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Response of Broiler Chickens to Triticale-Based Diets Supplemented with Microbial Enzymes (2. Microbial Profiles and Activities)

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Abstract
The microbial profiles and activities of microbes in the digesta from chickens on diets containing Bogong or Canobolas with or without inclusion of microbial enzymes were investigated on the 384 day-old male Ross 308 broiler chickens. There was a reduction in population of *C. perfringens* ($P < 0.01$) of the ileum as a result of interaction between cultivar and xylanase. On the other hand, in the ceca, the population of entero bacteria were reduced ($P < 0.01$) by the interaction between grain and phytase, as well as the interaction between grain, xylanase and phytase. Also, the inclusion of xylanase reduced the population of *C. perfringens* in the ceca. The cecal concentration of acetic acid ($P < 0.05$) and butyric plus isobutyricacid ($P < 0.01$) was increased in birds received Bogong and Canobolas diets. Supplementation of xylanase in Bogong diets increased (grain x xylanase, $P < 0.05$) the acetic acid and lactic acid concentration in ceca while the reversed was the case for Canobolas diets. The ileal and cecal pH was not affected by grain, the inclusion of xylanase and phytase or interactions between these factors. It can be concluded that diets containing Bogong or Canobolas diets supplemented with phytase and xylanase influenced the microbial profile and their activities in gastrointestinal tract which may be due to the variation in nutrient content of these two cultivars.

Introduction

In the trials conducted by South Australian Research and Development Institute, Bogong and Canobolas were found to be the highest yielding new cultivars of triticale (Crouch and Saunders, 2009). In the previous work (Widodo *et al.*, 2015), it shown that diets based on these two new cultivars were supported excellent production of broiler chickens. The primary constraint to the use of triticale in poultry diets is the presence of non-starch polysaccharides (NSP), especially xylans and arabinoxylans, as well as phytate, which together reduce nutrient digestibility (Antoniou and Marquardt, 1981).

The inclusion of xylanase and phytase in diets based on Bogong and Canobolas has already been shown to improve the nutritive value of such diets and improve the productivity of broiler chickens (Widodo *et al.*, 2011). Feed enzymes are known to reduce the bacterial activity in the ileum by reducing the amount of nutrient available for microbial fermentation (Silva and Smithard, 2002). Jamroz *et al.* (2002) suggested that the end-products of microbial fermentation (e.g., short-chain fatty acids; SCFA) in the chicken intestine may contribute energy to the host bird. In addition, Ricke (2003) and van der Wielen *et al.* (2000) reported that dietary

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enzymes may play an important role in regulating the gastrointestinal tract (GIT) microbial population. The concentrations of SCFA and lactic acid in the GIT depend on the cereal type and feed enzymes used (Jamroz *et al*., 2002; Silva and Smithard, 2002; Józefiak *et al*., 2004a; 2004b) and reflect to a certain extent the activity of the resident microflora (Engberg *et al*., 2002).

The aim of this work was to investigate how feeding of diets based on triticale, cultivar Bogong and Canobolas, and supplemented with xylanase and phytase influence the gastrointestinal ecosystems of broiler chickens in terms of microbial profiles, concentrations of organic acids and pH.

Materials and Methods

Experimental design and bird management

The experiment was approved by the Animal Ethics Committee of the University of New England, Australia (Approval No. AEC 10/098).

