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Effect of enzyme supplementation on the digestibility, blood biochemical and performance of crossbred gilts fed diets with increasing levels of rice bran

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Rice bran, the main byproduct of rice milling, is abundantly available in South-East Asian countries, including India and serves as a cheap source of energy in the feeding of livestock, particularly pigs. Higher levels of rice bran in diets of growing pigs lowered digestibility and performance (Soren *et al*. 2003, and Thirumurugan *et al*. 2008). Non-ruminants do not have fibre degrading enzymes hence digestibility of rice bran based diets, which is rich in fibre i.e. non-starch polysaccharides (NSP), cell wall and pentosans (Wang *et al*. 2008), is lower than the grain-based diets. Moreover, NSPs exhibited anti-nutritive activity, which may negatively affect the performance of pigs, when present in large quantity in the diets (Kerr and Shurson 2013). Therefore the use of exogenous fibre degrading enzymes may be an alternative means of improving the nutritional quality of feedstuffs containing higher level of NSP (Kerr and Shurson 2013).

Microbial enzymes are used as feed additives for many years in the animal feed industry (Wenk 2000). Supplementing enzymes in wheat bran and oat-based diets resulted in higher xylose and arabinose concentration and increased volatile fatty acid (VFA) concentration in the ileum of pig (O'Shea *et al*. 2010). Rice bran inclusion at higher level in the diet reportedly affected estrus in gilts (Soren *et al*. 2003). Restricting energy intake resulted in delayed puberty attainment and estrus in gilts (van Wettere *et al*. 2011). The hypothesis is that addition of non-starch polysaccharides degrading enzymes might be beneficial to improve the blood glucose level with the reduction of blood urea level or in other words if there is any advantage of adding those enzymes would be reflected on blood biochemical parameters. Thus, the present experiment was conducted to elucidate the possibility of improving growth and reproductive performances of Indian crossbred pigs fed on increasing level of RB-based diets by addition of feed grade enzymes.

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For the present study, 3 diets were formulated with different levels of rice bran (Table 1). Diet RB0 served as the control diet and all the diets contained similar concentration of CP. Commercial feed grade enzymes premixes (E) containing cellulase, ß-glucosidase, xylanase, α-amylase and protease with enzyme activities 1071.6 U, 469.1 U, 2316.7 U, 3274.33 U, and 114.5 U/g, respectively, were supplemented at 0.5 g/kg concentrate mixture.

For determining the enzyme activity in enzyme

Table 1. Ingredients and chemical composition (g/ kg DM basis) of the experimental diets

Ingredients	Diets		
	RB0	RB1	RB2
Ingredient composition			
Maize	350	175	0
Wheat bran	470	235	0
Rice bran	0	410	820
Soybean meal	100	100	100
Fishmeal	60	60	60
Mineral mixture*	15	15	15
Salt	5	5	5
Vitablend AD_3** (g/100 kg)	15	15	15
Chemical composition			
Organic matter	920	890	860
Crude protein	190	180	170
Ether extract	30	70	90
Crude fibre	60	120	160
Nitrogen free extract	640	530	440
Total ash	80	100	140
Acid insoluble ash	10	40	60
Digestible energy (kJ/kg)+	14.154	14.339	14.477
Digestible energy (kJ/kg)++	14.167	11.351	9.899

*Composition of mineral mixture: Calcium, 280 g/kg, phosphorus, 120 g/kg, iodine (as potassium iodide), 0.001 g/kg, copper, 0.0013 g/kg; dietary treatments RB0, RB1 and RB2 contain rice bran 0.0, 410 and 820 g/kg, respectively. ** Vitablend AD_3 contained vitamin A,7500 IU, vitamin D_3, 750 IU. Lysine, methionine, methionine + cystine and threonine content of the diet were 8.6, 3.2, 6.4 and 6.1 g/kg in RB0, 9.3, 3.4, 6.3 and 6.7 g/kg in RB1 and 10, 3.5, 7.3 and 7.4 g/kg in RB2, respectively.

