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Original Research Article

Nutrient utilisation and growth performance of broiler chickens fed standard or moderately reduced dietary protein diets with and without β-mannanase supplementation

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The use of reduced protein diets in broiler chicken production provides potential benefits for performance and environmental footprint of production. The effectiveness of β-Mannanase supplementation in wheat and soy based standard protein (SP) and reduced protein (RP) diets was tested for growth performance, nutrient utilisation and selected intestinal gene expression of broiler chickens. In a 2 × 2 factorial arrangement of treatments, two main factors included dietary protein (standard and reduced protein) and β-Mannanase supplementation (with or without). All diets contained phytase and carbohydrases (xylanase and glucanase). A total of 480 Ross 308 male off-sex day-old chickens were assigned to the four experimental diets in a 35-d study. Each diet was replicated 12 times with 10 birds per replicate. Using an additional 160 birds, separate apparent metabolizable energy (AME) and nutrient digestibility assays were undertaken for the 4 experimental diets from d 21 to 24 of age. Selected genes involved in gut integrity, inflammation and immune response were quantified using quantitative PCR assays. There was no interaction between β-Mannanase and dietary protein for any of the studied parameters except ileal viscosity. Enzyme had no effect on feed intake but tended to increase body weight gain (BWG) from d 0 to 35 of age ($P = 0.079$). Birds fed RP diet consumed more feed when assessed from d 0 to 35 of age ($P = 0.029$). At the same time, β-Mannanase tended to reduce feed conversion ratio independent of dietary protein ($P = 0.069$). β-Mannanase reduced ileal viscosity of the birds fed RP diet ($P < 0.001$). Reducing dietary protein increased nitrogen retention, nitrogen digestibility coefficient and digestibility coefficients of 11 amino acids ($P < 0.001$). β-Mannanase significantly improved digestibility coefficients of nitrogen and Arg, Gly, Thr, Lys, and Ile ($P < 0.05$). Dietary treatments had no effect on AME or gene expression of selected tight junction proteins, interleukin-10, interleukin-1β, mucin-2 and nuclear factor-kappa B. In conclusion, supplementation of β-Mannanase tended to improve feed efficiency and increased nutrient digestibility of broilers fed wheat-based diets independent of a moderate reduction in dietary protein. Complementary mode of actions of β-Mannanase for intestinal health requires further investigation.

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1. Introduction

The negative implication of non-starch polysaccharides (NSP) contents of cereal grains and leguminous plants is well known to the poultry industry. Depending on the composition of NSP, different type of NSP degrading enzymes have been used to target different substrates present in poultry diets leading to improved

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nutrient utilisation, energy metabolism and performance of broiler chickens.

β-Mannans are components of NSP present in feed ingredients in form of glucomannan and galactomannan and are polysaccharides of D-Mannose units connected by β-(1–4) glycosidic bonds (Kiarie et al., 2021). Compared with cereals, the higher concentration of β-Mannans in soybean meal is reported to be around 1.3% to 2.07% (Bach Knudsen, 1997). β-Mannans, similar to other fibre components are not digested by the endogenous enzymes in the gastrointestinal tract of monogastric animals. In addition to their negative effects on nutrient utilisation and growth performance, the host immune system can recognise β-Mannans as pathogen associated molecular patterns as they are similar to the mannose residues on the surface of most cells with multiple immune and biological functions (Kiarie et al., 2021; Arsenault et al., 2017). Hence, the presence of β-Mannans has been associated with activated immune response at the intestinal level causing energy wastage and retardation of animal performance (Arsenault et al., 2017).

The endo-1,4-β-D-mannanase hydrolyzes β-Mannans into smaller components including mannobiose, mannotriose, and mannose by cleaving the 1,4-β glycosidic bonds (Dhawan and Kaur, 2007; Ferreira et al., 2016). The advantage of supplementation of β-Mannanase on growth performance of broiler chickens is relatively well documented (Ferreira et al., 2016; Latham et al., 2018). However, most of these studies have been conducted using corn-based diets with less research on wheat-based diets. In addition, there is an increasing interest in use of reduced protein (RP) diets in broiler chickens. In the content of RP diets the benefit of β-Mannanase in wheat-based diets is less known particularly when the concentration of soybean meal is reduced as the main source of mannans.

The mode of actions of exogenous β-Mannanase in broiler chickens are multifaceted including indirect suppression of harmful microorganisms, effect on immune response, energy-sparing effect and reducing intestinal viscosity (Shastak et al., 2015). Research in pigs has shown beneficial effect of β-Mannanase on expression of tight junction proteins and therefore potential for enhancing gut integrity particularly in presence of xylanase (Tiwari et al., 2018). It was hypothesized that β-Mannanase may have similar effects in broilers fed wheat and soybean-based diet due to a possible low-grade intestinal inflammation caused by galactomannans in soybean meal and NSP in wheat.

Thus, the current project was conducted to evaluate the effectiveness of an exogenous β-Mannanase for improved growth performance and nutrient digestibility of broiler chickens under fed standard protein (SP) and RP diets. Selected gene expression of tight junction proteins and genes involved in intestinal inflammation were also undertaken to test possible mode of action of β-Mannanase for gut integrity.

