Partial replacement of commercial soybean meal with raw, full-fat soybean meal supplemented with varying levels of protease in diets of broiler chickens

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(Received 4 November 2016; Accepted 12 December 2016; First published online 21 December 2016)

Abstract

A 3 * 3 factorial study was used to evaluate the feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) of broilers fed on the test diets. The commercial soybean meal (SBM) was replaced by raw, full-fat soybean meal (RSBM) at 0, 10 or 20%, equivalent to 0, 30 and 60 g/kg of diet, respectively, and the microbial protease was also supplemented in diets at 0.1, 0.2 or 0.3 g/kg, equivalent to ~7500, 15000 and ~22500 PROT/kg of diet, respectively. Microbial phytase (1000 FYT/kg) was uniformly added to each diet, which was fed to six replicate groups, with nine birds per replicate. Samples of test-ingredients and test-diets, after mixing, were also subjected to chemical analysis, prior to being used, to assess the nutritional compositions, particularly anti-nutritional factors. As the result of this study, the analysed concentrations of trypsin inhibitors (TI) in the diets ranging between 1730.5 and 9913.2 TIU/g. Increasing the levels of RSBM in the diets reduced the FI, during 1 to 35 d of age. Except in the starter phase, increasing the level of RSBM had no significant effect on BWG. When protease was added to the diets, the BWG was significantly improved during the periods of 1 - 10 d, 1 - 24 d and 1 - 35 d. Feed efficiency was decreased by increasing the level of RSBM (1-10 d). Neither increasing levels of RSBM nor protease affected the yield of any meat part at d 35. The relative weight of the small intestine at d 10 increased with rising levels of RSBM, but was reduced when protease was added to the diets. In conclusion, commercial SBM could be replaced by RSBM up to 20% in diets for broilers when the test microbial protease is supplemented.

Keywords: Anti-nutritional factors**,** broiler chickens, carcass, growth performance, microbial protease, raw-full-fat soybean meal, trypsin inhibitors.

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Introduction

Soybean meal (SBM) is an excellent source of protein in diets for poultry (Pettersson & Pontoppidan, 2013). However, in addition to the fluctuation in supply and seasonal scarcity in some parts of the world, the price of SBM has been increasing over the years (Shi *et al*., 2012; Pettersson & Pontoppidan, 2013). It is therefore more effective to use full-fat SBM to replace both commercial SBM and the oil in the diets for broilers (Popescu & Criste, 2003). Full-fat soybean meal can be obtained either after heat treatment or from the raw full-fat soybean, which is known to be raw full-fat soybean meal (RSBM) (Erdaw *et al*., 2015a; Erdaw *et al*., 2015b).

The problem with RSBM, however, is the concentration of anti-nutritional factors (ANF) (Chen *et al*., 2013; Erdaw *et al*., 2016b). There are three well-known ANF in soybean - trypsin (protease) inhibitors (TI), lectins, and phytate (Chen *et al*., 2013; Pettersson & Pontoppidan, 2013), among which the most important in raw soybean meals are the TI (Newkirk, 2010; Dourado *et al*., 2011). Friedman & Brandon (2001) reported that protease inhibitors constitute approximately 6% of soya protein, while Pettersson & Pontoppidan (2013) stated that the amount of ANF altogether constituted approximately 5% of the CP fraction of SBM, and large amounts of ANF are concentrated in the seed hull.

The nutritive value of raw full-fat soybean is negatively affected by the presence of ANF (Liu *et al*., 1997; Erdaw *et al*., 2016a), especially by trypsin inhibitors and lectins (Newkirk, 2010). Protein digestibility could be reduced by a multi-faceted action of ANF, including nutrient binding and increase in gut viscosity (Ao, 2011). Various scholars (Liu *et al*., 1997; Newkirk, 2010; Erdaw *et al*., 2015a; Erdaw *et al*., 2015b) have reported that feeding raw SBM with high levels of trypsin inhibitors and lectins negatively affects pancreatic function, the growth of birds, and feed efficiency. Mogridge *et al.* (1996) reported that the consumption of raw

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science http://dx.doi.org/10.4314/sajas.v47i1.10

beans increased the size of the pancreas (0.80 vs 0.37% of live weight) and the duodenum (1.35 vs 1.0 % of live weight) and reduced feed consumption and growth of the chicks (66 vs 97 g/14 d). Similarly, ASA (2004) reported that diets based on raw beans reduced feed consumption and live weight, and decreased the feed conversion indices by 14, 35, and 53%, respectively.

