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The use of Donryu rats as a model for the humans in the formulation of dietary protein

F.A. Dawodu [1*] and K.O. Ajanaku [2]
*[1] Department of Chemistry (Industrial), University of Ibadan, Ibadan, Nigeria. [2] Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. *e-mail: fadawodu@yahoo.com*

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Abstract
The effects of brewery spent grain formulated diet on the performance of Donryu rats were investigated. The rats were allocated into 6 dietary treatment groups of 6 rats each and fed with diet containing graded levels of BSG 0, 3, 6, 9, 12 and 100%. The experimental feeding lasted for fifteen days. The BSG formulated diet was found to have a positive effect on the growth performance of the rats up to levels of 12% including the control (0%). The histopathological evaluation shows that 3–9% BSG could be used as protein supplement in human foods.

Key words: Brewery spent grains (BSG), dietary treatment, growth performance, histopathological evaluation.

Introduction
The making of beer generate by–products such as spent grains. Brewery spent grains (BSG) is a safe feed when it is used fresh or properly stored. It contains high protein of about 26–30% and crude fibre [12]. Wet spent grains spoil rather quickly and should be used fresh or stored in an airtight compartment. For longer storage, it may be ensiled in an airtight trench silo. Wet spent grain can be ensiled alone or in association with other feeding ingredients such as 2–3% molasses to ensure proper fermentation [8]. It can also be used with chopped root vegetable or legumes to feed domestic animals.

BSG has received little attention as a marketable commodity. Its disposal is often a problem. Its present disposal methods are no longer sustainable for the environment with devastating level of pollution. Therefore, the BSG waste management problems require developing new ways to use the spent grains considering the pressure it puts on environment and our health.

There has been various researches [1-3, 14] on alternate uses of brewery by–products and waste minimization from brewery processes. Most of these investigations were mainly on animal feed. There is also a growing interests in the use of BSG in human foods such as flower mixes, bread [4, 9, 10] and meat product [5]. However, not much has been studied in the area of histopathological effect in human foods when it is used as protein supplement.

This study was to evaluate the effect of dietary BSG on the growth performance of Donryu rats and then use the results to formulate protein supplement for human's free side effect on the physiological aspects of human body and also to increase resource utilization and eliminate pollution from these breweries spent grains.

Materials and Methods
Materials: Brewery spent grains (BSG) was obtained from the major breweries in Nigeria, namely; Nigerian Breweries Plc, Ibadan and Guinness Nigeria Ltd., Benin and Lagos. Maize, soyabean meal, wheat offal, fish meal, bone meal, salt, lysine, methionine, premix (Growers) and water were bought locally to prepare the rats feed. The Donryu rats were bought from Cocoa Research Institute of Nigeria (CRIN), Ibadan.

Methods: The BSG sample was dried at 40°C for about 24 hours in an electric oven. The dried BSG was milled to increase the surface area. The moisture content, ash content, crude fat, carbohydrate, crude protein, crude fibre and the nitrogen-free extract of the BSG were determined (Tables 1 and 2). The BSG was mixed with rats feed at levels of 0, 3, 6, 9, 12 and 100%. The 0% was used as the control. The control diet had no addition of the formulated diet.

The thirty six Donryu rats were allocated into six dietary treatment groups of 6 rats each and confined in individual cages during the experimental period. The cages were built for easy collection of the faeces and urine. The rats in the groups are four weeks old before the commencement of the experiments. The rats were weighed at the beginning of the experiment as zero day, fed according to their group levels and subsequently weighed at daily intervals throughout the 15 days experimental period.

On the sixteenth day, the rats were slaughtered using cervical dislocation method of euthanasia. Their blood was collected into two heparinized tubes for haematological studies; one tube contained ethylenediaminetetraacetic acid (EDTA) with calcium serves as anticoagulant in the blood samples. This was used for plasma parameter analysis. The second tube, which did not contain EDTA, was used for blood enzyme analysis. The red blood cell (RBC) and white blood cell (WBC) counts were determined using Neubauer haemocytometer. Packed cell volume (PVC) was determined using haematocrit centrifuge. Haemoglobin was determined by cyanomethaemoglobin method (MCH), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined according to the methods of Jain [7]. Data collected were subjected to statistical analysis of variance and means compared by the Duncan's multiple range test [13].