2. Materials and methods

2.1. Animal ethics statement

The Animal Ethics Committee of the Department of Primary Industry and Regions, Adelaide, South Australia approved all the experimental procedures of the study (PIRSA AEC 7/21).

2.2. Experimental design and diet preparation

Using a 2 × 2 factorial arrangement of treatments, two diets of SP and RP with and without β-Mannanase (Natupulse TS, 8000 TMU/g, BASF, Germany) were formulated to meet or exceed nutrient specifications of Ross 308 (2019). Ingredients used for the study were analysed for amino acid content and proximate analysis using Bruker MPA II Fourier transform near-infrared spectrometer (Bruker, MA, US) with OPUS 2 software prior to feed formulation (Format Solutions, NJ, US). Diets were wheat and soybean meal based with inclusion of whole canola seed as shown in Table 1. Table 2 contains measured amino acid concentrations of basal experimental grower and finisher diets. The amino acid contents were measured using acid hydrolysis methods conducted by Australian Proteome Analysis Facility (Macquarie University, NSW, Australia). A Leco TruSpec CNS analyser (LECO Corporation, MI, US) was used to quantify the nitrogen (N) content of the diets. The enzyme product was supplemented at 100 g per metric tonne according to the recommendation of the manufacturer (800 TMU/kg feed). All diets contained xylanase (560 TXU/kg feed), glucanase (250 TGU/kg feed) and phytase (1000 FTU/kg feed). For the phytase, the full matrix values were applied according to the manufacturer's recommendation. All grower diets included 5 g/kg titanium dioxide as an indigestible marker for digestibility assays. The experimental diets were prepared and pelleted using a cold-press pelleting machine at SARDI Feedmill, Roseworthy Campus except starter diets that were fed as mash.

2.3. Performance experiment

A 35-d growth performance study was conducted using 480 Ross 308 off-sex one-day-old male broilers (average weight, 38.8 g) in 48 raised floor pens placed in an environmentally controlled broiler shed located in SARDI poultry facilities at Roseworthy Campus, Roseworthy SA, Australia. Each of the 4 experimental treatments was replicated 12 times with 10 birds in each replicate. For the first 7 d of age, birds received a starter diet with or without β-Mannanase which was not reduced in protein to avoid performance losses. Birds that were to receive the enzyme after d 7 of age accordingly received the same enzyme in the first 7 d of age. From d 7 onwards, the treatments were split into SP and RP diets, with and without β-Mannanase supplementation, respectively. The 4 experimental grower diets were assigned to the birds from d 8 to 21 of age and finisher diets were offered from d 22 to 35 of age. Birds were weighed at d 0, 7, 21 and 35 of age. Feed intake was recorded throughout the experiment.

Birds had 24 h lighting in the first day of age followed by 8 h dark and 16 h light from d 2 until the end of the study. Wood shavings were used as bedding material. Feed and water were provided ad libitum throughout the study. Room temperature was kept around 31 °C for the first 2 d of age and then gradually decreased to 23 °C by d 21 thereafter it was kept constant for the remainder of the study.

2.4. Apparent metabolizable energy (AME) and amino acid digestibility assays

An additional 160 one-day-old birds (excluding any spare bird) were obtained at the same time and same conditions as the performance trial and were raised on floor pens and fed experimental diets similar to the performance study. The AME assay was based on standard total excreta collection method performed on grower diets fed to 8 replicate/cages of 5 birds each. Accordingly on d 18, birds were transferred to a total of 32 group cages and were adapted to the 4 experimental diets for 3 d before performing 4 d of total excreta collection from d 21 to 24 of age. At the conclusion of AME assays, on d 25, three birds per cage were euthanised and the ileal contents were collected and pooled. A sub-sample of fresh ileal content was centrifuged at 12,000 × *g* for 10 min at 4 °C to obtain the supernatant. The viscosity was measured on approximately 0.5 mL of thawed supernatant using a Brookfield DV-III viscometer at 25 °C with a CP 40 cone. The shear rate was from

Table 1
Composition and nutrient levels of basal experimental starter, grower, and finisher diets (as is basis, g/kg, unless specified).[1]

