

# EFFECT OF METABOLIZABLE ENERGY CONTENT AND ILEAL AMINO ACID DIGESTIBILITY OF SORGHUM-BARLEY BREWER'S SPENT GRAIN (SBBSG) ON GROWTH, CARCASS AND BLOOD PARAMETERS IN BROILERS

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**ABSTRACT:** The aim of this study was to determine the protein and amino acid digestibilities, and apparent metabolizable energy (AME) contents of sorghum-barley brewers' spent grain (SBBSG), use this information to formulate an appropriate diet for broilers, and determine the effect of SBBSG inclusion on growth, carcass and blood parameters. In experiment one fifteen individually housed broilers were used to determine the apparent digestibilities of nutrients. Another cohort of 15 broilers were raised alongside and nutrient digestibility of key nutrients in wheat bran determined. The AME of SBBSG (6.72 MJ/kg) was higher than previously assumed, whereas the digestibility values of some of the essential amino acids for SBBSG (Lys, Met, Phe, Thr and Val) were generally lower (42.93, 71.6, 55.47, 75.26, and 62.66%) than previously assumed. In experiment two, diets were formulated based on nutrient availability values from trial one and fed to two hundred and fifty day-old chicks. Birds were allotted to five diets (T1, T2, T3, T4 and T5, representing 0, 4, 8, 12 and 16% SBBSG, respectively). Average daily feed intake (ADFI) for birds on T1 and T2 were similar (105.6 and 102.3g) and these were higher ( $P < 0.05$ ) than ADFI for birds on T3 to T5 (92.2, 93.4 and 88.3 respectively). There were no treatment differences in average daily gain (ADG) and final body weight (FNBW). However as the level of dietary SBBSG increased, feed conversion efficiency (FCE) improved. Carcass parameters were not affected by the level of dietary SBBSG. Blood parameters were within acceptable ranges for broilers. It can be concluded that inclusion of SBBSG up to 16% in diets that are formulated based on nutrient availability, will have no adverse effects on broiler performance and blood profile. Results of this trial indicate that nutrient availability is key in effective feed formulation and broiler productivity.

**Keywords:** Sorghum-Barley Brewers' Spent Grain, Digestibility, Performance, Blood Profile

## INTRODUCTION

The broiler industry in Ghana faces severe challenges and its growth continues to be slow. Providing adequate and affordable nutrition for poultry and livestock poses a challenge to livestock production in developing countries like Ghana (FAO, 2011). The development of the poultry industry depends to a large extent on the availability of feedstuffs that are used or can be made suitable for use in poultry nutrition (Ondwasy et al., 2006). Feed costs alone can constitute up to about 70% of the total cost of production of poultry meat and eggs (Babiker et al., 2009). Feed prices in Ghana have been climbing primarily due to the fact that grains used in animal feed formulation, are the same grains that are used in human diets and in the breweries as well. The United States Department of Agriculture (USDA) estimates that in Ghana, the poultry industry consumes nearly 30 percent of all corn produced (USDA, 2013). The competition and consequently, increase in the cost of feed and animal production (FAO, 2011) necessitates a reduction in the use of whole grain and cereals in monogastric diets with increasing emphasis on the

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use of cereal and Agro-Industrial by-products. Nutrients in these by-products must be packaged in such a way that they can support monogastric production. Spent grain from the brewing industry is a typical example of such an ingredient.

The brewing industry in Ghana generates large quantities of brewer's spent grain. The composition of this by-product depends on the grain used in the brewing process. Generally however they tend to be high in some essential nutrients (including protein and essential amino acids and fats; [Mussatto et al., 2006](#)) but have somewhat limited use in monogastric diets because of high fibre levels ([Nortey et al., 2013](#); [Manu-Barfo et al., 2013](#)). Some of the larger breweries in Ghana use malted Sorghum as starting grains for their brewing operations. The resulting spent grain, Sorghum-Barley Brewer's Spent Grain (SBBSG), is unique and is used mainly by swine producers in the Kumasi Metropolis of Ghana as part of a wet-feeding regimen. Initial trials indicated that broilers fed SBBSG-based diets had improved growth rate, but the composition of this grain was mainly fat. Although the chemical composition of SBBSG, particularly amino acids, was determined and found to be high, its digestibility and availability to broilers was not determined ([Nortey et al., 2013](#)). As a result of this, in the above mentioned trials, digestibility values for brewers spent grain reported by [Donkor and Attoh-Kotoku \(2009\)](#) were used in the formulation of SBBSG based diets. Differences in digestibility values for different ingredients are important, as formulating diets on the basis of total nutrients rather than on digestible nutrients, resulting in wrong amino acid to Energy ratios, can result in excessive fat deposition ([Jackson et al., 1982](#)). According to the National Research Council (NRC), availability of nutrients in different feed ingredients and ultimately in compounded feed is essential for the optimal production of birds ([NRC, 2000](#)). Information on the ability of a ration to supply digestible rather than total nutrients is necessary for accurate diet formulation ([Ravindran, 2013](#)).

Digestibility of poultry diets is very important and can be a limiting factor governing energy and nutrient availability ([Adedokun et al., 2007](#)). Digestibility of nutrients present in a feed ingredient therefore needs to be quantified. However, there is no information on nutrient digestibility of SBBSG in poultry. The hypothesis of this study therefore was that, when diets are formulated based on available, rather than on total amino acid and energy availability, SBBSG can be included in diets for broilers without negatively affecting growth, carcass composition and the health of birds.

