Content list available at http://epubs.icar.org.in, www.kiran.nic.in; ISSN: 0970-6429

Indian Journal of Hill Farming

December 2023, Volume 36, Issue 2, Pages 208-215

Effect of feeding rice based brewers dried grain on bone development and nutrient digestibility in laying hens

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ARTICLE INFO

Article history:
Received: 04 May, 2023
Revision: 16 December, 2023
Accepted: 17 December, 2023

Key words: *Rice brewers dried grains, laying hen, bone, nutrient digestibility*

DOI: 10.56678/iahf-2023.36.02.28

ABSTRACT

An experiment was undertaken to study the response of feeding sun dried rice based brewers dried grain (RBDG) on bone development and nutrient digestibility of laying hens. For this, a feeding trial of 125 laying hens was conducted for 16 weeks (25th to 40th weeks of age) with completely randomized design divided into five treatments with 25 laying hens per treatment. Five experimental diets as per ICAR (2013) were prepared by incorporating rice brewers dried grain at inclusion level of (0, 10, 20, 30 and 40%) respectively by replacing maize and soybean meal from basal diet. The results revealed that the development of tibia bone at 40 weeks of age in term of bone morphometry such as length, proximal width, mid shaft width and distal width in mm, and tibia bone mineralization such as fresh bone weight, dried bone weight, bone moisture, total ash, calcium (Ca) and phosphorus (P) contents of laying hens did not differed significantly (P>0.05) among the treatment groups. The results also indicated that nitrogen intake and retained (g/b/d) did not differed significantly due to replacing maize and soybean meal with rice based brewer's dried grain. However, significantly (P<0.05) lower value of nitrogen voided was found at 0 to 20% than that observed at 40% inclusion level of rice based brewer's dried grain and also significantly lower nitrogen retention was observed at 40% inclusion level than those recorded at 0-20% RBDG. The calcium and phosphorus intake, voided, retained (g/b/d), and retention (%) did not differed significantly. Thus, it is concluded that rice based brewers dried grain (RBDG) can be safely incorporated in diet of laying hens at the inclusion level of 20% for better bone development and nutrient digestibility.

1. Introduction

The major challenges to the poultry industry in developing countries are the high prices of soybean meal and yellow corn, which are mainly used for formulation of poultry feed. The cost of feed is between 65 to 70% (Al-Sagheer *et al*., 2019) and 70 to 75% (Abd El-Hack *et al.,* 2015) of the total cost of production, as opposed to about 50 to 60% in developed countries (Saeed *et al*., 2017). Reducing the cost of feed is therefore an important target in the poultry industry. Increased feed costs and limited amounts of animal protein sources in poultry feed have led to the use of alternative plant proteins that make up some or all animal protein in feed (El Boushy *et al*., 2000; Vasso & Russ, 2007). Poultry and livestock production may continue to be ulcerative if costly conventional feedstuffs are not replaced with cheaper and available feedstuffs.

In this situation, brewery by-products like brewers' dried grains (BDGs) are worthy of consideration as potential non-conventional feed resources. Incorporating agro-industrial by-products in animal feed holds tremendous

