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Review Article

Potential Use of Bypass Protein and Fat in Dairy Animal Diets: Challenges and Opportunities

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ABSTRACT

During the lactation season, optimizing animal nutrition is crucial for dairy animals' productivity. To address the limitations of ruminal breakdown, nutritionists focus on providing high-energy, protein-rich feed that bypasses ruminal digestion. The primary challenge lies in the extensive breakdown of fat and protein, leading to inadequate nutrient availability for milk production. Dietary supplementation of protected fat, and protein play a key role to fulfill the nutritional demands, enhanced milk yield and composition of dairy animals. In the past, several studies have focused on the delivery of protective fats, proteins, and amino acids to minimize the adverse effects of rumen fermentation. Literature suggested that lactating animals' diets supplemented with protected fat and/or protein positively improved the milk production and composition in early lactating cows. Additionally, dietary crude protein adjusted at 14 to 18% further enhances milk yield and composition. The disparities in dietary elements, such as ruminal undegradable protein, ruminal degradable protein, and dietary crude protein, underscore the necessity for customized nutritional approaches. This review discusses and update the results of protected fats and proteins as a bypass method particularly highlights the effectiveness and economic viability for optimizing dairy animal nutrition to enhanced milk production and composition.

INTRODUCTION

Agriculture research has traditionally focused primarily on increasing productivity and production, with little attention paid to enhancing the nutrient profiles of food items. Therefore, research and agricultural techniques that could enhance the nutrient profile of food items are of interest to both farmers and scientists. The dairy industry's recent efforts to alter the protein and fat content of milk are one such example (Gawad *et al*., 2015).

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Energy (fats, carbohydrates) and proteins are the major nutritional factors most likely to limit dairy cattle rations. The relationship between energy and protein requirements for lactating ruminants is complex because there are two requirements to be met: one for the host animal and another for ruminal microbes (NRC, 2001). Moreover, because less fodder is being grown and there are fewer crop leftovers available led to there is a growing gap between the amount needed and what is available for feed and fodder. Under these circumstances, lactating animals potential for producing milk fluctuates and is not fully developed, resulting in lower productive performance (Mahajan *et al*., 2022). Additionally, unbalanced protein and energy levels might lead to metabolic problems such as ketosis and placenta retention (Bargo *et al*., 2002).

Adding fat or high-fat feedstuffs to increase the energy density and energy intake of diets for transition period dairy cattle is necessary to avoid negative energy balance, which is becoming common because maximal dry matter intake (DMI) does not occur until after peak milk production (Kholif and Ebeid, 2009). Moreover, feeding large amounts of ruminally unprotected fat (oil, tallow, etc.) may have detrimental effects on fiber digestibility in addition to depressing total milk protein by 0.1 to 0.2 percentage units, total milk N and casein N (Palmquist and Jenkins, 1982; DePeters and Cant, 1992). As reported by Magorzata *et al.* (2020), the oil released in the rumen may exert a negative impact of polyunsaturated fatty acids (PUFA) on ruminal microbial growth through bio-hydrogenation (BH) processes and cellulytic bacteria population may be decreased (Małgorzata *et al*., 2020). In the mammary glands, milk fat with fewer than 16 carbons is created from starch. Fatty acids (FA) with more than 16 carbons are created through de novo synthesis, whereas FA with 16 carbons is created through a combination of diet and de novo synthesis (Castañeda-Gutiérrez *et al*., 2007). Therefore, we should lessen the negative impact of fat on ruminal fermentation; the fat supplements must be largely inert in the rumen (Ramos-Morales *et al*., 2016). Previous investigations in this area discovered that feeding lactating cows with protected fat supplements would boost their diet's energy concentration and metabolic effectiveness (Ranjan *et al*., 2012). Feeding a protected lipid that has been chemically or physically altered to resist microbial biohydrogenation and ensures that unsaturated fatty acids reach the small intestine (Gawad *et al*., 2015).

Furthermore, when ruminants consume protein meals (highly degradable protein sources), such as soybeans and amino acids, they are rapidly degraded by proteolytic enzyme microbes in the rumen, leading to the production of ammonia at large scale, much of which is wasted as urea excreted through urine and feces, as well as the loss energy that an animal must expend in the liver to convert ammonia to urea and thus effect on milk protein content. To counter the negative impact of protein and fat in the rumen, a lot of publications have been done, and there are currently commercial products in livestock marketing like Magnapac and Megalac for protected fat and Smartin for protected AA (Donkii *et al*., 1989; NRC, 2001; Kholif *et al*., 2006a, b; Ebeid *et al*., 2007). The present state of the art will be discussed, including the type and role of protected fat and proteins in enhancing the milk yield, ruminal fermentation, digestibility coefficients, blood metabolites, and their effect on dairy animals' performance.

PROTECTED NUTRIENTS

The protected nutrients are defined as inert fats and proteins in the rumen that are resistant to hydrolysis and enable the digestion and absorption of these nutrients from the lower tract without passing through the rumen. Also known as bypass or runaway fat and proteins, they are more expensive per unit of protein and energy delivered compared to commodity proteins and fats (Mahendra *et al*., 2015). The Association of American Feed Control Officials described "rumen protected" as "a nutrient or nutrient (s) provided in such a form that gives an increase in the flow of that nutrient or nutrient (s), unmodified to the abomasum, yet available to the gut of an animal (Noel, 2000). Rumen-protected proteins are therefore found in protein-containing feeds that have undergone special processing to improve the amount of digestible RUP and reduce the amount of ruminal protein degradation.

Commercially accessible products vary in their protective technology forms and consequent level of protection from ruminal degradation (Ji *et al*., 2014; Mahendra *et al*., 2015). Additionally, how well the coating protects in the rumen environment depends on the type of coating material, the smoothness of the pellet surface, the hardness of the inner core pellet, the solubility of the active component and the size of the pellet (Wu *et al*., 2012). However, due to direct interaction with ration ingredients, variations in coating techniques and their efficacy may have an impact on resistance to surface deterioration and the release of fat and amino acids (AA) (Mahendra *et al*., 2015).

PROTECTED FAT

- A vital nutrient, dietary fat increases energy concentration in the diet and metabolic efficiency improves in animals, critical fatty acids, and fat-soluble vitamins.
- Elevated saturated fat diet consumption has been linked to coronary heart disease, atherosclerosis, and high cholesterol levels.
- An essential PUFA supplementation in protected form (especially linoleic acid (18:2n-6) and a-linolenic acid (ALA; 18:3n-3) in animals diets, which cannot be produced by human beings, they are necessary fatty acids that must be obtained through diet to the prevention of disease and the promotion of health is the diet's ideal fatty acid composition (Małgorzata *et al*., 2020).