The specific objectives of the present study were: I) to determine the AME and ileal digestibilities of amino acids in SBBSG, II) use the results obtained to formulate appropriate diets for broiler birds, and III) determine the digestibility of a comparable high-fibre ingredient (wheat bran). Results of the third objective were then compared to literature values and was intended to serve as a validation for the results obtained for the SBBSG digestibility values.

## **MATERIALS AND METHODS**

The animal protocol used followed principles and ethical procedures recommended by the Institutional Animal Care and Use Committee of the Noguchi Memorial Institute for Medical Research, University of Ghana. The experiments were conducted in two phases. The first phase consisted of a digestibility trial and the second phase was a feeding trial. The two experiments were carried out at the Livestock and Poultry Research Centre (LIPREC), College of Basic and Applied Sciences (CBAS) of the University of Ghana (UG). Digestibility values obtained in the first experiment were used to formulate appropriate diets containing varying levels of SBBSG for a growth trial. The growth performance and carcass characteristics of broiler chickens fed these diets were then evaluated.

The proximate chemical analysis of the feed ingredients and the compounded feed were done at the Nutrition Laboratory at the Department of Animal Science (DAS), CBAS- UG. Amino acid analysis was done at the Animal Science Department of the University of Illinois, United States of America. The analysis for protein and energy in the ileal digesta and the faecal samples of the individual birds were done at the Nutrition Laboratories of the Department of Animal Science (DAS), CBAS- UG and the Livestock and Poultry Research Centre (LIPREC).

### **Feed Ingredients**

The SBBSG was obtained from the Kaase plant of Guinness Ghana Brewery Limited (GGBL) in Kumasi, Ghana. Fresh material with an approximate moisture content of 95% was sun-dried for one week to a moisture content of 6%, ground through a hammer mill with a screen size of approximately 2mm, and stored until ready for use. The wheat bran was obtained from a local flour mill that sells this particular by-product on the open market to farmers.

### **Animals and housing**

One hundred mixed sex COBB day-old broiler chicks were used as the starting point out of which 30 were used for digestibility trials. They were initially placed in deep litter pens and fed a commercial broiler starter diet

from day one to day 21. On day 22 the birds were transferred to individual battery cages measuring 42 x 35 x 42 cm (L x B x H) where they were fed a broiler finisher diet till d 35.

#### Determination of apparent metabolizable energy in SBBSG and wheat bran

On d 36, an initial group of 30 individually housed broilers were used for the determination of apparent metabolizable energy contents in SBBSG and wheat bran (15 birds per ingredient). Birds were fed *ad libitum* on a broiler finisher diet for 1 week prior to force-feeding (Sibbald, 1986). Collection trays were fixed to the individual cages, the birds were fasted for 24 h and force-fed 60 g of the test ingredient. Excreta were collected daily from each bird for 48 h and the samples immediately placed on ice to help prevent microbes from multiplying. The samples were then freeze dried at a temperature of -18°C for 70h. Excreta samples were blended, ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored in plastic bags until analyses. Both the test ingredients and faecal materials were subsequently analysed for gross energy by a bomb calorimeter.

#### Determination of apparent ileal amino acid and nitrogen digestibility in SBBSG and wheat bran

Out of the remaining 70 birds that remained from the initial group, 30 male broilers (15 per test ingredient) of approximately equal weight were selected and used to determine apparent ileal and amino acid digestibilities. The test ingredients, SBBSG and wheat bran were mixed with minerals and a vitamin premix. Chromic acid (an indigestible marker) was incorporated into the diets at a level of 0.3% (Table 1). The birds were fed the test diets *ad lib* from day 35 for seven days. On day 42, the birds were euthanized by cervical dislocation and the gastro-intestinal-tract removed. The contents of the terminal ileum (half-way between the ileal-cecal junction and Meckel's diverticulum) were emptied by dissecting this region and collecting the digesta by gentle finger-stripping and flushing with 10 -15 mls of distilled/deionized water into a plastic bag following the method of Ravindran (2007).

The bags containing the 30 individual samples (15 per test ingredient) were immediately placed on ice, transported and freeze-dried. The freeze-dried samples were then allowed to equilibrate to room temperature, ground through a 0.75 mm sieve (Ultra-centrifugal mill ZM 200, Retsch GmbH & Co. KG, Haan, Germany) and stored at room temperature in a dry place prior to chemical analyses. Digesta samples were blended, and stored in plastic bags at 30°C until analysis for the major nutrients (protein, amino acids and energy). Digestibility of wheat bran was also conducted and the results compared to literature values. This was to serve as a validation for the results obtained for the SBBSG digestibility values.

#### Growth Performance and Carcass Parameters

**Animals, Housing and Diets.** A total of 250 mixed-sex Ross broiler day-old chicks were randomly allocated to five treatments, replicated five times with 10 birds per replicate in a Completely Randomized Design (CRD). In the first 4 wks, birds were housed in a brooder house in pens measuring 152 x 152 cm. Thereafter, they were transferred to cages measuring 55x 39 x 49 cm. The birds were individually weighed at the start of the experiment and subsequently on a weekly basis. Birds in each replicate were given a known amount of feed daily, and any leftover feed weighed the next day prior to the day's feeding in order to determine feed intake. The leftover feed was then discarded and fresh feed provided. Water was provided ad-lib. Standard routine vaccination protocols were strictly adhered to. The birds were fed with the experimental starter diet for 28d (Table 3) after which they were switched onto the finisher diet (Table 4) for the rest of the trial. The results of the digestibility trials and chemical analysis of the feed ingredients were used as the basis for formulating the diets. Diet one (T1) had zero SBBSG, while T2, T3, T4 and T5 had 4, 8, 12 and 16% SBBSG respectively.

