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REVIEW PAPER

Effects of Betaine Supplementation to Broiler Diets Under Heat Stress

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Abstract

Betaine is an osmoregulatory substance obtained from some natural plant materials such as sugar beet, regulating intracellular biochemical events by attracting water. Due to this important function of betaine in water balance, it may play an important role in maintaining the body water balance of birds under heat stress. On the other hand, since betaine is a good methyl donor, some essential nutrients such as methionine and choline can be saved in the diet, or they can serve as complementary substances in the deficiency of these nutrients. Previous studies have shown that the addition of betaine can make significant contributions to the nutrition of broiler chickens in heat stress situations. The effects that may occur, especially when the body needs methyl groups, may be more pronounced. Dietary betaine supplementation increases live weight gain and breast muscle efficiency, improves feed efficiency and reduces fat content in broilers. The osmoregulatory function of betaine, immune system disorders, low immune strength, reducing respiratory alkalosis, oxidative stress and intestinal microbiome changes caused by heat stress. In this study, some evaluations were made in terms of the use of betaine in broiler diets to meet the performance and physiological needs of animals.

Introduction

High environmental temperature is one of the factors that negatively affect broiler production. Feed intake decreases in birds exposed to heat stress, and as a result, the performance decreases as the chickens cannot consume enough nutrients to provide optimum performance. Since it is not possible to eliminate the economic losses related to productivity caused by heat stress due to physiological and metabolic changes, it will be possible to minimize these economic losses with structural and in-house breeding techniques and/or precautions to be taken in feeding (Kutlu *et al*., 1996). For this purpose, increasing the number of critical nutrients in the ration and supplementation of some feed additives is a common practice used by nutritionists.

One of the additives used to reduce the effect of temperature is betaine. Betaine is an osmoregulatory substance obtained from some natural plant materials such as wheat, alfalfa flour and sugar beet, regulating intracellular biochemical events by attracting water (Attia *et al.,* 2005). In the presence of sufficient betaine in the cell, osmotic balance continues without loss of water. This important function of betaine in water balance leads to a decrease in the effects of heat stress (Attia *et al.,* 2011). Betaine plays a role of methyl group donor, which is necessary in many vital functions. In this way, choline and methionine can be saved and thus economical diets can be prepared. Betaine can be tried due to its methionine-saving feature.

Studies have revealed different results regarding the digestibility and performance of betaine, immune response, and physiology of broilers in heat-stressed birds (Sakomura *et al*., 2013; Attia *et al.,* 2016; Park and Kim, 2017). In recent years, betaine has been used as a feed additive and is also available as betaine monohydrate, betaine hydrochloride and anhydrous betaine as a purified feed additive (Mishra *et al.*, 2020). Increasingly, betaine supplementation low-cost feed additives are being explored as potential amelioration strategies against heat stress.

In a significant part of our country, high temperatures in the summer season prevent especially broiler production, feed intake and live weight gain in broilers decrease and mortality rate increases. In this study, the effect of betaine, which is an osmoregulator in the diets as an anti-stress and preventing the harmful effects of high environmental temperature was emphasized. In addition, attention will be paid to the potential effects of betaine on nutrient digestibility and performance characteristics, immune responses and poultry physiology in heat stressed birds.

Effects of High Ambient Temperature on Broilers

High ambient temperature is one of the factors that negatively affect broiler production. If the ambient temperature rises above 28 ºC, it causes the deterioration of some physiological mechanisms. In this case, feed intake decreases, respiratory rate increases, and performance deteriorates due to decreased feed intake (Daghir, 1995). Based on the ambient temperature of 20-21°C, each 1°C increase in temperature between 5-35°C reduces the feed intake by 1.5 grams (NRC, 1981). The decrease in feed intake with the increase in ambient temperature is not linear, it is more pronounced at high ambient temperature. For example, at 20, 25, 30, 35 and 40 °C, the changes in feed intake were 0, 1.4, 1.6, 2.3 and 4.8% respectively, despite an increase in temperature by 1 °C (Daghir, 2008).

