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PIECING TOGETHER THE BETAINE PUZZLE

Many recognise the name, few so far can identify its properties, but the use of Betaine in animal feeds is receiving more and more attention, as the evidence begins to fit together.

by Erkki Virtanen

Known modestly as betaine, the compound glycine betaine, trimethyl glycine, is in simple terms a provitamin. Present in all living organisms, although to highly variable quantities, it functions as a source of methyl groups for methylation reactions. Recently, new applications for the use of betaine have been presented. These include sparing of methionine in poultry diets, reduced osmotic disorder and improved nutrient utilization under stress. The physiological basis for these responses cannot be found in text books for nutritionists, but a broader review to the recently published knowledge on betaine provides a better perspective.

The structure of betaine is represented in figure 1. The molecule is bipolar and contains three reactive methyl groups. It is the transfer of these methyl groups during enzyme-catalysed reactions that confers unique properties to betaine.

Betaine as a methyl donor

Betaine has been long known as a methyl donor by nutritionists and, consequently used as a partial replacement for choline. Choline is required as part of acetylcholine, the neurotransmitter. To fulfil its function as a methyl donor, choline has first to be converted in the mitochondria, and thus betaine, as a direct methyl donor may be more effective. Furthermore, many compounds in practical diets, including ionophores, may inhibit choline oxidation in mitochondria, resulting in its lower availability for methylation reactions.

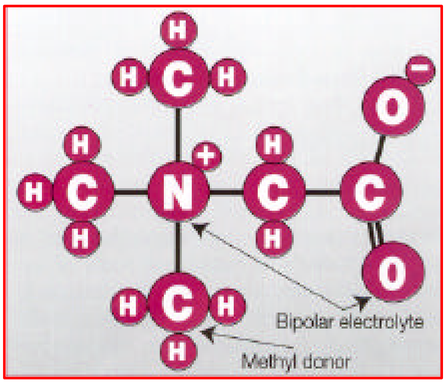

Figure 1 Structure of the betaine molecule. Arrows indicate metabolically active sites

While choline is generally considered as the major source of methyl groups in practical diets, published literature demonstrates a very variable availability of choline for methylation reactions. Early work of Stekol et al (1953) reported that the extent of methyl group incorporation from choline in chicks was only a fraction of that observed with either methionine or betaine. Accordingly, choline was markedly less effective than betaine to methylate homocysteine to methionine in chicks.

Specific requirements of choline are not accurately defined, but studies of Baker et al (1983) and Lowry et al (1987) indicate a level of 400-600 ppm. In commercial poultry feeds the amount of choline from raw materials varies normally within the range of 1000-1500 ppm of which 60-75% is available (Molitoris and Baker, 1976). Hence, in practical diets the specific requirement for choline is met with background choline. Several trials have indicated that replacement of supplementary choline with betaine has no adverse effects on performance in commercial poultry operations.

Betaine as an osmo-protectant

While most organisms contain betaine, few accumulate the compound in high concentrations. Plants belonging to the Chenopodiaceae family, of which sugar beet is the most familiar, are among the best known accumulators. Some microbes and marine invertebrates also contain high concentrations.

by Erkki Virtanen

The main reason for betaine accumulation in these organisms is to protect the cells from osmotic stress in conditions of drought or in high salinity. The osmoprotectant function of betaine has been extensively described in the literature regarding osmotic regulation of plants, microbes and animals, but

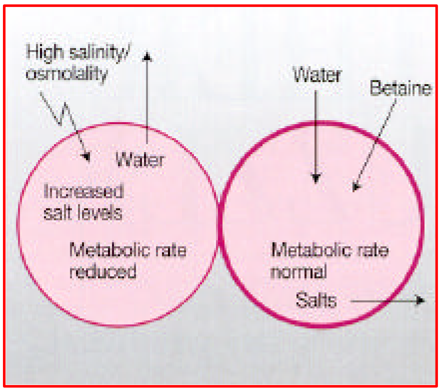

Figure 2 *Action of betaine as an osmo-protectant. Under osmotic stress, diffusion of water from the cell causes increased concentration of organic salts, and enzyme inhibition. Betaine prevents this by increasing osmotic strength of the cell*

has so far raised little attention among nutritionists. Figure 2 illustrates the mechanism of osmo-protection. In this function, betaine accumulates into cells and cell organelles exposed to osmotic stress, replaces inorganic ions and protects macromolecules from ionic inactivation.