**Table 1 - Composition of the diets used for the digestibility trial**

Ingredient	SBBSG-Based diet	Wheat Bran-based diet
SBBSG	95.7	0
Wheat Bran	0	95.7
Dicalcium Phosphate	1.5	1.5
Vitamin Premix <sup>a</sup>	1.00	1.00
Oyster Shell	1.00	1.00
Salt	0.50	0.50
Cr <sub>2</sub> O <sub>3</sub>	0.3	0.3
Total	100	100

<sup>a</sup> Premix provided the following per kg of the diet: vit. A, 7500 IU; vit. D<sub>3</sub>, 2200 IU; vit. E, 10 IU; vit. K, 1.73 mg; riboflavin, 2.5 g 2.5 g; cobalamin, 0.05 mg; pantothenic acid, 6 mg; niacin, 20 mg; choline, 240 mg; folic acid, 0.5 mg; Mg, 2.8 mg; Fe, 45 mg; Cu, 5.5 mg; Mn, 55 mg; Zn, 50 mg; I, 0.8 mg; Co, 0.2 mg

**Table 2 - Composition of broiler starter diets used for the growth trial**

Ingredients (%)	0% SBBSG*	4% SBBSG	8% SBBSG	12% SBBSG	16% SBBSG
Maize	51.50	53.50	54.50	55.50	56.50
Soybean meal	27	25.35	24.35	23.35	22.35
Wheat bran	16	12	8	4	0
SBBSG	0	4	8	12	16
Soybean oil	1	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.7	0.7	0.7	0.7	0.7
Vitamin premix <sup>a</sup>	0.6	0.6	0.7	0.7	0.7
Methionine	0.25	0.3	0.3	0.3	0.3
Lysine	0.25	0.3	0.3	0.3	0.3
NaCl	0.25	0.25	0.25	0.25	0.25
Limestone	2.5	2.5	2.5	2.5	2.5
Total	100	100	100	100	100
<b>Calculated analyses</b>					
ME, MJ/kg	11.5	11.5	11.6	11.73	11.86
Crude protein (%)	21.6	21.6	21.61	21.67	21.72
Crude fat (%)	4.43	3.9	3.85	3.8	3.8
Crude fibre (%)	3.4	3.6	3.8	3.97	4.15
Lysine (%)	1.3	1.32	1.3	1.28	1.26
Methionine (%)	0.5	0.56	0.57	0.58	0.59
Calcium (%)	1.14	1.16	1.16	1.16	1.16
Phosphorus (% total)	0.7	0.64	0.62	0.59	0.57

\* SBBSG: Sorghum-Barley Brewer's Spent Grain; <sup>a</sup>Premix provided the following per kg of the diet: vit. A, 7500 IU; vit. D<sub>3</sub>, 2200 IU; vit. E, 10 IU; vit. K, 1.73 mg; riboflavin, 2.5 mg; cobalamin, 0.05 mg; pantothenic acid, 6 mg; niacin, 20 mg; choline, 240 mg; folic acid, 0.5 mg; Mg, 2.8 mg; Fe, 45 mg; Cu, 5.5 mg; Mn, 55 mg; Zn, 50 mg; I, 0.8 mg; Co, 0.2 mg

**Table 3 - Composition of broiler finisher diets used for the growth trial**

INGREDIENTS (%)	T1 0% SBBSG*	T2 4% SBBSG	T3 8% SBBSG	T4 12% SBBSG	T5 16% SBBSG
Maize	55.25	56.80	57.80	58.80	59.80
Soybean meal	23.7	22.05	21.05	20.05	19.05
Wheat bran	16	12	8	4	0
SBBSG	0	4	8	12	16
Soybean oil	1	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.7	0.7	0.7	0.7	0.7
Vitamin premix <sup>a</sup>	0.6	0.6	0.6	0.6	0.6
Methionine	0.25	0.3	0.3	0.3	0.3
Lysine	0.25	0.3	0.3	0.3	0.3
NaCl	0.25	0.25	0.25	0.25	0.25
Limestone	2.5	2.5	2.5	2.5	2.5
Total	100	100	100	100	100
<b>Calculated analyses</b>					
ME, MJ/kg	11.56	11.59	11.72	11.85	11.98
Crude protein (%)	20.2	20.4	20.4	20.46	20.51
Crude fat (%)	4.44	3.9	3.85	3.8	3.75
Crude fibre (%)	3.4	3.58	3.76	3.94	4.11
Lysine (%)	1.21	1.24	1.22	1.20	1.18
Methionine (%)	0.5	0.55	0.56	0.57	0.57
Calcium (%)	1.15	1.15	1.15	1.15	1.15
Phosphorus (% total)	0.65	0.63	0.60	0.58	0.56