Item	Starter	Grower		Finisher	
		SP	RP	SP	RP
Ingredients					
Wheat	492.9	493.5	606.4	508.3	628.4
Barley	100.0	125.0	125.0	150.0	150.0
Canola seed	50.0	70.0	70.0	80.0	80.0
Soybean meal	311.1	258.4	132.1	214.0	99.5
Canola oil	9.2	16.7	0.8	21.5	2.1
L-Lysine HCL	2.6	2.3	6.0	1.9	5.2
DL-Methionine	2.8	2.3	3.3	1.9	2.7
L-Threonine	1.3	1.0	2.7	0.7	2.2
Sodium chloride	1.3	1.3		1.4	0.2
Sodium bicarbonate	4.1	4.1	6.0	3.9	5.7
Limestone	13.5	11.0	11.5	9.0	9.4
Mono-dicalcium phosphate	8.0	6.1	7.3	4.4	5.4
Phytase (Natugrain TS)	0.1	0.1	0.1	0.1	0.1
Carbohydrases (Natuphos E 10 000 G)	0.1	0.1	0.1	0.1	0.1
Choline chloride 60%	1.0	1.0	0.8	0.7	0.7
Titanium dioxide		5.0	5.0		
Premix[2]	2.0	2.0	2.0	2.0	2.0
Sand (inert filler)		0.1	9.3	0.1	0.1
L-Valine 98%			1.7		1.1
L-Arginine			3.3		2.7
L-iso-Leucine 98%			1.7		1.4
L-Leucine 98.5%			1.8		1.0
Glycine			3.1		
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Nutrient levels					
AME, MJ/kg	12.55	12.97	12.97	13.39	13.39
Protein	238.2	220.0	195.0	205.0	180.0
Protein (measured)		215.0	192.1	201.4	174.5
Starch	350.8	364.5	430.4	379.4	449.6
Fat	43.4	57.4	50.9	69.0	50.1
Dig Lys	12.8	11.5	11.5	10.3	10.3
Dig Met + Cys	9.5	8.7	8.7	8.0	8.0
Dig Met	5.9	5.2	5.6	4.6	4.9
Dig Arg	13.7	12.4	12.3	11.4	11.0
Dig Gly	8.4	7.8	9.2	7.2	5.9
Dig Ser	10.0	9.2	7.2	8.5	6.8
Dig Gly equivalent	15.8	14.6	14.6	13.6	11.0
Dig His	5.4	4.9	3.8	4.6	3.6
Dig Phe + Tyr	16.8	15.2	11.5	14.0	10.8
Dig Phe	10.3	9.4	7.3	8.7	6.9
Dig Thr	8.6	7.7	7.7	6.9	6.9
Dig Trp	2.7	2.5	1.9	2.3	1.8
Dig Tyr	7.1	6.4	4.7	5.9	4.4
Dig Val	9.7	9.0	8.7	8.4	7.8
Dig Ile	8.9	8.1	7.8	7.5	7.1
Dig Leu	15.4	14.1	12.7	13.1	11.3
Ca	10.1	8.7	8.7	7.5	7.5
Available P	4.8	4.4	4.4	4.0	4.0
K	9.6	8.6	6.0	7.8	5.5
Na	1.8	1.8	1.8	1.8	1.8
Cl	2.0	2.0	2.0	2.0	2.0
DEB, mEq/kg	266.3	241.1	174.5	221.3	162.6

SP = standard protein; RP = reduced protein; AME = apparent metabolizable energy; DEB = dietary electrolyte balance; Dig = digestible.

[1] The composition β-Mannanase (Natupulse TS) supplemented diets was exactly same except that with the tested enzyme was supplemented at the expense of sand at 0.100 g per kg of diet.

[2] Supplied per kilogram of diets: vitamin A 12,000 IU, vitamin D_3 3000 IU, vitamin E 25 mg, vitamin K_3 3 mg, vitamin B_1, 2 mg, vitamin B_2 6 mg, niacin 45 mg, pantothenate 15 mg, pyridoxine 5 mg, folate 1 mg, cyanocobalamin 16 μg, biotin 150 μg, Cu (sulfate) 10 mg, Fe (sulfate) 60 mg, I (iodide) 1 mg, Se 0.3 mg, Mn (sulfate and oxide) 120 mg, Zn 70 mg, antioxidant 20 mg.

5 to 500/s. The leftover samples were stored at −20 °C and then freeze-dried until used for further analyses. Jejunal tissues were also collected, snap-frozen in liquid nitrogen and stored in −80 °C until used for gene expression analysis. The weight of intestine, liver and fad pad were also recorded and expressed to the bird's body weight.

All ileal digesta samples were freeze-dried and ground through a 0.5-mm screen. The concentration of titanium oxide in the diet and ileal digesta was determined by the method of Short et al. (1996). Amino acid content of diet and digesta was determined by acid hydrolysis method. Samples were analysed for the amino acids by the Australian Proteome Analysis Facility, Macquarie University using similar methods previously described by Macelline et al. (2022). Nitrogen content of diets, digesta and excreta was analysed using a Leco analyser as per the method of AOAC (2005).

The apparent ileal digestibility (AID) coefficients of N and amino acids were calculated using the following formula:

Table 2
Measured concentration of amino acids (g/kg, as is) in basal experimental grower and finisher diets.