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potential in alleviating the high cost and insufficient supply of feed ingredients (Longe, 1985; Babatunde, 1989). BDG is available in large quantities throughout the year, but its main application has been limited to animal feeding. Brewery dried grain which contains 21-29% crude protein on dry matter and costing lower than rice bran and coconut oil cakes. Brewers' grains are a by-product of beer making industry. The nutritional value of brewers' grains varies depending on the grain used (barley, wheat, rice, or corn), the extent of the fermentation, and the type of fermentation process used. Brewers' grains are primarily fed to dairy cattle but have some nutritional value for poultry as well. The major problem limiting the use of dried brewers' grains in poultry rations is related to the grains' high fiber content. Brewer's dried grain is an excellent source of quality by-pass protein and digestible fiber, with good amino acid profile and it has high mineral and water soluble vitamin content. Spent grain or dried brewers' grain contains 93% dry matter (DM), 48.6% nitrogen free extract (NFE), 22.4% CP, 19.1% CF, 6.2% EE, 4% ash and 2360 kcal/kg of metabolizable energy (ME) (Longe *et al*., 1983). Dried brewers' grain is high in CP and ME, and could be used to reduce the quantity of soybean meal and maize grain in broiler chicken diets. Brewers dried grain is relatively cheap, readily available with little or no competition between humans, farm animals and industries. To date, very limited studies have been performed investigating bone development and nutrient digestibility in laying hens fed with diets containing RDBG. The hypothesis of this study was that RBDG feeding would improve the bone development and utilization of Nitrogen, Ca and P in laying hens. Therefore, the current trial was performed to evaluate the effect of various dietary inclusion levels of RDBG on bone development and nutrient digestibility in laying hens.

2. Materials and Methods

Experimental design

A feeding trial was undertaken in a completely randomized design with 125 no of Dahlem red hens of same age group which were randomly assigned into five groups (D_1 to D_5). Each laying hen was kept in individual cages (25 replicates) with separate feeding facility. The allocation of birds in each dietary treatment was based on body weight and 50% egg production at the start of the experiment. The experiment was conducted for 16 weeks (25^{th} to 40^{th} weeks of age). Out of five treatments, D_1 was maize-soybean based control diet (No inclusion of brewer dried grains) and the rest treatments were D_2-D_4 consisting four dietary inclusion levels (10, 20, 30 and 40%) respectively, of rice based brewer dried grains.

Experimental diets

A basal diet was prepared based on maize and soybean meal to meet the energy and protein requirement as per ICAR (2013) standard for laying hen. From this basal diet, five diets (D_1-D_5) were prepared by addition of 0, 10, 20, 30 and 40% of rice based brewer's dried grain to meet the energy and protein requirement for the laying hen during 25^{th} to 40^{th} weeks of egg production.

Table 1. Ingredients and nutrient composition of experimental diets containing different levels of RBDG for layers (25^{th} - 40^{th} wks) of age:

Feed Ingredients	Layer mash				
	D_1	**D_2**	**D_3**	**D_4**	**D_5**
Maize	65.74	60.02	54.55	47.25	44.96
DORB	2.70	5.00	6.80	6.70	4.90
RBDG	**0.00**	**10.00**	**20.00**	**30.00**	**40.00**
Soybean	18.20	12.10	6.00	3.00	0.60
Fish Meal	5.00	5.00	5.00	5.00	0.00
Shell Grit	3.20	3.10	3.10	3.10	5.00
Limestone	3.00	3.00	3.00	3.00	1.60
DCP	1.20	1.10	0.90	1.30	1.30
Methionine	0.19	0.13	0.10	0.10	0.14
Lysine HCL	0.22	0.00	0.00	0.00	0.95
TM Mix	0.15	0.15	0.15	0.15	0.15
Vitamin	0.15	0.15	0.15	0.15	0.15
Salt	0.15	0.15	0.15	0.15	0.15

Toxin binder	0.10	0.10	0.10	0.10	0.10
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Nutrient composition (%)					
Crude Protein	16.00	16.00	16.00	16.00	16.00
ME (Kcal/Kg)	2800	2800	2800	2820	2820
Lysine	0.88	0.88	0.88	0.88	0.95
Methionine	0.40	0.40	0.40	0.40	0.40
Calcium	3.00	3.00	3.00	3.10	3.10
Total Phosphorus	0.64	0.65	0.65	0.65	0.65
Available Phosphorus	0.40	0.40	0.40	0.40	0.42
Feed cost (Rs./kg)	22.95	20.30	17.98	16.36	15.52

Experimental protocol: The experimental birds (laying hens, n=125) were housed in specially designed individual laying cages with watering, feeding and egg collection facilities and reared under standard management conditions. The weighted amount of experimental diets was offered as mash *ad libitum* to respective groups of birds daily in the morning, and fresh and wholesome potable water was always made available to the birds throughout the experimental period of 16 weeks (25^{th} to 40^{th} weeks of age).

