Effect of betaine, a methyl group donor, on broiler chicken growth performance, breast muscle quality characteristics, oxidative status and amino acid content

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ABSTRACT
This study was aimed to investigate the effect of feed supplemented with betaine on broiler chickens’ growth and slaughter performance, breast muscle histomorphometric and physico-chemical properties, oxidative status and amino acid content. A total of 1000 1-day-old Ross 308 broiler chickens were divided into four treatments. Control group chickens were fed with standard compound diet (SCD), the chickens from experimental groups B1, B2 and B3 receiving SCD supplemented with 1 g/kg (B1), 2 g/kg (B2) and 3 g/kg (B3) betaine anhydrous, respectively. Each treatment had five replicate pens. Feeding test results showed that betaine reduced broilers’ mortality but increased feed conversion ratio (p < .05). Forty broiler chickens (5 weeks old) were slaughtered and slaughter performance showed that 2 g/kg betaine inclusion improved breast muscle percentage and yield (p < .05). Betaine dosage of 1 g/kg into feed increased breast muscle fibre areas (p < .05). Betaine affected some physicochemical properties: higher a* and the highest drip loss in B2; the highest cooking losses in B1, B2; the highest shear force and fat content in SCD; the highest amounts of ashes in B1, B2 (p < .05). Lower malondialdehyde levels were observed in all betaine-treated groups (p < .05), except B1 fresh meat samples. The highest total amino acid content and a greater amount of essential amino acids were obtained in SCD breast muscles (p < .05), except equally highest amounts of methionine were found in both SCD and B3 samples (p < .05). However, according to our study results, betaine, as a methyl group donor, in broiler chicken diets cannot replace methionine as an essential amino acid.

HIGHLIGHTS
• Betaine is known as functional nutrient in poultry nutrition, which can fulfil the function of a methyl donor.
• Betaine in animal feed is saving feed costs by replacing choline chloride and methionine.

Introduction
In recent decades, world demand for poultry has been growing rapidly. It is becoming increasingly important for consumers to get not only cheaper but also better quality poultry production (Wen et al. 2017). The main factors which determine the quality of poultry meat are genetic, environmental and feeding (Fletcher 2002). However, today’s modern broiler chickens grow much faster due to genetic selection and up to 6 weeks of age they are already attaining the right weight and size of the breast muscles (Fanatico et al. 2007). Such rapid growth can negatively affect the organoleptic and functional characteristics of broiler meat. In addition, with the current advances in genetics science, it is believed that a modern growing poultry suffers from greater stress, which may affect histological and biochemical muscle changes, which degrade certain meat quality indicators (Wen et al. 2017).

In order to accelerate bird growth, improve immune system performance, meat qualitative indicators and decrease oxidative processes, various feed additives are used in broiler chickens’ diets (Chand...
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et al. 2014; Abudabos et al. 2016; Alzawqari et al. 2016; Khan et al. 2017). Choline and betaine additives in animal nutrition are the main sources of methyl groups (CH₃) (Ratriyanto et al. 2009), but only betaine can act as a methyl group donor (Kettunen et al. 2001). Methyl groups are of vital importance in the metabolism of all animals because they cannot synthesise methyl groups and thus need to receive them in their diets. Therefore, betaine additive used as a methyl group donor is useful for enriching diet with the amino acid methionine, which plays an important role in the body’s protein synthesis (Sun et al. 2008). The main natural source of betaine is sugar beets (Lever and Slow 2010), but in order to replace methionine with more economical additive, several synthetic sources of betaine have recently emerged in the market, for example, betaine anhydrous.

A wide range of research has been carried out to assess the effect of methionine replacement to betaine on broiler chicken growth and carcass characteristics (Alzawqari et al. 2016; Khan et al. 2017). Research has shown that the use of betaine in poultry diet improves bird’s immunity, growth, and increases breast muscle mass (Pereira et al. 2010; Mervat et al. 2018). However, little attention has been paid to broiler chickens’ meat quality, oxidative status and composition of amino acids. Thus, the present study was designed to examine the effects of betaine on broiler chickens’ growth and slaughter performance, breast muscle histomorphometric and physicochemical properties, oxidative status and amino acid content.

Materials and methods

Research has been carried out in accordance with the Law of the Republic of Lithuania on the Care, Storage and Use of Animals. Complied with directives: Directive 2010/63/EU (European Union 2010) of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes and Directive 2007/43/EC (European Union 2007) which describes rules for the protection of chickens kept for meat production.