In rumen, there are two important microbial fermentation processes have occurred in fat metabolism: bio-dehydrogenation and lipolysis. Free fatty acids are released from esterified plant lipids as a result of lipolysis. Biohydrogenation follows, which reduces the number of double bonds.

Rumen biohydrogenation is the process by which rumen microbes change 70 to 90% of dietary unsaturated fatty acids into saturated fatty acids. This procedure is important for minimizing the toxic effects that unsaturated fatty acids have on the development of bacteria (Jenkins, 1993). This process is done through the dietary triglyceride being hydrolyzed into a free fatty acid and a glycerol molecule as the first stage of rumen biohydrogenation, and the microbial populations that are primarily responsible of rumen biohydrogenation are ciliate protozoa, anaerobic bacteria, and anaerobic fungi (Jenkins *et al*., 2008; Gawad *et al*., 2015). Subject to the availability of COOH (carboxyl group) on FA, the isomerase enzyme that catalyzes the hydrolysis will not catalyze this process (Ramos-Morales *et al*., 2016). PUFAs like C18:2n6c and C18:3n3 are also converted into saturated end products, such as C18:0, which can then be transported to the small intestine for absorption, be deposited in animal tissues (like the mammary gland), and be later used in products (like milk fat) by rumen biohydrogenation (Małgorzata *et al*., 2020). The following diagram is shown the pathway of fat and lipid digestion in the rumen and absorption into the small intestine and deposited in animal tissues (Fig. 1).

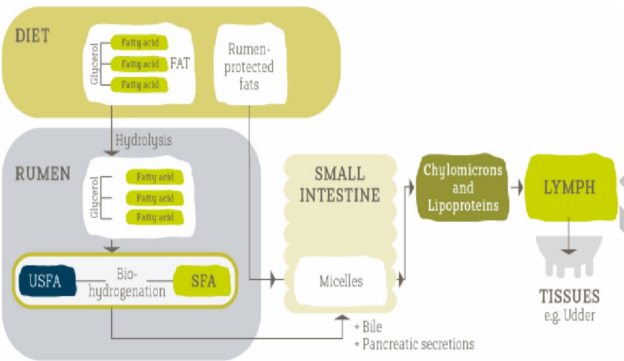

Fig. 1. Pathway of digestion and metabolism of fat in ruminants.

During late gestation (last two months) and early lactation (first 4 weeks), called the transition period, high-yielding cows and buffaloes are often in negative energy and nitrogen balance because maximal DMI does not occur until after peak milk production, and then due to their inability to obtain enough energy from their diets, they perform less productively as is shown in Figure 2. Additionally, animals were exposed to significant physiological changes and metabolic stress during the transition phase.

Moreover, feeding of free or unprotected fat above 1% level has a depressing effect on rumen cellulolytic microbial activity (may have detrimental effects on fiber digestibility) because such fats are generally negative due to physical and chemical changes in the microbial fermentation of feed. Therefore, to counter these issues, supplementation of high-quality nutrients delivered directly to the abomasum (bypass) serves the specific objective of enhancing energy or availability of protein during a time of high dietary requirement (early lactation).

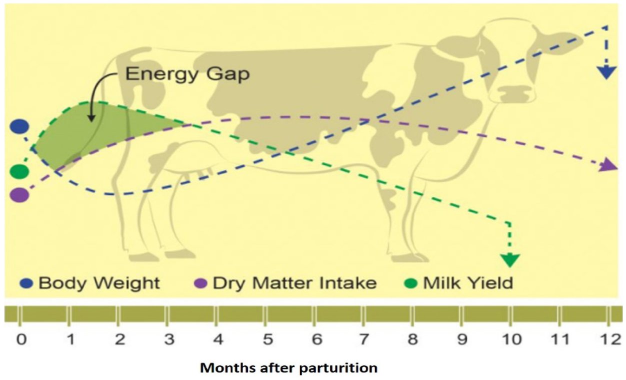

Fig. 2. Effect of energy diet on physiological changes and metabolic stress during the transition phase.

Types of protected fat in the rumen

Protected fat is available in different forms, and we summarize differences in coating protection methods of fat as follows:

1. *Whole oilseeds*: Whole oilseeds may reduce exposure to rumen bacteria involved in biohydrogenation, which may slow the rate of fatty acid release in the rumen (BH). Additionally, depending on the overall ration, processing such as roasting, rolling, crushing, or ground processing could be successfully fed to dairy animals, and increase utilization, depending on the total ration and may make handling easier (Knapp *et al*., 1991; Ward *et al*., 2002).
2. *Heat treatment*: Oilseed heat treatment may protect dietary fat from biohydrogenation by polymerizing the protein matrix encasing the fat droplets (Kennelly, 1996). This would result in higher polyunsaturated fatty acid concentrations reaching to the small intestine (Abel-Caines *et al*., 1998). The availability of soya oil in the rumen has been increased using the extrusion method. Heat-treated oilseeds, such as extruded oilseeds, may change the seed coat's ability to defend against oxidation, reduce the amount of polyunsaturated fatty acids subjected to bio-hydrogenation, or produce oxidation products. Any of these factors may have an impact on ruminal lipid digestion (Privé *et al*., 2010).
3. *Heat+chemical treatment*: To achieve the non-enzymatic browning reaction, cracked soybeans are heated with wood-processing sulfite liquor (lingo sulfonate) containing 40% xylose as a reducing sugar (Abel-Caines *et al*., 1998).
4. *Chemical treatment*: Formaldehyde-treated protein

protection of fats, despite the fact that formaldehyde-treated protein successfully protects fats from ruminal BH, this practice has developed in Australia around 1970, but has not been widely adopted. The limitations of using formaldehyde in feedstuffs, the cost of the process, and the inconsistent quality of the product are the most common criticisms (Gulati *et al.*, 2005; Carroll *et al.*, 2006).

