lower proportion of SFAs and a higher proportion of PUFAs like n-3 fatty acids are desirable. Before biohydrogenation there is isomerization in which the *cis*-12 double bond in USFA is converted to a *trans*-11 isomer. PUFAs are biohydrogenated to trans 18 : 1 fatty acid by a group of bacteria (e.g. *B. fibrisolvens* and *Clostridium proteoclasticum*), whereas another group of bacteria (e.g. *Butyrivibrio proteoclasticus* and *C. proteoclasticum*) completely saturate *trans* C-18:1 fatty acid to stearic acid[11]. The linoleic and linolenic acids in most diets are biohydrogenated to a great extent. Thus, there is an increased concentration of mono USFA and stearic acid in the rumen due to the metabolism of vegetable oils.

Effect of vegetable oil supplementation on milk and its fatty acid profile

About 400 different fatty acids in milk fat are contributed from dietary fat and the fatty acids produced from microbial activity in the rumen of the cows. Fatty acids with chain length C4-14 and some C16 are endogenously synthesized in the mammary gland, whereas the rest of the C16 fatty acids and C18 and longer chain fatty acids are obtained chiefly from diet[12]. Short-chain (constituting about 20% of total milk fatty acids), odd chain and branched chain fatty acids are also synthesized *de novo* in the mammary gland. A desirable component of milk fat is conjugated linoleic acid (CLA) that is produced in the rumen through incomplete biohydrogenation and *cis–trans* isomerization or *de novo* synthesis in the mammary gland (Figure 1). CLA has been considered a functional compound due to its significance reported for the health of animals and humans. Transvaccenic (C18:1, t-11) and rumenic (C18:2, c-9 t-11) acids are the main CLAs in milk fat. CLA has been reported to reduce body fat levels, improve glycaemic profile, resynthesize glycogen, modulate immune function, improve bone mineralization, prevent heart diseases, atherosclerosis, diabetes and cancer in experimental models[13]. In dairy cows, the probability of pregnancy had increased by 26% (91%) when CLA was supplemented at 10.1 g/day in early lactation compared to unsupplemented group (72%)[14]. In overweight conditions, CLA supplementation reduced body fat in humans[15]. Beneficial effects on the human immune function were also reported[16]. A dose of 3.0–3.5 g CLA per day had an anti-carcinogenic effect, whereas 15–20 g CLA per day was reported to inhibit fat synthesis and deposition in humans[17]. In postmenopausal women, lower serum CLA was found to be associated with a higher risk of breast cancer[18]. Apart from positive responses, several studies also reported adverse effects of CLA on human health.

In milk, a portion of CLA constitutes the biohydrogenation product of PUFA by ruminal microorganisms that are absorbed from the digestive tract, while the rest comes from the endogenous synthesis in the mammary gland from the absorbed vaccenic acid (Figure 2). Vegetable oils, along with increasing the energy density of the diet of high-yielders, become an important substrate for biohydrogenation. Due to their rich USFA profile, they could increase the content of CLA or CLA precursors in the rumen fluid. After absorption in the duodenum and small intestine, CLA and its precursor reach the mammary gland and different tissues where under the action of Δ9 desaturase enzyme, the CLA precursors are converted to CLA[19]. Approximately 64–78% of *cis*-9, *trans*-11 CLA appearing in milk fat is synthesized from trans vaccenic acid in the mammary gland[20]. Supplementation of oils rich in linoleic or linolenic acid at 4% of the diet increased the proportion of vaccenic acid and rumenic acid[21].

