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Role of Rumen Bypass Nutrients in Dairy Animal's Health and Productivity: A Review

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ABSTRACT

Ruminants have unique ability to convert poor quality feeds to high value animal protein. The rumen microflora degrades dietary nutrients into volatile fatty acids (VFA) and ammonia for synthesis of microbial crude protein. The microbial protein along with rumen undegradable nutrients reaches small intestine which is sufficient to meet the requirements of maintenance and low producing animals. However, in case of high yielders and physiological stress such as pregnancy, lactation, transition, *etc.* it becomes a limiting factor. Under above circumstances, rumen protected/bypass nutrients are effective in delivering nutrients directly to the intestine in utilizable and efficient form. Supplementation of rumen protected protein, fat and vitamins improves milk yield, reduces heat stress and boosts immunity. Certain rumen bypass fatty acids can also enhance quality of milk by reducing saturated fat content and also improve the reproductive performance in dairy animals. Thus supplementing nutrients in rumen protected form can result in quantity and quality production from ruminants especially under stressful conditions.

Key words: Health, Production performance, Protected nutrients, Ruminants.

Nutrient requirement of dairy animals under Indian conditions are being recently refined for precise feeding of animals to get better stimulus in production and reproduction performance in economically efficient way. Nutrient demands of high yielding dairy animals especially during early lactation, transition and heat stress conditions often exceeds to that supplied from microbial biomass synthesis and rumen fermentation. Furthermore, increased milk yield at early lactation is often associated with lower feed intake in milch animals. Peak milk yield occurs 6 to 8 weeks postpartum, while maximum feed intake lags behind peak milk yield by several weeks (Sharma *et al.,* 2021). The disparity between timings of maximum energy output as milk yield and feed intake in first 60 days of lactation renders varying levels of negative energy balance in farm animals. Under such condition, animals utilize their body reserves to support production which ultimately results in metabolic disorders (milk fever, ketosis *etc.*), weight loss and infertility (Grummer, 1991; Wankhade *et al.,* 2017). Considering above facts, transition nutrition has gained an important significance among researchers worldwide due to various metabolic disorders associated during this phase which determines the subsequent productive and economic losses (Remppis *et al.,* 2011). During transition, dairy animals require better energy supplements to minimize deleterious effects caused by reduced dietary intake and body weight losses (Katiyar *et al.,* 2019). Furthermore, extensive degradation of high quality nutrients in rumen often renders their availability to the host and leads to wastage of nutrients. Therefore, in order to provide quality and precise nutrition to dairy animals during periods of high nutrient demand, use of protected/ by pass dietary nutrients can be a promising strategy for enhancing both the quality and quantity of animal production.Various methods such as heat treatment, chemical treatment, encapsulation, selective manipulation of rumen metabolic pathways have been employed to protect or decrease degradation of nutrients in the rumen (Ganai *et al.,* 2019). These methods help the nutrients to bypass the rumen and increases the outflow of nutrients from rumen to intestine. Initially, dietary proteins were protected as these are the most expensive nutrient in ruminant's ration. However, in recent years nutrients like fat, vitamins, amino acids and probiotics (Lara *et al.,* 2006; Robinson, 2010) are also given to the animals in rumen protected form to obtain maximum productivity. The potential benefits and level of supplementation of various protected nutrients at

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different physiological conditions have been briefly reviewed in this article.

Rationale behind protection of dietary nutrients

The potential benefits of delivering nutrients directly to the intestine in a form available for direct absorption and utilization by the animals are; i) prevention of energy losses associated with degradation and fermentation of nutrients in the rumen, hence, sparing available energy for productive purposes, ii) ensuring particular nutrient will be available to the host animal in original form iii) taste masking of unpalatable substances and lastly, iv) preventing interaction among nutrients and its oxidation during storage.