5. *Fat supplementation in amide form*: Unsaturated fatty acids and primary amines, such as butylamine, react to form fatty acyl amides, which protect against ruminal biohydrogenation and lessen the disruption of ruminal fermentation. Fatty acid amides are only slowly converted to BH compounds because the BH process necessitates the availability of free COOH group in the substrate. This is likely due to the ruminal breakdown of the amide bond. However, the lack of commercial use for acyl amides as a protected fat supplement greatly restricts their ability to be used in dairy diets (Fotouhi and Jenkins, 1992a, b; Jenkins, 2000; Rodriguez de Fonseca *et al.*, 2001).
6. *Fat supplementation in calcium salt forms*: The "protected" calcium salt of fatty acids ruminally inert is the right term. Fatty acids that have a calcium ion attached to them as opposed to a glycerol backbone make up calcium salts of fatty acids (also known as calcium soap). They are less expensive than other options, Ca salts of fatty acids (Ca-FA), which are thought to be inactive in the rumen, provide a way to increase diets' energy density without disrupting the digestion of fiber. The pH range of the rumen is 6.2–6.8, however the calcium soaps are insoluble in the abomasum's pH range of 2-3. Fatty acid calcium salts are produced using the combination of NaOH and CaCl2 double breakdown and fusion process. Additionally, acid oil and other products such as acid oil (a byproduct of vegetable oil refining). Currently, CaSFAs are being manufactured commercially from palm fatty acids (Jenkins and Palmquist, 1982; Sukhija and Palmquist, 1990; Palmquist, 2009).
7. *Prill fat*: It is created by liquefying saturated fatty acid solution and spraying it into a chilled environment under pressure. Compared to the rumen's temperature of 38–39°C, saturated lipids like animal fats and palm oil have a higher melting point, at 50–60°C (Grummer, 1988). Because of this, PF is unaffected by ruminal temperature.
8. *A whey protein emulsion gel complex (WPEG)*: Unsaturated lipids can be safeguarded against rumen biohydrogenation by using heat-induced whey protein gelation. The process entails a series of actions, including the unfolding and restructuring of the protein molecules surface structures, the exposing of hydrophobic groups, and the activation of sulfhydryl groups. Following these occurrences, protein aggregates begin to form, which interact to create a three-dimensional protein network in the whey protein gels. Although it has been demonstrated that adding WPEG to milk can affect the FA pattern over time, the high-water content of WPEG raises concerns about how long it will last when stored, preventing WPEG from being used on a broad scale (Carroll *et al.*, 2006; Palmquist, 2009; Van Vuuren *et al.*, 2010).
9. *Microencapsulation*: It is characterized as the technology of packing liquid, solid and gaseous materials in tiny capsules that release their contents at controlled rates over extended periods of time. The animal business has employed encapsulation technology to transfer nutrients to the small intestine for absorption while protecting delicate substances (vitamins, minerals, amino acids, etc.) from destruction in the rumen or abomasum. Popular material in microencapsulation is sodium alginate and k-carrageenan as natural and safe polymers. They have been used for the fast immobilization of particulate enzymes because of their capacity to gel under relatively mild circumstances when exposed to calcium ions (Gawad *et al.*, 2015).
10. *Secondary plant metabolites*: Condensed tannins added to oils (fish-soybean oils blend) could successfully control the activity of rumen bacteria engaged in the BH process (Ramos-Morales *et al.*, 2016; Szczechowiak *et al.*, 2016).
11. *Nanoemulsified form of oil*: A nanoemulsion is a multiphase colloidal dispersion that is formed when one liquid is combined with another that is dispersed as nanoscale droplets. By including an oil in water nanoemulsion as a novel dietary component, nano-emulsified oil blends significantly reduced the rate at which polyunsaturated fatty acids are converted to saturated fatty acids in the biohydrogenation environment. When introduced directly to the rumen fermentation culture, nanoscale droplets of PUFA-rich oil blends would inhibit microbial processes including lipolysis and biohydrogenation (El-Sherbiny *et al.*, 2016; Ebeid *et al.*, 2020).

PROTECTED PROTEINS

Regarding mention above proteins are major nutritional factors most likely to limit dairy cattle ration, and unbalanced protein levels in dairy cattle diets might lead to metabolic problems. Therefore, to avoid those negative effects of protein in the rumen, the researchers suggested some methods resistant the hydrolysis the digestion in the rumen and absorption of these nutrients from the lower tract without passing through the rumen which is known as bypass or runaway proteins.

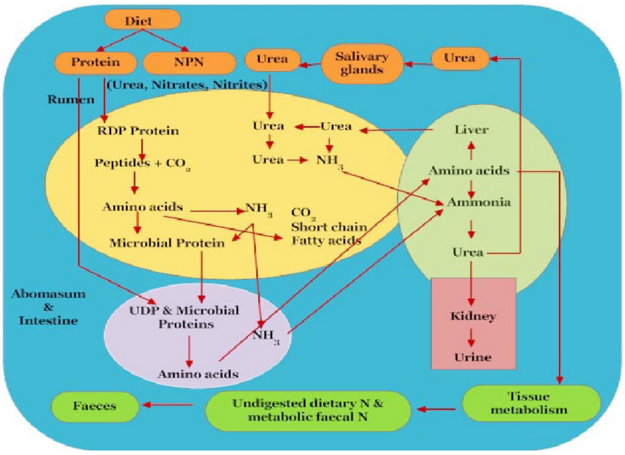

Fig. 3. Rumen digestion and absorption metabolic pathways of dietary protein.

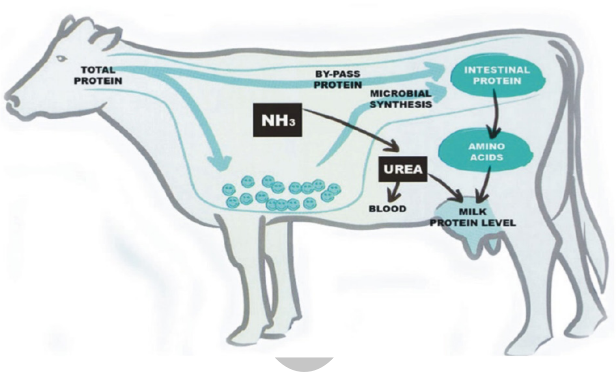

Fig. 4. Direct pathway to rumen-protected protein and absorption metabolic into ruminants.

The primary reason for making the coating process for protein, particularly amino acids, go back to highly degradable protein sources is that proteolytic enzymes produced by microbes in the rumen when ingested by ruminants, result in release of large-scale ammonia, which is wasted as urea excreted through feces and urine (Shelke *et al*., 2011, 2012). Even the animal must expend energy in the liver to convert ammonia to urea. Moreover, supplementing with crystalline AA is ineffective at delivering dietary AA to the intestinal absorptive site (Shelke *et al*., 2011). So, to improve the efficiency of protein consumption from highly degradable sources such as soybeans, cottonseeds, sunflower meals, etc., these proteins must be protected from excessive ruminal degradation and used as protected proteins (Shelke *et al*., 2012). Therefore, the highlighting the need for ruminally protected (RP) amino acids (AA) from these proteins in feed ingredients is highlighted because they are absorbed from the animal intestine for tissue protein synthesis as well as in liver through the process of gluconeogenesis (Walli, 2008; Swanepoel *et al*., 2010). Figure 3 shows the rumen and absorption metabolic pathways of dietary protein in ruminants, and Figure 4 summarizes the direct pathway to protected protein in the rumen.

