The Pharma Innovation Journal 2023; 12(2): 387-391

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 387-391

www.thepharmajournal.com
Received: 07-11-2022
Accepted: 16-01-2023

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Role of Betaine in ruminants

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DOI: https://doi.org/10.22271/tpi.2023.v12.i2e.18456

Abstract
Betaine is a ubiquitous natural compound found in plants and animals. The beneficial functions of Betaine are ascribed to its chemical structure. Betaine, as such, acts as an osmolyte, thus preventing cellular dehydration and denaturation of proteins and enzymes. This osmoprotectant property makes Betaine a useful feed additive under various stressful conditions. In addition, it functions as a methyl group donor in transmethylation reactions and for the synthesis of various compounds such as creatine and Carnitine. Further, Betaine, a trimethyl derivative of glycine, exhibits the characteristics of the amino acid glycine. Besides, it is widely known as a "carcass modifier" due to its lipotropic and growth-promoting effects. Due to its unique properties, Betaine plays an important role in amino acid and lipid metabolism. Thus, Betaine serves as an excellent natural feed additive in animal nutrition.

Keywords: Betaine, ruminants, osmoprotectant amino acid metabolism, lipid metabolism

Introduction
Betaine, the trimethyl derivative of the amino acid glycine, is a multifunctional compound found naturally in plants and animals in small quantities. It was first discovered in the juice of sugar beets (*Beta vulgaris*) in the 19th century. Animals obtain Betaine from either a dietary source or as a product of choline oxidation in the mitochondria. Chemically, Betaine, being a quaternary ammonium compound, is stable and nontoxic. The chemical structure of betaine is attributed to its two main functions. The first is as a methyl donor (via S-adenosyl-methionine (SAM)), thereby sparing methionine and increasing the available substrates for protein synthesis. Second, when not catabolized, Betaine serves as an organic osmoprotectant, accumulating within osmotically stressed cells. Further, it is widely known as a carcass modifier due to its lipotropic properties and the increased availability of methionine and cysteine for protein deposition. Due to its methyl donor and amino acid functions, Betaine is involved in protein and energy metabolism. Thus, the multifaceted benefits of Betaine make it a potential natural feed additive to support and enhance animals' health and performance.

Dietary source
Condensed molasses soluble is a rich source of betaine, while wheat, wheat bran, and wheat Middlings contain a moderate amount of betaine. However, most animal feed products are low in betaine content (Fernandez *et al.*, 2000) [9]. Therefore, animals can neither obtain a significant quantity of betaine from a dietary source nor by oxidizing choline in the body. Nevertheless, betaine is available as a feed additive in purified form. The most popular forms of feed-grade betaine are anhydrous betaine, betaine monohydrate, and betaine hydrochloride. Also, the purified form of betaine originates from the extraction of molasses solubles, which forms the dietary source of betaine.

Chemical structure
Betaine is a neutral chemical compound with a positively charged cationic functional group, such as a quaternary ammonium or phosphonium cation, and a negatively charged functional group, such as a carboxylate group. As a result, it exists as a zwitter ion.

Synthesis of Betaine

In plants, betaine is synthesised and accumulated in response to abiotic stressors and thus serves as an osmoprotectant. Betaine is formed from choline in two steps: First, choline is converted to betaine aldehyde by the enzyme choline monooxygenase (CMO), and then betaine aldehyde is converted to betaine by the NAD+-dependent enzyme betaine aldehyde dehydrogenase (BADH), (Fan *et al.,* 2006) [8]. While in animals, betaine is formed from the irreversible oxidation of choline from a dietary source or endogenous origin by choline dehydrogenase found in the inner membrane of mitochondria, primarily in the liver and kidney. In two-step FAD-dependent oxidative reactions, the enzyme choline dehydrogenase catalyses the conversion of choline to betaine, producing betaine aldehyde as an intermediate product (Fan *et al.,* 2006) [8].

Functions of Betaine

The chemical structure of betaine is responsible for its various functions (Fig. 1). Because of its osmotic activity and high water solubility, betaine exists as a dipolar zwitterion. As an osmolyte, betaine improves the function of the cellular Na+/K+ pump and reduces water loss under hyperosmotic conditions, thereby protecting cells from dehydration. Under osmotic and ionic stress conditions, variations in cellular water content affect its ion concentration, in turn affecting the intercellular proteins and enzymes. In such situations, betaine exhibits an osmoprotective role by accumulating in stressed cells, thereby substituting inorganic ions, thus protecting cellular enzymes and proteins, and in turn maintaining normal cell functions. The cells of the intestine are continuously exposed to osmotic stress due to the hyperosmotic content of the intestine; hence, the process of digestion and absorption of nutrients demands an osmoregulatory mechanism (Eklund *et al.,* 2005) [6]. Thus, the osmoregulatory function of betaine helps to sustain intestinal cell volume and water balance. Furthermore, betaine has been shown to stimulate the growth of intestinal cells, resulting in an increased surface area for nutrient absorption by the gut epithelium. Thus, the osmoprotectant role of betaine has a beneficial effect on nutrient digestion and absorption.

