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Utilization of enzyme incubated rape seed meal as a source of protein in commercial broiler diets

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ABSTRACT

Two experiments were conducted to study the possibility of utilizing enzyme incubated rape seed meal (RSM) in broiler diets (1 to 42 day of age). The RSM was anaerobically incubated with non-starch polysaccharide hydrolyzing enzymes (cellulase 1,500 IU, xylanase 250 IU and pectinase 125 IU per kilogram) for 300 minutes (processed RSM1, experiments 1 and 2) and 600 min (processed RSM2, experiment 2). Processed and raw RSM contained similar concentration of glucosinolates (275 and 288 mM/g, respectively). During the experiment 1, maize–soybean meal control diet (CD), two diets with raw (RRSM) and processed RSM (PRSM1) at 100 g/kg each were prepared. In the experiment 2, RRSM, PRSM1 and PRSM2 were included each at 3 levels (50, 100 and 150 g/kg). Each diet was offered to 9 replicates having 5 birds in each. Body weight gain (BWG), feed intake (FI), feed efficiency (FE), ready to cook (RTC) yield and breast weight were depressed by incorporating RRSM (experiments 1, 2). As a result of this study, PRSM1 significantly reduced FI, maintained BWG and improved FE compared to the control group. BWG was not affected by including PRSM1 and PRSM2 up to 100 and 50 g/kg, respectively but depressed at higher inclusion levels compared to the CD. The BWG in processed RSM fed groups were higher than the respective level of raw RSM fed groups. Retention of energy and protein were improved by incorporating processed RSM compared to those fed RRSM. Based on the data, it is concluded that enzyme (cellulase, xylanase and pectinase) incubated RSM for 300 minutes improved the nutritional value, which can be incorporated up to 100 g/kg in broiler diet without affecting performance and slaughter variables, which can be attributed to the improved retention of energy and protein in RSM based diets.

Key words: Broiler chicken, Enzyme incubation, Performance, Protein retention, Rape seed meal

The growth of broiler chicken farming primarily depends on the cost of maize and soybean meal (SBM) as the dietary sources of energy and protein, respectively. Increase in the price of SBM and swift change in global trade dynamics of this commodity resulted in shortage of protein source at an affordable price to the poultry farmers. Frequent occurrence of such crises opens opportunities to utilize alternate feed ingredients in place of SBM. Utilization of various alternate protein sources (sunflower meal, sesame cake, guar meal) were explored in poultry diets to economize the feed cost (Rama Rao *et al.* 2008, 2015). Rape seed meal (RSM) is a fairly good source of protein (35 to 40%), moderate source of energy (2200 kcal/kg) and rich in methionine + cysteine (1.61 to 1.75%) compared to SBM (Rama Rao *et al.* 2005).

Another alternate approach to improve the nutritional value of RSM is through supplementation of exogenous enzymes to diets containing the alternate protein source to hydrolyze the higher fibre levels present in the meal. Enzyme supplementation in poultry diets showed promising results (Rama Rao *et al.* 2014, O'Neill *et al.* 2014, Prakash *et al.* 2016) on the bird performance. Understanding the precise composition of NSP will help in targeting the substrate with appropriate NSP hydrolyzing enzyme combination, which will be effective in degrading cell wall polysaccharides. Therefore, incubation of RSM with NSP hydrolyzing enzymes (cellulase, pectinase and xylanase) for longer duration may facilitate fibre hydrolysis and improve the feeding value of RSM for poultry. In the present study, an attempt was made to utilize NSP enzymes incubated RSM as a source of protein in commercial broiler diets.

