Defatted Rice Bran Nonstarch Polysaccharides in Broiler Diets: Effects of Supplements on Nutrient Digestibilities

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The efficacy of bile salt, lipase, or fiber-degrading enzyme supplementation in the nonstarch polysaccharides (NSP) of defatted rice bran (DRB) diet on nutrient digestibilities and energy value was examined in 1- to 14-d-old chicks. Two types of NSP, Extracted-NSP (isolated from DRB, 15%/kg of diet) and Natural-NSP (given as intact DRB, 30%/kg of diet), were tested in this experiment. The 15%-Extracted- and the 30%-Natural-NSP provided a comparable amount of total-NSP concentration. The birds fed the Extracted-NSP diet had better weight gain and lower feed : gain ratio compared with those given the Natural-NSP diet, although fat digestibility did not differ between the two NSP diets. Addition of bile salt improved fat digestibility more than that of lipase or fiber-degrading enzyme in both NSP diets on d 7 ($P \le 0.001$). There was a significant NSP type x supplements on fat digestibility showing that the effect of the fiber-degrading enzyme during the first week of age was more profound ($P \le 0.01$) in diets containing the Extracted-NSP than those with the Natural-NSP. But, pancreatic lipase activity did not correlate well with fat digestibility. During the second week of age, the effect of bile salt on protein digestibility was inferior whereas the effect of lipase was superior in the two NSP diets (P < 0.05). The dietary ME_n values of the diets containing the Extracted-NSP were greater than those with the Natural-NSP (P< 0.001). Bile salt had the greatest effect among the three supplements on the ME_n (P< 0.001) of the NSP diets. Tibia ash content of the birds was adversely affected only by feeding diets containing the Natural-NSP. The current study suggested that bile salt appeared the most effective supplement in increasing fat digestibility and energy value of the diet containing considerable amounts of defatted rice bran-nonstarch polysaccharides.

Key words: defatted rice bran, nonstarch polysaccharides, nutrient digestibility, supplement

Introduction

When the dietary nonstarch polysaccharide (NSP)-containing cereals and their by-products are fed to chicks, fat digestibility is adversely affected (Choct et al., 1996; Dänicke et al., 1997; Dusel et al., 1998). This is not only because of the less secretions of the initial digestive enzymes (Krogdahl, 1985; Nir et al., 1993) and bile acids (Krogdahl, 1985) but also due to the binding property of NSP with bile acids

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(Normand and Ory, 1984; Smits et al., 1998).

Recently, defatted rice bran (DRB) has become more available than full-fat rice bran for poultry feed, but its considerable amount of NSP needs to be considered for the optimum use. A study with chicks showed a decrease in lipase activities when the chicks were fed a diet containing 7% of NSP extracted from DRB, and this adverse effect of NSP was ameliorated with xylanase supplementation (Adrizal and Ohtani, unpublished data). These authors also noted that the negative effect of NSP disappeared in a 15%-Extracted-NSP diet when the diet was supplemented with bile salt. It is likely that an increasing concentration of NSP up to 15% also causes an increasing other potential antinutritive NSP fractions such as β -glucans. Hence, supplemental xylanase in their study was possibly not sufficient to work on all NSP fractions to elicit its significant effect. Interestingly, the extracted form of NSP (15%) elicited a different effect from the natural form (DRB as intact, 30%) on fat digestibility, and the effect of xylanase did not effective to improve fat or protein digestibility of these diets in their study.

It seems that the inclusion of bile salt will improve fat digestibility of the Extracted-or Natural-NSP of DRB diet, but such an effect of supplemental xylanase is not evident. The assay done in our laboratory (Adrizal and Ohtani, unpublished data) showed that arabinose (0.42 mol%) and xylose (0.35 mol%) constituted the main sugars of the Extracted-NSP (insoluble-NSP + soluble-NSP) isolated from DRB followed with glucose (0.10 mol%), galactose (0.08 mol%), and mannose (0.05 mol%). These compositional values were comparable with that of the Natural-NSP (intact DRB). Fincher and Stone (1986) have also reported low concentration of β - (1,3 and 1,4)-D-glucans in rice bran. If the arabinoxylans and β -glucans of the total-NSP of DRB act as antinutritively as in the wheat-arabinoxylans (Pettersson *et al.*, 1990) and barley- β -glucans (Aman and Graham, 1987), perhaps the multiple fiber-degrading enzymes are more appropriate than the single enzyme to alleviate the negative effect of the NSP on fat digestibility. Boros *et al.* (1998) reported that the viscosity-reducing effects of fiber-degrading enzymes improved the nutritive values of wheat-, rye-, or barley-based diets.