Although treating the soybean seed by heat is the most common strategy to reduce the risks of ANF (Mayorga *et al*., 2011), heating may negatively influence the quality of the product (Căpriţă *et al*., 2010). Therefore, research (Costa *et al*., 2008; Dosković *et al*., 2013) has suggested that supplementation of poultry diets with exogenous enzymes may be effective. Microbial proteases are protein-digesting enzymes that are used in pig and poultry nutrition to break down the stored proteins and proteinaceous anti-nutrients in various plant materials (Ao, 2011; Barletta, 2011).

Supplementation of diets with microbial phytase increases the digestibility of CP and amino acids in plant-based proteins (Barletta, 2011; Guggenbuhl *et al*., 2012). The objectives of this study were to determine the composition of ANF, for example, trypsin inhibitors (TI), urease activities (UA) and nitrogen solubility index (NSI), in the test-ingredients and test-diets, and to evaluate the effects of microbial protease supplementation in diets containing varying levels of RSBM, with the possibility of improving the performance of broiler chickens.

Materials and Methods

The experiment was approved by the Animal Ethics Committee (Authority No: AEC14-005) of the University of New England (UNE), Australia, prior to commencement. The test raw soybean seed was purchased at harvest from a local producer in northern New South Wales, Australia. The raw full-fat seeds were cleaned and hammer-milled to pass through 2 mm sieve size and then used directly to partially replace commercial SBM. Commercial soybean meal (SBM) was obtained from Cargill (Australia) Pty Ltd in New South Wales. The crude protein content of the RSBM was about 38% (see Table 2), which looks lower than the value of its counterpart-SBM. The lower CP content for RSBM, when compared to its counterpart-SBM, might be due to the presence of the oil in it.

The experiment was a 3 * 3 factorial study, with 3 levels of RSBM (commercial SBM was replaced by RSBM at 0, 10, or 20%, equivalent to 0, 30, and 60 g/kg of the diet) and 3 levels of protease (0.1, 0.2, or 0.3 g/kg, equivalent to ~7500, 15000 and 22500 PROT/kg of diet, respectively) from *Nocardiopsis prasine* (Ronozyme® ProAct, DSM Nutritional Products, Australia Pty Ltd). Microbial phytase (1000 FYT/kg, DSM Nutrition Products) was uniformly supplemented across all the diets (Table 1). This experiment had no protease-free dietary treatment. The enzymes were added prior to pelleting at 65 °C. Sub-samples of the test-diets, containing RSBM and enzymes, were then collected and analysed for TI (AOAC, 2006a), protein solubility (KOH) (Araba & Dale, 1990), urease activity (UA) (AOCS, 2006a), and nitrogen solubility index (NSI) (AOCS, 2006b), and are summarised in Table 2 & 3.

The potential efficacy of Ronozyme® ProAct has been demonstrated at dose level of 15000 PROT/ kg feed in chickens for fattening (EFSA, 2009). The activity of 6-phytase is expressed in phytase units (FYT, equivalent to FTU). According to the Applicant, one FYT unit is defined as the amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute under reaction conditions with a phytate concentration of 5.0 mM at pH 5.5 and 37 °C (EFSA, 2016).

A total of 486 day-old Ross 308 male broiler chicks (average initial weight, 40.24 ± 0.097 g), were obtained from a local commercial hatchery (Baiada Poultry Pty. Ltd., Tamworth, Australia). Nine chicks, of uniform body weight, were allocated to each cage (600 x 420 x 23 cm), and each diet was fed to 6 replicate cages from 0 to 35 days of age in climate-controlled rooms. The main reason why the trail was ended at d 35, instead of allowing for a longer period, was to enable achieving the locally required marketable live weight of birds. The feeders were scrubbed and cleaned before diets were provided. The drinkers were also checked from time to time to ensure water supply and cleanliness. The excreta trays were scrubbed and cleaned whenever they were full. The temperature of the rooms was set at 33 °C for the first two days. This temperature was then gradually reduced to 24 °C at 19 days age and this was maintained for the remaining study period. For the first two days, 24 h light was provided. This was reduced to 23 h for the next six consecutive days, followed by 20 h light for the remaining days. Feed was provided *ad libitum* (crumbled for starters and pelleted for both grower and finisher), and the birds had free access to water.

