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Betaine: A potent feed additive for amelioration of adverse effect of heat stress in livestock and poultry

S V SINGH[1✉], ADITYA D DESHPANDE[2] and YALLAPPA M SOMAGOND[1]

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

This review encapsulates the beneficial effects of dietary betaine in livestock and poultry to ameliorate the adverse effect of heat stress. Betaine (trimethylglycine) has its own functional, nutritional and physiological roles. It is widely used in feed formulations as feed additive in livestock and poultry feed to increase performance. Beet (*Beta vulgaris*) is the main source of commercially available betaine. Betaine's molecular structure possesses N^+ and COO^-, so it acts as a zwitter ion. Main functions of betaine are as an osmolyte as well as a methyl group donor. These functions showed many valuable effects of dietary betaine to livestock and poultry. Betaine binds with water molecules in its hydration shell and prevents cellular dehydration. This function mainly helps to ameliorate heat stress in livestock and poultry. It is also used as growth promoter in pig farming. Betaine supplementation resulted in increased antioxidant enzyme levels to prevent cellular oxidative stress and ROS formation. Betaine supplementation increased milk yield as well as milk fat in lactating dairy animals. It also increased carcass quality and lean meat production by suppressing lipogenesis genes in meat animals. Also, betaine is stable, non-toxic, and a cost-effective feed additive. Therefore, it can be concluded that betaine is a potent feed additive for livestock and poultry farming.

Keywords: Antioxidant, Betaine, Growth, Heat stress, Methyl donor, Osmolyte

In the last two decades in India, animal husbandry has shifted towards intensive farming. Intensive animal farming is profitable as well as difficult to manage. Intensive farming has its pros and cons and demands a faster growth rate, early attainment of puberty, and high production in a stress-free environment. In the recent climatic scenario, environmental heat stress in tropical countries is a major challenge. It causes reduced growth in growing animals, a decrease in milk yield with the difference in milk constituents, oxidative stress and reproductive problems which ultimately leads to economic losses. So, many researchers had successfully tried for a nutritional strategy (Singh *et al.* 2021), dietary inclusion of astaxanthin and prill fat (Somagond *et al.* 2019, Somagond *et al.* 2020) and betaine (Deshpande *et al.* 2020); part of managemental interventions; a dietary betaine to livestock and poultry species to cope up against all above mentioned physiological problems.

In 1869, Scheibler isolated and discovered betaine from sugar beet (*Beta vulgaris*). Betaine is short-chain molecule, neutral in nature and trimethyl derivative of glycine. Betaine is also known as N,N,N-trimethylammonioacetate (Scheibler 1869). Betaine is a stable, non-toxic natural phytoconstituent present in plants, animals, and microorganisms (Craig 2004 and Zhao *et al.* 2018). Though the production performance of dairy animals can be increased through environmental modification, the cost of this technique is very high (DiGiacomo *et al.* 2016). The use of feed additives may be a cost-effective and easily accessible method to reduce heat stress in animals, and it can also improve the production performance of dairy animals.

Sources

Betaine was first discovered from the byproduct of beet industry and then found in various micro-organisms, marine animals, plants, and animals. Sugar beet is the main source of betaine, available commercially (Scheibler 1869). It is found in high concentration in wheat, lucerne meal, wheat bran, groundnut meal, wheat germ and spinach, as well as in microorganisms and aquatic invertebrates (Zhao *et al.* 2018). It is synthesized in body through the metabolism of choline (Craig 2004 and Zhao *et al.* 2018) or externally consumed through the dietary intake (Willingham *et al.* 2020).

Also, betaine is presented in different purified forms (betaine monophosphate, anhydrous betaine, and betaine

Present address: [1]Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana. [2]Physiology and Climatology Division, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh. ✉Corresponding author email: sohanvir2011@gmail.com

Table 1. Betaine concentration in feed constituents

Feed constituent	Betaine concentration (mg/kg)	Reference
Sugar beet molasses	116,000	Eklund *et al.* 2005
Wheat	3,960	Chendrimada *et al.* 2002
Lucerne meal	3,850	Westberg 1951
Wheat bran	2,675	Westberg 1951
Groundnut meal	2,520	Chendrimada *et al.* 2002
Barley	730	Kidd *et al.* 1997
Oats	590	Kidd *et al.* 1997
Fish meal	400	Kidd *et al.* 1997
Peas	160	Steinmetzer 1972

hydrochloride) for commercial use as feed additive (Saeed *et al.* 2017).