Table 1. Ingredients and nutrient composition (g/kg) of dietary treatments

Ingredients	B	BX	BP	BXP	C	CX	CP	CXP
Bogong	650.0	650.0	650.0	650.0	-	-	-	-
Canobolas	-	-	-	-	650.0	650.0	650.0	650.0
Soybean Meal	190.0	190.0	190.0	190.0	190.0	190.0	190.0	190.0
Soycomil K	69.4	69.4	69.4	69.4	61.3	61.2	61.2	61.2
L-Threonine	1.8	1.8	1.8	1.8	1.9	1.9	1.9	1.9
L-Lysine HCl	4.8	4.8	4.8	4.8	5.3	5.3	5.3	5.3
DL-Methionine	2.6	2.6	2.6	2.6	3.0	3.0	3.0	3.0
Sunflower oil	35.7	35.7	35.5	35.3	42.6	42.6	42.5	42.4
Limestone	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1
Dical. P	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8
Common Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline Cl-70%	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5
Xylanase	-	0.1	-	0.1	-	0.1	-	0.1
Phytase	-	-	0.2	0.2	-	-	0.2	0.2
Premix[1]	2.6	2.6	2.6	2.6	2.5	2.5	2.5	2.5
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient composition								
ME (Kcal/kg)	3033	3033	3033	3033	3081	3081	3081	3081
Crude protein	220.0	220.0	220.0	220.0	220.0	220.0	220.0	220.0
Crude fat	53.8	53.7	53.6	53.5	59.4	59.3	59.2	59.1
Crude fiber	25.5	25.5	25.5	25.5	25.2	25.2	25.2	25.2
Lysine	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Methionine	6.1	6.1	6.1	6.1	6.5	6.5	6.5	6.5
Met + Cys	10.5	10.8	10.8	10.8	11.3	11.3	11.3	11.3
Calcium	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Available P	5.2	5.2	5.2	5.2	5.6	5.6	5.6	5.6
Sodium	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chlorine	5.6	5.6	5.6	5.6	3.4	3.4	3.4	3.4

[1] Supplied per kg of diet (mg): vitamin A (as all-trans retinol): 3.6; cholecalciferol: 0.09; vitamin E (as d-α-tocopherol): 44.7, vitamin K_3: 2.0; thiamine: 2.0; riboflavin: 6.0; pyridoxine hydrochloride: 5.0, vitamin B_{12}: 0.2, biotin: 0.1, niacin: 50.1, D-calcium pantothenate: 12.0, folic acid: 2.0, Mn: 80.0, Fe: 60.0, Cu: 8.0, I: 1.0, Co: 0.3, and Mo: 1.0.

Bird management and treatments

The experiment was conducted in a 2 × 2 × 2 factorial arrangement. Eight experimental diets were formulated to study the effect of 2 cultivars of high-yielding triticale (Bogong and Canobolas), with or without xylanase and with or without phytase on intestinal microbial profile and activities. The experimental diets contained 65% of either Bogong or Canobolas. The microbial phytase, Quantum® 2500, which is a 6-phytase from *E. coli* (AB Vista, Marlborough, UK) was added to supply 500 FTU per kg diet. The xylanase preparation, Econase® XT, which contains thermostable endo-1,4-beta-xylanase, produced by *Trichoderma reesei*, was added to supply 160,000 BXU of xylanase activity. In all diets, titanium dioxide (TiO_2) was incorporated as an indigestible marker to enable assessment of nutrient digestibility. All diets were pelleted after mixing and fed up to 21 days of age. The experimental diets were formulated to meet the minimum Aviagen breeder recommendations

(Aviagen, 2007). The details of the ingredients and nutrients composition of all diets are described in Table 1. The dietary treatments were as follows: a diet based on Bogong without any enzymes (B); Bogong with the inclusion of xylanase (BX); Bogong with the inclusion of phytase (BP); Bogong with the inclusion of xylanase and phytase (BXP); Canobolas without enzymes (C); Canobolas with the inclusion of xylanase (CX); Canobolas with the inclusion of phytase (CP), and Canobolas with the inclusion of xylanase and phytase (CXP).

A total of 384 day-old male Ross 308 broiler chicks (Baiada Poultry Pty. Ltd, Tamworth, NSW, Australia), weighing 41.30 ± 0.35 g, were randomly assigned to eight treatments. Each of the treatments had six replicates, with 8 birds per replicate cage. The experimental chickens were raised in battery brooders, 60 × 42 × 23 cm, set up in a climate-controlled room. The birds were initially brooded at a temperature of 34°C, which was reduced gradually to 24 ± 1°C at 21 days of age when the feeding trial ended. Light was provided for 18 h per day throughout the trial. The birds had free access to water and feed.

