

ROLE OF PROTECTED AMINO ACIDS IN RUMINANT'S PHYSIOLOGY: A REVIEW

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ABSTRACT

This review was designed to shed light on the applications of rumen-protected amino acids (AA) and choline, undertaken by supplying ruminant's diet with rumen protected bypass products, and their effects on physiological aspects. AA and choline in a hydrogenated palmitic acid matrix, which provides optimum protection, undergo bypass to the rumen compartments, thereby avoiding the physical degradation in this part. Thus, AA and choline involves maximum release, high bioavailability and better absorption rate within alimentary tract. The balancing of ration formula with methionine, lysine, histidine, tryptophan and choline using a rumen protected form, may help to enhance production and permit for better flexibility during diet formulation. Rumen-protected AA and choline result in the most cost-effective solution when formulating dairy diets based balance. Supplementation of rumen protected essential AA and choline is performed to maintain a healthy status, high bioavailable source of these substances, and improve the efficiency of protein metabolism. To sum up, dietary supplementation of rumen protected AA and choline enhances their availability in the gastrointestinal tract and production, with a significant improvement of body conditions, compared to unprotected form. Therefore we recommend using this protected AA and choline continuously in diet formula of ruminants.

Key words: Rumen protected methionine, rumen protected lysine, rumen protected tryptophan, rumen protected choline, ruminants

INTRODUCTION

Amino acids (AA) are the building units of proteins, which represent main constituents of animal tissues. AA plays a major role in various important metabolic and biochemical activities in the living cells, in addition to their role in growth and reproduction (Wu, 2014). The availability of

AA and choline in food is crucial. It is important that ruminants consume sufficient amounts of AA to maintain their productivity and healthy status, thus the animals require a source of AA in the feed ingredients (Kung and Rode, 1996). Animals need both essential and non-essential AA with appropriate amounts for metabolic processes, whether to produce milk, build muscles or reproduction. The requirement of animals to specific AA and choline differs depending on the species, gender, diet and age, for instance, lysine (Lys) and methionine (Met) are standard first-limiting AA for dairy cows (Tedeschi *et al.*, 2015).

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Many problems related to deficient AA and choline in the diet of farm animals are expected when these substances are not provided in sufficient amounts, thus the animals body cannot supply adequate proteins to maintain numerous metabolic functions and growth (figure 1) (Wu, 2014). As a result of destruction of available uncoated AA and choline in diet by rumen microflora, the dietary supplementation of

rumen protected AA and choline is suggested to improve physiological functions, production and reproduction of animals (Ratika *et al.*, 2018). Therefore, this study aimed to explain the Rumen-protected technology application on ruminants physiology and effects of supplementation of protected AA and choline when they bypass small intestine without degradation in rumen.