Structure

Chemically, betaine is zwitter ion. It possesses N^+ and COO^-. It has a positively charged trimethylammonium group and a negatively charged carboxyl group (Fig. 1). Thus it does not require any counter ions to maintain electro-neutrality in the cytosol. Also, betaine is highly soluble in water (Scheibler 1869).

Functional Roles

Osmolyte: Betaine functions as an osmolyte and a chemical chaperone (Hammer and Baltz 2002). Osmolytes are organic molecules of small size that get accumulated in the cytoplasm during osmotic stress. The osmo-protective ability of betaine creates a hindrance for cellular dehydration during high ambient temperature. Betaine can bind and hold water molecules in the cytoplasm. So, it reduces vascular perfusion and prevents water loss from ECF during hyperthermia. It also minimizes epidermal dehydration via sweating. It improves kidney electrolyte balance and water with-holding efficiency (Hammer and Baltz 2002). Moreover, betaine functions as chaperone that assist the folding and unfolding of macromolecular structures and denatured proteins (Day and Kempson 2016).

Betaine supports maintaining the cytoplasmic osmotic pressure and permits cells to control the surface tension of water and stabilizes protein structure with its function (Craig 2004, Willingham *et al.* 2020). Betaine protects cells, cellular proteins, and some enzymes prone to osmotic stress

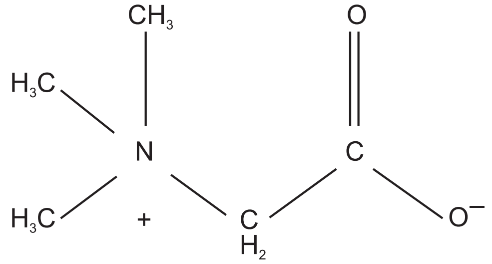

Fig. 1. Molecular structure of betaine (Zhao *et al.* 2018).

(Horio *et al.* 2001). Betaine's osmolyte function maintains the hydration of albumin protein (Courtenay *et al.* 2000), surrounds a single layer of water molecules around the albumin, and maintains haemoglobin solvation (Hundahl *et al.* 2003). In macrophages of the liver, betaine checks the hyper-osmolarity induced release of TNFα and initiation of prostaglandins formation with cyclooxygenase-2 (COX-2), and finally modulates immune functioning. The mRNA expression of betaine transporter is significantly increased by hyper-osmolarity (Zhang *et al.* 1996).

Methyl group donor: Betaine provides its methyl group to homocysteine and converts into methionine (Fig. 2). This reaction is catalyzed by an enzyme called betaine-homocysteine methyltransferase (BHMT) (Craig 2004).

Betaine can modify methylation process through BHMT. This controls levels of S-adenosyl-methionine as well as S-adenosyl-homocysteine. Some genetic mechanisms which can alter gene expression without altering the genetic code mainly depend on DNA methylation or of its histones, and also dietary supply of choline and some additional methyl group donors impacts these reactions of methylation (Zeisel 2017).

Methyl-tetrahydrofolate as well as betaine function as methyl group donors which are required for the methylation reaction of homocysteine to methionine (Stryer 1988). Activated methyl group donor; S-adenosylmethionine shifts a methyl group for the synthesis of phosphatidylcholine, creatine, as well as epinephrine. Also, choline releases from phosphatidylcholine which is reacted to betaine (Stryer 1988).

Physiological roles: The physiological roles of betaine are represented in Fig. 3.

Studies on cattle and buffalo: Supplementation of rumen-protected betaine (RPB) to lactating dairy cows showed increased ($P<0.05$) milk yield and milk fat % whereas significantly ($P<0.05$) lower milk somatic cell count (SCC) as compared to control and rumen-protected methionine (RPM) supplemented group. Also, NEFA, TG and BUN levels were significantly reduced ($P<0.05$) in the rumen-protected betaine (RPB) fed group (Wang *et al.* 2020, Cheng *et al.* 2020). Significantly higher ($P<0.05$) feed efficiency was found in cows supplemented with betaine (Cheng *et al.* 2020).