Types of protected protein

Several protection methods were used for reducing the breakdown of amino acids and proteins in rumen, such as heat processing, chemical treatment, encapsulation, and the use of amino acid analogs (Walli, 2008; Negi *et al*., 1989; Ebeid *et al*., 2007; Swanepoela *et al*., 2010; Kholif and Ebeid, 2009; Shelke *et al*., 2011, 2012; Sayed *et al*., 2021); the current survey will provide a summary of protein and amino acid protection methods as follows:

1. *Naturally protected*: The most of protein meals (soybean, sunflower, linseeds) are highly degradable in the rumen. However, tree forage has 50-70% total N in protected form and insoluble nitrogen in acids detergents fiber makes up 16–53% of the total nitrogen in these forages that are linked in tannins.
2. *Heat treatment*: Drying forage improves the proteins' level of protection. Different levels of heating are applied to the meals during oil extract from seeds, which partially explains discrepancies in the level of protection. Denaturation of the protein is brought about by thorough heating of the protein supplement, and it effectively prevents microbial fermentation in the rumen. Extruding seeds under high-pressure steam have produced results that are both protected and encouraging.
3. *Esophageal groove*: With young animals, it is a typical function and advantageous for liquid proteins. Age, liquid temperature, an animal's posture while drinking, the location of the delivery into the esophagus, and the liquid's chemical makeup all have an impact on how close the groove is.
4. *Chemical treatment*: Formaldehyde treatment proved an effective and affordable approach to safeguarding the protein source from highly rumen-degradable cakes at a rate of 3–4 kg of commercial formalin (37–40% HCHO, formalin) for every 100 kg of CP.
5. *Post rumen infusion (Fistula)*: This technique, which only produces data and information about the rumen degradation pattern at the research level, involves creating a surgically implanted fistula in the lower

tract of the intestine.

6. *Encapsulation*: Is typically carried out for proteins with high biological value and for certain amino acids that have a limited impact on feed consumption and milk production, such as methionine and lysine. Thus, they may be administered in the form of a capsule containing a mixture of fats or fatty acids, occasionally with the addition of carbonate, kaolin, lecithin, glucose, etc.
7. *Amino acids analogs*: Another potential strategy for amino acid bypassing the rumen is a structural modification of amino acids to produce resistance to ruminal breakdowns, such as Methionine hydroxy, N-acetyl methionine, DL-homocysteine thiolactone-HCl, and so forth.
8. *Feed processing*: Rumen could be increased or decreased by standard manufacturing practices for some grains. Disruption of the protein matrix may lead to an increase in ruminal protein degradation, whereas heat used or produced during grain processing can reduce ruminal protein breakdown.
9. *Lowering ruminal activity*: Antibiotics can be employed to slow down the rumen's protein breakdown.
10. *Metal complex*: Metal complexes or mineral chelates assume that they must be stable in the abomasum and rumen environment and be transferred to the small intestine intact. and are much better absorbed than inorganic forms. It is widely accessible and comes in different forms, including Zn-methionine, Zn-lysine, Cu-lysine, Mn-methionine, iron methionine, etc.
11. *Plant secondary compounds*: These are primary substances from secondary metabolism that are typically not used in metabolic processes. These substances, which include lignin, tannin, terpenoids, volatile essential oils, alkaloids, and others, have the potential to be utilized as protein protectants in the rumen. Tannin has received a lot of attention. Although it is thought to be antinutritional, it can be employed at lower levels in ruminant animals because it suppresses protein synthesis or reduces digestibility; nonetheless, it is harmful to monogastric organisms.
12. *Decreasing retention time in the rumen*: The reason comes from altering the physical form of feed or proteins such as particle size and concentrate-to-roughage ratio to shorten their stay in the rumen environment. Less exposure to enzymatic action results in less degradation of the feed or protein because it passes through the rumen more quickly.
13. *Slow-release urea (SRU)*: Coating granules of urea with substrates that can preserve them from quick breakdown is one strategy to increase the N synchronization with the microbial requirement. The primary purpose of SRU is to give a consistent supply of N to support the microbial protein syntheses; the addition of SRU to ruminant diets appears to be more than just a source of N for the rumen microbe.

LEVELS OF PROTECTED FAT AND PROTEINS SUPPLEMENT

At the specific level of production, the assessment of surplus fat or fat shortage in ration should enter some parameters production details such as: Milk yield (kg/cow/day), butter fat content in milk (%), estimated weight loss (kg/cow/day), total ration intake as DM (kg/cow/day), and total ration fat (% DM). Moreover, the previous reports concluded that the average dose ranged from 0.5 to 1.0kg in early lactating cows, while 0 kg mid-lactation cows and in general ranged doses 0.25 to 0.5 kg in early and mid-lactation cows. Theoretically, when extra dietary fat contributes about 7-8% of dietary fat on the dry matter basis or dietary metabolize energy (ME) of 7-8%, the efficiency of nutrient utilization is maximum for milk production (Naik, 2013). According to NRC (2001), the total dietary fat in the ration shouldn't be more than 6-7% of the DM and is found in roughly 3% of the dairy ration (a blend of cereals and forages). Additionally, Palmquist and Jenkins (1982) concluded that there is beneficial effect on milk production subjected to the addition of fat (3-5%) of total ration DM. Correspondingly, the production decreases when fat level exceeds 6% of total ration DM.

On the other side, dietary CP% for dairy cows is 16% on average. The average milk yield is predicted to increase by 0.75 kg/d if dietary CP one percentage unit is increased from 15 to 16 percent and by 0.35 kg/d if CP one percentage unit is increased from 19 to 20 percent. Moreover, feeding diets with incredibly high CP concentrations may boost milk production, but the consequences to the economy and environment must be weighed against those of lower CP diets (NRC, 2001). According to the NRC calculation, there would be increase in milk consumption by 2.1Kg/d by raising the dietary CP from 14-18%, but the increase predicted by the equation above is 2.8 kg/d, and a CP of 22%, the highest output of milk protein was attained. Additionally, the dietary RUP (2.2-11.9%; mean 5.5%), dietary RDP (4.6-18.2; mean 10.7%) and dietary CP (8.5-29.6%; mean 16.2%) are wide-ranging from diet to diet.