Amino acid	Grower		Finisher	
	SP	RP	SP	RP
His	5.7	4.7	5.4	4.5
Ser	10.1	8.0	9.4	7.7
Arg	12.4	11.8	11.6	10.9
Gly	9.0	9.7	8.5	7.1
Aspartic acid	17.9	12.5	16.5	11.8
Glutamic acid	47.5	42.3	45.6	41.1
Thr	8.4	7.9	7.8	7.3
Ala	8.2	6.4	7.7	6.2
Pro	14.9	13.7	14.5	13.5
Lys	11.5	10.8	10.7	10.3
Tyr	5.2	4.0	4.8	3.7
Met	4.4	4.4	3.7	3.9
Val	9.8	9.2	9.3	8.5
Ile	8.7	8.0	8.2	7.5
Leu	14.9	13.1	14.1	12.1
Phe	10.2	8.2	9.6	7.8

SP = standard protein; RP = reduced protein.

$$\text{AID coefficient} = \frac{(\text{NT/Ti})_\text{d} - (\text{NT/Ti})_\text{i}}{(\text{NT/Ti})_\text{d}},$$

where $(\text{NT/Ti})_\text{d}$ is the ratio of nutrient (NT) to titanium (Ti) in diet and $(\text{NT/Ti})_\text{i}$ is the ratio of nutrient (NT) to titanium (Ti) in ileal digesta. Nutrients were either protein (N) or amino acids (16 amino acids).

The AME was calculated as:

$$\text{AME (kcal/kg)} = (\text{GEI}-\text{GEE})/\text{FI},$$

where, GEI is gross energy intake, GEE is gross energy output of excreta (kcal/kg of DM), and FI is the feed intake (kg). The AME values were corrected for N (AMEn kcal/kg) by correcting N retention to zero using the factor of 36.54 kJ/g N retained in the body (Titus et al., 1959).

2.5. RNA extraction, cDNA synthesis and PCR assays

Jejunal tissue samples were processed for homogenisation and RNA extraction as per previously described procedures (Barekatain et al., 2021). The cDNA and PCR assays as well as the sequences of primers used in the study were the same as previously described methods by Barekatain et al. (2021) except primer for claudin 2 which its sequence can be found from Gong et al. (2020). The PCR conditions were as follow: an initial 10 min denaturation at 95 °C followed by 95 °C for 15 s, 40 cycles of 60 °C for 20 s, and 72 °C for 40 s. Two reference genes of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and TATA-box binding protein (*TBP*) were included in the analysis. Data of each targeted gene was divided by *TBP* as the most stable gene to express all the values relative to the reference gene. Four selected tight junction proteins included claudin 1, claudin 2, claudin 3 and zonula occludens-1 (*ZO-1*). Interleukin-1β (*IL-1β*), interleukin-10 (*IL-10*), mucin 2 (*MUC2*), nuclear factor-kappa B (*NF-κB*) and regulatory-associated protein of mTOR (*Raptor*) were also assayed.

2.6. Statistical analysis

All data of the study were analysed by General Linear Model of SAS (9.4). Data were tested for normal distribution. For normally distributed data, two-way ANOVA was used to assess the main effects of dietary protein, enzyme supplementation and their interaction. When a significant difference was detected, means were separated by Least Square Differences test. Nonparametric tests of Scheirer-Ray-Hare were undertaken for any data that were not normally distributed and could not be transformed. Not parametric test was undertaken using the statistical software package R (version 4.2.2 https://www.r-project.org/) and the R companion package. The $P < 0.05$ was set as the level of significance and a tendency was specified for $0.05 \leq P \leq 0.10$.

3. Results

3.1. Growth performance

Table 3 shows the growth performance of broiler chickens. There was no interaction between dietary protein and enzyme supplementation for growth performance parameters. During the first 7 d of age, enzyme had no significant effect on body weight gain (BWG), feed intake or feed conversion ratio (FCR). Dietary protein tended ($P = 0.060$) to increase FCR of birds from d 7 to 21 of age whereas enzyme tended ($P = 0.069$) to reduce it. From d 22 to 35 of age, reduction of dietary protein significantly increased feed intake ($P = 0.002$) and BWG ($P = 0.034$), while FCR remained unaffected. At the same time (d 22 to 35 of age), enzyme effect was not significant for performance parameters. When assessed from d 0 to 35 of age, BWG tended ($P = 0.079$) to increase by supplementation of β-Mannanase with no effect of dietary protein. At the same time, reducing dietary protein significantly increased ($P = 0.029$) feed intake in birds from d 0 to 35 of age while there was no effect of enzyme supplementation. From d 0 to 35 of age, β-Mannanase tended ($P = 0.072$) to decrease the FCR whereas reducing dietary protein significantly increased it ($P = 0.029$).

3.2. Nutrient digestibility, AME, AMEn and ileal viscosity

As shown in Table 4, no interaction was found between dietary protein level and β-Mannanase for N digestibility coefficient, N retention, AME and AMEn ($P > 0.05$). Reducing dietary protein ($P < 0.001$) and enzyme supplementation ($P = 0.039$) independently increased N digestibility coefficient and N retention. AME and AMEn were not affected by dietary protein or enzyme supplementation ($P > 0.05$). Dietary protein and β-Mannanase interacted ($P < 0.001$) for the ileal viscosity where enzyme only reduced the viscosity significantly in birds fed RP diet (Table 4).

Tables 5 and 6 illustrate the results of amino acid digestibility coefficients in birds at d 24 of age. Dietary protein and enzyme did not interact for any of the 16 amino acids ($P > 0.05$). The coefficients of digestibility of His, Asp and Ala were not influenced by dietary treatments ($P > 0.05$). The RP diet increased coefficients of digestibility of Arg ($P < 0.001$), Gly ($P < 0.001$), glutamic acid ($P < 0.001$), Thr ($P < 0.001$), Pro ($P < 0.001$), Lys ($P < 0.001$), Tyr ($P = 0.028$), Met ($P < 0.001$), Val ($P < 0.001$), Ile ($P < 0.001$), Leu ($P < 0.001$) and Phe ($P < 0.001$).

