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Review Article

Effects of Various Fat Supplements on Blood Metabolites and Plasma Hormones in Milch Animals: A Review

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Abstract

In dairy the stages of pregnancy and lactation especially the period of transition which is between late pregnancy (-3 weeks) and early lactation (+3 weeks) greatly modify the metabolism in animals and induce high stress and presents huge metabolic challenges in terms of energy balance, blood metabolites and hormonal changes. The loss in the Body Condition Score (BCS) is the consequence of the negative energy balance (NEB) seen just after parturition. The levels of non-esterified fatty acids and higher β-hydroxybutyrate concentrations are indicative of lipid mobilization and fatty acid oxidation. Average to high yielding dairy animals under NEB will mobilise the body reserves to support lactogenesis and milk production resulting in metabolic disorders and sub-optimal milk yield. Various forms of fats like bypass fat and prilled fat addition to the diets is a practicable option to alleviate NEB and to improve lactating animal performance. The present review discusses the effects of various fat supplements on blood metabolites and plasma hormones in milch animals.

Keywords: Blood Metabolites, Bypass Fat, Fats, Milch Animals, Prilled Fat, Prill Fat, Plasma Hormones

Introduction

Dairy animals having average to high milk yield on supplementation of the fat more specifically as bypass fat (BF) has shown good positive effect on lactation, nutrition and production performance parameters, more so evident at the early lactation. Some adverse effect may be noticed on supplementation of higher level of fat or bypass fat in animals diet which may be negative to health and productivity of animals due to increase in the level of lipid profile in blood and milk. Research reports on feeding of various forms of especially on blood biochemical profile i.e., blood metabolites and plasma hormones are scanty. Therefore, this review attempts to consolidate the work on the effects of different fat supplements on blood biochemical profile of lactating animals.

Effect of Various Fat Supplements on Blood Metabolites

Glucose

The plasma glucose levels increase before calving and then the glucose concentration declines to a minimum value between 11- and 25-days post-partum, consequent to the high glucose requirements for lactose synthesis (Kappel *et al*. 1984). Glucose forms the principal metabolic energy and is essential for essential organ function, milk production and foetal growth (Leblanc, 2010). Glucose is used as an energy source (McDonald *et al,* 2002), and is needed to provide reducing equivalents and serves as a source of glycerol for the production of triglycerides (Bines and Morant. 1983). Glucose stimulates fatty acids re-esterification in adipose tissue (Chilliard *et al.* 2000).

Work on Nili-Ravi buffaloes fed with four diets having i.e. control (no added fat) or added tallow, poultry fat or mustard oil @ 3% of dietary dry matter (DM) by Nawaz *et al.* (2012) had observed that the concentrations of glucose did not show any significant differences ($P>0.05$) in the blood of buffaloes on control versus diets fed containing different sources of supplemental fat. However, concentrations of these metabolites tended to be greater for buffaloes fed supplemental fat sources.

Tyagi *et al.* (2009a) conducted an experiment on feeding bypass fat supplement during the first quarter of lactation on milk production in crossbred cows. The control group was fed wheat straw, green maize and concentrate as per their requirements and the same ration along with 2.5% bypass fat in treatment group and recorded that the mean blood glucose concentrations were similar in both the groups being 58.64 and 57.82 mg/dl respectively.

Ranjan *et al.* (2012) investigated the effect of dietary supplementation of bypass fat (BF) on productive performance and blood biochemical profile of multiparous Murrah buffaloes (2-4th lactation) in early to mid-lactation divided in 3 homogenous groups T_1 (control), T_2, and T_3 with 5 animals in each group. The animals in T_1 were fed with a basal diet consisting of a concentrate mixture, green sorghum, and wheat straw as per requirements, while the animals in group T_2 and T_3 were fed with similar diets added with 0.7 % (100 g/day) and 1.4 % (200 g/day) of BF (on DMI basis) respectively, and recorded that serum glucose levels were not influenced by supplemental BF but increased ($P<0.01$) progressively with the advance in lactation trial.

Wadhwa *et al.* (2012) work on high yielding crossbred cows with control group (C) being offered *ad lib.* concentrate mixture and non-leguminous green fodder and the animals in the experimental group offered *ad lib.* control diet, supplemented daily with 150–200g BF recorded that the BF added diet group did not show any significant impact on blood profile except an increase in triglyceride (TG) level.