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MATERIALS AND METHODS

Incubation of RSM with NSP enzymes: Raw RSM (RRSM) was procured from the local market. The RSM was fed as such or anaerobically incubated with microbial enzymes at two different holding times to increase the nutrient digestibility in chicken. Briefly, moisture content of the RSM was increased to 25% with deionized water. The meal was subjected to steam cooking (55°C) for about

Table 1. Ingredient composition (g/kg) of diets (Experiment 1)

Ingredient	Starter (1-21 day)		Finisher (22-42 day)	
	Control	RSM diets	Control	RSM diets
Maize	526.3	482.0	602.3	558.9
Soybean meal (45% CP)	403.9	335.4	312.6	242.9
Soybean oil	31.06	42.74	47.76	59.15
Rape seed meal (36% CP)	0.00	100	0.00	100
Dicalcium phosphate	20.05	21.02	17.56	18.54
Shell grit	7.82	7.98	8.00	8.17
Salt	4.40	4.4	4.50	4.50
Premix[a]	4.50	4.50	4.50	4.50
L-Lysine HCl	0.00	0.21	0.88	1.55
DL-methionine	1.98	1.89	1.87	1.78

[a]Supplied per kg of diet: retinol acetate, 2.75 mg; cholecalciferol, 0.03 mg; α tocopherol, 10 mg; thiamin, 1 mg; pyridoxine, 2 mg; cyanocobalamine, 0.01 mg; niacin, 15 mg; pantothenic acid, 10 mg; riboflavin, 10 mg; biotin, 0.08 mg; menadione, 2 mg; choline, 650 mg; copper, 8 mg; iron, 45 mg; manganese, 80 mg; zinc, 60 mg; selenium, 0.18 mg; monensin sodium, 50 mg; hydrated sodium calcium aluminoailicate, 800 mg.

20 min. A mixture of enzymes containing cellulase 1,500 IU, xylanase 250 IU and pectinase 125 IU per kilogram was added to the steam cooked RSM and incubated at 37°C for 300 minutes (processed RSM1, experiments 1 and 2) or 600 minutes (processed RSM2, experiment 2). After incubation, the meals were heated to 70°C for drying them. The same heat treatment without the enzymes was applied to RRSM. The enzymes were procured from Advanced Bio-Agro Tech Ltd (Pune, India).

Experimental diets: Soybean meal (SBM), raw RRSM and processed RSM (PRSM1 and PRSM2) were analysed for crude protein and amino acids. The amino acid composition of the feed ingredients was measured at Evonik Pte Ltd (SEA), Singapore (Llames and Fontaine 1994). The feed samples were hydrolysed with 6 N HCl at 110°C for 18 h and were oxidised with performic acid prior to acid hydrolysis. Ninhydrin was used for colour development. Hydrolysis was done for 24 h with 3 N HCl for estimating the threonine. While for estimating tryptophan, the feed samples were hydrolysed with 2 N NaOH with paradimethyl amino benzaldehyde as the colouring agent. Glucosinolate (total) content in the RSM was estimated using titration methods suggested by McGhee *et al.* (1965). Maize-SBM based control diets (CD) for broiler starter (1–21 day) and finisher (22–42 day of age) phases were prepared using SBM as the major protein source. All the feed ingredients were ground independently using 3/16 and 1/4 inch mesh size for starter and finisher diets, respectively and were mixed as per the composition of the diets. In experiment 1, RRSM and PRSM1 were incorporated independently at 100 g/kg diet (Table 1). While in experiment 2, RRSM and both forms of PRSM (PRSM1 and PRSM2) were included at 50, 100 and 150 g/kg diet (Table 2). Energy, protein (including lysine and methionine), calcium and phosphorus levels were maintained uniform among diets in each phase. Levels of maize, SBM and soybean oil were altered to maintain desired level of nutrients in the diets.