\* SBBSG: Sorghum-Barley Brewer's Spent Grain; <sup>a</sup>Premix provided the following per kg of the diet: vit. A, 7500 IU; vit. D<sub>3</sub>, 2200 IU; vit. E, 10 IU; vit. K, 1.73 mg; riboflavin, 2.5 mg; cobalamin, 0.05 mg; pantothenic acid, 6 mg; niacin, 20 mg; choline, 240 mg; folic acid, 0.5 mg; Mg, 2.8 mg; Fe, 45 mg; Cu, 5.5 mg; Mn, 55 mg; Zn, 50 mg; I, 0.8 mg; Co, 0.2 mg

### Chemical Analyses

All major feed ingredients and experimental diets were ground to pass through a 1mm sieve in a hammer mill (Retsch SM100, F.Kurt GmgH Co). Samples for analysis were weighed using a top-loading electronic balance (ADAM AAA 250LE). Dry matter was determined overnight at 105°C in a Gallenkamp oven (method 930.15; AOAC, 2000). Crude protein analysis was done using the Kjeldahl method (method 942.05; AOAC, 2000). Samples were digested at 400°C for 3 hours with the Gerhardt Kjeldatherm (Germany) digester; distillation and titration were done using the Gerhardt Vapodest 50s (Germany) distillation unit. Amino acid analysis was carried out using ion-

exchange chromatography, following standard procedures (AOAC, 2000). First, the protein was hydrolyzed, followed by separation, identification, and finally quantification of the amino acids. The procedure involved sample hydrolysis in a 6 N HCl for 24 h at 110 °C under N atmosphere. For sulphur containing amino acids (methionine and cysteine), the performic acid oxidation was first carried out before acid hydrolysis. This step is to convert cysteine to cysteic acid and methionine to methionine sulfone, which are acid stable and can then be separated by chromatographic methods. Samples for tryptophan analysis were hydrolyzed using barium or sodium hydroxide to avoid the destruction of tryptophan by acid hydrolysis and to enhance its stability. The amino acids in the hydrolysate were then determined by HPLC after post-column derivatization (AOAC, 2000; 982.30 E). The chromic oxide concentration in feed, faeces and digesta were determined using the method described by Fenton and Fenton (1979).

The whole trial lasted 56d. Performance and feeding records were taken and summarized on a weekly basis. These included average daily feed intake (ADFI), average daily gain (ADG) and feed conversion efficiency (FCE). On day 56, the final body weights (FBW) were taken and five birds per replicate were randomly selected. Blood from each bird was quickly drawn from the wing vein using a 10ml syringe. About 3ml was transferred into a 4ml sample tube (Surgifield Medicals, Middlessex, England) using K<sub>3</sub> EDTA as an anticoagulant. For serum profiling, about 3ml of blood was transferred into a 5ml serum-separator vacuum tube. Both samples were analysed within one hour of collection for haematological and biochemical parameters using an Advia 120 analyzer.

The remaining 5 birds from each replicate were slaughtered by jugular venipuncture (Osei et al., 2010). They were de-feathered after scalding in boiling water and immediately transferred into a cold room where they were stored overnight at -4°C. The next day, the whole chilled carcasses were weighed. Weights of some internal organs (including liver, full and empty intestines, and gizzard) were taken. Abdominal fat which had solidified following the overnight chilling was carefully separated and the weight recorded.

#### Data analysis

Estimates of apparent nitrogen and amino acid digestibilities were determined using ileal samples and calculated from the dietary ratio of protein or amino acids to chromium relative to the corresponding ratio in the ileal digesta. Apparent metabolizable energy was determined according to the method used by Donkor and Attoh-Kotoku (2009). The formula used was as follows:

$$AME = (EI - EO)/FI$$

Where

EI = Gross energy in feed

EO = Gross energy in faeces

FI = Feed Intake (90 g, in this case)

Nutrient digestibility was calculated using the formula below;

$$\text{Digestibility\%} = \frac{100 - \{(\text{Concentration (CrO}_3\text{) in feed} \times \text{concentration (Nutrient) in digesta})\}}{\text{Concentration (CrO}_3\text{) in digesta} \times \text{concentration (Nutrient) in feed}} \times 100$$

#### Statistical analyses

All data collected were subjected to statistical analysis using the Generalized Linear Model (GLM) Procedure of Statistical Analysis System Institute (SAS, 2003). Significant differences among treatment means were separated using the Student Neuman-Keuls' (SNK) Test.

The model used was as follows;

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where,

Y<sub>ij</sub> is the observation

μ is the mean

D<sub>i</sub> is the effect of diet

E<sub>ij</sub> is the error term

## RESULTS

#### Nutrient composition

The analyzed nutrient composition of the SBBSG and wheat bran are shown in Table 4. Crude protein, crude fat, and organic matter (OM) levels were higher in SBBSG (26.95, 7.85 and 75.76%) than in WB (16.61, 3.55 and 70.88%). Essential amino acids (Lys, Met and Thr), and Crude fat levels were also higher in SBBSG (0.77, 0.44, 0.90 and 8.85% than in wheat bran were (0.66, 0.20, 0.50 and 3.55%). Generally there were wide variations between

some amino acid digestibilities in SBBSG. For the essential amino acids Lys, Thr, Met, Cys, Phe and Leu, apparent digestibilities were higher in Thr for SBBSG (42.93, 75.26, 71.58, 53.91, 55.47 and 55.9%) but similar or lower for the rest of them in WB (69.5, 67.2, 70.9, 70.8, 78.55 and 69.63%).

#### Nutrient digestibilities

The apparent amino acid digestibilities of the SBBSG and wheat bran are shown in Table 5.