Garg *et al.* (2012) conducted an experiment in Crossbred cows (n=24), yielding 8-14 kg milk/animal/day and divided into three groups. All animals were fed similar basal ration, comprising 14-15 kg oat green, 5-6 kg Lucerne green and 4-5 kg wheat straw. Concentrate mixture was given according to the level of milk production, to meet the maintenance and milk production requirements. In addition to basal ration, cows in group II were fed 100 g bypass fat supplement, whereas, cows in group III were fed 100 g bypass fat and 10 g rumen protected choline (RPC) supplement per animal per day. Blood glucose was not affected by the dietary treatments.

Singh *et al.* (2014) reported that in crossbred cows fed with prill fat@75 g/d for a period of 90 days during mid lactation that blood glucose levels were similar in prill fat supplemented as well as control group.

Overall, the blood glucose concentration remained within the normal range and no significant difference was

observed at any stage of experiments. The reason may be a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body does not allow appreciable change in glucose level.

Non-Esterified Fatty Acids (NEFA)

The fraction of plasma fatty acids that are not in the form of glycerol esters are called as NEFA and are characteristically made up of saturated or unsaturated aliphatic compounds with an even number of carbon atoms, this group of acids include palmitic, stearic and oleic acids. About 10% of the total blood fatty acids (FA) usually 0.5-2.0 mmol/l are non-esterified. The plasma concentration of NEFA increases in fasting, as fatty acids are released from adipose tissue as a metabolic fuel. Transition from pregnancy to lactation experiences a dramatic increase in nutrient requirement that cannot be met by feed intake alone and during last 3 weeks of pregnancy, nutrient demand by foetus is greatest (Bell, 1979).

Hayirili *et al.* (2002) observed 32% decrease in dry matter intake (DMI) during the final 3 weeks of gestation where in 89% of decline occurred during the final week of gestation. As a consequence, body reserves are mobilized for milk production and this demand causes a negative energy balance (NEB) in normal to high yielding milch animals. As the concentration of NEFA in blood increases around calving or in early lactation, more NEFA are taken up by the liver (Bell, 1979). Once taken up by liver it can be either completely oxidized to CO_2 to provide energy for the liver or partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues such as muscle and incorporated into milk fat. Other options may be reconverted to storage fat (triglycerides). Increased adipose tissue mobilization post-partum is correlated with increased NEB and higher levels of plasma NEFA mobilized from adipose tissue (Ferguson, 1996; Drackley, 1999).

The desirable concentration of NEFA for lactating cows is below 0.62 mmol/L in early lactation and below 0.10 mmol/l for cows in late gestation (Kraft and Dürr, 2005).

In the trial of Castaneda Gutierrez *et al.* (2009), feeding different peripartum dietary supplementations (different fatty acid (FA) supplements or propylene glycol) showed no effect on plasma concentration of NEFA compared with control animals. Increased NEFA mobilization during the period of NEB in early lactation and during under-nutrition is due to a decrease in FA re-esterification, together with lipolysis (Chilliard *et al.,* 2000).

Tyagi *et al.* (2009a) conducted an experiment on crossbred cows feeding BF supplement during the first quarter of lactation on milk production. The control group was fed wheat straw, green maize and concentrate as per requirements and the same ration along with 2.5% of BF in the treatment group and recorded that the mean blood NEFA concentrations were similar in both the groups. However, the NEFA concentration maintained at a higher level throughout the experimental period.

Garg *et al.* (2012) conducted an experiment in Crossbred cows (n=24), yielding 8-14 kg milk/animal/day and divided into three groups. All animals were fed similar basal ration, comprising 14-15 kg oat green, 5-6 kg lucerne green and 4-5 kg wheat straw. Concentrate mixture was given according to the level of milk production and maintenance requirements. In addition to basal ration, cows in group II were fed 100 g of BF supplement, whereas, cows in group III were fed 100 g BF and 10 g rumen protected choline (RPC) supplement/animal/day. NEFA in blood serum were reduced by 16.12 and 24.19% ($P<0.01$) in groups II and III, respectively.

