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Research Article

Effect of Fermented Wheat-Rice Based Distiller's Grains with Solubles (FDDGS) on Meat Quality and Serum Amino Acid Profile of Broilers

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Abstract

An experiment was conducted to investigate the effect of replacing high inclusion levels of distillers' dried grains with solubles (DDGS) with FDDGS on meat quality and serum amino acid profile in Chinese yellow broilers. A total of 24 42 days male Chinese Yellow broilers were randomly allotted to the FD or D treatments, each replicated 6 times, 2 birds per replicate, which received basal corn-soybean diet plus 20 % of fermented DDGS (D) or 20% of DDGS (FD) respectively. Broilers were euthanized at 70 d of age. The right half of each breast was evaluated for meat quality. Both breast and thigh meat were evaluated for proximate composition and fatty acid composition. Blood were sampled and serum were analyzed for free amino acid composition. The FD treatment had lower pH and drip loss in breast meat than those from the D treatment ($P<0.05$). No differences existed among proximate composition of breast and thigh meat from the FD and D treatments($P>0.05$). Myristic acid (C14:0) concentration of thigh muscles was lower for FD treatment when compared with D treatment ($P<0.05$). Serum concentrations of lysine, taurine, alpha-aminoadipic acid, glycine, 3-Methylhistidine were all lower in FD treatment than in D treatment ($P<0.05$). Meanwhile, serum phosphoserine concentration was greater in FD treatment compared with D treatment ($P<0.05$). In conclusion, replacing the high inclusion levels of DDGS in the diet with FDDGS can improve meat quality for broilers by decreasing pH and drip loss of breast meat, but the lack of advantages in serum amino acids implicated that the optimum amount of addition also needs further study.

Keywords: Fermented DDGS; Broiler; Fatty acid; Amino acid; Meat quality

Introduction

As ethanol production has expanded in recent years, the availability of dried distiller's dried grains with solubles (DDGS) as a feedstuff for poultry diets has increased. Due to high percentage of lignocellulose and low quality of protein [1], utilization of DDGS remains elusive problem in animal production. For poultry, the DDGS can be included in the diets by at least 10% to 15% without reducing product quality [2]. However, damnification of production efficiency factor appeared when concentration of DDGS exceeded 18% [3]. The 18 and 24% DDGS treatments yielded breast meat with higher pH values, higher shear force compared with control treatment [4]. Corn DDGS contains high levels of polyunsaturated fatty acids (PUFAs). Inclusion of DDGS in diets improved meat quality [5,6] by increasing proportion of PUFA. But PUFA are vulnerable to lipid peroxidation [7], feeding DDGS containing oxidized lipids may induce oxidative stress, alter immune function and, thus, negatively affect animal growth performance.

Solid-state fermentation has been utilized as a means of improving nutritional value of agricultural by-product. Fermentation loosens the structure of lignocellulose and makes available nutrients in DDGS more accessible to animals. The microorganisms also add nutrients to the substrate which might be inadequate in the untreated sample, such as lipid. Study in our laboratory showed that after fermentation, both crude fat and crude protein content were elevated and at the same time crude fiber content of DDGS declined (data not published).

Effect of fermentation on nutritional value improvement was confirmed in the past. However, if fermented DDGS can relieve negative effects on performance and meat quality when high inclusion levels of DDGS feed for broilers is still unclear. Therefore, the present study was conducted to investigate the effect of replacing FDDGS for high inclusion levels of DDGS on meat quality and serum amino acid profile in broilers.

Materials and Methods

The experimental design and procedures in this study were reviewed and approved by the Animal Care and Use Committee of the College of Life Sciences and Environment, Hengyang Normal University.