On day 21, three birds from each cage, were randomly selected, weighed and killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and removed. The contents of the ileum and ceca were squeezed and collected in plastic containers and pooled by cage for the determination of SCFA. Around 1 g of ileal and cecal contents were also collected separately into prepared McCartney bottles containing anaerobic broth for the enumeration of microbial populations. The McCartney bottles and plastic containers containing digesta samples for microbial profiles and SCFA concentrations, respectively, were kept at –20°C until they were analyzed.

Gut microbial population

Approximately 1 g of sample of either ileal or cecal digesta was placed into a pre-weighed McCartney bottle containing 8 mL of anaerobic broth and 1 mL of reducing (R) solution. After addition of sample to the anaerobic broth, the bottle with broth and sample was weighed again. Once the sample was weighed, the first dilution of the sample was calculated by subtracting the weight of the bottle and the broth plus the R solution from the final weight of the bottle containing the sample (this is the sample weight) and dividing the sample weight by the broth plus R solution. This calculation yield is the proportion of the original sample per mL of solution in the bottle. The suspension was homogenized for 2 min in CO_2-flushed plastic bags using a bag mixer (Interscience, St. Norm, France) and then serially diluted in 10-fold increments in anaerobic broth according to the procedure described by Miller and Wolin (1974) and Engberg *et al.* (2004). A 1 mL aliquot of the homogenized suspension was then transferred into 9 mL of anaerobic broth and serially diluted from 10^{-1} to 10^{-5} for ileum samples and 10^{-1} to 10^{-6} for the cecal samples. From the last three diluted samples, 0.1 mL of each sample was plated on the appropriate medium (10 mL) for enumeration of microbial populations.

The enumeration of anaerobic bacteria was done by following the method described by Miller and Wolin (1974) and Engberg *et al*. (2004). Total anaerobic bacteria were grown in anaerobic roll tubes containing 3 mL of Wilkins-Chalgren anaerobic agar (Oxoid, CM 0619) incubated at 39°C for 7 days. Lactic acid bacteria were enumerated after incubation on MRS agar (Oxoid, CM0361) under anaerobic conditions at 39°C for 48 h. Lactobacilli were enumerated on Rogossa agar (Oxoid, CM 0627) after anaerobic incubation at 39°C for 48 h. Enterobacteria were incubated on MacConkey agar (Oxoid, CM 0007) aerobically at 39°C for 24 h prior to counting. *Clostridium perfringens* (Cp) colonies were counted on Tryptose-Sulfite-Cycloserine and Shahidi-Ferguson Perfringens agar base (TSC & SFP) (Oxoid, CM 0587 OPSP) mixed with egg yolk emulsion (Oxoid, SR0047) and Perfringens (TSC) selective supplement (Oxoid, SR0088E) according to the pour-plate technique, where plates were overlaid with the same agar after spreading the inoculum and incubated anaerobically at 39°C for 24 h. An anaerobic Anaero Gen™ sachet (AN0025A, Oxoid Ltd, Hampshire, UK) was used to generate the anaerobic environment (<1% O_2 and 9–13% CO_2) for all anaerobically incubated agar plates. After incubation, colonies formed on the respective media were carefully counted, converted into logarithmic equivalents (log10) and expressed as numbers of colony-forming units (CFU) per gram of wet ileal or cecal digesta.

Measurement of organic acid

A modification of the analytical method described by Jensen *et al*. (1995) for the analysis of organic acids (SCFAs, lactic acid and succinic acid) concentrations was used. In general, frozen

ileal and cecal samples were thawed and homogenized by vigorous shaking. About 1 to 2 g of ileal and cecal digesta (wet weight) were accurately weighed into centrifuge tubes (placed on ice) and 1 mL internal standard (0.01 methyl butyric acid) was added and thoroughly mixed with a vortex mixer, followed by centrifugation at 25,700 × g at 5°C for 20 min in a Beckman model J2-21M Induction Drive Centrifuge with a JA-21 rotor. The supernatant (approximately 1 mL) was transferred into 8-mL vials (on ice). At this stage, the standards and blanks were prepared. One mL of the standard acid mixture was accurately transferred to the 8-mL vials. The blank was treated similarly (1 mL of Milli-Q water), and 0.1 mL of 0.1 M ethyl butyric acid was added to the standards and blank vials. Two mL of ether and 0.5 mL of concentrated HCl (36%) were then added to all the samples, the standard and the blank and the lids were tightened prior to vortexing for about 1 minute. After repeat vortexing, all the tubes were centrifuged at 2060 × **g** for 15 min at 5°C. Accurately, 400 µL of the supernatant were transferred into gas chromatograph (GC) vials (2 mL) and mixed with 40 µL of N-tert-butyldimethylsilyl-N-methyl trifluoroacetamide (MTBSTFA) or 50 µL MTBSTFA for the standard. The GC vials were tightly capped, vortexed and kept on a heating block at 80°C for 20 min and then left at room temperature for 48 h. After 48 h, concentrations of the different organic acids were determined, using a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA).