Birds and Management: A total of 135 (experiment 1) and 450 (experiment 2) C*obb* 400 (Venkateswara Hatcheries Pvt Ltd., Hyderabad, India) day-old broiler male chicks (day-old chick body weight was 41.2±0.26 g in experiment 1, 42.6±0.11 g in experiment 2) were distributed randomly into 27 and 90 stainless steel battery brooder pens (24 × 30 × 18"), respectively at the rate of 5 chicks in each pen. Ground maize was fed on day one and each experimental diet was allotted to 9 replicate groups (pens) randomly and fed *ad lib.* from day 2 to 42 d of age. Birds were vaccinated with Newcastle Lasota, IBD and Lasota on 8th, 15th, and 20th d of age, respectively. The brooder temperature was maintained at 35°C during week 1 followed by reduction

Table 2. Ingredient composition (g/kg) of diets (Experiment 2)

Ingredient	Starter (1-21 day), RSM (g/kg)				Finisher (22-42 day), RSM (g/kg)			
	0	50	100	150	0	50	100	150
Maize	525.9	503.6	481.6	459.9	613.7	592	570.2	548.5
Soybean meal (45% CP)	403.9	369.9	335.3	300.5	310.5	275.6	240.8	205.9
Soybean oil	31.01	37	42.7	48.4	38.1	43.8	49.5	55.2
Rape seed meal (36% CP)	0	50	100	150	0	50	100	150
Dicalcium phosphate	20.1	20.54	21	21.5	17.52	18	18.5	18.99
Shell grit	7.82	7.9	7.98	8.07	8.03	8.12	8.21	8.29
Salt	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Premix[a]	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
L-Lysine HCl	0	0	0.21	0.55	0.92	1.26	1.6	1.93
DL-methionine	1.98	1.93	1.89	1.85	1.86	1.82	1.78	1.74

[a]Supplied per kg of diet: retinol acetate, 2.75 mg; cholecalciferol, 0.03 mg; α tocopherol, 10 mg; thiamin, 1 mg; pyridoxine, 2 mg; cyanocobalamine, 0.01 mg; niacin, 15 mg; pantothenic acid, 10 mg; riboflavin, 10 mg; biotin, 0.08 mg; menadione, 2 mg; choline, 650 mg; copper, 8 mg; iron, 45 mg; manganese, 80 mg; zinc, 60 mg; selenium, 0.18 mg; monensin sodium, 50 mg; hydrated sodium calcium aluminoailicate, 800 mg.

of 2°C per week till week 4, after which the birds were reared at room temperature (22 to 30.2°C). Artificial heat was provided with incandescent bulbs. Uniform management practices were followed across the treatment groups from day 1 through 42 day of age. The experimental protocol was approved by the Institute Animal Ethics Committee (IAEC/DPR/13/14).

Traits measured: Body weight (BW) and feed intake (FI) were recorded at weekly intervals. BW gain (BWG) and feed efficiency (fee intake/BWG) were calculated. At the end of both experiments (43rd day of age), one bird from each replicate representing the mean BW of the treatment was selected and slaughtered by cervical dislocation to study the ready to cook (RTC) yield, relative weights of liver, breast and abdominal fat. During the experiment 2, two metabolic trials were conducted at 19–21 (starter) and 40–42 (finisher) day of age by housing two birds in a metabolic cage and maintaining nine replicates per treatment. Birds were conditioned on the respective diet in metabolic cages for 4 day before collection period. Total intake of dry matter and dry matter voided (excreta) per pen (two birds together) were measured and the ratio was expressed as the nutrient retention. Protein and gross energy contents in both excreta and feed were analysed using Kjeldhal method and bomb calorimeter, respectively (AOAC 2005). Retention of these nutrients was expressed as the amount retained per unit intake, which was expressed in per cent.

Statistical analysis: The data of the experiment 1 were subjected to one-way analysis of variance, while experiment 2 data were subjected to 3×3 factorial analysis by considering processing and inclusion level as main factors. The difference among various treatments was tested at P<0.05 with Duncan Multiple Range test.