Table 1 Ingredient and composition of basal starter (0 - 10d), grower (10 - 24d) and finisher (24 - 35d) diets

RSBM (g/kg of diet)	Basal diets								
	Starter (0 - 10 d)			Grower (10 - 24 d)			Finisher (24 - 35 d)		
	0	30	60	0	30	60	0	30	60
Ingredients (g/kg)									
Corn (rolled)	599.0	593.8	614.9	607.3	606.7	605.9	612.4	612.0	617.0
Soybean meal	300.0	270.0	240.0	300.0	270.0	240.0	300.0	270.0	240.0
Raw soybean meal	0.0	30.0	60.0	0.0	30.0	60.0	0.0	30.0	60.0
Meat meal	53.0	62.8	43.7	25.0	30.0	35.0	15.0	15.0	15.0
Limestone	10.4	10.0	10.0	8.9	8.3	7.9	9.3	10.0	9.4
Dical Phos	6.5	6.0	7.1	7.5	6.9	6.3	7.9	9.4	8.7
Canola oil	14.0	10.5	6.4	34.7	31.7	28.6	42.6	39.8	37.1
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
L-Lysine	3.0	3.0	3.5	2.4	2.3	2.2	0.5	0.5	0.5
DL-Methionine	3.0	2.2	2.8	3.3	3.3	3.4	3.0	3.5	3.0
Salt	2.1	2.0	2.0	2.5	2.5	2.5	2.0	2.0	2.0
L-Threonine	1.0	1.9	1.7	0.8	0.8	0.8	0.2	0.5	0.2
Sodium bicarbonate	1.2	1.0	1.1	0.76	0.70	0.60	0.2	0.2	0.2
Trace mineral, 0.75kg/mt[1]	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Choline Cl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75	0.5
Vitamins, 0.5 kg/mt[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phytase	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Nutrients (g/kg)									
ME poultry (MJ/kg)	12.59	12.59	12.59	13.18	13.18	13.18	13.39	13.39	13.39
Crude protein	220.6	220.0	220.0	218.8	212.5	212.7	204.6	202.1	199.6
Crude fat	37.4	37.2	37.2	55.1	56.6	58.1	61.9	63.3	64.6
Arginine	14.4	13.8	13.8	13.4	13.4	13.5	13.0	12.9	12.8
Lysine	14.0	13.9	13.9	12.7	12.7	12.7	10.9	10.8	10.7
Methionine	6.2	5.8	5.8	6.3	6.3	6.3	5.9	5.8	5.8
Methionine + cystine	9.4	8.9	8.9	9.4	9.4	9.4	8.9	8.8	8.7
Threonine	8.9	9.4	9.4	8.3	8.3	8.3	7.5	7.5	7.4
Calcium	10.7	10.7	10.7	9.0	9.0	9.0	8.5	8.5	8.5
Phosphorus (avail)	5.4	5.0	5.0	4.5	4.5	4.5	4.2	4.2	4.2
Sodium	1.9	1.6	1.6	1.6	1.6	1.6	1.2	1.2	1.2
Chloride	2.6	2.6	2.6	2.6	2.6	2.6	1.9	1.9	1.9
Choline	1.5	1.3	1.3	1.3	1.3	1.3	1.4	1.3	1.3

[1]Trace mineral supplied per kilogram of diet: Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based- carrier, 128 mg; mineral oil, 3.75 mg.
[2]Vitamin supplied per kilogram of diet: Vitamin A (retinol), 12000 IU; Vitamin D3(cholecalciferol), 5000 IU; Vitamin E (tocopheryl acetate), 75 mg, Vitamin K (menadione), 3 mg; thiamine, 3 mg;riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg;Vitamin B12 (cyanocobalamin), 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg. The dietary treatments were basal diets (SBM was replaced by RSBM at 0, 10 and 20%, equivalent to 0, 30 and 60 g/kg, respectively) and supplemented with proteas at 0.1, 0.2 of 0.3 g/kg, equivalent to ~7500, 15000 and ~22500 PROT/kg of diet, respectively.
RSBM= raw soybean meal. Dical= dicalcium; Choline Cl= Choline Cloride; TiO2= titanium dioxide.

