



Metabolic, Osmoregulatory and Nutritional Functions of Betaine in Monogastric Animals

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ABSTRACT : This review focuses on the metabolic and osmoregulatory functions of betaine and its impact on nutrient digestibility and performance in pigs and poultry. Betaine is the trimethyl derivative of the amino acid glycine, and is present in plant and animal tissue. It has been shown to play an important role in osmoregulation of plants, bacteria and marine organisms. Due to its chemical structure, betaine exerts a number of functions both at the gastrointestinal and metabolic level. As a methyl group donor, betaine is involved in transmethylation reactions and donates its labile methyl group for the synthesis of several metabolically active substances such as creatine and carnitine. Therefore, supplementation of betaine may reduce the requirement for other methyl group donors such as methionine and choline. Beneficial effects on intestinal cells and intestinal microbes have been reported following betaine supplementation to diets for pigs and poultry, which have been attributed to the osmotic properties of betaine. Furthermore, betaine potentially enhances the digestibility of specific nutrients, in particular fiber and minerals. Moreover, at the metabolic level, betaine is involved in protein and energy metabolism. Growth trials revealed positive effects of supplemental betaine on growth performance in pigs and poultry, and there is evidence that betaine acts as a carcass modifier by reducing the carcass fat content. In conclusion, due to its various metabolic and osmoregulatory functions, betaine plays an important role in the nutrition of monogastric animals. (**Key Words :** Betaine, Methyl Group Donor, Osmolyte, Nutrient Digestibility)

INTRODUCTION

Betaine supplementation to diets for livestock has increased during the last decade (Feng et al., 2006; Fernandez-Figares et al., 2008). Betaine, the trimethyl derivative of the amino acid glycine, is a naturally occurring compound, which is widely distributed in many plants and animal tissues. It is present in large quantities in aquatic invertebrates and sugar beets, but also in wheat, wheat products and lucerne meal (Kidd et al., 1997; Chendrimada et al., 2002). Common sources of betaine are sugar beets and their by-products such as molasses and condensed molasses solubles (Eklund et al., 2005). As a feed additive, betaine is also available in purified form and most commonly added to animal diets in the form of anhydrous betaine, betaine monohydrate and betaine hydrochloride (Kidd et al., 1997; Eklund et al., 2005). Betaine is stable and non-toxic (Yu et al., 2004).

Due to its chemical structure (Figure 1), betaine has a number of different functions both at the gastrointestinal and metabolic level (Eklund et al., 2005). Betaine donates its labile methyl group which can be used in transmethylation reactions for synthesis of substances like carnitine and creatine (e.g. Kidd et al., 1997). Therefore, the dietary supplementation of betaine may reduce the requirement for other methyl group donors such as methionine and choline (Siljander-Rasi et al., 2003). Betaine acts as an osmoprotectant in plants (e.g. Xing and Rajashekar, 2001), bacteria (e.g. Pichereau et al., 1999), and marine organisms (e.g. Clarke et al., 1994). In vertebrates, betaine is used by numerous tissues as an osmolyte (e.g. Law and Burg, 1991). Due to its osmotic properties, betaine may have the potential to improve the digestibility of specific nutrients (Eklund et al., 2006a, b). Furthermore, betaine is involved in protein and energy metabolism due to its methyl group donor function (Eklund et al., 2005). The animal's betaine need is strongly influenced by the concentration of other methyl group donors in the diet and the occurrence of osmotic stress in the intestinal tract or other organs. If the total betaine need cannot be met by endogenous metabolism, dietary betaine supplementation

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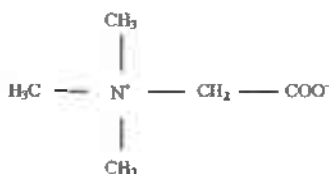


Figure 1. Chemical structure of betaine.

may be beneficial to maintain or to improve animal health and performance (Kidd et al., 1997). Studies on the dietary effect of betaine revealed variable results, both in nutrient digestibility and animal performance (Øverland et al., 1999; Attia et al., 2005; Eklund et al., 2006a, b). The objective of this paper is to review the metabolic and osmoregulatory functions of betaine and its impact on nutrient digestibility and performance criteria in pigs and poultry.

EFFECTS OF BETAINE ON ANIMAL PERFORMANCE AND CARCASS CHARACTERISTICS

Dietary betaine supplementation may affect animal performance and carcass characteristics, even though the effects are variable (Attia et al., 2005; Dunshea et al., 2007; Fernandez-Figares et al., 2008). Some of the investigated effects of betaine supplementation on performance of pigs and poultry are presented in Table 1 and 2, respectively. In some studies, the addition of betaine to the diet improved weight gain and feed conversion in pigs (e.g. Wray-Cahen et al., 2004; Yu et al., 2004; Dunshea et al., 2007), and poultry (e.g. Attia et al., 2005; Hassan et al., 2005), though the results of several other studies revealed minimal or no effect of betaine supplementation on animal performance (e.g. Esteve-Garcia and Mack, 2000; Feng et al., 2006). Dietary supplementation of betaine to a pig's diet improved weight gain and feed efficiency up to 15 and 8%, respectively (Wang and Xu, 1999; Zou et al., 2002; Yu et al., 2004; Huang et al., 2008). Moreover, betaine has been shown to improve feed efficiency of pigs housed under sub-optimal hygienic conditions (Spreeuwenberg et al., 2007). In poultry, dietary supplementation of betaine to diets with adequate methyl group donors improved weight gain and feed efficiency by approximately 3 to 15% (Hassan et al., 2005). Moreover, betaine may enhance performance of *Eimeria*-infected chicken indirectly, by support of the intestinal structure and function in the presence of coccidial infection, but also directly, by partial inhibition of coccidial invasion and development (Augustine et al., 1997; Matthews et al., 1997; Matthews and Southern, 2000). During periods of osmotic disturbance, caused by water salinity stress in broiler chickens, betaine is involved in the protection of intestinal epithelia, resulting in an improved growth and feed efficiency (Honarbakhsh et al., 2007a, b).

Furthermore, under heat stress conditions, supplementation of betaine has shown to enhance egg production and egg shell quality in laying hens (Ryu et al., 2002), and to improve weight gain of broilers (Farooqi et al., 2005). In contrast, Zulkifli et al. (2004) could not show any effects of betaine on weight gain and feed conversion in broilers reared under heat stress conditions.

