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Determination of Adequate Method for Protein Extraction from Rice Bran and the Substitution of Dried Skim Milk with Protein Concentrate from Rice Bran in Early Weaned Pigs

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ABSTRACT : The present study was conducted to determine a feasible method of protein concentrate extraction from rice bran (RBPC) and its effect as a substitution for skim milk in early weaning pig diets. An investigation to extract protein concentrate from full fat rice bran was undertaken to determine the best ratio of water and rice bran, the amount of NaOH and a HCl solvent to use in a simple paddle-type mixer with modified spinning to produce RBPC. The results stated that the best ratio for water mixing in the RBPC extraction process was 1:5 with 20 g NaOH and 30 min in a paddle-type mixer at 300 rpm. A mix of 250 ml 0.2 N HCl was optimum for neutralization and protein precipitation. After the fluid was spun out with a washing machine, the sediment was left for 12-14 hours to complete the filtration. One kilogram of rice bran could produce an average of 324.5 gram RBPC and it contained 3.40% ash, 496.48 kcal of GE/100 gram, 1.94% crude fiber, 28.20% ether extract, 7.64% moisture and 16.66% crude protein, respectively. A total of 45 crossbred piglets, weaned at 3 weeks of age were allotted into control diet (A) and dietary treatments formulated with a four different rates of RBPC substitution for skim milk at a percentage of 25 (B), 50 (C), 77 (D) and 100 (E) respectively, in a randomized complete block (RCB) design. All piglets had free access to feed and water until 8 week of age when the experiment ended. Feed intake, average daily gain, growth rate and feed efficiency were not affected by dietary treatments. Blood test parameters after completion of the growth trial indicated normal health. Even though the mean of cell hemoglobin concentration was significantly different between treatments ($p<0.05$) it was still within the normal range. The cost difference for BW gain of 100% RBPC substituted for skim milk in the weaning diet was approximately 35% lower than that of the control and the relative cost of production was 96.67, 92.85, 70.75 and 64.48% lower for the replacement of 25, 50, 75 and 100% of skim milk respectively. These results implied that this technology is feasible for use by small scale farmers to improve their self-reliance. (**Key Words :** Rice Bran Protein Concentrate, Early Weaning Pig Diet, Adequate Protein Extraction Method)

INTRODUCTION

Thailand is known as the main rice producer and exporter in the world. In the year 2010, rice in Thailand was harvested approximately 9.05 million ton, the co-/by products left such as broken rice, rice bran as well as hull are produced approximately 32.69 million ton after milling. Consequently these byproducts can be utilized as an ingredient of animal feed and some broken rice might be processed as flour and noodle for human consumption. Fresh or sole rice bran and extracted rice bran are mainly used as energy source in animal feed, however, currently rice bran oil after extraction is increasing popularity for human consumption and recognized to be a healthy products. The other nutritional quality of rice bran composed of approximately 12-15% protein and 3-4% lysine which is higher than that of rice endosperm protein or other cereal bran and legume, are still not fully use in animal feed. For a decade, about 80% of pigs in Thailand produced from intensive farming systems and 56% of these are from farms with over 1,000 pigs and was followed by small (50-200 pigs) and medium (201-1,000 pigs) farms. Large intensive farms are either integrated company owned (8.5%) or private independent (47.5%) farms (Cameron, 2000). Still, pig production in Thailand is being produced by large size farm, followed by medium size and the rest for small scale producer. As feed is known as the most variable expenditure in animal production, small farms may have high competitiveness because it is able to produce some feedstuffs by itself with lower production cost. To reduce

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the feed cost in mass production system or large scale farms, a complicate technology as well as a continuous processing with modern equipment should be utilized. In feed formulation for early weaning pig, skim milk was widely used because high activity of lactase can be maintained in gastrointestinal tract of post-weaning pig. However, because of expensive price of skim milk, many animal nutritionists tried to find out the alternative protein sources such as cooked soybean (Nongyao, 1974), steam and acid or base soaking (Uechiewchankit, 1975), germinated soybean (Tancho, 1988). Normally, new born piglets had their immunity by suckling colostrum which contained high levels of albumin and globulin. Coner et al. (1976) reported the nutritive value of rice bran protein concentrate (RBPC) extracted from fresh rice bran consisted of 24.7%, fat 36.4%, fiber 1.1%, ash 13.5% and starch 17.0% whereas those extracted from extracted rice bran had a value of 33.7, 8.2, 1.6, 17.0 and 25.5% respectively. Also, Juliano (1985) reported the RBPC had a similar protein percentage as in skim milk. A group of researchers (Bera and Mukherjee, 1989; Gnanasambandam and Hettiarachchy, 1995) was able to prepare rice bran protein concentrate/isolate and propose potential use as a food protein ingredient and also a weaning food. Therefore, RBPC had a potential to develop and substituted of skim milk in early weaning diet. The management of this technology could be access by develop the feasible method and manipulated the product as alternative protein at a lower cost in early weaning diet as well as serving the small scale farmer's self-reliance. The aims of this study were to investigate the appropriate method for rice bran extraction, and their affects to piglet performance when substitution at different levels for skim milk in the early weaning diet as well as cost return consideration.