The birds were provided with starter (0 - 10 d), grower (10 - 24 d) and finisher (24 - 35 d) diets (corn-soybean based) formulated to Aviagen standards for Ross 308. Mortality of birds was recorded whenever it occurred. On each of the 10, 24, and 35 days of age, one bird per replicate was randomly selected and killed using cervical dislocation. The weight of the internal organs, such as the gizzard, proventriculus, small intestine, pancreas, liver, spleen and bursa, was recorded on each of the 10, 24, and 35 days of age; the average live weight of the birds was recorded per cage. The leftover feed was also weighed to calculate the FI of the birds for each phase, and the FCR was computed for each cage using the BWG and the FI. Meat yield was measured at 35 days of age. Two birds were randomly selected from each cage and humanely killed by cervical dislocation, scalded, and plucked. The head, legs, and all visceral organs of the birds were then removed, and the dressed carcass was weighed. The main carcass parts, including the breast, drumsticks, thighs, wings, and neck, were then cut out and weighed to evaluate their weights, relative to dressed carcass weight. Meat parts were used with their bones (i.e. no deboned meat part was used in the data). The total N content of ingredient samples (e.g. RSBM, and commercial SBM) and diets containing RSBM was determined by the Dumas' combustion technique (LECO); Official Method 990.03 (Kjeldahl), and 984.13 (A-D), (AOAC, 2006b). The CP content of the sample was derived from the N content using a multiplication factor of 6.25. Available lysine was measured using Method 975.44 (AOAC, 2006c).

Descriptive statistics was used to analyse the data of nutritional composition of the test-ingredient and test-diets. One-way ANOVA and general linear model (GLM) of Minitab software version 17 (Minitab, 2013) were used to compare the mean values of treatments, and main factors (RSBM level and enzyme supplementation, as well as their interactions) of the feeding trail. The differences between the mean values were considered to be significant at *P* <0.05, and these mean values were also separated using Duncan's test.

Results

Nutrient composition and values of quality measuring paramters of RSBM in comparison to the commercial SBM are shown in Tables 2 and 3.

Table 2 Analysed values of nutrient composition and quality measuring parameters of raw soybean meal (RSBM) in comparison to the commercial soybean meal

	Composition (g/kg)							Quality measuring parameters			
	DM	CF	CP	EE	Total Sugars	Total starch	AME, MJ/kg	Avail. lysine	KOH, g/kg	TI, TIU/g	UA, ΔpH
RSBM	923.6	62.0	382.4	147.3	95.0	26.1	12.6	26.4	898.6	13498	2.1
SBM	914.8	37.9	422.9	19.2	107.6	37.0	9.0	28.4	794.4	5743	0.09
	Amino acid composition (mg/g)										
	Met	Lys	Iso	Leu	Cys	Thr	His	Arg	Val	Ala	Try
RSBM	5.6	26.6	17.9	31.0	6.0	16.1	10.9	32.9	18.7	17.3	4.7
SBM	6.2	29.0	20.5	35.6	6.0	18.2	12.1	32.9	21.9	20.3	6.5

DM = dry matter; CF = crude fibre; CP = crude protein; EE = ether extract; AME = calculated value of gross energy; KOH = protein solubility; TI = trypsin inhibitors; UA = urease activities.

As shown in Table 3 the values of TI, NSI and UA were increased from 1747 to 9913.2 TIU/g, 155.3 to 206.1 g/kg and 0.16 to 1.40 ∆pH, respectively as the level of RSBM increased in the diet.

The results of the gross response of the birds, in terms of FI, BWG and FCR, are shown in Table 4. During the period from 1 to 35 d, feed consumption was reduced (*P* <0.01) in line with increasing the level of RSBM in the diets, but this had no similar influence (*P* >0.05) in the early period (1 - 10 d or 1 - 24 d). Increasing the level of protease had no influence (*P* >0.05) on the FI over the entire trial period. The BWG of the birds, during 1 - 10 d was negatively affected (*P* <0.001) by rising an inclusion rate of RSBM in diets. The BWG (during 1 - 24 days) was reduced by up to 3.1% at the highest level of RSBM in the diet, although this was not statistically significant (*P* >0.05).

Table 3 Effect of replacing commercial soybean meal by raw soybean meal in broiler diet (g/kg)

	RSBM in diet (g/kg)		
	0	30	60
Available lysine	16.2	14.8	15.6
Nitrogen solubility index (NSI)	155.3	169.0	206.1
Protein solubility in KOH	670.3	502.5	603.4
Trypsin inhibitors (TIU/g)	1747.3	5881.2	9913.2
Urease activity (∆pH)	0.16	0.60	1.40

Table 4 Response of broiler chickens, in terms of feed intake (g/b), body weight gain (g/b) and feed conversion ratio (FCR) of between hatch and 10, 24 or 35 days of age