#### Growth performance

Growth traits of interest that were recorded included ADFI, ADG, FCE and FNBW (Table 6).

#### Average daily feed intake

Generally as the dietary level of SBBSG increased, ADFI reduced. For T1 and T2, ADFI was 105.59 and 102.30 g respectively. These values were different ( $P < 0.05$ ) from birds on T3 and T4 (92.92 and 93.35g) respectively. Birds on T5 consumed the least amount of feed (88.26g) and this was lower ( $P < 0.05$ ) than all the other treatments.

#### Average daily gain, feed conversion efficiency and final body weight

There was no dietary effect ( $P > 0.05$ ) on the average daily gain of birds. As the level of SBBSG increased in the diet, FCE tended to improve. Feed conversion efficiency for birds on T5 (0.55 g/bird/day) was similar ( $P > 0.05$ ) to FCE of birds on T3 and T4 (0.52 and 0.52 g/bird/day respectively) but better ( $P < 0.05$ ) than FCE of birds on T1 and T2 (0.50 and 0.50 g/bird/day respectively). Final body weight was not affected by treatment.

#### Carcass parameters

There were no significant differences ( $P > 0.05$ ) between all the carcass parameters measured as shown in Table 7.

#### Blood parameters

**Hematological parameters.** Table 8 shows the effect of diet on some blood hematological parameters. There was no dietary influence on any of the blood parameters

**Biochemical parameters.** Table 9 shows the effect of diet on some blood biochemical parameters. With the exception of alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT) and glucose where there were differences among treatments ( $P < 0.05$ ), all the other blood biochemical parameters were not affected by diet.

**Table 4 - Analyzed nutrient composition of SBBSG \*and Wheat Bran**

Item	SBBSG*	Wheat Bran
GE, kcal/kg	3,855	3,389
DM, %	84.37	89.55
CP, %	26.95	16.61
Ash, %	8.61	8.67
Crude fat, %	8.85	3.55
Crude fibre, %	14.75	9.2
OM <sup>1</sup> , %	75.76	70.88
P, %	0.90	1.32
Ca, %	0.86	0.60
<b>Amino acids, %</b>		
Arg	0.91	0.98
His	0.49	0.37
Ile	1.02	0.51
Leu	2.79	0.96
Lys	0.77	0.66
Met	0.44	0.20
Phe	1.33	0.57
Thr	0.90	0.50
Trp	0.10	0.12
Val	1.26	0.76
Ala	1.90	0.84
Asp	1.75	1.12
Cys	0.44	0.38
Glu	4.35	3.12
Gly	0.91	0.85
Pro	1.92	0.76
Ser	1.03	0.66
Tyr	0.79	0.46

\*Sorghum-Barley Brewer's spent Grain; <sup>1</sup> Organic matter

**Table 5 - Apparent metabolizable energy (MJ/kg) and ileal crude protein and amino acid digestibilities (%) in SBBSG and WB**

Item	SBBSG*	Wheat Bran
AME	7.22	6.01
Crude protein	73.57	73.55
Calcium	60.45	59.75
phosphorous	45.40	35.87
Arginine	79.66	78.30
Histidine	64.24	71.22
Iso Leucine	64.72	75.47
Leucine	55.9	69.63
Lysine	42.93	69.50
Methionine	71.58	70.9
Phenylalanine	55.47	78.55
Threonine	75.26	67.20
Valine	62.66	62.11
Tryptophan	55.38	70.2
Alanine	52.08	72.59
Asparagine	69.77	71.48
Cysteine	53.91	70.8
Glutamic acid	83.05	81.0
Glycine	69.22	70.4
Proline	52.5	71.42
Serine	76.89	70.8
Tyrosine	80.77	70.4

\*Sorghum-Barley Brewer's spent Grain; <sup>1</sup> Organic matter**Table 6 - Growth performance of birds fed different levels of SBBSG**

PARAMETER	T1 0%SBBSG <sup>1</sup>	T2 4%SBBSG	T3 8%SBBSG	T4 12%SBBSG	T5 16%SBBSG	SEM	P-VALUE
ADFI (g)	105.59 <sup>a</sup>	102.30 <sup>a</sup>	92.92 <sup>b</sup>	93.35 <sup>b</sup>	88.26 <sup>c</sup>	1.50	<0.01
ADG (g)	52.79 <sup>a</sup>	51.15 <sup>a</sup>	48.31 <sup>a</sup>	48.54 <sup>a</sup>	48.54 <sup>a</sup>	1.84	0.79
FCE	0.50 <sup>b</sup>	0.50 <sup>b</sup>	0.52 <sup>ab</sup>	0.52 <sup>ab</sup>	0.55 <sup>a</sup>	0.01	0.04
FNBW (kg)	2.95	2.86	2.71	2.72	2.72	.170	0.65

<sup>1</sup> Sorghum-Barley Brewer's Spent Grain: <sup>a,b,c</sup>, Means within the same row with different superscripts are significantly different (P<0.05);SEM: Standard error of the mean**Table 7- Carcass parameters of birds fed different levels of SBBSG**