RESULTS AND DISCUSSION

Raw and PRSM contained 36.42 and 35.20% crude protein, respectively which is lower (44.92%) than SBM (Table 3). Processing did not change the amino acid composition of RSM. Both raw and PRSM contained higher concentration of methionine and cystine compared to SBM, while the concentrations of other amino acids were lower in RSM compared to SBM. Both PRSM and RRSM contained similar concentrations of glucosinolates (275 and 288 mM/g, respectively).

Table 3. Analysed amino acid composition (g/100g) of soy bean meal (SBM) and raw, and processed Rape seed meal (RSM)

Nutrient	Soybean meal	PRSM1 & 2	RRSM
Crude protein	44.92	35.20	36.42
Methionine	0.58	0.64	0.66
Cystine	0.49	0.79	0.81
M+C	1.07	1.43	1.46
Lysine	2.68	1.68	1.65
Threonine	1.69	1.42	1.49
Tryptophan	0.58	0.47	0.50
Isoleucine	2.18	1.29	1.25
Leucine	4.01	2.33	2.21
Valine	3.11	1.65	1.59
Glucosinolates (mM/g)	nil	275	288

PRSM1 and 2, processed rape seed meal (incubation for 300 and 600 min, respectively); RRSM, raw rape seed meal.

Experiment 1–Performance: BWG was not affected by including either raw or PRSM1 in broiler diet compared to the SBM group during starter (1–21 day of age) phase (P>0.05) (Table 4). However, the FI was reduced (P<0.05) in broilers fed RSM based diets compared to the SBM control group. The FI in PRSM1 was significantly less than RRSM. The FE was higher (P<0.05) in PRSM fed groups compared to those fed either the SBM or RRSM based diets. Progressive decrease in the bird performance with RRSM in diet might be due to proportionate increase in glucosinolate content. Similarly, McNeill *et al.* (2004) reported lowered FI and impaired growth in chicken with RSM based diet. Considering the data of BWG and FE, it was evident that PRSM1 and PRSM2 could be included in broiler diet up to 100 and 50 g/kg, respectively without affecting the performance. Through inclusion of the PRSM beyond the above levels depressed the performance variables, the performance was better than those fed the respective levels of RRSM, which implies that the processing employed in the current study was effective in

Table 4. Performance of commercial broilers fed raw (RRSM) and processed (PRSM1) rape seed meal (Experiment 1)

Treatment	Week 3			Week 6		
	BWG (g)	FI* (g/bird)	FI/BWG*	BWG* (g)	FI* (g/bird)	FI/BWG*
SBM control	713.3	902.2[a]	1.266[a]	2042[a]	3310[a]	1.622[b]
RRSM	689.3	853.3[b]	1.238[a]	1758[b]	2989[b]	1.700[a]
PRSM1	715.7	735.9[c]	1.028[b]	1993[a]	3069[b]	1.541[c]
P	0.343	0.001	0.001	0.001	0.010	0.001
n	9	9	9	9	9	9
SEM	8.023	16.30	0.0216	34.66	47.42	0.0150

[abc]Means having no common superscript in a column differ significantly; *(P<0.01); BWG, body weight gain; FI, feed intake; RRSM, raw rape seed meal; PRSM1, processed rape seed meal (300 min). Initial body weights in (g); SBM control, 42.92±0.44; RRSM, 43.31±0.54; RRSM1, 42.80±0.62. Final body weights in (g). SBM control: 2084±48.9; RRSM, 1801±41.09; RRSM1, 2035±43.55.

Table 5. Slaughter variables (g/kg pre-slaughter live weight) in commercial broilers fed raw (RRSM) and processed (PRSM1) rape seed meal (experiment 1)

Treatment	RTC*	Breast*	Liver*	Abdominal fat
SBM control	763.9[a]	214.8[b]	16.26[b]	9.215
RRSM	724.4[b]	205.8[b]	18.96[a]	5.735
PRSM1	768.6[a]	237.3[a]	18.83[a]	7.638
P	0.001	0.001	0.010	0.363
n	9	9	9	9
SEM	5.262	3.768	0.445	0.981