There is some uncertainty regarding dairy cattle's AA needs. The factorial approach has been used to attempt to quantify the amount of AA that cattle are required to consume. His approach showed that, to attain the better content and yield of milk protein from the cows fed corn-based diets, there must be Lys and Met contribution about 7.0% and 2.5 % of total AA in duodenal digesta. Additionally, the optimal Lys and Met concentrations in MP (7.3% and 2.4%) for the combined tasks of maintaining and producing milk protein are within the range of those previously reported for milk protein (7.1 to 8.2% and 2.4 to 2.7 %, respectively). However, rumen protected amino acids (RPAA) mix was fed in order to provide 23 g/d of digestible Lys and 7 g/d of digestible met. Kholif and Ebeid (2009) added AA in buffaloes ration by 15 g protected methionine (Met) + 40g protected lysine (Lys). Therefore, to determine the quantity of RPAA feed additive be given to the basic feed, the amount of metabolizable protein that is deficient in the diet is converted into the amount of ruminally-protected methionine and/or lysine required to make up for this shortage, and their contribution to diet dry matter, including its range and average.

EFFECT OF PROTECTED FAT AND PROTEINS ON ANIMAL PERFORMANCE

Nutrient digestibility, feed intake and body condition score

On feed intake and digestibility coefficients, the impact of fat and protein supplementation in various forms, whether protected or not, was examined. In a study conducted by Swanepoela *et al.* (2010) discovered that supplementing ruminal protected lysine (RPL) did not affect early lactation cows' intakes of DM, OM, NDF, or CP. With the inclusion of RPL, there was a trend for the mid-lactation group to consume less DM. Early lactation cows receiving RPL supplementation had decreased CP digestibility, but neither group's a NDF digestibility (NDFD) was affected. On the contrary, when Tiwari *et al.* (2018) added 1.5 kg of heat- or formalin-treated soybean cake as a feed additive to nursing Jersey dairy cows' diets, the consumption of concentrate and straw increased. Additionally, previous studies found that feeding cows with a high dose of AA increased their feed intake by 3 to 4 kg per day. These trials enhanced the control ration with ruminally protected Lys and Met to deliver 27 g/d of Lys and 8 g/d of Met as readily accessible AA at the duodenum postoperatively and ruminally protected AA for 8 wk postpartum (Xu *et al*., 1998). Similarly, added ruminally protected Lys before calving increased body weight, and continued after calving with an increase of DMI (Fehlberg *et al*., 2020). Also, Elsaadawy *et al*. (2022) observed during the transition period in dairy cows, DMI was higher in cows fed a combination of rumen-protected Lys–Met than individual supplementation, and this in agreement with the reported by Tajaddini *et al*. (2021) in lactating goats' diet when fed on canola meal treated in formaldehyde.

On the other side, the supplement of protected fat rate 100g and 200g/ day in buffaloes ration did not influence feed intake (Ranjan *et al*., 2012). Moreover, other reports concluded that supplementation protected fat and proteins, either individually or their combination, did not have any effect on feed intake (Ebeid *et al*., 2007; Sharma *et al*., 2016; Katiyar *et al*. (2019), however, the body condition score (BCS) by bypass fat and proteins supplementation was increased changes, and the negative energy balance trended to reduction. Similarly, Shelke *et al*. (2012) found that feeding buffaloes a combination of protected fat and protein did not influence DMI. In the same context, adding protected fat in three different forms (prilled fat, prilled fat with lecithin and calcium soap) in male sheep diet did not affect the body weights, average daily gain, gain to feed ratio, and nutrients intakes and digestibilities (DM, OM, CP, and NDF), while ether extract and crude fiber differed with the treated treatment of rumen-protected fat (Behan *et al*., 2019).

However, others observed a reduction in DMI with fat supplementation, which may be due to buffaloes' high capacity for fiber digestibility compared to cattle, which is usually associated with a decrease in the dry matter intake as a consequence of disturbances in the cellulolytic bacterial activity that was caused a reduction in fibre digestibility (Ebeid *et al*., 2007; Sharma *et al*., 2016; Katiyar *et al*., 2019). While some reports found a reduction in DMI by supplementing dairy cows with protected fat, which may be caused by a disruption due to excessive fatty diet in cellulytic bacteria action (El-Sherbiny *et al*., 2016).

The NRC (2001) concluded that DMI is reduced by 2.5% by calcium soaps corresponding to percentage unit more the diet, Whereas, DMI was not affected by the triglycerides (TG) or hydrogenated fatty acids (hFA). This conclusion was in consist with the reported by Mane *et al*. (2017), who observed a reduction in DMI when protected fat (99%) was added to dairy Crossbred cow's ration at 10g fat/ kg milk production.

Rumen fermentation parameters

The rumen's digestion of non-lipid energy sources may be hampered by lipid supplementation in the diet of ruminants. Although inhibition of microbial protein activity and ammonia nitrogen (NH3-N) in the rumen fermentation by oil supplementation might lead to a reduction in total bacteria, protozoa, and methanogens in the rumen, increased acetate concentration without effect on total VFA concentration as reported by Ebeid *et al*. (2020). However, according to the available literature, increasing the rate of protein and carbohydrate oxidation or their degree of synchronization has a little additional benefit for microbial protein production when rumen fermentation is normal (NRC, 2001).

Moreover, in a variety of ratios, non- and protected maize oil supplementation had an impact on the *in-vitro* ruminal fatty acid profile, according to Anam et al. (2022). It is reported that the MUFA, PUFA, linolenic acid (18:3n-3) and the linoleic acid (18:2n6c) profile may be raised in rumen by combining the maize oil and skim milk powder (1:2) along the addition of formaldehyde, while the SFA profiles were decreased. On contrary, supplemented RPF (prilled fat, prilled fat with lecithin and calcium soap) in sheep diet did not affect ammonia nitrogen concentrations and total volatile fatty acids (VFA), wheras, acetate to propionate ratio, isobutyrate, VFA molar proportionate were decreased with prilled fat with lecithin supplemental although the treatments used identical amounts of propionate and butyrate (Behan *et al*., 2019). Additionally, fatty acids profile in the rumen had a positive response to supplementation of RPF in sheep diet on total SFA, USFA, total n-6, total n-3, USFA:SFA ratio and PUSFA:SFA concentrations (Behan *et al*., 2019). Moreover, adding protected fat in prilled fat form reduced total protozoa population while the population of Fibrobacter succinogenes and Ruminococcus albus significantly increased. However, calcium soap supplementation had a lower significantly in the population of Ruminococcus flavefaciens and total cellulolytic bacteria while higher total protozoa (Behan *et al*., 2019). On the other side, feeding cows on balanced rations containing bypass protein during early lactating crossbred cows reduced methane emissions by 13.5 and 17.9% (Hossain *et al*., 2017). Additionally, ruminal NH3-N was decreased by feeding formaldehyde-treated canola meal (0.8g/ 100g CP) without effect on ruminal pH in lactating goats diet, but the goats consumption of the low protein content with formaldehyde-treated (1.2g/100g CP) resulted in higher concentrations of TVFA (total ruminal VFA) and decrease acetate: propionate ratio (Tajaddini *et al*., 2021).