Take, for example, spinach, which is grown in saline soil. The accumulation of betaine at levels of up to 3% of their fresh weight enables the chloroplasts to photosynthesise in conditions where they would otherwise be prevented (McCue & Hanson 1990). As another example, the kidney of the rabbit accumulates betaine at a level of 0.5 % in inner medullary cells, enabling this animal to counteract the osmotic stress

The osmoprotectant function of betaine has so far raised little attention among nutritionists

Sugar Beet, a rich source of betaine, is the origin of commercial production

caused by concentrated urea (Yancey et al, 1982). When salmon liver mitochondria are exposed to osmotic stress, they display an active uptake of betaine. Under these conditions, metabolic activity is reduced to a much lesser degree than when betaine is not present (Björköy, 1991) Consequently, betaine supplementation has been widely adopted in fish feeding to relieve the osmotic stress of fish at seawater transfer.

Use of Betaine in animal diets

In mammals, the osmotic role of betaine is best known from its accumulation in renal medulla cells to help the cells tolerate osmotic stress connected with the production of hyperosmotic urine (Bagnasco et al, 1986). The mammalian kidney accumulates betaine mostly through active, sodium-dependent transport (Yancey, 1992). Synthesis of the compound from choline in the mitochondria does take place, but the rate of synthesis is not high enough to respond to the varying requirements for betaine, especially under stress.

A similar osmoprotective effect has been observed in animals which do not accumulate betaine through synthesis, when given betaine in the feed. Based on this evidence, and given betaine's role in the osmoregulation of many tissues and organisms, we proposed that the compound may have a stabilising function in cells suffering from osmotic and ionic imbalance.

The coccidiosis case

Our first area of extensive study was coccidiosis in poultry. Coccidiosis is one of the major intestinal diseases, and is associated with osmotic and ionic disorders. These are caused by the disease itself, but the ionophore drugs used widely to treat the disease can themselves cause ionic and osmotic changes. We looked at the effect of betaine on parasite development and growth performance, faecal water loss and nutrient digestion of coccidia-challenged chicks.

In a preliminary trial, betaine was given with various combinations of an ionophore coccidiostat, salinomycin to chicks inoculated at 14 days of age with a mixture of three Eimeria species, E. tenella, E. maxima and E. acervulina. Although betaine supplementation resulted in an overall reduction in lesion score and improvement in feed efficiency, no clear interaction was seen between betaine and salinomycin (Figure 3).

In a following trial we compared betaine to other methyl donors, methionine and choline. These were supplemented in equimolar amounts using all possible combinations. Results of the experiment are illustrated in figure 4. Of the three methyl donors, betaine alone affected lesion score significantly. This demonstrated that the response was not mediated through methylation reactions, which apparently have importance in the immune responses. However, it is important to note that like betaine, choline and methionine have specific metabolic functions where they are irreplaceable. Hence, a correct balance between betaine, choline and methionine is important.

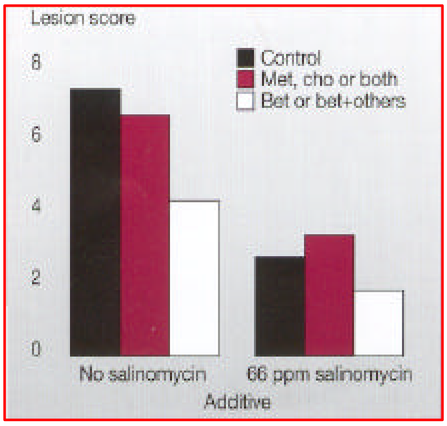

Figure 4 Effect of methyl donors on gut lesion score in coccidia-inoculated chicks

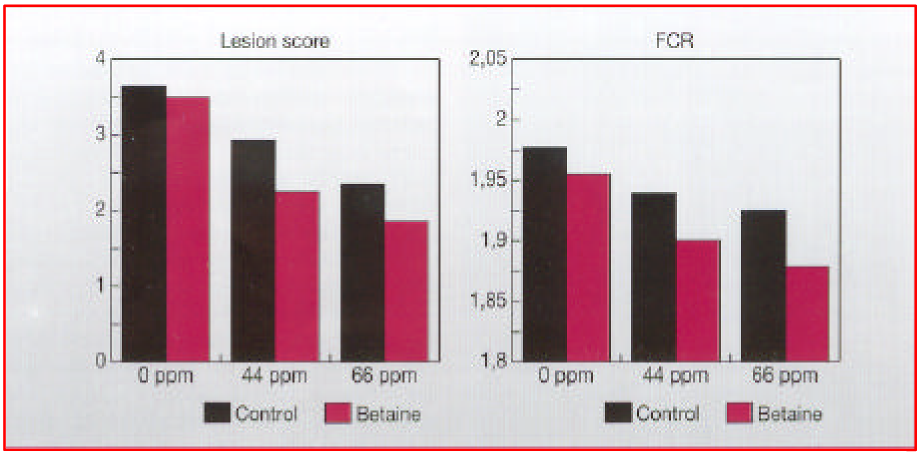

Figure 3 Effect of betaine on intestinal lesion score (at 21 days) and feed conversion ratio (it 45 days) in coccidia inoculated chicks