Intestinal pH

The pH of ileal and cecal digesta was measured on fresh samples collected at 21 d of age. The pH was determined by the modified procedure of Corrier *et al*. (1990). Around 1 g of the content was diluted in 9 mL of cold distilled MilliQ water. The suspension was mixed thoroughly with a stirrer and the pH was determined by insertion of a glass electrode (EcoScan 5/6 pH meter, Eutech Instruments Pte Ltd., Singapore).

Statistical analysis

All data were analyzed by ANOVA using the general linear model (GLM) procedure of Minitab® Version 16 (Minitab, 2010) for the main factors (cultivar, xylanase, and phytase) and the interactions between these three factors. The significance of difference between means was determined by Fisher's least significant difference (LSD) test, for which the significance level was set at $P < 0.05$.

Results

Gut microflora

The populations of total anaerobic bacteria (TAB), lactobacilli, and Enterobacteria in the ileal digesta were not affected ($P > 0.05$) by grain cultivar, inclusion of xylanase or phytase (Table 2). An interaction between grain cultivar, xylanase and phytase was significant ($P < 0.01$) in the case of the population of lactic acid bacteria (LAB), tending to rise with the inclusion of both enzymes. A grain × xylanase interaction ($P < 0.01$) was noticed on *C. perfringens* population, which in Canobolas-based diet was higher than in Bogong-based diet. Furthermore, there was an interaction ($P = 0.067$) between xylanase and phytase on the population of *C. perfringens*, although there were opposite trends inBogong- and Canobolas-based diets. In Bogong-based diets, the population of *C. perfringens* was less than on the control diet while it was higher in Canobolas-based diet.

In the cecaldigesta, the grain, xylanase and the phytase inclusion did not affect the population of TAB, LAB, and lactobacilli (Table 3). Meanwhile, the inclusion of xylanase decreased ($P < 0.01$) the population of *C. perfringens*. An interaction between grain and phytase was noticed in the population of enterobacteria ($P < 0.05$) and *C. perfringens* ($P = 0.055$). The population of enterobacteria was less in Bogong diet plus phytase than the Bogong diet without enzyme, however; it was opposite in the Canobolas diet, which the Canobolas diet plus phytase had higher population of enterobacteria than the Canobolas diet without enzyme. An interaction ($P < 0.01$) between grain, xylanase and phytase inclusion indicated that the population of enterobacteria that in Bogong diet decreased the population, on the contrary, it increased in Canobolas diet. The interaction between grain, xylanase and phytase inclusion tended ($P = 0.074$) to decrease the population of *C. perfringens* in both diets with the inclusion of xylanase and phytase.

Table 2. Bacterial counts (log_{10} CFU/g digesta) in ileal digesta of broiler chickens triticale-based diets with or without enzyme supplementation[1]