Experimental design

The feeding test was performed with 1000 Ross 308 line combination male broiler chickens, which were individually weighed and randomly assigned to four dietary treatments (250 broilers in each group) with five replicate pens of 50 broiler chickens in each. Each group of birds was kept in separate pens, which floor was covered with wood shavings litter and stocking density for all groups has not exceeded more than 33 kg/m² (Council Directive 2007/43/EC, European Union 2007). The poultry house temperature was initially maintained to 32°C and gradually decreased (2.5°C every week) to reach a constant temperature of 20–22°C in the fifth week. Light intensity was 40 lux at the first week and from the second week till the end of the trial – 20 lux. Lighting programme (light:dark cycle) at the first week was 23:1 h and from the second week till the fifth week – 18:6 h, respectively. The temperature and lighting programme were consistent with the recommendations of Aviagen Incorporation (2014). Birds in each pen had free access to feed from hanging feeders and freshwater from drinkers. Broiler chickens were fed ad libitum with a standard compound diet (SCD) and with diet supplemented with 1 g/kg (B1), 2 g/kg (B2) and 3 g/kg (B3) betaine anhydrous additive. The diets were formulated to meet the nutrient and energy requirements for Ross 308 broiler chicken (2014). Table 1 shows feed ingredient composition and nutrient content (1–35 days of age).

Body weight was recorded on 1 day of age of broiler chicken, after that with 1-week interval and later with 2-week interval till 5 weeks of age. The average daily gain (ADG), feed conversion ratio (FCR) and mortality rate were calculated for each experimental group. At the end of 5 weeks of the experiment, 10 birds from each group (total n = 40) were randomly selected and euthanised by using electrical stunning. Slaughter was carried out at a commercial slaughterhouse in accordance with established procedures which complied with the laws of the Republic of Lithuania (Order No. B1-866 of 31 October 2012 of the Director of the State Food and Veterinary Service on the approval of requirements for the keeping, care and use of animals for scientific and educational purposes [Valstybinės maisto ir veterinarijos tarnybos direktooriaus išsakymas dėl 012 m. spalio 31 d. Nr. B1-866 dėl mokslo ir mokymo tikslais naudojamų gyvūnų laikymo, priežiūros ir naudojimo reikalavimų patvirtinimo]) (Lietuvos Respublikos Seimas, 2012).

Breast muscle samples collection

Forty breast muscle samples (n = 10 birds/group) were collected post-mortem, dissected away, minced and stored at −80°C (as a fresh meat) and at −18°C (as a stored meat) for later malondialdehyde (MDA) analysis. Other meat quality indicators (physicochemical properties, amino acid content) were analysed on the fresh meat.
Table 1. Feed ingredient composition and nutrient content (1–35 days of age).

| Ingredient, % (fed basis) | Group   |
|--------------------------|---------|
|                          | SCD     | B1     | B2     | B3     |
| Soybean meal             | 32.25   | 34.16  | 34.13  | 34.10  |
| Maize                    | 30.00   | 30.00  | 30.00  | 30.00  |
| Wheat                    | 25.71   | 25.93  | 25.94  | 25.95  |
| Vegetable oil            | 2.90    | 2.87   | 2.89   | 2.91   |
| Limestone                | 1.45    | 1.45   | 1.45   | 1.45   |
| Monocalcium phosphate    | 0.93    | 0.93   | 0.93   | 0.93   |
| Lysine sulphate          | 0.46    | 0.46   | 0.46   | 0.46   |
| Wheat flour              | 0.30    | 0.30   | 0.30   | 0.30   |
| Threonine                | 0.19    | 0.19   | 0.19   | 0.19   |
| Sodium sulphate          | 0.18    | 0.18   | 0.18   | 0.18   |
| Sodium chloride          | 0.18    | 0.18   | 0.18   | 0.18   |
| Mineral premix           | 0.10    | 0.10   | 0.10   | 0.10   |
| Vitamin premix           | 0.03    | 0.03   | 0.03   | 0.03   |
| DL-methionine            | 0.48    | 0.38   | 0.28   | 0.18   |
| Betaine                  | 0.00    | 0.10   | 0.20   | 0.30   |