Furthermore, with three methyl groups, it acts as the methyl donor required for transmethylation reactions for the synthesis of numerous substances such as carnitine, creatine, phosphatidylcholine, and methyl purines, as well as methylated amino acids (Wang *et al.,* 2019; Brougham *et al.,* 2020) [16, 2], and thus spares methionine and choline for protein and phospholipid synthesis, respectively. In animals, sources of the methyl group are methionine, choline, and betaine. Among these, betaine is considered to be a more efficient source of methyl donor. During various methylation reactions that occur in cells, methionine donates its methyl group, thus converting to homocysteine through S-adenosyl methionine (SAM) and S-adenosylhomocysteine (SAH). Further, betaine provides a methyl group for the remethylation of homocysteine to methionine, mediated by the enzyme betainehomocysteine methyl transferase, primarily in the liver and kidney (Zhao *et al.,* 2018) [20]. Subsequently, betaine is reduced to dimethylglycine and further metabolized to glycine.

The quality of meat is determined by muscle pH, colour, muscle fat, and water holding capacity. The osmolyte nature of betaine prevents the loss of water from meat. Further, betaine increases the amount of creatine in muscle, which is a primary source of energy for muscle contraction. Thus, an increase in the availability of energy reserves prevents a rapid fall in muscle pH by delaying glycolysis and consequently lactic acid production in meat after slaughter, thus preventing denaturation of proteins and enzymes (Dong *et al.,* 2019) [15]. Additionally, betaine improves the myoglobin content of muscle by enhancing the concentration of glycine (a betaine metabolite) and succinyl-CoA. Finally, all these factors together improve the appearance, water-holding capacity, muscle tenderness, and meat quality.

Because of its methyl donor property, it has a lipotropic effect, preventing fatty liver and making it an excellent carcass modifier (Dong *et al.,* 2019) [15]. Betaine, a methyl group donor, participates in many methylation reactions, likely increasing the availability of methionine and cysteine for protein synthesis and choline for phospholipid synthesis. The addition of betaine to the diet enhanced carnitine levels, which are involved in the beta oxidation of fatty acids in the mitochondria, thus preventing fatty acid deposition in animals (Fan *et al.,* 2022) [7]. Carcass modifier, a betaine function, could also be due to betaine and its metabolite, glycine, stimulating the hypothalamus-pituitary to release growth hormone. Consequently, improved weight gain with increased protein deposition results in a leaner carcass. Further, choline is involved in the synthesis of VLDL, which prevents fat deposition in the liver as well as mobilizes fat from the liver.

In addition, as it is a derivative of glycine, it exhibits the properties of glycine as well. Due to both its methyl donor and its amino acid function, betaine is involved in protein and energy metabolism. Besides, it has excellent antioxidant properties, thus preventing oxidative stress and further apoptosis (Arumugam *et al.,* 2021) [1].

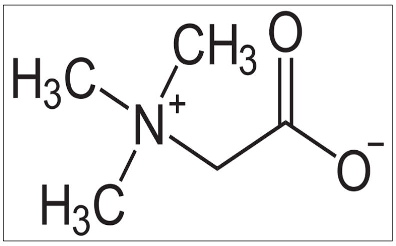

Fig 1: Chemical structure of Betaine

Betaine as rumen modulator

In the ruminant sector, the intensification of modern dairy farming and the genetic improvements for milk production have led to typical stressors such as hyperthermal and hyperosmotic stress caused by feeding as well as a high ambient temperature. Both stress conditions can lead to poor fermentation and subsequent adverse effects on ruminant productivity. In the rumen, betaine acts as a thermoprotectant for bacterial cells, and it serves as a methyl donor and a direct substrate for ruminal microbes that directly modulate ruminal fermentation (Mahmood *et al.,* 2020) [13]. It can also stabilise native protein structures and prevent molecular disintegration. These properties make betaine suitable for restoring microbial cell physiology disrupted by heat and osmotic stress. Betaine supplemented to grain-rich diets enhances microbial fermentation, leading to more SCFA production during heat stress, and it minimizes the effect of hyperosmotic stress by decreasing the acetate to propionate ratio by favouring propionate-producing microbes (Mahmood *et al.,* 2020) [13].