Treatments							
Grain	Xyl[2]	Phy[3]	Total Anaerobic	Lactic acid	Lactobacilli	Entero-bacteria	*C. perfringens*
Bogong	-	-	7.1	8.0^{abc}	7.1	4.6	4.3^{abc}
Bogong	+	-	7.1	8.2^{ab}	7.3	4.7	4.3^{abc}
Bogong	-	+	7.0	8.1^{abc}	7.1	4.8	4.5^{ab}
Bogong	+	+	7.1	7.8^{bc}	7.1	4.8	3.9^{c}
Canobolas	-	-	7.1	8.5^{a}	7.1	4.6	4.1^{bc}
Canobolas	+	-	7.1	7.6^{c}	7.1	4.8	4.8^{a}
Canobolas	-	+	7.0	8.2^{ab}	7.1	4.8	4.2^{bc}
Canobolas	+	+	7.0	8.4^{a}	7.1	5.0	4.5^{ab}
Pooled SEM[4]			0.08	0.07	0.08	0.04	0.07
Source of variation			Significance of treatment effect				
Grain			ns	ns	Ns	ns	ns
Xylanase			ns	ns	Ns	ns	ns
Phytase			ns	ns	Ns	ns	ns
Grain × Xylanase			ns	ns	Ns	ns	**
Grain × Phytase			ns	ns	Ns	ns	ns
Xylanase × Phytase			ns	ns	Ns	ns	0.067
Grain × Xylanase × Phytase			ns	**	Ns	ns	ns

[1] Each value represents the mean of 6 replicates; [2] Xylanase; [3] Phytase; [4] *SEM* = Standard error of means.
[a-c] Values with unlike superscripts within each column are significantly different at **P< 0.01; ns = not significant.

Table 3. Bacterial counts (log10 CFU/g digesta) in cecal digesta of broiler chickens fed triticale-based diets with or without enzyme supplementation[1]

Treatments							
Grain	Xyl[2]	Phy[3]	Total Anaerobic	Lactic acid	Lactobacilli	Entero-bacteria	*C. perfringens*
Bogong	-	-	8.8	9.4	7.8	8.1^{a}	6.1^{a}
Bogong	+	-	8.8	9.3	7.7	8.0^{abc}	5.8^{bc}
Bogong	-	+	8.7	9.4	7.9	7.8^{bcd}	6.0^{ab}
Bogong	+	+	8.7	9.3	7.9	7.8^{abcd}	5.9^{abc}
Canobolas	-	-	8.7	9.4	7.7	7.7^{d}	6.1^{a}
Canobolas	+	-	8.7	9.4	7.9	8.1^{a}	6.0^{a}
Canobolas	-	+	8.7	9.3	7.8	8.0^{ab}	6.0^{a}
Canobolas	+	+	8.7	9.3	7.8	7.8^{cd}	5.7^{c}
Pooled SEM[4]			0.05	0.04	0.04	0.04	0.03
Source of variation			Significance of treatment effect				
Grain			ns	ns	Ns	ns	ns
Xylanase			ns	ns	Ns	ns	**
Phytase			ns	ns	Ns	ns	ns
Grain × Xylanase			ns	ns	Ns	ns	ns
Grain × Phytase			ns	ns	Ns	*	0.055
Xylanase × Phytase			ns	ns	Ns	0.066	ns
Grain × Xylanase × Phytase			ns	ns	Ns	**	0.074

[1]Each value represents the mean of 6 replicates; [2]Xylanase; [3]Phytase; [4]*SEM* = Standard error of means.
[a-d]Values with unlike superscripts within each column are significantly different at *P< 0.05; **P< 0.01; ns = not significant.

Short-chain fatty acids, lactic acid and succinic acid

The concentrations of formic, acetic, butyric, lactic, succinic acids and lactic acid of ileal are shown in Table 4. There was no significant difference in concentrations of formic, lactic and succinic acids, but acetic acid concentration decreased (P <0.01) in birds on diets supplemented with phytase and was numerically reduced (P =0.066) by the inclusion of xylanase in both Bogong- and Canobolas-based diets.