Calculated analysis, % (unless stated otherwise)

|          | SCD  | B1   | B2   | B3   |
|----------|------|------|------|------|
| ME, MJ/kg| 13.23| 13.22| 13.21| 13.20|
| Crude protein | 21.60| 21.54| 21.47| 21.41|
| Crude fat | 6.91 | 6.88 | 6.90 | 6.92 |
| Crude ash | 6.17 | 6.17 | 6.17 | 6.17 |
| Crude fibre | 2.59 | 2.59 | 2.59 | 2.59 |
| Ca       | 0.88 | 0.88 | 0.88 | 0.88 |
| P        | 0.61 | 0.61 | 0.60 | 0.60 |
| Na       | 0.16 | 0.16 | 0.16 | 0.16 |
| Mg       | 0.08 | 0.08 | 0.08 | 0.08 |
| K        | 0.97 | 0.96 | 0.96 | 0.96 |
| Cl       | 0.17 | 0.16 | 0.16 | 0.16 |
| Lysine   | 1.34 | 1.34 | 1.34 | 1.34 |
| Methionine | 0.69 | 0.69 | 0.70 | 0.70 |
| Met + cysteine | 1.03 | 1.03 | 1.03 | 1.03 |
| Tryptophan | 0.27 | 0.27 | 0.27 | 0.27 |

Groups: SCD: standard compound diet; B1: standard compound diet + 1 g/kg betaine additive; B2: standard compound diet + 2 g/kg betaine additive; B3: standard compound diet + 3 g/kg betaine additive.

Histomorphometric assay of breast muscles

Histomorphometric properties were determined on 40 breast muscles (n = 10 breasts/group). One piece of muscle (1 × 1 cm) was taken from the same position of breast of each bird post-mortem and fixed in neutral buffered 10% formalin. Samples were processed by routine paraffin embedding technique, further, 4 μm thick tissue sections were cut with rotary microtome Leica RM 2235 (Leica Microsystems, Nussloch, Germany) and stained with haematoxylin and eosin. Prepared breast muscle histologic preparations were examined by using Olympus BX63 microscope (Olympus Corp., Tokyo, Japan), Olympus DP72 digital camera (Olympus Corp., Tokyo, Japan) and computer Image-Pro Plus programme system for Windows, version 7.0 (Media Cybernetics, Inc., Bethesda, MD, USA, 2009). Morphometrically measured cross-sectional areas of breast muscle fibres, measured in micrometres squared (μm²).

Physicochemical assay of breast muscles

Physicochemical properties were determined on 40 breast muscles (n = 10 breasts/group). The dry matter (DM) content of broiler chickens’ breast muscles was determined by the ISO 1442:1997 method (Lietuvos standartizacijos departamentas, 2000), while drying it at 105 °C to constant weight. Broiler chickens’ breast muscle pH values were determined with analyser Inolab 730 (WTW GmbH, Weilheim, Germany). Using the Chroma Meter CR-410 Colour Gauge (Konica Minolta, Inc., Osaka, Japan) broiler chickens’ breast muscle colour coordinates were defined in the same contrast colour space. Coordinates L*, a*, b* were measured in light reflectance mode (respectively, the coordinates of brightness, redness, yellowness on the CIE-LAB scale). The standard light source C, whose radiation is close to the average daylight, was used for the measurements. Before each measurement, the instrument is calibrated with a light trap and a white standard.

Drip loss and water binding were determined by loss of sample weight within 24 h (breast muscle samples were stored in special hanging bags at 4 °C) (Hamm 1986). Breast muscle cooking losses were determined using the meat boiling and weighing method when boiling in a circulating water bath for 30 min at 70 °C and weighing before and after cooking (Schilling 1966). The shear force of breast muscle was evaluated according to the Warner-Bratzler cutting power method (American Meat Science Association 2016).

The ash content was determined by burning breast muscle organic matter at 600–800 °C according ISO 936:1998 method (Lietuvos standartizacijos departamentas 2002); fat content – by Soxhlet method, extraction of the fat by chloroform for up to 8 h; the total protein content was determined by the Kjeldahl method (King-Brink and Sebranek 1993).