Regardless of dietary protein, β-Mannanase supplementation increased digestibility of Arg ($P = 0.037$), Gly ($P = 0.029$), Thr ($P = 0.017$), Lys ($P = 0.023$), Ile ($P = 0.035$) and tended to increase digestibility of Ser ($P = 0.074$), glutamic acid ($P = 0.068$) and Leu ($P = 0.070$).

3.3. Selective gene expression on jejunal tissues

Table 7 shows the data for relative mRNA expression of selected genes involved in gut integrity and inflammation. There was no interaction between dietary protein and β-Mannanase for any of the studied genes ($P > 0.05$). The relative mRNA expression of

Table 3
Growth performance of broiler chickens fed standard or reduced protein diets with and without β-Mannanase.[1]

Item		BWG, g/bird				Feed intake, g/bird				FCR			
		d 0 to 7	d 7 to 21	d 22 to 35	d 0 to 35	d 0 to 7	d 7 to 21	d 22 to 35	d 0 to 35	d 0 to 7	d 7 to 21	d 22 to 35	d 0 to 35
Treatment													
Protein	β-Mannanase												
SP	−	129	805	1375	2307	137	1025	1957	3116	1.063	1.274	1.425	1.353
SP	+	134	831	1438	2403	140	1052	1997	3189	1.053	1.266	1.391	1.328
RP	−		792	1459	2382		1034	2081	3255		1.306	1.428	1.367
RP	+		816	1457	2405		1039	2061	3240		1.275	1.415	1.347
SEM			7.8	11.7	16.6		8.9	14.3	21.0		0.0062	0.0076	0.0048
Main effects													
SP			818	1406^{b}	2355		1039	1977^{b}	3152^{b}		1.270	1.408	1.340^{b}
RP			804	1457^{a}	2394		1036	2071^{a}	3247^{a}		1.290	1.422	1.356^{a}
β-Mannanase	−		799	1416	2344		1029	2019	3185		1.290	1.426	1.359
	+		823	1447	2404		1045	2028	3214		1.271	1.403	1.337
***P*-value**													
Protein			0.367	0.034	0.246		0.893	0.002	0.029		0.061	0.342	0.029
β-Mannanase		0.175	0.121	0.203	0.079	0.162	0.378	0.747	0.491	0.489	0.069	0.560	0.072
Protein × β-Mannanase			0.962	0.176	0.274		0.548	0.302	0.302		0.083	0.606	0.804

BWG = body weight gain; FCR = feed conversion ratio; SP = standard protein; RP = reduced protein; SEM = pooled standard error of the mean.
[a,b]Means sharing not a common superscript differ significantly at the $P < 0.05$ level.
[1] For the first week of age, all birds received a standard starter diet with and without β-mannanase (Natupulse TS). Each treatment value represents the mean of 12 replicates.

Table 4
Nitrogen digestibility coefficient, nitrogen retention, AME and ileal viscosity of broiler chickens fed standard or reduced protein diets with and without β-Mannanase.[1]

Item		Nitrogen digestibility coefficient	Nitrogen retention, %	AME, MJ/kg	AMEn, MJ/kg	Viscosity, mPa•s
Treatment						
Protein	β-Mannanase					
SP	−	0.826	64.02	13.20	12.40	4.12^{b}
SP	+	0.849	65.28	13.28	12.45	3.90^{b}
RP	−	0.861	69.00	13.34	12.57	6.40^{a}
RP	+	0.869	70.43	13.29	12.49	4.50^{b}
SEM		0.0036	0.277	0.030	0.029	0.119
Main effects						
SP		0.837^{b}	64.6^{b}	13.24	12.42	4.01
RP		0.865^{a}	69.7^{a}	13.33	12.53	5.45
β-Mannanase	−	0.843^{b}	66.5^{b}	13.26	12.48	5.25
	+	0.859^{a}	67.8^{a}	13.28	12.47	4.20
***P*-value**						
Protein		<0.001	<0.001	0.207	0.078	<0.001
β-Mannanase		0.039	0.022	0.737	0.798	<0.001
Protein × β-Mannanase		0.334	0.881	0.263	0.230	<0.001

AME = apparent metabolizable energy; AMEn = nitrogen-corrected apparent metabolizable energy; SP = standard protein; RP = reduced protein; SEM = pooled standard error of the mean.
[a,b]Means sharing not a common superscript differ significantly at the $P < 0.05$ level.
[1] Each value represents the mean of 8 replicates for treatment effects and 16 replicates for main effects.