When the impact of the addition (500 g in diet) of linoleic acid-rich oils (cottonseed oil, soybean oil and corn oil) was studied in high-yielding HF cows (>34 l milk yield),

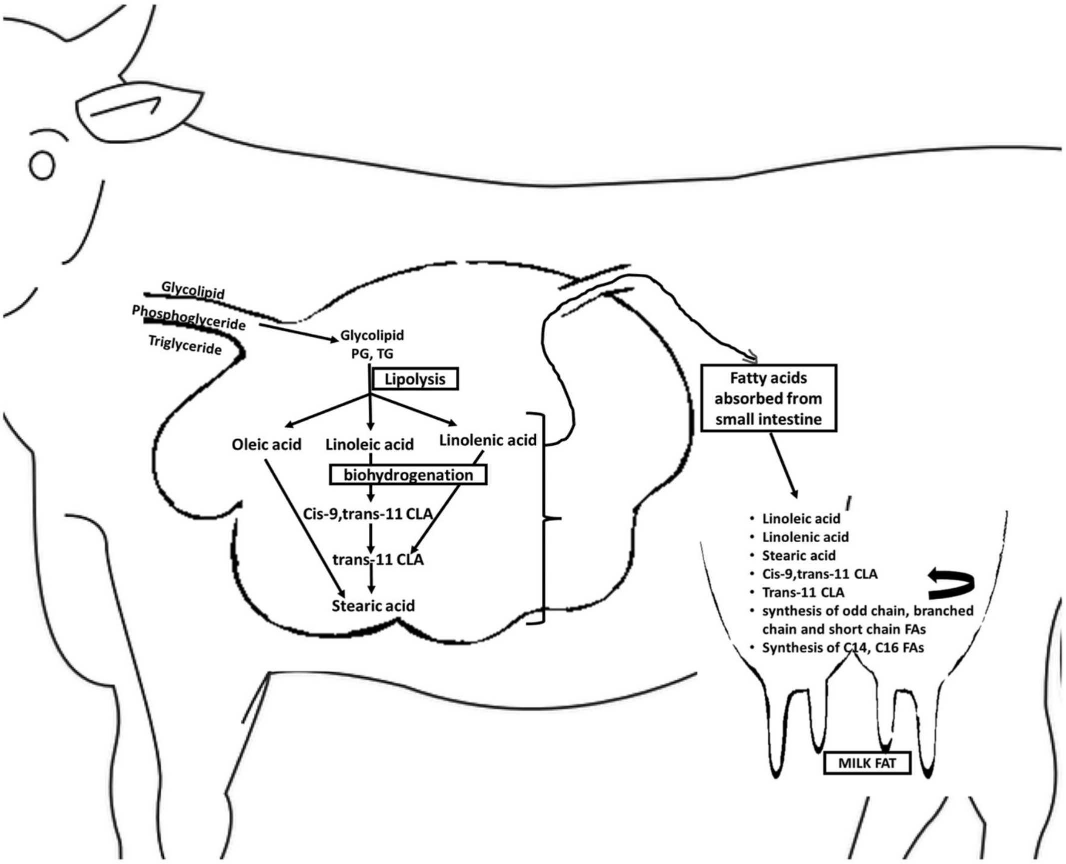

Figure 1. Milk fat composition of cattle less affected by dietary fat.