+Calculated; ++ Estimated.

premixes, 2 g of enzyme (E) sample was weighed into a 100 ml beaker; and 30 ml of buffer (phosphate buffer, pH 6.8) was added. It was dissolved with the help of a magnetic stirrer at 4°C, centrifuged and the supernatant was used for further enzyme activity analysis. Carboxymethyl cellulase (EC 3.2.1.4; endo- 1, 4-ß-glucanase) activity was determined (Miller 1959) with slight modification (pH adjusted to 6.8). The enzyme activity (U/g) was defined as nano mole of glucose formed/ minute. Xylanase (EC 3.2.1.8; endo- 1,4-ß-xylanase) activity was determined (Miller 1959) and one xylanase unit was defined as the nano mol xylose formed/ minute. The activity of ß- Glucosidase (EC 3.2.1.21; ß-D-glucoside glucohydrolase) was assessed by incubating p-nitrophenol ß-D- glucopyranoside as a substrate (Shewale and Sadana 1978), enzyme activity was expressed as nano-mole p-nitrophenol released/minute. Enzyme activity of α-amylase (EC 3.2.1.1; 1,4-α-glucanohydroxylase) was assessed by measuring the rate of releasing of reducing sugar during the incubation of substrate and the enzyme activity was expressed as nano-mole reducing sugar released/min. Protease activity was determined by azocasein method (Sastry *et al*. 1999) and one protease unit was expressed as mg hydrolyzed protein/ minute.

Crossbred (Landrace × local Indian) gilts (54) of 26.38±0.85 kg body weight and 25 weeks of age were selected and randomly divided into 6 groups in a completely randomized design in such a way that each treatment had three replicates of three animals each. The gilts in each replicate were housed separately in cemented-floor pens with arrangement for feeding, watering and a run. Each group was assigned to 1 of the 6 dietary treatments. The pigs were de-wormed and vaccinated as per the normal schedule of the farm. The diets were offered once daily in the morning in mesh form, with free access to *ad lib.* drinking water. Group feeding was employed for all the animals. Recording of feed offered and residues left were maintained daily for 112 days. The gilts were weighed individually at fortnightly intervals before offering feed in the morning. On attainment of 31 weeks of age, heat detection was started in all the gilts twice daily at 9.00 AM and 5.00 PM using a teaser boar for 90 days. Duration of oestrous cycle (days) was measured by recording the intervals (days) between 2 successive estrous. Oestrous duration (h) was also recorded whenever the gilts exhibited heat symptoms. The effect of the respective diet on duration of oestrous cycle (days), duration of estrous (h), heat symptoms and regularity of oestrous was studied for 3 consecutive cycles. Symptoms like swelling and reddening of the vulva, discharges of mucous, standing heat reflex, cock ear, etc. were considered for studying heat symptoms.

At the end of the experimental feeding, a metabolism trial of 6 days collection period, preceded by 7 days adaptation period to metabolism cages was carried out on 5 representative gilts per treatments (30) to study nutrient utilization. Daily feed offered, residues left and faeces and urine excreted was recorded on 24 h basis. Besides feeds and residues, an aliquot of faeces were dried at 100±1°C for 24 h for the estimation of DM. The dry samples were pooled for proximate analysis. Day-to-day fresh samples of faeces was preserved with 1: 4 sulphuric acid for nitrogen determination. Blood samples were collected initially and after 60 days of feeding. It was collected in the morning, 2h before feeding from 5 representative gilts in each treatment groups from the anterior venecava and serum was separated by centrifugation. The serum obtained was used for determination of glucose (Hultmann 1959), total protein (Doumas 1975) and albumin (Doumas *et al*. 1971). Serum globulin was obtained by subtraction of albumin from total serum protein. Serum urea (Wybenga *et al*. 1971) and cholesterol was estimated (Wybenga *et al*. 1970). All these parameters were estimated as per standard colorimetric methods using commercial reagent kits using a spectrophotometer.

Proximate principles of the feeds and faeces, viz. crude protein, ether extract, crude fibre and ash were analyzed (AOAC 1995). Gross energy (GE) content of the rations and faeces was determined by ballistic bomb calorimeter (CBB 330) using thermo-chemical grade benzoic acid (Soren *et al*. 2004). The data were subjected to one-way analysis of variance for all the parameters to ascertain the effect of rice bran and enzyme supplementation on the digestibility, blood parameter and performance of the gilts (Snedecor and Cochran 1989).