Different studies revealed considerable changes in carcass composition in pigs (Table 3) and poultry (Table 4) due to dietary betaine supplementation. Dietary betaine has been shown to exert positive effects on carcass characteristics of pigs by increasing carcass lean content, longissimus muscle area and loin depth, associated with a reduction in carcass fat content and back fat thickness, although without influencing performance (Cadogan et al., 1993; Smith et al., 1995; Yu et al., 2004; Huang et al., 2008). Studies in pigs revealed that dietary betaine reduced carcass fat content up to 18%, and raised the lean content of the carcass up to 8% (e.g. Wang and Xu, 1999; Feng et al., 2006). In growing pigs kept under a restricted feeding regime, betaine induced lower fat concentrations in the carcass, associated with a higher protein deposition (Fernandez-Figares et al., 2002). Following betaine supplementation to diets for poultry, several authors reported a reduction in abdominal fat weight, whereas breast meat yield was increased in broiler chicken (Zhan et al., 2006), turkeys (Noll et al., 2002) and meat ducks (Wang et al., 2004). In contrast, other studies did not show any effects of betaine supplementation on carcass characteristics in pigs (e.g. Fernandez-Figares et al., 2008) and poultry as well (Waldroup and Fritts, 2005).

The involvement of betaine in lipid metabolism offers an interesting perspective in meat production to satisfy consumer's needs for lean meat. Due to the reduction of carcass fat content and increase in carcass lean, betaine is often referred to as 'carcass modifier'. The mode of action of betaine as 'carcass modifier' may be related to its methyl group donor properties (Eklund et al., 2005). The improvement in carcass lean percentage may be attributed to a higher availability of methionine and cystine for protein deposition (McDevitt et al., 2000). An enhanced utilization of dietary amino acids for protein synthesis may result in fewer amino acids available for deamination and eventual synthesis of adipose tissue (Wallis, 1999). Accordingly, changes in hormone levels and growth factors involved in the regulation of fat synthesis and degradation, as well as lower activities of lipogenic enzymes have been observed following dietary betaine supplementation (Huang et al., 2006). Moreover, it is well known that betaine as a methyl group donor provides its methyl group for synthesis of lecithin, which facilitates the transport of fat through the body (Saunderson and MacKinlay, 1990). In addition, betaine may improve choline availability, thus providing

more choline for the synthesis of very low density lipoprotein. The production of very low density lipoprotein prevents the deposition of fat in the liver and accelerates the removal of fat from the liver (Yao and Vance, 1989). Another function of betaine in lipid metabolism is that it is associated with an enhanced synthesis of methylated

Table 1. Effect of supplemental betaine in the diet on performance traits of pigs

Animal	Betaine level (%)	Betaine effects	Reference
Gilts; 60-103 kg	0.13	-	Cadogan et al. (1993)
Grower-finisher pigs; 34-102 kg	0.10	-	Smith et al. (1994)
Barrows, gilts; 83-16 kg	0.13	-	Cera and Schinckel (1995)
Gilts; 60-104 kg	0.10	↑ ADG	Smith et al. (1995)
Lactating sows	0.20	-	Campbell et al. (1997a)
Barrows; 30 kg	0.13	↑ ADG ↑ ADFI	Campbell et al. (1997b)
Finisher pigs	0.20	↑ ADG	Urbanczyk (1997)
Barrows, gilts; 30-112 kg	0.20	-	Urbanczyk (1997)
Gilts; 55-110 kg	0.13	-	Matthews et al. (1998)
Pigs; 56-113 kg	0.11	↑ ADG ↓ FCR	Cromwell et al. (1999)
Pigs; 24-113 kg	0.11	-	Cromwell et al. (1999)
Pigs; 30-112 kg	0.20	-	Hanczakowska et al. (1999)
Pigs; >20 kg	1.05	-	Overland et al. (1999)
Barrows; 20-65 kg	0.15	↑ ADG, 10%	Wang and Xu (1999)
Gilts; 20-65 kg	0.15	↑ ADG, 15%	Wang and Xu (1999)
Barrows, gilts; >10 kg	0.08	↑ ADG, 12% ↑ ADFI, 9% ↓ FCR, 3%	Yu and Xu (2000)
Grower pigs; >21 kg	0.10	↑ ADG, 13.3%	Feng and Yu (2001)
Finisher pigs	0.10	↑ ADG, 5.7%	Feng and Yu (2001)
Barrows; 50-110 kg	0.25	-	Matthews et al. (2001a)
Piglets; 5-12 kg	0.06	↑ ADG, ADFI	Matthews et al. (2001b)
Barrows, gilts; 66-88 kg	0.13-0.30	↓ ADFI	Matthews et al. (2001c)
Finisher pigs; 53-113 kg	0.11	↓ FCR	Petty et al. (2001)
Barrows, gilts; 83-118 kg	0.13	-	Petty et al. (2001)
Barrows, gilts; >30 kg	0.25-1.00	↑ ADG ↓ FCR	Siljander-Rasi et al. (2003)
Weanling pigs	0.08	↑ ADG, 8.7%	Yu et al. (2001)
Grower pigs	0.10	↑ ADG, 13.2%	Yu et al. (2001)
Finisher pigs	0.18	↑ ADG, 13.3%	Yu et al. (2001)
Barrows; 30 kg	0.13-0.50	-	Fernandez-Figares et al. (2002)
Barrows, gilts; 83-116 kg	0.13	↓ ADFI	Lawrence et al. (2002)
Grower-finisher pigs	0.10	↑ ADG, 13% ↓ FCR, 8%	Zou et al. (2002)
Barrows; >46 kg	0.13	-	Schrama et al. (2003)
Boar; >64 kg	0.15	↑ ADG	Suster et al. (2004)
Gilts; 20-30 kg	0.13-0.50	↑ ADG ↓ FCR	Wray-Cahen et al. (2004)
Grower pigs; 20-64 kg	0.10-0.20	↑ ADG ↑ ADFI ↓ FCR	Yu et al. (2004)
Finishing barrows; >62.5 kg	0.13	-	Feng et al. (2006)
Barrows, gilts; 55-90 kg	0.13	↑ ADG	Huang et al. (2006)
Boar, gilts; >58 kg	0.13	↑ ADG, 8%	Dunshen et al. (2007)
Finishing pigs	0.13	↑ ADG, 5.5%	Huang et al. (2007)
Piglets	0.20	↓ FCR	Spreeuwenberg et al. (2007)
Gilts; 20-50 kg	0.50	-	Fernandez-Figares et al. (2008)
Barrows, gilts; 55-90 kg	0.13	↑ ADG	Huang et al. (2008)

-, No effect ($p > 0.05$); ↑, increase ($p < 0.05$); ↓, decrease ($p < 0.05$).