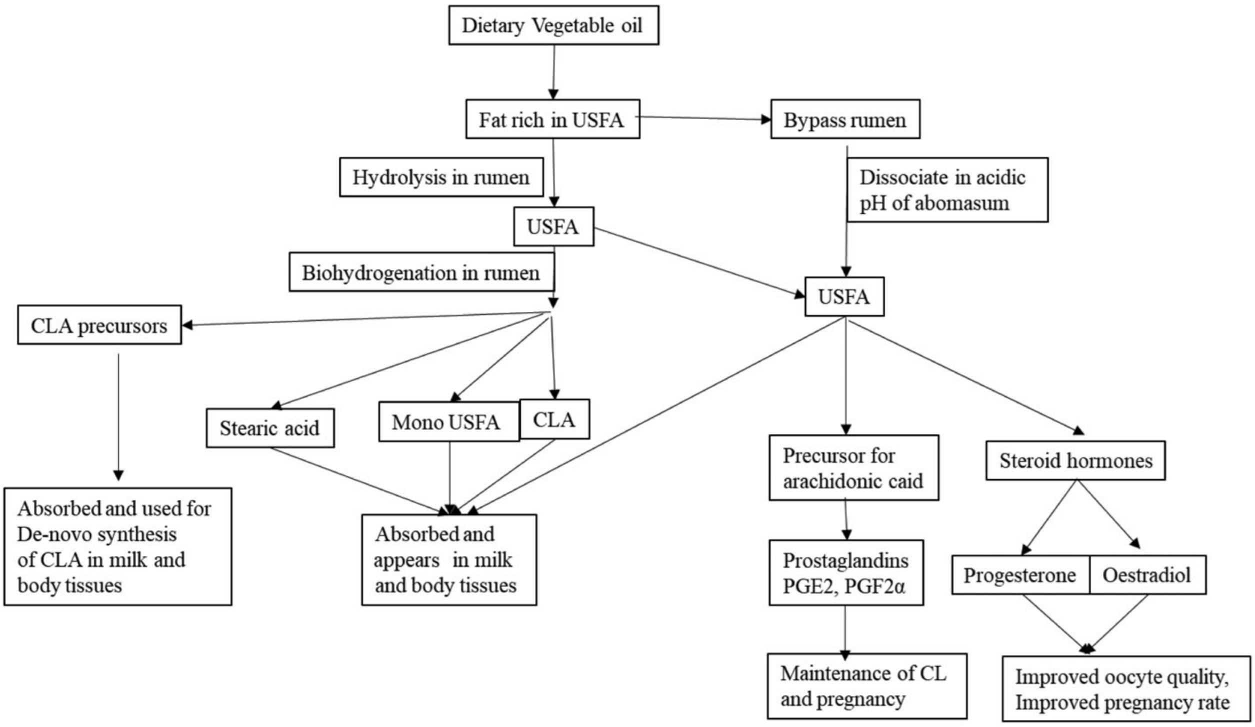

Figure 2. Pathway of impact of dietary fat on milk composition and reproduction in cattle.

cottonseed oil produced the highest milk yield, whereas soybean oil produced the highest CLA content (10.3 mg/g) in milk[2]. Zheng *et al.*[2] observed significantly lower contents of $C_{14:0}$ and $C_{16:0}$ in milk fat in response to all the supplemented vegetable oils, which could be due to altered rumen fermentation, resulting in decreased acetic acid production[22], the precursor for *de novo* synthesis of short and medium chain fatty acids in the mammary gland. Else, dietary long-chain fatty acids (LCFAs) might have competed with the newly synthesized short chain fatty acids (SCFAs) for triglyceride synthesis in the mammary gland[23], or possibly the LCFAs and/or CLA isomers negatively affected the mammary enzymes responsible for the synthesis of short and medium chain fatty acids[24].

Increased milk yield and milk fat%, and altered milk fatty acid profile (decreased proportion of short (C-8:0 to C-12:0) and medium chain (C-14:0 to C-16:0) fatty acids and increased proportion of long-chain (≥C-18:0) fatty acids) were observed on supplementation of either soybean or flaxseed oil[25]. At 2% level of inclusion, soybean oil (rich in linoleic acid) significantly enhanced vaccenic and rumenic acid levels in milk fat of dairy cattle compared to flaxseed oil, leading to the conclusion that linoleic acid was a better precursor of CLA in milk compared to linolenic acid[25]. Significant increases in the concentration of C18:2, C18:1 *trans*-11 and C18:2 *cis*-9 *trans*-11 CLA in milk and increased milk yield were observed on the inclusion of soybean oil in the diet of lactating dairy cows[26].

Adding oil/fat to the diet of high-yielding dairy cattle has shown variable effects on milk yield and milk fat composition. Though many studies reported positive effects, a few negative findings have also been reported. Decreased milk fat yield and fat concentration were observed on feeding oils rich in linoleic acid (corn oil and safflower oil); however, palm oil feeding showed an improved effect[3].

Addition of 5.7% fat in the diet of dairy cows (either linseed, extruded linseed or linseed oil) reduced milk yield due to the simultaneous reduction in fibrous carbohydrate digestibility and dry matter intake (DMI) in the experimental animals[27,28].

