Betaine: a promising antioxidant agent for enhancement of broiler meat quality

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Betaine: a promising antioxidant agent for enhancement of broiler meat quality

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Abstract

1. Antioxidant and methyl donor effects of betaine in experimental animal models have recently been demonstrated. The present study was therefore designed to examine the antioxidant effects of betaine on the antioxidant status and meat quality of breast muscles in broilers.

2. Cobb broilers were randomly divided into Control, Methionine low, Methionine low plus betaine, and Betaine groups.

3. The activity of the main antioxidant enzyme (glutathione peroxidase) in the Betaine and the Methionine low plus betaine groups significantly increased compared to the Methionine low and Control groups. Catalase and superoxide dismutase activities were significantly higher in the Betaine group compared to the Methionine low group, and lipid peroxidation was significantly higher in the Control and the Methionine low groups.

4. The present study indicates that adding betaine (1 g/kg) to a diet deficient in methionine can significantly improve antioxidant defences and meat quality, decreasing lipid peroxidation in the breast muscles of broiler chickens.

INTRODUCTION

It is well known that muscles contain various endogenous prooxidant and antioxidant systems and the lipid peroxidation process of meat results when antioxidant defences are overcome by peroxidation mechanisms (Decker and Zhimin, 1998; Gheisari and Motamedi, 2010). The glutathione antioxidant system plays a fundamental role in cellular defence against reactive oxygen species (ROS). The cellular tripeptide, GSH (γ-glutamyl cysteinyl glycine) reduces peroxidative damage by neutralising free radicals (Ganesan et al., 2011; Alirezaei et al., 2011). Glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), which are responsible for the destruction of peroxides, have a specific role in protecting tissues against oxidative damage (Sun et al., 2008; Ganesan et al., 2011). Reduction of the activity of these enzymes in oxidative stress may lead to the formation of $\text{O}_2^-$ and $\text{H}_2\text{O}_2$, which in turn can form the hydroxyl radical (OH) and bring about a number of harmful reactions, leading to lipid peroxidation (Kalra et al., 1988). Previous studies in mammals showed that ROS generated during metabolism were not adequately removed when the antioxidant defences were overcome by oxidative stress, resulting in lipid peroxidation (Vogt and Richie, 1993; Di Simplicio et al., 1997; Robinson et al., 1997; Zhang et al., 2008). Lipid peroxidation is directly linked to rancidity in...
meat, which has a considerable negative effect on meat quality (Ruff et al., 2003; Zhang et al., 2008). Malondialdehyde is a metabolite derived from lipid peroxidation and has been widely used as an indicator of oxidative damage and meat quality (Halliwell and Gutteridge, 1989; Frigg et al., 1990; Winston and Di Giulio, 1991; Zhang et al., 2008). Antioxidants delay or prevent lipid peroxidation by reducing free radical activities in meat (Gheisari and Motamedi, 2010), and the antioxidant supplementation of feed is an efficient method for increasing oxidative stability (Roginsky and Lissí, 2005; Gheisari and Motamedi, 2010).

Betaine is a common term for trimethylglycine, a substrate for betaine-homocysteine methyl transferase (BHMT) in the liver and kidney (Kettunen et al., 2001; Attia et al., 2009). It is often found in high concentrations in plants subjected to drought, which is due to the osmoregulatory properties of betaine (Kettunen et al., 2001; Attia et al., 2009). Previous studies indicated that betaine may play important roles such as improving growth performance, fat distribution (Wang et al., 2004; Sun et al., 2008; Attia et al., 2009), immune response, and act as a coccidiosis enhancer (Swain and Johri, 2000; Kettunen et al., 2001; Attia et al., 2009) in broilers. The betaine in maize and soybean meal was reported to be below the detectable level (Chendrimada et al., 2002), and was not detected in maize-soybean meal diet (Waldroup and Fritts, 2005; Attia et al., 2009). Betaine and folic acid are all considered as methyl donors and have been shown to compensate for the partial deficiency of labile methyl groups in maize-soybean-based diets (Matthews and Southern, 2000; Pillai et al., 2006; Dilger et al., 2007). The formation of methionine from homocysteine can occur either via betaine or via 5-methyl tetrahydrofolate (Craig, 2004; Alirezaei et al., 2010; Alirezaei et al., 2011, 2012). Previous studies have shown that both pathways are equally important and that betaine is a vital methylating agent (Barak and Tuma, 1983; Finkelstein and Martin, 1986; Alirezaei et al., 2012). Betaine transfers a methyl group via the enzyme BHMT to become dimethylglycine (Alirezaei et al., 2011). Methionine is one of the most limiting amino acids, playing a crucial role in body protein synthesis, and therefore it would be beneficial to spare its function as a methyl donor (Sun et al., 2008). It has been shown that choline must first be activated and then converted to betaine before the methyl groups are liberated to methylation cycles (McKeever et al., 1991; Sun et al., 2008). In contrast, betaine contains three methyl groups in its structure and donates these in several metabolic reactions (Sun et al., 2008; Alirezaei et al., 2012), therefore allowing it to be used as an effective compound to spare dietary methionine as a methyl donor.

