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Histopathological Studies of Utilization of Brewery Spent Grains Effect in Humans Food Chain

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Abstract

The brewing industries continuously generate lot of solid waste of which spent grains is a major by product. The present utilization method of Brewery Spent Grains (BSG) in food chain makes it imminently necessary to explore the adverse effect of the waste on humans. This paper focuses on investigating the effects of BSG formulated diet on haematological, biochemical, histological and growth performance using Donryu rats as model for the experiments. 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 15 % weight/weight. The results revealed that the 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 the threshold limit should not exceed 9 % of the grain. However, the histophatological study on the liver indicated a limit of 3 % exploitation of BSG in feed without serious adverse effect, hence blend range of 1-3 % will be appropriate for utilization in human food without adverse effect on liver organ.

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

1. Introduction

Brewery Spent Grains (BSG) generation constitute about 85 % of the total by-products of the brewing industry [1]. The utilization of the waste has little attention as a marketable commodity apart from primary use as feeds for ruminant and biomass; its disposal is often a problem. Presently, dumping has become the major means of disposal for BSG in developing countries and this is not sustainable because of the different levels of environmental pollution associated with the disposal method. Nutritionally, it was observed that the grain has residual protein level in the range of 26-30 % and crude fiber content up to 13 % [2]. The waste management problems for BSG then require to develop new ways to utilize the grains taking into account the adverse impact on environment and health.

Research advances on the importance of the fiber and protein content in food, flour mixes, bread and cookies as sourcing means alternative uses of brewery by–products and waste minimization from brewing processes [3-14]. However, the histopathological effect in human foods when used as protein supplement has not been investigated. In the view of this dearth, this study was undertaken to determine the histophatological effect of using BSG as dietary feed on human organs using Donryu rats as model for the experiments.

2. Materials and Methods

2.1 Methods

Brewery Spent Grains, BSG, (sorghum and barley mixture) was obtained from Nigerian Breweries Plc, Ibadan, Nigeria. The wet samples were sun dried and then dried at 40^{o}C for six hours in an electric oven, milled and proximate analysis was determined. Samples were analyzed for dry matter, ash, crude protein, crude fat and total nitrogen by AOAC method [15]. All sample analysis was performed on a thoroughly mixed batch of each feed ingredient. Maize, soybean meal, wheat offal, fish meal, bone meal, salt, lysine, methionine were used as additive to prepare rats feed and were obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The grains were mixed with rats feed at levels of 0, 3, 6, 9, 12 and 15 % w/w. The 0 % mix was used as control for the experiment. The Donryu rats for the experiment were obtained from the animal farm of Cocoa Research Institute (CRIN) Ibadan with life average weight range of 49.92 ± 5.69 g and were four weeks old. The ethnic use of the animals was also obtained from the Institute. Thirty six (36) rats were allocated into six dietary treatment groups of six rats each and confined into individual cages built for easy collection of the faeces and urine during the experimental period. They were fed according to their group levels with the BSG compounded feeds and

subsequently weighed at daily intervals on a short time study of fifteen days

On the sixteenth day of the experiment, human slaughtering using cervical dislocation method of Euthanasia was employed in the killing of the rats [16]. Their blood samples were collected into two heparinized tubes for the studies; first tube for the haematology tests while the second tube, stored at -20^{o}C, for the biochemical studies. The liver organs of the rats were collected, weighed and the microscopic slide were prepared and observed. 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, Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined according to the methods stated [17]. 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. All the chemicals and reagents used in the work were of analytical grade and the data collected were subjected to statistical analysis of variance and means compared by the Duncan's Multiple Range Test [18].

3. Results and Discussion

The proximate analysis of the Brewery Spent Grains (BSG) samples is presented in Table 1. The crude protein content was 23.19 % while the carbohydrate level was high with value of 51.38 %. The of nitrogen-free extract has 38.03 % value; with total nitrogen of 3.71 %. The high protein values observed in the BSG sample may be due to the water fraction leaching of water-soluble protein during the wort separation step which is one of the processes in brewing operations.

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

Analysis	Percentage Mean Amount (%)
Crude Protein	23.20
Crude Fiber	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 formulation for the feed diet preparation is shown in Table 2 while the average weight of the rats measured daily is shown in Table 3. The effect of BSG blend on the weight of the rats was commendable. The rats in 3 and 6 % BSG blend cages gave an average daily weight gain of 3.810 and 3.520 g respectively, which was higher than control (3.706 g). There was significance increase ($p<0.05$) in the daily average weight gained by the rats fed in all the groupings. This indicated 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 0.53 - 0.15 x Weight Gain in order to describe the relationship between the feed-efficiency and weight gain. The rats in the 15 % group experienced daily weight gain of 0.495 g/day, with their metabolic wastes concentration was very high and also toxic to inhale. The loss in the body weight might be due to low level of crude fat (2.79 %) in the feed. The feed conversion efficiency and fat supplementation agreed and this was in line with the literature study [19] and acceptable limit of 1 % to 6 % w/w of BSG blends can be taken as threshold. 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) hence the rats body weights are different from each other from the descriptive statistics.