Role of protected nutrients in dairy animals

Rumen protected protein and amino acids

Generally, ruminants meet their protein requirement from microbial protein synthesized in rumen and digestible rumen undegradable dietary protein (RUP). Dietary protein that is soluble in rumen (RDP) is hydrolyzed into peptides and amino acids by rumen microflora. The amino acids (AA) are further degraded to ammonia, organic acids and carbon dioxide. Ammonia produced serves as the primary nitrogenous base for microbial protein synthesis which provides nearly two-thirds to three-quarters of protein requirement in host animals (AFRC, 1992). Protein is the most expensive and one of the major limiting nutrient in the diets of dairy animals (Mahesh and Thakur, 2018), particularly during transition and summer stress due to inadequate dry matter (DM) intake. Low DM intake also decreases the availability of rumen fermentable energy to synthesize microbial protein. Under such circumstances, feeding a diet containing high rumen degradable protein is not a satisfactory strategy as >10% RDP in the diet is associated with delayed first ovulation or oestrous, decreased conception rate and increased number of days open (Tamminga, 2006). On the other hand, feeding RDP below the requirements for optimum rumen function can hamper microbial protein production, rumen degradation and nutrient availability to dairy animals (Clark *et al*., 1992). Increasing crude protein (CP) concentration in diet have also been associated with decreased conception or pregnancy rates upto 68.5% (Table 1) due to moreammonia production in the rumen resulting in increased blood urea nitrogen. Therefore, simply increasing the CP content of diet is not a suitable method to meet the protein requirement of animals. Hence, there is a need to balance protein content of ration with available dietary protein sources based on the content of rumen undegradable protein to meet the requirement of these animals. In this regards, efforts have been made to balance dietary protein requirement in ruminant's ration on the basis of either RDP and RUP or metabolizable protein (MP) conent, which can improve feed efficiency, reduce feed cost and N losses to the environment. Generally, amino acids (AA) are vital for synthesis of proteins, maintenance, growth, reproduction and production of animals. Therefore, balancing protein content of the diet on the basis of rumen-protected limiting AAs are more precise and can reduce feed cost and improve overall utilization of dietary protein (Robinson, 2010). Lysine and methionine are the most limiting essential AA for high yielding dairy animals followed by phenylalanine, isoleucine, threonine, histidine and arginine (Liu *et al.,* 2000). Concerning tropical field conditions, where animals mainly subsists on high roughage and maize based diets, methionine and lysine are considered to be the first two co-limiting amino acids (Gami *et al.,* 2017). Therefore, rations balanced for these AA, either alone or in combination; increases milk energy and yield, milk protein percentage, proportion of dietary N captured as milk N and milk/DM intake ratio.

Supplementation of rumen protected lysine (RPL) and methionine (RPM)

For optimum milk protein synthesis, NRC (2001) has recommended 7.2 and 2.4% (as%MP) of lysine and methionine respectively, in the diet of dairy cattle. Thus, for an adult cattle (400 kg body weight and 10 kg milk yield) and buffalo (600 kg body weight and 10 kg milk yield) available lysine and methionine requirements will be around 53.7 and 17.9 g/d and 70.6 and 23.5 g/d, respectively (NRC, 2001; ICAR, 2013). Amrutkar *et al.* (2015) reported that supplementation of 5 g RPM plus 20 g RPL; prepartum and 7 g RPM plus 60 g RPL; postpartum resulted in increased milk yield, fat, protein and lactose content in cross-bred dairy cows under Indian conditions. Similarly, Lara *et al.* (2006) reported that Holstein cows producing 35 kg milk per day requires 16 g ruminally protected

Table 1: Effect of rumen degradable protein on fertility in dairy cows.