The reaction of poultry to temperature changes is affected by many factors. Humidity of the atmosphere, wind velocity, and readiness of birds for acclimatization are among the most important (Daghir, 2008). In general, birds can maintain themselves over a relatively wide temperature range. This range is between 10-20°C and does not differ for broilers, layers, and turkeys (Mardson and Morris, 1987). Factors originating from the external environment or internal world of the living thing, and which aim to disrupt the hemostatic balance in body are called stress factors or stressors. All the biochemical, physiological, and behavioral changes that occur in the bodies of animals exposed to stress factors to reestablish the deteriorated hemostatic balance are called stress (Leeson, 1986). Increasing the amount of critical nutrients in diet and adding some additives to the diets to protect the birds raised in hot conditions from the negative effects of heat stress and to maintain their high performance is a practice frequently used by the breeders.

In hot environmental conditions, feed and nutrient intake decrease, the vessels on the body surface expand, respiratory rate increases, respiratory alkalosis occurs, blood ionic Ca level decreases, carbonic anhydrase enzyme activity decreases in kidney and eggshell glands, and Ca mobilization from bone stores decrease (Daghir, 1995). At the same time, a decrease in Ca intake causes weakening and breaking of bones during the production period and, accordingly, significant losses in animals (Koelkebeck *et al.,* 1993).

Betaine Supplementation in Broiler Diets

Betaine is widely found in animals and plants. Sugar beet molasses, obtained as a by-product during sugar production, is the most important source of betaine (Hruby, 2002). Betaine, its generic name is trimethylglycine, and it contains three methyl groups. Methyl groups, CH_3) are indispensable elements for life and are necessary for hundreds of metabolic reactions. Methyl groups are involved in the synthesis of phospholipids, the secretion of adrenal hormones, and the construction of RNA and DNA. Methyl groups cannot be synthesized in the animal body and must be taken from the diet. The most important sources of methyl groups in the diet are methionine, choline, and betaine. The amount of methyl groups requirement increases in the presence of heat stress and coccidiosis in poultry. Methyl groups have a very important function in the transmethylation cycle. The key product of the transmethylation cycle is S-adenosyl methionine, SAM). Tissue repair, DNA and RNA synthesis, formation of immune system response, synthesis of phospholipids is the result of only a small amount of activation of SAM (Virtanen, 1999).

Betaine is an osmoregulatory substance that can easily enter animal cells and regulates intracellular biochemical events by attracting water according to its density in the cell. Heat stress causes intestinal, liver and kidney cells to lose water, increasing the density of intracellular minerals, and as a result, disruption of the work of intracellular enzymes. In the presence of sufficient betaine in the cell, osmotic balance continues without loss of water. This important function of betaine in water balance leads to a decrease in the effects of heat stress. In addition, it is reported that betaine also contributes by saving the use of choline and methionine (Obeid, 2013). It has been reported that low protein and methionine in the diet were more effective in increasing serum albumin concentration with betaine supplementation (Park *et al.,* 2019). While body water increases proportionally in hot conditions, it decreases in cold conditions. Betaine is a good osmoregulatory and helps to maintain the water balance in the body in hot conditions. By maintaining the water balance in the cells, it not only maintains the balance of other minerals, but also prevents the disruption in enzyme activity

(Silversides *et al*., 1999). Another way that can be used as a means of maintaining body temperature within certain limits is to increase the water retained in the body. While adjusting the body temperature, the amount of water retained in the body changes, while the water retained in the body increases at high ambient temperatures, it decreases at low temperatures. This important function of betaine in water balance leads to a decrease in the effects of heat stress. In a study, in broilers under natural summer stress betaine addition 2 g/kg concentration caused a significantly higher feed intake and body weight gain and better feed conversion ratio, FCR). In the same study, lower heterophile count and higher lymphocyte count with betaine supplementation. Researchers concluded betaine supplementation to the broiler diets immunity may increase under heat stress environment (Chand *et al.,* 2017).