The addition of exogenous lipase to the NSP diet may also be expected to provide additional lipase for facilitating fat digestibility as its activity is impeded by dietary NSP (Normand and Ory, 1984). Kermanshahi et al. (1998 a, b) reported that under in vitro conditions that approximated the glandular stomach, bacterial or fungal lipase was more stable than porcine lipase. Nevertheless, an in vivo study needs to be done for further clarification of the efficacy of bacterial lipase with respect to the digestibility of fat (Kermanshahi et al., 1998b). Thus, for the present experimental purpose, inclusion of bacterial lipase in the NSP diet was deemed appropriate.

This study was aimed to compare the efficacy of bile salt, fiber-degrading enzyme, and bacterial lipase in minimizing the antinutritive effect of the dietary NSP of DRB (either in the extracted or in the natural form) and in improving nutrient digestibilities, especially fat, and energy value of the diet fed to chicks.

Materials and Methods

Extraction Procedure of Nonstarch Polysaccharides from Defatted Rice Bran

Firstly, 1.5 ml of α -amylase (E.C.3.2.1.1. [A3306, heat stable, reagents for the determination of total dietary fiber, Sigma Chemical Co., St. Louis, MO, USA]) was mixed with 101 of tap water in a stainless bucket followed with the addition of 1 kg of ground (<1.5 mm) DRB (Zen-Noh, Chita, Japan). The mixture was stirred in a water bath at 85 °C for 1 h. After cooling to 50 to 55 °C, while in a water bath, 5 ml of amyloglucosidase (E.C.3.2.1.3. [A9913, heat stable, reagents for the determination of total dietary fiber, Sigma Chemical Co., St. Louis, MO, USA) was added to breakdown starch. Immediately, 15 ml of 0.2% (w/v) pancreatin solution (P7545, from porcine pancreas, Sigma Chemical Co., St. Louis, MO, USA) was added to hydrolyze protein, and stirred overnight (16h) in a water bath at 50° to 55°C. The mixture was then centrifuged at 3000 × g for 30 min at room temperature. The supernatant was decanted (the residue was insoluble NSP), adjusted to four-time-volume of 80% (v/v) ethanol, and allowed to stand several hours at room temperature. The ethanol supernatant was discarded, and the precipitate was dried (soluble-NSP). The residue from the centrifugation was dried (insoluble-NSP) and combined with the dried soluble-NSP, and used as the Extracted-NSP material for diet formulation. The use of intact DRB in the diet was constituted the Natural-NSP.

The assay of the NSP sugars (sum of arabinose, xylose, mannose, galactose, and glucose) in the Extracted- and the Natural-NSP material was based on the procedure of Dusel *et al.* (1997) with a few modifications in the gas chromatograph (GC-14A, Shimadzu Co., Kyoto, Japan) condition. A capillary column with fused silica (Rtx-50, 30 m \times 0.25 mm; Shimadzu Co., Kyoto, Japan) was used with the column temperature of 235 °C (isothermal). Initial temperature of the GC was set at 200 °C for 2 min and held at final temperature (235 °C) for 8 min with the rate of temperature at 5 °C/min. The flow-rate of helium was set at 2.1 ml/min and split 1: 10. Sample was injected at 1 μl to the GC.