MATERIAL AND METHODS

Investigation the proper condition for extraction of RBPC

Fresh rice bran/ full fat rice bran samples of long grain rice (*Oryza sativa* L., *indica* subspecies), obtained after milling from rice cultivated in the eastern-central region of Thailand, was used in this study. The extraction step is a modification of previous experiments (Gnanasambandam and Hettiarachchy, 1995; Jiamyangyuen et al., 2005) with process of soaking fresh RB in the water (1^{st}) and extracted with Sodium hydroxide at certain time (2^{nd}) and (3^{rd}) precipitate with hydrochloric acid solution. The modified process was conducted with a simple method and tools as an attempted to obtained RBPC with an appropriate technology for small scale farmer. The extraction step included the investigation the proper ratio of rice bran sample and water (1), the amount of sodium hydroxide (NaOH) used for facilitate the alkaline extraction (2) and the amount of hydrochloric acid (HCl) to adjust pH and precipitate (3) after the slurry was spun out. The stainless paddle-type mixer with 300 rpm was used to agitate the water-rice bran mixture, the filter bag made from four layers cloth with a linen in 2^{nd} and 3^{rd} layer and cheesecloth at inner and outer layers. The washing machine was modified to use as a spinner to flush out the slurry. To obtain the proper condition, a trial was set a mixture of rice bran:water ratio to 1:2.5, 1:5 or 1:10 w/v, with the different amount of NaOH (10, 20 or 30 g) tested the appropriate alkaline extraction. Then, the supernatant was adjusted with different amount of 0.2 N HCl of 200, 250 and 400 ml to test the better condition for neutralization. The precipitate was done by suspended overnight. The final product, which is called rice bran protein concentrate (RBPC), was then sundried for 2-3 d and the amount obtained was measured. The percent yield was calculated as follows;

$$\text{Percent yield} = \frac{\text{Weight of RBPC}}{\text{Weight of rice bran}} \times 100$$

The preliminary trial was performed in triplicate repeated combination. After selection the best condition indicated by percent yield, that process was repeated 10 times for a consistency. Then, RBPC was collected and analyzed for chemical composition (AOAC, 1990) by The Central Laboratory (Thailand) Co., Ltd. and expanding production for mixing in the dietary treatments for the weaning pig diets.

Experimental design, diet and measurements

A total of 45 crossbred piglets (Landrace×Duroc), weaned at 3 weeks of age with an average initial body weight of 7.09±0.07 kg were allotted into 5 treatments with 3 replicates and 4 animals per pen, according to randomized complete block (RCB) design. Five dietary treatments included; the control diet (A) and dietary treatments formulated with a four different rates of RBPC substitution on skim milk at a percentage of 25 (B), 50 (C), 77 (D) and 100 (E), respectively. The composition of ingredients in all dietary treatments was shown in Table 1. The nutrients in experimental diets were met animal's requirement as recommended and formulated by Suwanwajokkasikit Animal Research and Development Institute (SARDI), Kasetsart University, Thailand. All piglets were raised in concreted floor pen and openness housing. The piglet was free access to feed and water until 8 weeks of age. The body weight and feed intake were recorded at the beginning and the end of trial of 5 week duration. At the end of experiment, blood samples were collected from piglets' vena cava and taken for analyzing the blood profile to diagnose their health conditions by College of Veterinary Science's

Table 1. Feed ingredients and chemical composition of the control diet and different rate of RBPC substituted for skim milk diets in early weaning pig diets