Ingredient and chemical composition of the experimental diets are presented in Table 1. With the increase in rice bran (RB), the CF content of the diet increased by 45% and 60% while the ether extracts content also increased by 60% and 69% in RB1 and RB2, respectively, due to higher oil content of the RB. Total ash and acid insoluble ash (AIA) contents of the diets also increased by 28 and 42% in RB1 and 72 and 81% in RB2, respectively. The CP contents of the diets were almost similar in all the groups. Calculated digestible energy of the experimental diets were 14.154, 14.339 and 14.477 MJ/kg but on actual estimation the energy was found to be diluted to 14.167, 11.351 and 9.899 MJ/kg for RB0, RB1 and RB2, respectively. The lower body weight in RB2 was accompanied with reduction in feed intake. The average daily gain (ADG) was similar for RB0, RB0E, RB1and RB1E group while it was lower (P<0.001) in RB2 and RB2E groups. The gilts fed 410 g/kg rice-bran based diets were able to maintain live weight gain similar to that of control (Table 2). But gilts in RB2 and RB2E groups failed (P<0.01) to maintain their body weight. Feed or DM intake was similar in RB0, RB0E, RB1 and RB1E groups but the group fed rice bran at 820 g/kg consumed less (P<0.01) feed/DM in comparison to those in RB0 and RB1. Therefore, the lower body weight in RB2 and RB2E was accompanied with reduction in feed intake.

Feed conversion efficiency was lowest (P=0.007) in RB2 and RB2E group (Table 2). The efficiency of protein and energy utilization did not differ due to feed enzyme supplementation. Crude protein intake per unit gain was highest (P=0.028) in 820 g/kg RB fed gilts, and enzyme supplementation did not further improve its efficiency. The

Table 2. Growth, dry matter intake, daily gain and nutrient conversion efficiency (kg/MJ intake per kg gain)

Parameters	Treatments							
	RB0	RB0E	RB1	RB1E	RB2	RB2E	SEM	P-value
Body weight (kg)								
Initial	26.18	26.33	25.76	26.48	25.88	26.03	0.840	0.994
Final	66.6a	63.9a	60.8a	63.9a	50.5b	49.8b	1.60	0.002
Average daily gain (g)	361a	336a	313a	333a	220b	216b	10.6	<0.001
Dry matter intake (kg/ day)	1.7a	1.58ab	1.6a	1.7a	1.3bc	1.27c	0.05	0.021
Feed intake (kg/day)	1.9a	1.8ab	1.8ab	1.9a	1.5c	1.4bc	0.06	0.017
Feed: gain	5.2b	5.4b	5.7b	5.7b	6.7a	6.7a	0.17	0.007
CP: gain	0.87b	0.91b	0.95ab	0.94ab	1.08a	1.09a	0.025	0.028
DCP: gain	0.60	0.63	0.61	0.58	0.66	0.62	0.012	0.485
DE: gain	15.5	16.4	14.3	13.9	14.3	14.4	0.32	0.196
ME: gain	14.5	15.3	14.6	13.2	13.5	12.9	0.31	0.181
TDN: gain	3.2	3.4	3.2	3.1	3.2	3.2	0.06	0.869

[a,b,c] Means with different letters in a row differ significantly, SEM: standard error of the means; RB, rice-bran; E,enzyme; Dietary treatments RB0¸ RB1 and RB2 contain rice bran 0.0, 410 and 820 g/kg, respectively. Enzyme supplementation @ 0.5 g/kg.