Animals and Dietary Treatments

One hundred and eighty 1-d-old broiler male chicks (Chunky), obtained from a local company (Yamamoto Hatchery, Minokamo, Japan), were assigned into 18 pen-groups of 10 chicks per pen. As shown in Table 1, diets, a combination of three supplements (bile salt [B], lipase [L], and fiber-degrading enzyme [E]) and two types of NSP (Extracted-NSP and Natural-NSP), were assigned to pen-groups from 1 to 14 d of age. Extracted-NSP (15%) and Natural-NSP (30%) supplied approximately 9.2% and 9.4% (DM) of NSP/kg of diet, respectively. Sodium-taurocholate (Becton Dickinson and Company, Sparks, MD, USA) was used at 0.5% in the diet. Lipase (from *Pseudomonas sp.*, Karlan Research Products Co., Santa Rosa, CA, USA) was used at 0.05%. It was premixed with DRB and soybean oil (0.8, 2, and 97.2%, respectively) before inclusion in the diet to facilitate easy mixing with other dietary ingredients. The fiber-degrading enzyme (GRINDAZYMETM GP 5000, Danisco Cultor, Brabrand, Denmark) was used at 0.05%. All diets were formulated to contain

Table 1. Ingredient and nutrient composition of experimental diets

Ingredients	$\begin{array}{c} Extracted-\\ NSP+B^{\scriptscriptstyle \dagger} \end{array}$	Extracted- NSP+L	Extracted- NSP+E	Natural- NSP+B	Natural- NSP+L	Natural- NSP+E
			% (air-dr	y basis)	****	
Soy protein	8.92	8.93	8.93	6.31	6.25	6.25
Yellow corn	34,12	35.11	35.11	28.44	29.32	29.32
Soybean meal	23.39	23.19	23.19	18.04	17.99	17.99
DRB-NSP	15.00	15.00	15.00		_	_
Defatted rice bran	_		_	30.00	30.00	30.00
DL-methionine	0.35	0.35	0.35	0.30	0.30	0.30
Oil^2	11.74	11.39	11.39	10.75	10.43	10.43
Dicalcium posphate	1.84	1.84	1.84	0.97	0.97	0.97
CaCO ₃	1.41	1.41	1.41	2.03	2.03	2.03
Vitamin mix ³	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ⁴	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.23	0.23	0.23	0.16	0.16	0.16
Celite	2.00	2.00	2.00	2.00	2.00	2.00
Na-taurocholate	0.50	_	_	0.50		_
Lipase ⁵	_	0.05	_	_	0.05	****
GRINDAZYM ^{TM 6}	_		0.05	_	_	0.05
Calculated values :						
ME _n (kcal/kg)	3000.02	3000.02	3000.02	3000.02	3000.01	3000.01
Fat (%)	13.32	13.00	13.00	12.30	12.01	12.01
Protein (%)	22.00	22.00	22.00	22.00	22.00	22.00
Lys (%)	1.28	1.27	1.27	1.22	1.22	1.22
Met+Cys (%)	0.92	0.92	0.92	0.91	0.91	0.91
Ca (%)	1.01	1.01	1.01	1.06	1.06	1.06
Non-phytate P (%)	0.45	0.45	0.45	0.48	0.48	0.48
Na (%)	0.20	0.20	0.20	0.20	0.20	0.20

¹B: bile salt; L: lipase; E: xylanase.

3,000 kcal ME_n/kg of diet and 22% CP and complied with the National Research Council (NRC, 1994) requirement. Celite^R (Celite Corp., purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as a marker to determine apparent (excreta) digestibilities of fat and protein.

Feed intake, body weight gain (BWG), and feed: gain (F:G) ratio were recorded at 1 to 7 and 8 to 14d of age. After recording body weight (BW) and collecting excreta (7 and 14d), two selected birds per pen were killed by decapitation (approved by the Federation of Animal Science Societies, 1999). Pancreatic tissue was

² A blended oil of soybean oil and palm oil (50:50).

 $^{^3}$ Provided per kg of diet:vit. A (palmitate), 10,000 IU; cholecalciferol, 3,000 IU, vit. E (DL-\$\alpha\$-tocopherol), 30 IU; vit. K_3, (menadione), 2 mg; vit. B_{12}, 0.01 mg; biotin, 0.20 mg; choline, 550 mg; folacin, 1.10 mg; niacin, 35 mg; pantothenic acid, 10 mg; pyridoxine, 3.50 mg; riboflavin, 4 mg; thiamin, 1.50 mg; and ethoxyquin, 40 mg.

⁴Provided per kg of diet:copper, 6 mg;iodine, 0.50 mg;iron, 50 mg; manganese, 60 mg; selenium, 0.15; and zinc, 40 mg.