Diet	CP (%)	RDP (%)	Service per conception	Pregnancy rate (%)	Author(s)
Low RDP	23.0	16.5	2.1	50.0	Chapa *et al.* (2001)
Medium RDP	23.3	18.7	1.8	68.4	
High RDP	25.8	21.2	2.3	50.0	
Low RDP	20.5	11.1	1.5	54.5	Garcia-Bojalil *et al.* (1998)
High RDP	20.7	15.7	1.2	50.0	
Low RDP	17.5	10.7	1.4	68.5	Sklan and Tinsky (1993)
High RDP	17.5	11.5	1.7	52.8	

CP- Crude protein; RDP- Rumen degradable protein.

methionine in a day. Furthermore, it was observed that decreasing CP content of ration from 18.5 to 16% had no negative impact on animal performance when supplemented with 10.2 g of RPM plus 16.0 g of RPL in diet. Therefore, supplementation of protected lysine and methionine not only improves animal performance, but also reduces the CP content of the diet which in turn reduces cost of feeding (Table 2). However, supplementation of excessive amounts of lysine or methionine can cause detrimental effects on animal performance due to improper ratio of absorbable methionine and lysine (Piepenbrink *et al*., 1996).

Rumen protected fat

By pass fat/inert fat plays a crucial role in balancing energy density in high yielding dairy animals. These are those dietary fats which resists ruminal lipolysis and bio-hydrogenation but gets assimilated in the lower gut. Whole oil seeds, when fed without processing except drying have natural rumen bypass properties due to their hard outer seed coat; however, during mastication there is physical breakdown of seed coat and results in poor rumen inertness. Various techniques have been established to obtain rumen inert fatty acids like prilled fats, calcium salt of fatty acids *etc.* Prilled fat (PF) is prepared by liquefaction and spraying solution of saturated fatty acids under pressure into a cooled atmosphere. These fats have a higher melting point *viz.* 50-60°C in contrast to rumen temperature (38-39°C), therefore, PF is not degraded in the rumen environment. Calcium salts of fatty acid are generally produced by fusion and double decomposition method. These calcium soaps are insoluble in rumen pH (6.2-6.8) but soluble in abomasum (pH 2-3). Calcium salts of fatty acids are most commonly used as they are economical.

Supplementation of bypass fat in ruminants

Dietary fat spares energy as it is more efficient than VFA or protein in terms of generating ATP. It also lowers heat increment, incorporates preformed fatty acids (FA) into milk fat and optimizes forage fiber intake as well as rumen function by substituting rapidly fermentable carbohydrate. Diet of dairy animals generally contains about 3% fat and it is recommended that total dietary fat in ration should not exceed 6-7% of the DM intake (NRC, 2001). Palmquist (1991), stated that first 3% fat of total DM intake in ruminants should be provided through various oilseeds while, that in excess of 3% should be supplied as inert fat. It is further stated that ration of high yielding dairy animals should contain 4-6% fat including fat from natural feeds, oilseed and bypass fat in equi proportions. Incorporation of prilled fat up to 9% of dietary DM had no adverse effect on nutrient utilization in buffaloes (Sharma, 2004). Supplementation of prilled fat up to 75 g from 45 days prepartum till parturition and 150 g till 70th day of lactation showed beneficial effect in transition cattle (Yadav *et al.,* 2015). Similar amount of PF feeding to Murrah buffaloes resulted in 17% increase in milk yield under field conditions (Khan, 2015). The effect of supplemental bypass fat on milk yield is also associated with breed, parity, stage of lactation, level of supplementation and nature of protected fat supplemented. Fat is the most variable component in milk when subjected to dietary changes. Inclusion of dietary bypass fat increases milk fat in lactating cows (Purushothaman *et al.,* 2008; Yadav *et al*., 2015). Tyagi *et al.* (2009) observed that supplementation of bypass fat at 2.5% of DMI increased milk production, persistency of lactation and proportion of unsaturated FA in milk fat without effecting DMI and nutrient digestibility. Pappritz *et al.* (2011) found reduction in dry matter intake by 11 and 16% in cows fed rumen protected conjugated linoleic acid (CLA) supplements CLA-1 and CLA-2 respectively, during first 7 weeks of treatment as compared to control. Likewise, the calculated energy balance for these two CLA groups was also lower than control. During later stage of lactation, in case of highest CLA supplementation there was reduction in milk fat content by 0.7%. Kliem *et al.* (2013) also found similar milk yield with linear reductions in milk fat and protein content along with dry matter intake when the content of Ca salts of mono and poly unsaturated fatty acids diets were increased in the diet. In tropical feeding scenario, about 200-300g bypass fat has been recommended in the daily diet of the lactating crossbred cows while, in transition cow (milk yield of 5000 L /lactation) and buffalo (milk yield of 3000 L/lactation) bypass fat supplementation of 300 to 500 g/day should be given 10 days prepartum to 30-50 days post-partum (Sirohi *et al.,* 2010; Garg *et al.,* 2012; Wadhwa *et al.,* 2012). Supplementation of bypass fat is a rational approach, however, supplementation should be adopted after due consideration of their cost-benefit ratio.