In animals, it is found mainly in the liver and functions as a methyl donor. Betaine transfers three methyl groups to homocysteine to produce methionine. When all three methyl groups are transferred, betaine normally forms the amino acid glycine. Thanks to the production of methionine in the liver, betaine diet saves some methionine and reduces the diet cost (Virtanen, 1999; Hruby, 2002). However, there are literatures reporting that betaine has little methionine-sparing effect. In addition, the possible sparing effect of betaine on methionine may interfere with lipid metabolism (Kermanshahi, 2001). Betaine participates indirectly in the synthesis of carnitine, required for the transport of long-chain fatty acids to the mitochondrial inner membrane for oxidation, thus resulting in a leaner carcass. Betaine has the potential to be used to reduce abdominal fat, which negatively affects consumer preferences.

Methionine has two main functions in poultry. One of them is protein synthesis and the other is required in methylation to produce S-adenosyl methionine, SAM). The requirement for methionine for methylation increases during heat stress. In this case, the amount of methionine can be reduced by adding betaine to provide the methyl groups required for the regeneration of methionine from homocysteine. Also, while methionine contains only one methyl group, betaine contains three methyl groups. In this respect, betaine is a methyl donor three times more effective than methionine. Considering as a methyl group donor, methionine is saved by adding betaine to the diet and this methionine saved can be used in protein synthesis. Another sparing effect of betaine is on choline. While providing methyl groups from choline, choline must first be converted to betaine. This conversion rate varies, but averages around 55%. Therefore, betaine is more effective in providing methyl donors (Obeid, 2013).

When poultry are exposed to heat stress or coccidiosis, body cells are exposed to osmotic stress. In this case, due to loss of extracellular fluids and increase in salt concentration or amount of solutions, intracellular fluid goes out of the cell (Cronje, 2007). This event causes the cell volume to shrink and if the water loss is not corrected quickly, the cell dies. In addition, the increase in the salt concentration and the amount of solutions in the intercellular fluid disrupts the cell metabolism. This deterioration is tried to be compensated by the membrane ion pump, and the water balance inside and outside the cell is tried to be reestablished. Since a very high amount of energy is used during this process, the rate of energy use increases and thus the amount of energy used for growth and production decreases. Betaine is a compound with an osmolyte function; thus, it prevents dehydration of water from the cell and contributes to the provision of water and ion balance of the cell by preventing the deterioration of cell functions. In this case, since the membrane ion pump is not used, the energy of the living share is saved and the use of energy for growth and production increases (Remus, 2001; Hruby, 2002).

Effect of betaine on nutrient digestibility

Betaine, due to its osmoregulatory function; supports intestinal microbes by balancing osmotic pressure in the digestive system (Ratriyanto *et al.*, 2009). In this way, it has been stated that carbohydrates such as cellulose, which cannot be digested by poultry, may be effective in reducing the negative effects (Eklund *et al.*, 2005). It is reported that dietary betaine supplementation may increase dry matter, crude protein, crude fiber, and crude ash digestibility in laying quail (Ratriyanto *et al.,* 2017). The increase in fiber digestibility increases the production of short-chain fatty acids, thereby improving mineral absorption. Moreover, the increase of short-chain fatty acids throughout the gut may result in increased absorption capacity and thus digestive capacity of the intestinal epithelium (Butzner *et al.,* 1994). The addition of betaine to the broiler diet kept under high temperature increases the jejunum, ileum, and total small intestine length, thereby positively affecting nutrient digestion and absorption (Ratriyanto *et al*., 2009; Ratriyanto *et al.,* 2014). After dietary betaine supplementation to poultry housed in high ambient temperature conditions, improvements in dry matter or organic matter digestibility have been demonstrated (Ratriyanto *et al.,* 2014). Addition of betaine increased crude protein digestibility by up to 11.4% (Awad *et al.*, 2014). In broilers, supplementation of betaine in diet, 0, 500, 1000 and 2000 mg/kg) increased villus width, duodenal and muscle wall thickness and serosal layer thickness, improved ileum length and jejunum. The optimal dose of betaine was offered as 500 mg/kg (Sun *et al.,* 2019). High temperature impairs the intestinal barrier function in broilers, resulting in decreased productivity. The addition of betaine to the diet improved the duodenal mucosal expressions of Claudin-4 and ZO-1. It revealed that dietary betaine addition improved ceacal microbial community and intestinal barrier function in broilers at high ambient temperature (Liu *et al*., 2021).