Table 4. Concentrations of various short-chain acids (µmol/g wet digesta) in ileal contents of broiler chickens on triticale-based diets with or without enzyme supplementation[1]

Treatments						
Grain	Xyl[2]	Phy[3]	Formic	Acetic	Lactic	Succinic
Bogong	-	-	0.31	1.6^{ab}	22.6	0.35
Bogong	+	-	0.34	1.4^{abc}	17.1	0.24
Bogong	-	+	0.31	1.2^{bc}	16.0	0.83
Bogong	+	+	0.31	1.0^{c}	19.2	0.44
Canobolas	-	-	0.43	1.8^{a}	15.3	0.19
Canobolas	+	-	0.30	1.4^{abc}	11.9	0.34
Canobolas	-	+	0.37	1.2^{bc}	12.4	0.35
Canobolas	+	+	0.25	0.9^{c}	13.8	0.11
Pooled SEM[4]			0.015	0.08	1.08	0.07
Source of variation			Significance of treatment effect			
Grain			ns	ns	ns	ns
Xylanase			ns	0.066	ns	ns
Phytase			ns	**	ns	ns
Grain × Xylanase			ns	ns	ns	ns
Grain × Phytase			ns	ns	ns	ns
Xylanase × Phytase			ns	ns	ns	ns
Grain ×Xylanase × Phytase			ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates; [2]Xylanase; [3]Phytase; [4]*SEM* = Standard error of means.
[a-c]Values with unlike superscripts within each column are significantly different at **P< 0.01; ns = not significant.

Table 5. Concentration of various organic and mineral acids (µmol/g wet digesta) in cecal contents of broiler chickens on high-yielding triticale-based diet with and without enzymes[1]

Treatments								
Grain	Xyl[2]	Phy[3]	Acetic	Propionic	Butyric + Isobutyric	Valeric + Isovaleric	Lactic	Succinic
Bogong	-	-	76.2^{c}	7.2^{bc}	19.8^{bc}	1.6^{b}	9.1^{ab}	25.2
Bogong	+	-	133.3^{ab}	6.4^{bc}	30.8^{ab}	1.8^{ab}	19.4^{a}	38.5
Bogong	-	+	104.2^{bc}	9.8^{ab}	21.7^{bc}	2.0^{ab}	4.7^{b}	34.8
Bogong	+	+	169.3^{a}	12.5^{a}	37.2^{a}	2.7^{a}	18.7^{a}	51.2
Canobolas	-	-	103.8^{bc}	7.4^{bc}	13.4^{c}	1.6^{b}	7.4^{ab}	52.5
Canobolas	+	-	107.5^{bc}	4.6^{c}	18.6^{c}	1.1^{b}	1.2^{b}	30.5
Canobolas	-	+	100.6^{bc}	6.9^{bc}	17.9^{c}	1.6^{b}	2.0^{b}	33.7
Canobolas	+	+	98.2^{bc}	3.7^{c}	20.2^{bc}	1.2^{b}	1.2^{b}	35.7
Pooled SEM[4]			7.09	0.63	1.74	0.13	1.72	3.54
Source of variation			Significance of treatment effect					
Grain			ns	**	**	*	**	ns
Xylanase			*	ns	**	ns	ns	ns
Phytase			ns	ns	ns	ns	ns	ns
Grain × Xylanase			*	0.081	ns	0.058	*	ns
Grain × Phytase			ns	*	ns	ns	ns	ns
Xylanase × Phytase			ns	ns	ns	ns	ns	ns
Grain × Xylanase × Phytase			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates; [2]Xylanase; [3]Phytase; [4]*SEM* = Standard error of means.
[a-c]Values with unlike superscripts within each column are significantly different at *P< 0.05; **P< 0.01; ns = not significant.

Various organic and mineral acids concentration

In the cecal contents, the organic acids recovered were acetic, propionic, butyric and isobutyric, valeric and isovaleric, as well as lactic and succinic acids (Table 5). The grain factor affected (P < 0.01) the concentration of propionic, butyric plus isobutyric and lactic acid, which the concentration of that acids was higher in Bogong diets than in Canobolas diets. In addition, the concentration of valeric plus isovaleric acid was higher (P < 0.05) in Bogong diets than in Canobolas diets. The inclusion of xylanase in both diets significantly increased acetic acid concentration (P < 0.05) and butyric plus isobutyric concentration (P < 0.01). There was no effect (P > 0.05) of phytase inclusion on the concentration of organic acids.