A metabolic trial lasting 3 days was carried out at 30^{th}-32^{th} day (5th weeks of experimental period) employing 10 birds per treatment and following the method of Hill and Anderson (1958), in order to study the digestibility of nitrogen, calcium and phosphorous. The feed consumed by each dietary group was recorded and the droppings voided over the same period were collected quantitatively. The droppings collected were dried for 4-5 days in oven at 60±5 ^{0}C till a constant weight was attained which represented the net faecal output. The representative sample of test diets and excreta samples were ground and stored in air tight containers for further analysis for nitrogen, phosphorous (AOAC, 1990) and calcium (Talapatra *et al.,* 1940). The intake, excreted and retained amount of nitrogen, calcium and phosphorous were calculated g/b/d basis and % retention of nitrogen, calcium and phosphorous were calculated on the basis of total intake.

At the end of feeding trial, 50 laying hens (10 birds/ treatment) were randomly slaughtered and left tibia bones were collected for estimation of tibia bone morphometry and bone mineralization. The bones were identified individually for each separate treatment and their adhere muscles together with connective tissues had been thoroughly removed manually by the help of scissors. The bones were dipped in boiling water for 5 minutes to remove remaining pin and soft tissues. For the measurement of bone length and width the method described by Collins and Moran (1999) was followed. Maximum width of the mid shaft and of each epiphysis (proximal and distal width) as well as the total length of the tibia bone was measured with vernier calipers.

In order to study the tibia bone mineralization, the left tibia used for observation of morphometry was subjected to study of tibia mineralization. Each tibia was defatted by dipping them for 16 hrs in petroleum spirit (boiling point 60-80 ºC) using the Soxhlet apparatus and dried before ashing. The weight of each tibia was recorded before and after drying in hot air oven for overnight and the bone moisture percentage was estimated as per (AOAC, 1990). The dried and defatted tibia bone was ash (dry ashing) in muffle furnace at 550ºC for 12 hrs and the bone ash percentage was estimated (AOAC, 1990). The Calcium and phosphorus content was estimated in each tibia bone sample as per the method described by Talpatra *et al*. (1940) and AOAC (1990).

The data obtained through the experiment subjected to statistical analysis in a completely randomized design as per standard procedures of Snedecor and Cochran, (1989) and significant means differences were tested as per Duncan's multiple range test (Duncan, 1955).

3. Results and Discussion

Tibia bone morphometry

The results pertaining to various tibia bone morphometry traits of laying hens as affected by replacing maize, soybean and fish meal with rice based brewer's dried grain at 40^{th} weeks of age have been presented in Table 2. The mean tibia bone morphometry at 40^{th} weeks of age in term of length , proximal width, mid shaft width and distal width (mm) of laying hens did not differed significantly (P>0.05) due to inclusion of different levels of rice based brewer's dried grain.

Present results get strengthen by work of Swiatkiewicz and Koreleski (2007) who performed a trail with 20% rye and corn DDGS. Result indicated that layer diets containing 20%

DDGS, did not affect performance or egg shell thickness, density and strength, elasticity and stiffness of the tibia and humorous bones of laying hens. Swiatkiewicz *et al.* (2014b) found that there were no statistically significant differences in the bio-mechanical and geometrical parameters of tibia and femur bones of laying hens fed diets with or without high level of DDGS (200g/kg) diet and authors concluded that high dietary level of DDGS (200 g/kg) had no negative effect on bone quality in laying hens. Swiatkiewicz *et al.* (2014a) reported that incorporation of DDGS into broilers diet decreased bone breaking strength of femur bones but had no influence on weight and geometrical indices of femur and weight, biomechanical and geometrical measurements of tibia bone. Authors also suggested that experimental additives did not affect the biomechanical and geometrical measurements of bone of broiler fed diet containing a high level of DDGS, except for feed enzyme which increased significantly bone breaking strength, yielding load and stiffness of femur.