MDA content assay

MDA content assay was conducted on 40 fresh breast muscles (n = 10 breasts/group) and 40 breast muscles after storage (n = 10 breasts/group). MDA content of the samples was tested at two time intervals: 24 h and 3 months following the broiler chickens’ slaughter. MDA content in broiler chickens’ breast muscles was
determined by high performance liquid chromatography method described by Mendes et al. (2009). For this purpose, a high pressure gradient HPLC system Varian ProStar (Varian, Inc., Palo Alto, CA, USA) was used, consisting of two ProStar 210 pumps, automatic sampling module Prostar 410 and Prostar 363 fluorescence detector. The separation of the MDA–2-thiobarbituric acid (MDA-TBA) compound was performed by HPLC using a 5 μm particle size, 250 mm long and 4.6 mm internal diameter Gemini C18 (Phenomenex, Inc., Torrance, CA, USA) chromatographic column. The mobile phase consisting of 50 mM KH₂PO₄, methanol and acetonitrile with a mixing ratio of 72:17:11 was supplied with 1 mL per 1 min increments. MDA-TBA compound was identified and quantified by measuring the fluorescence at Eₓ 525 nm – Eₘ 560 nm wavelengths. The sample injection volume was 10 μl. Data collection and evaluation were performed by using Galaxy Workstation operating system (Varian, Inc., Palo Alto, CA, USA). The MDA-TBA compound was quantified by comparison between peak area of MDA-TBA compound in sample and peak area of this compound in standard solution.

**Amino acid content assay**

Amino acid content was determined on 40 breast muscles (n = 10 breasts/group). Hydrolysis of samples for amino acid analysis was proceeded as described in Commission Regulation (EC) No. 152/2009 which describes laying down the methods of sampling and analysis for the official control of the feed. Amino acid assay was performed by AccQ Tag technology (Waters Corp., Milford, MA, USA). For amino acid analyses in samples, Shimadzu low pressure gradient HPLC system (Shimadzu Corp., Kyoto, Japan) consisted of solvent delivery module LC-10ATVP, auto-injector SIL-10ADVP, column oven CTO-10ACVP, spectrofluorometric detector RF-10AXL, system controller SCL-10AVP, online degasser DGU-14A was used. For HPLC system control and data collection was used Workstation LC Solution (Shimadzu Corp., Kyoto, Japan). Amino acid derivatives were separated on Nova-Pak C18, 4 mm, 150 × 3.9 mm chromatography column (Waters Corp., Milford, MA, USA) by temperature 37 °C. 10 mL of derivatives were injected into separation. Separated derivatives were detected at Eₓ 250 nm – Eₘ 395 nm wavelengths. A gradient flow was used for the separation of amino acid derivatives. Flow rate was set at 1 mL/min. Amino acids were identified by the retention times as compared to the retention times of the amino acid standard solution. The results were calculated by measuring the peak areas of the sample and standard solution for each amino acid.

**Statistical analysis**

Data analysis was performed by SPSS for Windows, version 25.0 (IBM SPSS Inc., IL, USA, 2017). One-way analysis of variance (ANOVA) test post hoc (Fisher’s least significant difference test) was conducted to detect differences among groups. A calculated p value of less than .05 was considered statistically significant.

**Results and discussion**

**Growth and slaughter performance**

The effect of feed supplemented with betaine on broiler chickens’ growth performance is presented in Table 2. According to Ross 308 male broiler chicken,
performance objectives (Aviagen Incorporation 2014), which indicate the performance achievable under good management and environmental conditions when feeding recommended nutrient levels, our trial broiler chickens were growing a little slower till 3 weeks. Nevertheless, on 5 weeks of life broiler chickens in all groups reached performance objectives and even weighted about 100 g more. The same tendency reflected in ADG results. However, there were no significant differences between SCD and betaine groups comparing broiler chickens’ body weight and ADG during whole growing period ($p > .05$). FCR in betaine groups tended to increase depending on betaine inclusion: the more betaine was added to feed, the higher feed conversion was achieved ($p < .05$). FCR of whole growing period (1–5 weeks) in all experimental groups was consistent with FCR values given in Aviagen Instruction for Ross 308. However, this parameter was higher ($p < .05$) in all betaine groups in comparison to SCD group. Research of Chand et al. (2017) showed that betaine supplemented to broiler diet improved FCR by reducing it. It shows that our study FCR results did not coincide with results obtained by other scientists. In our study, the lowest mortality rate was observed in B1 group with 1 g/kg betaine inclusion ($p < .05$). Other treatment results did not exceed the usual mortality rate for broiler chickens, which is 1% per week. However, this indicator was lower in 1 g/kg and 2 g/kg betaine supplemented groups compared to SCD ($p < .05$).

After evaluating slaughter performance on 35 days of age of broiler chicken, results showed that betaine additive did not have significant effects on slaughter weight, carcass weight, carcass yield, breast muscle length and thickness ($p > .05$) (Table 3). Nofal et al. (2015) in his study reported that betaine can significantly improve breast muscle yield, when their feed was supplemented with betaine at levels of 0.1% or 0.2%. During our experiment, the highest breast percentage was found in B2 group and a bit lower in SCD and B1 groups’ samples ($p < .05$). Our study results were in line with Nofal et al. (2015) reported data. The widest breast muscles were measured in B3 samples, lower width breast muscles were measured in B1 samples ($p < .05$). The highest breast muscle yield was obtained in B2 samples, the lowest in SCD samples ($p < .05$). Similar results were reported in El-Shinnawy’s (2015) study, when broilers fed with feed supplemented with betaine showed a significantly higher percentage of breast muscle yield.