[a,b]Means having no common superscript in a column differ significantly *(P<0.01). RTC, ready to cook yield; RRSM, raw rape seed meal; PRSM1, processed rape seed meal (300 min).

improving the nutritional value of RSM in chicken diet. Though the calculated glucosinolate concentration in groups fed 100 g/kg RSM was similar in broilers fed both RRSM and PRSM1 (27.5 and 28.8 M/kg, respectively), performance depression was observed in RRSM group compared to the PRSM1, which implies that incubating RSM with enzymes might be responsible for improving the performance of broilers fed PRSM1 compared to RRSM fed birds.

Meat yield (RTC and breast) was reduced in broilers fed RRSM and PRSM2 compared to the SBM control group (Tables 5,7). The lower meat yield in these groups might be due to the reduced feeding value of raw and longer incubation of RSM as evident from the reduced retention of energy and protein in these groups. Retention of energy and protein during both starter and finisher phases was also significantly lower in RRSM or PRSM2 compared to the CD fed broilers. The meat yield in groups fed PRSM1 up to 150 g/kg was similar to those fed the SBM CD, which followed the same trend for energy and protein retention.

Liver weight increased progressively with the level of RSM in broiler diet; highest liver weight was observed at 150 g/kg followed by 100 and 50 g/kg, which were heavier than the SBM control group. Increased liver weight might be due to the increased fat deposition in liver, which was associated with reduced bird performance (Amerah *et al.* 2015). Hepatic enlargement in birds fed RSM was reported in the literature (Rama Rao *et al.* 2005). Toxic principles present in the RSM might have interfered with fat metabolism leading to fat accumulation and enlargement of liver (Newkirk and Classen 2002).

Table 6. Performance parameters of commercial broilers fed graded levels of raw (RRSM) and processed (PRSM) rape seed meal (experiment 2)

RSM	Level, g/kg	Week 3			Week 6		
		BWG* (g)	FI* (g/b)	FI/BWG*	BWG* (g)	FI* (g/b)	FI/BWG*
SBM		778.9[a*]	969.2[a]	1.246[d]	2294[a]	3765[a]	1.641[cd]
RRSM	50	701.4[cd]	873.2[cd]	1.245[d]	1952[c]	3215[c]	1.648[cd]
RRSM	100	417.7[f]	561.0[f]	1.342[b]	963[f]	1823[f]	1.895[b]
RRSM	150	306.1[g]	440.6[g]	1.441[a]	664[g]	1497[g]	2.258[a]
PRSM 1	50	758.9[a]	947.2[ab]	1.249[d]	2269[ab]	3597[b]	1.586[e]
PRSM 1	100	772.9[a]	990.4[a]	1.283[cd]	2275[ab]	3711[ab]	1.632[de]
PRSM 1	150	716.4[bc]	911.1[bc]	1.272[d]	2193[b]	3571[b]	1.629[de]
PRSM 2	50	741.5[ab]	944.4[ab]	1.274[d]	2252[ab]	3680[ab]	1.634[de]
PRSM 2	100	670.9[d]	832.1[d]	1.241[d]	1683[d]	2847[d]	1.695[c]
PRSM 2	150	544.8[e]	718.3[e]	1.320[bc]	1236[e]	2358[e]	1.909[b]
SBM		778.9[a]	969.2[a]	1.246[c]	2294[a]	3765[a]	1.641[c]
RRSM		475.1[d]	624.9[c]	1.343[a]	1193[c]	2179[d]	1.934[a]
PRSM 1		749.4[b]	949.6[a]	1.268[bc]	2246[a]	3626[b]	1.616[c]
PRSM 2		652.4[c]	831.6[b]	1.278[b]	1724[b]	2962[c]	1.746[b]
	0	778.9[a]	969.2[a]	1.246[c]	2294[a]	3765[a]	1.641[c]
	50	733.9[b]	921.6[b]	1.256[bc]	2158[b]	3498[b]	1.623[c]
	100	620.5[c]	794.5[c]	1.288[b]	1641[c]	2794[c]	1.741[b]
	150	522.5[d]	690.0[d]	1.345[a]	2294[d]	2475[d]	1.932[a]
SEM		4.254	5.090	0.004	9.691	16.93	0.006
P-Value							
RSM		0.001	0.001	0.001	0.001	0.001	0.001
Levels		0.001	0.001	0.001	0.001	0.001	0.001
RSM×Levels		0.001	0.001	0.001	0.001	0.001	0.001