Table 5
Amino acid digestibility coefficients of broiler chickens fed standard or reduced protein diets with and without β-Mannanase.[1]

Item		His	Ser	Arg	Gly	Aspartic acid	Glutamic acid	Thr	Ala
Main effects									
SP		0.850	0.815	0.873^{b}	0.798^{b}	0.796	0.901^{b}	0.793^{b}	0.811
RP		0.861	0.825	0.898^{a}	0.853^{a}	0.799	0.926^{a}	0.835^{a}	0.818
β-Mannanase	−	0.849	0.812	0.879^{b}	0.815^{b}	0.789	0.909	0.803^{b}	0.808
	+	0.862	0.828	0.892^{a}	0.835^{a}	0.805	0.918	0.825^{a}	0.820
SEM		0.0041	0.0044	0.0029	0.0043	0.0049	0.0023	0.0045	0.0044
***P*-values**									
Protein		0.194	0.256	<0.001	<0.001	0.754	<0.001	<0.001	0.186
β-Mannanase		0.138	0.074	0.037	0.029	0.127	0.069	0.017	0.336
Protein × β-Mannanase		0.196	0.418	0.391	0.333	0.233	0.105	0.427	0.220

SP = standard protein; RP = reduced protein; SEM = pooled standard error of the mean.
[a,b]Means sharing not a common superscript differ significantly at the $P < 0.05$ level.
[1] Each value represents the mean of 8 replicates for treatment effects and 16 replicates for main effects.

Table 6
Amino acid digestibility coefficients of broiler chickens fed standard or reduced protein diets with and without β-Mannanase.[1]

Item		Pro	Lys	Tyr	Met	Val	Ile	Leu	Phe
Main effects									
SP		0.871[b]	0.863[b]	0.824[b]	0.939[b]	0.819[b]	0.831[b]	0.841[b]	0.851[b]
RP		0.901[a]	0.893[a]	0.843[a]	0.951[a]	0.857[a]	0.872[a]	0.875[a]	0.872[a]
β-Mannanase	−	0.879	0.871[b]	0.833	0.943	0.829	0.834[b]	0.852	0.856
	+	0.893	0.885[a]	0.844	0.947	0.846	0.859[a]	0.864	0.867
SEM		0.0031	0.0028	0.0040	0.0018	0.0039	0.0035	0.0032	0.0032
***P*-value**									
Protein		<0.001	<0.001	0.028	<0.001	<0.001	<0.001	<0.001	<0.001
β-Mannanase		0.299	0.023	0.162	0.326	0.126	0.035	0.070	0.220
Protein × β-Mannanase		0.317	0.446	0.705	0.181	0.291	0.249	0.277	0.258

SP = standard protein; RP = reduced protein; SEM = pooled standard error of the mean.
[a,b]Means sharing not a common superscript differ significantly at the $P < 0.05$ level.
[1] Each value represents the mean of 8 replicates for treatment effects and 16 replicates for main effects.

Table 7
Relative mRNA expression of selected gene in jejunum of broiler chickens fed standard or reduced protein diets with and without β-Mannanase.[1]

Items		Claudin 1	Claudin 2	Claudin 3	*ZO-1*	*IL-10*	*MUC 2*	*IL-1β*	*NF-κB*	*Raptor*
Main effects										
SP		0.462	1.637	0.457	0.897	0.298	0.474	0.492	1.798	0.685
RP		0.438	1.436	0.470	0.869	0.336	0.533	0.559	1.835	0.679
β-Mannanase	−	0.448	1.595	0.479	0.878	0.588	0.507	0.521	1.748	0.680
	+	0.451	1.479	0.448	0.888	0.360	0.487	0.530	1.887	0.684
SEM		0.0165	0.0548	0.0165	0.0214	0.0810	0.0223	0.0260	0.0411	0.0252
***P*-value**										
CP		0.461	0.078	0.712	0.512	0.708	0.290	0.201	0.662	0.913
β-Mannanase		0.937	0.288	0.347	0.829	0.054	0.637	0.860	0.103	0.927
Protein × β-Mannanase		0.331	0.615	0.331	0.804	0.614	0.581	0.281	0.756	0.501

ZO-1 = zonula occludens-1; *IL-10* = interleukin-10; *MUC 2* = mucin-2; *IL-1β* = inleleukin-1β; *NF-κB* = nuclear factor- κB; *Raptor* = regulatory-associated protein of mTOR; SP = standard protein; RP = reduced protein; SEM = pooled standard error of the mean.
[1] Each value represents the mean of 16 replicates for main effects.

claudin 1, claudin 3, *ZO-1*, *MUC2*, *IL-1β* and *Raptor* was not affected by either dietary protein or enzyme supplementation ($P > 0.05$). Reducing dietary protein tended ($P = 0.078$) to downregulate the relative mRNA expression of claudin 2 independent of β-Mannanase supplementation. With no effect of dietary protein, β-Mannanase tended ($P = 0.054$) to reduce *IL-10* relative mRNA expression.

3.4. Relative weight of organs and fat pad

As shown in Table 8, there was no interaction between dietary protein and enzyme for any of the organs or fat pad weights ($P > 0.05$). The relative weights of gizzard, duodenum and fat pad were not affected by dietary treatments ($P > 0.05$). Feeding birds with RP diets, regardless of enzyme, led to a heavier liver ($P < 0.001$) and a lighter ileum ($P < 0.001$) than birds fed SP diets. Supplementation of β-Mannanase reduced ($P = 0.017$) the relative weight of jejunum independent of dietary protein.