Administration of betaine has been shown to exert a significant role within tissue as a methyl donor, which in turn may be used for the synthesis of methionine, carnitine, phosphatidylcholine, and creatine, substances which play a key role in protein and energy metabolism in the cells (Craig, 2004; Alirezaei et al., 2012). In this regard, betaine significantly improved the breast meat yield and growth performance of broilers (Sun et al., 2008; Attia et al., 2009). However, its antioxidant effects on breast meat quality have not yet been explored. We recently demonstrated the methyl donor and antioxidant properties of betaine in experimental animal models (Alirezaei et al., 2010; Alirezaei et al., 2011). Thus, the present study was designed to examine the antioxidant and nutrient effects of betaine on meat quality in Cobb broiler chickens.

MATERIALS AND METHODS

Materials

Betaine (Betafin®, 96%) was obtained from Biochem Company (Brinkstrasse 55, D-49393 Lohne, Germany). GPx and SOD kits were obtained from Randox®, Antrim, UK. All chemicals used were of analytical grade.

Animals

A total of 58 5-d-old Cobb broiler chickens, previously vaccinated against infectious bronchitis at the hatchery, were purchased from Fars Poultry Company (Fars Company, Shiraz, Iran). The birds were weighed and stratified and 48 randomly distributed into 4 equal groups. The control group (C, which received a commercial basal diet formulated to meet the nutritional requirement of the birds according to the NRC (1994) recommendation), Methionine low group (ML, control group diet except methionine content lowered from 5 to 4.6 g/kg starter and 4.2 to 3.5 g/kg grower diet) (Sun et al., 2008), Methionine low plus betaine group (ML + B, Methionine low group diet plus betaine, 1 g/kg of the diet), and Betaine group (B, control group diet plus betaine 1 g/kg of the diet) (Attia et al., 2009). The diets were supplemented with a commercial vitamin and mineral premix and 5000 mg/kg of the diet by choline chloride 50%. The experiment was conducted into two phases: starter phase (1–21 d) and grower phase (22–49 d). All birds were fed on a maize-soybean meal basal diet (Table 1 and 2). All groups were fed for 49 consecutive days, food and water were provided ad libitum and weight gain and food consumption were determined at weekly
of 23 L:1D and the temperature was gradually decreased to 20°C by day 21. There was no coccidiostat included in the diets. The birds were kept in 4 separated and cleaned pens with wood shavings. No signs of coccidiosis were apparent and mortality was low (1 out of 48 birds). Hence, the potential positive effects of betaine under a coccidiosis challenge played no role in the present experiment. One day after the last treatment the birds were starved for 3 h, slaughtered, plucked, bled and weighed. The breast muscles were dissected away, carefully cleaned of adhering materials such as blood and stored at −70°C for later (within 2 months) antioxidant analysis. Meat proximate composition (crude protein, fat, moisture and ash) was analysed on the fresh meat.

Muscle preparation for protein measurement, TBARS detection and enzyme assay

Breast muscles were thawed and manually homogenised via liquid nitrogen in cold phosphate buffer (pH 7.4, 0.1 M) containing 5 mM EDTA and the debris removed by centrifugation at 5000 g for 10 min (Centrifuge 5415 R; Rotofix 32 A, Germany) (Kheradmand et al., 2010). Supernatants were recovered and used for protein measurement, antioxidant enzyme activities, and TBARS concentration. With respect to the different moisture contents of the muscle tissues in the control and treatment groups (due to the osmoregulatory effect of betaine), we decided to express activity of the antioxidant enzymes in order of unit/mg protein. Protein content of tissue homogenates was determined using the colorimetric method of Lowry with bovine serum albumin as a standard (Lowry et al., 1951).