Tibia bone mineralization

The results revealed that the tibia bone mineralization at 40th weeks of age in terms of tibia fresh bone weight, dried bone weight, bone moisture percentage, total ash percentage, calcium and phosphorus contents in tibia bone of laying hens did not differed significantly (P>0.05) due to replacing maize, soybean and fish meal with rice based brewer's dried grain (Table 3).

Present results get support from previous observation reported by Deniz *et al.* (2013) who noticed that there is no significant difference in tibia crude ash between hens fed diets without or with 100 g DDGS/kg. Similarly, non-significant effect of DDGS on phosphorus retention was reported by (Masa'deh *et al.*, 2011, 2012). Swiatkiewicz *et al.* (2014a) reported that incorporation of DDGS into broilers diet decreased bone breaking strength of femur bones but had no influence on weight and geometrical indices of femur and weight, biomechanical and geometrical measurements of tibia bone. Authors also suggested that experimental additives did not affect the biomechanical and geometrical measurements of bone of broiler fed diet containing a high level of DDGS, except for feed enzyme which increased significantly bone breaking strength, yielding load and stiffness of femur. Contrary to our finding, Angel *et al.* (2005) reported that the incorporation of probiotics with low calcium and phosphorus levels in broilers' diets significantly increased the retention of calcium and phosphorus along with tibia breaking strength and ash content. The beneficial influence of probiotic bacteria on tibia breaking strength and mineralization in broilers chicks, attributed by higher assimilation of calcium in the bones, was also demonstrated when a diet with standard levels of calcium and phosphorus was used (Panda *et al.*, 2008). Some of the studies suggested that the utilization of calcium and phosphorus decreased in layers fed diets with high levels of DDGS (Swiatkiewicz and Koreleski, 2007; Thacker and Widyaratne, 2007; Leytem *et al.*, 2008), which could negatively affect the mineralization and quality of bones of highly performing laying hens.

Table 2. Tibia osteo-morphometry of laying hens as influenced by feeding different levels of rice based brewers dried grain

Diets	RBDG (%)	Length (mm)	Proximal width (mm)	Mid shaft width (mm)	Distal width (mm)
D_1	0	110.20	20.20	7.70	11.40
D_2	10	113.50	19.60	7.40	11.60
D_3	20	114.50	20.60	7.50	12.30
D_4	30	111.40	19.20	7.35	12.00
D_5	40	115.40	21.20	8.05	11.80
Pooled SEM		0.99	0.49	0.13	0.26
P value		NS	NS	NS	NS

NS- Non- significant

Table 3. Tibia bone mineralization of laying hens as influenced by feeding different levels of rice based brewers dried grain

Diets	RBDG (%)	Fresh bone weight (g)	Dry bone weight (g)	Bone moisture (%)	Total ash (%)	Calcium (%)	Phosphorus (%)
D_1	0	7.08	4.80	31.79	45.88	22.07	11.34
D_2	10	8.03	5.22	34.83	46.04	23.50	11.98
D_3	20	8.71	5.54	34.56	43.90	22.75	12.54

D_4	30	7.30	4.77	35.02	49.93	25.33	14.92
D_5	40	8.89	5.70	34.10	49.61	24.63	13.75
Pooled SEM		0.26	0.16	1.39	1.18	0.70	0.64
P value		NS	NS	NS	NS	NS	NS

NS- Non- significant

Nutrient Digestibility:

Excreta moisture and nitrogen retention

Data on excreta moisture content (%) and nitrogen retention in laying hen as influenced by replacing maize and soybean meal with rice based brewer's dried grain have been presented in Table 4. The excreta moisture content, nitrogen intake and nitrogen retained (g/b/d) did not differed significantly (P>0.05) due to replacing maize, soybean and fish meal with rice based brewer's dried grain. However, nitrogen voided and nitrogen retention% differs significantly among the groups. Significantly (P<0.05) lower value of nitrogen voided was found at 0 to 20% inclusion levels than that observed at 40% inclusion level of rice based brewer's dried grain. However, nitrogen voided at 30% inclusion level of rice based brewer's dried grain was found statically similar to those having 0, 10, 20 and 40% inclusion level. Significantly lower nitrogen retention was observed at 40% inclusion level of RBDG than those recorded at 0-20% RBDG. However, nitrogen retention observed at 30% RBDG was found intermediary.

Present results get strengthen with work reported by Khalili *et al.* (2011) who noticed that digestibility of DM and protein did not differ among broilers fed diet containing 5-25% BDG at different stages of growth and authors also suggested that illeal protein digestibility was similar in broilers fed 20% BDG. Swain *et al.* (2012) also reported that retention of dry matter and protein did not differ among Vanaraja chicks by partially replacing maize, soybean and deoiled rice bran with BDG at 10 and 20% inclusion levels. Masa'deh *et al.* (2012) reported that feeding up to 12.5% DDGS had no negative effect on growth performance and N retention in laying hens. Swiatkiewicz *et al.* (2014a) reported that at 42 day, incorporation of DDGS in broiler diet had negatively affected (P<0.05) apparent digestibility of dry matter and organic matter, metabolizable energy content of the diet and retention of nitrogen. Dinani (2018) reported that nutrient utilization in term of nitrogen retention, dry matter and energy metabolizability, protein efficiency ratio and energy efficiency ratio were significantly lower at 15% rice DDGS level and higher at 7.5% level as compared to control and other dietary rice DDGS levels in broiler diets. Denstadli *et al.* (2010) reported that a significant reduction in the protein digestibility as BDG replaced the wheat and soy-based control diet, probably due to the insoluble properties of the BDG protein. Josson and Carre (1989) reported that the decrease of apparent protein digestibility with increasing fiber contents might be which increase the endogenous losses apparent digestibility. Present results disagree with results of Masa'deh *et al.* (2011) prepared diets containing 0, 5, 10, 15, 20 and 25% DDGS to laying hens from 24-46 weeks (phase I) and reported greater nitrogen retention in hens fed 25% DDGS levels in diets. Reza *et al.* (2013) also reported that illeal digestibility, value of protein significantly increased by inclusion of 5, 10, 15, and 25% BSG replacing soybean meal in broiler ration. Esonu *et al.* (1999) who reported that digestibility of DM, protein and ether extract did not differ significantly in broiler chicks fed diet containing maize sorghum based brewer's dried grains (BDG) at 15 - 30% level. Gupta (2016) reported that there were no significant differences in nitrogen intake, nitrogen retain (g/b/d), nitrogen retention and DMM at 5, 7.5 and 10% levels of rice based DDGS in comparison to control diet during 26^{th} to 35^{th} week of age, either addition of DDGS sole or in combination with enzyme.

Calcium retention

Present results revealed that calcium intake, calcium voided, calcium retained (g/b/d) and % calcium retention did not differ significantly due to replacing maize, soybean and fish meal with rice based brewer's dried grain (Table 5) . Present results are in agreement with previous work reported by Dinani (2018) who reported that nutrient utilization in term of calcium retention did not show any significant (P> 0.05) differences at 15% rice DDGS level as compared to control and other dietary rice DDGS levels. Contrary to our finding, Swiatkiewicz *et al.* (2014a) reported that at 42 day, incorporation of DDGS into the broiler diet had negatively affected (P<0.05) retention of calcium. Alabi *et al. (*2014) reported that increased in the dietary level of BDG without commercial enzyme supplementation significant decreased (P<0.05) in nutrient digestibility. There were significant (P<0.05) interaction between dietary levels of brewers' dried grain and commercial enzyme on nutrient digestibility and gastro-intestinal tract characteristics of broiler chicks.

