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Original Research Article

Effects of extruded rice bran based diets on the performance, intestinal microbiota and morphology of weaned pigs

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Abstract

Reducing the cost of production while maintaining or increasing the productivity has been a major challenge of pig industry in Nigeria. This gives rise to the use of processing techniques that improve the utilization of relatively cheap feed ingredients. Extrusion cooking process has the potential of improving the digestibility and absorption of rice bran by solubilizing high fibre, deactivating anti-nutritional factors and stabilizing lipolytic enzyme activities in rice bran. This study was conducted to evaluate the effects of extruded rice bran based diets on the performance, intestinal microbiota and morphology of weaned pigs. Forty-eight female Large White × Landrace weaned pigs were randomly allotted to three treatments consisting of a control (T1) with 30% inclusion level of raw rice bran; T2 with 30% inclusion level of extruded rice bran extruded at 100 °C, T3 with 30% inclusion level of extruded rice bran extruded at 120 °C. The final body weight, weight gain, and feed conversion ratio were significantly ($P < 0.05$) higher in pigs on T3 compared with the control group. Morphology data for the small intestine showed that T3 had higher villi (604.15 µm 10^{-6}) and crypt depth (153.86 µm 10^{-6}) in comparison with the other treatments. Extrusion cooking processing techniques had a positive impact on body weight gain and feed conversion ratio of the experimental animals as well as improved their intestinal microbiota and morphology.

Keywords: animal nutrition; rice waste; improved animal production; cost effectiveness; *E. coli; Salmonella* spp.

INTRODUCTION

The pig industry is one of the most lucrative sectors of animal production in Nigeria. However, religious practices and high cost of production have been major limitations for pig production in the country (Tewe 1997; Babayemi and Bankole 2009). These have limited the production of pigs in the large scale. To reduce costs, the use of alternative and inexpensive feed ingredients is necessary (Babayemi and Bankole 2009). Rice bran, a relatively inexpensive feed ingredient is a by-product of the rice milling industry which has the potential of being used as an alternative to grains in pig production. It contains 15 – 22% oil, 11 – 17% protein, 6 – 14% fibre, 10 – 15% moisture, and 8 – 17% ash (Sharif et al. 2014).

The factors inhibiting the efficient and effective utilization of rice bran include; presence of anti-nutritional factor such as phytic acid (Farrell 1998), trypsin inhibitor and haemagglutinin (Benedito and Barber 1978), high oil content and lipolytic enzymes, which predisposes it to being easily oxidized during storage at room temperature (Linfield 1985), as well as high fibre content (Ersin et al. 2006). Chae and Lee (2002) reported that feeding rancid rice bran led to decreased growth performance in pigs. Adebiyi et al. (2014) noted that a good gut health contributes to an optimal performance in animals.

Extrusion cooking is a high-temperature and short time thermal/mechanical pre-treatment feed and feedstuffs processing technique, which involves applying heat treatment in the presence of moisture. It is an effective processing method causing physico-chemical and nutritional modifications of the feed constituents, such as permanently denaturing lipases (Ramezanzadeh et al. 1999). Extrusion cooking is also effective in stabilizing oxidative rancidity in rice bran (Shin et al. 1997). This has both economic and environmental importance as this can lower production costs and gives an opportunity for the conversion of a low-quality agricultural by-product to a high value feed source. The use of agricultural wastes and by-products as animal feeds in order to reduce cost of production and as an effective means of handling their disposal is encouraged (Adejumo et al. 2017a; Adejumo et al. 2017b; Adetunji and Adejumo 2017). The temperature reached by the feed during extrusion cooking can be as high as 200 °C but the exposure time at this elevated temperature is very short (5 s to 10 s). For this reason, extrusion cooking processes are often called HTST (High Temperature / Short Time) (Harper 1979).

