



Original Research Article

Betaine improves growth performance, liver health, antioxidant status, breast meat yield, and quality in broilers fed a mold-contaminated corn-based diet



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ABSTRACT

Betaine has been demonstrated to improve growth performance and antioxidant status of animals under various stress conditions. However, there is no literature on the effects of betaine in animals exposed to mycotoxins, which are among the most prevalent contaminants in feed. Therefore, this study was conducted to evaluate the influence of dietary betaine on broilers fed a diet based on mold-contaminated corn (MCC). A total of 192 Ross 308 male broiler chicks at 1 d of age were randomly divided into 4 groups with 6 replicates and fed an MCC-based diet supplemented with 0, 250, 500, and 1,000 mg/kg betaine, respectively. Betaine increased average daily gain (linear, $P = 0.030$) and decreased feed conversion ratio (linear, $P = 0.027$) of broilers during d 1 – 21, and decreased feed conversion ratio during d 22 – 42 (linear, $P = 0.012$; quadratic, $P < 0.001$) and d 1 – 42 (linear, $P = 0.003$; quadratic, $P = 0.004$), whereas feed intake was not affected. Total cholesterol (linear, $P = 0.024$), alanine aminotransferase (quadratic, $P < 0.001$) and alkaline phosphatase (linear, $P = 0.007$; quadratic, $P = 0.025$) activities in serum were decreased by betaine. Betaine linearly increased breast muscle yield ($P = 0.003$) and pH_{24 h} ($P = 0.008$), and decreased drip loss ($P = 0.022$). Betaine increased (linear, $P = 0.025$; quadratic, $P = 0.016$) total superoxide dismutase activity in breast muscle and reduced malondialdehyde content in serum (linear, $P = 0.006$), liver (quadratic, $P = 0.006$) and breast muscle (linear, $P = 0.003$). Moreover, the zearalenone concentrations in breast muscle were linearly decreased by betaine ($P = 0.006$). It was concluded that betaine could improve growth performance, liver health, antioxidant status, and breast meat yield and quality, and reduce zearalenone residue in broilers fed the MCC-based diet, especially at 500 or 1,000 mg/kg.

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1. Introduction

Mycotoxins are hazardous metabolites of molds (fungi) that are usually found in feed. They are known to exert toxic effects in animals and humans. Mycotoxigenic fungi infect crops in the field and

during storage. Until now, over 300 mycotoxins have been found, and a handful of mycotoxins are considered important from an economic point of view (Streit et al., 2013; Adegbeye et al., 2020). For example, aflatoxins (particularly aflatoxin B₁ [AFB₁]), deoxynivalenol (DON), zearalenone (ZEN) are among the most common mycotoxins in corn, which is the most important feed grain. According to a survey of mycotoxin levels in the samples of feed and feed raw materials, nearly 90% of the samples contained at least one mycotoxin and 64% of the samples were found to be co-contaminated (Gruber-Dorninger et al., 2019). Mycotoxin contamination results in the loss of nutritive value of feedstuffs and leads to mycotoxicoses, which vary from acute diseases with a high morbidity to chronic disorders with a reduced animal productivity (Bryden, 2012). The degree of toxicity mainly depends on the toxins present, dosage, duration of exposure, and a variety of other factors,

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such as animal species, age, and nutrition (Andretta et al., 2011). Mycotoxin contamination levels are usually not high enough to cause an overt disease but may cause an array of metabolic disturbances. Mycotoxin ingestion promotes the generation of free radicals and induces oxidative damages to DNA, proteins, and lipids (Doi and Uetsuka, 2011). In addition, mycotoxins may accumulate in animal products, compromising the safety of humans (Zaki et al., 2012). Therefore, there is a need to develop methods to reduce the adverse effects of mycotoxins in feed. Currently, clays, plant extracts, and mycotoxin-degrading bacteria and enzymes have been used in feed to deal with mycotoxin problems (Adegbeye et al., 2020).