Harvatine and Allen (2005) conducted an experiment on multiparous Holstein cows in mid to late lactation and treatments were 2.5% added dietary FA from saturated FA (SAT) or unsaturated FA (UNS) sources (SAT = prilled hydrogenated free FA (Energy Booster 100); UNS = calcium salts of palm FA (Megalac). Results of experiment showed that all cows decreased plasma NEFA when fed SAT compared with UNS. Saturated FA treatment decreased NEFA over 20% compared with UNS (89.3 vs. 115.5 Eq/L respectively ($P<0.001$).

Fukumori *et al.* (2012) determined the effects of calcium salts of long-chain fatty acids (CLFAs) and rumen-protected methionine (RPM) on plasma concentrations of NEFAs in lactating cows. Cows were fed corn silage-based diets with supplements of CLFAs (1.5% added on dry matter basis), RPM (20 g/d), CLFAs plus RPM, and without supplement. Plasma concentrations of NEFAs were increased with CLFAs but increases of plasma concentrations of TG and total cholesterol were moderated by CLFAs plus RPM. Compared with cows without CLFAs, plasma concentrations of NEFA in cows fed CLFAs were higher ($P<0.001$), In cows fed RPM diets plasma

NEFA tended to be lower (P=0.15)

It can be inferred from the various experiments that non supplemented groups had higher concentration of plasma NEFA because milch animals mobilised more body reserves in order fulfil energy needs and comparatively lower levels of NEFA is due to the positive impact of fat supplementation in the diet.

Beta Hydroxy Butyrate (β-HBA)

Measurement of β-HBA content is a sensitive tool to determine energy balance. Normal plasma β-HBA level ranges from 0.5 to 0.9 mmol/l and values >1.2-1.4 mmol/L lead to subclinical cases of ketosis (Duffield, 2002). Carlson *et al.* (2006) described β-HBA as a key indicator of hepatic ketogenesis as a result of influx of NEFA into the liver. The desirable concentration of BHB for lactating cows is below 1.00mmol/L in early lactation and below 0.60mmol/L in late gestation (Whitaker *et al.,* 1983).

β-HBA indicates the completeness of oxidization ("burning") of fat in the liver. Ketone bodies (β-HBA, acetone and acetoacetate) are intermediate metabolites of oxidation of fatty acids; as the supply of NEFA to the liver exceeds the ability of liver to completely oxidize the fatty acids to supply energy, the amount of ketone body production increases. Ketone bodies can be used by muscle as an alternative fuel source to glucose, sparing glucose for milk production (Herdt, 2000a). However, ketone production does not result in as much net energy release as does complete oxidation of fatty acids. Additionally, increasing concentrations of ketones are thought to suppress feed intake.

These ketone bodies are produced from the metabolism of NEFA and volatile fatty acid (propionate, butyrate and acetate produced by rumen fermentation) in ruminant and out of these butyrate is converted to β-HBA in the rumen epithelium and the liver (McDonald *et al.,* 2002). Hence, the presence of ketone bodies in the body fluid is normal to certain degree, whereas high concentration of ketone bodies indicate that adaptability of metabolism is exceeded and whole-body homeostasis cannot be maintained (Baird, 1982).

The excessive mobilization of body fat around parturition lead to increased circulation of free fatty acids in dairy cows. These free fatty acids are then oxidized to βHBA by βHB dehydrogenase or spontaneously decarboxylized to acetone and acetoacetate, which are considered as ketone bodies (Brumby *et al.,* 1975; Baird, 1982; Herdt, 2000). Increased β-HBA concentration reveals incomplete oxidation of NEFA in tricarboxylic acid cycle during NEB (Grummer, 1993; Doepel *et al.,* 2002). Ketone body concentration in blood can also indicate energetic stress and rate of lipid mobilization in the animal. Increased mobilization of body tissues in early lactation is generally associated with an increased production and concentration of ketone bodies (Ingvartsen and Andersen, 2000). There is a limit to the amount of mobilized FA that can be oxidized in liver. When this limit is reached, TG accumulates within the hepatocytes, and acetyl-coenzyme A is converted to ketone bodies (Goff and Horst, 1997).

Plasma BHBA levels were not significantly different in control and prilled fat supplemented group during mid lactation when supplemented group fed prilled fat @ 75 g/d (Singh *et al.*, 2014).

