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

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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. Data collected were subjected to statistical analysis of variance and means compared by the Duncan’s multiple range test.

Results and Discussion
The proximate analysis of the brewery spent grains (BSG) samples and the total percentage of crude protein in each feed formulation 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>23.1985</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>12.8500</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.7900</td>
</tr>
<tr>
<td>Moisture content</td>
<td>6.1400</td>
</tr>
<tr>
<td>Ash content</td>
<td>16.9925</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>51.3862</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>3.7118</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>38.0290</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Feed formulation</th>
<th>Total crude protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% (without BSG)</td>
<td>21.6000</td>
</tr>
<tr>
<td>3% BSG inclusion</td>
<td>22.0600</td>
</tr>
<tr>
<td>6% BSG inclusion</td>
<td>22.4500</td>
</tr>
<tr>
<td>9% BSG inclusion</td>
<td>22.7600</td>
</tr>
<tr>
<td>12% BSG inclusion</td>
<td>23.2400</td>
</tr>
<tr>
<td>100% (BSG only)</td>
<td>23.2000</td>
</tr>
</tbody>
</table>

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 that fat supplementation significantly improved feed conversion efficiency.

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

<table>
<thead>
<tr>
<th>Feed formulation</th>
<th>Weight (g)</th>
<th>Weight (g)</th>
<th>Weight (g)</th>
<th>Weight gain g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>15th day</td>
<td>difference</td>
<td></td>
</tr>
<tr>
<td>0% (without BSG)</td>
<td>52.55±2.250</td>
<td>108.15±4.351</td>
<td>55.60±2.301</td>
<td>3.707</td>
</tr>
<tr>
<td>3% BSG</td>
<td>55.50±2.700</td>
<td>112.65±3.750</td>
<td>57.15±1.050</td>
<td>3.810</td>
</tr>
<tr>
<td>6% BSG</td>
<td>49.55±3.350</td>
<td>102.40±3.70</td>
<td>52.85±0.350</td>
<td>3.523</td>
</tr>
<tr>
<td>9% BSG</td>
<td>49.15±3.451</td>
<td>85.15±5.550</td>
<td>36.00±1.199</td>
<td>2.400</td>
</tr>
<tr>
<td>12% BSG</td>
<td>44.10±3.900</td>
<td>57.70±4.40</td>
<td>13.60±0.500</td>
<td>0.907</td>
</tr>
<tr>
<td>100% (BSG only)</td>
<td>48.65±4.100</td>
<td>17.45±2.801</td>
<td>-31.20±2.801</td>
<td>2.080*</td>
</tr>
</tbody>
</table>

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.
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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0% BSG</th>
<th>3% BSG</th>
<th>6% BSG</th>
<th>9% BSG</th>
<th>12% BSG</th>
<th>100% BSG</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (%)</td>
<td>10.9</td>
<td>13.8</td>
<td>13.8</td>
<td>7.6</td>
<td>11.6</td>
<td>12.8</td>
<td>16.1 ±0.4</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>31.7</td>
<td>39.0</td>
<td>38.0</td>
<td>30.0</td>
<td>24.0</td>
<td>30.7</td>
<td>40.6 ±0.12</td>
</tr>
<tr>
<td>RBC (x10^6/mm³)</td>
<td>3.68</td>
<td>5.52</td>
<td>4.78</td>
<td>3.80</td>
<td>4.50</td>
<td>3.85</td>
<td>8.21±0.14</td>
</tr>
<tr>
<td>MCV (V³)</td>
<td>95.0</td>
<td>87.0</td>
<td>83.0</td>
<td>82.0</td>
<td>85.0</td>
<td>91.0</td>
<td>56.2 ±0.6</td>
</tr>
<tr>
<td>MCH (µg)</td>
<td>33.0</td>
<td>32.0</td>
<td>29.0</td>
<td>27.0</td>
<td>30.0</td>
<td>32.0</td>
<td>14.7– 15.9</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>6</td>
<td>20</td>
<td>2</td>
<td>4</td>
<td>26</td>
<td>12</td>
<td>10-55</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>94</td>
<td>80</td>
<td>96</td>
<td>74</td>
<td>88</td>
<td>40–90</td>
<td></td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Platelets (x10^9/mm³)</td>
<td>155</td>
<td>198</td>
<td>210</td>
<td>180</td>
<td>184</td>
<td>168</td>
<td>54.5 ± 13.6</td>
</tr>
<tr>
<td>WBC (x10^3/mm³)</td>
<td>5.0</td>
<td>7.2</td>
<td>7.1</td>
<td>5.2</td>
<td>5.0</td>
<td>6.2</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>ALP (g/l)</td>
<td>25.0</td>
<td>30.2</td>
<td>30.0</td>
<td>29.8</td>
<td>27.5</td>
<td>21.3</td>
<td>43.2 ± 0.38</td>
</tr>
<tr>
<td>GOT (g/l)</td>
<td>6.6</td>
<td>7.2</td>
<td>7.1</td>
<td>7.0</td>
<td>6.8</td>
<td>5.7</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>AP (g/l)</td>
<td>4.6</td>
<td>3.6</td>
<td>4.4</td>
<td>4.6</td>
<td>4.8</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>TP (g/l)</td>
<td>0.73</td>
<td>0.66</td>
<td>0.69</td>
<td>0.64</td>
<td>0.65</td>
<td>0.71</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>ALB (g/l)</td>
<td>0.39</td>
<td>0.43</td>
<td>0.41</td>
<td>0.40</td>
<td>0.38</td>
<td>0.38</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>GLB (g/l)</td>
<td>0.34</td>
<td>0.23</td>
<td>0.28</td>
<td>0.24</td>
<td>0.27</td>
<td>0.33</td>
<td>NA</td>
</tr>
</tbody>
</table>

References
3Bi-Yu, Chen-Chaoven, Chiu-Wenshy 1998. Wet brewer’s grain or bean curd pomace as partial replacement of soya bean meals lactating cows. J. Animal Feed Science and Technology 5:120-128.