Betaine is a common term for trimethylglycine, a naturally occurring compound that can be found in plants and animals. Due to its chemical structure, betaine shows the characteristics of a dipolar zwitterion with osmoprotective properties. It has been reported that betaine supplementation can mitigate the osmotic stress induced by diarrhoea and coccidiosis in animals (Kidd et al., 1997). Betaine provides its labile methyl groups for the methylation of homocysteine to methionine. The methyl groups are also required for the synthesis of several substances, such as creatine and carnitine, which play an important role in the oxidation of fatty acids (Eklund et al., 2005). Therefore, it can be expected that betaine may spare methionine and improve carcass quality in animal production (Sun et al., 2008). Previous studies have indicated that dietary betaine supplementation may improve the growth performance and carcass traits of animals (Leng et al., 2016; Mendoza et al., 2017; Chen et al., 2018). Moreover, betaine is considered as a promising antioxidant agent (Alirezai et al., 2012a; Zhang et al., 2016). It may alleviate oxidative damage in animals under various stress conditions (Balkan et al., 2005; Alirezai et al., 2012b; Wen et al., 2019). However, to our knowledge there is no literature on whether betaine can alleviate mycotoxin-induced toxicity and oxidative damages in animals. Therefore, this study was designed to evaluate the effects of betaine on broilers fed a diet based on mold-contaminated corn (MCC), which is a common ingredient and happens to be a significant source of mycotoxins.

2. Materials and methods

2.1. Experimental design

The procedures involving animals in this study were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee (SYXK (Su)2017-0007).

The MCC used in this study was kindly provided by a local feed company (Fengda Feed Co., Ltd., Linyi, China). The pelleted betaine (96% anhydrous) was provided by Skystone Feed Co., Ltd. (Yixing, China). A total of 192 Ross 308 male broilers at 1 d of age were randomly divided into 4 groups with 6 replicates of 8 chicks each, and fed an MCC-based mash diet (Table 1) supplemented with 0, 250, 500, or 1,000 mg/kg betaine, respectively. The concentrations of AFB₁ and ZEA in the starter (d 1 to 21) and the finisher (d 22 to 42) diets (Table 2) were analyzed by an enzyme-linked immunosorbent assay kit (R-Biopharm AG, Darmstadt, Germany). The variation of mycotoxin concentrations between the starter and the finisher diets was due to the different batch of MCC provided by the feed company. The betaine concentrations in the 4 groups were 7.8, 261.2, 493.8, and 982.5 mg/kg for the starter diets and 6.9, 243.6, 509.1, and 1,014.2 mg/kg for the finisher diets as analyzed by high-performance liquid chromatography (Chendrimada et al., 2002). Broilers were raised under a 23L:1D lighting program in 3-layer cages. The temperature of the experimental room was set at 33 °C initially for 3 d and then decreased to 20 °C by lowering 2 to 3 °C per week. Broilers had free access to mash feed and water. Feed

Table 1

Ingredients and nutrient content of the basal diet (as-fed basis, g/kg unless otherwise stated).

Item	Day 1 to 21	Day 22 to 42
Ingredients		
Mold contaminated corn	570	620
Soybean meal	326	280
Corn gluten meal	30	20
Soybean oil	30	40
Dicalcium phosphate	20	16
Limestone	12.3	13.0
L-Lysine HCl	3.2	3.1
D,L-Methionine	1.5	1.1
Sodium chloride	3.0	3.0
Vitamin and mineral mix ¹	4.0	3.8
Calculated nutrient content		
Metabolizable energy, MJ/kg	12.5	13.0
Crude protein	210	193
Lysine	12.2	11.0
Methionine	5.0	4.3
Methionine + Cystine	8.6	7.6
Calcium	10.1	9.3
Available phosphorus	4.6	3.9
Analyzed nutrient content		
Dry matter	863	875
Gross energy, MJ/kg	16.4	16.9
Crude protein	216	187
Ether extract	56.5	64.1
Crude fiber	35.2	28.3
Lysine	11.6	10.2
Methionine	4.7	3.8
Methionine + Cystine	8.5	7.2

¹ The premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 3,000 IU; vitamin E, 30 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 600 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B₁₂, 0.013 mg; Fe, 80 mg; Cu, 8 mg; Mn, 110 mg; Zn, 65 mg; I, 1.1 mg; and Se, 0.3 mg.

Table 2

Mycotoxin concentrations (μg/kg) in the diets.