A grain × xylanase interaction ($P < 0.05$) indicated that xylanase inclusion increased acetic acid and lactic acid concentration in Bogong diets but decreased in Canobolas diets. Meanwhile, the interaction between grain and xylanase ($P = 0.081$) and ($P = 0.58$) affected the concentration of propionic acid and valeric plus isovaleric acid, respectively. In addition, an interaction ($P < 0.05$) between grain and phytase inclusion was noticed in the concentration of propionic acid, which in Bogong diet with phytase was higher than Bogong diet without enzyme, on the other hand, in Canobolas diet plus phytase, the concentration was less than the Canobolas diet without enzyme.

Intestinal pH

There were no significant effects of grain, the inclusion of xylanase and phytase or interactions between these factors for both ileal and cecal pH (Fig. 1); however, the pH of the cecal content tended ($P = 0.07$) to be higher on Canobolas than Bogong-based diets. Furthermore, the cecal pH was numerically reduced by the inclusion of xylanase as well as the combination of supplemental xylanase and phytase.

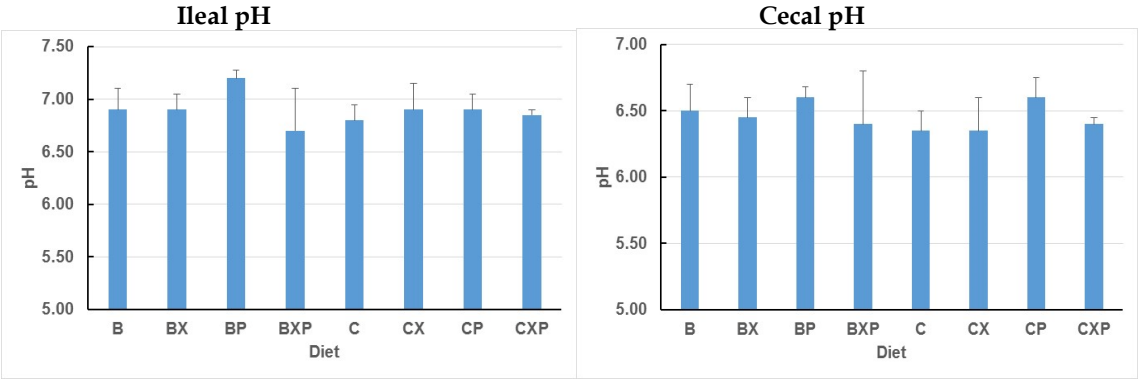

Figure 1. pH of ileal and cecal contents of broiler chickens on triticale-based diets with or without enzymes supplementation.

Bogong without any enzymes (B); Bogong with the inclusion of xylanase (BX); Bogong with the inclusion of phytase (BP); Bogong with the inclusion of xylanase and phytase (BXP); Canobolas without enzymes (C); Canobolas with the inclusion of xylanase (CX); Canobolas with the inclusion of phytase (CP) and Canobolas with the inclusion of xylanase and phytase (CXP)

Discussion

Microbial profiles and activities

In this study, the interaction between grain, xylanse and phytase significantly influenced the count of LAB in ileum. Birds fed the Canobolas diets supplemented with both enzymes showed the highest count of LAB population in the ileum compared to Bogong diet with both enzymes. This result indicates that grain cultivar rather than enzyme type influenced the LAB population in ileum and similar observation was reported in a previous study where NSP composition in grain ingredients influenced the GIT microbial profile (Apajalahti *et al.*, 2004). The presence of high concentrations of both soluble and insoluble NSP, especially arabinose and xylose in Canobolas variety may be the reason for this increase LAB concentration. Soluble NSP increases the digesta viscosity whereas insoluble NSP reduce digesta passage rate by slowing down intestinal motility and hindering the access of endogenous enzymes for respective substrate (Bedford and Schulze, 1998) may lead to increase in microbial population in the GIT (Hubener *et al.*, 2002). In contrast, the lowest number of LAB population in the CX diets than Canoboloas diet with or without both enzymes is partly consistent with results of other studies (Engberg *et al.*, 2004) where xylanase supplementation not only increased LAB population in ileum but also the lactic acid concentration in ceca. However, this is not completely true for the present study as the concentration of lactic acid in ceca was low in the all Canobolas diets than Bogong diets. The reason behind this is not clear.