	Rate of RBPC substitution (%)				
	A~0%	B~25%	C~50%	D~75%	E~100%
Broken rice	51.49	51.22	50.95	50.50	50.29
Rice bran, fine	5.00	5.00	5.00	5.00	5.00
Soybean meal	13.16	13.73	14.30	14.89	15.46
Extrude soy bean	13.00	13.00	13.00	13.00	13.00
Skimmilk	5.00	3.75	2.50	1.25	0.00
Tallow	2.50	2.20	2.00	1.70	1.40
RBPC*	0.00	1.25	2.50	3.75	5.00
Fish meal	7.00	7.00	7..00	7.00	7.00
DCP	2.00	2.00	2.00	2.00	2.00
Salt, NaCl	0.35	0.35	0.35	0.35	0.35
L-lysine	-	0.03	0.05	0.07	0.10
Vit-min premix[1]	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition[2] (%)					
Crude protein	21.00	21.00	21.00	21.00	21.00
Gross energy	3,408.54	3,407.47	3,406.4	3,409.86	3,408.78
Ether extract	6.61	6.65	6.70	6.84	6.88
Crude fiber	2.76	2.82	2.88	2.94	3.00
Calcium	1.10	1.08	1.07	1.05	1.04
Phosphorus, avai.	0.68	0.67	0.66	0.65	0.64
Lysine	1.26	1.24	1.22	1.20	1.17
Methionine	0.67	0.67	0.66	0.65	0.64
Cost (Baht/kg)*	24.31	21.89	19.54	19.32	15.03

* RBPC 23.56 Baht/kg (0.74$ approximately).

[1] Vit-min premix: provided per kg diet: Vitamin A, 16,000 IU; Vitamin D_3, 3,200 IU; Vitamin E, 35 IU; Vitamin K_3, 5 mg; Riboflavin, 6 mg; Calcium pantothenic acid, 16 mg; Niacin, 32 mg; d-Biotin, 128 μg; Vitamin B_{12}, 20 μg; Fe, 281 mg; Cu, 288 mg; Zn, 143 mg; Mn, 49 mg; I, 0.3 mg; Se, 0.3 mg.

[2] Calculated value.

laboratory, Kasetsart University at Kampangsaen. Analysis of variance (ANOVA) technique was used to compare mean differences of samples and a significance was set at $p<0.05$. If the difference in mean existed, multiple comparisons were performed using the Duncan's Multiple Range Test (Snedecor and Cochran, 1989). The price of each ingredient in dietary treatments was calculated based on local market price during March-April 2009, for the formula cost and the benefit of cost return.

RESULTS AND DISCUSSION

The effects of varying levels of water to bran ratio, amount of sodium hydroxide, and hydrochloric acid solution on protein extracted yield from fresh rice bran in the first trial are given in Table 2. The effect of 3 rice bran-water ratio (1:2.5, 1:5.0 or 1:10 w/v) on extracted amount from fresh rice bran (FRB) was 21%, 33.0% and 23.0%. The amount obtained from adding 10/20/30 g to 3 rice bran-water mixture ratio were 28.0, 32.0 and 32.0% respectively. Hydrochloric acid 0.2 N at 200, 250 and 400 ml could extract 22.0, 32.0 and 32.0% RBPC, respectively. Varying levels of water mixture ratio, amount of NaOH in facilitate alkaline extraction and quantity of HCl solution did not

Table 2. Comparison amount of RBPC obtained from several combination of the full fat rice bran-water ratio, amount of NaOH and HCl solution used for proper alkaline extraction

Items	RBPC (g/kgRB)
Full fat RB:water ratio	
1:2.5	210±10.0
1:5.0	330±10.0
1:10.0	230±10.0
NaOH (g)	
10	270±17.3
20	320± 20.0
30	320±17.3
HCl, 0.2 N (ml)	
100	220±10.0
250	320±20.0
400	320±10.0