Parameter	T1 0% SBBSG <sup>1</sup>	T2 4% SBBSG	T3 8% SBBSG	T4 12% SBBSG	T5 16% SBBSG	SEM	P-Value
Chilled wt./ bird (g)	2584.7	2596.3	2461.0	2515.3	2528.3	79.5	0.75
Carcass wt./ bird (g)	2323.7	2308.5	2215.3	2260.7	2251.9	74.9	0.84
Viscera wt./ bird (g)	261.01	287.87	245.67	254.6	276.3	26.8	0.81
Fat wt./ bird (g)	32.2	26.07	24.93	26.73	25.00	2.9	0.37
Fat(% of chilled weight)	1.23	0.99	1.01	1.07	1.01	0.1	0.49
Fat(% of carcass weight)	1.37	1.12	1.20	1.19	1.12	0.1	0.53

<sup>1</sup> Sorghum- Barley Brewer's Spent Grain; SEM: Standard error of the means, SEM: Standard error of the means**Table 8 - Effect of diet on blood hematological parameters of birds**

Parameter	T1 0% SBBSG <sup>1</sup>	T2 4% SBBSG	T3 8% SBBSG	T4 12% SBBSG	T5 16% SBBSG	SEM	P-Value
WBC (10 <sup>9</sup> /L)	267.1	270.9	291.2	264.8	259.7	8.19	0.105
RBC (10 <sup>12</sup> /L)	1.84	1.79	2.57	1.81	1.72	0.22	0.079
HGB (g/dL)	7.42	7.54	9.00	6.60	7.24	0.95	0.503
HCT (%)	20.60	23.12	30.34	23.58	21.34	2.49	0.084
MCV (fL)	123.1	128.7	118.3	123.1	124.3	2.71	0.158
MCH (pg)	40.20	42.10	42.04	40.52	41.86	0.94	0.466

<sup>1</sup>Sorghum-Barley Brewer's Spent Grain; WBC: white blood cell, RBC: red blood cell, HGB (g/dL): heamoglobin (gram per deciliter), HCT: hematocrit, MCV (fL): mean corpuscular volume (femtoliters), MCH (pg): mean corpuscular haemoglobin (pigograms); SEM: Standard error of the mean

**Table 9 - Effect of diet on blood biochemical parameters of birds**

Parameter	T1 0% SBBSG	T2 4%SBBSG	T3 8%SBBSG	T4 12%SBBSG	T5 16%SBBSG	SEM	P-Value
ALP (U/L)	3785.1 <sup>ab</sup>	3254.7 <sup>b</sup>	4546.1 <sup>a</sup>	3978.3 <sup>ab</sup>	3778.7 <sup>ab</sup>	268.31	0.043
AST (U/L)	10.62	10.32	11.06	19.14	9.8	2.36	0.059
GGT (U/L)	16.20 <sup>b</sup>	18.17 <sup>b</sup>	15.90 <sup>b</sup>	24.84 <sup>a</sup>	27.06 <sup>a</sup>	1.86	<0.05
T. Bilirubin (µmol/L)	2.52	3.22	2.68	2.57	2.74	0.49	0.86
Protein (g/L)	40.79	42.33	42.09	39.76	40.33	2.76	0.955
Albumin (g/L)	14.05	15.76	15.92	14.65	15.26	0.91	0.589
Urea (mmol/L)	0.46	0.36	0.60	0.58	0.42	0.11	0.534
Creatinine (µmol/L)	15.70	13.58	18.76	17.44	16.84	1.89	0.403
Glucose (mg/dL)	260.78 <sup>a</sup>	238.58 <sup>b</sup>	260.22 <sup>ab</sup>	258.90 <sup>ab</sup>	280.82 <sup>ab</sup>	7.67	0.0187
UricAcid (µmol/L)	243.16	169.02	239.86	153.08	220.81	31.81	0.188
Cholesterol (mmol/L)	2.46	2.68	2.80	2.78	2.72	0.15	0.535
Triglycerides (mmol/L)	0.76	0.64	0.94	1.02	0.80	0.11	0.172
HDL (mmol/L)	1.14	1.32	1.12	1.22	1.18	0.08	0.632
LDL (mmol/L)	1.02	1.08	1.26	1.12	1.16	0.09	0.527

ALP: alkaline phosphatase, AST: aspartate aminotransferase, GGT: Gamma-glutamyltransferase, T. Bilirubin: total bilirubin, HDL: high density lipoprotein, LDL: low density lipoprotein, U/L: units per liter, µmol/L: micromoles per liter, g/L: gram per liter, mmol/L: millimoles per liter, mg/dL: milligram per deciliter; SEM: Standard error of the mean

## DISCUSSION

### Chemical Analysis

In initial trials using SBBSG (Nortey et al., 2013; Manu-Barfo et al., 2013) in diets for both layers and broilers, the authors made certain assumptions with regards to nutrient, particularly amino acid content. These assumptions had been based on work done by Donkoh and Attoh-Kotoku (2009), using BSG and on values obtained in other scientific literature (NRC, 1994). The analyzed results of wheat bran used in this trial were compared to the analyzed results of Donkor and Attoh-Kotoku (2009), to serve as a check and a validation for the protocols. The results indicated similarities in the crude protein and most amino acid values. Of the essential amino acids Lys, Met, Cys, Thr, Val and Leu, analyzed results were 0.66, 0.20, 0.38, 0.50, 0.75 and 0.96% which were comparable to cited literature values of 0.66, 0.22, 0.34, 0.48, 0.79 and 0.98% respectively. Generally the crude protein content of SBBSG was higher than BSG (26.95 vs. 20.7% respectively). Chemical composition and amino acid profile can vary according to the variety of grain, harvest time, malting and mashing conditions (Mussatto et al., 2006). The higher protein content of the SBBSG translated to slightly higher values for most of the amino acids. Comparing SBBSG used in this trial to BSG (Donkor and Attoh-Kotoku, 2009), Lys, Met, Cys, Thr and Val and Leu values were 0.77, 0.44, 0.44, 0.90, 1.26, 2.79 and 0.76, 0.39, 0.52, 0.72, 1.21 and 1.64% respectively. Protein values for BSG as reported by Westendorf and Wohlt (2002) ranged from 21 to 29%. Similarly, Adama and Ribadu (2003) and Olorunnisomo et al., (2006) reported protein values for BSG similar to what was reported for SBBSG in this study. However it is clear from this study that similarities in protein content between SBBSG and similar brewery by-products, did not translate into similarities in amino acid contents. In monogastric feed formulation however, a greater emphasis needs to be placed on amino acid composition and availability, and not on protein content per se (Dalibard et al., 2014)