**Histomorphometric properties of breast muscles**

Diameter and density of muscle fibre directly affect the meat tenderness on a tissue basis, which is also an important index for the assessment of muscle tenderness (Sarsenbek et al. 2013). The higher the histomorphometric indexes of the muscles, the more tender the meat. Such a connection and correlation between muscle fibre characteristics and tenderness of meat were demonstrated by several scientists in their previous studies (Sifre et al. 2005; Zhang et al. 2007; An et al. 2010). In our recent study, after evaluating betaine influence on breast muscle fibre area, significant differences were observed between SCD and betaine supplemented groups (Table 4). 1 g/kg betaine additive inclusion in feed improved broilers breast muscle fibre areas, which were significantly larger compared to SCD ($p < .05$).

**Physicochemical properties of breast muscles**

The effect of feed supplementation with different levels of betaine additive on broiler chickens’ breast muscle physicochemical properties was evaluated during the present test (Table 5). The results showed that

| Group | Indicator | SCD | B1 | B2 | B3 | SEM | $p$ Value |
|-------|-----------|-----|----|----|----|-----|-----------|
|       | Slaughter weight, g | 2633.80 | 2672.80 | 2609.20 | 2636.40 | 102.36 | .708 |
|       | Eviscerated weight, g | 1800.15 | 1823.15 | 1808.79 | 1821.10 | 67.26 | .737 |
|       | Breast muscles, % of slaughter weight | 24.55<sup>a</sup> | 24.60<sup>b</sup> | 26.25<sup>b</sup> | 25.31<sup>b</sup> | 0.79 | .048 |
|       | Dimensions of breast muscles, cm | | | | | | |
|       | Length | 18.02 | 17.84 | 17.30 | 17.70 | 0.79 | .377 |
|       | Width | 10.12<sup>ab</sup> | 9.02<sup>a</sup> | 9.34<sup>ab</sup> | 10.22<sup>b</sup> | 0.58 | .050 |
|       | Thickness | 3.38 | 3.18 | 3.14 | 3.20 | 0.30 | .436 |
|       | Yield, % | 68.34 | 68.21 | 69.43 | 69.09 | 1.14 | .301 |
|       | Breast muscles | 35.93<sup>a</sup> | 36.08<sup>ab</sup> | 37.77<sup>b</sup> | 36.63<sup>b</sup> | 0.88 | .049 |

Groups: SCD: standard compound diet; B1: standard compound diet + 1 g/kg betaine additive; B2: standard compound diet + 2 g/kg betaine additive; B3: standard compound diet + 3 g/kg betaine additive.

<sup>a,b</sup>Means in a row with different superscripts differ ($p < .05$).

SEM: standard error of means.
Table 4. Effect of feed supplemented with betaine on histomorphometric properties of fresh broiler chickens’ breast muscles.

| Indicator | SCD | B1 | B2 | B3 | SEM | p Value |
|-----------|-----|----|----|----|-----|---------|
| Breast muscle fibre length, µm² | 72.93<sup>a</sup> | 85.01<sup>b</sup> | 81.21<sup>b</sup> | 80.11<sup>b</sup> | 4.50 | .002 |

Groups: SCD: standard compound diet; B1: standard compound diet + 1 g/kg betaine additive; B2: standard compound diet + 2 g/kg betaine additive; B3: standard compound diet + 3 g/kg betaine additive. SEM: standard error of means.

Table 5. Effect of feed supplemented with betaine on physicochemical parameters of fresh broiler chickens’ breast muscles.