RSBM[1] g/kg	Protease g/kg	Feed intake (days)			Body weight gain (days)			FCR (days)		
		1 - 10	1 - 24	1 - 35	1 - 10	1 - 24	1 - 35	1 - 10	1 - 24	1 - 35
	0.1	253.6	1894.7	3897.9	238.1	1411.2	2453.3	1.08	1.36	1.60
0	0.2	261.6	1753.8	3669.6	242.8	1414.3	2474.6	1.07	1.29	1.48
	0.3	267.0	1817.8	3776.0	245.1	1442.0	2562.4	1.07	1.25	1.47
	0.1	264.4	1839.1	3581.4	231.7	1383.1	2348.7	1.09	1.33	1.53
30	0.2	267.6	1747.2	3418.6	241.8	1349.4	2350.7	1.10	1.30	1.46
	0.3	263.3	1734.2	3502.4	242.8	1407.5	2475.2	1.07	1.24	1.42
	0.1	259.9	1735.7	3458.2	219.7	1356.4	2348.4	1.16	1.32	1.48
60	0.2	245.7	1820.6	3586.0	220.1	1361.3	2437.1	1.14	1.31	1.47
	0.3	271.1	1786.1	3529.4	235.9	1418.7	2484.6	1.14	1.26	1.45
Pooled SEM		2.01	15.42	34.73	1.62	8.63	21.43	0.012	0.011	0.022
Main effects										
0		260.7	1822.1	3780.9[a]	242.0[a]	1422.5	2499.3	1.07[b]	1.30	1.52
30		265.1	1773.5	3500.8[b]	238.8[a]	1379.9	2391.6	1.08[b]	1.29	1.47
60		258.9	1780.8	3524.5[b]	225.2[b]	1378.8	2424.2	1.15a	1.29	1.47
	0.1	259.3	1823.2	3631.0	229.8[b]	1383.6[ab]	2381.3	1.11	1.34[b]	1.54
	0.2	258.3	1773.9	3551.5	234.9[ab]	1375.0[b]	2420.8	1.11	1.30[ab]	1.47
	0.3	267.1	1779.3	3602.6	241.3[a]	1422.7[a]	2508.7	1.09	1.25[a]	1.45
Sources of variation										
RSBM		NS	NS	**	***	NS	NS	***	NS	NS
Protease		NS	NS	NS	**	*	0.06	NS	**	NS
RSBM x protease		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b,c] Means bearing uncommon superscript within a column are significantly different;
[1]RSBM = raw soybean; NS= nonsignificant; *P <0.05;**P <0.01;***P<0.001; SEM = pooled standards error of means(SBM was replaced by RSBM at 0, 10 and 20%, equivalent to 0, 30 and 60 g/kg, respectively) and supplemented with proteas at 0.1, 0.2 of 0.3 g/kg, equivalent to ~7500, 15000 and ~22500 PROT/kg of diet, respectively.

Supplementation of the diet with increasing levels of protease improved the BWG during 1 - 10 d (*P* <0.01) and 1 - 24 d (*P* <0.05). Body weight gain also tended (*P* =0.06) to improve over the period from 1 to 35 d in response to protease supplementation. The birds fed on diets containing the highest level of the RSBM grew poorly (*P* <0.01) during the early assessed period (1 - 10 d). Feed efficiency was also reduced by between 0.8 and 3.3%, but this was not significantly affected (*P* >0.05) during 1 - 24 d or 1 - 35d.

Table 5 The relative weights of meat parts (weight of meat part (g)/weight of a carcass (kg)) of broiler fed different diets at 35 d of age

RSBM1 (g/kg)	Protease (g/kg)	Dressed (%)	Relative Weights				
			Breast	Thigh	Drumstick	Wing	Neck
	0.1	75.8	347	139	129	94	54
	0.2	77.6	349	138	127	97	57
	0.3	75.5	344	138	126	96	51
	0.1	75.6	341	135	130	104	54
30	0.2	75.0	344	138	122	102	48
	0.3	75.8	343	136	124	95	53
	0.1	76.4	343	137	127	97	59
60	0.2	75.1	336	139	127	95	54
	0.3	75.1	344	138	125	99	52
Pooled SEM		0.33	2.10	1.21	1.22	1.23	1.33
Main effects							
0		76.3	347	138	127	96	54
30		75.5	343	136	126	100	52
60		75.6	341	138	126	97	55
	0.1	75.9	344	137	129	98	56
	0.2	75.9	343	138	125	98	53
	0.3	75.5	344	137	125	97	52
Sources of variation							
RSBM		NS	NS	NS	NS	NS	NS
Protease		NS	NS	NS	NS	NS	NS
RSBM x protease		NS	NS	NS	NS	NS	NS

Feed efficiency was improved (*P* <0.01) during the 1 - 24 d period in response to increasing the level of protease supplementation. During any of the assessed periods (1 - 10 d, 1 - 24 d or 1 - 35 d), no significant interaction effect was observed on the FI, BWG or FCR between protease and RSBM. There was also no significant (*P* >0.05) difference in the mortality of the birds between treatment groups during the entire study period. Only 23 birds died out of 486, and this was not treatment-specific.