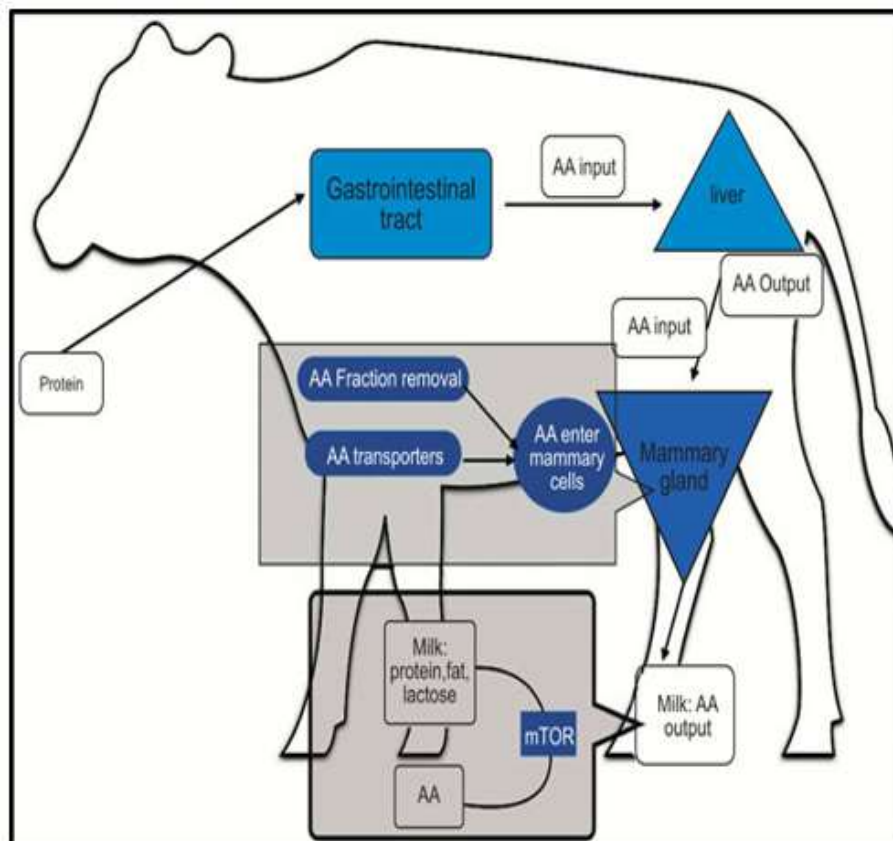


Figure 1: fate of dietary Crude Protein in ruminants (Wang *et al.*, 2019)

Rumen-protected AA technology (microencapsulation technology)

In order to prevent a deficiency of AA and choline in ruminant diets they can obtain it from dietary manipulations, and adaptation the coating forms in adequate quantities of the various balanced diets (Ben Salem, 2010). The major challenge of AA and choline supplementation to ruminants as a complementary feed is the rumen: by the presence of normal flora that ferment any unprotected feed, these substances will be degraded by the enzymes produce by rumen microflora (Matthews *et al.*, 2019). The

microencapsulation technique includes using the ingredients (AA) or choline with spray freeze coat of a specific layer of hydrogenated palmitic fatty acid matrix to make it able to bypass the rumen and resists to physical damage that occurs in the rumen. Thus, the hydrophobic layer of this fatty acid prevents the protected AA and choline form microbial damaging in the rumen, giving the maximum digestibility and efficient absorption within the intestine, after they release in the lumen of gastrointestinal tract (A.O.A.C., 2005) (figure 2).

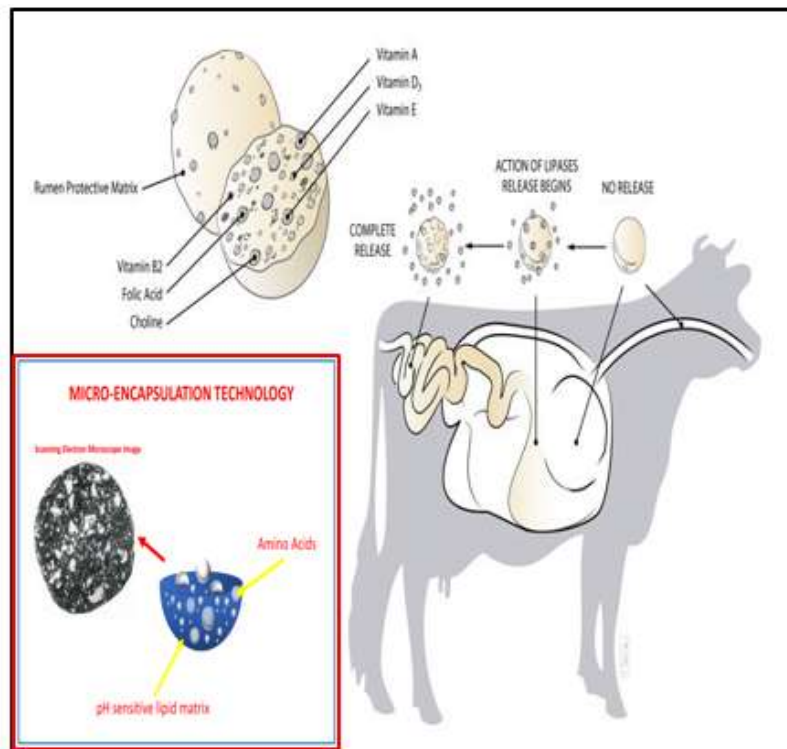


Figure 2: Microencapsulation technology of protected AA, choline and vitamins with a lipid matrix

