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HISTOLOGICAL STUDIES OF BREWERY SPENT GRAINS IN DIETARY PROTEIN FORMULATION IN DONRYU RATS

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ABSTRACT

The increasing production of large tonnage of products in brewing industries continually generates lots of solid waste which includes spent grains, surplus yeast, malt sprout and cullet. The disposal of spent grains is often a problem and poses major health and environmental challenges, thereby making it imminently necessary to explore alternatives for its management. This paper focuses on investigating the effects of Brewery Spent Grain formulated diet on haematological, biochemical, histological and growth performance of Donryu rats. The rats were allocated into six dietary treatment groups and fed on a short-term study with diet containing graded levels of spent grains from 0, 3, 6, 9, 12 and 100% weight/weight. The outcome demonstrated that formulated diet had a positive effect on the growth performance of the rats up to levels of 6% inclusions, while the haematological and biochemical evaluation revealed that threshold limit should not exceed 9% of the grain. However, the histological study on the liver indicated a limit of 3% inclusion in feed without serious adverse effect. Thus invariably showing that blend between ranges 1-3% is appropriate for the utilization of the waste in human food without adverse effect on the liver organ. The economic advantage accruing from this waste conversion process not only solves problem of waste disposal but also handle issues of malnutrition in feeding ration.

Keywords: Brewery spent grains, waste utilization, donryu rats, dietary treatment.

INTRODUCTION

Brewery Spent Grains (BSG) is one of the voluminous solid residuals that remain after the mashing process. It has received little attention as a marketable commodity apart from being used primarily as ruminant feed and its disposal is often a problem. Its present disposal methods which include dumping, use as animal feed and biomass are no longer sustainable for the environment with devastating level of pollution. Nutritionally, the grain is far from spent since the residual protein level is in the range of 26-30% and crude fibre content up to 13% (Qzturk *et al*., 2002). BSG is a safe feed when it is used fresh or properly stored. The wet grain spoils quickly and should be used fresh or stored in an air tight compartment. However, BSG may vary with barley variety, time of harvest, characteristics of hops and other adjunct added as well as brewing technology. The waste management problems then require developing new ways to use the spent grains considering the adverse impacts on environment and health.

There have been advances on the importance of fibre in diets as well as protein being used as supplements in food (Trowell *et al*. (1975). Other researches Bays (1977); Tacon and Ferms (1978); Ahn (1979); Enweremade *et al*. (2008); and Bi-Yu *et al*. (1998) also focused on alternative uses of brewery by – products and waste minimization from brewery processes. There was also a growing interest in the use of BSG in human foods such as flour mixes, bread and meat product (Morgan *et al*., 1984; Chiou *et al*., 1995; Kellems and Church, 1998; Finley and Hanamoto, 1980). However not much has been carried out in the area of histopathological effect in human foods when used as protein supplement. In the light of the above findings, this study has been undertaken to determine the effect of using BSG as dietary feed and the histological effect it will have on human organs if utilized.

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MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were of analytical grade.

Sources of Materials

Brewery Spent Grains was obtained from Nigerian Breweries Plc, Ibadan, Nigeria. The spent grain was a mixture of sorghum and barley. Maize, soyabean meal, wheat offal, fish meal, bone meal, salt, lysine, methionine and premix (Growers) were obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria to

prepare the rats feed. The rats for the experiment were obtained from the animal farm of Cocoa Research Institute (CRIN) Ibadan having life average weight range of 49.92 ± 5.69g. The rats were in the family of Donryu species and were four weeks old before the commencement of the experiments. The ethnic use of the animals was also obtained from the Institute.

Treatment of Brewery Spent Grains Sample

The BSG samples were collected in wet form, sundried and later dried at 40^{o}C for six hours in an electric oven. It was then stored in an airtight container till the time of use. The dried BSG was milled to increase the surface area. The moisture content, ash content, crude fat, crude protein, crude fibre and the nitrogen-free extract of the BSG were determined. The BSG were mixed with rats feed at levels of 0, 3, 6, 9, 12 and 100% w/w. The 0% was the control, while the 100% serves as the extreme use of BSG alone.

Treatment of Animals

The thirty six (Donryu) rats were allocated into six dietary treatment groups of six rats each and confined into individual cages during the experimental period. The animals were free from externals and internal parasites. The study was conducted during the rainy season and the cages were built for easy collection of the faeces and urine. They were weighed at the beginning of the experiment as zero (0) day; fed according to their group levels with the BSG compounded feeds and subsequently weighed at daily intervals in a short time study of fifteen days.

