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Efficacy of Xylanase Supplementation Produced from *Thermoascus aurantiacus* SL16W in Diet on Thai Native Chicken Performance

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Abstract: The aim of this study was to determine the efficacy of xylanase supplementation in the diet on Thai native chicken performance. 36 birds (12 birds /group) were divided into three groups. The first group was administered a control diet and the second and third group were fed an experimental diet with two different levels, 10g/kg and 30g/kg, respectively. Live weight, feed intake, survivability the primary toxicity effect on organ weights and plasma biochemistries were recorded and evaluated. The results indicate that xylanase supplementation improves Thai native chicken performance by increasing live weight and decreasing feed conversion efficiency (FCE) and has no effect on survivability. Xylanase supplementation lead to a decreased blood urea nitrogen (BUN) level in 30g/kg diet, but was a slightly increased serum glutamic pyruvic transaminase (SGPT) levels. In addition xylanase supplementation had no effect on the internal organs. Therefore, it can be concluded that xylanase supplementation efficiently can improve Thai native chicken.

Key words: *Thermoascus aurantiacus*, Xylanase, NSPs, exogenous enzyme, animal feed enzyme

Introduction

T. aurantiacus SL16W can produce high quality of thermostable xylanase. It has been shown that it can potentially be used in the development of industrial enzymatic diet supplement as a hydrolysis agent of non-starch polysaccharides (NSPs) which are an important problem in monogastric animal digestion system. Most of the feed ingredients of standard diets contain non-digested part (Hemicellulose) and some anti-nutritive factors which inhibit food intake utilization and growth (Bhat, 2000). NSPs have a negative effect on feeding digestibility (Bedford, 2000). Recently, xylanase was used successfully in the diet of monogastric animals (e.g. pigs and poultry) that lack enzymes for the hydrolysis of NSPs (Choct *et al.*, 1995). Nevertheless, many microbial strains (e.g. fungi, bacteria and actinomycetes) can produce xylanase any times (Kalogerris *et al.*, 2003). But, the microbial fungal strains have attracted the attention of researchers in recent years due to their potential application in the animal feeding industry because so far only pelleted food is widely used. Food production processes undergo high temperatures to yield pelleted food. The property of the enzyme for these applications is an important criterion. The suitable enzyme for using in feed industry should have high thermal stability to withstand the high temperature during the manufacturing process and subsequently be active in the gastrointestinal tract of the animal (Marquardt *et al.*, 1996; Bhat, 2000; Bedford, 2000). Thus, the thermophilic fungus is the best microbial strain for these properties. In this study we used *Thermoascus aurantiacus* SL16W isolated from

soil samples in Chiang Mai province, Thailand. It has been reported that this strain produces high quality of thermostable xylanase when grown in SSC (solid state culture) (Kasinubon, 2004). This enzyme is characterized by low pH and heat tolerance, therefore exhibiting the potential for use as an industrial feed enzyme. Kongbuntad *et al.* (2004a; 2004b) investigated that crude xylanase extracted from *T. aurantiacus* SL16W had no toxic effects on albino rats by *in vivo* toxicity tests. However, the effect of xylanase as a diet component extracted from *T. aurantiacus* SL16W has not yet been hitherto studied. Therefore, we investigated the efficacy of xylanase supplement in diet on Thai native chicken performance. The factors measured were weight gain or loss, feed conversion efficiency, survival and toxic effect on internal organ and blood biochemistry.

Materials and Methods

Microbial strain preparation: The fungal strain used in this study was *T. aurantiacus* SL16W. It was isolated from a soil sample in Chiang Mai province, Thailand and kindly provided from Assoc. Prof. Dr. Saisamorn Lumyong, Department of Biology, Faculty of Science, Chiang Mai University. The fungus was grown on potato dextrose agar (PDA) at 45-50°C for 3 to 4 days and stocked at 4°C. Stock cultures were transferred to fresh medium every 10 to 15 days.

Solid state culture (SSC) preparation

Carbon sources preparation: The fresh corncob was cut and washed with tap water. It was boiled three times for 30 minutes removing the sugar content by decanting

Table 1: Composition and proximate analyzed of SSC inoculated with *T. aurantiacus* SL16W