Methionine (Met)

Met is a sulphur-containing AA which is a constituent of most proteins. It is one of the essential AA in the diet of vertebrates, and considered as the substrate for cysteine and taurine. The sulfur group is a distinctive characteristic of this AA, and because it contains sulfur, it acts as a source of all AAs specially those containing sulfur. Met plays a crucial function in the building of proteins to the body. The sulfur atoms contribute to formation of cysteine and the biosynthesis of glutathione, thus Met acts as an antioxidant. In the liver, Met is adenylated and then converted into S-adenosyl Met (SAM). SAM is then converted to s-adenosyl homocysteine (SAH) and with the presence of B6, the SAH is converted to cystathionine and then cysteine (Yin *et al.*, 2019). Indeed, Met is also one of the limiting AA that is required for dairy ruminants to produce milk due to it involves in transmethylation reactions and synthesis of milk protein (Girard *et al.*, 2010).

Protected methionine

Extensive investigations during last few years showed that diet supplements with encapsulated form of Met lead to improve milk content by increasing milk protein and fat. Exactly calculating AAs content in feed permits nutritionists to optimize the entire protein composition in diet. Tetsuo *et al.* (2019) proved that feeding dairy cattle with a diet rich with crude protein did not influence milk production compared to a diet rich with rumen-protected methionine (RPMet). The reason of improving milk production more than crude protein is that RPMet acts to reduce nitrogen excretion and improves nitrogen utilization (Panel *et al.*, 2011). In addition, increase in milk production when supplement provided with RPMet through increasing dry matter intake (DMI) that enhances liver function (Batistel *et al.*, 2017). It was noted that there is a close hormonal relationship between MET and prolactin hormone PRL, that is associated with milk production, it was found that milk production is induced by the increasing the prolactin receptors expression within the substantia nigra and hypothalamus of the elderly animals after 30

days of treating with S-adenosyl-L - Met. Furthermore, the addition of the Met showed an increase in the number of receptors in the hypothalamus membranes (Muccioli and Di Carlo, 1989). Also, Supplementation with rumen protected Met plus lys will improve the daily gain in calves (Sai *et al.*, 2014).

Met is an important AA that affects animal fertility, and it is known that the lack of Met in nutritional formula leads to a lack of fertility and even may affect fetal development during pregnancy or loss of pregnancy (abortion) because many of the AA are condensed in the ovary, uterus and in the fluid that surrounds the fetus (Lucy *et al.*, 2014). Peñagaricano *et al.* (2013) proved in recent study dramatic changes in gene expression of ruminant embryos when they fed on RPM during lactation. Supplementation of RPM would improve embryonic growth by measuring Pregnancy-specific protein B (PSPB) level and is expanding the size of embryonic and amniotic vesicles, thus elevate the pregnancy

rate with declination of abortion rate (Toledo *et al.*, 2017).

Met and choline each have been the focus of a number of transition cow studies over the past 10-15 years. Interestingly, these two AA are metabolically related due to the fact that both compounds can serve as methyl donors, that is important in many biological activities in cells is that dependent on the methyl groups. This relationship between Met and choline is well studied in non-ruminants however, in ruminants, especially dairy cows fed more on these two components as well as there is a insufficient evidence that Met can significantly alternative for choline (Pinotti *et al.*, 2005); quite, each of these AA has distinct effects on the transition cows (figure 3). In the pre-ruminant lambs are getting abundance of choline from the milk, but in grown ruminants only minor amounts of methyl group nutrients are existing from the diet and Met synthase responsible for production of Met methyl groups from one-carbon pieces provided by tetrahydrofolate (choline) (Girard *et al.*, 2010).