Restoring performance during coccidia challenge

It seemed apparent that dietary betaine affected gut status and feed efficiency in a similar fashion. Further work was therefore carried out to look at the effect of betaine on nutrient digestibility in coccidia-inoculated chicks. Chicks were orally inoculated with a mixture of three Eimeria species at 14 days of age, and digestibility of dry matter, protein, fat, lysine and carotenoid was determined. As can be seen from figure 5, addition of betaine to the diet of birds resulted in a lower faecal water content and higher digestibility of these nutrients when betaine was added to the diet. Consequently, the feed conversion efficiency of betaine-supplemented chicks was significantly improved. Although mostly effective in controlling serious outbreaks, anticoccidial treatment often does not bring feed conversion efficiency to the level of non-challenged chicks. In these circumstances which are frequently present under commercial operations, betaine addition seems to be an effective way to recover much of the "lost" feed efficiency. Betaine has been tested in combination with most commercial anticoccidials. Improved feed conversion efficiency was seen in inoculated birds treated with those anticoccidials which effectively depressed feed conversion efficiency. However, no improvement was seen with anticoccidials which did not have a depressing effect on performance. Results can be seen in figure 6. It should be stressed that these data were results from a single trial with specific Eimeria strains, and do not demonstrate the general efficacy of the studied anticoccidials.

The simple extraction of sugar beet to yield molasses is the starting point for betaine production. Using chromatography the molasses is separated into individual components based on size and physical properties. An almost pure solution of betaine is collected at the outlet point, and is then concentrated and crystallised into pure betaine.

Response may be multi -faceted

Mechanisms of the effect are not fully known. While the methylation functions appear to have little importance, only sparse evidence so far can be found on the osmoprotectant function in the chick's gut. An interesting piece of work on the effect of betaine on water retention in turkey gut has been carried out by Dr. Ferket at North Carolina State University. Trials with turkeys suffering from "flushing" problem indicated that betaine supplementation can markedly decrease water loss in diarrhoeic animals (Figure 7). Although not yet demonstrated, it would appear that gut epithelial cells can accumulate

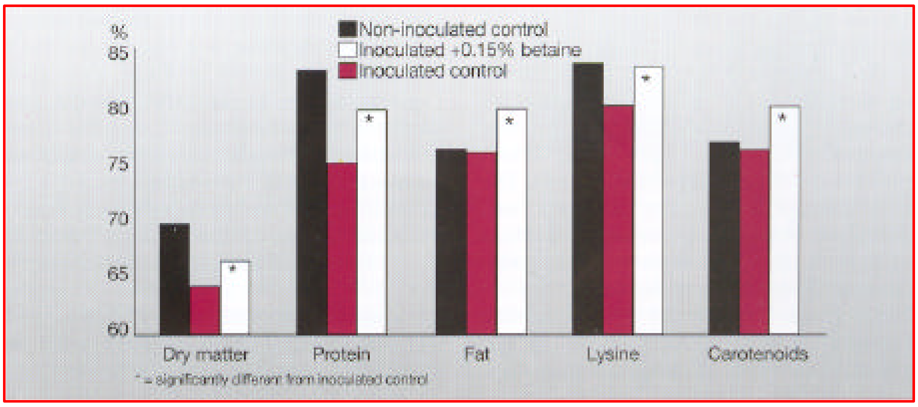

Figure 5 Digestibility of nutrients with or without betaine during coccidiosis challenge (measurements taken at 21 days)

betaine through active uptake, resulting in better water retention, lower potassium accumulation and alleviation of urea inhibition as in the kidney medulla.

Studies of the effect of betaine on in vitro and in vivo development of Eimeria species have been initiated at the United States Department of Agriculture. Preliminary data indicate that betaine has no direct inhibiting effect on the sporozoite invasion in vitro. However, in vivo betaine, supplemented chicks had markedly less sporozoites in the intestinal cross-section (Dr. Augustine, personal communication).