There was a reduction in milk yield on supplementation of additional 5.7% fat in the diet of dairy cows from either linseed, extruded linseed or linseed oil[29]. Reduction in milk yield was also reported when cows were fed soybean oil at 5% of DMI[30]. No significant effect of supplementation of vegetable oil (linseed) at varying levels (2.5%, 5.1% and 7.7% DM) on milk yield of grazing dairy cattle was reported[31]. With a low level of inclusion of soybean oil or linseed oil (2.3% in the diet), there was a decrease in milk fat% but the fatty acid profile was suitably altered, resulting in a decreased proportion of SFAs, short and medium chain fatty acids, and increased proportion of mono and poly USFAs, n-3 USFA and CLA[4]. Similarly, decreased milk fat% and milk yield but improved rumenic (1.71 g/100 g fatty acids) and vaccenic acid (4.14 g/100 g fatty acids) components of milk fat were reported with an oleic and linoleic acid-rich fatty acid diet[32]. Under similar experimental conditions, varying effects of different vegetable oils on milk yield and fatty acid content have been reported[33]. Milk fat content increased on the addition of linseed oil or rapeseed oil at 1.5% of the diet, but decreased on the addition of sunflower oil, while CLA content increased by 16% (ranging from 8.8 to 10.5 g/d) in the milk of rapeseed and sunflower seed oil supplemented groups[33]. Decreased milk fat% despite improved CLA content on vegetable oil supplementation was attributed to the higher production of *trans*-10, *cis*-12 CLA on a USFA-rich diet that is expected to decrease the mRNA expression of acetyl CoA carboxylase, fatty acid synthetase, lipoprotein lipase and other enzymes involved in the uptake and transport of circulating fatty acids and synthesis of fat in the mammary gland[34]. Little effect of including oil as whole rapeseed on milk yield was reported, but milk fat content decreased significantly ($P < 0.01$)[35]. A quadratic increase in milk fat content (3.44%, 3.60%, 3.56%, 2.86% and 2.93%) with increased levels of soybean oil (no additional oil, 0.5%, 1%, 2% and 4% oil) was observed that suggested significance of the level of fat inclusion on milk fat content[5]. However, the effect was positive until the USFA level adversely affected the rumen bacteria population to the extent that hydrogenation by rumen cellulolytic bacteria was halted.

Effect of vegetable oil supplementation on reproduction in dairy cattle

Fertility is influenced by factors such as genetic makeup, management practices, environmental conditions, and nutrition status, among which the nutrition plays the most important role in attaining successful reproductive function. An inadequate supply of nutrients, viz. energy, protein, vitamins and minerals or their combinations in the diet, has been associated with suboptimal reproductive performance. Energy is the most important nutritional factor for reproductive function in animals[36]. A sufficient energy balance of pregnant cows in late gestation is critical to attaining early postpartum heat and subsequent pregnancy. The effect of the addition of fat in the diet to optimize reproductive function has been an area of enduring interest among researchers. Fatty acids and cholesterol are substrates for the synthesis of reproductive hormones. Dietary fat expresses the effect either through improving the levels of reproductive hormones and/or increasing energy availability. The effect of fat on reproduction is significantly affected by the concentration of USFA than the quantity of fat intake[37]. Fat rich in PUFAs has positively influenced the growth of ovarian follicles, corpus luteum (CL) function and postpartum reproductive performance by providing increased precursors for the synthesis of reproductive hormones and prostaglandins (PGs)[38]. Among different classes of PGs, prostaglandin PGF2α and PGE2 are chiefly produced by bovine endometrium. PGF2α is luteolytic in action, whereas PGE2 has a reverse action on luteolysis. Therefore, a balance between the concentration of PGF2α and PGE2 is necessary for the maintenance of the corpus luteum and pregnancy in cattle[39]. The proposed mechanism through which USFA affect reproduction is given in Figure 2.