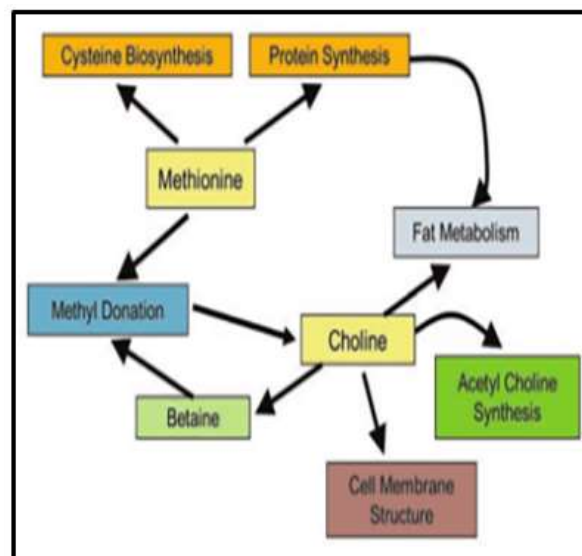


Figure 3: Correlation and metabolic functions of choline and Met

Interestingly, there is an improvement of immune function of the cows that fed on rumen-protected Met because, as they showed greater phagocytic function of the neutrophils harvested at 21 days postpartum (Osorio *et al.*, 2013). In addition, Met acts to

support animal performance by methyl donation for choline production, and choline supplementation promotes to extra betaine metabolite for the creation of sulfur-containing AA (figure 4) (Ardalan *et al.*, 2011).

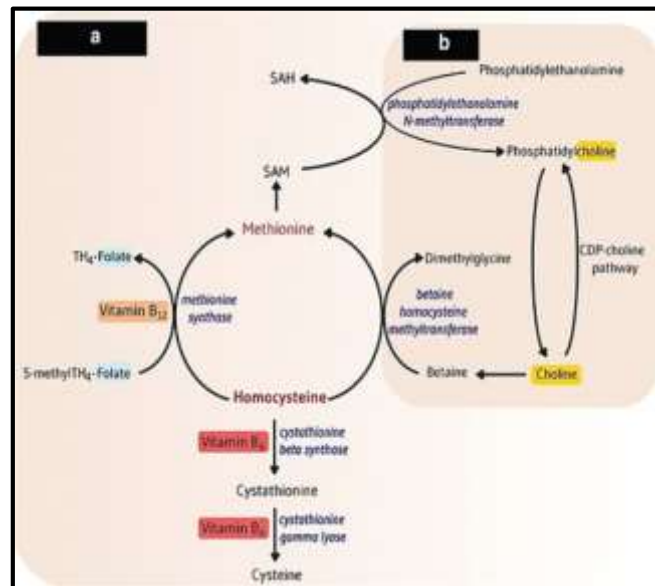


Figure 4: Path of homocysteine and formation essential AA (Abbasi *et al.*, 2017)

In another study and During the transition phase (negative energy balance in dairy cattle), fed animals on RPC and RPMet showed an elevation of vitamin E level in plasma associated with increasing of total antioxidant capacity while malondialdehyde values may decrease (Sun *et al.*, 2016).

Lysine (Lys)

Lys is an essential AA that must be existing in the feed. Lys is crucial for the creation of entire proteins, and is involved for the conservation of the proteins structure such as collagen and elastin, which construct all connective tissues such as skin, tendons, and bones. Lys is also a precursor to carnitine that required for lipids metabolism and energy. Furthermore lys may improve the immune system and perform cardiovascular system. Lys is one of those AA that the body is unable to synthesize it therefore; it must be obtained from dietary sources. Supplementation of lys acts to improves physiological functions such as protective effects from hypertension by preventing precipitation of lipoprotein-(a) to the arterial walls (Vuvor *et al.*, 2017). Lys promotes the protein synthesis and increases milk production by increasing the viability of the epithelium tissue in the udder, whereas protein degradation was inhibit with in these cells, for this reason, lys is crucial in cattle diet for milk production (Lin *et al.*, 2018).

Lys was first isolated from casein protein in milk; because β -Casein is one of the important milk proteins with high nutritional value, lys additive with other AA such as Met to the diet of early lactating cows will significantly increase β -casein expression in the bovine mammary epithelial cells via stimulation of the rapamycin complex 1 signaling pathway (Gao *et al.*, 2015).