Table 3. Effect of enzyme supplementation on the total tract apparent digestibility (%)

Parameters	Treatments							
	RB0	RB0E	RB1	RB1E	RB2	RB2E	SEM	P-value
Dry matter	69.81a	71.45a	55.83b	55.08b	44.17c	44.08c	2.357	<0.001
Organic matter	73.67a	74.26a	60.63b	59.59b	47.03c	48.21c	2.343	<0.001
Crude protein	70.01a	70.07a	64.90ab	61.70b	64.03ab	59.73b	1.119	0.014
Ether extract	70.14	73.41	77.81	71.33	79.03	79.01	1.498	0.328
Crude fibre	30.73a	31.43a	19.67bc	23.38b	19.21c	18.78c	1.202	<0.001
Nitrogen free extract	79.70a	80.33a	68.26b	67.64b	51.42c	53.29c	2.409	<0.001
Total carbohydrates	74.75a	75.41a	57.49b	57.65b	39.51c	40.13c	3.052	<0.001

[a,b,c] Means with different letters in a row differ significantly; SEM: standard error of the means; RB, rice-bran; E, enzyme; Dietary treatments RB0¸ RB1 and RB2 contain rice bran 0.0, 410 and 820 g/kg, respectively. Enzyme supplementation @ 0.5 g/kg.

conversion efficiency of other nutrients (DCP, ME and TDN) was similar among the gilts fed different diets. Increasing level of RB depressed (P<0.001) the digestibility of all the nutrients except EE, which was found to be similar in all the dietary treatments (Table 3). The CP digestibility was similar in RB1, RB1E, RB2 and RB2E diets while it was higher in RB0 (control) diet. Increasing trend in the digestibility of ether extract in RB2 reflected the better quality of fat (high oleic and linoleic acid) in RB (Warren and Farrell 1990). Enzyme supplementation could not further improve the digestibility of DM, OM or fibre in the present study. Our findings are contrary to the earlier report (Alu *et al*. 2009, and Ngoc *et al*. 2011), where enzyme supplementation improved digestibility of nutrients in high fibrous diets. However, enzyme supplementation in diet with 410 g/kg rice bran showed only 15% improvement in CF digestibility vis-à-vis un-supplemented diet.

Although, the overall duration (days) of estrous was similar in all the dietary treatments, but significant (P<0.05) effect of enzyme was observed during the third oestrus cycle (Table 4). Inclusion of RB at higher level affected the growth, sexual maturity and hormonal profile leading to irregular estrus (Dyck 1988, Soren *et al*. 2003). Oestrus duration (hours) in all the oestrus cycles significantly (P<0.05) decreased on enzyme supplementation. Blood glucose level decreased (P<0.001) due to inclusion of graded levels of rice-bran, but enzyme supplementation increased (P<0.001) its level. Lower glucose level with the increased level of rice-bran was due to a decrease in nitrogen-free extract content. Enzyme supplementation improved the blood glucose level and the effect of enzyme was evident in RB2 diet. A higher VFA concentration with more propionate level in small intestine (Haberer *et al*. 1999) have been reported in growing pigs fed high fibrous diet with NSP degrading enzymes supplementation. Pigs fed rice bran exhibited higher serum urea levels (P<0.001) indicating increased protein breakdown (Soren *et al*. 2003), but enzyme supplementation lowered (P<0.001) serum urea levels in both 410 and 820 g/kg RB fed groups, indicating better utilization of protein in high fibrous diets (Yin *et al*. 2001). The cholesterol lowering effect of RB was seen in RB1 group where rice-bran replaced maize by 50%.

Table 4. Effect of enzyme supplementation on reproductive performance of crossbred gilts

Parameters	Treatments							
	RB0	RB0E	RB1	RB1E	RB2	RB2E	SEM	P-value
Estrus duration (days)								
Oestrous-1	20.3	19.8	18.3	21.9	21.1	20.2	0.69	0.782
Oestrous-2	19.8	18.9	18.1	18.9	20.0	19.6	0.29	0.439
Oestrous-3	18.8	19.2	18.3	19.6	16.4	20.1	0.42	0.158
Overall	19.6	19.3	18.2	20.3	19.2	20.0	0.35	0.642
Oestrus duration (hours)								
Oestrous-1	70.7a	48.0b	64.0ab	49.3b	54.7ab	50.7b	2.76	0.096
Oestrous-2	73.3a	53.3b	62.7ab	50.7b	50.7b	48.0b	2.59	0.030
Oestrous-3	77.3a	49.3bc	65.3ab	58.7abc	53.3bc	41.3c	3.00	0.006
Overall	73.7a	50.2bc	64.0ab	52.9bc	52.9bc	46.7c	2.25	0.002

[a,b,c] Means with different letters in a row differ significantly; SEM: standard error of the means; RB,rice bran; E,enzyme; Dietary treatments RB 0, RB1 and RB2 contain rice bran 0.0, 410 and 820 g/kg, respectively. Enzyme supplementation @ 0.5 g/kg.