Milk yield and composition

Most studies reported that the use of fat and protein supplementations in dairy cattle diet has helped to provide a dietary strategy to offset the negative energy balance that occurs in the transition period. Furthermore, supplementing lactating cows diets with protected fat and protein will raise the concentration of energy and protein, improve metabolic efficiency, and prevent protein breakdown and microbial biohydrogenation in the rumen (Shelke *et al*., 2012). According to recent research, feeding buffaloes with bypass one month before the parturition at 100g/ head/day and during lactation, 20g/kg milk production is better in terms 6% FCM yield and higher milk supply (Prajapati *et al*., 2022).

However, Ranjan *et al*. (2012) found that FCM yield was higher when adding 200g per day of protected fat at the rate in buffaloes ration than control; however, milk yield and milk composition were not influenced. Many other studies have also noted a notable increase in whole milk yield in dairy cows fed bypass fat (Ramteke *et al*., 2014; Desai *et al*., 2017; Atkare *et al*., 2018). Moreover, lysine and methionine, which are typically regarded as the first limiting and second-limited AA for milk protein synthesis, have been explored as another type of ruminally protected protein in dairy cows (Xu *et al*., 1998). Supplementation of 40 g/d of Lys and 13 g/d of Met in dairy cow's feed improved significantly milk output and the percentage of milk protein and fat during the first 8 wk of lactation (Xu *et al*., 1998). Furthermore, adding ruminal-protected amino acids (lysine) (41 g of lysine/cow/d) to the TMR for high-yielding milk cows during early and mid-lactation did not affect milk, true protein or lactose yields (Swanepoela *et al*. 2010). Also, it was noticed that milk fat concentration and milk fat yield is reduced when ruminal-protected AA were fed. However, milk urea N level was raised. The blood plasma level of AA was decreased except for lysine in response to the RPL feeding suggesting that initially the lysine was limiting and later that supplementation increase the utilization of other AA and absorption increased. However, Tiwari *et al*. (2018), who fed heat treatment of soybean cake led to a 2.4 kg increase in milk output and was determined to be superior to formalin treatment. Similarly, Tajaddini *et al*. (2021) fed lactating goats canola meal treated in formaldehyde due to an increase in average daily milk, FCM, ECM yields and milk yields of milk composition.

In the line, Throat *et al*. (2016) stated that rapeseed meal (heat treated; bypass protein) supplementation at 1Kg in crossbred cow's diet led to an increase in daily milk yield of 20-21 kg milk which was in line with Aasiwal *et al*. (2015), who also experimented on lactating Jersey cows in their different parties at different stages of lactation (early, mid and late parity groups lactating cows. Similarly, cows fed protected lysine during pre and postpartum periods or just during the prepartum period produced higher quantities of milk that were energy-corrected (ECM), milk fat, milk true protein, milk casein, and milk lactose (Fehlberg *et al*., 2020). Also, in a recent study, Elsaadawy *et al*. (2022) compared rumen-protected Lys, Meth or their combination with control, and noticed that lactose; milk total protein as well as nitrogen efficiency was increased with treated groups compared to control, whereas milk urea nitrogen was lower for other treatments. However, supplemental AA in combination form increased ECM yield. Moreover, they noted that transition cows consuming AA produced more milk at its highest peak of lactation and did so more quickly.

Other researches have reported (Ebeid *et al*., 2007); Kholif and Ebeid, 2009; Waghela *et al*., 2016; Katiyar *et al*., 2019), supplementation of protected nutrients (fat or proteins) in an individual or their combination form in lactating buffaloes during the early and mid-lactation. They have similar results with adding protected nutrients either individually or their combination for FCM yield and milk protein, while actual milk yield did not change. However, milk fat content is enhanced in buffaloes (Desai *et al*., 2017), and in dairy cows with bypass fat supplementation. Higher milk fat in animals receiving protected fat supplements could be the result of more FA being available in intestine for the absorption as they are being protected from degradation in rumen. As a consequence, the FA directly incorporates them into milk fat after being absorbed from the intestine (Katiyar *et al*., 2019). However, in his review, Naik (2013) found that feeding on protected fat improved the postpartum recovery of the body weight and BCS and reproductive performance of the dairy animals, increasing milk output by 5.5-24.0%. Moreover, Mane *et al*. (2017) noted that positive response with actual daily milk, FCM yields and milk fat% when fed on protected fat and protein in individual or combination form.

The increased availability of amino acids in the context of enough energy might have generated a better balance of precursors for milk synthesis and an improved protein energy balance, resulting in an increase in milk output (Mane *et al*., 2017). Also, supplementing with protective protein and fat before delivery may lessen the unfavorable impacts of a negative energy balance, which may have improved lactation efficiency. In a previous review by Apelo *et al*. (2014), it was discussed how better prediction systems need may be created by comprehending the kinetics of EAA metabolism in mammary and splanchnic tissues. They concluded that adding individual EAA to reduced crude protein diets would boost gross nitrogen efficiency to over 30%. Moreover, numerous investigations on the effects of supplementary fat and protein in dairy animals' diets came to the conclusion that it had a variable effect (positive, negative, or did not affect) on milk production and composition. They claimed that various factors, such as the baseline diet, lactation stage, energy balance, diet's composition in fat and protein, and the amount of supplemental fat and protein, could interact with supplementing.