Protected vitamins

Vitamins are generally encapsulated for extending their shelf life, preventing interaction of vitamins in premixes and

Table 2: Effect of rumen protected methionine and lysine on production performance of dairy cows.

Animal	Supplementation		DMI (kg/d)	Milk yield (kg/d)	Milk composition		Author(s)
	Lysine (g/d)	Methionine (g/d)			Fat (%)	Protein (%)	
Karan Fries	5	20	Increased	Increased	Higher	Higher	Amrutkar *et al.* (2015)
Holstein Friesian	16	6.5	Decreased	No effect	No effect	No effect	Watanabe *et al.* (2006)
Holstein Friesian	16	10.2	No effect	Increased	Higher	Higher	Socha *et al.* (2005)
Holstein Friesian	50	15.9	Decreased	Decreased	Decreased	Decreased	Robinson *et al.* (2002)

DMI- Dry matter intake.

protecting from damages during processing. Ascorbic acid (Vitamin C), pyridoxine and folic acid are most susceptible to loss of activity during feed manufacturing (Killeit, 1994). Another major reason to encapsulate vitamins is to prevent rumen degradation; choline, niacin and folic acid and riboflavin are highly susceptible to ruminal degradation (Santschi *et al.,* 2005). Rumen microflora produces appreciable amounts of B vitamins which is sufficient to meet the maintainance requirements (NRC, 2001). However, under intensive livestock farming, it has been observed that folic acid, pantothenic acid and pyridoxine are limiting B vitamins in dairy animals. Recently various studies have reported about the beneficial effects of vitamin B supplementation, *i.e.* niacin, biotin or its complex on dairy animal's health and productivity (Li *et al.,* 2016; Kaur *et al.,* 2019) especially during transition, peak lactation or environmental stress. Kaur *et al.* (2019) reported that supplementation of protected vitamin B complex improved endometrial mRNA expression of genes involved in immune system, steroid hormone regulation and nutrient transportation in pregnant animals and thus directly affects fertility. The effect of various B vitamin supplementation on health and productivity of dairy animals are discussed below.

Supplementation of niacin in ruminants

The importance of niacin (nicotinic acid or vitamin B_3) in intermediate metabolism of nutrients and mitochondrial respiration is well established. Nicotinic acid (niacin) causes intense skin flushing which increases peripheral heat loss (Gille *et al.,* 2008). Niacin is also involved in prostaglandin D synthase activity of Langerhans cells (Maciejewski *et al.,* 2006) which increases the concentration of prostaglandin D causing increased skin vascularity and sweating rate (Zimbelman *et al.,* 2010) and thereby alleviating heat stress in dairy animals. Most studies pertaining to niacin supplementation showed that doses upto 36 g/d had little or no impact on animal's performance under heat stress conditions (Schwab *et al.,* 2005; Lohölter *et al.,* 2013). This could be due to fact that most dietary niacin is degraded in the rumen. Santschi *et al.* (2005) observed that only 3 to 10% of unprotected niacin escaped ruminal degradation and was insufficient to produce any beneficial effect. On the other hand, Zimbelman *et al.* (2013) reported that supplementation of rumen-protected niacin (RPN) at a dose of 12 g/day was sufficient to reduce heat stress and increase milk yield in lactating dairy cows. Guo *et al.* (2017) reported that supplementation of 24.8 g vitamin C, 21.1 g niacin, 137 g K_2 SO_4 and 44.8 g gamma butyric acid were helpful in reducing heat stress and improving production performance of dairy animals. Furthermore, Havlin *et al.* (2016, 2018) reported that high level of RPN could be harmful in transition animals as it blocks lipolysis to such an extent that it leads to severe negative energy balance. Effects and dose of protected niacin under various physiological conditions have been summarised in Table 3. Based on the available literature it can be concluded that high level of RPN is harmful for early lactating animals and a dose upto 3.5 g/d is optimum for this stage, while, during heat stress a dose of 12 g/d can be recommended for mid and late lactating animals.

