



## Performance, Egg Quality and Nutrient Utilization by Laying Birds Fed *Penicillium chrysogenum* Degraded Brewer Dried Grain

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### Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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### ABSTRACT

The sub-Saharan African feed industry is encumbered with competitors for the conventional feed ingredients. The competition most often increases the prices of these ingredients and invariably the cost of animal production. The use of agro industrial by-products after fungal biodegradation can be of help in combating this challenge. This study was carried out to assess the effect of *Penicillium chrysogenum* degraded brewer's dried grains (BDG) on the performance, digestibility and egg quality of layers. Degraded and Undegraded brewers dried grains (BDG) were used to formulate the rations for laying birds for 12 (twelve) weeks. A total of two hundred and fifty two (252) laying birds that were thirty (30) weeks old were randomly allocated to the diets. There were seven treatments and treatment 1 was the control with 0% brewer dried grains (BDG) inclusion level. The degraded and the undegraded BDG were used at 3, 5 and 7% inclusion levels. Thirty six (36) birds were

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allocated to each of the treatments with three (3) replicates at 12 birds each. *Penicillium chrysogenum* was inoculated on BDG by Solid State Fermentation method for seven days and was used as the degraded sample. The proximate analysis of undegraded BDG was compared with degraded BDG. The Crude protein, ash, ether extract of degraded brewers dried grain rose from 25.67 to 31.57%, 9.87 to 12.45%, and 6.87 to 7.35% respectively while crude fibre reduced from 15.93 to 10.39%. Results on the performance of the birds revealed that there were significant ( $P<0.05$ ) differences in feed intake, weight gain, feed conversion ratio and hen-day production. Egg quality characteristics also revealed a significant ( $P<0.05$ ) differences in egg weight and shell thickness. The results showed that *Penicillium chrysogenum* was able to enhance the feeding value of BDG and this impacted positively on the feed consumption, body weight gain, feed conversion ratio and egg quality characteristics.

**Keywords:** Brewer dried grains; *Penicillium chrysogenum*; layers.

## 1. INTRODUCTION

The escalating cost of feeding has brought about the need to look inward for alternative sources of feed materials. There is competition between humans and livestock for the use of conventional feed ingredients. For instance, maize, which is the major source of energy in animal feed, is also a major ingredient in the manufacture of many food products for human consumption such as cornflakes, indomie and pasta. This is a challenge that livestock farmers have at hand now and the bulk of this is on the monogastrics, especially poultry that are reared intensively [1,2,3]. This suggests that they primarily depend on the expensive and at times, unaffordable conventional feed ingredients. From the foregoing, it is evident that the farmers may need to consider the possibility of using alternatives- the non conventional feed ingredients (agro industrial by-products). Agro industrial by products (AIBs) are secondary products derived from processing of agricultural products, that is, the waste products which are obtained after farm produce are processed. In a bid to overcome this major constraint, several studies have been carried out on the utilization of agro industrial by products and crop residues. However, AIBs are characterized by low digestibility, low level of nitrogen, protein and minerals. Hence, to improve the digestibility and the nutritive value of these by-products, various processing methods have emerged, including biodegradation. Notable agro industrial by products are: citrus pulp, wheat offal, plantain peel, groundnut cake, palm kernel cake, brewers dried grain, sugarcane bagasse, rice husk and corn cobs. Many methods have been used to improve the usability of these AIBs namely: chemical, biological and physical [4,5,6]. For this work, a biological technique (solid state fermentation) was adopted using *Penicillium chrysogenum*. SSF refers to aerobic microbial transformation of solid materials. Bacteria, yeast

and fungi can grow on solid substrates and find application in solid substrate fermentation processes, although filamentous fungi are best adopted and are dominated in research works, this is because most fungi are capable of degrading a wide variety of substrate. *Penicillium chrysogenum* is specie of fungus in the family *Trichomaceae*. It is common in temperate and subtropical regions and can be found on salted food products, but it is mostly found in indoor environments, especially in damp or water-damaged buildings. It has rarely been reported as a cause of human disease. It is the source of several  $\beta$ -lactam antibiotics, most significantly penicillin. Other secondary metabolites of *Penicillium chrysogenum* include roquefortine C, meleagrins, chrysogins and xanthocillins. Like many other species of the genus *Penicillium*, *Penicillium chrysogenum* usually reproduces by forming dry chains of spores from brush-shaped conidiophores. Brewers' grains are the solid residue remaining after the processing of dried cereal grains for the production of beer and other malt products. Though barley is the major cereal that is being used for brewing, beers can also be made from other cereals such as: wheat, maize, rice and sorghum. Brewers' grains are obtained at the end of the brewing process [7]. This work was carried out to assess the biodegradation effect of *Penicillium chrysogenum* on BDG using solid state fermentation technique and also to determine the effect of feeding biodegraded brewer dried grain on the performance of commercial layer.