### Nutrient digestibility

The AME of the wheat bran that was used as a validation was 6.01MJ/kg and this was comparable to the values obtained by Donkoh and Attoh-Kotoku (2009) who obtained a value of 5.80MJ/kg. For the SBBSG, an AME value of 7.22MJ/kg was obtained. This value was much higher than what was assumed in the trial by Nortey et al. (2013) and by Manu-Barfo et al. (2013). The higher AME level of the SBBSG used in this trial compared to what was obtained for BSG (Donkor and Attoh-Kotoku, 2009), could be a direct result of the fat content (8.85% vs 6.01%). Essential amino acids cannot be synthesized by poultry, therefore they must be provided in the diets of the birds at the right levels and proportions (Kim et al., 2008). The ileal digestibilities of some essential amino acids like Lys, Met and Cys (42.93, 71.58 and 53.91 %) were 43.58, 13.13 and 15.89% lower than the assumed values of 76.1, 82.4 and 64.1% obtained in the trial by Donkoh and Attoh-Kotoku (2009) and which was used in an earlier broiler trial (Nortey et al., 2013).

Generally nutrient digestibility is a result of several factors including the age of the birds, processing of the ingredient and the presence of anti-nutritive factors (ANF) like fibre (Schulze et al., 1994; Lenis et al., 1996; Slominski et al., 2004). The crude fibre content of the SBBSG used in this study was 15.66 (Table 4). This is high when compared to what was obtained by Madubuike and Obidimma (2009). The two major grains from which SBBSG was obtained are sorghum and barley. The major ANFs found in barley are arabinoxylans, and beta glucans (Aalto et al., 1988; Andersson et al., 1999; Zijlstra et al., 1999), while those found in sorghum are phytic acid, trypsin and amylase inhibitors (Mohammed et al., 2010). Research indicates that high amount of dietary fibre reduces nutrient availability to poultry and swine (Schulze et al., 1994; Lenis et al., 1996; Slominski et al., 2004).

It may therefore be speculated that the reduced digestibilities of some amino acids observed in SBBSG was a direct result of the presence of these ANFs.

### Growth performance

**Average daily feed intake (ADFI).** Several factors affect ADFI in broilers. Feed intake is influenced by stocking density, water supply, fibre, the nature and the palatability of the feed, energy content of the feed, health status of the birds and stress (Ferket and Gernat, 2006). With the exception of feed, all the other conditions were held constant. Generally as the level of SBBSG increased in the diet, feed intake reduced. The results were similar to the findings of Boudouma (2010) who observed a reduction in the feed intake when diets containing high levels of Brewers' spent grain (> 13 %) were presented in the mash form for broilers. On the other hand the results were in disagreement with reports from Ash and Akoh (1992), Opara (1996), Omekan (1994), Esonu et al. (2002) who reported increased feed intake as the fibre level in the diet increased. As the level of fibre increases in a diet, monogastrics tend to increase their feed intake in an attempt to meet their nutrient (particularly energy) needs (Leeson and Summers, 1997). This is because increasing fibre levels tend to dilute the energy density of the feed. This phenomenon has been observed in broilers by Adeyanju et al. (1976) and Alemawor et al. (2010) and in layers by Umar Faruk et al. (2010). In this trial, the birds were fed *ad-lib*, and it was expected that birds would consume more of T5 than T1 or T2, due to an increase in dietary SBBSG, and consequently an increase in the fibre content. Dietary fibre, in addition to diluting the energy content of the feed, is known to affect rate of passage in the gastrointestinal-tract (GIT) of birds (Montagne et al., 2003; Ferket and Gernat, 2006; Saki et al., 2011).

Sorghum Barley Brewers Spent Grain contains high levels of NSPs. In an earlier trial, Manu-Barfo et al. (2013) determined that broiler grower diets containing 4% and 16% SBBSG contained 9.39 and 12.26% total NSP's respectively. Of the 9.39% NSP contained in the 4% SBBSG diet, 2.21 % was insoluble, while 7.18% was soluble. However the insoluble vs soluble NSP in the 16% SBBSG diet was 5.57 and 6.68% respectively. It can be seen that SBBSG contains relatively more insoluble than soluble fibre. According to Jørgesen et al. (1996) water insoluble NSPs tend to reduce transit time in the GIT. A reduction in transit time, will result in a longer period of satiety which will ultimately result in a reduction ADFI (De Leeuw et al., 2008; Rochell et al., 2012). It is also possible that the higher fat content of SBBSG compared to wheat bran, may have contributed to the lower intakes of the diets containing SBBSG.