Supplementation of biotin in dairy animals

Biotin, a water soluble B complex vitamin is present in the diet of dairy animals and also synthesized in the rumen.

Table 3: Effects of rumen protected niacin supplementation in the diet of lactating animals.

Animal	Dose	Results	Author (s)
Holstein cows (-2 days prior calving to 28 days in milk)	0, 3.5, 7 and 14 g RPN	Supplementation of 3.5 g/day RPN decreased ketosis prevalence from 36% to 20% without affecting NEFA concentration and also increaed DM intake and milk production as compared to control. However, 14 g RPN supplementation had no effect on ketosis prevalence, NEFA concentration and lowered milk production.	Havlin *et al.* (2016 and 2018)
Holstein cows over 100 days in milk (DIM)	15 g/d of RPN	Overall there was no effect of RPN on milk production but milk fat content was increased. Nevertheless, animals supplemented with RPN showed higher trends in milk yield at higher ambient temperature and THI.	Pineda *et al.* (2016)
Holstein cows mid lactating	0, 4, 8, or 12 g/d RPN	During heat stress period there was no effect of RPN on sweating rate and core body temperature, however there was increased water intake in animals supplemented with RPN.	Rungruang *et al.* (2014)
Holstein cow early lactating and mid lactating	19 g/d RPN *i.e.* about 6 g/d intestinal absorbable niacin	There was decreased respiration and pulse rate during heat stress period in animals with supplementation of RPN. No effect was observed on dry matter intake and milk production but its supplementation reduced milk fat content in early lactating cows.	Wrinkle *et al.* (2012)

NEFA- Nonesterified fatty acids, THI- Temperature humidity index, RPN- Rumen protected niacin.

However, its bioavailability depends on the type of diet offered to the animals. Diets rich in protein contain more biotin but high roughage and low concentrate diet is more favorable for ruminal synthesis of biotin (Singh *et al*., 2011). Apart from its role in synthesis of keratin and epidermal tissues of hoof-horn, biotin acts as a cofactor of different enzymes required for glucose and fatty acid synthesis and impaired activity of these enzymes leads to metabolic disorders during lactation and transition. The effect of biotin supplementation on production performance of dairy animals have been reported by various workers (Majee *et al.,* 2016; Rosendo *et al.,* 2004) and are further illustrated in Table 4. Dietary supplementation of approximately 20 mg of biotin increases milk yield with no effect on milk constituents. However, research on B vitamins supplementation is limited due to previous conception that B vitamin synthesized by rumen microflora is sufficient to meet the requirements in ruminants. Although, recent studies have suggested that lactating ruminants requires B vitamin supplementation at different physiological stages to optimize health and productivity. Therefore, future research should focus on dietary factors which helps in modifying ruminal synthesis of B vitamins, their dose and form (rumen protected or unprotected) of supplementation at different physiological conditions in dairy animals.