ADFI = Average daily feed intake; ADG = Average daily gain; FCR = Feed conversion rate.

Table 2. Effect of supplemental betaine in the diet on performance traits of poultry

Animal	Betaine level (%)	Betaine effects	Reference
Broilers	0.05-0.15	↑ ADG ↓ FCR	Virtanen and Rosi (1995)
Broilers	0.08	↑ ADG ↓ FCR	Virtanen and Rosi (1995)
Broilers, unsexed	0.15	↑ ADG ↓ FCR	Augustine et al. (1997)
Broilers	0.10-0.50	↑ ADG ↓ FCR	Matthews et al. (1997)
Broilers	0.10	↓ FCR	Matthews et al. (1997)
Broilers	0.15	↓ FCR 0-14 d	Teeter et al. (1999)
Broilers	0.10	↑ ADG 21-35 d	Teeter et al. (1999)
Broiler; female	0.10	↑ ADG ↓ FCR	Waldenstedt et al. (1999)
Broilers	0.08	↑ ADG ↑ Total plasma protein	Matthews and Southern (2000)
Broilers; female	0.05	-	Esteve-Garcia and Mack (2000)
Broilers; male	5-10	-	Zulkifli et al. (2004)
Broilers; male	0.05-0.10	-	Pirompud et al. (2005)
Broilers; male			Waldroup and Fritts (2005)
0-14 d	0.10	-	
0-35 d	0.10	↓ FCR	
0-42 d	0.10	↓ FCR	
0-49 d	0.10	-	
Broilers; unsexed	0.10	↑ ADG (under heat stress)	Farooqi et al. (2005)
Broilers; unsexed	0.04-0.07	↑ ADG ↓ FCR ↑ Feather weight	Attia et al. (2005)
Broilers; unsexed	0.07-0.14	↑ ADG ↓ FCR	Hassan et al. (2005)
Broilers; unsexed	0.28	-	Pillai et al. (2006)
Broilers; male			Waldroup et al. (2006)
0-14 d	0.10	-	
0-35 d	0.10	↓ FCR	
0-42 d	0.10	↓ FCR	
0-56 d	0.10	-	
Broilers; male	0.05	↑ ADG ↓ FCR	Zhan et al. (2006)
Broilers; unsexed	0.05-0.10	↑ ADG ↓ FCR	El-Husseiny et al. (2007)
Broilers; male	0.08-0.23	↑ ADG ↓ FCR	Honarbaksh et al. (2007a, b)
Turkeys	0.10	-	Remus (2001)
Turkeys	0.09	↑ ADG (8 and 11 weeks)	Noll et al. (2002)
Turkeys	0.09	-	Noll et al. (2002)
Meat ducks; female	0.50	↑ ADG ↓ FCR	Wang et al. (2004)
Layer, ISA	0.04-0.08	↑ Egg production ↑ Concentration of VLDL ↑ Vitellogenin	Lu and Zou (2006)
Layer, ISA brown	0.03-0.12	↑ Egg weight ↑ Serum estradiol ↑ Melatonin	Park et al. (2006)

-, No effect ($p > 0.05$); ↑, increase ($p < 0.05$); ↓, decrease ($p < 0.05$). ADG = Average daily gain; FCR = Feed conversion rate.

Table 3. Effect of supplemental betaine in the diet on carcass characteristics of pigs

Animal	Betaine level (%)	Betaine effects	Reference
Gilts; 60-103 kg	0.13	↓ Backfat thickness	Cadogan et al. (1993)
Grower-finisher pigs; 34-102 kg	0.10	-	Smith et al. (1994)
Barrows, gilts; 83-116 kg	0.13	-	Cera and Schinckel (1995)
Gilts; 60-104 kg	0.10	↑ Loin depth	Smith et al. (1995)
Pigs; >20 kg	1.05	-	Overland et al. (1999)
Barrows, gilts	0.20	↓ Backfat thickness	Urbanczyk (1997)
Gilts; 55-110 kg	0.13	↑ Carcass length	Matthews et al. (1998)
Pigs; 56-113 kg	0.11	-	Cromwell et al. (1999)
Pigs; 24-113 kg	0.11	-	Cromwell et al. (1999)
Barrows; 20-65 kg	0.15	↑ Dissected lean of carcass, 3% ↓ Backfat thickness, 18%	Wang and Xu (1999)
Gilts; 20-65 kg	0.15	↑ Dissected lean of carcass, 8% ↑ Longissimus dorsi, 39% ↓ Backfat thickness, 11%	Wang and Xu (1999)
Barrows; 50-110 kg	0.25	-	Matthews et al. (2001a)
Barrows, gilts; 66-88 kg	0.13-0.30	↑ Carcass length, fat free lean ↓ 10 th rib backfat ↓ Cooking loss ↓ Carcass fat	Matthews et al. (2001c)
Finisher pigs; 53-113 kg	0.11	↑ Leanness carcass	Petty et al. (2001)
Barrows, gilts; 83-118 kg	0.13	-	Petty et al. (2001)
Barrows, gilts; >30 kg	0.25-1.00	↑ Carcass weight	Siljander-Rasi et al. (2001)
Weanling pigs	0.08	↓ Dissected fat, 13% ↑ Dissected lean of carcass, 4%	Yu et al. (2001)
Grower pigs	0.10	↓ Dissected fat, 10% ↑ Dissected lean of carcass, 7%	Yu et al. (2001)
Finisher pigs	0.18	↓ Dissected fat, 12.5% ↑ Dissected lean of carcass, 3%	Yu et al. (2001)
Barrows; >30 kg	0.13-0.50	↑ Carcass protein ↑ Protein deposition rate ↑ Lean gain efficiency ↓ Carcass fat, 10% ↓ Fat depth, 26% ↓ Viscera weight	Fernandez-Figares et al. (2002)
Barrows, gilts; 83-116 kg	0.10	↓ Fat depth	Lawrence et al. (2002)
Barrows; >64 kg	0.15	↑ Lean tissue deposition ↑ Lean meat yield	Suster et al. (2004)
Grower pigs; 20-64 kg	0.10-0.20	↑ Carcass lean, longissimus muscle area ↓ Carcass fat, fat depth	Yu et al. (2004)
Finishing barrows; >62.5 kg	0.13	↓ Carcass fat, 8.1% ↓ 10 th rib backfat thickness, 8.8%	Feng et al. (2006)
Barrows, gilts; 55-90 kg	0.13	↑ Carcass lean, longissimus muscle ↓ Carcass fat, backfat thickness	Huang et al. (2006)
Boar, gilts; >58 kg	0.13	↑ Lean tissue, 5%	Dunsha et al. (2007)
Gilts; >65 kg	0.02-0.06	↑ Saturated fatty acids ↓ Unsaturated fatty acids	Hur et al. (2007)
Gilts; 20-50 kg	0.50	-	Fernandez-Figares et al. (2008)
Barrows, gilts; 55-90 kg	0.13	↑ Carcass lean, loin muscle area ↓ Carcass fat, backfat thickness	Huang et al. (2008)

-, No effect ($p>0.05$); ↑, increase ($p<0.05$); ↓, decrease ($p<0.05$).