Measurement of GPx activity

The activity of glutathione peroxidase (GPx) was evaluated with Randox® GPx detection kit according to the manufacturer’s instructions. GPx catalyse the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance was measured spectrophotometrically (Varian Australia, Pty Ltd) against blank at 340 nm. One unit (U) of GPx was defined as 1 µmol of oxidised NADPH per min. The GPx specific activity was expressed as milliunit per milligram of tissue protein (mU/mg protein).

Measurement of SOD activity

The activity of superoxide dismutase (SOD) was evaluated with the Randox® SOD detection kit according to the manufacturer’s instructions. The role of SOD is to accelerate the dismutation of superoxide to form hydrogen peroxide. The activity of SOD was determined using a spectrophotometric assay and measured at 405 nm. The activity of SOD was calculated as units per mg of protein using the molar absorbance coefficient of 43.6 M⁻¹ cm⁻¹ at 405 nm.
of the toxic superoxide ($O_2^-$), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity is then determined by the degree of inhibition of this reaction. One unit of SOD is that which causes 50% inhibition in the rate of reduction of INT under the conditions of the assay. The SOD specific activity was recorded at 505 nm and through a standard curve, and expressed as unit per milligram of tissue protein (U/mg protein).

**Measurement of CAT activity**

Tissue catalase activity was assayed using the method described by Claiborne (1986). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H$_2$O$_2$, and a 20-50 μl sample. The reaction was initiated by the addition of H$_2$O$_2$ and absorbance changes were measured at 240 nm (25°C) for 30 s. The molar extinction coefficient for H$_2$O$_2$ is 43.6 M$^{-1}$ cm$^{-1}$. The CAT specific activity was expressed as the unit defined as 1 μmol of H$_2$O$_2$ consumed per min per milligram of tissue protein (U/mg protein).

**Measurement of TBARS content**

The 2-thiobarbituric acid (TBA) assay was carried out according to the extraction method described by Vyncke (1975), with some modifications: the meat sample (1-50 g) was homogenised (Ultra Turrax T-25, Staufen, Germany) with 6 ml of a 7.5% trichloroacetic acid solution including 0.1% propylgallate and 0.1% EDTA for 45 sec at 13,500 RPM and the homogenate filtered through filter paper, 589.3. The extract (2 ml) was mixed with 0.02 M thiobarbituric acid (2 ml), heated and cooled as described by Vyncke (1975). The absorbance was measured at 532 and 600 nm using a CARY 3 UV-visible spectrophotometer (Varian Australia, Pty Ltd), and the absorbance difference, A$_{532}$ - A$_{600}$, was calculated with A$_{600}$ correcting for sample turbidity. TBARS was calculated using 1,1,3,3-tetraethoxypropane (TEP)/malonic aldehyde as a standard and expressed as nmol/mg protein of muscle tissue.

**Analysis of meat**

The ground meat was analysed for crude protein, fat, moisture, and ash contents according to the methods of AOAC International (AOAC, 1984) (Table 3).

**Statistical analysis**

All results are presented as Mean ± standard error of mean (S.E.M.). Data were compared by one-way analysis of variance (ANOVA) with Tukey’s post hoc analysis. A calculated P value of less than 0.05 was considered statistically significant. Statistical analysis was performed using the Graphpad PRISM version 5 (Graphpad Software Inc., San Diego, CA). All variables were previously tested for normal distribution and homogeneous variances by Leveni’s statistic test.

**RESULTS**

The mean values (±S.E.M.) of the glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activity of the breast muscles are presented in Figures 1–3, respectively. GPx activity was significantly higher in the B group than in the C and ML groups. The ML+B group had GPx levels similar to the B treatment and significantly higher than ML treatment. SOD activity was significantly lower in ML treatment compared to the other treatments ($P<0.05$). Although SOD activity in the C and ML+B treatments was lower than in the B treatment, this reduction was not statistically significant ($P>0.05$). CAT activity in the B group was significantly higher than in the other groups; thus it was indicated that betaine is not able to increase CAT activity in the ML+B group near to that of the B group.