USFA in vegetable oils may compete with arachidonic acid for binding to prostaglandin H synthase, thus inhibiting the activity of the enzyme necessary for PGF2α synthesis[40]. Another mechanism for inhibition of PG synthesis due to supplemental linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is possibly due to the inhibition of cyclooxygenase enzyme activity[41].

Higher progesterone level during the luteal phase is associated with enhanced fertility. Higher progesterone at the time of artificial insemination (AI) and subsequently higher pregnancy rate in cows were observed on feeding 0.75 kg of linseed compared to sunflower seed[42]. Researchers have also reported better efficacy of linseed oil over soybean oil in improving the plasma progesterone level and pregnancy rate on the first AI in cows[4]. *In vitro* study revealed that in comparison to n-6 PUFA, supplementation of n-3 PUFA resulted in enhanced oocyte quality, increase in blastocyst rate, good quality blastocyst rate (freezable blastocyst) and improved fatty acid composition of oocytes, especially those involved in cell membrane structure[43]. Conversely, the buffering ability of ovary oocytes against the effects of fluctuation in plasma fatty acid profile in lactating dairy cows has also been demonstrated. Dietary supplementation of fatty acids, viz. rumen inert fat (palmitic and oleic acid rich), soybean (linoleic acid) and linseed (linolenic acid) revealed no change in major fatty acids in the granulosa cells, though the plasma and milk fatty acid profiles were directly influenced by the source of dietary fatty acids[44].

IGF-1 has a positive effect on cell proliferation, transformation and differentiation. Thus it has been used as an indicator for improvement of the breeding efficiency of cattle[45,46], though levels beyond the threshold limit have been reported to have a negative impact on the pregnancy rate[47]. Many studies also reported comparable effects of vegetable oils with varying fatty acid profiles (n-3 and n-6) on IGF-1 concentration. Higher plasma IGF-1 concentration in cows receiving fat from either soybean or linseed[48] was due to the direct relationship of plasma IGF-1 level with energy balance and pregnancy[49]. Similar effects on supplementing sunflower oil have been reported in cows[50]. Improved rate of pregnancy has also been reported with corn oil-supplemented diet[51]. When energy was limited in high-yielding cattle, increased luteinizing hormone (LH) secretion was observed through fat supplementation resulting in the growth of preovulatory follicles increased in size as well as numbers[40]. An increase in the number of small follicles in cows and heifers[52], and enhanced levels of plasma oestradiol in cattle[48] have been reported on supplementation of fat from linseed. However, high oestradiol levels in the plasma in response to extruded flaxseed did not alter its corresponding concentration in follicular fluid of preovulatory follicles in cows[52]. Lower oestradiol level in follicular fluid despite higher plasma concentration was possibly attributed to the prevention of steroid metabolism in the liver due to inhibition of a variety of cytochrome P450 enzymes under the action of PUFAs[53]. Soybean oil has been reported to reduce oestradiol concentration in the follicular fluid of beef cows[54]. During pro-estrus, under the influence of high oestradiol, there is increased secretion of gonadotropin-releasing hormone (GNRH) that stimulates the pituitary gland to release LH and trigger the LH surge[55].