Table 4. Effect of supplemental betaine in the diet on carcass characteristics of poultry

Animal	Betaine level (%)	Betaine effects	Reference
Broilers	0.05-0.15	↓ Percentage fat ↑ Breast yield	Virtanen and Rosi (1995)
Broilers	0.08	↑ Breast yield	Virtanen and Rosi (1995)
Broilers; female	0.05	↑ Carcass yield	Esteve-Garcia and Mack (2000)
Broilers; male			Waldroup and Fritts (2005)
0-14 d	0.10	-	
0-35 d	0.10	-	
0-42 d	0.10	↑ Dressing percentage	
0-49 d	0.10	-	
Broilers; unsexed	0.04-0.07	↑ Carcass yield ↑ Feather weight ↑ Protein muscle tissue	Attia et al. (2005)
Broilers; unsexed	0.07-0.14	↓ Abdominal fat ↑ Serum total protein	Hassan et al. (2005)
Broilers; male	0.05-0.10	↑ Breast yield	Pirompud et al. (2005)
Broilers; male			Waldroup et al. (2006)
0-14 d	0.10	-	
0-35 d	0.10	-	
0-42 d	0.10	↑ Breast yield	
0-56 d	0.10	↑ Breast yield	
Broilers; male	0.05	↑ Breast yield ↓ Abdominal fat	Zhan et al. (2006)
Turkeys	0.10	↑ Breast yield	Remus (2001)
Turkeys	0.09	↑ Breast yield	Noll et al. (2002)
Meat ducks; female	0.50	↓ Abdominal fat ↑ Breast yield	Wang et al. (2004)

-, No effect ($p > 0.05$); ↑, increase ($p < 0.05$); ↓, decrease ($p < 0.05$).

compounds in liver and muscle such as carnitine and creatine (Xu and Zhan, 1998; Gu and Li, 2003; Zhan et al., 2006). Carnitine is directed to the transport of long-chain fatty acids across the inner membrane of mitochondria where fatty acid oxidation takes place (Stryer, 1988; Gu and Li, 2003; Wang et al., 2004). Accordingly, increased hormone-sensitive lipase activity has been observed in pigs (Huang et al., 2006) and poultry (Zhan et al., 2006) following dietary betaine supplementation. Moreover, Zou et al. (1998) found that betaine enhanced lipase activity and decreased the concentration of triacylglycerols and cholesterol in serum of laying hens. This is in agreement with results obtained by Skomia and Gagucki (2003) who observed lower levels of poly-unsaturated fatty acids and higher levels of mono-unsaturated fatty acids in meat and back fat of growing-finishing pigs. In a more recent study, Huang et al. (2008) reported that the reduction in fat deposition in pigs due to betaine supplementation might result from a decrease in the rate of lipogenesis by adipose tissue, resulting from a reduction in the activities and gene expression of lipogenic enzymes, as evidenced by the decrease in fatty acid synthase mRNA expression.

METABOLIC EFFECTS OF BETAINE

Betaine as a methyl group donor

There are several reviews describing the underlying mechanisms involved in the regulation of methyl group transfer (e.g. Kidd et al., 1997; Simon, 1999). In this review, the main focus will be on betaine's function as methyl group donor. Methionine, choline and betaine are the most important carriers of preformed, transferable methyl groups in diets for livestock. However, methionine is used for protein synthesis, whereas choline predominantly serves for the formation of cell membranes and neurotransmitters (e.g. Stryer, 1988). Dietary betaine may be directly used as methyl group donor, whereas choline needs to be converted to betaine in a two-step enzymatic reaction occurring mainly in the mitochondria of liver cells (Kidd et al., 1997). The methyl group transfer (Figure 2) depends on the activation of methionine to S-adenosyl methionine, which transfers its methyl group to an acceptor for the synthesis of various substances such as creatine, carnitine, phosphatidylcholine and epinephrine (Stryer, 1988; Kidd et al., 1997). During this reaction, S-adenosyl methionine is

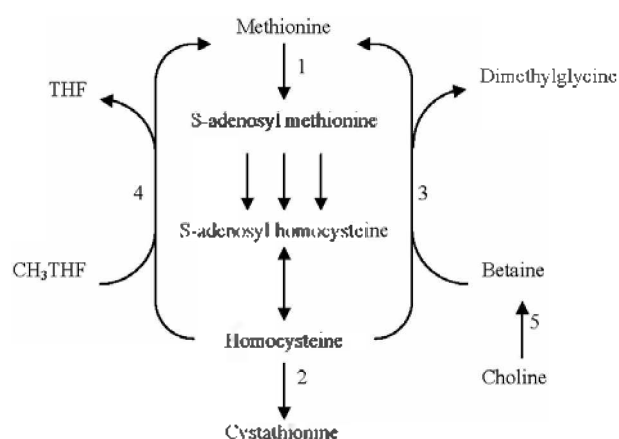


Figure 2. Pathway linking methionine, choline and betaine metabolism. The enzymes assayed are 1) methionine adenosyltransferase (EC 2.5.1.6); 2) cystathionine β -synthase (EC 4.2.1.22); 3) betaine-homocysteine methyltransferase (EC 2.1.1.5); 4) 5-methyltetrahydrofolate methyltransferase (EC 2.1.1.3); and 5) choline oxidase (EC 3.1.1.27). THF, tetrahydrofolate; CH₃THF, methyltetrahydrofolate (adapted from Saunderson and Mackinlay, 1990).