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

Ingredients	Diets					
	% 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: Weight gained 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

Table 4: Haematological Studies of BSG Blended Feed in rats.

Parameter / Blends	0% BSG	3% BSG	6% BSG	9% BSG	12% BSG	15% BSG
Hb (g%)	10.9 ± 0.2	13.8 ± 0.6	13.80 ± 0.2	7.6 ± 0.3	11.6 ± 0.4	12.8 ± 0.1
PCV (%)	31.7 ± 1.22	39.0 ± 1.18	38.0 ± 2.17	30.0 ± 1.11	24.0 ± 1.42	30.7 ± 2.23
RBC (10^6/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
MCV (U^3)	95.0 ± 6.10	87.0 ± 4.20	83.0 ± 3.02	82.0 ± 5.40	85.0 ± 3.25	91.0 ± 2.50
MCH (Ug)	33.0 ± 1.22	32.0 ± 3.10	29.0 ± 3.12	27.0 ± 1.12	30.0 ± 2.72	32.0 ± 1.11
MCHC (%)	33.0 ± 2.10	37.0 ± 2.11	36.0 ± 1.10	35.0 ± 3.11	34.0 ± 3.40	34.2 ± 3.25
Neutro (%)	6.0 ± 0.15	20.0 ± 0.55	2.0 ± 0.18	4.0 ± 0.10	26.0 ± 0.49	12.0 ± 1.20
Lympho (%)	94.00 ± 5.35	80.0 ± 6.82	98.0 ± 6.82	96.0 ± 4.57	74.0 ± 3.45	88.0 ± 5.10
Eosino (%)	0	0	0	0	0	0
Mono (%)	0	0	0	0	0	0
Baso (%)	0	0	0	0	0	0
Platelets (10^3/ m^3)	155 ± 11.2	198 ± 10.3	210 ± 12.3	180 ± 13.1	184 ± 10.1	1680 ± 11.1
WBC (10^3/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

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.

In Tables 4 and 5, the haematological and the biochemical studies of the rats used for the experiment were respectively presented. The normal Packed Cell Volume (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 x 10^6/mm^3 as against the control 3.68±0.42 (10^6/mm^3); White Blood Cell counts (WBC) has high value of 7.20 and 7.10 (10^3/mm^3) in 3 % and 9 % respectively.

Platelets had the highest value of 210±12.32 (10^6/mm^3) in 6 % as against the observed 155.00±11.20 (10^6/mm^3) 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 anti infection properties which is present in the blood. It was also observed that the 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. The rats fed with 12 % blend had a low value in PCV, AP , Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), lymphocytes, 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. 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 histopathology 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.

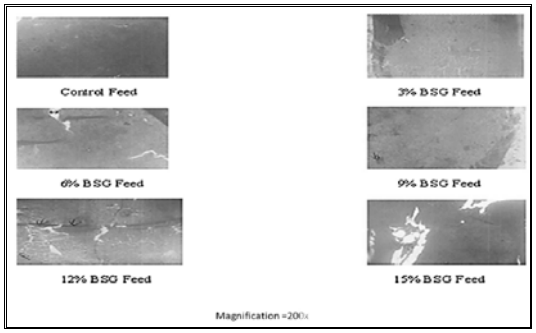

Plate 1: Microscopic view of rats' liver fed with compounded feeds

Table 5: Biochemical Studies of BSG Blended Feed in rats.

Parameter/ Blends	0%	3%	6%	9%	12%	15%
ALP (IU/L)	250 ± 35.1	302 ± 30.1	300 ± 12.2	298 ± 16.3	275 ± 28.2	213 ± 30.1
GOT (IU/L)	66.0 ± 5.40	72.0 ± 4.90	71.0 ± 3.20	70.0 ± 2.50	68.0 ± 4.50	57.00 ± 3.11
GPT (IU/L)	46.00 ± 4.22	36.00 ± 3.61	44.0 0 ± 1.84	46.00 ± 2.61	48.00 ± 2.22	46.00 ± 2.62
AP (g/dL)	65.00 ± 4.80	68.00 ± 2.56	67.0 0 ± 1.68	66.00 ± 3.22	59.00 ± 3.81	62.00 ± 1.12
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
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
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
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); NV = Normal Value; NA = Not Available

The histopathology study of the liver of the rats used as experimental model is shown in Plate 1. The observed cases in 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 also 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 also 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 overall inference of the histopathology result suggests that the utilization up to 3% concentration in confectioneries will not have adverse effect on human liver as long as the concentration of the blend is kept minimal.

4. Conclusion

This study has demonstrated the utilization that BSG blends from 1-3 % range can be good protein supplement in human food such as confectioneries. It has also established 3 % exploitation of the spent grains as the threshold limit by virtue of the observed histological effect on the rats liver hence blends between ranges 1-3 % becomes appropriate for utilization in human food without adverse effect on the liver organ. This can now be said to provide an additional utilization alternative to the disposal of brewery spent grains worldwide.

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