## 2. MATERIALS AND METHODS

*Penicillium chrysogenum* was obtained from stock culture cultivated on Potato Dextrose Agar (PDA) slant at Microbiology Laboratory, Bowen University Iwo, Nigeria. A piece of mycelia of *Penicillium chrysogenum* was subcultured on potato dextrose agar (PDA) in Petri dishes and

incubated at 30°C for 4 days under aseptic condition as described by [8]. 150 g of BDG was placed in each of four, 250ml conical flasks corked with cotton wool and sterilized at 121°C for 15 minutes. Sterile water was added to raise the moisture of the meals as described by [9]. About 10 mm of inocula (*Penicillium chrysogenum*) was used to inoculate each of the BDG containing flasks under aseptic condition and then incubated in the oven for 5 days at 30°C. Each of these 150 g samples was then poured into 10 kg sterilized and moistened brewer dried grains and mixed thoroughly. It was then incubated in the bigger bag for 7 days. The sample was oven dried after 7 days at 70°C and the dried sample was then kept for the preparation of diets and laboratory analysis. A total of two hundred and fifty two (252) Isa Brown laying birds that were thirty (30) weeks old were randomly allocated to the diets. Degraded and undegraded BDG were used to formulate rations for laying birds for 12 (twelve) weeks. There were seven treatments and treatment 1 was the control with 0% brewer dried grains (BDG) inclusion level. The degraded and the undegraded BDG were used at 3, 5 and 7% inclusion levels. Thirty six (36) birds were allocated to each of the diets with three (3) replicates at 12 birds each. Feed intake and body weights were recorded weekly. Vaccination programme was strictly followed and isocaloric and isonitrogenous diets were formulated. At the end of the twelfth week of the experiment, metabolic trial was carried out. The birds were offered known quantities of the respective experimental diets. Nine birds per treatment (3 birds per replicate) with close average weights were used for this purpose. The birds were allowed three days of adjustment to the cage before faecal collection. Feed intake was measured during the same period of 3 days at 24 hours interval. Faeces for each replicate was weighed and placed in aluminum foil and dried to constant weight at 80°C. Water was offered *ad-libitum* and appropriate vaccination, medication and requisite management practices were strictly adhered to. Three eggs were selected per replicate on the 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> week respectively for egg quality assessment. Yolk colour was evaluated using Roche colour fan. Shell thickness was determined using the micrometer screw gauge. Diameter and the height of the yolk were determined by using the vernier calipers. From the obtained result, the value of yolk index was calculated. Proximate composition of the diets and faeces was analyzed by the methods of [10]. Data collected

were analyzed statistically using the Analysis of Variance (ANOVA) technique of [11]. Where statistical significant differences were observed, the treatment means were compared according to [12].

### 3. RESULTS AND DISCUSSION

Table 1 shows the effect of biodegradation on BDG using *P. chrysogenum* as seen in changes in levels of crude protein, ash, crude fibre, gross energy and detergent fibres. There were 18.69, 28.78, 9.64 and 18.44% increase in crude protein, ash, gross energy and dry matter respectively. Besides, there were 28.76, 51.24 and 27.00% reduction in crude fibre, ether extract and cellulose respectively. The reduction seen in the crude fibre, detergent fibre and cellulose content in degraded BDG after fermentation could be possibly traced to production of extra cellular enzymes which may include xylanase, amylase and cellulases released into the BDG by the fungus. This is done with the motive of using the BDG as carbon source or substrate [13]. Furthermore, fungi are known to have the ability to colonize the carbon source for optimum utilization of the available nutrients in carbon source. Subsequently, they manufacture and produce considerable quantities of extra cellular enzymes that can affect the hydrolysis of nutrients to materials that can penetrate the fungi via the cell membrane to increase the fungal metabolic activities and this process will promote development of the fungus. Hence, development in growth and continuous multiplication of fungal body mass as single cell protein (SCP) may partly be responsible for the increase in the protein content after biodegradation [8,14]. The process of solid state fermentation, apart from adding to the protein content, also adds monosaccharides into the medium owing to hydrolysis of unavailable non starch polysaccharides. This implies the production of enzymes in the medium that empowers them to break complex polymers [15, 16,17]. This probably further sheds light on the reasons for reduction in crude fibre content. This result was in accordance with the works of [13]. In their work, the results obtained showed that fermentation of pineapple waste using *Aspergillus niger* and *Trichoderma viride* via the technique of solid state fermentation significantly ( $P<0.05$ ) enriched the nutrient content of the wastes particularly increasing the crude protein and ash content while lowering the crude fibre content. Furthermore, in the works of [14], it was reported that after biodegradation of citrus pulp