Results and Discussion

The proximate analysis of the brewery spent grains (BSG) samples and the total percentage of crude protein in each feed formation are presented in Tables 1 and 2 respectively. The high protein values observed in the sample may be due to the protein rest and washing operation of the grains.

Table 1. Proximate analysis of the brewery spent grains (BSG) samples.

Parameter	Mean content (%)
Crude protein	23.1985
Crude fibre	12.8500
Crude fat	2.7900
Moisture content	6.1400
Ash content	16.9925
Carbohydrate	51.3862
Total nitrogen	3.7118
Nitrogen–free extract	38.0290

Table 2. Total percentage of crude protein in each feed formulation.

Feed formulation	Total crude protein (%)
0% (without BSG)	21.6600
3% BSG inclusion	22.0600
6% BSG inclusion	22.4500
9% BSG inclusion	22.7600
12% BSG inclusion	23.2400
100% (BSG only)	23.2000

Table 3 shows the effect of dietary addition of BSG on the body weight and feed consumption of the growing rats. The 3% BSG for formulated diet yielded the highest body weight while the least was observed in 100% BSG diet. It was also observed that increasing levels of dietary BSG resulted in decreased body weight and body weight gains for each group and feed consumption of the rats. The gain in weight during the experimental feeding period implied a high feed efficiency of rats.

Table 4 reveals that the average weight gain of the growing rats in cages of 0, 3, 6, 9 and 12% is in the range of 0.90-3.81g per day while rats in cage of 100% BSG gave a daily body weight loss of 2.08 g. The loss in the body weight might be due to low level of fat in the feed. These findings agreed with the results [6, 11] that fat supplementation significantly improved feed conversion efficiency.

Table 4. Determination of weight gain by rats for each feed formulation per day.

Feed formulation	Weight (g) 0 day	Weight (g) 15th day	Weight (g) difference	Weight gain g/day
0% (without BSG)	52.55±2.250	108.15±4.551	55.60±2.301	3.707
3% BSG	55.50±2.700	112.65±3.750	57.15±1.050	3.810
6% BSG	49.55±3.350	102.40±3.70	52.85±0.350	3.523
9% BSG	49.15±4.351	85.15±5.550	36.00±1.199	2.400
12% BSG	44.10±3.900	57.70±4.40	13.60±0.500	0.907
100% (BSG only)	48.65±4.100	17.45±6.901	-31.20±2.801	2.080*

± Standard error of the mean value. * Weight loss per day.

Table 5 shows a significant increase in haemoglobin concentration (Hb) and red blood counts (RBC), while there is a drop at higher concentration from rats fed with 9% BSG. This was also observed in PCV indicating positive nutritional quality of this formulated diet. There was a significant increase in white blood cell counts (WBC), platelets, mean corpuscular haemoglobin concentration (MCHC), glutamate oxalacetate transamine (GOT), acid phosphatase (AP) and albumin (ALB) parameters at 3, 6 and 9% BSG compared with control. This shows that the resistance of the rats to infection in 3, 6 and 9% BSG was very high and that there is a direct actions of antibodies attacking the antigenic invaders due to antibodies properties that is present in the blood. Platelet counts in all the percentage feeds were of high value indicating a positive sign in the stoppage of blood during bleeding. The blood histopathology properties of rats fed with 0, 3, 6, 9, 12 and 100% BSG were significantly different at the 0.01 level (2-tailed) of 99% confidence interval compared to the control.

Table 3. Mean ±standard error body weight of rats for each day (g) in dietary treatment groups.