The use of 10%, 20% and 30% extruded rice bran in broiler diets, compared with raw and roasted rice bran, increased weight gain and feed consumption (Mujahid et al. 2004). Similarly, previous research indicated that feeding of extruded rice (cooked rice) had the ability to protect young pigs against diarrhoea, increase CATTD (coefficient of total tract apparent digestibility) and average daily gain (ADG) (Mathews et al. 1999; Pluske et al. 2003; Mateos et al. 2006). Diarrhoea is often experienced by piglets after weaning and it is a multifactorial condition associated with proliferation of *ß*-haemolytic strains of *Escherichia coli* in the small and large intestine (Hampson, 1994). Although previous studies reported the beneficial effects of extrusion cooking process of rice bran on the performance of monogastric animals (chicks, broilers and pigs; Mateos et al. 2006; Vicente et al. 2008), the adequate temperature of extrusion of rice bran for optimal performance is yet to be substantiated. We hypothesised that processing of rice bran and inclusion in pig nutrition could help reduce the public health implications that could arise from indiscriminate disposal of these wastes, improve animal performance as well as reducing the cost of animal production. Therefore, this study was aimed at assessing the effect of 30% inclusion of raw, 100 °C and 120 °C extruded rice bran in weaner piglet diet on their growth performance and intestinal conditions.

MATERIALS AND METHODS

The experiment was carried out at the Piggery Unit of Teaching and Research Farm, University of Ibadan, Nigeria.

Processing of extruded rice bran

Freshly milled rice bran (of unknown variety) was procured once from a commercial feed miller in Bodija Market in Ibadan, Nigeria. A locally fabricated single-screwed extruder with a 20 mm die and a 305 rpm screw speed was used to extrude the material. It was subjected to a regulated high temperature of 100 °C and 120 °C for 15 seconds. The material was then allowed to cool to room temperature.

Experimental animals and diets

Forty-eight healthy female (Large White × Landrace) 8 weeks old weaned pigs were purchased from a reputable (farm with adequate record keeping) source. The sample size was determined using the formula, sample size = [confidence level of 95% multiplied by standard deviation of the outcome interest]^2/error unit (Sullivan, 2018). The result was however narrowed down to 16 animals per treatment owing to budgetary consideration and available animals of similar status in the herd. All female animals were used to avoid sex variation in the observation. The pigs were randomly (through numbers from a hat) allotted to three dietary treatments with four replicates per treatment and four pigs per replicate in a completely randomized design (Adeniji 2008). The extruded rice bran was formulated with other ingredients to meet the nutritional requirement of the animals. The proximate analysis of the test ingredients was carried out at the Agricultural Biochemistry and Nutrition Laboratory, Department of Animal Science, University of Ibadan, Ibadan. The pigs were fed *ad libitum*. The animals were housed according to groups and were fed twice a day (Giang et al. 2004; Kaensombath et al. 2013). The study lasted for seven weeks. The experimental diets were:

Treatment 1 (Control): 30% incorporation of raw rice bran

Treatment 2: 30% incorporation of 100 °C extruded rice bran

Treatment3: 30% incorporation of 120 °C extruded rice bran.

Data collection

At the beginning of the experiment, the pigs were weighed and subsequently weighed weekly. Feed intake was recorded daily (feed offered – feed left over); feed conversion ratio (FCR) was calculated as quantity of feed consumed per unit weight gained over the same period. The animals were weighed weekly using DV-201-pig weighing crate (Danvaegt, Denmark), accurate to 1.0 kg (Carter et al. 2017). The pigs were labelled with indellible marker but the initial status of the intestinal and external parasites was not known. However, the pigs were treated with levamisole and ivermectin against internal and external parasite before the commencement of the experiment.