Under normal physiological conditions, betaine is converted in the rumen to acetate, which is the building block for milk fat synthesis. Under hyperosmotic rumen conditions, betaine shifted the SCFA towards more propionate, a glucogenic precursor required for lactose synthesis and responsible for milk yield (Mahmood *et al.,* 2020) [13]. Thus, in this way, betaine can support milk production during heat and hyperosmotic stress.

Metabolism of betaine

Mitchell *et al*. (1979) [14] conducted both *in vitro* and *in vivo* experiments using [1-^{14}C] betaine, [^{15} N] betaine, and [methyl-14C] betaine to study the metabolism of betaine. When [1–14C] betaine was incubated with rumen contents or added directly to the rumen, radioactivity was recovered in acetate and CO_2. While incubating [methyl 14 C] betaine with rumen content produced radioactivity in trimethylamine, methane, and CO_2, it did not produce radioactivity in trimethylamine, methane, or CO_2. As a result, the methane and carbon dioxide produced were almost certainly the result of a demethylation reaction involving betaine or trimethylamine. This demonstrated that rumen microbes were capable of degrading betaine. Further, the rate of utilization of betaine was found to be approximately 5 moles/ml/h, and the estimated turnover rate and $T_{1/2}$ were 45% per hour and 1.5 hours, respectively. These results suggested that betaine was metabolized more rapidly in the rumen.

Effect of betaine on ruminant performance

In ruminants, under normal physiological conditions, betaine also has a positive influence on fermentation; it increases total volatile fatty acid (VFA) production and apparent total tract nutrient digestibility and consequently increases average daily gain because betaine serves as a source of ruminal available nitrogen, substrate, and methyl donor for ruminal microbial growth, especially fibrolytic microbes (*Ruminococcusalbus*, *Fibrobactersuccinogenes*, and protozoa), and has a positive effect on the activity of their enzymes, thereby increasing microbial fermentation rate (Wang *et al.,* 2020) [17]. Therefore, betaine under both normal physiological and stress conditions has a positive influence on milk performance in ruminants. Several studies have found that supplementing lactating dairy cows with betaine can increase milk yield by 5-12%. Inclusion rates of 15–100 g/d were used in the different studies, and the highest increase was seen with 100 g/d of betaine (Wang *et al.,* 2010) [18]. Increasing milk fat (3.27 to 3.43%; Wang *et al.,* 2010), lactose (5.01 to 5.16), and protein (2.99 to 3.12%, respectively) (Zhang *et al.,* 2014) [19]. Also in dairy goats, there is a positive effect of betaine on milk performance and composition. Betaine supplementation of 4 g/kg resulted in an increased milk yield from 1.47 to 2.0 kg/d in the 4th month of lactation. Milk fat percentage (from 4.4 to 4.69% in the 5th month of lactation) and SCFA concentration were also increased with betaine inclusion.

Betaine alleviates heat stress and improves animal performance

A lot of animals live in environments with temperatures that exceed their thermal comfort zone, leading to heat stress. Heat stress is a typical condition where it is important for animals to regulate their water balance. By its ability to act as a protective osmolyte, betaine helps to maintain cell function and volume and thereby relieves heat stress, as indicated, for example, by lower rectal and skin temperatures in sheep (Di Giacomo *et al.,* 2016) [4]. Betaine at 2 g/day has been shown to effectively reduce basal metabolic rates in sheep under both thermal neutral and heat stress conditions by decreasing energy loss through heat produced by processes such as respiration (Di Giacom o*et al.,* 2016) [4]. However, a high dose of betaine (4 g/d) increased metabolic heat load, as evidenced by elevated rectal, skin, and respiratory temperatures and heat rates. This indicated the dose-dependent responses to dietary betaine supplementation in sheep. Similarly, supplementation of betaine at 25 g/d/animal had higher animal performance as indicated by higher DMI, feed conversion efficiency, body weight gain, plasma GH, and IGF-1 levels in heat stress. Karan fries heifers at a rate of 50 g/d/animal (Lakhani *et al.,* 2019) [11]. This positive effect of betaine would be due to its osmolyte property, which increases the water retention of both the gut and muscle tissues and supports the ionic pump function of intestinal cells under dehydration, which reduces energy expenditure and makes it available for production purposes. Thus, betaine, with its osmoprotective property, supports intestinal cells and the growth of intestinal microbes, thereby improving nutrient digestibility. High plasma GH and IGF-1 may be due to the positive effect of betaine on the somatotropic axis, which increases plasma GH, which in turn acts on the liver to release plasma IGF-1 (Lakhani *et al.,* 2019) [11]. Also, Zhang *et al.* (2014) [19] reported the beneficial effect of betaine at 15 g/d/animal in combating heat stress in dairy cows and improving their performance and productivity. Besides, betaine improves the antioxidant status with increased plasma catalase, glutathione peroxidase, superoxide dismutase, and decreased malondialdehyde levels in stressed animals (Zhang *et al.,* 2014; Cai *et al.,* 2021) [19, 3].