Animals and experimental treatments

A total of 24 Chinese yellow male broilers, with an initial body weight of 1.46±0.21kg, were randomly assigned to FD or D treat-

ments. Each had 6 replicates with 2 birds per replicate. Treatment FD or D received 80% basal diet plus 20% fermented DDGS or DDGS respectively. Basal diet (Table 1) was formulated to meet NRC (1994) nutrient recommendations. DDGS was kindly provided by Yanjing beer company (Yanjing, Hengyang, China), it was by-product of beer production industry, mainly residues of wheat and rice. Fermented DDGS was manufactured in our laboratory using *Aspergillus niger, Saccharomyces cerevisiae*, *Bacillus subtilis* co-fermentation on DDGS. The gross energy, protein, fat, crude fiber, ash content of material fermented DDGS were 4902kcal/kg, 32.41%, 4.15%, 7.21%, 3.47% respectively, and corresponding numerical of material DDGS were 5180kcal/kg, 24.90%, 3.92%, 14.67%, 3.99% respectively. Birds were housed in wire-floored, suspended cages, consumed feed and water on an *ad libitum* basis. Experiment was performed between April and May and lasted 28 days. Housing conditions were controlled by a natural ventilation system and there were two fans in the room. Natural lighting was applied during experiment.

Table 1: Calculated ingredient composition of basal diet for broilers, as-fed basis.

Ingredients, %	Basal Diet
Corn	58.75
Soya bean meal 43% CP	26.40
Extruded full-fat soybean	9.30
Soybean oil	1.35
Limestone	0.90
Dicalcium phosphate	1.96
Premix[1]	1.00
Salt	0.34
Total	100
Calculated Values[2]	
ME, kcal/kg	2950
CF, %	2.30
Crude fat, %	5.40
CP, %	19.50
Ca, %	0.85
P, %	0.67
Digestible P, %	0.45
Salt, %	0.37
Lys, %	1.06
Met+Cys, %	0.70
Thr, %	0.81
Trp, %	0.24

[1]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 8065 IU; vitamin D_3 as cholecalciferol, 1580 IU; vitamin E as DL-alpha tocopheryl acetate, 15 IU; riboflavin, 7.8mg; vitamin B_{12}, 16μg; D-pantothenic acid as D-calcium pantothenate, 12.8mg; niacin, 75mg; folic acid, 1.62mg; biotin, 0.27mg; Choline chloride, 509mg; Cu, 12mg as copper sulfate; Fe, 60mg as ferrous sulfate; I, 2.25mg as ethylenediamine dihydriodide; Mn, 80mg as manganese sulfate; Se, 0.15mg as sodium selenite; and Zn, 90mg as zinc sulfate.

[2]Amino acids are indicated as standardized ileal digestible AA.

Sample collection

At 28 of the experiment, blood samples were taken from the wing vein and centrifuged (2000 × g,15min) at room temperature, and then the serum was harvested and stored at -80 ^{0}C until analysis. At the end of the 4-wk feeding period, 6 broilers from each treatment were randomly selected and euthanized by cervical dislocation. After harvest, abdominal fat, crureus, breast muscles, oesophagus, glandular stomach, muscular stomach, duodenum, jejunum, ileum, cecum, colorectum, bursal, spleen, liver were separated and weighted. Breast and thigh muscles were sampled from the right side of carcass, a portion of them were stored at -4 ^{0}C to measure shear force and others at -80 ^{0}C until analysis.

Assessment of meat quality

Subjective breast muscle color scores on a scale from 1 to 5 (1=pale pinkish gray to white, and 5=dark purplish red) compared with standard colorimetric board (Minolta, Osaka, Japan) were determined. A hand-held pH meter (Russell CD700, Russell pH Limited) was used to measure the pH of breast samples at 45min postmortem. Drip loss of breast was determined by a suspension method, that is, the breast muscle was weighed and placed in a Whirl-Pak bag, suspended in a 4 ^{0}C cooler for 24h, reweighed, and drip loss was calculated as follows: % drip loss=[(initial weight-final weight)/initial weight]×100%. The fresh breasts were weighed and baked in a steamer to a final internal temperature of 77 ^{0}C. Internal chicken breast temperatures were determined using thermometer. Cooked breasts were cooled to ambient temperature, and reweighed. Cooking loss was calculated as a percentage of lost weight based on the pre-cooking weight of breast. Breasts that were used for cooking loss determinations were used for shear force determinations. To measure the firmness, breasts were cut into squares (20×10×10 mm) and then subjected to the measurement of shear force using C-LM3B Tenderness meter (Nanjing, China). Three shear force values were recorded and averaged.