Microbial load and intestinal morphology

The total microbial count, *E. coli* and *Salmonella* spp. were cultured from fresh pig intestinal contents after 49 days of feeding. The digests were serially diluted according to the procedure described by De Man et al. (1960). The bacterial counts were determined using the pour plate technique. The agar plates were allowed to solidify, were incubated at 37 °C for 24 h and the bacterial colonies were counted in plates using

Table 1. Ingredient composition of experimental diets

Ingredients	RRB diet(g/100g)	ERB at 100 °C (g/100g)	ERB diet at 120 °C (g/100)
Maize	20.70	20.70	20.70
Rice bran	30.00	30.00	30.00
Groundnut cake	15.00	15.00	15.00
Palm kernel cake	30.00	30.00	30.00
Bone meal	1.50	1.50	1.50
Limestone	1.50	1.50	1.50
Premix	0.50	0.50	0.50
Salt	0.80	0.80	0.80
Total	100.00	100.00	100.00
Calculated composition			
Dry matter (%)	85.42	85.42	85.42
Energy (kcal/kg)	2617.34	2617.34	2617.34
Crude protein (%)	17.82	17.82	17.82
Calcium (%)	1.20	1.20	1.20
Phosphorus (%)	1.28	1.28	1.28
Crude fibre (%)	8.5	8.5	8.5
Proximate composition of test ingredients on dry matter basis			
Nutrients (g/100g)	**RRB**	**ERB (100 °C)**	**ERB (120 °C)**
Dry matter	86.00	92.10	92.89
Protein	6.88	5.92	5.67
Ash	7.18	7.02	7.05
Fibre	26.70	24.51	24.32
Ether extract	3.19	3.10	3.20

RRB = Raw rice bran; ERB = Extruded rice bran

an optical counter. For morphology, samples of ileum from six pigs per treatment (two animals per replicate) were collected after euthanasia. About a 15 cm segment was cut from the ileum and was placed in 10% buffered formalin. Measurements of crypt depth, villus height, thickness of *tunica muscularis* and goblet cells were determined from the tissues.

Experimental design and statistical analysis

The experimental design was a completely randomized design while all the data were analysed using one-way analysis of variance of SAS (Adeniji 2008; SAS, 2012; Nguye et al. 2012). Significant means were separated using Duncan's Multiple Range Test of same software.

RESULTS

Performance characteristics of pigs fed extruded rice bran based diet

The performance indicators of the experimental animals are presented in Table 2. Feed intake showed no significant difference while the final body weight, body weight gain and the feed conversion ratio were significantly ($P < 0.05$) different. Extruded rice bran diets resulted in higher final weight and body weight gain than the control group. The FCR was also lower

Table 2. Performance of weaner pigs fed extruded rice bran based diet

Indicator	T1	T2	T3	SEM	P-value
Initial body weight (kg)	18.25	19.42	19.94	0.13	0.1906
Final body weight (kg)	11.87^{c}	12.47^{b}	12.93^{a}	0.05	0.0001
Weight gain (kg)	6.38^{b}	6.95^{a}	7.01^{a}	0.11	0.0143
Feed intake (kg)	24.50	25.04	24.67	0.19	0.2024
FCR	3.85^{a}	3.60^{b}	3.52^{b}	0.06	0.0228

Mean with different superscripts within the same row are significantly different. $P = 0.05$.
T1 = Diet + Raw rice bran diet (g/100); T2 = Diet + 100 °C extruded rice bran diet (g/100 g); T3 = Diet+ 120 °C extruded rice bran diet (g/100g); SEM: Standard error of mean

Table 3. Microbial count of weaner pigs fed extruded rice bran based diet

Bacteria (CFU×10^5/ml)	T1	T2	T3	SEM	P- value
E. coli	6.63^a	5.72^b	5.40^b	0.01	0.0024
Salmonella	6.81^a	6.03^{ab}	4.94^b	0.04	0.0469

T1 = Diet + Raw rice bran diet (g/100); T2 = Diet + 100 °C extruded rice bran diet (g/100 g);
T3 = Diet + 120 °C extruded rice bran diet (g/100 g); SEM: Standard error of mean