In comparison with control group animals, betaine supplementation to lactating HF cows at high environmental temperature increased average daily milk production ($P<0.001$). Also, dietary betaine increased milk protein levels ($P<0.001$) and milk fat concentration ($P<0.001$) (Dunshea *et al.* 2019).

During heat stress conditions (THI≥72), supplementation of betaine to lactating cows (T) showed significantly higher ($P<0.05$) DMI, milk production, and milk fat % as compared to control (C). Somatic cell count (SCC) was higher ($P<0.05$) in the C group as compared to T group. The levels of volatile fatty acids (VFA) in the T group were significantly higher ($P<0.05$) than that of the C group. The concentration of serum total antioxygenic

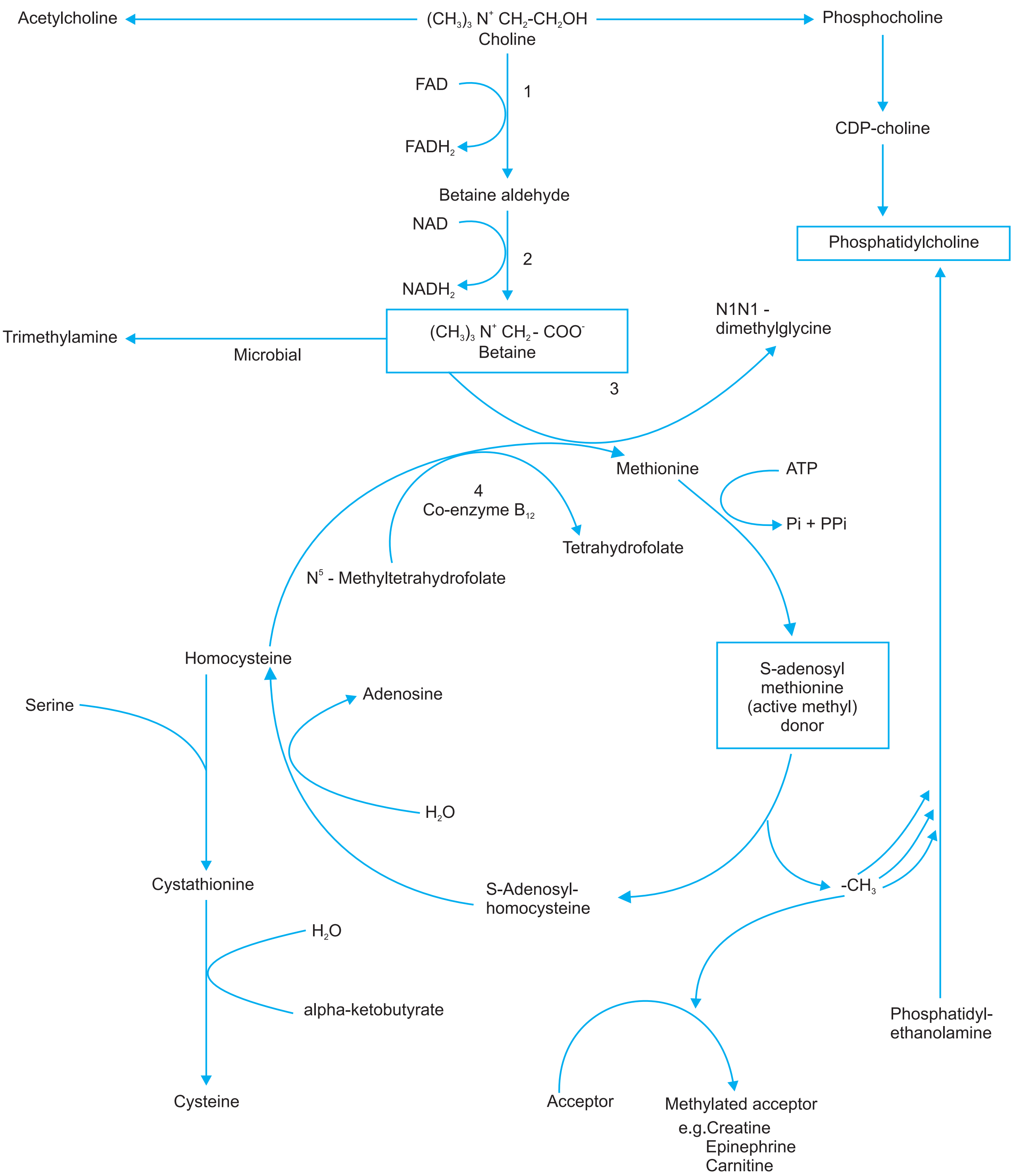

Fig. 2. Betaine metabolism pathway (Saeed *et al.* 2017).