*[a b c d r f g]Means having no common superscript in a sub-column differ significantly *(P<0.01);
BWG, body weight gain; FI, feed intake; RRSM, raw rape seed meal; PRSM1 and PRSM2, processed rape seed meal (300 and 600 min, respectively).

Experiment 2: The interaction between processing method and level of RSM inclusion significantly (P<0.05) influenced the performance (BWG, FI and FE), RTC yield, relative weight of breast and liver, and retention of energy and protein. The relative weight of abdominal fat was not affected (P>0.05) by the treatments.

Performance: The interaction between the level and processing of RSM on BWG at 21 day of age indicated that the weight gain decreased progressively with increased levels of RSM in broiler diet irrespective of processing methods employed (Table 6). The BWG was lower (P<0.01) in RRSM fed groups even at the lowest inclusion level compared to the SBM group. The FE in broilers fed PRSM1 and 2 was similar to the SBM control group, except those fed PRSM2 at 150 g/kg, which was significantly lower than those fed the CD. Improved performance of broilers fed enzyme incubated RSM might be due to fibre hydrolyzing ability of exogenous enzymes. Similarly, improved FE was also reported (Rama Rao *et al.* 2014) in chickens fed diet supplemented with exogenous enzyme. The performance of broilers fed PRSM2 particularly at higher inclusion levels (100 and 150 g/kg) was significantly lower than those fed PRSM1 at the same inclusion levels in diet. Thus the data suggest the negative influence of longer incubation period employed (PRSM2) while processing the RSM up to 600 min compared to 300 min used for PRSM1. The exact mechanism of performance depression with longer incubation period was not clear. However, it is presumed that the longer incubation period might have exerted negative influence on bird performance through release of products from NSP substrates. The relation between dose response or time duration on the bird performance was well discussed by O'Neill *et al.* (2014). It could be that with longer incubation, the additional cell wall hydrolysis of RSM becomes compromised by the subsequent production of mono-saccharides on longer incubation. In support of such hypothesis, Damen *et al.* (2012) showed that the *in vitro* molecular size reducing ability of some NSP enzymes is dependent on dose and time of incubation. The reduced performance of broilers fed PRSM2, which was incubated for longer (600 min) duration might be due to excess release of monosaccharides, which might have hindered the bird performance due to either their reduced absorption capacity or the increased energy cost of their excretion (O'Neill *et*

Table 7. Slaughter variables (g/kg pre-slaughter live weight) in commercial broilers fed raw (RRSM) and processed (PRSM) rape seed meal (Experiment 2)