Table 4. Gut Morphology of weaner pig fed extruded rice bran based diet

Parameter (µm (10^{-6}))	T1	T2	T3	SEM	P-value
Villus height	486.68^b	500.36^b	604.15^a	11.17	0.0006
Crypt depth	128.94^b	144.89^a	153.86^a	4.19	0.0154
Muscular thickness	614.38^a	452.71^b	392.78^c	13.34	0.0001
Villus width	154.28	192.05	191.03	14.50	0.1917

Means with different superscripts within the same row are significant different. *P* = 0.05,
T1 = Diet + Raw rice bran diet (g/100); T2 = Diet + 100 °C extruded rice bran diet (g/100 g);
T3 = Diet + 120 °C extruded rice bran diet (g/100 g); SEM = Standard error of mean

for extruded rice bran diets. For feed intake, T2 had a significantly higher value compared to T1 and T3, respectively. The highest value for final body weight was observed at T3. Consequently, T3 had the highest value for weight gain. The FCR also had the highest value in T1 compared to T2 and T3.

Microbial count of weaner pig fed extruded rice bran based diet

Table 3 presents the bacteria count (CFU×10^5/ml) for *Escherichia coli* and *Salmonella* spp. For *E. coli*, significant differences were observed between T1, T2 and T3 while T2 and T3 were not different. Microbial count for *Salmonella* spp. also showed a significant difference between T1 and T3 with no difference between T2 and the other treatments. Consequently, *Salmonella* spp. observed the highest count in T1. *E coli* observed the highest count in TI.

Gut morphology of weaner pig fed extruded rice bran based diet

The result from Table 4 on gut morphology of weaner pigs fed extruded rice bran based diet showed that the indicators measured were significantly different except for villus width where no difference was observed among the treatments. The values observed for villus height ranged from 486.68 µm 10^{-6} (T1) to 604.15 µm 10^{-6} (T3). Crypt depth was significantly different, with values ranging from 128.94 µm 10^{-6} (T1) and 153.86 µm 10^{-6} (T3), tunica muscularis thickness had a mean range value of 392.78 to 614.38 (µm 10^{-6}) and the villus width had a mean range value of 154.28 to 191.03 (µm 10^{-6}). The values obtained for villus height for 100 °C extruded rice bran and the control diet were similar. Pigs on 120 °C extruded rice bran had the highest values. Crypt depth was higher for pigs fed extruded rice bran, but extruded rice bran resulted in lowered tunica muscular thickness.

DISCUSSION

Extrusion processing of rice bran at 100 °C and 120 °C had a significant effect on weight gain and feed conversion ratio of weaner pigs when compared to those fed raw rice bran. This could be a result of the improvement in the crude protein, increased fibre digestibility as well as increased villus height and crypt depth which might have increased the nutrient absorption. This is in line with the findings of Zare-Sheibani et al. (2015) who reported that birds feeding on 20% and 30% extruded rice bran had a higher body weight gain and lower feed conversion ratio. Rice bran is easily oxidized during storage (Linfield et al. 1985). It was reported that feeding rancid rice bran can be a cause of digestive disorders (Yokochi 1972). Rancidity also reduces palatability of feeds (Godber et al. 1993). Extrusion process has been observed to inactivate the enzymes and thereby prevent rancidity. This might have accounted for the reduced feed intake of our weaner pigs fed raw rice bran compared to those fed extruded rice bran at 120 °C which agrees with the findings of Chae et al. (2002) who reported that feed intake was reduced in broiler chicks fed rice bran compared to control broilers fed defatted or extruded rice bran. It may be said that extrusion of rice bran ameliorates reduced palatability of rice bran caused by rancidity and thus, improves weight gain and feed conversion ratio.

Giang et al. (2004) reported that processing of sweet potato as partial replacement for rice bran in pigs did not improve average daily gain and feed conversion ratio of the animals when compared with the control group with rice bran. However, diets with sweet potato meal and sweet potato silage at 40% inclusion level resulted in higher average daily gain and better feed conversion ratio compared to diets with sweet potato meal and sweet potato silage at 60% inclusion rate. The results of the study conducted by Nguyen et al. (2012) revealed a significantly improved pig performance and protein gain by extra methionine supplementation of 0.2% lysine +0.1% dl-methionine and 0.1% lysine + 0.05% dl-methionine in the pig diets containing ensiled cassava leaves as the main protein source with synthetic amino acids, dl-methionine alone or with L-lysine.