Parameters	Proximate analysis of solid state culture	
	Non-inoculated	Inoculated
Xylanase activity (unit/ml)		179-190
Heavy metals (mg/kg)		
-Pb	NT	<0.03
-Cu	NT	0.53
-Cd	NT	0.02
-Fe	NT	0.84
-As	NT	0.01
Mycotoxin (as aflatoxin)		
-Aflatoxin type B1	ND	ND
-Aflatoxin type B1	ND	ND
-Aflatoxin type G2	ND	ND
-Aflatoxin type G1	ND	ND
Proximate analyzed		
-Dry matter (DM) (g/kg)	36.19	29.11
-Crude protein (g/kg DM)	9.7	10.55
-Crude fat (g/kg DM)	1.48	0.73
-Crude fiber (g/kg DM)	7.03	4.9
-Ash (g/kg DM)	2.17	2.44
-Calcium (g/kg DM)	0.24	0.07
-Phosphorus (g/kg DM)	0.43	0.51
-Energy cross dry matter (cal/kg)	4430	4515
-Ash in acid (AIA) (g/kg DM)	0.11	0.13

LOD (Limit of Detection) for aflatoxin type B1=0.05 µg/kg, B2 = 0.09 µg/kg, G1 = 0.24µg/kg and G2 = 0.07µg/kg, ND = Not Detected, NT = Not Test

the water. Subsequently, the corncob was dried in a hot air oven at 80°C until a constant dry weight was achieved. The dried corncob was ground up in a Hammer mill and passed through a 0.7 mm diameter sieve by a meshing instrument. b) Inorganic nitrogen source: Ammonium phosphate was used as the nitrogen source. The concentrations were calculated to provide 0.06 g nitrogen per gram of corncob. SSC samples were determined for proximate analysis and randomly selected from other flasks at day 0 (non-cultivated) and day 7 (cultivated) (Table 1). As mycotoxins may result from contaminated raw materials; enzyme preparation process and/or mycotoxins may be produced after incubation. Therefore, the mycotoxins of the SSC were determined as only of four types (B1, B2, G1 and G2) randomly from other flasks at day 7 of inoculation. The results of aflatoxin were measured by immunoaffinity columns and the HPLC method based on Sharma and Marquez (2001) with the cooperation of the Laboratory Center for Food and Agricultural Products Co., Ltd.: LCFA and Laboratory Service Department Chiang Mai office, Thailand. The limit of detection (LOD) of aflatoxin type B1, B2, G1 and G2 are 0.05, 0.09, 0.24 and 0.07 µg/kg respectively. Analyzed results did not confirm of aflatoxin in the crude xylanase. These analyses show that the crude enzyme preparation processes did not develop microbial contamination and that this fungus strain did not produce mycotoxins when cultured in the SSC (Table 1).

Enzyme assays: Xylanase activity was determined using 0.5% oat spelt xylan (Sigma, USA), carboxymethyl cellulose and locust bean gum in 100 mM citrate buffer pH 4.8 as substrate. The mixture of substrate and crude enzyme was incubated at 60°C for 10 minutes consequently, the release of reducing sugars was determined using the 3, 5-dinitro salicylic acid (DNS) method with D-xylose as standard sugars. One unit (U) of enzyme activity was required to liberate 1 µmole of product per minute under assay condition.

Experimental design and procedures: Two-week old Thai native chicken, were obtained from Chalernpokkapun Co., Ltd.; CP company, Thailand. The experiments were conducted at the Huai Hong Khrai Royal Development Study Center, poultry livestock research unit, Chiang Mai province, Thailand. The birds were reared on the floor providing floor space 1, 000 cm²/bird. Fresh and dry rice husk were used as litter at a layer of about 3-5 cm. The pen was equipped with one tube feeder and one tube water. The birds were exposed to 24 hours light per day throughout the experiment. The diet was formulated to meet nutritional recommendations (NRC, 1994) and was fed in mashed form. All diets were formulated for two phases (starter diet, 2 to 5 week and finisher diet, 6 week to finish). The amount of feed ingredients and nutrient requirement were the same in all diets but they differed in enzyme supplement concentration. The control diet contained no enzyme. The other two diets contained two different levels of SSC, 10 g/kg and 30g/kg, respectively. All experimental birds were generally treated and managed throughout the experimental period. Complete Randomize Design (CRD) was used as experimental design. The total birds were divided into three groups and were assigned to three dietary treatments. Each diet was fed to 36 birds, all birds in each treatment group were chosen at random applying a complete randomized design. Live weight, feed intake, survival were recorded to calculate the FCE and other factors. At the end of the experimental period, potentially toxic effects were evaluated and the toxicity effect assessed. The internal organs were rapidly removed and weighted. The relative organ weights were calculated by g/ % kg body weight (bw) of each bird. Furthermore, blood biochemistry, SGPT; ALT, serum glutamic oxaloacetic transaminase(SGOT; AST), BUN and creatinine(Crea) concentrations were measured. Both data were measured by an automated method (Synchron C5X, Beckman) with the cooperation of Clinical Chemistry Department, Faculty of Associated Medical Science, Chiang Mai University, Thailand.