Preparation of Blood Samples

On the sixteenth day of the experiment, the rats were humanely slaughtered using cervical dislocation method of Euthanasia Klaunberg *et al.* (2004). Their blood samples were collected into two heparinized tubes for the studies; one tube contains ethylene diaminetetracetic acid (EDTA) with calcium serving as anticoagulant in the blood samples for the haematology tests while the second tubes, which did not contain EDTA, were stored at -20^{o}C for the biochemical studies. The internal organs of the rats (liver, heart and kidney) were collected and weighed. The microscopic slide of the liver organs were then prepared and observed.

Analysis of Heamatological and Biochemical Parameters

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 cyanmethemoglobin method (MCH), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined according to the methods of Jain (1986). Glutamate Pyruvate Transaminose (GPT), Glutamate Oxaloacetate Transaminase (GOT), Globulin (GLB), Albumin (ALB) and Alkaline Phosphatase (ALP) were analysed spectrophotometrically by using commercially available diagnostic kits.

STATISTICAL ANALYSIS

The data collected were subjected to statistical analysis of variance and means compared by the Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULT AND DISCUSSION

The proximate analysis of the Brewery Spent Grains (BSG) samples is presented in table 1. The carbohydrate level was high with value of 51.38% and coupled with the 38% of nitrogen-free extract and crude protein of 23.19% gives a clue of a balanced formulation if the BSG is compounded as feed blends. The high protein values observed in the sample was due to the protein rest and washing operation of the grains during the brewing process.

Table 1. Proximate Analysis of the Brewery Spent Grains (BSG) Samples.

Analysis	Percentage Mean Amount (%)
Crude Protein	23.20
Crude Fibre	12.85
Crude Fat	2.79
Moisture Content	6.14
Ash Content	16.99
Carbohydrate	51.39
Total Nitrogen	3.71
Nitrogen – Free Extract	38.03

The ration formulation for the feed diet is shown in table 2 while the effect of spent grains on the weight of the rats and the average weights of the rats are shown in tables 3 and 4. The effect of BSG blend on the weight of the rats was commendable. The 3 and 6% BSG blends gave an average daily weight gain of 3.810 and 3.520g respectively, which was higher than the value of 3.706g obtained for the control (0% BSG). There was significance increase ($p < 0.05$) in the average weight gained by the rats fed in all the groupings except in the 100% group. This implied a high feed-efficiency due to increased level of blending of the spent grains. The statistical analysis shows that the results fits a linear model of 0.532024 - 0.154247 x Weight Gain in order to describe the relationship between the feed-efficiency and weight gain. This also revealed 84.58% of the variability in feed-efficiency while the correlation coefficient indicated a strong relationship between the variables. Since the p-value is < 0.05, there is a statistically

Table 2. Ration formulation of the treatment feed diets (g/100g).

Ingredients	Diets					
	1	2	3	4	5	6
	% BSG inclusion in diets					
	0%	3%	6%	9%	12%	15%
Maize	2.64	2.57	2.49	2.41	2.33	2.25
BSG	0.00	0.07	0.15	0.23	0.31	0.39
Soybean Meal	1.20	1.20	1.20	1.20	1.20	1.20
Palm Kernel Cake / Wheat offal	1.60	1.60	1.60	1.60	1.60	1.60
Blood meal	0.4	0.4	0.4	0.4	0.4	0.4
Bone meal	0.04	0.04	0.04	0.04	0.04	0.04
Salt	0.04	0.04	0.04	0.04	0.04	0.04
Lysine	0.03	0.03	0.03	0.03	0.03	0.03
Methionine	0.03	0.03	0.03	0.03	0.03	0.03
Premix[1]	0.02	0.02	0.02	0.02	0.02	0.02
Total (g)	6.00	6.00	6.00	6.00	6.00	6.00

[1]Contained vitamins A (10,000,000iu); D (2,000,000iu); E (35000iu); K (1900mg); B_{12} (19mg); Riboflavin (7,000mg); Pyridoxine (3800mg); Thiamine (2,200mg); D pantothenic acid (11,000mg); Nicotinic acid (45,000mg); Folic acid (1400mg); Biotin (113mg); and trace elements as Cu (8000mg); Mn (64,000mg); Zn (40,000mg); Fe (32,000mg); Se (160mg); I_2 (800mg); and other items as Co (400mg); Choline (475,000mg); Methionine (50,000mg); BHT (5,000mg) and Spiramycin (5,000mg) per 2.5kg

Table 3. Mean body weight of Rats for each day (Grammes).