have significant effects on protein extraction from FRB (p>0.05). Rice bran protein concentrate was extracted efficiently by treated combination ranging from 21.0 to 33.0%. However, the highest yield was observed using the ratio of rice bran and water 1:5, 20 g NaOH and 250 ml HCl, and selected to expand the production to mix in weaning diet. After repeated 10 times for consistency, this combination was followed by suspending overnight approximately 12-14 hrs before 2-3 d sundried. The average yield of 32.4% obtained in this study was similar with Connor et al. (1976) that could obtained 33-38% and 44% RBPC (Theerakulkiat et al., 2006) when rice bran was extracted with alkaline solution. In addition, they reported the extraction with extracted rice bran had a percentage of protein and yield more than from a full fat rice bran. Monkolkanchanasiri (2002) reported the best condition with ratio of RB and water as 1:4 w/v, pH 9.5 and 45 min and in a paddle-type mixer at 500 rpm mixer, obtained 44.7% from extracted RB and 64.8% from full fat RB. However, Shih (2003) stated that alkaline extraction is effective method but carefully control is needed to avoid too high pH, otherwise the protein would be changed to harmful metabolites. This common extraction methods used in this study is feasible as rice bran protein consisted of albulmin: globulin: glutelin at the ratio of 36:5:22 with mainly albumin and globulin (Juliano, 1985). Albumin is water soluble protein and its ion is enough, consequently there was no bonding or attachment with disulfide bond. Furthermore, this subunit was soluble in slight saline as they had sufficient ion. Prolamin and glutelin had a property as highly hydrophobic proteins that insoluble in the water (Damodaran, 1996). In addition, NaOH solution could cleavage hydro bond and disulfide bond which resulted in reduce their binding and size of molecules enough for soluble in the water (Hamada, 1997).

In present study, the nutrient composition of RBPC extracted from full fat rice bran included 3.49% ash, 4964.8 kcal of GE/kg, 1.94% crude fiber, 28.20% ether extract and 16.66% crude protein. The higher in crude fat and lower percentage of protein in RBPC was under expected as lower than in the previous studied of Monkolkanchanasiri (2002), who reported the concentration of protein obtained from full fat RB extraction was 31%. The difference in nutrient quantity of RBPC might be derived from their originate varieties. Hamada (1997) extracted protein from different varieties of rice such as Bengal, Cypress, Della, Mars, Maybelle and Toro-2 stated that rice varieties affected to different composition of protein and obtained the most amount RBPC from Mars rice (73.7%) and the least from Toro-2 rice (56.1%). These data implied the optimum condition need to further investigation and improvement of the process as well as test with extracted rice bran rather than full fat rice bran.

Growth performance of the piglets including body weight and gain, feed intake and feed per gain ratio was not significant difference among treatments (p<0.05, Table 3). The final BW of the piglet fed with 75 to 100% substitution RBPC (D, E) had 7.21% and 8.92% differed from control treatment (A) resulted in approximately 10.95% and 14.85% lower ADG. These data derived from lower feed intake approximately 10-11% than control. Rice bran protein concentrate has a protein efficiency ratio (PER) of 2.0-2.5 which is not substantially lower than of casein (2.5) and also rice bran protein is very digestible (Wang et al., 1990). This finding implied to the beneficial cost from RBPC substitution diet for weaning pigs. In addition, almost of their blood characteristics showed a comparable to normal profile and was no difference among treatments with an exception of mean cell hemoglobin concentration (MCHC), demonstrated that the inclusion of RBPC did not affected to health condition (Table 4). All piglets had a normal health in appearance, even though MCV (mean cell volume) and MCH (mean concentration hemoglobin) were slightly low in all treatments but those values were within normal range, indicated nearly anemia incidence cause by lower consumption of iron. The lower MCHC (p<0.05) was found with the 75% RBPC substitution and a trend to lower for higher substitution rate. Antinutrition factors such as

Table 3. Comparison on growth performance of piglet fed the control diet and different rate of RBPC substituted for skim milk in early weaning pig diets

Characteristics	Control	Rate of RBPC substitution (%)				SEM
	A~0	B~25	C~50	D~75	E~100	
Number (head)	9	9	9	9	9	
Period of trial (d)	35	35	35	35	35	
Initial body weight (kg)	7.07	7.09	7.04	7.07	7.22	0.03
Final body weight (kg)	20.40	17.34	17.09	18.93	18.58	0.60
Average BW gain (kg)	13.33	10.26	10.04	11.87	11.35	0.60
ADG (g/d)	380.86	293.14	286.86	339.14	324.29	17.01
Average feed intake (kg)	21.79	17.95	18.91	19.33	19.44	0.63
Feed intake per day (g/h/d)	622.57	512.86	540.29	552.29	555.43	18.10
Feed conversion ratio	1.63	1.75	1.88	1.63	1.71	0.05

Table 4. Blood profile concerning health status of piglet fed the control diet and different rate of RBPC substituted for skim milk in early weaning pig diets