Statistical analysis: Statistical tests were performed using SPSS software version 11. 5. The data were analyzed by analysis of variance (ANOVA) to test the

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Table 2: Percent of ingredient used in Thai native chicken starter diet, phase I

Materials	Percent of ingredient used in Thai native chicken starter diet (0 to 5 week)		
	Control diet(CD) (without enzyme)	CD+10g enzyme	CD+30g enzyme
Corn meal	52	52	50
Rice brain	12	11.9	11.9
Soybean meal(45% cp)	25.1	25.1	25.1
Fish meal (66% cp)	6	5.99	5.65
Shell meal	0.8	0.8	0.8
Dicalcium phosphate	0.4	0.4	0.4
Tallow	3	2.11	2.45
DL-methionine	0.1	0.1	0.1
Salt	0.35	0.35	0.35
Enzyme ¹	-	+	++
Vitamin and mineral premix ²	0.25	0.25	0.25
Total	100	100	100
Crude protein (%)	20	20	20
Metabolizable energy (ME) kcal/kg	3150	3150	3150
Calcium (%)	0.07	0.07	0.07
Phosphorus (%)	0.51	0.51	0.51

¹+ =CD+10g enzyme/kg, ++ =CD+30g enzyme/kg. ²The vitamin and mineral premix provided/kg of diet: Vitamin A 15,000,000IU, VitaminD₃ 3,000,000IU, VitaminE 25,000IU, VitaminK 5,000mg, VitaminB₁ 2,500mg, VitaminB₂ 7,000mg, VitaminB₆ 25mg, pantothenic acid 12,000mg, nicotinic acid 35,000mg, folic acid 1,000mg, biotin 15mg, choline chloride 250,000mg, Mn 60,000mg, Zn 45,000mg, Cu 1,600mg, I 400mg and Fe 80,000mg.

Table 3: Percent of ingredient used in Thai native chicken finisher diet, phase II

Materials	Percent of ingredient used in Thai native chicken finisher diet (6 to finish week)		
	Control diet(CD) (without enzyme)	CD+10g enzyme	CD+30g enzyme
Corn meal	54.7	54.7	54.7
Rice brain	15	15	15
Soybean meal(45% cp)	19.5	19.5	19.5
Fish meal (66% cp)	6	5	3
Shell meal	0.7	0.7	0.7
Dicalcium phosphate	0.5	0.5	0.5
Tallow	3	3	3
DL-methionine	0	0	0
Salt	0.35	0.35	0.35
Enzyme	-	+	++
Vitamin and mineral premix	0.25	0.25	0.25
Total	100	100	100
Crude protein (%)	18	18	18
Metabolizable energy (ME) kcal/kg	3150	3150	3150
Calcium (%)	0.07	0.07	0.07
Phosphorus (%)	0.51	0.51	0.51

¹+ =CD+10g enzyme, ++ =CD+30g enzyme. ²The vitamin and mineral premix provided/kg of diet: Vitamin A 15,000,000IU, VitaminD₃ 3,000,000IU, VitaminE 25,000IU, VitaminK 5,000mg, VitaminB₁ 2,500mg, VitaminB₂ 7,000mg, VitaminB₆ 25mg, pantothenic acid 12,000mg, nicotinic acid 35,000mg, folic acid 1,000mg, biotin 15mg, choline chloride 250,000mg, Mn 60,000mg, Zn 45,000mg, Cu 1,600mg, I 400mg and Fe 80,000mg.

effect of the factor or treatments. The Duncan's Multiple Rang Test was used to compare means. The level of significance used was $P \leq 0.05$.

Results and Discussion

Effect on growth performance: The results of growth performance of chicken (Table 4) showed that the live weight of chicken in both xylanase treatment groups increased significantly with respect to the control group ($P \leq 0.05$) during 28 days of the experimental. However, there was no significant difference in the body weight between two xylanase supplement levels (10g and

30g/kg feed). This shows that the lower enzyme concentration can be used in industrial diet preparation. In addition, no deaths occurred in any experimental groups. This indicates that xylanase diet supplement in diet has no negative effect on survival. This study suggests that xylanase supplement could improve growth in chickens. This finding agrees with similar results in the literature, as illustrated by Steinfeldt *et al.* (2003) who reported that xylanase supplementation of wheat-based diets improved the performance in broiler chicken for which, body weight increased but feed intake was not influenced by enzyme addition. While, Wu *et al.*

Table 4: Growth performance of Thai native chicken as a result of different concentration of enzyme in the diet