In experiment of Casteneda-Gutierrez *et al.* (2009), peri parturient cows fed different FA supplements or propylene glycol showed a decrease in BHB concentrations compared with control animals.

Triglycerides

As components of dairy cow rations, triglycerides (TG) are mostly broken down in the rumen to release the fatty acids and the glycerol, unless the triglyceride is extremely saturated (e.g., a highly hydrogenated tallow, grease, or vegetable oil).

Castenada-Gutierrez *et al.* (2009) observed no differences in hepatic TG content for cows fed different FA supplements or propylene glycol three weeks postpartum compared with control animals. Plasma triglycerides (TG) concentration was similar in both control and bypass fat supplemented group of cows, as TG have high metabolic rate of utilization like glucose which might be the reason behind unaltered TG levels between the two groups (Tyagi *et al.,* 2009b). The mean plasma triglyceride concentration in control and prill fat fed (PFG) cows during mid lactation was (31.47±2.04 mg/dl) and (25.96±1.24 mg/dl) respectively and were not significantly different (Singh

et al., 2014).

Wadhwa *et al.* (2012) reported the performance of high yielding crossbred cows on supplementation of bypass fat (BPF). The animals in the control group (C) were offered *ad lib.* concentrate mixture and non-leguminous green fodder and the animals in the experimental group were offered *ad lib* control diet, supplemented daily with 150–200g BPF. The dietary supplementation of BPF did not show any significant impact on blood profile except on triglyceride (TG) level, which was improved ($P<0.01$) in the BPF supplemented group

Nawaz *et al.* (2012) conducted an experiment in Nili-Ravi buffaloes fed four diets either no added fat or had tallow, poultry fat or mustard oil at 3% of dietary dry matter and observed that triglycerides and total blood lipids did not vary significantly due to feeding different fat sources. It was concluded that fat sources studied can be fed safely at 3% of dietary dry matter to early lactating buffaloes.

Ranjan *et al.* (2012) investigated the effect of dietary supplementation of bypass fat on blood biochemical profile of lactating Murrah buffaloes. The animals in T_1 were fed with a basal diet as per requirements, while the animals in group T_2 and T_3 were fed with same ration supplemented with 0.7 % (100 g/day) and 1.4 % (200 g/day) bypass fat (on dry matter intake (DMI) basis) respectively. The dietary supplementation of BPF did not show any significant impact on blood profile except on triglyceride (TG) level,

Barley and Baghel (2009) reported that supplementation of bypass fat in Murrah buffaloes increased weekly average milk yield as well as fat content and serum triglyceride content. The increased energy supply to the animals in negative energy balance was responsible for increased milk yield and availability of low-density serum triglyceride in plasma led to increased fat content and serum triglyceride levels.

Fukumori *et al.* (2012) determined the effects of calcium salts of long-chain fatty acids (CLFAs) and rumen-protected methionine (RPM) on plasma concentrations of triglyceride (TG). Plasma concentrations of triglyceride were increased with CLFAs, but increases of plasma concentrations of TG was moderated by CLFAs plus RPM. These results indicate that the addition of methionine to cows given CLFAs decrease plasma concentrations of Triglycerides.

Fukumori *et al.* (2013) elucidated the effects of medium-chain fatty acids (MCFAs) on plasma triglycerides in lactating dairy cows. Treatments consisted of diets supplemented without (control) or with calcium salts of MCFAs (MCFA-Ca; 1.5% dry matter). The concentrations of triglycerides in plasma increased in cows fed the MCFA-Ca diet.

Plasma Total Cholesterol

Cholesterol concentration in the blood plasma ranges from 120 to 220 mg/dL (McDonald *et al.*, 2002). Cholesterol is mostly produced in the small intestinal epithelium to transport dietary lipid; therefore, lower concentrations in blood of the feed restricted animals might be expected because of lower DMI (Doughlas *et al.*, 2006). It is also the precursor of steroid hormones (McDonald *et al.*, 2002). The mean plasma cholesterol, HDL and VLDL cholesterol concentration in control and prill fat fed (PFG) cows during mid lactation were not significantly different (Singh *et al.*, 2014).