The gut microbial counts for *Escherichia coli* and *Salmonella* spp. in weaner pigs fed raw and at 100 °C and 120 °C extruded rice bran were significantly affected. Weaning is a major critical period of pig rearing because of increased susceptibility to gut disorders, infections and diarrhoea. Weaning involves complex psychological, social, environmental and dietary stresses that interfere with gut development and adaptation. The immediate effect of weaning is a dramatic reduction in feed (energy) intake leading to undernutrition and a transient growth check. Intestinal alterations often observed in post-weaning piglets include changes in villus/crypt morphology and in brush border enzyme activities and implication of enteric pathogens (*Escherichia coli* and rotaviruses) have also been addressed (Pluske et al. 1997). In a research conducted by Mohana Devi et al. (2015) on weanling pigs, it was reported that lower incidence of diarrhoea documented might be due to fewer viable *E. coli* in the intestine, which made the authors to conclude that extruded rice can lower viable *E. coli* counts in the intestine and decrease the incidence of diarrhoea. Furthermore, it has been reported that feeding cooked rice might protect young pigs against diarrhoea, increase coefficient of apparent total tract digestibility (CATTD) and average daily gain (ADG) (Mathews et al. 1999; Pluske et al. 2003; Mateos et al. 2006; Vicente et al. 2008). The result obtained in the present study may be attributed to the effect of the high temperature from the extrusion process on the bacteria count.

Taller villi indicate more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus. It has been demonstrated that villus height and crypt depth are a direct representation of the gut function and health. Uni et al. (1995) suggested that development of intestinal morphology could reflect the health status of the GI tract of an animal. New epithelial cells are produced in the intestinal mucosal crypts and migrate along with the villi to the top. The crypt depth showed corresponding increase as the villus increases because long villi indicate a faster multiplication of the base of the crypt which migrates faster to the tip of villi (Nordstorm and Dahlqvist 1973). The crypt can be regarded as the villus factory, and a large crypt may be an indication of a fast tissue turnover and a high demand for new tissue (Yason et al. 1987). A decrease in either villus height or crypt may lead to a reduction in nutrient absorption. The results presented in the present study revealed that on 120 °C extruded rice bran based diets had significantly higher villi and crypt depth, which signifies a positive effect of extrusion on the gut integrity of the pigs as compared to those pigs on raw rice bran diet. This is in agreement with Foltyn et al. (2013) who observed increased villus height and crypt depth with increasing level of extruded diets in broilers. These findings are consistent with the growth performance of the birds, which indicates an established relationship between gut health and good growth performance.

CONCLUSION

Results of the present study confirmed the potential of extrusion treatment as an efficient processing technology in increasing the quality of rice bran for swine nutrition. Extruded rice bran at 120 °C up to 30% inclusion improved weaner pigs' performance in terms of body weight gain, and feed conversion efficiency. It is evident from this study that the digestibility and absorption of rice bran by weaner pigs can be improved by extrusion techniques at 100 °C and 120 °C as a result of an improvement in the intestinal morphology. The results of this study also show that extruded rice bran at 100 °C and 120 °C lowered viable intestinal *E. coli* counts, which may decrease the incidence of diarrhoea in post-weanling pigs. Further investigation on the effects of extruded rice bran based diets on the intestinal morphology and microbiota of weaned pigs is recommended.

ETHICS APPROVAL

The feeding trial was approved by Animal Care Ethics Committee, Department of Animal Science, University of Ibadan, Nigeria, and the experiment was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

CONFLICTING INTERESTS

We declare that we have no conflicting interest regarding this manuscript.

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