degraded to S-adenosyl homocysteine and subsequently to homocysteine which competes for two different metabolic pathways. Firstly, during the transsulfuration pathway, homocysteine can be irreversibly transformed to cystathionine and afterwards to cysteine which, in turn, can be utilized for protein synthesis. Secondly, homocysteine can be re-methylated to form methionine, either via the betaine pathway through betaine-homocysteine-methyltransferase (BHMT; EC 2.1.1.5) or by means of the tetrahydrofolate pathway (involving folates and vitamin B12) through methyltetrahydrofolate-homocysteine-methyltransferase (THFMT; EC 2.1.1.3), (Finkelstein and Martin, 1984; Xue and Snoswell, 1985; Snoswell and Xue, 1987; Kidd et al., 1997; Lewis, 2003). The BHMT specifically catalyses the transport of the preformed labile methyl group from the betaine molecule to homocysteine (Eklund et al., 2005). The methyl group transfer results in the transformation of betaine to dimethylglycine which still contains two methyl groups. These methyl groups can be split off via oxidation as one-carbon fragments. During this reaction, dimethylglycine is degraded to sarcosine and finally to glycine. The one-carbon fragments may be used to synthesize methyl groups *de novo* in the form of methyltetrahydrofolate, which are transferred via the enzyme THFMT to homocysteine to form methionine (Snoswell and Xue, 1987; Eklund et al., 2005).

Following betaine supplementation, the activity of BHMT increased in pigs fed diets which were either adequate (Emmert et al., 1998) or deficient in their methionine content (Feng et al., 2006). Similar effects were observed in broiler chickens fed methionine adequate or

deficient diets (Emmert et al., 1996). These results suggest that pigs and poultry may have a specific requirement for preformed labile methyl groups. Increasing dietary betaine supplementation levels from 0.08 to 0.13% to diets deficient in methionine but adequate in total sulfurous amino acids or to diets deficient both in methionine and total sulfurous amino acids increased total plasma protein concentration in broiler chickens, indicating a higher total remethylation rate (El-Husseiny et al., 2007). Moreover, addition of betaine to diets for poultry deficient both in methionine and cystine or adequate in methionine but deficient in cystine increased total homocysteine remethylation through both BHMT and THFMT pathways. However, based on the difference in molecular weight of methionine formed through BHMT and THFMT, the THFMT pathway seems to account for most of the changes in total remethylation (Pillai et al., 2006). It is important to note that the diets used by Pillai et al. (2006) contained a lower level of cystine (0.31%) compared with previous experiments in poultry (0.48% cystine) (e.g. Emmert et al., 1996). Dietary cystine has been shown to lower the activity of cystathionine beta-synthase, thereby decreasing the conversion of homocysteine to cystathionine and subsequently to cystine. Consequently, dietary cystine increases the availability of homocysteine for remethylation (Yamamoto et al., 1995). Moreover, the magnitude of homocysteine remethylation due to betaine supplementation was more pronounced in situations of methionine rather than sulfurous amino acids deficiency (El-Husseiny et al., 2007). Obviously, the dietary cystine level has a major impact on the degree of homocysteine remethylation.

Methionine sparing effect of betaine

The indispensable amino acid methionine and its metabolites are involved in multiple fundamental biological processes including protein deposition and the synthesis of S-adenosyl methionine (Finkelstein and Mudd, 1967). S-adenosyl methionine can either be utilized by multiple transmethylation reactions or be metabolized to spermidine and spermine (Finkelstein, 1998). Methionine provides sulfur for the synthesis of cysteine via the reaction of serine with homocysteine to form cystathionine (Kidd et al., 1997). In addition, methionine is important for cellular and humoral immunity, with the methionine requirement for immune response being even higher than that for growth (Tsiagbe et al., 1987).

Both, the protein synthesis and the formation of S-adenosyl methionine compete for the available methionine (Finkelstein, 1998). Therefore, any alternative methyl group donors may either substitute methionine as a methyl group donor, or provide the methyl groups which are necessary for the conversion of homocysteine to methionine (McDevitt et al., 2000). However, the potential of dietary betaine to spare

part of the methionine in the diet is variable and subject to considerable controversy (Campbell et al., 1995; Matthews et al., 2001b; Zhan et al., 2006).

Observations in finishing pigs fed sulfurous amino acid deficient diets indicate that betaine could replace a portion of methionine based on growth and feed conversion (Campbell et al., 1995). Accordingly, Yu and Xu (2000) reported that addition of betaine or methionine to the basal diet improved weight gain, feed intake and feed conversion by 11.8, 8.7 and 2.8%, respectively, compared with a basal diet without methionine supplementation. In contrast, Emmert et al. (1998) reported that betaine did not improve growth traits in growing pigs fed diets marginally deficient in methionine which is in agreement with the results of other studies in pigs (Alaviuhkola and Suomi, 1990; Matthews et al., 2001b). Moreover, Eklund et al. (2006a) showed that the supplementation of DL-methionine to a basal diet deficient in methionine and low in compatible osmolytes was more efficient in improving N retention than the replacement of DL-methionine by betaine originating from betaine monohydrate or condensed molasses solubles.

In contrast to pigs, the methionine sparing effect of betaine in poultry has been more thoroughly investigated. Some studies revealed that betaine could be as effective as methionine in promoting growth and feed efficiency in broilers (Pesti et al., 1979; Zhan et al., 2006) and starter ducks (1-21 d) (Wang et al., 2004) fed diets marginally deficient in methionine. Interestingly, Virtanen and Rosi (1995) and Virtanen and Rumsey (1996) concluded that supplementation of betaine to a broiler diet marginally deficient in methionine is more effective in promoting growth and feed efficiency than methionine. According to Garcia et al. (1999), the relative bioavailability of betaine compared with methionine is 50-67% in broiler chickens, based on weight gain and feed conversion. Accordingly, Attia et al. (2005) showed that in slow growth type chickens, supplementation of either 0.07% betaine or 0.05% methionine improved weight gain and feed conversion compared with the basal diet marginally deficient in methionine. In this study, dietary levels of methionine could be lowered from 0.42% to 0.37% or even to 0.32% provided that the diet was supplemented with betaine. In a more recent study, the addition of 0.05 to 0.08% betaine to a diet containing 0.33% methionine improved weight gain and feed efficiency of broilers, whereas betaine supplementation to a diet containing 0.45% methionine, did not affect performance (El-Husseiny et al., 2007). However, the results obtained could not be compared with a basal diet without supplemental betaine. On the other hand, Schutte et al. (1997) reported that supplementation of methionine to a basal diet deficient in methionine was more efficient in improving growth performance and breast meat yield of broiler chickens, which is in agreement with other studies in

poultry (McDevitt et al., 2000; Esteve-Garcia and Mack, 2000).