Chemical analysis

Fresh meat samples were freeze-drying and then ground with soybean milk machine. Lipid concentrations of muscles were analyzed by Soxhlet extraction procedure using petroleum ether as extraction agent (Soxhlet method, method 991.36; AOAC [8]). Crude protein concentrations of muscles were determined in accordance with Kjeldahl method (Nitrogen concentration ×6.25, method 968.06; AOAC [8], using CNS-200 carbon, nitrogen and Sulphur analyser (LECO Corporation, St, Joseph, MI)). Tissue fatty acid composition was determined as follows. Briefly, total lipid were extracted using chloroform: methanol (2:1, v/v) as extraction

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reagent. The fatty acid methyl esters (FAMEs) were prepared using a mixture of boron-trifluoride, hexane, and methanol (35:20:45 v/v). FAME profiles were determined by Agilent 7890A gas chromatography equipped with SP-2560 column (100m× 250μm × 0.2μm; Agilent Technologies Inc., Santa Rosa, CA). The gas chromatography conditions were as follows: column oven temperature was set at 45 ^{0}C for 4 min, raised to 175 ^{0}C at 13 ^{0}C/min, held at 175 ^{0}C for 27min, increased from 175 ^{0}C to 215 ^{0}C at 4 ^{0}C/min, and held at 215 ^{0}C for 35min. The injector and detector temperatures were maintained at 250 ^{0}C. The carrier gas (hydrogen) flow rate was 30mL/min. By comparing the FAME profile of the samples with those of FAME standards (Sigma Chemicals Co., St. Louis, MO), the fatty acids were calculated as percentage of the total fatty acids. Serum amino acid concentrations were analyzed using oxidation analysis method on an Applied Biosystems 3200Q TRAP LC/MS/MS system equipped with RP-C18 column (150mm length, 4.6mm diameter, 5mm particle size). Amino acid and fatty acid composition were tested in Subtropical Institute of Chinese Academy of Sciences.

Statistical analysis

The effects of the fermented DDGS were analyzed by one-factor GLM using SAS 8.2 software package (SAS Inst. Inc., Cary, NC). Differences between mean values were determined using Tukey test at the level of $P<0.05$.

Results

Carcass traits

Table 2: Relative organ weights of broilers fed diets supplemented with DDGS or FDDGS from 42 to 70 days of age.

Item[a], %	FD	D	SEM	*P*-Value
abdominal fat	1.62	2.29	0.14	0.052
crureus	5.61	5.21	0.12	0.144
breast muscles	4.72	4.90	0.29	0.763
oesophagus	0.17	0.24	0.02	0.085
glandular stomach	0.56	0.44	0.04	0.214
muscular stomach	2.11	2.46	0.15	0.273
duodenum	0.87	0.75	0.02	0.052
jejunum	0.55	0.47	0.03	0.220
ileum	0.47	0.46	0.02	0.812
cecum	0.40	0.53	0.03	0.100
colorectum	0.76	1.09	0.04	0.004
bursal	0.10	0.10	0.01	0.872
spleen	0.23	0.15	0.02	0.055
liver	1.86	1.75	0.09	0.602
small intestine	1.88	1.69	0.04	0.060
large intestine	1.15	1.62	0.04	0.001

aValues calculated as weight of organ divided by body weight. D: 80% basal diet plus 20% wheat-riced based distillers'dried grains with solubles (DDGS), FD: 80% basal diet plus 20% fermented DDGS.