Effect of betaine on poultry performance

Betaine is effective to decrease harmful effects of high temperature and heat stress during the growing period of broilers. Heat stress caused a reduced daily feed intake and daily body weight gain and impaired FCR. Betaine may decrease the respiratory rate and increased the jejunum transepithelial resistance (Shakeri *et al*., 2018). Liu *et al*., 2019) showed that addition dietary betaine in heat stress condition may positively effect of healing of long-term heat exposure decreased growth performance, carcass yield and digestive function. Bogdanov *et al*. (1976), addition of vitamin B_{12} or 0.2% betaine to the starting broiler diets increased weight gain, however, addition of 0.2% betaine to the finishing diets did not affect on the weight gain.

Matthews *et al*. (1997) conducted three studies investigating the effects of adding 0, 0.1 and 0.5% betaine and monensin to the diets of broilers infected or not infected with coccidiosis oocytes. In the first study, they reported that the live weight, live weight gain and feed intake decreased in the infected animals and the addition of 0.1% betaine improved these characteristics in the infected animals, but the live weight gain and feed intake decreased in the uninfected animals due to the betaine contribution. While the effect of 0.5% betaine supplementation on feed intake and live weight gain was found to be insignificant, it increased FCR in infected and uninfected animals. In the second study, the addition of 0.1% betaine caused a tendency to improve FCR in uninfected animals, but in the third study, betaine supplementation in the diets of uninfected animals caused a reduction in feed injury. Garcia *et al.,* 2000), low protein relatively high energy, 17% crude protein, CP) and 3.3 kcal/g metabolic energy, ME)) and high protein lower energy, 24% HP, 3.0 kcal/g ME) diets added 1.5% methionine and in the two studies they conducted for 0-21 days in which they investigated the effect of 0.65% betaine addition, the addition of methionine and betaine to the diet containing 24% HP caused the improvement of body weight and FCR in both studies. Only in one study, adding methionine to a diet containing 17% HP improved performance. In both studies, neither methionine nor betaine supplementation affected abdominal fat. Although both additives increased feathering, they did not significantly affect the feather ratio as % of live weight. As a result, the researchers concluded that the ratio of protein to methionine in the diet did not significantly affect the carcass fat and the activity of betaine could not be used as a lipotropic agent. Matthews and Southern (2000), in their two studies, investigated the effect of 0 or 0.075% betaine supplementation in the diet in coccidiosis-infected and uninfected broilers, it was found that betaine supplementation caused a decrease in daily body weight gain in uninfected animals, but it did not increase live weight gain in infected animals. In the second study, there was an improvement in feed intake and body weight gain due to the contribution of betaine. McDevitt *et al*. (2000) investigated that the substitution of betaine for methionine, reported that the addition of betaine did not have a compensatory effect in chickens fed a methionine deficient diet, and the body weight decreased, while the FCR increased. However, they reported that betaine caused an increase in breast ratio. Kermanshahi (2001) reported that the use of 0, 25, 50, 75 and 100% betaine in the diet instead of methionine did not have a significant effect on feed intake and FCR at 0-3 and 0-7 weeks, but on body weight gain, breast weight and abdominal fat decreased. It has been reported that the hypothesis that betaine can be used effectively instead of methionine is not supported based on these research findings.