**Average daily gain (ADG) and FCE.** Feed intake influences the body weight gain and feed conversion efficiency (Ferkert and Gernat, 2006). However in this trial, there was no effect of dietary SBBSG level on ADG, although a numerical trend towards slight reductions were observed. Thus the expected drastic reductions in ADG that normally accompany a reduction in feed intake were not observed in this trial. The absence of this difference resulted in better feed efficiency as the level of SBBSG increased in the diet. In the present trial, nutrient availabilities, as opposed to total nutrient content were used to formulate diets to meet the nutrient requirements for COBB500 broiler birds (COBB, 2015). This was possible following the determination of nutrient availabilities in SBBSG in the digestibility trial. According to Cavalcanti and Behnke (2004), and Dalibard et al. (2014), formulating diets for monogastrics animals based on nutrient availabilities results in better feed efficiency and growth rates. The lack of any effect on ADG when ADFI was reduced could be due to the fact that hens were more efficient at utilizing the available nutrients when feed intake was reduced. This phenomena has also been observed by Sklan et al. (2003) and Alvarado et al. (2007).

**Carcass parameters.** There were no dietary effects on any of the carcass parameters studied. Generally as the level of dietary fibre increases and energy is diluted, ADFI increases. This can cause an increase in intestinal weight of birds (Sulieman and Abd-Ra-Mabrouk, 1999; Nortey et al., 2013) and is a compensatory effect that is intended to support the extra feed consumed. In this study no such effect was observed and it may be a direct result of the fact that there was no corresponding increase in ADFI with increased dietary fibre levels. Earlier studies using SBBSG (Manu-Barfo et al., 2013) in broiler diets, resulted in an increase in the abdominal fat of birds fed 12 and 16% SBBSG. However in that trial, assumed digestibility values based on earlier work by Donkor and Attoh-

Kotoku (2009) had been assumed. For this particular trial, and using this special SBBSG, real ileal digestibilities of nutrient were determined and used in formulating the broiler diets. A precise ratio of dietary ME to amino acid is required to control fat accumulation in broiler finishers and maintain superior carcass characteristics (Bregendahl et al., 2002) and in the absence of such precise information, there was an excess accumulation of fat as was observed by Manu-Barfo et al. (2013). In this trial there was no excess abdominal fat accumulation by birds fed any of the SBBSG treatments because a precise knowledge of nutrient availabilities had been determined and used in dietary formulation.

### **Blood hematological and biochemical properties**

Changes and deviations from the norm in blood hematological properties are an indication of a physiological disorder that may not yet be exhibiting external clinical signs (Togun et al., 2007). For this trial there were no differences in any of the hematological parameters studied. Also the values obtained were within the normal ranges for broilers as was reported by Bowes et al. (1989), Ikhimioya et al. (2000) and Talebi et al. (2005).

There were some observed differences in some of the biochemical indices that were studied. These included alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT) and glucose.

The Gamma-Glutamyl Transpeptidase test is used to detect liver disease and bile duct obstructions. An increased GGT level indicates that an individual's liver is being damaged. Normal ranges for GGT have been reported in healthy chicken from 21.28 to 37.32 units per liter (U/l) (Silva et al., 2007), 7.78 to 16.07 U/L (Café et al., 2012), and 23.56 to 27.79 U/L (Kuttappan et al., 2012).

Thus although there existed some differences in GGT levels in this trial, all the values were within the normal range. The level of glucose in the blood at any time may be influenced by the time it is measured post-prandial and also on the type of carbohydrate in the diet (Wheeler and Pi-Sunyer, 2008). Thus the level of glucose in the blood may be slightly higher immediately post prandial than when the individual took a meal a few hours prior, and is in a state of hunger. Also easily digestible carbohydrates will result in quicker spikes in blood levels of glucose compared to slower digestible carbohydrates. The differences in blood glucose levels of birds in this trial were within the normal range of 200 – 500mg/dL (Jain, 1993; Thrall, 2007) and the observed differences in blood glucose levels could be due to time post-prandial, and/or activity level of the birds immediately pre-sampling. All other biochemical parameters were not different, indicating that the birds were not sick

## **CONCLUSIONS**

It is concluded from this study that:

- The AME of SBBSG is slightly higher, while amino acid content and digestibilities are generally lower than earlier studies reported
- When the basis of feed formulation is on nutrient availability and not on total nutrient content, SBBSG can be included in diets for broilers up to 16% without negatively affecting performance.
- There are no negative health effects on broilers when SBBSG is included in broiler diets up to 16%

## **DECLARATIONS**

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### **Authors' Contributions**

TNN is the Principal Investigator who designed the trial, formulated the feed and supervised the laboratory work and all aspects of the experiments. RJ carried out the day-to-day work on the farm, conducted the laboratory work and put together the draft version of the manuscript. AAN participated in the design of the study, helped with the statistical analysis and helped in coordinating and drafting the manuscript.

### **Ethics**

The animal protocol used followed principles and ethical procedures recommended by the Institutional Animal Care and Use Committee of the Noguchi Memorial Institute for Medical Research, University of Ghana

### Consent to publish

We the authors of this manuscript, titled "Effect of metabolizable energy content and ileal amino acid digestibility of sorghum barley brewers spent grain on growth, carcass and blood parameters in broilers" do hereby give consent for this manuscript to be published

### Availability of data and materials

Not applicable

### Competing interest

The authors declare that they have no competing interests.

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