Ingestion of FDDGS decreased the percentages of colorectum and large intestine when compared with D treatment ($P<0.05$) (Table 2). Percentages of abdominal fat, oesophagus tended to be lower and percentages of duodenum, spleen, small intestine tended to be higher when broiler fed with FD diet compared with D diet ($P<0.10$). No differences existed respected to percentages of crureus, breast muscles, glandular stomach, muscular stomach, jejunum, ileum, cecum, bursal, liver of broilers that were fed different diets ($P>0.10$).

Meat quality

No differences existed between FD and D treatments with respect to the subjective incarnadine score, cooking loss, shear force, protein and fat content of breasts and thighs ($P>0.05$) (Table 3). Breasts meat from broilers that were fed FDDGS had lower pH and lower percentage of drip loss than those from the D treatment($P<0.05$).

Table 3: Meat quality of broilers fed diets supplemented with DDGS or FDDGS from 42 to 70 days of age.

Item[a]	FD	D	SEM	*P*-Value
Subjective incarnadine score	1.80	2.25	0.16	0.197
pH value	5.30	6.00	0.14	0.046
drip loss, %	0.96	1.48	0.11	0.048
cooking loss, %	26.72	26.23	0.89	0.794
shear force, kg of force/c^{m}2	3.69	3.88	0.21	0.659
Protein Content, %				
thigh muscles	0.46	0.52	0.02	0.273
breast muscles	0.62	0.58	0.01	0.324
Crude Fat Content, %				
thigh muscles	7.92	8.91	0.32	0.082
breast muscles	3.32	3.54	0.22	0.560

aD: 80% Basal Diet Plus 20 % Wheat-Riced Based Distillers' Dried Grains with Solubles (DDGS); FD: 80% Basal Diet Plus 20% Fermented DDGS

Fatty acid composition

Only one out of the 15 fatty acids that were detected in broiler thighs differed ($P<0.05$) in proportion between FD and D treatments (Table 4). Thighs from the FD treatment had a lower proportion of myristic acid (C14:0) than thigh meat from the D treatment

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(P<0.05). Feeding FDDGS also had a tendency to decrease (P<0.10) elaidic acid (C18:1n9T) compared with D treatment.

Table 4: Fatty acid profile of chicken thigh meat from broilers fed diets supplemented with DDGS or FDDGS from 42 to 70 days of age.

Fatty Acid[a], %	FD	D	SEM	*P*-Value
Myristic (C14:0)	0.68	0.75	0.01	0.032
Palmitic (C16:0)	23.42	24.59	0.52	0.299
Heptadecanoic (C17:0)	0.29	0.30	0.01	0.765
Stearic (C18:0)	9.11	9.09	0.25	0.965
Arachidic (C20:0)	0.12	0.09	0.01	0.131
Palmitoleic (C16:1)	3.46	3.41	0.16	0.868
Elaidic (C18:1n9T)	0.16	0.19	0.01	0.095
Oleic (C18:1n9C)	37.06	37.25	0.88	0.916
Eicosenoic (C20:1 n9)	0.53	0.47	0.02	0.191
Linoleic (C18:2n6C)	23.57	22.17	0.98	0.498
γ-Linolenic (C18:3n6)	0.22	0.21	0.02	0.779
Homo-γ-Linolenic (C20:3n6)	0.26	0.31	0.03	0.401
Arachidonic (C20:4n6)	0.14	0.13	0.02	0.672
α-Linolenic (C18:3n3)	0.75	0.87	0.04	0.195
DHA (C22:6n3)	0.22	0.21	0.02	0.663

aD: 80% Basal Diet Plus 20% Wheat-Riced Based Distillers' Dried Grains with Solubles (DDGS); FD: 80% Basal Diet Plus 20% Fermented DDGS

Serum metabolites

Feeding FDDGS decreased (P<0.05) the concentration of lysine, taurine, alpha-aminoadipic acid, glycine, 3-Methylhistidine in serum compared with D treatment (Table 5). Meanwhile, serum concentration of phosphoserine was greater for broiler fed with FDDGS compared with DDGS (P<0.05). However, there was no difference in serum concentration of histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, arginine, urea, aspartic acid, serine, glutamic acid, sarcosine, alanine, β- alanine, citrulline, α-aminobutyric acid, cysteine, cystathionine, tyrosine, β- aminoisobutyric acid, γ- aminobutyric acid, ethanolamine, hydroxylysine, ornithine, 1-methylhistidine, carnosine, anserine, proline, hydroxyproline between the treatments (P>0.05).