L-Lys is an essential AA that is available as a supplement for animal feed. Therefore, L-Lys is synthesized to increase growth of *Corynebacterium glutamicum*, the highly important character for its ability to produce L-glutamate and L-lys, which are required in animal diet, also it build up for the creation of almost entirely the L-amino acids with commercial form such as L-isoleucine, L-valine, L-threonine and L-serine (Junior *et al.*, 2016).

Protected lysine

To improve the dairy cow production, rumen-protected lysine (RPL) is supplemented in a protected form due to its sensitivity to the acidic environment of the first part of the digestive tract; hence, coated lys will be effectively absorbed in the small intestine, yield positive effect on milk production (Lee *et al.*, 2015).

Lys and Met can synthesize L-carnitine in the mitochondria, cytosol, and lysosome by several-steps; firstly lys is methylated to form ϵ -N-trimethyl lys by methyltransferase, derived from Met, secondly, it released carnitine. L-carnitine is synthesized in the liver and transported by the bloodstream to cardiac and skeletal muscles, which play a role in fatty acid oxidation. The rate of L-carnitine biosynthesis depends on lys in the supplemented diet, which may increase endogenous L-carnitine synthesis (Rebouche, 2014). Since carnitine is essential for mitochondrial fatty acids oxidation, this allows adequate energy supplies from fat by fat break down in the cell and observe energy from fat reserves, however the disorders of carnitine biosynthesis occur when inadequate lys in the diet or inability to bypass rumen. This may lead to insufficient energy supply and raises the possibility of brain and liver dysfunction (Longo *et al.*, 2016). In addition, the added lys may have a significant immunological role by increasing serum zinc level, CD4 receptors, thus both zinc and lys act to activate thymulin to improve the process of differentiation and maturation of T- lymphocytes (Sugeng *et al.*, 2015). Researchers improved Infusion mixture of the complete AA resulted in an increase in the plasma concentrations of several essential AA, glucagon and decreased growth hormone and depressed dry matter intakes (Weekes *et al.*, 2006). In addition, the supplementation of both rumen-protected Met and rumen-protected lys enhance weight gain of growing calves and improve the efficiency of feed utilization, with an increase in the digestibility of nutrients, which leads to decrease costs (Gavade *et al.*, 2019). Also, diet containing protected lys causes elevation of milk production in lactating cows (Liu *et al.*, 2016).

Histidine (His)

Structurally, containing dipeptides (carnosine, anserine and balenine) has numerous therapeutic properties,

physicochemical. Histidine acts as buffering system inside cells (homeostasis of acid–base), and regulates calcium. Physiologically, histidine is strongly dependent on non-oxidative forms of energy production fuelled to their skeletal muscles, and it is considered as a precursor of histamine in addition to the vital role in inflammatory agents and immune response (Dolan *et al.*, 2019; Andersen *et al.*, 2015).

Protected histidine

The researchers reported that deficiency in digested His in the feed causes decrease in the concentration of hemoglobin in cows. Also, an additional confirmation showed that low dietary his impair milk production and milk protein in dairy cows, but feeding on diets rich in rumen-protected histidine (RPHis) has the potential to reverse these effects. (Giallongo *et al.*, 2016; Giallongo *et al.*, 2017). Therefore, supplementation of diet with RP-His increases linearly with milk yield and milk protein synthesis. This may suggest that plasma his has exceeded the requirement with extra nitrogen converted to urea in the liver. Also, the dipeptides (carnosine, and anserine) of RP-His caused increased concentrations of AA in the muscle (Zang *et al.*, 2019). Animals treated with rumen-protected His will affect positively on quality products of beef cattle (Sandberg *et al.*, 2017).

Tryptophan (Trp)

There are two types of this AA: L and D-tryptophan and the difference between them are the direction of the molecule. Trp is an essential AA, which is used as building units for many proteins. It is considered as a precursor to the serotonin (acts as a neurotransmitter, melatonin and Niacin (vitamin B3). Also, trp plays vital roles in cytoskeleton in addition to the action by anchoring membrane proteins within the cell membrane (Slominski *et al.*, 2002). The physiological effect of trp is same to the therapeutic effects of the melatonin, because it stimulates the secretion of melatonin from the pineal gland, and because the melatonin

has indirect effects of gonadal function by its influence on gonadotropin-releasing hormone and then for the reproduction of the animals (Sánchez *et al.*, 2008).