Supplementation of protected choline in dairy animals

Choline, also known as trimethyl ethanolamine is an essential component of mammalian diet. Major role of choline is synthesis of phosphatidylcholine and very low density lipoproteins (VLDL). Choline deficiency is associated with fatty liver due to reduced export of triglycerides from liver and VLDL. During periparturient period high amount of energy is required for maintenance of body tissues and milk production, thereby forcing mobilization of body fat reserves to satisfy energy needs. As a consequence, the level of non-esterified fatty acids (NEFA) is increased in plasma which leads to hepatic lipidosis. A schematic representation showing mechanism of action (MOA) of choline in lactating dairy animals is presented in Fig 1. Furthermore, choline and methionine metabolism are closely associated (Fig 2) and around 28% of absorbed methionine is diverted towards

Table 4: Effect of biotin supplementation in the diet of lactating animals.

Author(s)	DMI(kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)
Rosendo *et al.* (2004)	No effect	No effect	No effect	No effect
Bergsten *et al.* (2003)	-	Increased	-	-
Majee *et al.* (2003)	Increased	Increased	No effect	No effect
Zimmerly and Weiss (2001)	No effect	Increased	No effect	No effect
Fitzgerald *et al.* (2000)	-	No effect	decreased	No effect

DMI- Dry matter intake.

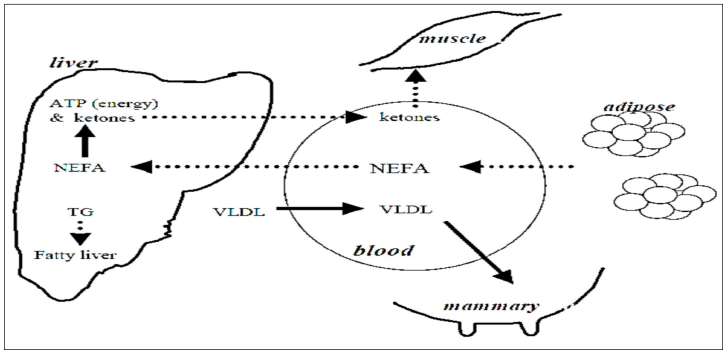


Fig 1: Mechanism of choline action in transitional dairy cow; Adapted from Donkin (2002).

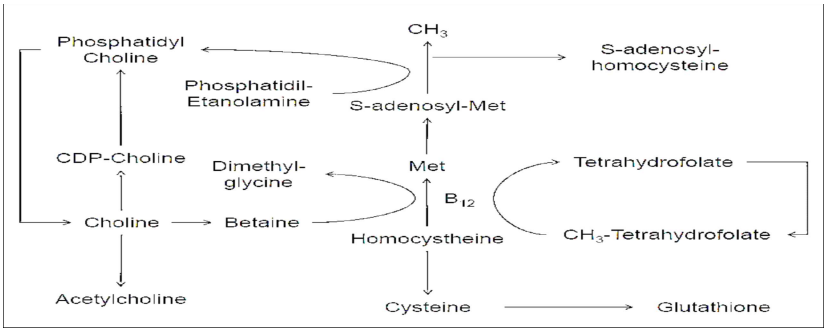


Fig 2: Relationship between choline and methionine; Adapted from Donkin (2002).

Table 5: Effect of supplementing rumen protected choline on animal performance.

Animal	Dose	Results	Author(s)
Karan-Fries multiparous	54 g/day RP-choline 40 days before and 120 days after calving	Concentration of TG and VLDL was lower, while phosphatidylcholine and vitamin E were higher in RP-choline supplemented group than control.	Amrutkar *et al.* (2015)
Jaffarabadi buffaloes	15 g/day RP-choline chloride and 150 g by-pass fat	Buffaloes supplemented with 15 g/d RP-choline chloride along with 150 g by pass fat group had 18% higher milk yield.	Garg *et al.* (2012)
Holstein Friesan	60 g/day RP-choline 3 wks pre calving to 6 wks post calving	Choline supplementation decreased the concentration of liver triacylglycerol during the first 4 wks after parturition.	Zom *et al.* (2011)
Holstein Friesan primi and multiparous (21-91 days in milk)	40 g/day of RP-choline	Multiparous cows supplemented with protected choline had 14.3% higher milk yield than control; while in primiparous cows no significant effect was observed.	Davidson *et al.* (2008)
Holstein Friesan	0, 30, 60 or 90 g/d RP-choline during transition period	Plasma concentrations of nonesterified fatty acids (NEFA), triglycerides and cholesterol were reduced in cows supplemented 30 g/day of RP-choline.	Xu *et al.* (2006)