Results of carcass yield and the meat parts of birds at 35 days of age are shown in Table 5. Increasing the level of RSBM in diets decreased (not significantly) the breast meat and drumstick weight by 1.7 and 0.8%, respectively. Increasing the protease level (0.1 to 0.3 g/kg) in the diets reduced the relative weight of drumsticks by 1.0% at 35 d of age. No meat part was affected by interaction between the main factors (RSBM x protease).

As shown in Table 6, the weight of bursa was influenced (*P* = 0.05) by interaction between the main factors (RSBM x protease) in diets, but this interaction had no significant (*P* >0.05) influence on the weight of any other internal organs at 10 d of age. Birds fed diets containing no RSBM (% of RSBM /kg of diets) had the lowest weight of bursa at 10 d of age. Except for the small intestine (P <0.05) and pancreas, increasing the level of RSBM in the diets had no influence (*P* >0.05) on the weight of other internal organs, including the gizzard and proventriculus (G+P), liver, heart, bursa, and spleen at 10 d of age. The weight of the small intestine was also reduced (*P* <0.01) in response to increasing the inclusion rate of microbial protease in diets, particularly at 0.3 g/kg. Increasing the level of protease in diets, had no (P >0.05) influence on the weight of any other internal organs.

Table 6 Weights of visceral organs (g/100 g of body weight) of broiler chickens on different diets at 10 days of age

RSBM[1] g/kg	Protease g/kg	S. intestine	Pancreas	Liver	G+P	Heart	Bursa	Spleen
0	0.1	8.5	0.510	4.3	4.0	0.98	0.15	0.09
	0.2	8.0	0.513	4.0	3.7	0.89	0.15	0.08
	0.3	7.0	0.607	4.3	3.7	0.90	0.16	0.08
30	0.1	8.7	0.645	4.3	3.8	0.10	0.20	0.09
	0.2	7.9	0.590	4.0	3.7	0.94	0.17	0.06
	0.3	8.1	0.605	3.9	3.9	0.99	0.17	0.08
60	0.1	8.7	0.692	4.3	4.0	0.82	0.17	0.09
	0.2	8.7	0.733	3.8	3.7	0.89	0.17	0.08
	0.3	8.3	0.705	4.0	3.7	0.90	0.19	0.08
Pooled SEM		0.02	0.02	0.26	0.28	0.02	0.01	0.00
Main effects								
0		7.8^{b}	0.543^{c}	4.2	3.8	0.92	0.15	0.08
30		8.2^{ab}	0.613^{b}	4.1	3.8	0.96	0.18	0.08
60		8.6^{a}	0.710^{a}	4.0	3.8	0.87	0.18	0.08
	0.1	8.6^{a}	0.616	4.3	3.9	0.92	0.17	0.09
	0.2	8.2^{ab}	0.612	3.9	3.7	0.91	0.16	0.07
	0.3	7.9^{b}	0.639	4.0	3.8	0.93	0.17	0.08
Sources of variation								
RSBM		*	***	NS	NS	NS	NS	NS
Protease		**	NS	NS	NS	NS	NS	NS
RSBM x protease		NS	NS	NS	NS	NS	P=0.05	NS

[a,b,c] Means bearing uncommon superscripts within a column are significantly different at NS= non-significant;* P <0.05; **P <0.01; [1]RSBM = raw soybean meal; SEM= pooled standard error of means; S. intestine= small intestine (duodenum + jejunum and ileum were weighed with their contents); gizzard and provetriculus were emptied (no content inside); G+P = gizzard + proventriculus. (SBM was replaced by RSBM at 0, 10 and 20%, equivalent to 0, 30 and 60 g/kg, respectively, and supplemented with proteas at 0.1, 0.2 of 0.3 g/kg, equivalent to ~7500, 15000 and ~22500 PROT/kg of diet, respectively.)

Increasing the inclusion rate of RSBM, as a main factor had no impact (P >0.05) on the weight of any internal organ at 24 d of age. Supplementation of diets with microbial protease decreased (P <0.01) the weight of the small intestine, but no other organ weight was affected (P >0.05).

As shown in Table 7, neither inclusion of RSBM, protease supplementation, or their interaction had any effect (P >0.05) on visceral organ weights at 24 d, but the weight of the pancreas was reduced (P <0.001) by increasing supplementation of RSBM at both 10 and 24 d of age.