Day	Body weight (g) of rats in dietary treatment groups					
	0% BSG	3% BSG	6% BSG	9% BSG	12% BSG	100% BSG
0	52.55±2.250	55.50±2.700	49.55±3.350	49.15±4.351	44.10±3.900	48.65±4.100
1	52.95±4.751	55.75±3.851	51.00±5.201	51.40±3.901	45.45±5.020	46.00±3.951
2	52.35±3.150	56.45±3.551	55.85±4.251	54.10±6.001	46.35±5.651	41.80±3.751
3	55.85±1.850	57.93±3.826	60.00±4.101	59.65±5.751	47.05±6.451	38.35±3.301
4	58.55±0.650	70.80±4.201	63.80±4.301	61.75±6.051	48.30±4.801	37.35±4.601
5	63.30±2.200	83.15±5.510	68.50±4.301	64.25±5.051	50.55±4.551	34.90±4.351
6	66.10±2.500	86.30±3.001	71.10±4.801	68.10±4.301	51.35±5.051	32.70±3.351
7	69.95±3.050	90.15 ±3.751	74.25±4.351	70.90±4.701	51.40±5.801	29.60±3.751
8	75.50±4.001	93.95±3.451	75.80±4.801	74.70±4.001	52.60±4.401	30.00±3.751
9	80.30±4.101	95.70±3.501	83.05±3.451	78.45±3.351	52.55±4.401	29.60±3.751
10	84.50±5.101	98.65±4.451	85.15±3.151	79.15±2.150	53.45±4.851	25.20±3.851
11	88.50±5.351	99.15±4.451	90.30±2.100	81.85±0.850	54.50±4.101	22.95±3.001
12	93.65±4.951	105.25±3.051	94.00±2.700	83.70±3.101	55.20±4.501	20.35±3.401
13	99.70±6.201	108.65±3.051	95.90±0.900	84.25±3.451	55.45±3.651	17.85±2.701
14	102.10±4.701	112.60±1.10	97.70±2.300	84.90±2.500	56.15±2.350	17.50±2.951
15	108.15±4.551	112.65±1.750	102.40±2.700	85.15±2.550	57.70±2.400	17.45±2.901

Table 5. Histopathology values of rats fed with formulated feeds.

Parameter	Dietary treatment group						
	0% BSG	3% BSG	6% BSG	9% BSG	12% BSG	100% BSG	Normal value
Hb (%)	10.9	13.8	13.8	7.6	11.6	12.8	16.1 ±.0.4
PCV (%)	31.7	39.0	38.0	30.0	24.0	30.7	40.6 ±0.12
RBC (x10^6/mm^3)	3.68	5.52	4.78	3.80	4.50	3.85	8.21±0.14
MCV (V^3)	95.0	87.0	83.0	82.0	85.0	91.0	56.2 ±0.6
MCH (μ/g)	33.0	32.0	29.0	27.0	30.0	32.0	14.7– 15.9
MCHC (%)	33.0	37.0	36.0	35.0	34.0	34.2	32.4± 0.4
Neutrophil (%)	6	20	2	4	26	12	10-55
Lymphocytes (%)	94	80	98	96	74	88	40–90
Eosinophil (%)	0	0	0	0	0	0	0
Monocytes (%)	0	0	0	0	0	0	0
Basophil (%)	0	0	0	0	0	0	0
Platelets (x10^3/mm^3)	155	198	210	180	184	168	54.5 ± 13.6
WBC (x10^3/mm^3)	5.0	7.2	7.1	5.2	5.0	6.2	5.3 ± 0.5
ALP (g/l)	25.0	30.2	30.0	29.8	27.5	21.3	43.2 ± 0.38
GOT (g/l)	6.6	7.2	7.1	7.0	6.8	5.7	7.3 ± 0.4
AP (g/l)	4.6	3.6	4.4	4.6	4.8	4.6	NA
TP (g/l)	0.73	0.66	0.69	0.64	0.65	0.71	0.65 ± 0.02
ALB (g/l)	0.39	0.43	0.41	0.40	0.38	0.38	0.43 ± 0.01
GLB (g/l)	0.34	0.23	0.28	0.24	0.27	0.33	NA

Hb Haemoglobin concentration (%); PCV Packed cell volume (%); RBC Red blood cell count (x 10^6/mm^3); WBC White blood cell count (x10^3/mm^3); MCV Mean corpuscular volume (V^3); MCH Mean corpuscular haemoglobin (μg); MCHC Mean corpuscular haemoglobin concentration (%); ALP Alkaline phosphate (g/l); GOT Glutamate oxalacetate transaminase (g/l); ALB Albumin (g/l); GLB Globulin (g/l); TP Total protein (g/l); NA Not available
Normal value was obtained as literature control from blood parameters result of don rat strain by NBRP rat Kyoto in Japan.

Conclusions

In this study, it has been shown that 3 and 6% BSG could be used as protein supplement in human foods with 9% BSG as the maximum limit. In the light of the above, BSG formulated diet in the 3-9% is a good supplement to human foods as well as to animal feed. Therefore BSG disposal as industrial wastes into the Nigerian ecosystems, would be reduced to the minimum bearable if not completely eliminated; an important advantage in developing economies. The use of BSG as food supplement would also help to reduce the number of people suffering from micronutrient deficiency related disease in developing nations.

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