Days/Blends	0%	3%	6%	9%	12%	15%	100%
0 day (g)	52.55 ±2.250	55.50 ±2.700	49.55±3.350	49.15 ±4.351	44.10 ±3.900	42.29 ±2.100	48.65 ±4.110
1st (g)	52.95 ±4.751	55.75 ±3.851	51.00 ±5.201	51.40 ±3.901	45.45 ±5.020	42.13 ±3.220	46.00 ±3.951
2nd (g)	52.35 ±3.150	56.45 ±3.551	55.85 ±4.251	54.10 ±6.001	46.35 ±5.651	43.26 ±2.510	41.80 ±3.751
3rd (g)	55.85 ±1.850	57.93 ±3.826	60.00 ±4.101	59.65 ±5.751	47.05 ±6.451	43.12 ±4.280	38.35 ±3.301
4th (g)	58.55 ±0.6501	70.80 ±4.201	63.80 ±4.301	61.75 ±6.051	48.30 ±4.801	43.64 ±2.110	37.35 ±4.601
5th (g)	63.30 ±2.20	83.15 ±5.51	68.50 ±4.301	64.25 ±5.051	50.55 ±4.551	44.32 ±3.320	34.90 ±4.351
6th (g)	66.10 ±2.50	86.30 ±3.001	71.10 ±4.801	68.10 ±4.301	51.35 ±5.051	44.24 ±4.110	32.70 ±3.351
7th (g)	69.95 ±3.050	90.15 ±3.751	74.25 ±4.351	70.90 ±4.701	51.40 ±5.801	45.13 ±4.350	29.60 ±3.751
8th (g)	75.50 ±4.001	93.95 ±3.451	75.80 ±4.801	74.70 ±4.001	52.60 ±4.401	45.35 ±2.600	30.00 ±3.751
9th (g)	80.30 ±4.101	95.70 ±3.501	83.05 ±3.451	78.45 ±3.351	52.55 ±4.751	45.68 ±3.140	26.10 ±3.651
10th (g)	84.50 ±5.101	98.65 ±4.451	85.15 ±3.151	79.15 ±2.150	53.45 ±4.851	46.32 ±2.101	25.20 ±3.851
11th (g)	88.50 ±5.351	99.15 ±4.451	90.30 ±2.10	81.85 ±0.850	54.50 ±4.101	47.01 ±5.210	22.95 ±3.001
12th (g)	93.65 ±4.951	105.25 ±3.051	94.00 ±2.700	83.70 ±3.101	55.20 ±4.501	48.15 ±2.130	20.35 ±3.401
13th (g)	99.70 ±6.201	108.65 ±3.051	95.90 ±0.90	84.25 ±3.451	55.45 ±3.651	48.98 ±3.323	17.85 ±2.701
14th (g)	102.10 ±4.701	112.60 ±1.10	97.70 ±2.30	84.90 ±2.50	56.15 ±2.350	49.32 ±2.220	17.50 ±2.951
15th (g)	108.15 ±4.551	112.65 ±1.750	102.40 ±2.70	85.15 ±2.550	57.70 ±2.40	49.71 ±3.170	17.45 ±2.901

significant relationship between feed-efficiency and weight gained at the 95.0% confidence level. It was observed that increasing levels of BSG in the diet resulted in decreased body weight with threshold limit in the range of 9% (Fig. 1).

The rats in the 100% BSG group experienced daily weight loss of -2.080 g per day, and their metabolic wastes concentration was very high and toxic to inhale. The loss in the body weight might be due to low level of crude fat (2.79%) in the feed. These findings agreed with the reports of Ironkwo and Oruwari (2004) who reported that fat supplementation significantly improve feed conversion efficiently. The acceptable limit is in the range of 1% to 6% w/w of the blends. For obese patient, the range between 9-12% is a good recommendation. The statistical significance of the mean body weight of the rats ($p<0.01$) when compared with the control (0%) in all groupings was significantly difference at the 0.01 level (2-tailed). Therefore their body weights are different from each other from the descriptive statistics.