Choline sparing effect of betaine

Choline is a precursor of betaine, and is essentially required for a number of physiological functions such as membrane synthesis or formation of acetylcholine (Simon, 1999). Choline can act as a methyl group donor and contribute to methylation reactions, provided it is converted to betaine in the mitochondria (Kidd et al., 1997). According to studies in pigs (Tiihonen et al., 2001; Siljander-Rasi et al., 2003) and poultry (Saarinen et al., 2001), the oxidation of choline to betaine may be a rate-limiting step in the synthesis of betaine, as betaine is more efficient in increasing liver betaine levels in comparison to an equi-molar amount of choline. Moreover, betaine can only substitute for the methyl group donor function of choline because betaine cannot be converted to choline for the formation of substances such as phosphatidyl choline (Lowry et al., 1987; Dilger et al., 2007).

Emmert et al. (1998) supplemented diets severely deficient in methionine but adequate in choline with either 0.34% betaine or 0.30% choline to supply the same amount of methyl groups. They obtained a similar growth response for both treatments in comparison to the control indicating that betaine can spare choline, presumably by supplying the portion of choline required as methyl group donor (Emmert et al., 1998). In contrast, replacing choline with betaine did not lead to an improved growth performance in finishing pigs fed a corn-soybean meal diet, as was observed for choline (Hall et al., 1997).

The addition of betaine to a choline-free diet did not improve growth performance of broiler chickens (Dilger et al., 2007). Moreover, when the combination of betaine and choline is supplemented to a choline-free basal diet, 50% of total dietary choline can be substituted by betaine, while the remaining 50% must be supplied as choline, to obtain similar growth performance. In contrast, according to results obtained in other studies, only 25% of total dietary choline in diets for broilers could be replaced by betaine (Lowry et al., 1987; Hassan et al., 2005). Furthermore, betaine was as effective as choline in improving breast meat yield in male broiler chickens, regardless of the dietary methionine content (Waldroup et al., 2006; Dilger et al., 2007). In laying hens, supplementation of betaine to diets with no added choline could maintain laying hen performance during peak production (Hruby et al., 2005).

A higher efficacy of betaine as methyl group donor in poultry than in pigs may be attributed to considerable differences in the choline requirement between pigs (0.03-0.06%; NRC, 1998) and poultry (0.075-0.13%; NRC, 1994). Based on these values, it is obvious that commercially available feed ingredients provide sufficient choline to fulfil

these requirements for pigs (NRC, 1998). However, total choline content may not always represent the amount of choline available for oxidation to betaine since most of the choline is bound to phospholipids (e.g. Emmert and Baker, 1997; Zhang and Wilson, 1999). A higher efficacy of betaine as methyl group donor in poultry than in pigs may be partly due to the use of ionophore coccidiostats in poultry diets which inhibit the activity of choline oxidase (Tyler, 1977). Under this condition, betaine supplementation might be required for an adequate supply of labile methyl groups.

BETAINE AS AN OSMOPROTECTANT

Osmoregulation

Osmoregulation is the ability of a cell to maintain its structure and function by regulating movement of water in and out of the cell (Kidd et al., 1997). Changes in cell water volume are known to change intracellular ionic strength, which may affect the conformation of proteins and enzymes in the cell (Biggers et al., 1993). For example, a slight increase in the volume transforms cells into a more anabolic state, whereas the reverse may happen with loss of water (Häussinger, 1998). Therefore, water homeostasis is an important factor for cells exposed to different osmotic conditions (Klasing et al., 2002).

The osmoregulation has evolved to utilize organic compounds for osmotic support (Dawson and Baltz, 1997). Organic osmolytes belong to a few classes of organic molecules including amino acids and their derivatives (e.g. betaine, carnitine) and methylamines (Biggers et al., 1993; Pichereau et al., 1999). Organic osmolytes are highly soluble molecules which, in contrast to inorganic salts, can reach high intracellular concentrations without disturbing vital cellular functions, such as DNA replication and cellular metabolism (Kempf and Bremer, 1998). The term "osmoprotectant" or "compatible osmolyte" has been coined for molecules which can be accumulated in large amounts in cells and protect against osmotic stress (Landfald and Strom, 1986). Betaine is considered to be the most effective osmoprotectant among other organic osmolytes such as glycine, proline, glutamine and taurine (Chambers and Kunin, 1985; Dawson and Baltz, 1997; Hammer and Baltz, 2002).

Betaine as an osmoprotectant for the intestinal cell

Intestinal cells always have to cope with variable osmotic media since the luminal content of the intestine is hyperosmotic in relation to blood plasma (Mongin, 1976). Moreover, the process of nutrient digestion and absorption requires osmolytic protection mechanisms since intestinal cells mediate the exchange of water, small solutes such as

ions, nutrients and macromolecules between plasma and intestinal fluid. Betaine is thought to be an important organic osmolyte for the control of the osmotic pressure inside the intestinal epithelial cells (Hochachka and Somero, 1984). Dietary betaine enhances the concentration of betaine in the intestinal epithelium (Klasing et al., 2002; Clow et al., 2008). The Na⁺ dependent active transport system of betaine is present in the duodenum and jejunum of broiler chickens, indicating that betaine is involved in the osmoregulation of the small intestine (Kettunen et al., 2001a). Along the small intestine, the osmotic pressure in the duodenum is higher than in the ileum (Mongin, 1976). Accordingly, the highest level of betaine was found in the duodenum, whereas the betaine concentration in the ileum was very low (Kettunen et al., 2001a; Klasing et al., 2002).