Wadhwa *et al.* (2012) reported the performance of high yielding crossbred cows on supplementation of bypass fat (BPF). The control group (C) animals were given *ad lib.* concentrate mixture and non-leguminous green fodder and the animals in the experimental group were given *ad lib.* control diet, supplemented daily with 150–200g BPF. The dietary supplementation of BPF did not show any significant impact on blood cholesterol. The non-significantly higher cholesterol level may have favourable effect on the synthesis of reproductive hormones and in turn may affect the reproductive performance of animals in the BPF supplemented group.

Nawaz *et al.* (2012) conducted an experiment in Nili-Ravi buffaloes fed four diets either contained no added fat or had tallow, poultry fat or mustard oil at 3% of dietary dry matter, and observed that total cholesterol did not vary significantly due to feeding different fat sources.

Ranjan *et al.* (2012) investigated the effect of dietary supplementation of bypass fat on blood biochemical profile

of lactating Murrah buffaloes in multiparous buffaloes (2–4 lactation) of early to mid-lactation. The animals in T_1 were fed with a basal diet consisting of a concentrate mixture, green sorghum, and wheat straw as per requirements, while the animals in group T_2 and T_3 were fed with same ration supplemented with 0.7 % (100 g/day) and 1.4 % (200 g/day) bypass fat (on dry matter intake (DMI) basis) respectively. It was reported that Supplementation of bypass fat to lactating buffaloes increased ($P<0.01$) serum cholesterol level without any alteration in triglyceride level. HDL cholesterol (good cholesterol) was higher ($P<0.05$) in group T3 than T1 and T2. LDL cholesterol (bad cholesterol) was also higher ($P<0.05$) in T3 than T1 but at par with T2 which was an indication of improvement in the proportion of good cholesterol than bad cholesterol with an increase in the dose of supplemental bypass fat from 0.7 to 1.4 % of the diet. The serum lipid profile of animals fed with bypass fat was also progressively increased ($P<0.01$) with the advance in lactation trial.

Garg *et al.* (2012) conducted an experiment in Crossbred cows (n=24), yielding 8-14 kg milk/animal/day and divided into three groups. All animals were fed similar basal ration, comprising 14-15 kg oat green, 5-6 kg lucerne green and 4-5 kg wheat straw. Concentrate mixture was given according to the level of milk production, to meet the maintenance and milk production requirements. In addition to basal ration, cows in group II were fed 100 g bypass fat supplement, whereas, cows in group III were fed 100 g bypass fat and 10 g rumen protected choline (RPC) supplement per animal per day. There was reduction ($P<0.01$) in cholesterol levels in blood serum in animals of groups II and III, as compared to group I.

Fukumori *et al.* (2012) determined the effects of calcium salts of long-chain fatty acids (CLFAs) and rumen-protected methionine (RPM) on plasma concentrations of cholesterol. Plasma concentrations of total cholesterol were increased with CLFAs alone, but increases of plasma concentrations of TG and total cholesterol were moderated by CLFAs plus RPM. These results indicate that the addition of methionine to cows given CLFAs causes decrease in plasma concentrations of TG and total cholesterol.

Fukumori *et al.* (2013) conducted study to elucidate the effects of medium-chain fatty acids (MCFAs) on plasma in lactating dairy cows. Treatments consisted of diets supplemented without (control) or with calcium salts of MCFAs (MCFA-Ca; 1.5% dry matter). The concentrations of total cholesterol in plasma increased in cows fed the MCFA-Ca diet.

Effect of Various Fat Supplementation on Plasma Hormones

Growth Hormone (GH)

The best-known function of GH is promotion of longitudinal growth. GH stimulates protein anabolism and promotes lipolysis (Copeland and Nair, 1994). The most prominent metabolic effect of GH is a marked increase in lipolysis at adipose tissues and free fatty acid levels in blood. During fasting and other catabolic states, GH predominantly stimulates the release and oxidation of free fatty acid. Among protein hormones GH and prolactin have been demonstrated to accelerate immunity (Redelman *et al.,* 2008). GH supplementation in hypophysectomised rats restored most of the impaired immune function (Takada *et al.,* 1991*),* suggesting that GH plays an important role in the development of immune function.

Plasma level of growth hormone varied between 6.0-8.8ng/ml respectively during pre and postpartum period in Holstein cows (Block *et al.,* 2001). Mallick (2010) recorded 11.6±1.8 ng/ml on day 14 ante partum, gradually increased to 14.7±3.1ng/ml on the day of parturition followed by decline to 11.6±1.6ng/ml on day70 postpartum in peripartum KF cows.