Table 4. Weight gain by rats for each feed formulation per day.

Feeding	Weight at 0 day (g)	Weight at 15th day (g)	Weight difference (g)	Weight gain/day
0%	52.55 ±2.250	108.15 ±4.551	55.60	3.706
3%	55.50 ±2.700	112.65 ±1.750	57.15	3.810
6%	49.55 ±3.350	102.40 ±2.70	52.85	3.520
9%	49.15 ±4.351	85.15 ±2.550	36.00	2.400
12%	44.10 ±3.900	57.70 ±2.400	13.6	0.907
15%	42.29 ±2.100	49.71 ±3.170	7.42	0.495
100% BSG	48.65 ±4.100	17.45 ±2.901	-31.20	2.080*

* Weight loss

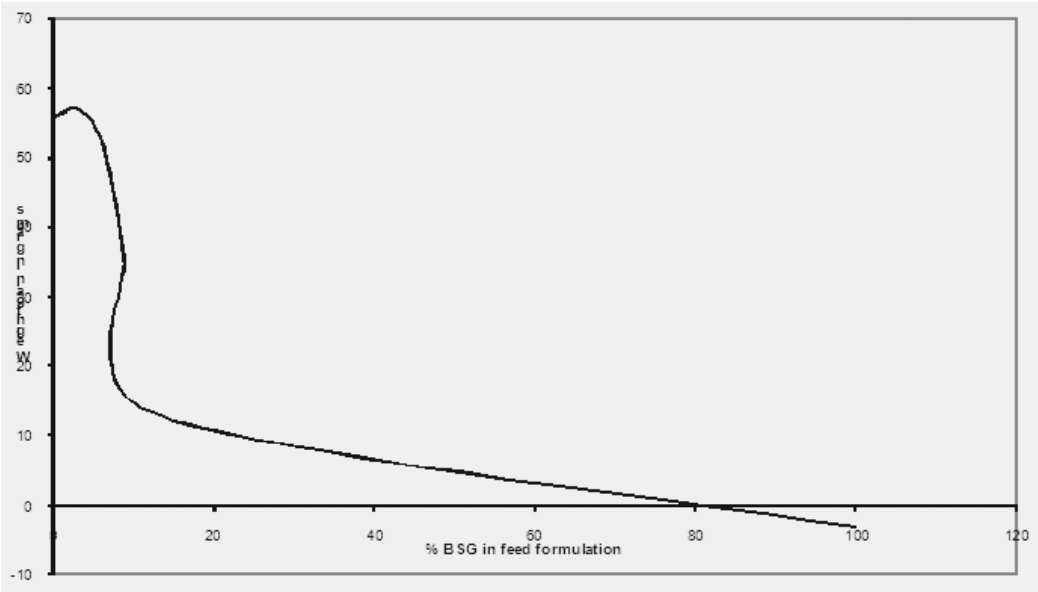

Fig. 1: Graph showing weight gain by rat against % BSG in the feed formulation.

Table 5. Mean internal organ weight (grammes) of the rats.

Blends	Internal organs			
	Liver	Kidneys	Lungs	Heart
	weight (g)	weight (g)	weight (g)	weight (g)
0%	5.41 ±0.11	0.970 ±0.05	0.86 ±0.060	0.53 ±0.025
3%	4.54 ±0.06	0.75 ±0.01	0.80 ±0.01	0.46 ±0.035
6%	5.01 ±0.59	0.91 ±0.04	1.30 ±0.13	0.42 ±0.005
9%	4.87 ±0.035	0.91 ±0.07	1.09 ±0.01	0.51 ±0.01
12%	5.11 ±0.685	0.75 ±0.255	1.03 ±0.175	0.51 ±0.005
15%	4.42 ±0.535	0.77 ±0.450	1.10 ±0.295	0.39 ±0.710
100% BSG	2.01 ±0.685	0.69 ±0.090	0.52 ±0.030	0.25 ±0.035

The result of the mean internal organs weight of the rats fed with the blends is presented in table 5. The mean liver weight for the control group was 5.41 ± 0.11 g, while those fed with only BSG dropped to 2.01 ± 0.685 g. The same was observed for the mean kidney weights, while others were within the range of the control 0.970 ± 0.05 g. Those fed in 100% BSG have their kidney weight reduced to 0.69 ± 0.09 g. Generally, the rats fed with 100% BSG have their liver, kidneys, lungs and heart weight reduced when compared with the control and other groups, because of the absence of enrichment that could sustain the blend. This implies that, if the blend is appropriately enriched, there could be a positive response as it was observed in the other groupings.