⁵ Supplied per kg of diet 3,318 units of lipase.

⁶ Supplied per kg of diet: 6,000 U of xylanase, 2,500 U of s-glucanase, and 5 U of pectinase.

excised, placed in liquid nitrogen, and stored at $-30\,^{\circ}\mathrm{C}$ for later lipase analysis. Left tibias of the two chicks per pen were also removed for ash determination. Sample Treatments and Chemical Analyses

Dry matter of feed, excreta, and tibia was determined by drying samples in a forced-draft oven at 105 °C for 16 h, cooled in dessicator, and weighed. Dried tibias were then extracted with diethyl ether for 6 h, ashed in a furnace at 600 °C for 24 h, cooled in dessicator, and recorded as ash weights. Gross energy of feed and excreta samples was determined using a bomb calorimeter (Model P-202 CS-4PJ, Shimadzu Co., Kyoto, Japan). Nitrogen contents in feed and excreta were analyzed with the macro-Kjeldahl method of the Association of Official Analytical Chemists (AOAC, 1980; section 7.015), and the ME_n values of the diets were determined using the formula of Hill and Anderson (1958). Fat contents in feed and excreta were extracted according to AOAC (1980; section 7.056). Lipase activity was analyzed following the procedure of O'Sullivan et al. (1992) and expressed as unit/g pancreas where one unit represents mg naphthol released in 10 min at 37 °C. Celite (acid insoluble ash, AIA) in feed and excreta was determined by the method of Vogtmann et al. (1975) for the digestibility coefficients of nutrients (DC):

Statistical Analysis

Data were analyzed by two-way classification of ANOVA (Campbell, 1989) according to age or age period, to identify the effect of NSP type, supplement, and the interaction of these two components.

Results

The total NSP concentration (DM basis) in the Extracted-NSP was approximately 68.1% (60.7% insoluble- and 7.4% soluble-NSP) and in the Natural-NSP (intact DRB) was 33.7% (27.2% insoluble- and 6.5% soluble-NSP) (Table 2). The molar ratios of arabinose to xylose in the Extracted-NSP and in the Natural-NSP were 1.20 and 1.21, respectively. The Extracted-NSP contained greater protein (22.1 vs 13.8%) and fat (2.3 vs 1.7%, DM) than the Natural-NSP.

In the age periods of 1 to 7 and 8 to 14 d, amount of feed consumed by chicks did not differ among the dietary groups (Table 3). However, BWG of the chicks fed the Extracted-NSP diet was greater than that of fed the Natural-NSP diet at the periods of 1 to 7 d (93 vs 85 g; P < 0.05) and 8 to 14 d of age (222 vs 201 g/bird; P < 0.001). The NSP type effect was also apparent on F: G ratios of the two dietary groups at 1 to 7 d (1.04 vs 1.09; P < 0.05) and 8 to 14 d of age (1.26 vs 1.40; P < 0.001).

Fat digestibility of the Extracted-NSP diets did not vary from that of the Natural-NSP diets at d 7 (92.4 vs 91.6%) and d 14 (87.1 vs 85.3%) (Table 4). However, supplements apparently affected fat digestibility on d 7 (P<0.001) and 14 (P<0.05), at which bile salt appeared to have a superior effect. The interaction effect between NSP

Table 2.	Chemical composition of the nonstarch polysaccharide (NSP) of the Extracted-
	and the Natural-NSP of defatted rice bran and their NSP sugar concentrations

D 1 4 66	Chemical Composition									
Feedstuffs	Insoluble-NSP	Soluble-NSP	Total-NSP ¹	Pro	tein	Fat				
			% (DM)							
Extracted-NSP	60.7	7.4	68.1	22	2.1	2.3				
Natural-NSP ²	27.2	6.5	33.7 13.8		3.8	1.7				
	NSP (Total) Sugar Concentrations									
	Ara	Xyl	Man	Gal	Glu	Ara : Xyl				
	(mol %) ³									
Extracted-NSP	0.42	0.35	0.05	0.08	0.10	1.20				

Ara: arabinose; Xyl: xylose; Man: mannose; gal: galactose; Glu: glucose; Ara: Xyl= molar ratio of arabinose and xylose.