Items [1]	Control A~0	Rate of RBPC substitution (%)				Normal range
		B~25	C~50	D~75	E~100	
RBC (10^3 cm)	6.670	6.970	7.098	6.592	6.963	5.66-9.55
WBC (10^6 cm)	15.472	16.418	14.719	17.791	18.200	8.23-26.10
HB (g/dl)	10.505	10.505	11.283	10.533	10.633	9.82-15.74
HCT (%)	33.605	32.277	34.738	33.388	33.222	33.25-49.10
MCV (fL)	50.666	46.555	49.055	50.611	48.000	45.91-63.09
MCH (Pg)	15.877	15.007	15.933	15.983	15.277	15.12-18.58
MCHC* (%)	31.288^b	32.494^a	32.511^a	31.516^b	31.911^{ab}	27.63-34.40
RDW	29.388	28.461	27.177	26.877	28.022	12.3-18.5
PLT (10^3 cm)	7.181	6.851	6.936	8.158	7.542	6.30-15.59
MPV (10^3 cm)	6.738	5.855	5.888	6.000	5.944	7.5-10.5

[1] WBC = White blood cell; RBC = Red blood cell; HB = Hemoglobin; HBC = Hematocrit; MCV = Mean cell volume; MCH = Mean cell hemoglobin; MCHC = Mean cell hemoglobin concentration; RDW = Red cell distribution width; MPV = Mean platelet volume; PLT = Platelet. * $p<0.05$.

such as phytin (phytate), trypsin inhibitor, oryzacystatin and haemagglutinin-lectin in the rice grain are concentrated in the bran fraction (embryo and aleurone layer). These factors are proteins except phytin are subjected to heat denaturation. Its phosphate groups readily complex with cations such as calcium, zinc and iron and with protein. It is heat stable and is responsible for the observed poorer mineral balance of subjects fed brown rice diets (bran included) in comparison to that of subjects fed milled rice diets. Rice-bran lectin (heamagglutinins) binding could cause agglutinate mammalian red blood cell (http;//www.fao.org/docrep/t0567e/T0567E0g.htm).However, Oureshi et al. (2002) reported the high quantity of γ-oryzanols (3mg/gRB), and phytosterols, polyphenols and vitamin E, both in tocopherol and tocotrienol form. These finding demonstrated rice bran would have a beneficial characteristics as an antioxidant that could reduce free radicals in the body consequently it helps to relieve oxidative stress. Riewklang (2002) analyzed the extracted RBPC extracted by mixed enzymes, showed amino acid composition in similar to casein and egg protein. These substance as well as amino acid pattern might affect to maintain growth performance as well as their health status.

Calculation cost for producing RBPC with alkaline extraction (exclude labor and energy cost) was 23.56 Baht/kg (approximately 1.36$) is low compared to skim milk (180 baht/kg, 5.63$). In Table 5, the cost difference for BW gain from control diet is quite high as 1.32, 2.82, 11.58 and 13.92 Baht/kg (0.04; 0.09; 0.36; 0.44$) resulted to relative cost was 96.67, 92.85, 70.75 and 64.48 for the weaning pig fed diets substituted skim milk with RBPC at 25, 50, 75 and 100%, respectively.

IMPLICATION

With the technology management in this study focus on self-reliance, the adequate process of extraction to produce RBPC was suggested. Even though the amount and protein

Table 5. Comparison of cost production in piglets fed the control diet and different rate of RBPC substituted for skim milk diets

Items	Control A~0	Rate of RBPC substitution (%)			
		B~25	C~50	D~75	E~100
Final weight (kg)	20.40	17.34	17.09	18.93	18.58
Average BW gain (kg)	13.33	10.26	10.04	11.87	11.35
Feed conversion ratio	1.63	1.75	1.88	1.63	1.71
Average daily feed intake (kg)	21.79	17.95	18.91	19.33	19.44
Feed price (Baht/kg)*	24.31	21.89	19.54	17.22	15.03
Cost for BW gain (Baht/h)	529.71	392.93	369.50	332.86	292.18
Cost for BW gain (Baht/kg)	39.63	38.31	36.80	28.04	25.70
Cost difference (Baht/kg)		1.32	2.82	11.58	13.92
Relative cost	100	96.67	92.86	70.75	64.48

* Skim milk price 180 Baht/kg, RBPC 23.56 Baht/kg, 1$ = 32.00 approximately.

concentration obtained from the present study was quite lower than that of expectation, growth performances and health status of piglets did not differed among dietary treatments that substituted with different levels of RBPC to skim milk. In addition, relative cost is flavor to 100% RBPC substitution rate as lower in approximately 35% comparison to control gave a potential as alternative feed protein for weaning pig diet. The profitable application data of RBPC are scared. However, knowledge gained about RBPC should be provided for a better processed control, and utilized a wider range in weaning pig diet.

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