In tables 6 and 7, the heamatological and the biochemical studies of the rats used for the experiment were respectively presented. The normal packed cell volume

Table 6. Haematological studies of BSG blended feed in Rats.

Parameter / Blends	0% BSG	3% BSG	6% BSG	9% BSG	12% BSG	15% BSG	Normal Value
Hb (g%)	10.90 ± 0.2	13.8 ± 0.6	13.80 ± 0.2	7.60 ± 0.3	11.60 ± 0.4	12.8 ± 0.1	16.1 ± 0.4
PCV (%)	31.70 ± 1.22	39.00 ± 1.18	38.00 ± 2.17	30.00 ± 1.11	24.00 ± 1.42	30.70 ± 2.23	40.6 ± 0.27
RBC ($x10^6/mm^3$)	3.68 ± 0.42	5.52 ± 0.22	4.78 ± 0.45	3.80 ± 0.34	4.50 ± 0.23	3.85 ± 0.54	8.21 ± 0.14
MCV (U^3)	95.00 ± 6.10	87.00 ± 4.20	83.00 ± 3.02	82.00 ± 5.40	85.00 ± 3.25	91.00 ± 2.50	56.2 ± 0.6
MCH (Ug)	33.00 ± 1.22	32.00 ± 3.10	29.00 ± 3.12	27.00 ± 1.12	30.00 ± 2.72	32.00 ± 1.11	14.7 - 15.9
MCHC (%)	33.00 ± 2.10	37.00 ± 2.11	36.00 ± 1.10	35.00 ± 3.11	34.00 ± 3.40	34.20 ± 3.25	32.4 ± 0.4
Neutro (%)	6.00 ± 0.15	20.00 ± 0.55	2.00 ± 0.18	4.00 ± 0.10	26.00 ± 0.49	12.00 ± 1.20	10 – 55
Lympho (%)	94.00 ± 5.35	80.00 ± 6.82	98.00 ± 6.82	96.00 ± 4.57	74.00 ± 3.45	88.00 ± 5.10	40 – 90
Eosino (%)	0	0	0	0	0	0	0
Mono (%)	0	0	0	0	0	0	0
Baso (%)	0	0	0	0	0	0	0
Platelets ($x10^3/mm^3$)	155.00 ± 11.20	198.00 ± 10.30	210.00 ± 12.32	180.00 ± 13.16	184.00 ± 10.10	168.00 ± 11.12	54.5 ± 13.6
WBC ($x10^3/mm^3$)	5.00 ± 0.11	7.20 ± 0.14	6.60 ± 0.17	7.10 ± 0.26	5.20 ± 0.13	6.20 ± 0.10	5.3 ± 0.5

Platelets($10^3/mm^3$), Neutrophil (%) Eosinophil (%); Lymphocytes (%); Monocytes (%); Basophil (%) Hb = Haemoglobin, concerntration (g%); PCV = Packed cell volume (%), RBC = Red Blood Cell Counts ($x10^6/mm^3$), WBC = White Blood cell count ($x10^3/mm^3$), MCV = Mean Corpuscular Volume (U^3) , MCH = Mean corpuscular Haemoglobin (UUg); MCHC = Mean Corpuscular Haemoglobin Concentration (%). IU/L = International unit per litre;

Table 7. Biochemical studies of BSG blended feed in Rats.