Betaine exerts an osmoprotective effect by accumulating in cell organelles and in cells exposed to osmotic and ionic stress, thereby replacing inorganic ions, and thus protecting enzymes as well as cell membranes from inactivation by inorganic ions (Petronini et al., 1992). Compatible osmolytes such as betaine increase the cytoplasmic volume and free water content of the cells at high osmolarity, and thus permit cell proliferation under stress conditions (Csonka, 1999). Additionally, betaine serves as a stabilizer of protein and cell components against the denaturing effects of high ionic strength (Kempf and Bremer, 1998). The presence of betaine in the intestinal tissue of pigs may reduce the energy requirement for ion-pumping, hence lowering the energy requirement for maintenance and providing more energy for intestinal cell proliferation (Siljander-Rasi et al., 2003). Accordingly, the accumulation of betaine increased water-binding capacity of the intestinal cells and promoted changes in the structure of the intestinal epithelium (Kettunen et al., 2001a). Moreover, the tensile strength of coccidian-challenged chickens (Remus and Quarles, 2000) and the tensile strength of the proximal ileum in pigs was improved (Siljander-Rasi et al., 2003). Fernandez-Figares et al. (2002) showed that supplemental betaine at a level of 0.13% increased small intestinal weight of pigs, whereas at higher levels a decrease in the weight of the small intestine was reported. According to Xu and Yu (2000), the villus height was increased in the duodenum of weaned pigs, the villi were more uniform and a 52% higher activity of proteolytic enzymes was observed following betaine supplementation. Furthermore, supplemental betaine resulted in a decrease in the crypt:villus ratio in both coccidian-infected and healthy chickens (Kettunen et al., 2001b), and the lesion score was reduced in coccidian-infected chickens as well (Virtanen and Rosi, 1995). Moreover, increased intestinal cell proliferation provides an enlarged surface for nutrient absorption. Since processes of nutrient absorption are

dependent on an intact gut epithelium, the osmotic characteristics of betaine may contribute to an improved nutrient digestibility (Eklund et al., 2006a, b).

Betaine as an osmoprotectant for intestinal microbes

The cytoplasmic membrane of microbes is permeable to water but not to other metabolites, and therefore, changes in the environmental osmolarity increase the flux of water across the cytoplasmic membrane (Csonka, 1989; Glaasker et al., 1996). The cytoplasmic water content in microbes affects the activities of protein and other biological macromolecules, which, in turn, determines the ability of the microbes to proliferate (Csonka, 1989). Microbes maintain an osmotic pressure in the cytoplasm that is higher than that of their surrounding environment by varying the concentrations of compatible osmolytes, in order to support a constant turgor pressure (Glaasker et al., 1996). The accumulation of compatible osmolytes not only allows the cells to withstand osmotic stress but also expands the ability of microorganisms to colonize ecological niches that are otherwise strongly inhibitory for their proliferation (Kempf and Bremer, 1998). Betaine is an effective osmolyte in many Gram-positive and Gram-negative bacteria, which can be accumulated by *de novo* synthesis or by transport from the environment (Csonka, 1989). The accumulation of betaine is stimulated by osmotic stress, as it has been observed in *Enterobacteriaceae* (e.g. Le Rudulier and Bouillard, 1983; Csonka, 1989; Pichereau et al., 1999), *Lactobacillus acidophilus* (Hutkins et al., 1987; Glaasker et al., 1996) and *Bacillus subtilis* (Boch et al., 1994). Perroud and Le Rudulier (1985) found that the intracellular concentration of betaine maintained by *Escherichia coli* was proportional to the osmolarity of the medium, and the addition of betaine alleviated the inhibitory effects of osmotic pressure. Moreover, in media with high salt concentrations, exogenous betaine stimulated growth rate of various members of *Enterobacteriaceae*, including *E. coli* (Le Rudulier and Bouillard, 1983). Similar to intestinal cells, intestinal microbes are exposed to various osmotic conditions in the gastrointestinal tract as well (Eklund et al., 2006b). Thus, betaine may support intestinal microbes in coping with osmotic stress due to its ability to maintain cell turgor in media of high osmolarity (Csonka, 1989).

Effect of betaine on nutrient digestibility and intestinal microbial fermentation

The effect of betaine on nutrient digestibility is presented in Table 5. The osmoprotective properties of betaine are likely to influence nutrient digestibility by supporting intestinal cells as well as growth and survival of intestinal microbes. Several studies showed improvements in ileal or total tract digestibility of dry matter or organic matter in pigs (Xu and Yu, 2000; Eklund et al., 2006a, b;

Mosenthin et al., 2007; Ratriyanto et al., 2007a) and poultry (El-Husseiny et al., 2007). These increases in dry matter or organic matter digestibilities correspond to higher digestibilities of other nutrients. For example, supplemental betaine improved ileal (Eklund et al., 2006b) and total tract crude protein digestibility in piglets (Xu and Yu, 2000). In poultry, increasing dietary betaine levels from 0.05 to 0.10% improved crude protein digestibility as well (El-Husseiny et al., 2007). Moreover, the supplementation of betaine to a basal diet, which was deficient in methionine and sulfurous amino acids and low in compatible osmolytes such as choline and betaine, improved total tract amino acid digestibilities in piglets (Eklund et al., 2006a). Furthermore, in studies with piglets, the addition of betaine to a diet adequate in methionine and sulfurous amino acid contents (NRC, 1998), enhanced ileal (Eklund et al., 2006b) and total tract (Ratriyanto et al., 2007b) digestibility of several amino acids. Improved amino acid digestibilities such as for lysine and methionine have been observed in broiler chickens fed diets supplemented with betaine during coccidial infection (Remus et al., 1995; Augustine and Danforth, 1999). Higher digestibilities of crude protein and amino acids following betaine supplementation may result from osmotic support of intestinal cells (Eklund et al., 2005; 2006b). In contrast, other studies showed a decrease in ileal digestibility of several amino acids in pigs after betaine supplementation, which may indicate enhanced microbial assimilation of protein and amino acids (Eklund et al., 2006b).

Betaine supplementation increased ileal and total tract crude fiber, neutral detergent fiber and acid detergent fiber digestibilities in pigs ranging between 4.3 and 17.9% (Eklund et al., 2006a, b; Ratriyanto et al., 2007a, b). Increasing dietary betaine supplementation levels from 0.05 to 0.08% to sulfurous amino acid adequate diets improved crude fiber digestibility in broiler chickens as well (El-Husseiny et al., 2007). Since intestinal cells of pigs and poultry do not produce fiber degrading enzymes, the improvement in the digestibility of fiber along the digestive tract suggests that betaine stimulates the microbial fermentation of fiber components in the gastrointestinal tract (Eklund et al., 2006a, b). Accordingly, ileal and fecal ornithine concentrations tended to be higher (Ratriyanto et al., 2007b) or were increased (Eklund et al., 2006a) due to dietary betaine supplementation in piglets, indicating higher cell counts of Gram-positive bacteria. Other observations revealed a tendency for higher ileal diaminopimelic acid concentrations and increased fecal diaminopimelic acid concentrations following dietary betaine supplementation, indicating enhanced cell counts of Gram-negative bacteria in the digestive tract of piglets (Mosenthin et al., 2007; Ratriyanto et al., 2007b). However, according to other reports, betaine did not influence ileal (Eklund et al., 2006b)