Milk amino acids and fatty acids profile

Dietary makeup affects milk's fatty acid and amino acid content. The effect of protected fat and/or protected AA supplementation to lactating animal's diet on FAs and AA in milk was reported by Kholif and Ebeid (2009); Mervat *et al*. (2009). They found that animals whose diets included protected fat and/or protected AA had lower percentages of milk's short- and medium-chain fatty acids. Animals fed treated meals compared to controls had the highest methionine, leucine, and lysine levels. Additionally, the AA-treated group outperformed the other groups in terms of valine, total essential AA, aspartic acid, glutamic acid, and essential/non-essential AA ratio. However, Carder and Weiss (2017) found that adding rumen-protected Lys and Meth to dairy cow diets did not alter the FA composition. However, increasing the concentration of CP from 16.1 to 18.9% did alter the milk FA composition and resulted in decrease concentration of different trans-FA including trans-10, trans-10 18:1, cis-12 18:2 as well as branched-chain FA, which the researchers hypothesized, are a reflection of microbial protein, rumen microbial population, rumen fermentation, however, postabsorptive synthesis also takes place, which may have contributed to an increase in milk fat production. On contrary, the type of protein and protection could be affected by milk fatty acids composition as reported by Tajaddini *et al*. (2021), who discovered that feeding untreated canola meal and/or canola meal treated with formaldehyde (1.2g/ 100g CP) increased C16:0 and total medium-chain FA in lactating goats fed, but canola meal treated with formaldehyde (0.8g/ 100g CP) decreased C17:0, C15:0, C12:0, C10:0, C8:0, C6:0, C4:0, and total short-chain in milk. Additionally, the report of NRC (2001) concluded that infused AA may have boosted the de novo production of C4 to C16 fatty acids, notably the C16 fatty acids, according to the analysis of milk fat for fatty acids. It is widely known that branched-chain AA and arginine are taken up by the mammary gland in amounts that are significantly greater than their direct output in milk protein. They can also be converted to non-essential AA or used as energy sources in the mammary gland.

Blood metabolites

Supplementation of CaSFAs consisting of a mixture of LCFAs and UFA based on palm oil FA distillate in milking Murrah buffalo's diets increased the serum cholesterol level more than control. However, the level of HDL (high density lipoprotein; good cholesterol) was higher ($P<0.05$) than LDL (low density lipo-protein; bad cholesterol) with increase dose of bypass fat by 200g as compared to 100g/day (Ranjan *et al*., 2012).

Swanepoela *et al*. (2010) observed that, except for lysine, blood plasma levels of the majority of AA fell when RPL was administered, indicating lysine was initially a limiting factor and that its replacement resulted in an increase in the absorption and utilization of other AA. During the first eight weeks of lactation, blood serum levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), triglycerides, and nonesterified fatty acids (NEFA) were lower when dairy cows received supplements of Met (13 g/day) and Lys (40g/day) (Xu *et al*., 1998), however, they noticed variations in treatment doses for Leu, Lys, Met, Glu, and Tyr at 8 weeks after delivery in whole blood AA concentrations. Cows fed diets supplemented with RP Lys at any dosage of supplementation had the greatest coccygeal concentrations of Lys and this is consistent with reported by Fehlberg *et al*. (2020), who discovered a rise in the plasma Lys concentration and hypothesized that prepartum supplementation of protected Lys would show that the prepartum supply of intestinally accessible Lys is important for postpartum function. Moreover, Elsaadawy *et al*. (2022) supplemented AA had no effect on serum total protein concentration, while during lactation period; variations were noticed in β-hydroxybutyrate with a reduced level of AA groups. The difference diminished as lactation progressed.

Additionally, the review study has shown that the activation of signaling pathways by EAA, energy substrates, and hormones controls local blood flow, tissue EAA extraction, and milk protein synthesis rates. These study discoveries would make it possible to modify dairy diets to increase mammary uptake of EAA and decrease splanchnic tissue degradation. Systems used to determine the nutritional needs of dairy cattle take EAA requirements as a whole into account as metabolizable protein (MP) and assume a set efficiency of MP usage for milk protein (Apelo *et al*., 2014). Furthermore, rumen-protected branch-chain amino acid (RP-BCAA) supplementation promotes the mTOR (mechanistic target of rapamycin) pathway and increases protein synthesis, which regulates glucose balance and protein metabolism (Yoon, 2016; Lynch and Adams, 2014). According to recent studies, feeding rumen-protected branched chain AA (85gm of 48% L-isoleucine, 91gm of 67% L-valine and 375gm of 37% L-leucine) along PG (propylene glycol) as energy source will lessen the risk of liver dysfunction brought on by excessive NEFA mobilization during early lactation (Yepes *et al*., 2021). In comparison to control, they discovered that cows in the RP-BCAA and RP-BCAAPG groups had reduced liver triglyceride levels and decreased aspartate aminotransferase and glutamate dehydrogenase activity throughout the first 21 days in milk (DIM). However, during the first 35 DIM, RP-BCAA and RP-BCAAPG had higher blood concentrations of insulin, methionine, and glucagon compared to control. In order to minimize hepatic lipidosis in dairy cows during early lactation, the use of RP-BCAA in combination with PG may be a viable approach (Yepes *et al*., 2021).

However, plasma Lys concentration was unaffected by MPAA. They suggested that feeding cows higher-protein diets of metabolizable protein (MPAA) enhanced plasma His and Met concentrations because the elevated plasma Met concentrations support an increased supply of that AA (Carde and Weiss, 2017). According to higher milk protein concentrations, cows given MPAA may have taken up more Lys via their mammary glands. Unknown factors may have prevented the MPAA therapy from increasing plasma Lys; however additional protected Lys and Met feeding frequently produces outcomes that are comparable (Lee *et al*., 2015). Additionally, feeding MPAA decreased the plasma isoleucine (Ile) concentration, suggesting that tissues were using Ile more efficiently and that Ile might be limited (Carde and Weiss, 2017). Furthermore, cows fed the control diet had higher plasma concentrations of 3-methylhistidine than cows fed the higher-protein diets, which could be a sign of muscle catabolism. Skeletal muscle could be helpful as protein reserve as it act as major protein depot. Due to very high requirement of protein in early lactation of dairy cows, the cows may mobilize muscle tissue to meet up the protein requirement. In early lactation, the breakdown of muscle is considered important as it function to provide AA for synthesis of milk protein and gluconeogenesis. There could be reducing muscle catabolism in early lactation due to increase dietary MP (Carde and Weiss, 2017).

FEED EFFICIENCY AND ECONOMICS

Ranjan *et al*. (2012) found that feed efficiency (milk yield/kg DMI) was higher in the group with the addition of 200g protected fat in buffaloes ration than control. This indicated to buffaloes fed with bypass fat which is 200 g/day of the diet were economically more efficient. Similar to this, Savsani *et al*. (2017) observed an increase in daily profit per buffalo due to bypass fat feeding as well as an improvement in daily return per buffalo. Additionally, better feed conversion, higher daily profit per animal when feeding buffaloes with bypass fat at a rate of 100 g/head/day one month before parturition and 20 g/kg milk production during the early lactation as reported by (Prajapati *et al*., 2022). However, according to a different study by Ebeid *et al*. (2007), providing protected lipids and/or amino acids to lactating buffaloes is not economically possible. However, doing so with highly lactating cows, whose protein and energy requirements are fairly high, maybe more profitable.