Betaine as carcass modifier

Betaine is known as an excellent carcass modifier due to its methyl donor property. While betaine is rapidly degraded to acetate in the rumen, only a small amount escapes. Hence, it is necessary to supply betaine in rumen-protected form to enhance its absorption in the small intestine. A comparative study between unprotected betaine and rumen-protected betaine conducted in lamb showed that supplementation of rumen-protected betaine improved lamb meat quality by retarding pH increase by reducing lactic acid accumulation, thereby reducing denaturation of protein and preventing water loss (Ekiund *et al.,* 2005) [6]. In addition, rumen-protected betaine improved the contents of meat-flavoring amino acids and essential amino acids compared to unprotected betaine. Thus, supplementation of rumen-protected betaine at 2.2 g/d/lamb proves to be economical and beneficial in improving growth performance, meat quality, and the PUFA and amino acid content of meat (Dong *et al.,* 2009) [15].

Effect of betaine on twin survivability

Brougham *et al*. (2020) [2] reported that maternal dietary betaine supplementation during late gestation can reduce lamb mortality, which may improve the productivity and welfare of sheep.

Flocks. Improved twin lamb survivability was observed in ewes supplemented with 4 g/d betaine during the second half of pregnancy compared to 2 g/d betaine throughout pregnancy due to increased creatine production. The increased survivability of twin lambs may be due to the ability of lambs from 4 g/d betaine supplemented ewes to contact and suckle the udder faster than lambs from 2 g/d supplemented ewes. The improved lamb vigour could be attributed to increased production of creatine in response to dietary betaine, which in turn enhanced ATP levels and thus prevented hypoxic damage to the neonatal brain during lambing. Whereas, lambs from ewes on 2 g/day of betaine throughout pregnancy reported improved post-natal growth at weaning due to enhanced pre-natal intestinal cell growth and survival. Similarly, prepartum maternal betaine supplementation has been beneficial in enhancing immunity, as indicated by increased total protein and plasma globulins in new-born calves (Wang *et al.*, 2019) [16]. The increased plasma TP and globulin concentrations of calves are because of maternal methyl donor supplementation. Maternal dietary supplementation with methyl donors could programme the health of offspring through the epigenetic regulation of the DNA molecule and cell signalling (Liu *et al.*, 2013; Ji *et al.*, 2016) [12, 10], which might improve the capacity for globulin absorption in the intestine to improve the immunity of newborn calves. Furthermore, betaine supplementation during gestation has been shown to reduce stress in calves during calving, as evidenced by lower glucose concentrations, which are positively correlated with cortisol levels in betaine supplemented calves (Vannucchi *et al.*, 2015) [15]. In addition, the greater plasma SOD concentrations 2 h after birth in the study showed that the betaine-supplemented calves were in a state of less stress (Vannucchi *et al.*, 2015) [15].

Conclusion

Betaine is a natural feed additive with immense potential to support and enhance animal health and production performance under various stressful conditions. Due to its osmolyte and methyl donor properties, betaine is essential for both rumen microbes and animals under stress conditions. Betaine supplementation of lactating dairy cows at 15-100 g/d can increase milk yield by 5-12%. Betaine, at 2 g/d and 15 g/d, is a useful dietary additive for combating heat stress in sheep and cows, respectively. Due to the rapid degradability of betaine in the rumen, post-ruminal effects such as lipotropic and methionine sparing are affected. Hence, betaine at 2.2 g/lamb/d in rumen-protected form is beneficial for lean meat production. Further, the dose of betaine in an animal depends on the feed interactions, physiological status of the animal, and its environment. As a result, using betaine as an alternative methyl source reduces the dietary supply of choline and methionine, as well as the feed cost associated with their use.

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