Table 8
Relative weight of organs and fat pad (g/100 g BW) in broiler chickens fed standard or reduced protein diets with and without β-Mannanase.[1]

Items		Gizzard	Liver	Duodenum	Jejunum	Ileum	Fat pad
Main effects							
SP		1.75	2.09[b]	0.81	1.68	1.12[a]	0.93
RP		1.78	3.09[a]	0.82	1.59	1.03[b]	1.01
β-Mannanase	−	1.76	2.82	0.84	1.69[a]	1.09	0.94
	+	1.77	2.92	0.79	1.57[b]	1.07	1.00
SEM		0.028	0.038	0.024	0.026	0.017	0.027
***P*-value**							
Protein		0.596	<0.001	0.745	0.105	<0.001	0.166
β-Mannanase		0.875	0.190	0.365	0.017	0.566	0.248
Protein × β-Mannanase		0.155	0.542	0.079	0.286	0.354	0.208

SP = standard protein; RP = reduced protein; SEM = pooled standard error of the mean.
[a,b]Means sharing not a common superscript differ significantly at the $P < 0.05$ level.
[1] Each value represents the mean of 16 replicates for each main effect.

4. Discussion

Birds grew to the expected Ross 308 performance standards at d 35 of age regardless of the dietary treatments with a superior FCR compared with the breed standard at the time (Aviagen, 2019b). The lack of interaction between β-Mannanase and dietary protein may be most likely because only a moderate reduction of dietary protein was adopted for RP diet and as such the compositional difference between RP and SP diets may have not been large enough to trigger different response to the supplemented β-Mannanase. The tendency for approximately 2 points of FCR improvement in birds resulted from β-Mannanase supplementation is in agreement with a Meta-analysis performed by Kiarie et al. (2021). Similarly, the lack of β-Mannanase effect on feed consumption concurred with the same review paper (Kiarie et al., 2021). Noteworthy, the effects of β-Mannanase were studied in presence of phytase (1000 FTU/kg feed), xylanase and glucanase already included in all the diets which may have limited the magnitude of the response to this enzyme considering a range of response to feed enzymes being additive, sub-additive or synergistic (Ravindran, 2013). Albeit only through a tendency, the beneficial effect of β-Mannanase on BWG still appeared to be more pronounced in SP diet with higher level of soybean meal due to higher substrate

availability. In hindsight, the recovery of the supplemented enzymes should have been undertaken in this study to confirm the intended activity of the supplemented enzymes in particular β-Mannanase. At the time of writing, such analysis was no longer feasible due to significant time passed from when the experiment was conducted. Nevertheless, the enzymes were added as per recommended dosage and other analysis of the diets including amino acids points to the accuracy of the feed preparation process. Noteworthy, increased feed intake and BWG resulted from reducing dietary protein highlights the benefits of including synthetic amino acids that are more readily available for absorption in the birds. A moderate reduction in dietary protein (2 to 3 percentage units) can be of benefit to the body weight of broilers as also shown in a previous study using wheat-based diets (Barekatain et al., 2019). The slight increase in FCR of birds fed RP diet may have been most likely the reflection of increased feed intake and possibly structural difference of the diets rather than nutrient utilisation that was positively affected. Accordingly, there were independent improvements in N digestibility coefficient, N retention and 11 amino acid digestibility coefficients in birds fed RP diets compared with SP diets. These observations can be explained by assumed 100% digestibility of synthetic amino acids more available in RP diet compared with SP diet (Liu et al., 2021). The improvement in amino acid digestibility was observed even though the diets were formulated based on digestible amino acid basis. Such observations are consistent with other studies as reviewed by Chrystal et al. (2020). Another probable cause for increased amino acid digestibility would be the attenuation of endogenous amino acid flows as dietary protein is reduced (Ravindran et al., 2008). Further, the higher concentration of soybean meal and associated β-Mannans in SP diet can have negative impact on nutrient digestibility compared with RP diet.

In this study, β-Mannanase supplementation improved N and some amino acid digestibility coefficients regardless of the level of dietary protein. Although not in wheat-based diets, our results are in agreement with some other studies (Ferreira et al., 2016; Yaqoob et al., 2022). The benefits of β-Mannanase supplementation for nutrient utilisation are perceived to be associated with reduced viscosity, release of trapped nutrients by increasing cell permeability, increased digestive enzyme activities as well as improvement in morphological architecture of the gastrointestinal tract (Yaqoob et al., 2022).

One of the main modes of action of β-Mannanase is to reduce intestinal viscosity that can in return enhance nutrient absorption and intestinal ecosystem (Latham et al., 2018). In our study, β-Mannanase interacted with dietary protein and thereby was able to reduce intestinal viscosity in birds fed RP diet that had higher level of wheat compared with standard protein diet. The exact mechanism for this interaction cannot be explained by the data of this experiment. Nevertheless, the digesta viscosity of birds fed SP diets supplemented with β-Mannanase was still numerically lower than unsupplemented diet and it may be possible that an increased wheat dietary level and its arabinoxylan concentration in RP diet may have been the main cause of the observed interaction for ileal viscosity.