with *Aspergillus niger*, the crude protein, gross energy and ash of the degraded pulp rose from 14.10 to 16.14, 2.88 to 3.79 kcal/kg, 58.21 to 61.31% and 4.61 to 5.11% respectively while the crude fibre content reduced from 8.15 to 6.41%. The results of feed intake, weight gain, feed conversion ratio and relative cost benefit are shown in Table 3. Feed intake, weight gain and feed conversion ratio showed significant ( $P<0.05$ ) differences. The highest feed intake (173.94 g/b/d) was observed in treatment 4 that had 7% degraded brewer dried grain (DBDG) and the highest weight gain was also observed in the same treatment (68.43 g/b/d). The best feed conversion ratio and relative cost benefit (2.11 and 4.23) were also noticed in treatment 4. It has been reported by various researchers that feeding of biodegraded agro industrial by-products will have a positive effect of reducing digesta viscosity along the gastro intestinal tract [18,19,20]. According to [21], agro industrial by products are characterized by non starch polysaccharides which are known to be parts of the anti nutritive factors that can increase the intestinal viscosity, impair endogenous enzymes actions and absorption of nutrients. [22] also reported that viscosity occasioned by the non starch polysaccharides can reduce the digestion and absorption of nutrients. The NSPs form gels when they get in touch with water; they raise the diet volume by water retention in the gastrointestinal tract causing a decrease in feed intake. Improvement in feed intake becomes attainable when there is exogenous reduction in the NSPs via enzymic hydrolysis occasioned by fungal biodegradation. There was better body weight gain by the birds fed degraded BDG because more nutrients were available to the birds. The Feed conversion ratio value decreased with the treatments placed on degraded BDG and this indicate better utilization of the feeds by the birds. It showed that the birds were able to use the obtained nutrients for maintenance and productivity. The relative cost benefit speaks about the profitability of the used ingredients. From the obtained results it is clear that it is economically expedient for the farmers to use degraded brewer dried grains. This shows that farmers stand to make more profit if degraded AIBs are considered as feed ingredient for their layers. In Table 4, significant ( $P<0.05$ ) differences were also expressed in nutrients digestibility. It is was observed that layers placed on treatments with degraded BDG had better digestibility of Ether extract, crude protein, crude fibre and ash than others placed on control and

undegraded BDG. In the works of [23], it was stated that the improvement in digestibility and nutrient utilization may possibly be due to the reduction on energy losses owing to caloric increment and to volatile fatty acids in excreta which occurs due to poor microbial fermentation. A considerable quantity of starch can maneuver to large intestine and can be hydrolyzed through microbial activities. This can lead to poor metabolic use in comparison to feeding the birds with degraded AIBs which enhances glucose utilization in the small intestine [24]. Furthermore, the improvements recorded in digestibility may be explained as the ability of *P. chrysogenum* to produce enzymes like phytase which increases the bioavailability of phytate phosphorus and this may invariably lead to improvement in the bioavailability of other minerals that are susceptible to chelation such as Zn, Mn, Ca, Cu and Fe [25]. Table 5 showed egg quality characteristics of layers fed undegraded and degraded brewer dried grains. Significant ( $P<0.05$ ) differences were also observed in egg weight, shell thickness and hen day production. 88.45 g, 0.38 mm and 71.22% were the highest values recorded for egg weight, shell thickness and hen-day production and they were observed in treatments with degraded BDG. The use of fungi degraded AIBs in feeding layers can increase egg production, egg weight and shell thickness as a result of better availability, digestibility and utilization of nutrients by the birds. Shells of eggs obtained from birds on DBDG were thicker possibly owing to availability of minerals like calcium and phosphorus [26,27, 28,29].