The initiation of lactation is a time of rapid adaptation. The dramatic increase in energy requirements of high-producing dairy cows during this period requires homothetic control of metabolism to direct endogenous and dietary nutrients to the mammary gland for lactogenesis (Drackley, 1999). The gluconeogenesis is highly prioritized by the cow and is under hormonal regulation mainly by insulin, glucagon and growth hormone (Aschenbach *et al.,* 2010). Growth hormone (GH) plays a central role in this process, inhibiting lipid storage in adipose tissue and increasing blood flow to the mammary gland, among other effects.

It is probable that the effect of GH is to preferentially partition nutrients to the mammary gland at the expense of other tissues. Therefore, regulation of GH secretion has been the focus of intensive research for years (McMahona

et al., 2001).

Thomas and Williams (1996) examined ovulatory responses following FSH treatment in beef heifers fed dietary fat supplements expected to produce differential effects on serum insulin concentrations and follicular recruitment patterns. Diets fed were control diet or to 1 of 2 fat-supplemented diets consisting of soybean oil (polyunsaturated fatty acids) or animal tallow (saturated fatty acids) and were fed until ovariectomy between experimental days 35 and 45. The rate of ovulation was established at ovariectomy five days after the superovulatory estrus (at day 35 to 45 of experiment). Both soybean oil and animal tallow diets increased ($P<0.015$) the number of medium-sized follicles and increased ($P<0.02$) serum concentrations of GH relative to the control diet.

The mean plasma GH concentration in control and prill fat fed (PFG) cows during mid lactation were significantly higher in treatment group fed prilled fat @75 g/d and the respective values were1.87±0.12 ng/ml and 2.29±0.13 ng/ml (Singh *et al.* 2014).

Harvatine and Allen (2005) conducted an experiment on multiparous Holstein cows in mid to late lactation and treatments were 2.5% added dietary FA from saturated FA (SAT) or unsaturated FA (UNS) sources (SAT = prilled hydrogenated free FA (Energy Booster 100); UNS = calcium salts of palm FA (Megalac). Plasma growth hormone concentrations were not affected by treatment.

Becú-Villalobos *et al*. (2007) investigated the effect of fat supplementation on plasma levels of hormones related to metabolism, in cows in early lactating cows. Animals were given no fat or else 0.5 or 1.0 kg of partially hydrogenated oil per day in addition to their basal diet from day 20 before the expected calving date to day 70 postpartum and was observed that no differences were observed in the plasma GH value between times or between dietary groups.

Ghrelin

Ghrelin is a hormone secreted from the digestive tract of many animals wherein it acts as a signal of hunger and therefore may stimulate feed intake. Ghrelin is a 28-amino acid, octanoylated peptide which is secreted primarily by cells in the abomasum in ruminants (Huang *et al.,* 2006). Ghrelin is called the "ultimate anabolic hormone" because it causes the body to consume and store energy (Litwack *et al.,* 2008). Ghrelin concentrations increase prior to scheduled meals (Sugino *et al.,* 2002) and in response to fasting (Wertz-Lutz *et al.,* 2006) in ruminants, and feeding suppresses ghrelin secretion.

Some workers suggested that leptin acts in opposing fashion to ghrelin and is expressed at lower concentrations during states of positive energy balance and increased during negative energy balance (Dimaraki and Jaffe, 2006; Gottero *et al.,* 2004; Nogueiras *et al.,* 2008). Because of this close relationship, it was also determined that both ghrelin and leptin may play roles in regulating reproduction.

Fukumori *et al.* (2012) reported the effects of calcium salts of long-chain fatty acids (CLFAs) and rumen-protected methionine (RPM) on plasma concentrations of ghrelin, and pancreatic hormones in lactating cows. Cows were fed corn silage-based diets with supplements of CLFAs (1.5% added on dry matter basis), RPM (20 g/d), CLFAs plus RPM, and without supplement. CLFAs augmented plasma ghrelin concentration, and the Ghrelin concentration with CLFAs plus RPM was the maximum among the treatments. It can be inferred that addition of methionine to cows given CLFAs raised the plasma concentrations of Ghrelin. The means of plasma ghrelin concentration were higher ($P<0.001$) in cows fed CLFAs than in cows not fed CLFAs. There were interactions between CLFAs and RPM for ghrelin ($P=0.002$). CLFAs plus RPM increased plasma ghrelin but RPM alone did not show such effects.