Protected tryptophan

It is reported that L-trp considered as an important AA in lambs and cattle. Thus, supplementation of Trp in a rumen protected form has beneficial influences on growth performance and health of beef cattle (Lee *et al.*, 2019). Researchers mentioned that intravenous administration of L-trp leads to enhance in the secretion of gastrointestinal hormones and results in good meat quality in ruminants, but supplementation of encapsulated L-trp to safely passing from the rumen to intestine gives more desirable effects on the secretion of gastrointestinal hormones to give high absorption rate inside the intestinal cells (Lee *et al.*, 2019). The gastrointestinal hormones that increased after oral administration of RPL-trp are cholecystokinin and pancreatic α -amylase, which leads to an increase in the activity of the hormones in the duodenal fluids, as well as increasing the digestion rate of starch in the small intestine, Which is useful for increasing muscle mass and, hence body weight (Lee *et al.*, 2020). The influence of dietary supplementation of RPL-trp was also observed on livestock during exposure to cold weather as a stress factor, when RPTrp enhances growth performance keeping the homeostasis and immune responses in beef steers in cool circumstances by elevating glucose level and reducing adipogenic gene expression (Lee *et al.*, 2019).

Choline

Choline was firstly discovered at 1849 by German chemist named Adolph Strecker. After that in 1867 Bayer described the chemical construction of choline (McDowell, 1989). It can be found freely in the biological tissues. Choline is a quaternary ammonium base (trimethylamine 2-hydroxyethyl) $[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{OH}]$, also called as trimethyl ethanolamine and it is considered as a nutrient with an amino

acid-like metabolism. It is a vitamin-like substance (In the past, choline was classified as a vitamin B; however, because it is not directly involved in enzyme systems, it has since been described as a quasi-vitamin. Choline acts as an essential nutrient for mammals when an insufficient supplementation of Met and folate in the diet. Vitamin B12 is also involved in this process. The dynamic interactions among Met, vitamin B12, and folic acid introduced the conception of choline as a vitamin-like structure. Choline has two functional properties; the first, as a methyl donor, when it is considered as a source of biological synthesis of methyl and its compounds. Secondly, it involves lipids metabolism, such as the transport of fats, as a fat-enhancing agent. Based on these functions, choline is a major source for energy and protein metabolism in mammals (Sathiyabarathi *et al.*, 2016).

Protected choline

Although choline is not AA but extensively provide in protected from like fed supplement due to their crucial role in many metabolic and physiological functions. In the rumen choline is breakdown by a large number of microbes, Therefore, the availability of choline is decreased and then not enough to absorb. To bypass this step the choline is available in protected from by coating it with a layer of fatty acids to avoid destruction by the enzymes of rumen microbes (Ardalan *et al.*, 2010; Pawar *et al.*, 2015). After choline reaches the small intestine and because it has a positively charged molecule thereby not freely cross the plasma membrane of enterocytes, so will need carrier; and then for absorption, choline must be hydrolyzed by phospholipases in the intestine, after the fatty acids are broken down by the digestive enzymes, thereby making the choline free and easily absorbable in beneficial from (Blanco and Blanco, 2017).

Choline is necessary for synthesis of the neurotransmitter, acetylcholine, in addition,

it acts as a source for acetylcholine after being transported in the neural cells, by carrier proteins, where it will eventually combine with acetyl CoA in the form the neurotransmitter acetylcholine (Wessler and Kirkpatrick, 2008).

The acetylated volatile fatty acids (VFA) increase when the protected choline is given and with it, the ability to digest increases with increasing pH in rumen. In addition, organic materials, raw protein, raw fibers, ether extract and nitrogen-free extract increased significantly in rumen (Mohsen *et al.*, 2011). The Rumen protected choline RPC supplementation also reduces the exposure to hepatic fatty infiltration, thereby preventing fat accumulation in the liver and avoiding fatty liver syndrome, that usually occurs in animals after birth as a result of an increase in the requirement for energy due to an elevation of fatty acids transportation to mammary gland (Zom *et al.*, 2011). Furthermore the RPC works to reduce the incidence of clinical ketosis (Ensink and Kampf, 2013).