In this study, β-Mannanase or dietary protein had no effect on AME or AMEn of the diet. This observation may largely be explained by the fact that the diets used in the study had been formulated based on Ross 308 specification (Aviagen, 2019a) which have been higher than the revised specification later released for the same breed of birds (Aviagen, 2022). β-Mannanase supplementation has been shown to be more effective in uplifting AME in birds fed low energy diets compared with those fed high energy diets (Kiarie et al., 2021).

Independent decrease in relative weight of jejunum in birds fed diets supplemented with β-Mannanase corroborates the results obtained for other carbohydrases used in wheat and soybean meal-based diets (Gao et al., 2008). Presence of β-Mannans and in broader terms, NSP increase viscosity of the intestinal content, decreases substrate diffusion as well as digestive enzyme activities that can lead to significant changes in structure and function of digestive organs. To adapt to such changes, hypertrophy of digestive tract occurs to increase digestive functions and secretory mechanisms (Gao et al., 2008). Addition of β-Mannanase counteracts such processes and as such a reduced relative weight of intestinal segments can be explained.

No effect on AME of diets highlights the possibility of the effect of β-Mannanase on other mode of actions such as gut health, immune response or intestinal microbial profile. As β-Mannans or β-Galactomannans are present on the surface component of various pathogens, they can induce the innate immune system in a process that is energy consuming and wasteful. This phenomenon is often referred to as feed induced immune response (FIIR) (Arsenault et al., 2017). In our study, we undertook selective mRNA expression on jejunal tissue for genes involved in gut integrity, inflammation and mucin production to ascertain any direct effect of β-Mannanase on gut integrity at least at molecular level. Some positive effects of β-Mannanase on biological pathways related to intestinal barrier including tight junctions and adherent junctions have been documented in broilers fed corn-based diets using kinome analysis (Arsenault et al., 2017). Tight junction proteins control paracellular permeability and are highly dynamic and constantly remodelled in response to physiological development or mucosal inflammation (Capaldo et al., 2014). These proteins respond to a wide range of stimuli, stressors and antinutritional factors and nutrients with possible implications for intestinal inflammation. In the present study, β-Mannanase did not significantly change the expression of tight junction proteins either barrier forming tight junction proteins (claudin 1 and claudin 3) or pore-forming (claudin 2). There is no direct study to compare these results in birds fed wheat-based diets.

The expression of *IL-10* was undertaken due to its role in the regulation of gut integrity and in particular tight junction proteins (Al-Sadi et al., 2010). However, there was still a tendency to reduce mRNA expression of *IL-10*, an anti-inflammatory cytokine, in birds fed diets supplemented with β-Mannanase. This is the first time that IL-10 is studied with regards to β-Mannanase supplementation in broilers. IL-10 has protective functions against intestinal inflammation models such as colitis (Wei et al., 2020) and it was not expected for β-Mannanase to reduce *IL-10* expression. In the present study, there was not strong evidence of FIIR due to lack of changes in expression of the selected tight junction proteins, IL-1β and NF-κB in response to changing the level of soybean meal and protein in the diets. Therefore, further analysis on quantification of the protein rather than mRNA is warranted to better explain the effect of β-Mannanase on intestinal functions. It may also be possible that the birds used in the study may have been well adapted to the dietary treatments in particular enzyme that was present in the diet for the entire period of study. Given that the wheat comprised around 50% of the standard grower diet and more than 60% of the RP diet, it is probable that all the birds may have had an underlying gut dysbiosis to a level that a moderate reduction in soybean meal could not induce changes in tight junction proteins or possible intestinal inflammation to a statistically significant level. Future studies need to specifically study the microbiota composition in birds fed wheat-based diets supplemented with β-Mannanase. β-Mannanase activity can produce a range of oligosaccharides and free sugars that may have prebiotic effects

when used as fermentation substrates in the hindgut and affect the intestinal microbiota composition (Bortoluzzi et al., 2019).

5. Conclusion

It can be deduced that supplementation of β-Mannanase benefits nutrient utilisation of broiler chickens and tends to improve feed efficiency regardless of a moderate reduction in dietary protein and in presence of phytase and carbohydrases that are commonly added to wheat-based diets. In our study, no clear association of gut integrity related genes with dietary protein or enzyme supplementation was found. This may indicate that mode of action of β-Mannanase in birds fed wheat-based diets may have been associated with mechanisms not specifically studied here including changes in microbiota composition, indirect prebiotic effects or other metabolic and immune pathways.

Credit author statement

Reza Barekatain: Conceptualization, Investigation, Formal Analysis, Data Curation, Resources, Project administration, Writing-Original Draft. **Leon Hall:** Funding acquisition, Methodology, Writing-Review & Editing. **Peter V. Chrystal:** Conceptualization, Methodology, Writing-Review & Editing. **Anna Fickler:** Methodology, Writing-Review & Editing.

Declaration of competing interest

Leon Hall and Anna Fickler are employees of the BASF company that funded the project. Other authors have no completing interest to declare.

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