Item	Betaine, mg/kg			
	0	250	500	1,000
Day 1 to 21				
Aflatoxin B ₁	110.18	105.86	119.35	94.37
Zearalenone	146.4	107.28	125.38	100.11
Day 22 to 42				
Aflatoxin B ₁	35.06	44.66	33.84	31.65
Zearalenone	789.73	797.19	829.61	845.19

consumption per cage was recorded weekly. At the end of the experiment, the broilers in each cage were weighed, and average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated.

2.2. Sample collection

One broiler per replicate was randomly selected and sacrificed by cervical dislocation. Blood samples (5 mL each) were taken from the jugular vein and centrifuged at 3,000 × g at 4 °C for 15 min. Then the serum was frozen at –20 °C for further analysis. Eviscerated yield was determined by removing the feathers, head, feet, abdominal fat, and all viscera. Abdominal fat percentage and breast muscle yield were calculated based on eviscerated weight.

2.3. Assay of serum metabolites

The concentrations of total cholesterol, triglyceride, glucose, total protein, albumin, alanine aminotransferase (ALT), aspartate

aminotransferase (AST), and alkaline phosphatase (ALP) in serum were determined using the analytical kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Meat quality assay

At 45 min and 24 h after slaughter, the meat color of breast muscle samples, including lightness (L^*), redness (a^*) and yellowness (b^*), was measured using a colorimeter (Minolta CR-10, Konica Minolta, Tokyo, Japan). The pH value of the samples was determined using a pH meter (HI9125, HANNA Instruments, Italy). Drip loss and cooking loss of the breast muscle samples at 24 h post-mortem were determined as described by Lu et al. (2017). Briefly, the breast muscle samples were placed in a Ziplock bag and stored in a refrigerator at 4 °C. After 24 h, the samples were wiped with absorbent paper and weighed. Then, the packed samples were heated in a water bath until the internal temperature reached 77 °C. After cooling under running water, the samples were weighed again. Cooking loss was calculated as the percentage of weight loss after cooking.

2.5. Oxidative status

The breast muscle and liver samples were homogenized with ice-cold physiological saline solution (1:9, wt/vol) for 1 min, each in an ice water bath. Then the homogenate was centrifuged at $5,000 \times g$ at 4 °C for 10 min to collect the supernatant as previously described (Wen et al., 2020). The assay of malondialdehyde (MDA), glutathione peroxidase (GPX), and total superoxide dismutase (T-SOD) in the supernatant and serum were performed using analytical kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Mycotoxin assay

The concentrations of AFB₁ and ZEA in the samples of breast muscle and liver were determined using an enzyme-linked immunosorbent assay kit according to the procedures described by the manufacturer (R-Biopharm AG, Darmstadt, Germany). The data were expressed on the basis of fresh tissues.

2.7. Statistical analysis

All data were analyzed as one-way ANOVA using polynomial contrasts to determine the linear and quadratic effects of betaine addition levels via SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Data were presented as means and standard error of the mean, and significance was set at $P < 0.05$.

3. Results

3.1. Growth performance

Dietary betaine increased ADG (linear, $P = 0.030$) and decreased FCR (linear, $P = 0.027$) of broilers during d 1 – 21, and decreased FCR during d 22 – 42 (linear, $P = 0.012$; quadratic, $P < 0.001$) and d 1 – 42 (linear, $P = 0.003$; quadratic, $P = 0.004$) (Table 3). The ADFI was not significantly affected by betaine throughout the experiment. Mortality showed no significant differences among groups (Data not shown).

3.2. Serum metabolites

Dietary betaine supplementation decreased total cholesterol (linear, $P = 0.024$), ALT (quadratic, $P < 0.001$), and ALP (linear,

Table 3

Effects of betaine on growth performance of broilers fed a mold-contaminated corn-based diet.¹