In lactating animals, RPC supplementation increases milk production, firstly by increasing the digestibility and total VFA concentration, and decreasing ammonium. Secondly, by increasing the intestinal supply of choline, which improves the milk production by approximately 7% compared to controls (Leiva *et al.*, 2015). There is evidence that casein increases in milk after giving RPC, and in this way choline works to increase the milk protein, fats and lactose. Thus, protected choline positively affects milk components (Grummer, 2011). Hence, the protected choline has positive effects on lactation particularly once feeding is began previous to calving and continues at the beginning of lactation (Grummer, 2011). When the body is exposed to negative energy balance, supplementation of protected choline to diet is essential to provide energy through export the triglycerides (TG) from the liver. Therefore RPC reduces TAG in the liver of dairy cows,

thus increases milk yield and enriches it with fats (Arash *et al.*, 2016). Choline acts as a major lipotropic factor in ruminants during lactation because it diminishes the extreme fat deposit in liver cells, with detectable buildup in milk production and its constituents (fat, lactose, total solids and protein) (Leiva *et al.*, 2015).

CONCLUSIONS

Dietary supplementation of rumen protected AA can promote production and reproduction performance in cattle and sheep. However, many researchers proposed that increasing total AA availability in the small intestine leads to a desirable effect via enhancing a variety body functions.

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دور الاحماض الامينية المحمية في فلسجة المجترات: مراجعة

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تم تصميم هذه المقالة لتسليط الضوء حول دور تقنية الاحماض الامينية المحمية والكولين المحمي من الكرش وتطبيقاتها من خلال توفير المكملات الغذائية من هذه الاحماض الامينية المحمية والكولين المحمي والتي تتحمل ظروف الكرش في الحيوانات المجتررة وتأثيرها على المعايير الفسلجية لهذه الحيوانات. تخضع الاحماض الامينية والكولين للرش بطبقة مصفوفة من الحامض الدهني بالماتيك المهدرج بوجود التبريد وذلك لتوفير الحماية المثلى وقدرة تجاوز حجات الكرش وذلك لتجنب تحطم هذه الاحماض الامينية والكولين فيها، وبالتالي هذه الطريقة توفر امكانية تحرر الحد الأقصى منها داخل الامعاء والتوافر البيولوجي العالي لها ومعدل امتصاص أفضل داخل القناة الهضمية. يساعد توازن الاحماض الامينية المحمية مثل الميثيونين اللايسين ، الهستدين والتربتوفان والكولين المحمي من خلال استخدامها بالشكل المحمي على تحسين الإنتاج والسماح بمرونة أكبر عند تصميم علائق الحيوانات. إضافة الاحماض الامينية والكولين المحمي من بيئة الكرش لها فعالية ومرونة من حيث التكلفة عند تصميم علائق الحيوانات المنتجة للحليب معتمدة على اساس توازن الأحماض الأمينية والكولين فيها. تساهم المكملات الغذائية من هذه الاحماض الامينية والكولين بشكها المحمي في الحفاظ على الحالة الصحية للحيوان كما ان هذه المواد لها معدل اتاحة عالي كونها مصدر بيولوجي، فضلا عن تحسين كفاءة البروتين القابل للاستقلاب. نستدل من هذه الدراسة إلى أن المكملات الغذائية من الاحماض الامينية والكولين المحمي من بيئة الكرش تؤدي إلى تعزيز توافر هذه المواد ودورها في أداء الجهاز الهضمي وإنتاجيته مع تأثير تحسين قيم حالة الجسم مقارنة بشكلها غير المحمي لذلك نوصي باستخدام الاحماض الامينية والكولين المحمي بشكل مستمر في تحضير علائق المجترات.

الكلمات المفتاحية: الميثيونين المحمي ، اللايسين المحمي ، التربتوفان المحمي ، الكولين المحمي، المجترات