Parameter/ Blends	0%	3%	6%	9%	12%	15%	Normal Value
ALP (IU/L)	250.00 ± 35.10	302.00 ± 30.12	300.00 ± 12.20	298.00 ± 16.30	275.00 ± 28.20	213.00 ± 30.11	43.2 ± 0.38
GOT (IU/L)	66.00 ± 5.40	72.00 ± 4.90	71.00 ± 3.20	70.00 ± 2.50	68.00 ± 4.50	57.00 ± 3.11	7.3 ± 0.4
GPT (IU/L)	46.00 ± 4.22	36.00 ± 3.61	44.00 ± 1.84	46.00 ± 2.61	48.00 ± 2.22	46.00 ± 2.62	NA
AP (g/dL)	65.00 ± 4.80	68.00 ± 2.56	67.00 ± 1.68	66.00 ± 3.22	59.00 ± 3.81	62.00 ± 1.12	NA
TP (g/dL)	7.30 ± 0.57	6.60 ± 0.77	6.90 ± 0.83	6.40 ± 0.10	6.50 ± 0.22	7.10 ± 0.45	0.65 ± 0.02
ALB (g/dL)	3.90 ± 0.71	4.30 ± 0.90	4.10 ± 0.21	4.00 ± 1.00	3.80 ± 0.51	3.80 ± 0.60	0.43 ± 0.02
GLB (g/dL)	3.40 ± 0.55	2.30 ± 0.11	2.80 ± 0.30	2.40 ± 0.41	2.70 ± 0.12	3.30 ± 0.45	NA
ALB/GLB RATIO	1.15 ± 0.01	1.87 ± 0.12	1.46 ± 0.11	1.67 ± 0.55	1.41 ± 0.31	1.15 ± 0.50	

ALP = Alkaline phosphatase (IU/L); g/dL = gramme per deciliter; GOT = Glutamate Oxalacetate Transaminase (IU/L); ALB = Albumin (g/dL); GLB = Globulin (g/dL); ALB/GLB = Albumin – Globulin ratio; GPT = Glutamate Pyrovate Transaminase (IU/L); TP = Total Protein (g/dL); AP = Acid Phosphatase (IU/L).

(PCV) of the Donryu rat was in the range of 36 – 54 %. The lower end of the range is normal in juveniles, but not in adult rats. The rats fed with 3% and 6% BSG blends experienced a significant increase in haemoglobin concentration of 13.8 ± 0.6 and 13.8 ± 0.2 g% respectively. The observed value for packed cell volume (PCV), 39.00 ± 1.18% for 3% blend and 38.00 ± 2.17% for 6% blend; red blood cell counts (RBC) was 4.78 ± 0.45 $10^6/mm^3$ as against the control 3.68 ± 0.42 $10^6/mm^3$; white blood cell counts (WBC) was in the range of 7.20 – 7.10 $10^3/mm^3$ in 3% to 9% respectively.

Platelets had the highest value of 210 ± 12.32 $10^6/mm^3$ in 6% as against 155.00 ± 11.20 $10^6/mm^3$ observed in the control. Mean corpuscular haemoglobin concentration (MCHC) of the entire group was higher than the control group. Alkaline phosphatase (ALP), glutamate oxalacetate transminase (GOT), acid phosphatase (AP), and albumin (ALB) also showed significant increase as compared with the 0% BSG blend. The resistance of the body system to infection in 3% and 6% rats' blood was high because there are direct actions of antibodies attacking the antigenic invaders, due to antibodies or anti infection properties that is present in the blood. Blood of the rats fed with 9% BSG blend had a reduced haemoglobin concentration, packed cell volume, but there was high value in WBC, lymphocytes, platelets, alkaline phosphates and albumin, compared with the 0% blend.

Table 8. Histopathology result of rats' liver.

Formulation	Histology	
	Sinusoids	Central Vein
0%	Normal	No visible lesions
3%	Normal	Mild periportal lymphocytic infiltrates
6%	Widening	Epithelia lining affected
9%	Almost disappeared	Epithelia lining affected
12%	Inflammatory	Hepatitis
15%	Compacted	Hepatitis