| Indicator | SCD | B1 | B2 | B3 | SEM | p Value |
|-----------|-----|----|----|----|-----|---------|
| DM, %     | 25.92 | 26.59 | 25.39 | 26.99 | 1.27 | .277 |
| pH        | 6.03<sup>a</sup> | 5.97<sup>a</sup> | 5.98<sup>b</sup> | 6.18<sup>b</sup> | 0.05 | .001 |
| Colour by coordinate L<sup>a</sup> | 53.01 | 54.71 | 56.69 | 56.88 | 2.16 | .091 |
|          | a<sup>a</sup> | 15.82<sup>b</sup> | 18.32<sup>b</sup> | 18.72<sup>b</sup> | 15.23<sup>b</sup> | 1.63 | .048 |
|          | b<sup>b</sup> | 10.08 | 8.95 | 11.34 | 11.45 | 1.62 | .145 |
| Drip loss, % | 2.22<sup>a</sup> | 4.24<sup>a</sup> | 4.60<sup>b</sup> | 2.45<sup>a</sup> | 1.01 | .018 |
| Water binding, % | 62.04 | 6.73 | 60.38 | 62.78 | 2.53 | .356 |
| Cooking losses, % | 19.40<sup>a</sup> | 19.73<sup>a</sup> | 25.55<sup>b</sup> | 21.26<sup>b</sup> | 2.66 | .034 |
| Shear force, kg/cm² | 1.26<sup>a</sup> | 0.90<sup>b</sup> | 0.87<sup>b</sup> | 1.12<sup>b</sup> | 0.16 | .026 |
| Fat, %     | 3.31<sup>a</sup> | 3.27<sup>b</sup> | 3.15<sup>b</sup> | 3.02<sup>b</sup> | 0.57 | .042 |
| Ash, %     | 2.68<sup>a</sup> | 2.70<sup>b</sup> | 2.70<sup>b</sup> | 2.70<sup>b</sup> | 0.19 | .041 |
| Protein, % | 21.35 | 21.05 | 20.96 | 22.78 | 1.13 | .127 |

Groups: SCD: standard compound diet; B1: standard compound diet + 1 g/kg betaine additive; B2: standard compound diet + 2 g/kg betaine additive; B3: standard compound diet + 3 g/kg betaine additive. SEM: standard error of means.

The nutritional value of broiler chickens’ breast muscles was determined. Compared to SCD samples, lower fat contents were found in B1 and B3 breast muscle samples (p < .05). The highest and equal amount of ashes was determined in B1, B2 samples, slightly lower amount in B3 (p < .05). Comparing our results with other author’s research there is no clear trend in the composition of breast muscle nutrition value, when broilers diet is supplemented with betaine. Alirezai et al.’s (2012) study results showed that fat and ash contents in betaine group were slightly lower compared to control, however, obtained data were not significant.

**MDA content**

Oxidation reactions are considered to be one of the main reasons for meat and meat products spoilage (Silva et al. 2018). Often for these reactions in meat is responsible MDA, which is one of the major aldehyde derivatives of lipid peroxidation, and it is a by-product of the lipid peroxidation processes (Smith et al. 2005). Our research showed that feed supplemented with different levels of betaine affects broiler chickens’ breast muscle oxidative stability (Table 6). Betaine supplementation in feeds slow down the oxidation processes in breast muscles: significantly lower lipid oxidation rates were observed in all betaine groups, except B1 group’s samples of fresh meat. After fresh breast muscle assay, results showed significantly lower MDA levels in B2 and B3 samples, compared to SCD (p < .05). Besides, lipid oxidation processes are inevitable during meat storage and our research results show that betaine inclusion significantly decreases lipid oxidation processes in breast muscles, compared to SCD. After 3 months of storage in a freezer (−18°C), it was found that the levels of MDA in B1, B2 and B3 samples were significantly lower, compared to SCD samples (p < .05). The same study as ours was conducted by Alirezai et al. (2011) and Fu et al. (2016), but the results obtained by these scientists...
**Table 6.** Effect of feed supplemented with betaine on malondialdehyde (MDA) content in fresh and stored broiler chickens’ breast muscles.

| Group    | Period | SCD | B1  | B2  | B3  | SEM | p Value |
|----------|--------|-----|-----|-----|-----|-----|---------|
| MDA in fresh meat, μmol/kg | | | | | | | |
| 0.85<sup>a</sup> | 0.63<sup>b</sup> | 0.57<sup>b</sup> | 0.44<sup>b</sup> | 0.11 | <0.00 | |
| MDA after storage, μmol/kg | | | | | | | |
| 2.05<sup>a</sup> | 0.89<sup>b</sup> | 1.11<sup>b</sup> | 0.82<sup>b</sup> | 0.24 | <0.00 | |

**Groups:** SCD: standard compound diet; B1: standard compound diet + 1 g/kg betaine additive; B2: standard compound diet + 2 g/kg betaine additive; B3: standard compound diet + 3 g/kg betaine additive.

<sup>a,b</sup>Means in a row with different superscripts differ (p < .05). SEM: standard error of means.