Table 7 The weights of visceral organ (g/100 g of body weight) of broiler chickens on different diets at 24 d of age

RSBM[1] (g/kg)	Protease (g/kg)	S. intestine	Pancreas	Liver	G+P	Heart	Bursa	Spleen
0	0.1	4.4	0.230	2.6	1.9	0.72	0.16	0.10
	0.2	5.2	0.220	2.7	1.9	0.73	0.15	0.10
	0.3	4.6	0.245	2.6	1.9	0.72	0.20	0.10
30	0.1	5.5	0.295	2.5	2.0	0.65	0.18	0.09
	0.2	5.2	0.315	2.7	2.1	0.74	0.17	0.08
	0.3	5.4	0.305	2.6	2.0	0.79	0.17	0.09
60	0.1	5.4	0.357	2.5	2.1	0.71	0.21	0.10
	0.2	5.5	0.340	2.6	2.2	0.75	0.16	0.11
	0.3	5.7	0.327	2.9	2.0	0.76	0.16	0.08
Pooled SEM		0.11	0.01	0.01	0.03	0.02	0.01	0.00
Main effects								
0		4.7	0.232[c]	2.6	1.9	0.73	0.17	0.10
30		5.4	0.305[b]	2.7	2.0	0.72	0.18	0.09
60		5.5	0.341[a]	2.6	2.1	0.75	0.18	0.10
	0.1	5.1	0.294	2.6	2.0	0.70	0.18	0.10
	0.2	5.3	0.292	2.6	2.1	0.74	0.16	0.10
	0.3	5.2	0.292	2.7	1.9	0.75	0.18	0.09
Sources of variation								
RSBM		NS	***	NS	NS	NS	NS	NS
Protease		NS	NS	NS	NS	NS	NS	NS
RSBM x protease		NS	NS	NS	NS	NS	NS	NS

[a,b,c] Means bearing uncommon superscripts within a column are significantly different;
[1]RSBM = raw soybean meal; S. intestine = small intestine (duodenum + jejunum and ileum with the contents); SEM = pooled standard error of means; NS = non-significant; G+P = gizzard + proventriculus.(SBM was replaced by RSBM at 0, 10 and 20%, equivalent to 0, 30 and 60 g/kg, respectively and supplemented with proteas at 0.1, 0.2 of 0.3 g/kg, equivalent to ~7500, 15000 and ~22500 PROT/kg of diet, respectively.)

Discussion

The values of TI, UA and NSI increased in line with increase in levels of RSBM in the diets. These results probably represent the first such data on raw soybeans of Australian origin. Results of this study are also evident on impacts of increasing levels of RSBM supplementation on the concentration of selected ANF in diets of non-ruminant animals. This information may help to know which level of ANF concentration could adversely affect the animals and thereby could help to intervene that reducing the negative impacts of ANF. The results agree with those of Frikha *et al.* (2012) who reported that protein solubility, TI, and UA correlate with the amounts of unheated soybeans in the diet. During the early periods (1 - 10 d and 1 - 24 d), increasing the level of RSBM in the diets had no influence on the FI, but the FI was decreased when considered over the entire trial period (1 - 35 d). This result partially agrees with the result of other researchers (Mogridge *et al*., 1996; ASA, 2004), who reported that birds fed on diets with RSBM experienced low FI. The reason for the reduction in feed consumption in the current study might be due to the negative impact of the ANF, particularly the TI in the RSBM. Feed passage rate, although not assessed in this study could be slowed down by poor pancreatic function in the presence of the TI. Overall, this result gives evidence that when the age of birds advances, the adverse impact of ANF on FI was reduced, which shows that birds may be adopting ANF in diets in the later stages of their growth. The reduction in feed intake directly affected the BWG and feed efficiency, especially in the starter phase (1 - 10 d). Previous researchers (ASA, 2004; Palacios *et al*., 2004; Romero and Plumstead, 2013) have shown that the performance of non-ruminant animals is affected by concentration of dietary TI. Although microbial enzymes, including protease were included right from hatch, they were not able to completely eliminate the negative effects of TI and other ANF that are present in RSBM during the starter phase.

The birds tended to adjust to the factors with age, possibly as digestive function improved or in response to the supplemental enzymes. Body weight gain of birds was therefore not affected when assessed over 1 - 24 d or 1 - 35 d. It has been reported (Rao *et al*., 2013) that broilers adapt to some ANF in the diet, especially with advance in age. It is anticipated that supplemental microbial protease could complement the activities of endogenous proteases. This assumption is based on the fact that increasing the level of the microbial protease in the diets improved the BWG of the birds in the periods of 1 - 10 and 1 - 24 d, and also tended to improve in the period 1 - 35 d. The mechanism on how the microbial protease could reduce the adverse impact of ANF on birds might be through many ways, for example by breaking the larger molecules into smaller ones, which could be at harmless position for birds.