RSM	Level (g/kg)	RTC*	Breast*	Liver*	Abdominal fat
SBM	0	804.9^{a}	242.7^{a}	16.22	14.27
RRSM	50	789.3^{a}	226.5^{ab}	19.44	10.74
RRSM	100	741.3^{c}	187.1^{c}	21.51	5.05
RRSM	150	735.8^{c}	180.5^{c}	23.97	9.13
PRSM 1	50	804.6^{a}	242.9^{a}	16.84	10.63
PRSM 1	100	801.0^{a}	244.3^{a}	17.07	11.79
PRSM 1	150	803.8^{a}	243.1^{a}	17.28	9.97
PRSM 2	50	790.0^{a}	222.7^{b}	19.15	10.72
PRSM 2	100	761.4^{b}	216.7^{b}	20.79	7.69
PRSM 2	150	736.5^{c}	187.4^{c}	24.83	8.89
SBM		804.9^{a}	242.7^{a}	16.22^{b}	14.27
RRSM		755.5^{b}	198.0^{b}	21.64^{a}	8.31
PRSM 1		803.1^{a}	243.4^{a}	17.06^{b}	10.80
PRSM 2		762.7^{b}	208.9^{b}	21.59^{a}	9.10
	0	804.9^{a}	242.7^{a}	16.22^{c}	14.27
	50	794.7^{a}	230.7^{b}	18.48^{b}	10.70
	100	767.9^{b}	216.0^{c}	19.79^{b}	8.18
	150	758.7^{b}	203.7^{d}	22.03^{a}	9.33
SEM		1.841	1.859	0.324	0.412
P-Value					
RSM		0.001	0.001	0.001	0.063
Levels		0.001	0.001	0.001	0.065
RSM×Levels		0.001	0.001	0.110	0.093

$^{a\,b\,c\,d}$Means having no common superscript in a sub-column differ significantly *(P<0.01) ; RRSM, raw rape seed meal; PRSM1 and PRSM2, processed rape seed meal (300 and 600 min, respectively).

Table 8. Nutrient retention (g/100 g intake) in commercial broilers fed raw (RRSM) and processed (PRSM) rape seed meal (Experiment 2)

RSM	Levels	19-21 day of age		40-42 day of age	
	(g/kg)	Energy*	Protein*	Energy*	Protein*
SBM	0	86.92^{a}	71.81^{a}	72.40^{b}	50.40^{b}
RRSM	50	87.72^{a}	72.28^{a}	71.79^{bc}	49.83^{bc}
RRSM	100	81.77^{c}	66.17^{b}	63.04^{d}	41.36^{d}
RRSM	150	75.85^{d}	61.79^{c}	52.47^{e}	34.87^{e}
PRSM 1	50	88.71^{a}	72.06^{a}	75.42^{a}	52.63^{a}
PRSM 1	100	86.75^{ab}	70.93^{a}	73.67^{ab}	50.94^{ab}
PRSM 1	150	87.21^{a}	71.75^{a}	73.44^{ab}	50.76^{b}
PRSM 2	50	86.58^{ab}	71.18^{a}	72.51^{b}	50.47^{b}
PRSM 2	100	89.04^{a}	72.74^{a}	69.91^{c}	48.13^{c}
PRSM 2	150	84.50^{b}	66.84^{b}	60.85^{d}	41.13^{d}
SBM		86.92^{a}	71.81^{a}	72.40^{b}	50.40^{a}
RRSM		81.78^{b}	66.75^{c}	62.43^{d}	42.02^{c}
PRSM 1		87.56^{a}	71.58^{ab}	74.18^{a}	51.44^{a}
PRSM 2		86.70^{a}	70.25^{b}	67.76^{c}	46.58^{b}
	0	86.92^{ab}	71.81^{a}	72.40^{a}	50.40^{a}
	50	87.67^{a}	71.84^{a}	73.24^{a}	50.98^{a}
	100	85.85^{b}	69.94^{b}	68.87^{b}	46.81^{b}
	150	82.52^{c}	66.79^{c}	62.25^{c}	42.25^{c}
SEM		0.445	0.403	0.745	0.588
P-Value					
RSM		0.001	0.001	0.001	0.001
Levels		0.001	0.001	0.001	0.001
RSM×Levels		0.001	0.001	0.001	0.001

$^{a\,b\,c\,d\,e}$Means having no common superscript in a sub-column differ significantly *(P<0.01).

al. 2014). Longer incubation period (600 min) might have favoured higher or near complete degradation of pentosans and other NPS components of RSM, by exo-acting enzymes, far from making useful sugars available, might be detrimental to performance (O'Neill *et al.* 2014). The authors also inferred that hydrolysis of all cell wall components in poultry diets may not be beneficial and there was a need to control the NSP hydrolysis with enzymes for optimum efficacy. Therefore, under the condition of the present experiment, incubation of RSM with the NSP hydrolyzing enzymes will be optimum at 300 min and 600 min was detrimental to the nutritional value of RSM. Improved feeding value of RSM observed with enzyme incubation could also be due to the decomposition of glucosinolate with microbial enzymes (Tripathi and Mishra 2007).