Item	Betaine, mg/kg				SEM	P-value	
	0	250	500	1,000		Linear	Quadratic
Day 1 to 21							
ADG, g	35.43	34.46	36.01	39.35	0.70	0.030	0.100
ADFI, g	55.14	49.49	50.63	53.74	1.15	0.767	0.067
FCR	1.56	1.44	1.41	1.37	0.03	0.027	0.515
Day 22 to 42							
ADG, g	70.88	71.74	75.76	70.77	1.26	0.754	0.267
ADFI, g	149.90	134.75	137.38	139.24	2.28	0.133	0.053
FCR	2.12	1.88	1.81	1.97	0.03	0.012	<0.001
Day 1 to 42							
ADG, g	53.15	53.10	55.89	55.06	0.76	0.244	0.805
ADFI, g	100.50	91.46	93.37	95.05	1.43	0.242	0.057
FCR	1.87	1.72	1.67	1.73	0.02	0.003	0.004

ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

¹ $n = 6$ per treatment group.

$P = 0.007$; quadratic, $P = 0.025$) activities in serum, but did not affect the other serum metabolites tested (Table 4).

3.3. Carcass traits and meat quality

Dietary betaine linearly increased breast muscle yield ($P = 0.003$), but did not affect eviscerated yield and abdominal fat percentage (Table 5). Betaine had no effect on meat color, but linearly increased pH_{24 h} value ($P = 0.008$) and decreased drip loss ($P = 0.022$) of breast muscle. There was no difference in cooking loss.

3.4. Oxidative status

Betaine elevated T-SOD activity in breast muscle (linear, $P = 0.025$; quadratic, $P = 0.016$) but not in serum or liver (Table 6). The MDA contents in serum (linear, $P = 0.006$), liver (quadratic, $P = 0.006$) and breast muscle (linear, $P = 0.003$) were decreased by betaine. The GPX activity did not differ among groups.

3.5. Mycotoxin concentrations in liver and breast muscle

The concentrations of AFB₁ in liver and breast muscle were not affected by betaine, but ZEA concentrations in breast muscle were linearly decreased ($P = 0.006$) by betaine (Table 7).

4. Discussion

The data showed that dietary betaine supplementation linearly increased ADG and decreased FCR during d 1 – 21, and it linearly and quadratically decreased FCR during d 22 – 42 and d 1 – 42. Our finding indicated that betaine could promote the growth rate of broilers during early growth period and improve feed efficiency throughout the experiment. Similar results were obtained by a previous research using normal corn (Chen et al., 2018), in which dietary betaine increased body weight gain and decreased FCR of broilers. Some studies have also shown the positive effects of betaine on the growth performance of broilers under stress conditions, such as coccidiosis, heat exposure, and transportation (Fetterer et al., 2003; Shakeri et al., 2018; Chen et al., 2020). This might be due to the improved retention of dietary nutrients in response to supplemental betaine (Eklund et al., 2005). The osmoregulatory function of betaine supports intestinal health and enhances cell activity, thereby improving nutrient digestibility

Table 4
Effects of betaine on serum metabolites of broilers fed a mold-contaminated corn-based diet.¹

Item	Betaine, mg/kg				SEM	P-value	
	0	250	500	1,000		Linear	Quadratic
Total protein, g/L	62.64	58.60	53.62	60.59	1.939	0.529	0.171
Albumin, g/L	19.12	20.00	20.12	18.21	0.522	0.589	0.206
Total cholesterol, mmol/L	3.54	3.30	3.05	2.26	0.200	0.024	0.474
Triglyceride, mmol/L	1.03	0.94	1.00	0.93	0.068	0.706	0.951
Glucose, mmol/L	12.79	12.53	12.82	11.75	0.325	0.361	0.552
ALT, U/L	3.57	1.69	1.99	2.81	0.200	0.095	<0.001
AST, U/L	26.63	29.35	29.96	27.76	0.747	0.549	0.116
ALP, U/L	28.94	15.28	15.82	15.97	1.791	0.007	0.025

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase.