There are reports in literature (Yadav & Sah, 2005; Barletta, 2011, Erdaw *et al*., 2015a) which indicate that microbial proteases are able to break down both stored proteins and proteinaceous anti-nutrients in diets. During the period from 1 to 24 d of this study, feed efficiency was improved in response to supplementation with microbial protease, but this response was weaker during the 1 - 10 d or 1 - 35 d.

Protease supplementation has been shown to improve poultry performance (Cowieson & Adeola, 2005; Rada *et al*., 2014), although most previous tests were conducted on diets containing only commercial SBM. As reported by scholars (Ravindran, 2013; Amerah, 2015), the mechanisms how the BWG and feed efficiency of birds were improved in the current study when supplementing their diets with microbial protease might also be due to the multi-faceted contribution of this enzyme, including complementarity effects with endogenous enzymes, contribution to reducing the gut viscosity, and by increasing the effectiveness of host (endogenous) enzymes and helping to alter the feed passage rate of the animals.

There was no relative meat part of the birds that was affected by partially replacing the commercial SBM with the tested level of RSBM. Most of the measures, including the dressing percentage, breast, thighs and necks, were not affected by inclusion of protease, but the relative weight of drumsticks was increased. These results are in line with the body weight of birds assessed at d 1 - 35, which was not influenced due to supplementing RSBM in diets. This result partially disagrees with Rada *et al.* (2014), who reported that the meat parts of broilers were positively affected by the addition of microbial enzymes but the differences may be due to the nature of basal diets.

The small intestine and pancreas were significantly heavier in response to inclusion of RSBM in the diet. This result partially agrees with other researchers (Mogridge *et al*., 1996; Mayorga *et al*., 2011; Erdaw *et al*., 2015b) who reported that birds fed diets containing RSBM had heavier pancreas and duodenum. Organs associated with digestion and/or absorption may strive to maintain their functions through increase in size although this may be achieved by different mechanisms. As it was confirmed by the same authors (Erdaw *et al*., 2016c), the pancreas was the first vital organ that was being adversely affected by TI in RSBM. The duodenum was also found curling the pancreas, which most likely exposed that the two organs shared some physiological uncertainty. Therefore, duodenum could be the next adversely affected part of the small intestine, in this current study.

Regardless of the level of RSBM in the diets, increasing the inclusion rate of protease significantly reduced the weight of the small intestine during the starter phase. Ao (2011) reported that enzyme supplementation can reduce the negative impact of anti-nutrients. The duodenum was particularly affected in this study because it is the first part of the small intestine and holds the pancreas in place. It is likely that any change in the size of the pancreas would extend to this region of the small intestine. Other visceral organs were largely unaffected possibly because they are not directly involved with digestion or physical contact with the diet. This current result might show an effective strategy to reduce the adverse impacts of ANF, particularly TI on digestive organs of chicks at early age.

When increasing the level of the microbial protease in diets, the BWG was improved and weight of small intestine reduced, but no influence on weight of pancreas at 10 d of age. The results of this study showed that due to the supplementation of microbial enzymes in diets, the vital organs (e.g. pancreas and small intestine) might help to improve the physiological functions (secreting the functional enzymes and changing the intestinal structures) of birds, which generally enables an increase of the nutrient absorption (small intestines) and thereby leads to improved performance of birds, particularly at early age. These all might be mainly influenced by the supplements of microbial protease

Conclusion

Although some of the dietary treatment groups contained the TI concentration beyond the threshold level for non-ruminant animals, supplementation with microbial protease slightly enabled the birds to tolerate up to 20% of the RSBM, replacing the commercial SBM without greatly compromising bird productivity. The RSBM reduced growth during the early period (1 - 10 d), but over time, all treatment groups achieved almost the same BWG. A cost-benefit analysis may be needed, to ascertain the economic benefits, if any, of the inclusion of some RSBM in the diet for broiler chickens.

Acknowledgements

The financial support by DSM, Singapore and University of New England, Australia are gratefully acknowledged. The opinions expressed by the author are his own and do not, in any way, reflect on the sponsor.

Authors' Contributions

MME and PAI were in charge of the experimental design, implementation and writing the manuscript. All authors participated in interpreting and reviewing the results of the study.

Conflict of Interest Declaration

There are no conflicts of interest.

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