Lipid peroxidation concentration (TBARS content) was significantly higher in the ML group than in those receiving the B and ML+B treatments ($P<0.05$). However, significant decreases were observed in lipid peroxidation for both groups that received the B and ML+B treatments.
compared to controls ($P<0.05$), and the C group indicated a slightly lower TBARS content compared to the ML group also (Figure 4).

Table 3 shows the effects of betaine and methionine on biochemical indicators of muscle composition. Overall, the treatment with betaine improved all of the breast indicators, although the effects were not significant ($P>0.05$). Betaine had no significant effect ($P>0.05$) on the ash and moisture of the breast muscles in any of the groups. There was no significant difference in muscle composition among the treatments. There was a tendency for the betaine-treated birds to have lower fat content ($P=0.072$) and for ML group to have lower crude protein content ($P=0.065$). One possible reason for the lack of statistical significance in effects on meat composition may be the small sample size ($n=48$) of broilers.

The reduction in methionine in the diet significantly decreased the weight gain of the broilers in the ML group ($P<0.05$). In contrast, the addition of betaine to the C and ML diet significantly increased the weight gain ($P<0.05$). The weights of the broilers in the groups that received betaine were higher than those in of the
controls, however, the enhanced weights were not statistically significant ($P > 0.05$). Indeed, the addition of betaine to the control diet had no significant effect on weight gain in broilers (Figure 5).

**DISCUSSION**

The present study demonstrated for the first time that betaine can act as an antioxidant agent in low-methionine diet-induced oxidative stress for broilers, and it is suggested that it does so by promoting the main antioxidant enzyme activities including GPx, CAT, and SOD, subsequently decreasing lipid peroxidation in breast muscles. Our observation for antioxidant enzymes, TBARS concentration and weight gain in the betaine-treated broilers supports the idea that betaine is associated with antioxidant and methyl donor properties through its involvement in cell membrane stabilisation and homocysteine remethylation (Zhang et al., 2008; Alirezaei et al., 2011). This experiment revealed that supplementation of betaine (1 g/kg of diet) to the diet of 10% methionine deficiency increased growth performance in broilers. Thus, it appears that betaine in a marginally methionine deficient diet could lead to an equivalent growth response in broilers, and that betaine could spare a small portion of methionine (Sun et al., 2008).

To evaluate the effects of betaine on antioxidant status in Cobb broiler chickens, the breast muscle activity of some antioxidant enzymes and the concentration of TBARS as a lipid peroxidation marker were evaluated. The result of oxygen radical formation is well-known to damage DNA, RNA, protein and lipids, subsequently damaging various cellular compartments (Gheisari and Motamedi, 2010; Alirezaei et al., 2011). Membrane-associated polyunsaturated fatty acids are readily attached by the hydroxyl radical in a process that leads to the peroxidation of lipids, which can affect meat quality (Ruff et al., 2003). It has been clearly indicated that lipid peroxidation significantly increases by accumulation of $H_2O_2$ in a concentration-dependent manner (Garcia et al., 2005). Cells are able to defend themselves against the damaging effects of oxygen radicals by way of their own antioxidant mechanisms, including enzymatic and non-enzymatic antioxidant systems (Ross, 1988; Sehirli et al., 2008). GPx and CAT are two key antioxidant enzymes that can decompose hydrogen peroxide to water (Turner and Lysiak, 2008; Kheradmand et al., 2010). SOD, another antioxidant enzyme in cells, rapidly converts the superoxide anion ($O_2^-$) to less dangerous hydrogen peroxide ($H_2O_2$). GPx and CAT can then decompose $H_2O_2$ to water (Kheradmand et al., 2009; Kheradmand et al., 2010). Although $H_2O_2$ is not a particularly reactive product, it can be reduced to a highly reactive metabolite hydroxyl radical (Peltola et al., 1994). With respect to antioxidant enzyme activities, our data showed a significant increase in antioxidant defense status in the breast muscle of the betaine-treated broilers, which suggests a decrease in the ROS generation rate (Alirezaei et al., 2011). In the present study, betaine caused significant increases in the GPx activity for both groups that received betaine compared to the C and ML groups. Betaine supplementation also significantly reduced TBARS concentrations, suggesting betaine acted as an antioxidant, possibly by scavenging ROS free radicals. On the other hand, the muscle TBARS content decreased in the betaine-treated broilers, suggesting betaine possessed antioxidant effects and preserved the cellular antioxidant stores. In accordance with GPx activity, other antioxidant enzymes increased in the B group compared to the ML group. In this setting, antioxidant properties of betaine decreased tissue damage arising from lipid peroxidation in heat stress of broilers (Attia et al., 2009). Further, we recently demonstrated that homocysteinaemia that was induced via a methionine low diet might potentiate the toxic effects of ROS. In addition, the autooxidation of homocysteine is known to generate ROS (Alirezaei et al., 2011).