According to Tiwari *et al*. (2018) feeding dairy cow's on heat treatment of soybean cake led to an increase in net income and was determined to be superior to formalin treatment and control. On contrary, Elsaadawy *et al*.

(2022) supplemented AA (Lys and Meth) in individual or combination form and did not affect feed efficiency. However, the economics of feeding protected fat with or without protein has been reported by Mane *et al*. (2017). They found the net return over feed cost through actual daily milk and 4% FCM yields were 17.31 (fat +protein), 13.08 (protein only) and 26.38% (fat only) percent higher than control animals. Also, it was found that treating canola meal with formaldehyde as a bypass protein can be a cost-effective way to increase dietary protein and milk production in lactating goat diets (Tajaddini *et al*., 2021). Furthermore, the main challenge has also been to determine which processing methods or treatment options will increase digestible RUP or fat after rumen bypass to a degree that justifies the cost of the procedure, with the least amount of loss of amino acids or fatty acids, and with an increase in feed efficiency with the addition of protected nutrients (NRC, 2001).

CONSIDERATIONS FOR THE SELECTION OF PROTECTED FATS AND PROTEINS

Due to their unique and specialized features and possible impacts on biological processes, feeding protected proteins and fat supplements in dairy diets requires careful consideration (Gawad *et al*., 2015). Therefore, when introducing a protected fat and protein supplement into a dietary regimen, various factors need to be taken into account. These elements consist of:

1. The rumen-inert dry fat is used to avoid interference with rumen function, measured the degree of protection as a function of the ratio of free fatty acids to triacylglycerols, the rate of ruminal lipolysis, and the accumulation of trans-octadecenoic acids produced by the biohydrogenation pathway.
2. The kind of fatty acids added, particularly unsaturated fatty acids (C18), which have a greater capacity to block microbial cellulolytic activity and lead to the generation of trans isomers.
3. The rumen's level of inertness or protection; The detrimental effect on ruminal metabolism and milk production is lessened with increased protection and inertness.
4. The most important aspects of evaluating commercial inert fats are their effects on intake and their digestibility. Where should be an increase of digestibility; as the proportion of unsaturated fatty acids into intestinal digestion and absorption of long chain fatty acids.
5. Hydrogenated triglyceride utilization has decreased as a result of their poor digestion. The two most often employed kinds of inert fats are calcium soaps of palm and prilled saturated free fatty acids, both of which are very easily digested.
6. The amount, composition, and degree of ruminal digestibility and protection all affect how much of the fatty acids are transported into milk fat, which ranges from 50% to 60%. Fat supplements are administered in an effort to enhance calorie intake, which could increase the amount integrated into milk.
7. The impact of different fats on DMI should be given more weight when evaluating them. It is abundantly known from prior scientific research and practical experience that calcium soaps routinely have a deleterious impact on DMI.
8. The possible effects on the lipogenesis of the mammary glands. With higher levels of trans C18:0 fatty acids, delta-9-desaturase and lipogenesis activity may be suppressed. Supplements containing C16:0 to C18:0 fatty acids diminish the production of C6:0 to C14:0.
9. Additionally, qualities that are thought to be desirable for supplements that contain protected proteins. The National Dairy Development Board cited the following benefits of feeding bypass protein to ruminants in Singh *et al*. (2019). Protected proteins must offer a high level of crude protein.
10. The ideal amino acid ratio in diets increases their availability, particularly the first scarce amino acids lysine and methionine in dairy animals.
11. To maximize protection, roughly 70–75% of the protein should be in a form that can transmit the rumen (UDP).
12. In the small intestine, around 80% of the rumen's non-degradable protein should be digestible and relatively inexpensive source of protein could be used for dairy animals.

PRACTICAL ADVICE FOR ADDING SUPPLEMENTAL FATS

Drackley (2007) set some recommendations when using the fat in dairy animals' diets as follows:

- The maximum quantity of fat in the entire ration shouldn't be more than 7% of the DM, and 6% or less is typically sufficient.
- Under requirement conditions, 2-3% additional fat should generally be the most necessary.
- If the diet is predominantly composed of alfalfa hay or silage, the initial 1.5 to 2% can come from high-quality tallow (very saturated, low free fatty acid concentration). A commercial inert fat product should be used for any additional amounts.
- When TMR particle size is on the border or when

diets are based on forages that contain more than 2/3 (DM basis) corn silage, we would add no more than 0.225g of tallow or grease.

- The quantity of tallow that will be tolerated without significant reduction in feed intake or milk fat content is expected to be considerably less when diets also include cottonseed, soybeans, and distillers grains.
- The cows should once again be the ultimate decision-makers in this case. Decrease the amount of distillers' grains or, more likely, cottonseed or soybeans to reduce the amount of vegetable oil if the amount of milk fat falls or is low.
- Until the fat test returns to the intended target, commodity fats should be eliminated or gradually replaced with commercial inert fats.
- Even though the cost of commercial fat may be considerable, adding a high-quality commercial fat product usually has a positive economic impact due to the rise in milk fat test percentage of 2 to 3 tenths of a percentage unit.

CONCLUSION AND FUTURE PROSPECTS

The nutritional requirements of dairy animals, particularly dairy cows, play a crucial role in their milk production. Supplementing the diet with protected or bypass fat, protein, and amino acids to meet the nutritional requirements of dairy cows are the strategy to improve the productive performance of dairy animal. The integration of protective measures in delivering essential nutrients not only enhances milk yield but also contributes to the overall well-being of dairy animals. Moving forward, a holistic understanding of the intricate balance between nutritional supplementation and physiological processes will be essential for advancing the efficiency and sustainability of dairy farming practices.

Even with the current resources, there is still a wide area for improvement in the output levels of dairy cows. It demands an increase in the efficiency of nutrient availability as well as the sustainable use of feeds and fodders. Therefore, one such technique to boost the effectiveness of feeding dairy cattle has been proposed bypass feed technology. Moreover, to determine the supplementary impact of bypass fat and proteins on dairy animals fed different types of basal diets at varied productive levels and lactation stages, more study is required. Additionally, future research should focus on discovering the mode of action of protected fats and proteins and their ability to minimize nitrogen pollution and reduce methane emissions in the rumen and the environment.

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