¹ n = 6 per treatment group.**Table 5**
Effects of betaine on carcass traits and breast meat quality of broilers fed a mold-contaminated corn-based diet.¹

Item	Betaine, mg/kg				SEM	P-value	
	0	250	500	1,000		Linear	Quadratic
Eviscerated yield, %	68.45	68.84	68.46	69.13	0.31	0.586	0.835
Breast muscle yield, %	29.72	30.46	33.02	32.72	0.46	0.003	0.495
Abdominal fat percentage, %	1.22	1.08	1.06	0.90	0.06	0.101	0.937
L* _{45 min}	47.64	46.87	48.75	48.27	0.42	0.345	0.867
a* _{45 min}	5.00	5.22	5.53	5.18	0.15	0.551	0.394
b* _{45 min}	13.40	13.15	13.76	13.05	0.32	0.884	0.728
L* _{24 h}	51.82	51.96	52.80	51.44	0.36	0.927	0.321
a* _{24 h}	5.99	5.72	5.85	6.12	0.11	0.625	0.262
b* _{24 h}	15.36	15.27	15.20	15.06	0.26	0.705	0.967
pH _{45 min}	6.30	6.33	6.34	6.39	0.02	0.162	0.819
pH _{24 h}	5.80	5.81	5.85	5.96	0.02	0.008	0.228
Drip loss, g/kg	32.28	27.99	29.95	26.02	0.85	0.022	0.907
Cooking loss, g/kg	276.42	269.98	265.05	264.66	4.02	0.295	0.722

L* = lightness; a* = redness; b* = yellowness.

¹ n = 6 per treatment group.**Table 6**
Effects of betaine on oxidative status of broilers fed a mold-contaminated corn-based diet.¹

Item	Betaine, mg/kg				SEM	P-value	
	0	250	500	1,000		Linear	Quadratic
T-SOD							
Serum, U/mL	172.35	177.65	199.61	187.47	4.28	0.066	0.282
Liver, U/mg protein	167.30	172.37	187.00	184.18	4.07	0.093	0.626
Breast muscle, U/mg protein	69.39	91.22	100.94	89.49	3.86	0.025	0.016
GPX							
Serum, U/mL	687.58	657.10	692.42	697.74	18.42	0.709	0.650
Liver, U/mg protein	45.06	45.50	46.55	45.64	1.05	0.780	0.766
Breast muscle, U/mg protein	16.23	16.52	17.10	16.28	0.69	0.893	0.716
MDA							
Serum, nmol/mL	3.23	2.78	2.24	2.27	0.14	0.006	0.338
Liver, nmol/mg protein	1.11	0.71	0.58	0.81	0.07	0.074	0.016
Breast muscle, nmol/mg protein	0.64	0.47	0.21	0.30	0.06	0.003	0.156

T-SOD = total superoxide dismutase; GPX = glutathione peroxidase; MDA = malondialdehyde.

¹ n = 6 per treatment group.**Table 7**
Effects of betaine on mycotoxin concentrations (µg/kg) in liver and breast muscle of broilers fed a mold-contaminated corn-based diet.¹

Item	Betaine, mg/kg				SEM	P-value	
	0	250	500	1,000		Linear	Quadratic
Aflatoxin B ₁							
Liver	2.20	1.81	2.15	2.24	0.08	0.485	0.113
Breast muscle	2.17	2.05	2.24	2.18	0.07	0.752	0.849
Zearalenone							
Liver	8.72	6.90	7.18	6.39	0.40	0.069	0.515
Breast muscle	0.55	0.47	0.34	0.32	0.03	0.006	0.690

¹ n = 6 per treatment group.

(Metzler-Zebeli et al., 2009). Furthermore, because the diets were marginally deficient in methionine and cystine in this study, the response in ADG and FCR might also be explained by the methionine-sparing effect of betaine, which has been reported in previous studies (Zhan et al., 2006; Sun et al., 2008). However, the effects of betaine on broiler performance were relatively small and not significant in another study (Esteve-Garcia and Mack, 2000). The inconsistency might be attributed to the methionine-deficient diet (2.5 to 3.2 g/kg) used in their study. It is known that betaine cannot replace methionine in protein synthesis; therefore the efficacy of betaine in broiler diets is related to dietary methionine levels.