Schutte *et al.* (1997), in a study they conducted to determine the usability of betaine instead of methionine in male broiler chicks, investigated the effects of adding 0% 0.05 and 1% methionine and 0% and 0.04% betaine to the diets, or both, on performance and carcass characteristics. Daily live weight gain and feed efficiency improved significantly with increasing methionine supplementation. However, the effect of betaine supplementation on growth was found to be insignificant, but it slightly increased the feed efficiency. Waldenstedt et al. (1999), in a study investigating the effect of betaine in coccidiosis-infected broilers, added 0 or 1 g/kg betaine and 0 or 70 ppm narasin to the control diet. In narasin-free and uninfected animals, the addition of betaine alone increased the live weight by 5.7, 5.4 and 5.6% on the 22nd, 29th and 36th days, but the effect on feed intake and feed efficiency was not significant, and the combination with narasin did not have a positive effect. In the study, the effect of betaine on Eimeria oocytes or lesions in the intestine was found to be insignificant.

Effect of betaine on poultry meat quality

Shakeri *et al*. (2019) reported that broilers under heat stress, were exposed to either thermoneutral or cyclic heat stress conditions, in respiratory alkalosis conditions, betaine supplementation increased carcass quality traits. Betaine generally improved growth rates and product quality. The physiological and metabolic responses of broilers to control thermoregulation during heat stress divert energy from efficient production in addition to increased morbidity and mortality. Therefore, heat stress conditions, recovery strategies may cause an increase the poultry meat production in summer season. In a study, the effect of different concentrations of betaine in quail diets, the feed intake decreased with betaine added groups compared to the control, however, the highest body weight gain, carcass and breast yield were noticed with betaine supplementation at the rate of 2.25 g/kg. In addition, intestinal weight and length were significantly higher in the same concentration of betaine group (Arif *et al.,* 2021). In the another study, investigating the effects of dietary betaine supplementation on meat quality and muscle fatty acid composition in slow-growing broilers;

better weight gain, FCR and meat color redness and yellowness in betaine-fed chickens 24 hours post-slaughter. Supplementation linearly reduced cooking loss and drip loss from the udder muscle. Muscle flexibility was improved, and sensitivity was increased. While intramuscular total monounsaturated fatty acids, MUFA) and polyunsaturated fatty acids, PUFA) increased and saturated fatty acids, SFA) decreased. Betaine increased total superoxide dismutase, SOD), glutathione peroxidase, GPx) activities, and reduced hydroxyl radical scavenging activity and glutathione/oxidized glutathione ratio. It increased the activity of total antioxidant capacity, T-AOC) in chest muscle. Also, supplementation up-regulated mRNA expression levels of blood and antioxidant markers. In conclusion, an additional 1000 mg/kg betaine may be recommended for slow-growing broilers (Yang *et al*., 2021).

Supplementation of betaine to the diet of broilers under heat stress, reduced redness, a* value) of the pectoral muscle was restored with betaine, which tends to reduce the drip loss of the pectoral muscle. The betaine-fortified diets tended to increase moisture content of meat in chickens under humic acid but decrease the crude protein content of pectoral muscle. In addition, betaine resulted in higher SOD and glutathione content and GSHPx activities, but lower malondialdehyde, MDA) content, in the pectoral muscles of broilers exposed to heat stress. Dietary betaine supplementation attenuated the adverse effects of heat stress on some meat quality characteristics and oxidative status of broiler chickens (Wen *et al.,* 2019). In a study conducted to determine the effect of increasing levels of betaine, 0, 250, 500 and 1000 mg/kg) on growth performance and carcass characteristics in broilers, higher body weight gain and lower FCR in broilers supplemented with high dose betaine, 500 or 1.000 mg/kg). Also, the 1000 mg/kg betaine supplemented group increased breast muscle efficiency and decreased relative abdominal fat weight (Chen *et al.,* 2018).