**Table 7.** Effect of feed supplemented with betaine on amino acid content in fresh broiler chickens’ breast muscles, g/kg DM (when it contains 95% DM).

| Group | Amino acid | SCD | B1  | B2  | B3  | SEM | p Value |
|-------|------------|-----|-----|-----|-----|-----|---------|
| Aspartic acid | | 72.79<sup>a</sup> | 69.09<sup>b</sup> | 69.11<sup>b</sup> | 71.50<sup>b</sup> | 1.62 | <0.036 |
| Threonine | | 35.57<sup>a</sup> | 34.43<sup>b</sup> | 33.66<sup>b</sup> | 34.14<sup>b</sup> | 0.81 | <0.031 |
| Serine | | 29.43<sup>a</sup> | 28.33<sup>b</sup> | 27.09<sup>b</sup> | 28.75<sup>b</sup> | 0.66 | <0.043 |
| Glutamic acid | | 118.09<sup>a</sup> | 111.66<sup>b</sup> | 111.72<sup>b</sup> | 115.43<sup>b</sup> | 2.38 | <0.010 |
| Proline | | 26.22<sup>a</sup> | 24.13<sup>b</sup> | 25.27<sup>b</sup> | 23.86<sup>b</sup> | 1.08 | <0.044 |
| Glycine | | 35.20<sup>a</sup> | 33.47<sup>b</sup> | 34.14<sup>b</sup> | 34.17<sup>b</sup> | 0.73 | <0.031 |
| Alanine | | 44.60<sup>a</sup> | 42.96<sup>b</sup> | 42.64<sup>b</sup> | 43.15<sup>b</sup> | 0.93 | <0.052 |
| Valine | | 39.45<sup>a</sup> | 36.75<sup>b</sup> | 37.35<sup>b</sup> | 38.26<sup>b</sup> | 0.93 | <0.010 |
| Methionine | | 39.19<sup>a</sup> | 35.75<sup>b</sup> | 37.19<sup>b</sup> | 39.19<sup>b</sup> | 1.10 | <0.006 |
| Isoleucine | | 38.79<sup>a</sup> | 36.26<sup>b</sup> | 36.58<sup>b</sup> | 37.54<sup>b</sup> | 0.92 | <0.014 |
| Leucine | | 60.20<sup>a</sup> | 56.79<sup>b</sup> | 59.08<sup>b</sup> | 58.16<sup>b</sup> | 1.95 | <0.101 |
| Tyrosine | | 28.27<sup>a</sup> | 27.18<sup>b</sup> | 26.94<sup>b</sup> | 27.77<sup>b</sup> | 0.81 | <0.121 |
| Phenylalanine | | 30.32<sup>a</sup> | 28.47<sup>b</sup> | 28.56<sup>b</sup> | 29.23<sup>b</sup> | 0.61 | <0.008 |
| Histidine | | 33.22<sup>a</sup> | 31.32<sup>b</sup> | 30.85<sup>b</sup> | 32.60<sup>b</sup> | 1.21 | <0.067 |
| Lysine | | 69.08<sup>a</sup> | 65.82<sup>b</sup> | 66.21<sup>b</sup> | 68.75<sup>b</sup> | 1.92 | <0.108 |
| Arginine | | 53.77<sup>a</sup> | 51.94<sup>b</sup> | 51.10<sup>b</sup> | 52.36<sup>b</sup> | 1.10 | <0.027 |
| Total | | 754.20<sup>a</sup> | 714.37<sup>b</sup> | 718.40<sup>b</sup> | 734.86<sup>ab</sup> | 14.59 | <0.015 |

**Groups:** SCD: standard compound diet; B1: standard compound diet + 1 g/kg betaine additive; B2: standard compound diet + 2 g/kg betaine additive; B3: standard compound diet + 3 g/kg betaine additive.

<sup>a,b</sup>Means in a row with different superscripts differ (p < .05). SEM: standard error of means.

The amino acid content differed. In the Fu et al.’s (2016) study, higher MDA levels were determined in betaine groups and lower in control. However, data obtained by this author were statistically unreliable. In our experiment, the positive effects of betaine on the reduction of oxidative processes were determined in Alirezaei et al.’s (2011) study. In her study, significantly lower amounts of MDA were found in betaine groups and higher in control. The mechanism of antioxidant effects of betaine occurs against oxidative damage and lipid oxidation processes increasing by restoring S-adenosyl methionine. By restoring S-adenosyl methionine, it contributes to an enhancement in the supply of substrate needed for the synthesis of glutathione (GSH), which protect the cells from reactive metabolites and reactive oxygen species (Alirezaei et al. 2011). Our results showed that MDA content decreased in breast muscles of betaine-treated broiler chickens, suggesting that betaine possessed antioxidant effects and preserved the cellular antioxidant stores.