NEFA- Nonesterified fatty acids; RP-choline- Rumen protected choline.

synthesis of choline. Supplying choline directly to dairy animals may enhance synthesis of phosphatidylcholine and increase VLDL synthesis along with methionine sparing effect (Santos and Lima, 2007). In ruminants, dietary choline as choline stearate and choline chloride is degraded upto 98.0 and 98.6%, respectively in rumen thereby necessitating the use of rumen protected choline (RP-choline). Various studies have been reported that increasing the intestinal supply of choline in rumen protected form (Table 5) have improved the hepatic fat export during transition period, reduced the risk of metabolic disorders in multiparous periparturient dairy cows (Zom *et al.,* 2011) and increased milk production (Amrutkar *et al.,* 2015; Baldi and Pinotti, 2006). On the basis of previous studies, a dose of 15 to 60 g/day of rumen-protected choline is optimum for enhancing productivity and reducing incidences of metabolic disorders in dairy animals.

Protected probiotics

Probiotics are defined as "live microbial feed supplements that beneficially affects the host by improving its intestinal microbial balance" (Fuller, 1992). They mainly belong to the genera *Lactobacillus* and *Bifidobacterium* (Ojha *et al.,* 2018, 2020, 2022). However, strains of *Bacillus, Pediococcus* and some live yeasts have also shown probiotic attributes. In ruminants, most widely used probiotics are yeast and *Aspergillus oryzae* which directly influences rumen fermentation. However, scare information is available regarding the viability and postruminal effect of live yeast. The biggest challenge of probiotic feeding in adult ruminants is to protect them from extensive rumen degradation and its postruminal delivery with original potency. To overcome this constraint, probiotics are encapsulated to prevent dissolution in rumen pH and permit slower release in lower gastrointestinal tract. Microencapsulation is the usual method for protecting sensitive probiotics from oxygen and gastrointestinal transit (Corbo *et al.,* 2011). Furthermore, these potential probiotics can also beneficially alter the intestinal microflora of ruminants and augment their productivity. Seyama *et al.* (2016) reported that in lactating Holstein cows, oral administration of encapsulated *Lactobacillus coryniformis* subsp. *torquens* (JCM1099) (3×10^{11} colony forming unit) survived in rumen and were slowly released in lower gastrointestinal tract. Likewise, Jiao *et al.* (2017) also observed that supplementing rumen encapsulated active dried yeast (EDY; 3.5 g/d) exhibited postruminal activity and improved whole tract organic matter digestibility. Till date, literature regarding the effect of protected probiotics in adult ruminants is scarce and further experiments are required to validate its role.

CONCLUSION

Protection of nutrients makes it possible to deliver nutrients at specific sites in gastrointestinal tract for better utilization and prevents interaction of nutrients in manufactured feed or rumen. Feeding of encapsulated/by pass nutrients is beneficial for dairy animals during transition and peak lactation because of greater nutrient demands. Furthermore, use of encapsulated fatty acids makes it possible to produce milk with lower amounts of saturated fats. Supplementation of protected vitamins like niacin and choline have improved milk yield, liver function, reduced heat stress, enhanced immunity and health performance in animals. Thus, it can

be concluded that supplementing nutrients in the protected form can be a strategy for quality dairy production especially under stressful conditions like transition, peak lactation and heat stress. Future researches should mainly focus on supplementation (doses and deliverable forms) of protected B vitamin complexes and probiotics in dairy animals at different physiological conditions along with dietary factors which can modify or enhance the efficacy of these nutrients.

Conflict of interest

The authors declare no conflict of interest.

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