Fukumori *et al.* (2013) further elucidated the effects of medium-chain fatty acids (MCFAs) on plasma ghrelin concentration in lactating dairy cows. Five early-lactating Holstein cows were randomly assigned to 2 dietary treatments in a crossover design with 2-wk periods. Treatments consisted of diets supplemented or not (control) with calcium salts of MCFAs (MCFA-Ca; 1.5% dry matter). Plasma ghrelin concentrations were higher in cows fed the MCFA-Ca diet. In conclusion, dietary MCFAs increase the plasma ghrelin concentrations in lactating dairy cows.

Leptin

Leptin is a 16-kDa protein hormone synthesized almost exclusively by White Adipose Tissue (WAT). In ruminants and other animals, leptin synthesis is regulated positively by adiposity, and negatively by under nutrition (Spiegelman and Flier, 2001) and is involved in the regulation of energy homeostasis.

Low concentration of plasma leptin during under nutrition induces increased appetite as well as neuroendocrine adaptations responsible for metabolic adaptations and energy conservation (Schwartz *et al.,* 2000). The plasma leptin is reduced abruptly around the time of parturition in lactating cows (Block *et al.,* 2001). From early to mid-pregnancy increased circulating leptin levels which remained elevated until late pregnancy has been reported (Liefers *et al.,* 2003) in cattle.

Leptin affects both fat deposition and LH concentrations and it could play an important role in the processes occurring during the lactation period in dairy cows. During early lactation, cows are in a state of negative energy balance and fat stores are first used for lactation, maintenance, and growth with reproductive processes receiving the lowest priority (Mwaanga and Janowski, 2000). The negative energy balance suppresses the LH pulse frequency, resulting in a delayed first ovulation (Jolly *et al.,* 1995). The hormone which is one of the adipose endocrine signals, also affects release of gonadotropin hormones, with implications in reproduction (Chagas *et al.* 2007).

In ruminants, no effect of fat ingestion on leptin levels could be consistently demonstrated (Chilliard *et al.,* 2001), whereas in 1 study the leptin concentration was shown to increase in lambs given fat supplementation by means of rumen bypass (Yildiz *et al.,* 2003). The mean plasma leptin concentrations in control and prill fat fed (PFG) cows during mid lactation were (2.66±0.16ng/ml) and (2.92±0.04ng/ml) respectively and were non-significantly higher in treatment group (Singh *et al.,* 2014).

Becú-Villalobos *et al.* (2007) investigated the effect of fat supplementation on plasma levels of hormones related to metabolism, with special attention to leptin, in cows in early lactation. 34 lactating cows were given no fat or else 0.5 or 1.0 kg of partially hydrogenated oil per day in addition to their basal diet from day 20 before the expected calving date to day 70 postpartum and was observed that the mean leptin plasma levels increased between 30 and 60 DIM ($P < 0.01$), but no significant differences were observed between the treatment groups. Positive correlations ($P< 0.05$) between leptin level and BCS were found at 30 and at 60 DIM.

Insulin

Insulin is a peptide hormone with a molecular weight of 5080 Daltons, composed of 51 amino acid residues and is produced in the pancreas of mammals from the beta cells in the Islets of Langerhans. Insulin encourages the uptake of glucose and the synthesis of steroid hormones (McDonald *et al.,* 2002). Measuring insulin levels can help to quantify the metabolic and physiological responses to different feeding levels (Carlson *et al.,* 2006). Recently insulin has been characterized as a satiety hormone because it down regulates intake with exogenous administration while maintaining blood glucose levels experimentally (Woods *et al.,* 2000).

The insulin concentration for the different fortnight was on higher side in bypass fat supplemented group, but the differences between the groups were non-significant (Tyagi *et al.* 2009b). Fat supplementation has a sparing effect on glucose hence, it was hypothesized that through feedback mechanism, the level of insulin would increase. The mean plasma Insulin concentration in control and prill fat fed (PFG) cows during mid lactation were found to be (17.39±0.96µIU/ml) and (17.72±1.58µIU/ml) respectively and were not significantly different (Singh *et al.,* 2014).