Table 5. Effect of supplemental betaine in the diet on nutrient digestibility in pigs and poultry

Animal	Betaine level (%)	Betaine effects	Reference
Pigs			
Pigs; 42-50 kg; total tract	0.13	-	Overland et al. (1999)
Piglets; 5 weeks; total tract	0.08	↑ DM, CP	Xu and Yu (2000)
Barrows; 13.5-15.1 kg			Eklund et al. (2006a)
Total tract (Bet)	0.30	↑ OM, CA, NDF, ADF, NFE, AA, minerals	
Total tract (CMS)	0.30	↑ CA, NDF, ADF, NFE, minerals	
Barrows; 8 weeks; 8.5-10.6 kg			Eklund et al. (2006b)
Ileal (Bet)	0.25	↑ CA, NDF, ADF ↓ EE, several AA	
Ileal (CMS)	0.25	↑ DM, CA, CP, NDF, ADF, AA	
Total tract (Bet)	0.25	↑ DM, CA, NDF, ADF ↓ EE	
Total tract (CMS)	0.25	↑ CA, NDF, ADF	
Barrows; 5 weeks; initial BW 9.7 kg			Mosenthin et al. (2007)
Ileal	0.45	↑ DM, ↑CF (trend)	
Total tract	0.45	↑ CF (trend)	
Barrows; 5 weeks; initial BW 9.7 kg			Ratriyanto et al. (2007a)
Ileal	0.15-0.60	↑ OM, CF, glycine	
Total tract	0.15-0.60	↑ Proline	
Barrows; 5 weeks; initial BW 9.7 kg			Ratriyanto et al. (2007b)
Ileal	0.15-0.60	↑ CF	
Total tract	0.15-0.60	↑ AA	
Grower pigs; 30 kg; total tract	0.50	-	Fernandez-Figares et al. (2008)
Poultry			
Broilers	0.15	↑ CP, EE, lysine, carotenoid	Remus et al. (1995)
Broilers	0.15	↑ Methionine	Augustine and Danforth (1999)
Unsexed broilers	0.04-0.07	-	Attia et al. (2005)
Unsexed broilers	0.07-0.14	-	Hassan et al. (2005)
Unsexed broilers	0.05-0.10	↑ OM, CP, EE, CF, NFE	El-Husseiny et al. (2007)

-, No effect ($p > 0.05$); ↑, increase ($p < 0.05$); ↓, decrease ($p < 0.05$)

DM = Dry matter; OM = Organic matter; CA = Crude ash; CP = Crude protein; EE = Ether extracts; CF = Crude fiber; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; NFE = Nitrogen-free extracts; AA = Amino acids; BW = Body weight.

Ileal = Ileal digestibility; Total tract = Total tract digestibility; Bet = Betaine monohydrate; CMS = Condensed molasses solubles.

and fecal diaminopimelic acid concentrations (Eklund et al., 2006a) but alleviated fecal diaminopimelic acid levels in piglets (Eklund et al., 2006b). In poultry, supplemental betaine decreased the total number of bacteria in the crop, associated with an increase of Gram-positive bacteria such as *Enterococci* (Kettunen et al., 1999).

A higher microbial fermentation activity due to betaine supplementation is also reflected in higher levels of microbial fermentation products in ileal digesta of pigs such as ileal lactic acid (Ratriyanto et al., 2007b) and short-chain fatty acids (Mosenthin et al., 2007). However, according to these authors there was no effect on fecal short-chain fatty acid concentrations. Similar to pigs, higher contents of short-chain fatty acids have been observed in the ileal and cecal contents of broiler chickens (Kettunen et al., 1999).

The improvement in fiber digestibility following betaine supplementation is associated with higher crude ash and mineral digestibilities (Eklund et al., 2006a, b). The fiber

fraction holds nutrients which may be released during microbial fiber degradation (Wenk et al., 1993; Aulrich and Flachowsky, 1997). Additionally, short-chain fatty acids, originating from microbial fiber fermentation, may promote nutrient absorption due to electrophysiological changes in the enterocytes, resulting in improved mineral absorption (Butzner et al., 1994) and reduced endogenous secretion of minerals (Krishnan et al., 1999).

Observations on the effect of betaine on ether extract digestibility revealed inconsistent results. In piglets, betaine tended to improve digestibility of ether extract compared with a basal diet deficient in methionine and sulfurous amino acids and low in the content of compatible osmolytes (Eklund et al., 2006a). In contrast, Overland et al. (1999) reported that supplemental betaine did not enhance the total tract digestibility of ether extract in methionine and sulfurous amino acids adequate diets for grower pigs. According to other reports, supplemental betaine decreased

ether extract digestibilities in methionine and sulfur amino acids adequate diets both at the ileal and fecal level (Eklund et al., 2006b). On the other hand, a higher ether extract digestibility in broiler chickens was observed, when 0.05 or 0.1% betaine was added to a methionine-deficient diet (El-Husseiny et al., 2007). According to Rorvik et al. (2000), an improvement in ether extract digestibility could be due to an increased bile acid secretion. Moreover, any increase in microbial short-chain fatty acid levels along the digestive tract may improve the absorption capacity of the intestinal epithelium, thus increasing the digestive capacity (Butzner et al., 1994). On the other hand, microbial activity is associated with deconjugation of bile acids which are required for lipid digestion and absorption, thus resulting in lower ether extract digestibility (Jönsson et al., 1995).

CONCLUSION

Over the past decades, numerous studies have been performed to investigate potential effects of betaine supplementation on animal performance. Due to its chemical structure, betaine has osmoprotective properties, thus protecting intestinal cells and microbes and hence counteracting performance losses during heat stress and coccidiosis. Thus, application of betaine under sub-optimal conditions and exposure to osmotic stress may have a positive impact on livestock production. Moreover, both intestinal cells as well as intestinal microbes are exposed to various osmotic conditions in the gastrointestinal tract. The osmotic properties of betaine may be essential to support intestinal growth, function and increased cell proliferation. Furthermore, betaine promotes intestinal microbes against osmotic variations and improves microbial fermentation activity, which in turn, may enhance nutrient digestibility. As a methyl group donor, betaine is involved in methylation reactions and has the potential to improve the availability of methionine and choline. Therefore, betaine spares other methyl group donors such as methionine and choline. Other potential benefits of the inclusion of betaine in monogastric animals' diets are reductions in carcass fat content associated with increases in the lean carcass percentage which may be of interest to satisfy consumer needs.

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