Table 5: Serum free amino acid profile of broilers fed diets supplemented with DDGS or FDDGS from 42 to 70 days of age.

Item[a], μmol/L	FD	D	SEM	*P*-Value
EAA				
Histidine	6.74	9.07	0.89	0.265
Threonine	13.07	19.82	2.12	0.184
Valine	8.03	9.03	0.78	0.572
Methionine	5.96	4.22	0.46	0.123
Isoleucine	4.41	4.50	0.26	0.874
Leucine	10.19	12.49	0.98	0.310
Phenylalanine	8.61	9.12	0.61	0.707
Lysine	12.23	19.88	1.04	0.014
Arginine	21.83	24.84	1.73	0.443
NEAA				
Taurine	9.59	12.96	0.30	0.002
Urea	4.95	4.14	0.56	0.522
Aspartic acid	5.61	5.23	0.51	0.738
Serine	35.12	32.53	1.74	0.510
phosphoserine	2.29	1.72	0.10	0.035
Glutamic acid	13.83	17.76	1.16	0.162
Sarcosine	0.62	0.88	0.05	0.065
α- aminoadipic acid	0.19	0.39	0.03	0.012
Glycine	16.88	26.10	1.34	0.018
Alanine	33.20	29.48	1.67	0.335
β- Alanine	1.73	2.11	0.18	0.357
Citrulline	1.22	1.52	0.11	0.266
α- Aminobutyric acid	1.46	1.84	0.21	0.435
Cysteine	3.65	4.41	0.40	0.410
Cystathionine	0.90	1.15	0.10	0.273
Tyrosine	13.15	11.20	1.29	0.501
β- Aminoisobutyric acid	0.91	1.05	0.11	0.554
γ- Aminobutyric acid	0.13	0.13	0.02	1.000
Ethanolamine	1.08	1.22	0.14	0.657
Hydroxylysine	0.12	0.19	0.00	0.050
Ornithine	1.25	1.37	0.07	0.470
1-Methylhistidine	6.31	4.33	1.05	0.407
3-Methylhistidine	1.33	2.24	0.15	0.032
Carnosine	2.22	3.40	0.30	0.116
anserine	4.22	7.94	0.75	0.057
Proline	12.50	16.99	1.00	0.078
Hydroxyproline	2.65	5.30	0.59	0.076

aD: 80% Basal Diet Plus 20% Wheat-Riced Based Distillers' Dried Grains with Solubles (DDGS); FD: 80% Basal Diet Plus 20 % Fermented DDGS

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Discussion

Meat quality

Table 6: Fatty acid profile of chicken breast meat from broilers fed diets supplemented with DDGS or FDDGS from 42 to 70 days of age.

Fatty Acid[a], %	FD	D	SEM	*P*-Value
Myristic (C14:0)	0.58	0.64	0.02	0.182
Palmitic (C16:0)	23.33	24.05	0.36	0.384
Heptadecanoic (C17:0)	0.26	0.27	0.01	0.915
Stearic (C18:0)	9.54	10.02	0.39	0.589
Arachidic (C20:0)	0.10	0.10	0.01	0.639
Palmitoleic (C16:1)	2.70	2.75	0.27	0.942
Elaidic (C18:1n9T)	0.14	0.16	0.01	0.122
Oleic (C18:1n9C)	32.30	33.16	1.05	0.710
Eicosenoic (C20:1 n9)	0.90	0.75	0.20	0.729
Linoleic (C18:2n6C)	20.89	19.58	0.89	0.515
γ-Linolenic (C18:3n6)	0.18	0.17	0.01	0.780
Homo-γ-Linolenic (C20:3n6)	0.37	0.47	0.08	0.588
Arachidonic (C20:4n6)	7.27	5.97	0.61	0.353
α-Linolenic (C18:3n3)	0.61	0.73	0.06	0.384
DHA (C22:6n3)	0.80	0.68	0.14	0.691