0.08

0.09

1.21

0.10

0.33

Natural-NSP

0.40

Table 3. Feed intake, BWG, and feed: gain (F:G) ratio of broiler chicks fed dietary 15% extracted-nonstarch polysaccharide supplemented with bile salt (Extracted-NSP+B), lipase (Extracted-NSP+L), or fiber-degrading enzyme (Extracted-NSP+E), or dietary 30% defatted rice bran supplemented with bile salt (Natural-NSP+B), lipase (Natural-NSP+L), or fiber-degrading enzyme (Natural-NSP+E) from 1 to 14 d of age

Diet	Feed	Intake	В	WG	F : G Ratio		
Diet	1 to 7 d	8 to 14 d	1 to 7 d	8 to 14 d	1 to 7 d	8 to 14 d	
		g/t		g : g			
Extracted-NSP+B	102	286	99	228	1.03	1.26	
Extracted-NSP+L	95	275	90	218	1.05	1.26	
Extracted-NSP+E	92	276	90	219	1.03	1.26	
Natural-NSP+B	92	279	86	204	1.07	1.37	
Natural-NSP+L	93	274	86	192	1.08	1.43	
Natural-NSP+E	93	288	84	206	1.11	1.40	
SEM ¹	2	5	3	4	0.03	0.02	
Sources of variances:							
NSP type	NS	NS	*	***	*	***	
Supplementary compound	NS	NS	NS	NS	NS	NS	
Interaction	NS	NS	NS	NS	NS	NS	

NS: not significant; *: P < 0.05; ***: P < 0.001.

type and supplements (particularly lipase and fiber-degrading enzyme) on fat digestibility was detected at d 7 (P < 0.01). At this age, the effect of lipase was more apparent when presented in the Natural-NSP than with the Extracted-NSP diet (92.7 vs 91.1%; P < 0.05). Contrastingly, the effect of fiber-degrading enzyme was more pronounced

¹Represents as a neutral NSP (uronic acids were not analyzed in this experiment).

² Defatted rice bran as intact.

³The molar proportion weights.

¹Means of three pens per treatment with 10 chicks (d 1 to 7) or eight chicks (d 8 to 14) per pen.

Table 4. Apparent digestibilities of fat and protein, dietary energy values (ME_n), tibia ash percentages, and pancreatic lipase activities of broiler chicks fed dietary 15% extracted-nonstarch polysaccharide supplemented with bile salt (Extracted-NSP+B), lipase (Extracted-NSP+L), or fiber-degrading enzyme (Extracted-NSP+B), or dietary 30% defatted rice bran supplemented with bile salt (Natural-NSP+B), lipase (Natural-NSP+L), or fiber-degrading enzyme (Natural-NSP+E) from 1 to 14 d of age

Diet	Fat		Pro	Protein		ME_{n}		Tibia ash		Lipase activity	
	d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14	
	% (DM)				(kcal/kg) ⁱ		% (fat-free)		(U/g of pancreas)		
Extracted-NSP+B	94.4	88.6	64.0	60.4	3,209	3,195	37.4	39.1	47	62	
Extracted-NSP+L	91.1	85.8	64.8	65.0	3,109	3,116	36.5	39.5	45	82	
Extracted-NSP+E	91.7	86.9	65.7	65.9	3,067	3,048	36.9	39.4	44	62	
Natural-NSP+B	93.5	87.7	63.0	61.2	3,124	3,105	36.2	38.0	49	74	
Natural-NSP+L	92.7	83.5	64.9	63.4	2,967	2,980	36.3	39.2	63	62	
Natural-NSP+E	88.7	84.8	61.4	61.1	2,969	2,980	36.0	38.5	72	88	
SEM^2	0.5	1.3	1.0	1.2	25	25	0.4	0.4	7	9	
Sources of variances	:					(P)	·				
NSP type	NS	NS	NS	NS	***	***	*	*	*	NS	
Supplementary compound	***	*	NS	*	***	***	NS	NS	NS	NS	
Interaction	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	

NS: not significant; *: P < 0.05; **: P < 0.01; ***: P < 0.001.

when included in the Extracted-NSP than in the Natural-NSP diet (91.7 vs 88.7%) at this age. At d 7, feeding chicks the Natural-NSP diets resulted in increased pancreatic lipase activities than feeding those the Extracted-NSP diets (61 vs 47 U/g of pancreas; P < 0.05) (Table 4) at this age. Only the supplementary compound elicited its effect on protein digestibility (d 14; P < 0.05) (Table 4). Interestingly, the supplementation of bile salt in the two NSP diets showed an inferior effect among the three supplements on protein digestibility, whereas the effect of lipase was superior in the two NSP diets at this age (P < 0.05).