The increase in CAT and SOD activity in our study for betaine-treated broilers correlates well with the decrease in TBARS content as a lipid peroxidation marker in the breast muscle tissue. Malondialdehyde is one of the major aldehyde derivatives of lipid peroxidation and it is a
by-product of the lipid peroxidation process (Smith et al., 2005; Alirezaei et al., 2011). To this extent, oxidative stress and lipid peroxidation was decreased in broilers receiving betaine supplementation. It is widely accepted that CAT and SOD, which are responsible for the destruction of peroxides, have a very specific role in protecting tissues against oxidative damage (Ganesan et al., 2007b; Alirezaei et al., 2011). In the present experiment, the unpaired electron present in the hydroxyl free radical may have been trapped and subsequently dismutated by betaine (Saravanan and Prakash, 2004; Alirezaei et al., 2011). However, the protective effect of betaine against methionine low-induced oxidative stress observed in this study may also be associated with the restoration of S-adenosyl methionine (SAM), which contributes to an increase in the supply of substrate needed for the synthesis of glutathione that protects the cell from reactive metabolites and ROS (Ganesan et al., 2007a; Alirezaei et al., 2011).

The positive influence of 1 g betaine/kg diet on meat quality observed in the present study may also be due to the increasing percentage of crude protein, while the percentage of fat and ash decreased. The exact mechanism affected by betaine on carcass composition is not clear. One report did indicate that betaine improved breast meat yield (McDevitt et al., 2000). In contrast, abdominal fat yield as well as the fat content in the liver was reduced, whereas fat content in the breast was increased by the supplementation of betaine, (Zhan et al., 2006; Sun et al., 2008). In the present study, with respect to breast composition, there was a decrease in the fat content of the breast meat, although it was not significant.

The ability of betaine to spare some of the methionine needs in broiler diets is subject to considerable controversy. Esteve-Garcia and Mack (2000) evaluated the potential replacement value of methionine by betaine in a methionine-deficient diet. There were no significant interactions between betaine and methionine; methionine supplementation significantly improved body weight and feed to gain at 21 and 41 d, and significantly increased breast yield at d 41. However, the addition of betaine significantly improved carcass yield with no significant change in other carcass parameters such as breast yield or abdominal fat (Esteve-Garcia and Mack, 2000; Sun et al., 2008). It was concluded that betaine could not be a substitute for methionine deficiency, but it may improve carcass composition, especially breast meat yield (Esteve-Garcia and Mack, 2000). The present study showed that up to 10% of dietary total methionine could be replaced by betaine without negatively affecting the growth performance of the broilers.

As previously mentioned, this is the first in vivo study to show that replacement of 10% methionine by betaine in broiler diets results in an increase in the main antioxidant enzyme activities and decreased lipid peroxidation in breast muscle, subsequently improving meat quality. Betaine is a methylating agent like SAM and it also spares methionine via the BHMT pathway (Waldroup et al., 2006; Alirezaei et al., 2011). Therefore, betaine may have antioxidant and nutrient effects against oxidative damage in broiler diets. In addition, betaine may have some advantages compared to the methionine application, as numerous methylation reactions involve methionine, which is then converted to SAM, the methyl group donor, and then to homocysteine (Esteve-Garcia and Mack, 2000; Waldroup et al., 2006; Alirezaei et al., 2011). Both methionine and homocysteine are potentially toxic to cells, and both may accumulate if methyl donors or methyl acceptors are deficient (Waldroup et al., 2006). Alternative methyl donors such as betaine or choline may alleviate the dietary needs of methionine by taking the place of methionine as a methyl donor or by providing the methyl group necessary for the conversion of homocysteine to methionine (Waldroup et al., 2006; Alirezaei et al., 2011). Although, betaine was demonstrated in the present paper as a potential antioxidant agent for the prevention of methionine low-induced oxidative stress in the broiler diet and improved antioxidant defence as well as meat quality, further studies should be performed to evaluate the nutrient effects of betaine principally on meat quality with a larger sample size of broilers.

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