Harvatine and Allen (2005) conducted an experiment on multiparous Holstein cows in mid to late lactation and treatments were 2.5% added dietary FA from saturated FA (SAT) or unsaturated FA (UNS) sources (SAT = prilled hydrogenated free FA (Energy Booster 100); UNS = calcium salts of palm FA (Megalac). Saturated FA increased insulin over 25% compared with UNS (12.8 vs. 10.1 µIU/mL, $P<0.001$).

Fukumori *et al.* (2012) studied effects of calcium salts of long-chain fatty acids (CLFAs) and rumen-protected methionine (RPM) on plasma concentrations of pancreatic hormones in lactating cows fed corn silage-based diets with supplements of CLFAs (1.5% added on dry matter basis), RPM (20 g/d), CLFAs plus RPM, and without supplement. Plasma concentrations of glucagon-like peptide-1, glucagon, and insulin were decreased with CLFAs, whereas adding RPM moderated the decrease of plasma glucagon concentration by CLFAs. Compared with cows fed non-CLFAs, plasma concentrations of insulin in cows fed CLFAs were lower ($P=0.012$). Plasma insulin

concentration tended to be higher (P=0.092) in cows supplemented with RPM than those without RPM. Fukumori *et al.* (2013) elucidated the effects of medium-chain fatty acids (MCFAs) on plasma ghrelin concentration in lactating dairy cows. Treatments consisted of diets supplemented or not (control) with calcium salts of MCFAs (MCFA-Ca; 1.5% dry matter). Plasma insulin concentrations decreased in cows fed the MCFA-Ca diet.

Insulin like Growth Factor-1 (IGF-1)

Insulin like growth factor-1 is produced principally by liver as an endocrine hormone. The target tissues also produce IGF-1 as paracrine /autocrine hormone. IGF-1 is also called somatomedin C, and has anabolic properties and helps the somatic growth and cellular metabolism. IGF-1 release is stimulated by GH and can be reduced during the mal nutrition and growth hormone (GH) insensitivity, absence of GH receptors, or signalling pathway failure of post GH receptors.

IGF-1 in the circulation is released from the liver in response to growth hormone coupling GH receptors, with systematic IGF-1 negative feedback to pituitary to regulate GH release. Although dietary fat supplementation has been shown to increase serum concentration of GH, concentrations of IGF-1 in serum remained unchanged (Ryan *et al.* 1995; Thomas *et al.* 1993).

Becú-Villalobos *et al.* (2007) investigated the effect of fat supplementation on plasma levels of hormones related to metabolism, in cows in early lactating cows. Animals were given no fat or 0.5 or 1.0 kg of partially hydrogenated oil per day in addition to their basal diet from day 20 before the expected calving date to day 70 postpartum and have recorded that the mean IGF-I plasma levels increased between 30 and 60 DIM ($P< 0.01$), but no significant differences were observed between the treatment groups, although the mean IGF-I concentration tended to diminish with increasing fat.

Singh (2015) was recorded that the IGF-1 levels were not influenced by fat feeding in spite of increased GH concentrations in an experiment on fifteen early lactating Murrah buffaloes, divided into three groups of five animals each having average milk yield up to 5 kg/d (T1) and the two groups (T2 and T3) of buffaloes having milk yield of 9-10 kg/d fed with balanced ration as per Kearl (1982) feeding standards. The third group (T3) was supplemented with prilled fat @ 75 g/day/animal in addition to basal ration from 6th day of postpartum to 45th day and 150 g/d/animal till the experimental period of 120 days. It was inferred that the GH hormone elicits its effect on milk yield through IGF-1 and there is uncoupling of GH-IGF1 axis in early lactation in which IGF-1 concentration does not increase in spite of increase in plasma GH (Ronge *et al.*, 1988; McGuire *et al.*, 1995a).

Conclusion

From existing literature, it can be concluded that various levels of fat supplements in the diet of milch animals is very important to reduce the negative energy balance without adversely affecting the blood biochemical profile. Supplementation of various fats gives additional benefit to the dairy animals. Additional research is necessary to specifically elucidate type of fat to be fed along with level of inclusion at different productive levels and stages of lactation.

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Conflict of Interests

There is no conflict of interest.

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