capacity (T-AOC) in T group was significantly improved ($P<0.05$). Also, the MDA and SOD of the T group were higher ($P<0.05$) than the C group. Also, the GSH-Px levels of T group were uppermost ($P<0.05$). Compared to C, T group had significantly higher ($P<0.05$) serum glucose. Cows given dietary betaine showed decreased serum BHBA and NEFA levels (Shah *et al.* 2020). RPB supplementation during the transition period increased fat mobilization in post-partum dairy cows with improved immune status of new-born calves by increasing total proteins and globulins concentrations (Wang *et al.* 2019).

Pre-treatment of betaine to mammary alveolar cells (MAC) culture increased total antioxidant status, SOD, and CAT and reduced ROS accumulation. Bax/Bcl-2 ratio (a

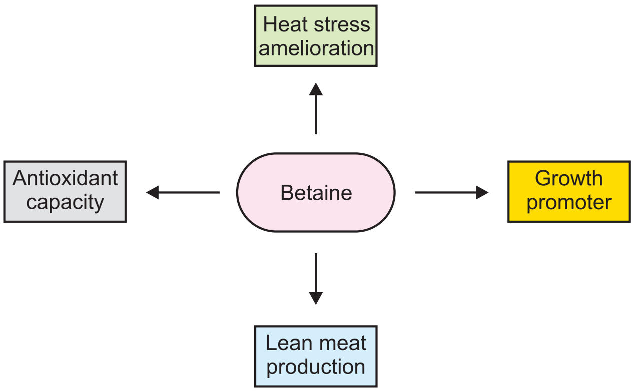

Fig. 3. Physiological roles of betaine.

marker of apoptosis) and Nrf-2/HO-1 (marker of oxidative stress) expression of heat treated MAC were decreased with dietary betaine (Li *et al.* 2019).

A decrease in rectal temperature and increase of feed intake, milk yield, GPx, and SOD activity was observed in Karan Fries (KF) cows supplemented with dietary Betaine during heat stress (Raheja 2017). A significant decrease was observed in RT, RR, ST and an increase in DMI, ADG, TBARS, Catalase, GPx, and SOD activity with increased total protein level in KF heifers fed with dietary betaine during hot dry as well as hot humid conditions (Lakhani 2018).

Dietary betaine supplementation proved as a potent growth promoter by lowering the levels of plasma cortisol and NEFA and enhancing the ADG, and DMI of buffalo heifers. Also, dietary betaine supplementation increased plasma growth hormone (GH) levels with reduced physiological responses (Deshpande *et al.* 2020). Feeding of betaine hydrochloride to lactating buffaloes under heat stress increased DMI and milk fat % whereas physiological responses like RT, RR and PR were significantly ($P<0.01$) reduced as compared to control group animals (Shankhpal *et al.* 2019).

Studies on sheep and goat: Dietary betaine improved serum lipid profile, immunity and increased testicular antioxidant of *Hu* sheep, which can be regulated via the mTOR signalling (Cai *et al.* 2021). The meat quality of lambs [Longissimus dorsi (LD) muscle] improved after supplementation of rumen-protected betaine (RPB) (Dong *et al.* 2020).

Supplementation of rumen-protected betaine to lambs increased ADG, GSH-Px, SOD and CAT in the LD, and T-AOC with the activities of SOD, CAT, and GSH-Px in the liver whereas decreased levels of serum MDA, LD and liver (Yu *et al.* 2020).

In comparison with the control group, ewes supplemented with rumen-protected betaine, methionine and choline diet showed higher milk fat % and total solids (TS) levels. Also, the significantly higher ferric reducing ability of plasma (FRAP) values was found. A significant increase was observed in the growth of lambs nursing ewes of treatment group, compared to control (Tsiplakou *et al.* 2017). Betaine supplementation to goats was shown to have a down regulating effect on the heat shock proteins (HSPs), signifying a role of betaine (chemical chaperone) on heat stress amelioration (Dangi *et al.* 2016).