**Table 1. Proximate and detergent fibre analysis of undegraded and degraded brewer dried grain (g/100 gDM)**

Parameter	Undegraded BDG	Degraded BDG
Dry matter	81.43	90.12
Crude protein	25.67	31.57
Ether extract	6.87	3.35
Ash	10.96	15.39
Crude Fibre	12.45	8.87
Gross Energy (Kcal/kg)	4.91	6.02
Cellulose	7.22	5.27
Hemicellulose	5.84	3.41
Neutral detergent fibre	2.51	1.11
Acid detergent fibre	2.28	1.20
Acid detergent lignin	6.20	4.12

**Table 2. Gross compositions of the experimental diets (g/100gDM)**

Ingredients	Treatment1 (0% BDG)	Treatment2 (3% DBDG)	Treatment 3 (5% DBDG)	Treatment 4 (7% DBDG)	Treatment 5 (3%UBDG)	Treatment 6 (5% UBDG)	Treatment 7 (7% UBDG)
Maize	42.00	40.00	39.00	38.00	39.0	40.00	41.00
Rice Bran	24.00	23.00	22.00	21.00	24.00	22.00	20.00
GNC <sup>1</sup>	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Fish meal	2.60	2.60	2.60	2.60	2.60	2.60	2.60
Wheat offal	6.00	6.00	6.00	6.00	6.00	6.00	6.00
UBDG <sup>2</sup>	0.00	0.00	0.00	0.00	3.00	5.00	7.00
DBDG <sup>3</sup>	0.00	3.00	5.00	7.00	0.00	0.00	0.00
Oyster shell	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Premix	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ME(Kcal/kg)	2628.8	2637.1	2625.8	2629.7	2628.9	2634.1	2628.6
Crude protein	16.43	16.44	16.51	16.34	16.55	16.41	16.42

UBDG<sup>2</sup>=undegraded brewer dried grains; DBDG<sup>3</sup>=degraded brewer dried grains. GNC<sup>1</sup>=Groundnut cake \*Vitamin mineral premix supplied the following vitamins and trace elements per kg diet: Vitamin/Mineral Premix to provide the following per kg of feed: Vit. A 12500IU, Vit. D<sub>3</sub> 2500IU, Vit. E 40 mg, Vit. K<sub>3</sub> 3 mg, Vit. B<sub>1</sub> 3 mg, Vit.B<sub>2</sub> 5.5 mg, Niacin 5.5 mg, Calcium Pantothenate 11.5 mg, Vit B<sub>6</sub> 5 mg, Vit B<sub>12</sub> 0.025 mg, Folic Acid 1 mg, Biotin 0.08 mg, Mn 120 mg, Choline Chloride 500 mg, Fe 100 mg, Zn 80 mg, Cu 8.5 mg, I 1.5 mg, Co 0.3 mg, Se 0.48 mg and Antioxidant 120 mg

**Table 3. Performance characteristics of experimental layers fed undegraded and degraded brewer dried grains**

Composition	T1 (0% BDG)	T2 (3% DBDG)	T3 (5% DBDG)	T4 (7% DBDG)	T5 (3% UBDG)	T6 (5% UBDG)	T7 (7% UBDG)	SEM
Feed intake (g/b/d)	141.97 <sup>b</sup>	152.72 <sup>b</sup>	170.99 <sup>a</sup>	173.94 <sup>a</sup>	132.51 <sup>c</sup>	131.64 <sup>c</sup>	131.70 <sup>c</sup>	3.51
Weight gain (g/b/d)	50.88 <sup>c</sup>	65.54 <sup>b</sup>	67.72 <sup>a</sup>	68.43 <sup>a</sup>	46.65 <sup>d</sup>	46.35 <sup>d</sup>	45.87 <sup>d</sup>	2.01
FCR	2.79 <sup>b</sup>	2.33 <sup>c</sup>	2.20 <sup>c</sup>	2.11 <sup>d</sup>	2.84 <sup>a</sup>	2.84 <sup>a</sup>	2.91 <sup>a</sup>	0.09
Hen-day production (%)	67.93 <sup>b</sup>	68.41 <sup>b</sup>	70.36 <sup>a</sup>	71.22 <sup>a</sup>	64.34 <sup>c</sup>	64.11 <sup>c</sup>	61.52 <sup>d</sup>	1.34
Mortality	0	0	1	0	1	0	0	0.00
Relative cost benefit (%)	0.00	3.74	4.19	4.23	3.66	3.66	3.42	-----