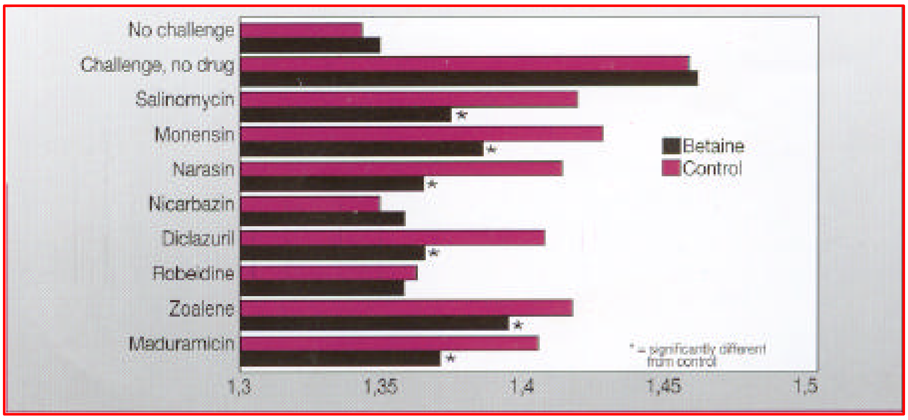

Figure 6 Effect of betaine on 3-week FCR in coccidia-challenged chicks fed with various coccidiostats

The betaine/methionine interaction

While the physiological connection between betaine and methionine has long been known, very few practical studies regarding the methionine sparing effect of betaine have been carried out until recently. Work of Pesti et al (1979) indicated that broiler chicks grown to 3 weeks of age and fed with a practical corn-soya diet did not require more than 0.37% methionine or 0.73% total sulphuric amino acids (TSAA) if methyl donors were available at high level. However, no data were available as to the efficacy of betaine versus methionine to promote growth and feed efficiency with slightly methionine-deficient diets. To date, the methionine-sparing effect of betaine has not been commonly acknowledged by the nutritionists in the poultry industry.

We have conducted several trials feeding the chicks with a basal corn-soya diet containing 75-80% of the NRC recommended methionine requirement (TSAA 85-90% of NRC), supplemented with DL-methionine or betaine. The trials have been carried out in practical floor-pen conditions with built-up litter. Interestingly, under these conditions, betaine has been more efficient than methionine in promoting growth and feed efficiency when added into the basal diet, although the diets have been adequate in choline. The betaine versus methionine response appears to be to some extent diet dependent: betaine had markedly less methionine-sparing effect in diets with rapeseed and peas as main protein sources. More importantly, coccidiosis challenge appears to be a major factor in the betaine methionine relationship. While feed efficiency in response to methionine is pronounced in completely coccidia-free conditions, even a low level of challenge has reduced methionine response and increased betaine response. Consequently, betaine has been a more efficient way than methionine to supplement slightly methionine-deficient diets in practical conditions with coccidial challenge, mostly without clinical symptoms of coccidiosis. It should be stressed that a major part of the dietary methionine and TSAA requirement must be met by sulphuric amino acids per se. Betaine supplementation of diets more deficient in sulphuric amino acids than those mentioned here results in poor responses in growth performance. In our trials we found that betaine use resulted in a reduced stress-induced loss of performance when used together with an ionophore coccidiostat for coccidia challenged chicks. Further trials in the U.S. showed that betaine also had similar effects in chicks not treated with ionophores. We concluded that betaine improves nutrient absorption in a coccidia-stressed gut, while choline and methionine do not have a similar effect.

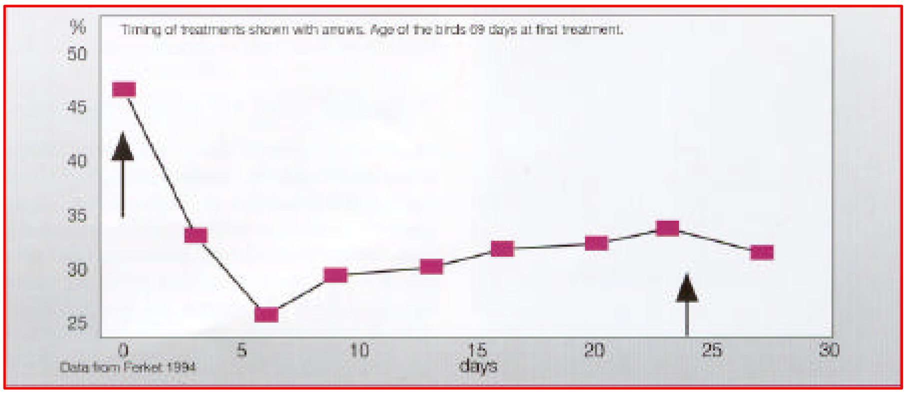

Figure 7 Effect of adding betaine to drinking water on average litter moisture in six turkey houses suffering from 'flushing' problems

Renewed opportunity for Betaine in animal feeds

While betaine as a methyl donor can stimulate methionine synthesis and thus increase its availability, its osmoprotectant function in the gut under pathogen challenge may be more important for the observed methionine-sparing effect. Osmotic disturbance is associated with several kinds of stress in animals, including gut disorders and heat stress. Utilization of organic osmolytes to alleviate these problems has in the past received little attention. However, the culmination of recent data to support the stabilising role of betaine during metabolic stress creates new opportunities for betaine in animal feeding

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