[a]D: 80% Basal Diet Plus 20% Wheat-Riced Based Distillers' Dried Grains with Solubles (DDGS); FD: 80% Basal Diet Plus 20% Fermented DDGS

Feeding FDDGS to Chinese Yellow broiler caused breast meat to be less acidic, which generally indicates higher quality meat. There was no other study in how FDDGS affect pH value of meat. Schilling et al. [4] found that feeding 12-24% corn DDGS resulted in higher average pH 24h than in the control and 6% corn DDGS treatment. But other reports showed that corn DDGS addition did not affect pH value of breast meat in broilers [5,9]. Higher pH may be related to altered glucose utilization in birds affected by DDGS that results in glycogen depletion. Early reports showed that drip loss was decreased after broilers ingestion DDGS [4,5], but there was contrary conclusion [9]. Present study confirms that fermented DDGS can improve the water-holding capacity (WHC) further Table 6. It is well known that the majority of water in the cell is held in myofibrils and that most water is retained (steric) by capillary forces generated by the arrangement of thick and thin filaments within the myofibril [10]. On the other hand, changes in chemical composition such as decrease in muscle fiber number Mazzoni et al. [11] and increase in fat content are likely to play a major role in reduction of WHC. As fat content of breast muscle was not affected by FDDGS addition, the reason for higher WHC ability in FD group could be ascribed to differences in muscle fiber, but it needed further study. Fatty acid composition of the feed is the most important determinant of the fatty acid composition in the resulting meat [12]. The proportions of monounsaturated fatty acid of thigh meat decreased and the proportions of polyunsaturated fatty acid increased when diet supplementation with 15% DDGS [6]. DDGS inclusion (200g/kg) in the diet reduced the concentration of stearic acid and behenic acid in thigh meat [2]. An increased proportion of PUFA is an indicator of increased susceptibility to oxidation, increasing the level of DDGS in the diets could make thigh meat more susceptible to oxidation. The use of FDDGS was failure to avoid such damage because only myristic acid (C14:0) concentration of thigh muscles was significantly lower than that in the D group.

Amino acid metabolism

Fermentation increased digestibility of essential amino acids and other useful nutrients Shi et al. [13] and promoted muscle protein metabolism [14]. But protein concentrations of both breast and thigh muscle were not affected by fermentation in our study. Both lysine, α-aminoadipic acid, phosphoserine engaged in emotional regulation. Lysine itself is major essential amino acid for broilers, and it is important in emotional regulation and the response to stress [15]. α-aminoadipic acid is a responsible factor of antisocial behavioural organization including aggression and impulsivity. Serotonin and other neurotransmitters promote antisocial behaviours such as aggressive behavior, impulsivity, hostile behaviours [16]. But their generation needs existence of pyridoxal-5-phosphate (P5P) [17,18]. Increase in serum phosphoserine can be an indicator of P5P deficiency. It suggests that stress behavior was more likely to happen in FD group as serum lysine and α-aminoadipic acid levels was lower and serum phosphoserine level was higher in this group. This could ascribe to lower fiber in FDDGS, previous studies have reported beneficial effects of fiber on satiety because of its large bulk.

Conclusion

In conclusion, the positive effects of diets containing FDDGS on meat quality of broilers showed that this processed source of protein can serve as an appropriate alternative for diet for broiler chickens. But the lack of advantages in serum amino acids implicated that the optimum amount of addition also needs further study.

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