**Amino acid content**

Different cultivation technologies can be used for animal husbandry to increase inadequate amino acid levels in feeds, which may affect bird growth and body development (Conde-Aguilera et al. 2013). Levels of amino acids in the poultry diet are primarily influenced by the chemical composition of the feed, but further accumulation into bird tissues can be affected by environmental factors, size of the feed pellet, age and sex of the bird. All these parameters can alter the consumption of amino acids and their subsequent metabolic processing. Table 7 shows the effect of feed supplemented with betaine on amino acid content in fresh breast muscles. The highest total amino acid content obtained in SCD samples and lower in B1, B2 samples (p < .05). The predominant amino acids in the samples were glutamic and aspartic, which were mainly identified in SCD samples, lower amounts were determined in B1, B2 samples (p < .05). The highest threonine and serine contents were also found in SCD samples but lower in B2 samples (p < .05). Higher levels of proline were detected in SCD samples as well and lower in B3 samples (p < .05). In SCD group, higher amounts of glycine and alanine were detected, compared to betaine groups. B1 samples were lower on glycine and B2 samples lower on alanine (p < .05). As in the most samples, the highest levels of valine, isoleucine and phenylalanine were obtained in SCD, compared to B1 and B2 groups’ samples (p < .05). Lower levels of arginine were detected in B2 breast muscles compared to SCD (p < .05).

Methionine is one of the most limiting amino acid, playing decisive role in body protein synthesis, consequently it would be beneficial to spare its function as a methyl group donor (Sun et al. 2008). Noteworthy, that the structure of betaine consists of three methyl groups, which are donated during several metabolic reactions, allowing it to use as an effective compound to spare methionine as methyl donor group (Alirezaei et al. 2011). The mode of action of betaine as a methyl group donor in remethylation permits methionine to be directed towards protein synthesis. Further betaine transfers a methyl group via the enzyme betaine-homocysteine methyltransferase (BHMT) to become dimethylglycine (Alirezaei et al. 2011), thus continuing the methionine production and transmethylation cycle. In our recent study, no significant increase of this essential amino acid was detected. Equally highest amounts of methionine were found in...
SCD and B3 groups and lower amount in B1 breast muscle samples ($p < .05$). Furthermore, during our study, 16 amino acids that are the basic components of the body’s proteins were determined. Eight of them are essential, as they cannot be synthesised endogenously via metabolic pathways and thus must be provided by dietary sources. After identifying the amounts of amino acids, which are essential for human nutrition (threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine and lysine), higher amounts of it were detected in SCD group compared to betaine groups. In both ours and Fu et al.’s (2016) studies, the amounts of aspartic acid, threonine, serine, proline, glycine, phenylalanine and total amino acids in betaine groups significantly decreased depending on the betaine additive dosage. Likewise, similar results were obtained by Conde-Aguilera et al. (2013), who claimed that the lack of amino acids affected the muscle composition of broiler chicken, when their diet was supplemented with betaine. The data obtained by us and other scientists shows that lower inclusion of betaine additives cannot particularly increase the amount of methionine, as an essential amino acid, in the final product. Esteve-Garcia and Mack (2000) suggested alike that betaine cannot replace methionine as a methyl group donor and as essential amino acid in protein synthesis.

Conclusions

It is concluded that diets supplemented with betaine can increase FCR at all betaine levels in feed: the higher betaine inclusion in feed, the higher FCR. Therefore, based on broiler chickens’ mortality rate results, we can conclude that 1 and 2 g/kg betaine inclusion can reduce mortality and improve health status of broiler chickens. 1 g/kg betaine inclusion into feed can improve breast muscle tenderness by increasing breast muscle fibre areas. Betaine supplementation at all levels slows down breast muscle oxidative processes by decreasing MDA content in it. This improvement can be attributed to betaine ability to have antioxidative properties and ability to preserve the cellular antioxidant stores. However, according to our study, betaine, as a methyl group donor, in broiler chicken diets cannot particularly increase the amount of methionine and cannot replace it as an essential amino acid, which coincides with other scientist’s research.

Ethical approval

No ethical committee permission was required as slaughter was carried out in commercial slaughterhouse and breast muscle samples were collected post-mortem.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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