Bypass vegetable fat

The fatty acid nutrition in cattle is intended to improve the energy status of the animals, fatty acids profile of milk fat and improve reproductive function by including appropriate fat sources in the diet. With regard to the milk fatty acid profile, the improved concentration of fatty acids of n-3 series and CLA and low levels of medium-chain saturated fatty acids are beneficial[8]. Medium-chain fatty acids are produced *de novo* in the mammary gland, whereas n-3 fatty acid, CLA and CLA precursors are of dietary origin, illustrating the significance of additional fat in the diet. However, there is a limit to the addition of fat based on the production level, feed intake, forage quality, weather, cattle breed and individual variation. Limit of fat inclusion is 3% of DMI in the case of dairy animals, beyond which a decrease in dry matter (DM) and fibre digestibility is anticipated[56]. Incorporation limit of fat in ruminant diets could go up to 16–20% of metabolizable energy intake[57]. However, fats rich in PUFAs may adversely affect fermentation patterns and exert toxic effects on rumen fibrolytic bacteria and protozoa in the rumen, subsequently lowering the DM digestibility[58]. Meta-analysis revealed reducing milk fat by adding unprotected fat, especially those rich in USFA[59]. The adverse effects of additional fat cannot prevent the inclusion of fat in the diet since the genetic potential of high-yielding cattle is unachievable with the conventional roughage–concentrate diet. Therefore, bypassing the additional fat is likely the effective strategy to optimize the production potential of high-yielding dairy cattle. The degree of bypassing of fat differs based on the nature of fatty acids, processing methods, etc. Micronized flaxseed (12.6%)-based diet improved milk yield and milk fat%, whereas extruded flaxseed at the same level in the diet had an adverse effect on the dairy cattle[60], possibly due to the failure of extrusion and effectiveness of the micronization process in protecting flaxseeds from ruminal digestion[61]. Inclusion of crushed flaxseed (6.5%) in the diet of multiparous crossbred cattle during the transition phase improved milk yield and concentration of α-linolenic acid (6.37% of total fatty acids) and omega-3 fatty acids and decreased short-chain fatty acids[62] owing to the higher passage rate of crushed flaxseed bypassing the rumen. In rumen pH, Ca salt of long-chain fatty acid (Ca-LCFA) is inert, but the fatty acids are dissociated in the acidic pH of the abomasum and efficiently absorbed from the small intestine. Ca-LCFA from canola oil (high oleic acid and moderate linoleic acid), soybean oil (high linoleic acid and moderate oleic acid), and linseed oil (high linolenic acid and moderate linoleic acid) increased the CLA content in milk fat by 4–6 times, with a higher increase for soybean oil and linseed oil compared to canola oil[63]. Researchers could not observe any significant effect of vegetable oil supplementation in the diet on fatty acid composition of granulosa cells and subsequently follicle numbers and post-fertilization development of oocytes *in vitro*[44]. However, improved oocyte quality manifested by an improved proportion of zygotes developed to the blastocyst stage was observed in increasing the levels of Ca-LCFA of palm oil in the diet of lactating dairy cows[64]. The analysed data suggested the positive effect of saturated fatty acids and negative effects of USFAs on milk fat%[59]. There was positive impact of encapsulated plant oils on milk fat% compared to other fat sources, viz. animal fat, encapsulated animal fat, Ca-LCFA of palm oil, oilseeds and plant oils. Prill fat is prepared by spraying a mixture of fatty acids of vegetable origin under pressure into a cooled atmosphere to liquify them. The product constitutes more than 85% palmitic acid and thus has a high melting point. Only a few studies have been conducted to examine its effect on lactation and reproductive performance. Supplementation of 2% prilled fat increased milk yield and milk fat% compared to the non-supplemented group[65]. In another study, prill fat supplementation augmented milk yield compared to control without altering the milk composition, plasma metabolites and hormone levels in dairy cows[66]. More studies must be conducted to compare prill fat with other fats for its bypass potential and subsequent effects in dairy cattle.

Summary

Addition of fat is necessary for high yielders, and scientific utilization can augment productivity in dairy cattle. Fatty acids are substrates for the synthesis of reproductive hormones. Fats rich in PUFAs positively influence the growth of ovarian follicle, CL function and postpartum reproductive performance. They adversely affect the fermentation pattern and exert toxic effects on rumen fibrolytic bacteria and protozoa in the rumen, subsequently lowering the DM digestibility. Adding oil/fat to the diet of dairy cattle has delivered variable responses for milk yield and milk fat composition. Thus, the use of fat for high-yielders should be done scientifically to produce favourable responses in them. Fat should be supplemented at the level where non-significant negative effects on the ruminal fermentation will occur, and to avoid depression in DMI or milk fat depression. Using bypass fat and other newer technologies may help maximise dairy animal productivity.

Conflict of interest: The authors declare that there is no conflict of interest.

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