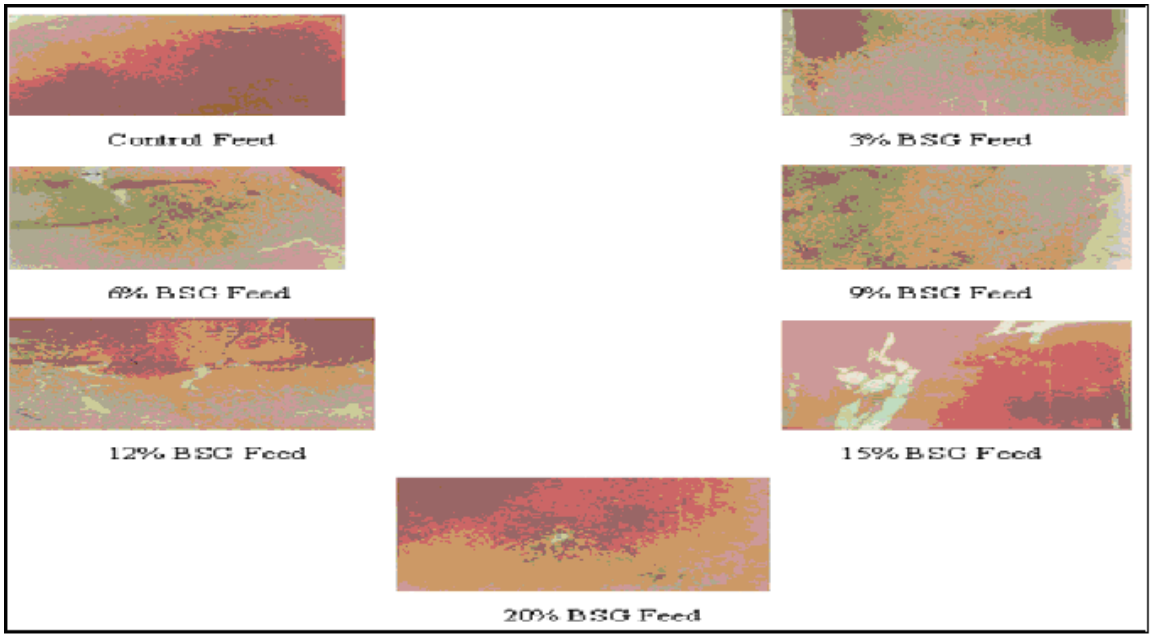

Plate 1. Microscopic view of rats' liver fed with BSG compounded feeds.

The rats fed with 12% blend had a low value in PCV, MCV, MCH, lymphocytes, AP, but increased value in ALP, Hb, RBC and WBC in comparison with the control. The 15% blends had a reduced MCV, MCH, ALP and lymphocytes values but an increased PCV, Hb, RBC, MCHC, WBC and platelets values. This result revealed that BSG presence activates oxygen in the blood cell thereby making the haemoglobin in the red blood cell inactive, a state which is termed hypoxia, and this enhances increase in the production of red blood cell counts and haemoglobin stimulating synthesis. It was observed that the histopathology result varies with the weather, climate, and region under which the experiment was carried out. The temperate and tropical region may have slight difference from each other in their blood result. In spite of this, it was observed that the rats fed with high concentration was adversely affected, hence low concentration of the blend is appropriate without side effects. Eosinophils count, monocytes and basophils counts are not significantly different in the blood of the rats, hence no significant change. The statistical significance of the histophatology on blood of the different blends at the 0.01 level (2-tailed) of 99% confidence interval showed significant difference, but they are not significantly different at 0.05 level (2-tailed) at 95% confidence interval, $p<0.05$, when compared with the control.

The histopathology study of the liver of the rats used as experimental model is shown in Plate 1 and Table 8. This revealed that the control blend cell looks normal, the central vein was seen and there was no visible lesion, the sinusoids were normal and the epithelium lining remained. In the 3% blend; the cell appeared normal but the sinusoids became larger and the space between sinusoids was bigger when compared to the control. There were mild periportal lymphocytic infiltrates noticed in the central vein. In 6% blend, the sinusoids became widened which encloses the central vein and the epithelia lining was affected too. The sinusoids almost disappeared and the hepatic cells were affected when the 9% blend was observed. For 12% blend, the central vein was seen and

the sinusoids experienced a hepatitis alteration. The sinusoid was more compacted and there was serum hepatitis in the central vein in the categories of 15% blend. The histological study of the kidney also showed that the kidneys of the control, 3, 6, 9 and 12% are all normal, while the kidney of 100% blend had a nephrotoxic effect, which is fatty degradation in the cellular tubules and glomerular region. This could be attributed to high protein, though the basis was not understood and the phenomenon that it was due to high protein was not confirmed. It could be due to some other substances that are present in BSG. The summary of the histopathology result suggest that the use up to 3% concentration will not have adverse effect on human liver and that the concentration of the blend should be kept minimal.

CONCLUSION

In this study, it has been shown that blends from 1-3% BSG could be used as protein supplement with 3% BSG as the threshold limit by virtue of the histological effect on rats liver. Thus invariably showing that blend between ranges 1-3% is appropriate for the utilization of the waste in human food without adverse effect on the liver organ. These levels of incorporation will reduce the number of people suffering from micronutrient deficiency related disease in developing nations as well as propose an additional utilization alternative to the disposal of brewery spent grains worldwide.

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