At 7 and 14 d of ages, the Extracted-NSP diets showed greater ME_n values (P < 0.001) than the Natural-NSP diets (3128 vs 3020 and 3136 vs 3022 kcal/kg, respectively) (Table 4). The increment contribution of bile salt supplementation compared to that of other two supplements on the ME_n values in the two NSP diets was 4.6% (P < 0.01) at d 7 and 3.9% (P < 0.001) at d 14. The effects of lipase and fiber-degrading enzyme on the energy values of the diets were comparable in the two NSP diets.

Feeding chicks the Natural-NSP diets resulted in reductions (P < 0.05) in tibia ash percentages compared to feeding those the Extracted-NSP diets at d 7 (36.9 vs 36.2%) and 14 (39.3 vs 38.6%) (Table 4).

¹ Air-dry basis.

 $^{^2}$ Means of three pens per treatment with 10 chicks (d 7) or eight chicks (d 14) per pen except for tibia ash and pancreatic lipase activities (n=2 for d 7 and 14)

Discussion

The uses of the Extracted- and the Natural-NSP in the present experiment supplied 92 and 94 g (DM) of NSP/kg of diet, respectively. So, this difference can be treated as of marginal consequence if the rice bran NSP which was predominated by arabinose and xylose, acts as antinutritive as wheat-arabinoxylans (Pettersson *et al.*, 1990).

The greater BWG of the Extracted- than that of the Natural-NSP groups of chicks which was also followed by the better F:G ratio implied a more severe effect of the total-NSP when presented in the natural form. Data of the dietary ME_n values of the Extracted-NSP diets also supported the fact that the antinutritive effect of the extracted form was not as great as in the natural form. However, the greater ME_n values of the Extracted-NSP than the Natural-NSP diets did not correlate well with fat or protein digestibility data, the reason of which cannot be explained from the available data in the present experiment.

Bile salt showed the most favorable effect among the supplementary compounds in improving fat digestibilities of the Extracted- and the Natural-NSP diets. This result confirms the finding of Danicke *et al.* (1997) that the reduced effect of the diet containing considerable amount of NSP (rye-based diet) fed to the chicks on fat digestibility was caused by the reduced available concentration of bile acids for the emulsification of particularly saturated fatty acids. Thus, the less effect of lipase addition on fat digestibility and pancreatic lipase activities in the Extracted-NSP chicks further indicated that bile salt was the first limited available compound for the favor of fat digestibility.

The increasing activities of lipase in the chicks fed the Natural-NSP diets (d 7) could be due to the temporal responses of the pancreas because their activities resembled that of the Extracted-NSP groups as chicks aged to 14 d. Besides, this increment did not significantly alter fat digestibility of the diets at d 7. The improved fat digestibility due to the supplementation of bile salt, which failed to correlate well with pancreatic lipase activities of the chicks, remains unclear.

The present results are in agreement with the finding Adrizal et al. (1996), which showed a reduction in tibia ash percentage when chicks were fed the Natural-NSP (30%-DRB) diet. The failure of the supplements to increase the tibia ash percentage from its binding with NSP (Houston, 1972) in the present experiment was possibly because of none of the supplements had a direct effect on the releases of minerals. Although Ravindran et al. (1999) did not measure the bone ash of the chicks, the results of their studies demonstrated that the supplementation of the associated enzyme, phytase, alone or in combination with xylanase, improved the growth of the chickens fed a wheat-based diet.

The current study suggested that bile salt appeared the most effective supplement to increase fat digestibility and energy value of the diets containing considerable amounts of defatted rice bran-nonstarch polysaccharides.

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