a, b, c, Means in the same row with different superscripts differ significantly ( $P < 0.05$ ); UBDG=undegraded brewer dried grains; DBDG=degraded brewer dried grains. FCR=Feed conversion ratio; SEM= Standard Error of Mean

**Table 4. Nutrient digestibility of layers fed undegraded and degraded brewer dried grains**

Composition (%)	T1 Control	T2 3%DBG	T3 5%DBG	T4 7%DBG	T5 3%UBDG	T6 5%UBDG	T7 7%UBDG	SEM
Dry matter	74.34	74.23	74.77	74.82	74.31	74.86	75.05	1.02
Fat	35.27 <sup>b</sup>	65.03 <sup>a</sup>	65.58 <sup>a</sup>	65.17 <sup>a</sup>	62.16 <sup>c</sup>	62.72 <sup>c</sup>	61.22 <sup>c</sup>	1.23
Crude protein	71.82 <sup>c</sup>	72.41 <sup>b</sup>	73.06 <sup>b</sup>	75.28 <sup>a</sup>	72.04 <sup>b</sup>	71.74 <sup>b</sup>	70.36 <sup>c</sup>	0.59
Crude fibre	58.08 <sup>d</sup>	61.02 <sup>c</sup>	63.18 <sup>b</sup>	65.22 <sup>a</sup>	58.58 <sup>d</sup>	57.36 <sup>d</sup>	55.22 <sup>e</sup>	1.11
Ash	58.38 <sup>b</sup>	59.44 <sup>b</sup>	59.61 <sup>b</sup>	62.82 <sup>a</sup>	57.68 <sup>c</sup>	57.63 <sup>c</sup>	56.89 <sup>c</sup>	1.85

a, b, c, Means in the same row with different superscripts differ significantly ( $P < 0.05$ ); UBDG=undegraded brewer dried grains; DBG=degraded brewer dried grains; SEM= Standard Error of Mean

**Table 5. Egg quality characteristics of layers fed undegraded and degraded brewer dried grains**

Composition (%)	T1 Control	T2 3%DBG	T3 5%DBG	T4 7%DBG	T5 3%UBDG	T6 5%UBDG	T7 7%UBDG	SEM
Egg weight (g)	66.67 <sup>ab</sup>	67.67 <sup>a</sup>	67.98 <sup>a</sup>	68.45 <sup>a</sup>	65.87 <sup>b</sup>	62.38 <sup>c</sup>	63.44 <sup>c</sup>	1.22
Shell thickness (mm)	0.35 <sup>b</sup>	0.37 <sup>a</sup>	0.37 <sup>a</sup>	0.38 <sup>a</sup>	0.33 <sup>c</sup>	0.32 <sup>c</sup>	0.33 <sup>c</sup>	0.002
Yolk colour	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
Yolk height (cm)	1.40	1.55	1.55	1.56	1.40	1.39	1.39	0.02
Shell weight (g)	5.64	5.64	5.65	5.65	5.64	5.64	5.63	0.05
Yolk diameter (cm)	3.78	4.18	4.07	4.00	3.88	4.08	4.08	0.02
Yolk index	0.37	0.37	0.38	0.39	0.36	0.34	0.34	0.04

a, b, c, Means in the same row with different superscripts differ significantly ( $P < 0.05$ ); UBDG=undegraded brewer dried grains; DBG=degraded brewer dried grains; SEM= Standard Error of Mean

#### 4. CONCLUSION

Biodegradation of brewer dried grains with *P. chrysogenum* increased the level of crude protein, ash and gross energy and reduced the fibre fractions. Layers placed on treatments with degraded BDG performed better in terms of feed intake, weight gain and feed conversion ratio. Besides, birds fed degraded BDG also indicated better nutrient digestibility and this impacted well on the egg quality characteristics of layers.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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