Schutte *et al.* (1997), conducted a research to determine the availability of betaine instead of methionine in broiler chicks, and showed that supplementation of 0, 0.05 and 0.1% methionine and 0 and 0.04% betaine to the diets, on performance and carcass characteristics. Breast meat increased by 1.5% with the addition of 0.05% methionine, while it increased by 0.3% with the addition of 0.04% betaine. As a result, researchers reported that using betaine instead of methionine in broiler diets is not adequately supported to save methionine.

The effects of betaine on physiology and immune responses

Heat stress alters the normal physiological status of organs such as the small intestine. Radiant heat loss causes a reduction in blood flow due to skin redistribution and causes organ dysfunction.

In heat-stressed chickens, supplementation with betaine decreased the concentration of Evans Blue Dye in kidney and muscle with betaine. Therefore, betaine can improve broilers' tolerance to heat stress and Evans Blue Dye may be a useful tool to investigate the effects of heat stress on broiler organ dysfunction, Shakeri *et al.* (2020). They stated that betaine influenced the metabolic rates of birds by reducing respiratory rates and rectal temperature under heat stress condition in broilers (Nofal *et al.*, 2015). Heat stress causes rapid respiration, respiratory alkalosis, increased loss of electrolytes in the urine, and resulting in increased blood pH (Borges et al., 2004). In this way, betaine may reduce heat stress by maintaining blood electrolyte levels and stabilizing osmotic pressure (Nofal *et al.,* 2015; Park and Kim, 2017). Thanks to osmotic functions of betaine causing in reducing the temperature of body of chickens under heat stress by reducing dehydration (Zulkifli *et al*., 2004). Also, increasing water retention under heat stress improves the thermal dissipation capacity through peripheral blood and evaporation, helping the birds control their body temperature *(Dai et al.,* 2009).

In a study, the effect of different doses of betaine supplemented diet on blood parameters in Japanese quail was determined. As a result, betaine supplementation showed growth hormones, higher high-density lipoprotein, LDL) insulin and thyroxine and lower alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase and LDL concentration. In this way, dietary betaine supplementation positively affect growth performance, carcass traits and blood chemistry of Japanese quail (Arif *et al*., 2021).

In another study, to determine the effects of betaine supplementation on the performance, antioxidant status and biochemical parameters of broiler chickens exposed to cold stress, 600 mg/kg betaine supplementation was performed on plasma glucose, uric acid, lactate, lactate dehydrogenase, total antioxidant status, red blood cell, hemoglobin. No significant dietary effects were determined on performance, blood hematological and biochemical parameters, including heterophile-lymphocyte and hematocrit ratios. However, while liver MDA level and SOD activity decreased, liver GPx and serum MDA levels decreased. In general, it was determined that betaine may have beneficial effects on the antioxidant status of chickens exposed to cold stress (Nasiroleslami *et al.,* 2018). It shows that heat stress reduces thyroid activity (Haldar *et al.*, 2015) and, however, betaine supplementation can cause increase thyroid activity in chickens (Haldar *et al.*, 2015; Nofal *et al.*, 2015; Attia *et al,* 2016). The activity of thyroid hormone decreases at high temperatures, and this causes a decrease in bird performance. In a study investigating the effects of dietary betaine supplementation on antioxidant ability in slowgrowing broilers; increased GPx and total SOD activities, GSH level, reduced glutathione/oxidized glutathione ratio and scavenging activity of hydroxyl radicals in betaine fed chickens. Also, supplementation up-regulated mRNA expression levels of blood and antioxidant markers. In conclusion, an additional 1000 mg/kg betaine has been recommended for slow-growing broilers (Yang *et al*., 2021).

Conclusion

As a result, it may be possible to add methyl group donor to the diet with the use of betaine in broiler feeds. In this way, an insurance duty may be provided to compensate for the deficiencies of some nutrients such as methionine and choline. It is a very strong assumption that the addition of betaine will have positive effects in the case of some nutrients such as methionine and choline in the diets. In addition, with the additive of betaine, it can have a positive effect on the improvement of health status and changes in other physiological needs in cases of illness, and it will be possible to reduce the negative effects of heat stress by maintaining the water balance in heat stress.

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