Dietary betaine linearly decreased total cholesterol concentration in serum. Our finding was similar to the data of He et al. (2015), who found that betaine decreased total cholesterol concentration in serum of broilers under heat stress. This might be explained by the inhibitory effect of betaine on the hepatic mRNA expression of 3-hydroxy-3-methylglutaryl-CoA reductase, which is the rate-controlling enzyme in cholesterol biosynthesis (Leng et al., 2016). Betaine also decreased ALT activity quadratically and ALP activity linearly and quadratically in serum, which were generally considered as the markers of liver injury. Our data were similar to the data of Konca et al. (2008), who demonstrated that betaine decreased the serum ALT activity of broilers exposed to heat stress. Thus, it can be inferred that betaine might improve the liver health of broilers fed MCC. Betaine has been demonstrated to ameliorate liver injury in mice under stress conditions (Kim and Kim, 2002; Ji and Kaplowitz, 2003; Wang et al., 2010). The hepatoprotective activity of betaine might be explained by the enhancement of antioxidant capacity through the regulation of sulfur amino acid metabolism (Jung et al., 2013).

Among the carcass traits tested, only breast muscle yield was linearly increased by betaine. Similar data were reported by Zhan et al. (2006), who used normal corn in their study. These data suggested that betaine might promote the muscle growth of broilers irrespective of MCC feeding. The promotion might be due to upregulated myogenic gene expression induced by betaine (Chen et al., 2018). However, some studies found that betaine supplementation reduced the abdominal fat percentage of broilers under normal or heat stress conditions (Zhan et al., 2006; He et al., 2015). The discrepancy might be related to different feed ingredients and environment between these studies. Dietary betaine supplementation linearly increased the pH_{24 h} value and decreased drip loss of breast muscle, which was in agreement with our previous research (Wen et al., 2019). The negative effects of mycotoxin on meat quality of broilers and the ameliorative effect of dietary intervention were demonstrated previously (Fan et al., 2013). Thus, the present study suggested that betaine supplementation might improve meat quality of broilers fed MCC. This might be due to alleviated muscle anaerobic glycolysis and improved antioxidant status in response to betaine (Alirezai et al., 2012a; Chen et al., 2020).

Dietary betaine linearly and quadratically increased T-SOD activity in breast muscle, and linearly or quadratically decreased MDA contents in serum, liver, and breast muscle. The data supported our above inference that the improved antioxidant activity might be involved in the regulation of liver injury and meat quality by betaine. An previous research showed that betaine supplementation ameliorated oxidative status of broilers exposed to heat stress (Wen et al., 2019). It was also reported that betaine pretreatment mitigated hepatotoxicity and oxidative stress in the liver of rats treated with lipopolysaccharide (Balkan et al., 2005). It might be attributed to the enhanced nonenzymatic antioxidant defenses by betaine through the methionine cycle (Alirezai et al., 2011; Zhang et al., 2016). However, it is not clear why there was no effect of betaine on T-SOD activities in serum and liver or GPX activities in serum, liver, and breast muscle.

The concentrations of AFB₁ did not differ among all the groups, but the concentrations of ZEA in breast muscle were linearly decreased by betaine supplementation, which was in parallel with the decreased MDA content in breast muscle. This implied that the reduction of ZEA residue might be associated with reduced lipid peroxidation. However, the mechanism through which betaine reduced ZEA residue is unclear. It was reported that dietary intervention might decrease mycotoxin residues in animal tissues possibly by increasing the activity of uridine diphosphate glucuronyl transferases, which are mainly synthesized in liver and induce

the biotransformation and degradation of mycotoxins and their metabolites (Obol'skiĭ et al., 1998; Wang et al., 2012). Thus, it might be speculated that betaine reduced ZEA residue by improving liver health, resulting in the enhanced synthesis of uridine diphosphate glucuronyl transferases.

5. Conclusion

This study indicated that dietary betaine supplementation linearly or quadratically improved growth performance, liver health breast muscle yield and meat quality in broilers fed an MCC-based diet. Moreover, the antioxidant status of serum, liver, and breast muscle was improved by betaine, which also reduced ZEA residue in breast muscle. Overall, the optimal addition level of betaine was 500 or 1,000 mg/kg.

Author contributions

Chao Wen: Conceptualization, Funding acquisition, Methodology, Data curation, Writing-Original draft preparation. **Rui Chen:** Formal analysis, Visualization. **Yueping Chen:** Investigation. **Liren Ding:** Project administration. **Tian Wang:** Writing-Reviewing and Editing. **Yanmin Zhou:** Supervision.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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