Phosphorus retention

In present results the phosphorus intake, phosphorus voided, phosphorus retained (g/b/d) and % phosphorus retention did not differ significantly due to replacing maize, soybean and fish meal with rice based brewer's dried grain (Table 6). Present findings get support

from earlier observation reported by Dinani (2018) who reported that nutrient utilization in terms of phosphorus retention did not show any significant (P> 0.05) differences at 15% rice DDGS level as compared to control and other dietary rice DDGS levels. Contrary to our finding, Masa'deh *et al.* (2011) reported greater phosphorus retention in hens fed diet containing 25% DDGS levels in diets than those recorded at its lower levels of inclusion. He also observed that feeding up to 12.5% DDGS had no negative effect on P retention and P output decreased as DDGS levels increased. Alabi *et al.* (2014) reported that increase in the dietary level of BDG without commercial enzyme supplementation significantly decreased (P<0.05) in digestibility of nutrients. Authors concluded that there were significant (P<0.05) interaction between dietary levels of brewer's dried grain and commercial enzyme on nutrient digestibility and gastro-intestinal tract characteristics of broiler chicks.

Table 4. Excreta moisture and Nitrogen utilization of laying hens as influenced by feeding different levels of rice based brewers dried grain

Diets	RBDG (%)	Excreta DM (%)	Nitrogen intake (g/b/d)	Nitrogen voided (g/b/d)	Nitrogen retained (g/b/d)	Nitrogen retention (%)
D_1	0	19.93	3.04	1.25^{a}	1.79	58.53^{b}
D_2	10	20.19	3.02	1.26^{a}	1.75	57.85^{b}
D_3	20	19.12	3.12	1.26^{a}	1.86	59.76^{b}
D_4	30	22.16	3.07	1.62^{ab}	1.45	47.44^{ab}
D_5	40	24.51	3.10	1.72^{b}	1.37	44.06^{a}
Pooled SEM		1.01	0.04	0.07	0.07	2.16
P value		NS	NS	P<0.05	NS	P<0.05

Values bearing different superscripts within column differ significantly (P<0.05),
NS- Non-significant

Table 5. Calcium utilization of laying hens as influenced by feeding different levels of rice based brewers dried grain

Diets	RBDG (%)	Calcium intake (g/b/d)	Calcium voided (g/b/d)	Calcium retained (g/b/d)	Calcium retention (%)
D_1	0	3.56	2.02	1.54	42.85
D_2	10	3.53	2.01	1.53	42.98
D_3	20	3.65	2.02	1.63	44.61
D_4	30	3.60	2.21	1.39	38.87
D_5	40	3.63	2.28	1.35	36.89
Pooled SEM		0.05	0.07	0.07	1.89
P value		NS	NS	NS	NS

NS- Non-significant

Table 6. Phosphorus utilization of laying hens as influenced by feeding different levels of rice based brewers dried grain

Diets	RBDG (%)	Phosphorus intake (g/b/d)	Phosphorus voided (g/b/d)	Phosphorus retained (g/b/d)	Phosphorus retention (%)
D_1	0	0.42	0.27	0.15	35.98
D_2	10	0.41	0.26	0.15	37.68
D_3	20	0.43	0.27	0.16	36.84
D_4	30	0.42	0.27	0.15	34.57
D_5	40	0.42	0.28	0.14	34.03
Pooled SEM		0.005	0.006	0.006	1.302
P value		NS	NS	NS	NS

NS- Non-significant

4. Conclusion

In conclusions, based on the present findings, rice based brewers dried grain can safely be incorporated into a layer diet at the inclusion level of 20% to inhance nutrients utilization and skeletal growth.

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