In the ceca, the population of Enterobacter was reduced in birds fed Bogong diets with phytase supplementation than those fed the Canobolas diet with phytase and this can be explained by the variation in P level in

aforementioned diets. It has been reported that phytase can regulate bacterial growth in intestine but its effect strictly depends on dietary Ca and P levels or Ca:P ratio (Ptak *et al*., 2015). A study reported that supplementation of phytase to low P diet improved the absorption of available phosphorus and Ca in distal ileum and reduced the availability of Ca and P in the ceca (Metzler-Zebeli *et al*., 2008). Because of the involvement of Ca and P in bacterial growth, the reduction of these two minerals consequently reduces the proliferation of certain bacteria (Metzler-Zebeli *et al*., 2010).

Regardless of grain cultivar, phytase supplementation significantly reduced the acetic acid concentration in ileum. In contrast, Ptak *et al*. (2015) reported that phytase supplementation increased the concentration of lactic and acetic acids in ileum only with a diet containing reduced level of Ca (8 g/kg) and digestible P levels (2.69 g/kg). It has been reported that formation of calcium phosphate complex (Ptak *et al*., 2015) or low P concentration could reduce the fermentation process in the GIT (Komisarczuk *et al*., 1987; Smulikowska *et al*., 2010). Therefore, it is possible that the highest level of dietary Ca (11.1 g/kg) and P (5.2 and 5.6 g/kg in Bogong and Canobolas, respectively) in the diets of the present study could facilitate the formation of Ca-phosphate complex, resulting in depletion of available P due to impaired phytase activity and subsequently reduce the microbial fermentation process in the ileum. The concentration of lactic acid in ceca was increased in birds fed Bognog diets with xylanase or xylanase + phytase than on Canobolas diets. The presence of high concentration of lactic acid in ceca indicates that xylanase supplementation did not completely digest the NSP, therefore, undigested carbohydrate from ileum would reach the ceca and be fermented by cecal microflora (Choct *et al*., 1999; Bedford, 2000).

Although SCFA produced in the ceca can yield only relatively small amounts of energy, they can provide other benefits. It has been reported that high fermentation activity in the ceca of chickens correlates with a lower pH, which has the potential to inhibit some pathogenic bacteria (Russell, 1992; van der Wielen *et al*., 2000). In addition, McHan and Shotts (1993) reported the toxic effect of SCFA on some enterobacteria such as *Salmonella typhimurium*. Using *in vitro* technique, they showed that the presence of SCFA could reduce the numbers of these bacteria by up to 50–80% but there was no such trend observed in the present study. In addition, the presence of certain SCFA in the chicken gut, such as lactic and acetic acids, have been associated with a decrease in the survival and adherence of *Salmonella enterica*, *E. coli* and *C. perfringens* (Engberg *et al*., 2002; Engberg *et al*., 2004; Bjerrum *et al*., 2005). Józefiak *et al*. (2004b) reported that lactic acid is the main by-product of carbohydrate fermentation, which is produced by lactic acid bacteria. Lactic acid has been found to be important to broiler gut health and human food safety because its presence can inhibit enterobacteria (Bjerrum *et al*., 2005). In this study, it was found that the population of enterobacteria and *C. perfringens,* which are considered to be risk groups, is generally smaller than that of lactobacilli that are regarded as beneficial bacteria; however, the increased population of enterobacteria and *C. perfringens* was undoubtedly affected by the supplemental enzymes on the diets. There were no symptoms of necrotic enteritis in this experiment that could be connected to the population of *C. perfringens*. Moreover, the mortality on this experiment was negligible, less than 1% and occurring in the first 7 d with no indications of infectious diseases.

Conclusion

The lactic acid bacteria and *C. perfringens* in the ileum and enterobacteria and *C. perfringens* in the ceca were significantly affected by the dietary treatments. Furthermore, concentrations of organic acids in the digesta were affected by grain type and enzyme supplementation. The population of gram negative bacterial in the digesta was significantly reduced by the inclusion of xylanase and the combination of xylanase and phytase; however, the values were higher on the Bogong-based diets than on the Canobolas-based diets. It is not known if this difference contributed in any way to the difference in performance.

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