Table 5. Effect of increasing level of rice-bran and enzyme supplementation on blood biochemical of gilts

Parameters	Treatments							
	RB0	RB0E	RB1	RB1E	RB2	RB2E	SEM	P-value
Glucose (mg/100 ml)								
Initial	101.4	102.5	105.7	108.1	100.0	104.6	0.89	0.060
Final	109.0ab	119.8a	107.7ab	101.3b	70.7c	116.7a	4.17	<0.001
Cholesterol (mg/100 ml)								
Initial	103.4	102.5	104.3	103.0	106.0	104.0	0.47	0.372
Final	137.3a	132.2b	99.9c	100.9c	135.0ab	138.2a	4.08	<0.001
Total protein (g/100 ml)								
Initial	8.3	7.9	8.2	8.1	8.0	8.1	0.09	0.797
Final	8.2	8.2	8.5	8.8	8.3	8.5	0.10	0.492
Albumin (g/100 ml)								
Initial	4.0	3.9	4.0	3.9	3.8	4.1	0.04	0.271
Final	3.8b	4.1b	4.2b	4.2b	4.6a	4.2b	0.06	<0.001
Globulin (g/100 ml)								
Initial	4.3	4.0	4.3	4.1	4.2	4.0	0.09	0.934
Final	4.4	4.2	4.2	4.6	3.7	4.3	0.11	0.272
Urea (mg/100 ml)								
Initial	20.9	22.6	21.5	21.8	23.4	20.8	0.35	0.227
Final	21.2d	23.4c	30.6b	24.9c	33.6a	25.4c	1.05	<0.001

[a,b,c] Means with different letters in a row differ significantly; SEM: standard error of the means; RB, rice bran; E,enzyme; Dietary treatments RB 0, RB1 and RB2 contain rice bran 0.0, 410 and 820 g/kg, respectively. Enzyme supplementation @ 0.5 g/kg.

Meager improvement in the performance of crossbred gilts by the supplementation of commercial feed grade enzymes in rice-bran based high fibre diets is suggestive of inadequate/insufficient enzyme activity for effective hydrolysis of dietary fibres and their subsequent release to simple sugar. Commercial enzyme preparations so far available in market are mostly fabricated for ß-glucans and arabinoxylans present in barley, oat, rye and wheat, and thus may not be applicable for other NSP compounds. Thus, it can be concluded that rice bran could safely be included to an extent of 410 g/kg in the diet of growing Indian crossbred pigs. Addition of commercial feed enzyme preparation in diets with rice bran was not beneficial. Further studies are needed to explore or fabricate the specific enzyme(s) formulae required for rice bran based diets and their effective application rate with respect to dietary level of rice bran.

SUMMARY

Influence of increasing levels (0.0–RB 0, 410 g/kg-RB1 and 820 g/kg-RB2) of rice bran (RB) along with or without feed enzyme (E) supplementation was studied for growth, nutrient utilization, blood biochemical and oestrous in crossbred gilts for 112 days. Pigs (54) were randomly divided into 6 groups and each treatment was replicated 3 times and 3 gilts were housed together in a pen to form the experimental unit. The enzyme complex contained cellulase, ß-glucosidase, xylanase, α-amylase and protease. Feed intake, growth rate, feed: gain was similar but decreased due to the increase in dietary level of rice bran in

RB2. DM intake, ADG and feed utilization efficiency was similar in the control and 410 g/kg rice bran incorporated diets, while it reduced significantly in RB2 diet in comparison to control. The digestibility of all the nutrients, except ether extract (EE) was reduced as the proportion of RB increased in the diets. The groups fed rice bran at higher level had lower serum glucose level than any other diets. Enzyme addition to RB2 improved blood glucose level than control. Enzyme supplementation decrease the duration of estrous (h) in gilts fed on RB based diets. It was concluded that addition of feed enzymes did not improve the performance of crossbred gilts fed on diets containing higher level of rice bran.

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