The reduced nutritional value of RSM with longer incubation (600 min) was also evident in our present study. Reduced retention of energy (84.5 vs 87.21%) and protein (66.84 vs 71.75%) was observed in broilers fed PRSM2 at 150 g/kg during starter (19–21 day of age) and all levels (60.85 vs 73.44%, 69.91 vs 73.67%, 72.51 vs 75.42% energy; 41.23 vs 50.76, 48.13 vs 50.94, 50.47 vs 52.62% protein at 50, 100 and 150 g/kg diet, respectively) during finisher phase (40–42 day of age) compared to those fed the respective level of PRSM1. Reduced retention of energy and protein in broilers fed RRSM and PRSM2 particularly at 100 and 150 g/kg inclusion in diet compared to the control group which might be due the reduced weight gain and FE (Amerah *et al.* 2015).

Similar to our present experiment, McNeill *et al.* (2004) reported significant growth depression in broiler fed RSM at 100 g/kg in diet. Contrarily, weight gain and FE were not affected in our previous experiment when the conventional RRSM was included up to 18.67% in broiler diets (Rama Rao *et al.* 2005). Similarly, higher inclusion levels (28–32%) were reported to support the bird performance. The reduced performance in the current study with 50 g/kg RRSM might be due to higher glucosinolate content in RRSM (288 mM/g) compared to that used in our previous (Rama Rao *et al.* 2005) study (156 mM/g). Reasons for the performance variation reported in relation to level of RSM inclusion might be due to variations in cultivar, residual oil content, processing conditions of the RSM (Newkirk and Classen 2002) or variation in reference protein used in the CD (Banday and Verma 2003).

Retention of energy and protein in general reduced progressively with inclusion of either RRSM or PRSM2 (Table 8). Retention of these nutrients was not affected due to inclusion of PRSM1 up to 150 g/kg except the protein retention at 100 g/kg PRSM1. As the utilization of energy and protein in PRSM1 was similar to the SBM control group, the performance of broilers fed PRSM1 was almost similar to those fed the CD. Progressive reduction in retention of energy and protein with inclusion level of PRSM2 also suggest the limitation of utilizing this RSM incubated for longer duration at higher inclusion levels (100 and 150 g/kg) in diet. Literature on the effect of enzyme incubation on the feeding value of RSM was limited. However, supplementation of exogenous enzymes to canola meal based diets resulted in a significant improvement in the digestibility of soluble NSP in the jejunum and thereby improved dietary energy utilization (Kocher *et al.* 2000, Amerah *et al.* 2015). Similarly, enzyme (xylanase, amylase and protease) supplementation in maize-SBM diet improved the BWG and FE in broilers, which was attributed to increased ileal digestibility of protein and energy. Enzyme (cellulase, pectinase, xylanase, glucanase, galactanase, mannanase) addition significantly increased NSP digestibility (from 11.1 to 30.1%) and TMEn value (4,172 vs 4,740 kcal/kg) of canola seed (Meng *et al.* 2005, Choct 2006).

Based on the data, it was concluded that the incubation (37°C for 300 min) of rape seed meal with enzymes (cellulase 1,500 IU, xylanase 250 IU and pectinase 125 IU per kg) improved the nutritional value of the RSM based diets as its inclusion improved the utilization of energy and protein. Further, it can be incorporated up to 100 g/kg in the diet without affecting performance and slaughter variables in broiler chicken.

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