Dietary betaine supplementation significantly ($P<0.001$) decreased the RT, ST and RR of merino ewes exposed to heat stress environment (DiGiacomo *et al.* 2016). Dietary betaine and sulphate to ewes altered ($P<0.05$) DMI. Daily milk production as well as milk fat % were significantly ($P<0.05$) increased by betaine and sulphate supplementation. Dietary betaine and sulphate significantly ($P<0.01$) increased the daily growth of the wool. Dietary betaine with sulphate supplementation significantly ($P<0.01$) increased the level of wool sulphur content (Nezamidoust *et al.* 2012).

Feeding of betaine supplemented diets to lactating goats increased the short-chain fatty acids (C6 : 0 to Cl0 : 0) and significantly ($P<0.05$) increased the medium-chain fatty acids (C12 : 0 to C15 : 0) in milk fat. Therefore, dietary betaine can be considered valuable for producing good quality milk for human consumption (Fernandez *et al.* 2009). Dietary supplementation of betaine to goats during the summer days increased the level of milk fat % at late lactation. Lower levels of triglycerides and ketone bodies were observed in the betaine-fed group (Fernandez *et al.* 2009).

Studies in pigs and poultry: In weaned pigs, digestibility of dry matter (DM) and crude protein (CP) was enhanced by 4.2% and 6.4% respectively in the dietary betaine fed group (Albuquerque *et al.* 2017). Dietary betaine to sows during pregnancy reduces hepatic lipogenesis in newborn piglets by epigenetic as well as glucocorticoid receptor (GR) mediated mechanisms (Cai *et al.* 2016). Betaine supplementation to finishing pigs reduced carcass features which are typical indicators of carcass fatness (Sales 2011). Betaine supplementation to finishing pigs significantly ($P<0.01$) increased lean carcass proportion and loin muscle area and significantly ($P<0.01$) reduced carcass fat and average back fat thickness (Huang *et al.* 2008).

Betaine maintains water molecules movement in intestinal epithelial cells and manages osmoregulation in the intestine of broiler chicks (Amerah and Ravindran 2015). Dietary betaine in broilers significantly improved ($P<0.05$) ADG and feed conversion ratio (FCR). Dietary betaine significantly reduced ($P<0.05$) cortisol concentrations and muscle MDA levels. Dietary betaine significantly reduced ($P<0.05$) muscle drip loss, lactate content, and lactate dehydrogenase with significantly improved ($P<0.05$) muscle glutathione level and GPX enzyme activity (Chen *et al.* 2020).

Betaine supplementation is advantageous during the high metabolic demand of nutrients in rapid growth in chicken broilers (Kidd *et al.* 1997). Dietary betaine supplementation significantly increased ADG and FCR in chicken broilers. Dietary betaine significantly improved the breast muscle gain and reduced abdominal fat (Zhan *et al.* 2006). Heat stress amplified abdominal as well as subcutaneous fat, whereas the dietary betaine supplementation significantly (P≥0.05) reduced fat deposition, giving lean muscle yield. Weight gain in heat-stressed chicken was significantly decreased at 28, 35 and 42 days of age. Betaine has a constructive effect in improving the growth of broiler chicken (He *et al*. 2015).

The activity of the GPx in the betaine supplemented group (B) and the ML+B group (Methionine low group diet plus betaine) were significantly increased compared to the ML (Methionine Low Diet) and C (Control diet) groups of broiler chicken. Catalase and SOD were significantly higher in the B group compared to the ML group. Lipid peroxidation was significantly greater in the C and the ML groups. The glutathione antioxidant system plays an essential role in cellular defense against free radicals. GSH (γ-glutamyl cysteinyl glycine) reduces pro-oxidative damage by counteracting free ROS (Alirezaei *et al.* 2012).

Conclusion

From the above review, it can be concluded that dietary betaine supplementation has wide benefits in livestock and poultry farming. It showed ameliorative effects on heat stress and worked as growth promoter, lean meat production, improved digestibility and reduced ROS production via increased antioxidant enzymes. Finally, betaine proved itself as a potent feed additive for livestock and poultry feed.

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