Variable	Age (day)	Dietary mixed enzyme			LSD significance
		Control diet (CD) (with out enzyme)	CD+10g enzyme	CD+30g enzyme	
Live weight (kg/bird)	Day old	0.225±0.03	0.242±0.03	0.235±0.04	0.416 ^{NS}
	14	0.466±0.09	0.488±0.05	0.481±0.06	0.353 ^{NS}
	28	0.672±0.13 ^a	0.718±0.06 ^b	0.711±0.10 ^b	0.330 [*]
	42	1.037±0.17	1.105±0.09	1.125±0.12	0.478 ^{NS}
	56	1.292±0.18 ^a	1.459±0.16 ^b	1.368±0.19 ^b	0.264 [*]
Feed intake (kg/bird)	14	0.828±0.04	0.801±0.08	0.841±0.05	0.734 ^{NS}
	28	1.036±0.12	1.020±0.01	1.053±0.19	0.714 ^{NS}
	42	1.370±0.04	1.350±0.12	1.410±0.10	0.774 ^{NS}
	56	1.601±0.26	1.602±0.26	1.601±0.26	0.832 ^{NS}
	70	1.734±0.11	1.745±0.23	1.670±0.20	0.989 ^{NS}
FCR (Feed: Weight)	14	3.68	3.30	3.57	0.242 ^{NS}
	28	2.22 ^a	2.09 ^b	2.18 ^b	0.038 [*]
	42	2.03 ^a	1.88 ^b	1.98 ^b	0.032 [*]
	56	1.54 ^a	1.45 ^a	1.17 ^b	0.021 [*]
	70	1.34 ^a	1.19 ^b	1.22 ^b	0.052 [*]
Survivability (%)	14	100.00	100.00	100.00	ND
	28	100.00	100.00	100.00	ND
	42	100.00	100.00	100.00	ND
	56	100.00	100.00	100.00	ND
	70	100.00	100.00	100.00	ND

ND=No Death of bird. ^{a, b, NS}Mean in row bearing different superscripts are significantly different (*P ≤ 0.05)

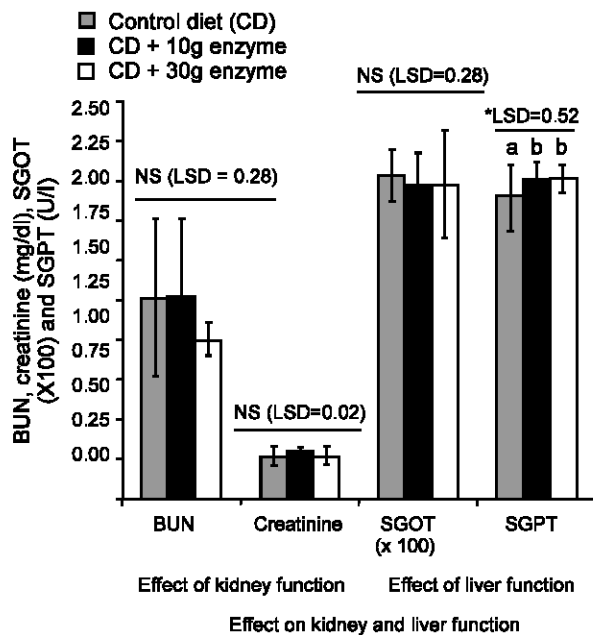


Fig. 1: BUN, creatinine, SGOT and SGPT of Thai native chicken treated with CX form mixed diet. a, b, S, NS Mean in top bearing different superscripts are significantly different (*P≤0.05)

(2003) reported on xylanase supplementation in ground wheat diets and whole wheat diet and the result suggested a gain in weight with both wheat forms, but improvements in ground wheat diets were greater than

those in whole wheat diets and feed intake in ground wheat diets increased, but decreased it in whole wheat diets.

FCE have relative among body weight increasing and feed intake reducing are indicated utilization of animal feed (Marquardt *et al.*, 1996; Bedford, 2000; Bhat, 2000; Wu *et al.*, 2003). Many reports suggest that the xylanase can be an improved growth performance due to xylanase functioning in the hydrolysis of arabinoxylan and starch (Heldt-Hansen, 1997). Hydrolases are the main class of enzymes used in monogastric feed. Hew *et al.* (1998) demonstrated that exogenous xylanase significantly improved ileal nitrogen and amino acid digestibilities, and apparent metabolizable energy (AME) in wheat for broiler chickens. Bhat (2000) demonstrated that the use of hydrolases eliminates ANF present in grains; degrade certain cereal components in order to improve the nutritional value of feed and to supplement animals' own digestive enzymes.

Effect on plasma biochemistry: At the end of the experiment period, blood samples were collected and then plasma biochemistry of BUN, crea, SGOT and SGPT of was examined (Fig. 1). The results show that BUN levels were not significantly different to those of the control diet group, but xylanase supplemented diet, 30 g/kg feed group was lower to control diet group. Also, creatinine and SGOT levels were found in all groups similar to those of the control diet group. Conversely, the SGPT level in xylanase supplemented diet was significantly higher than the control diet group (P≤ 0.05).

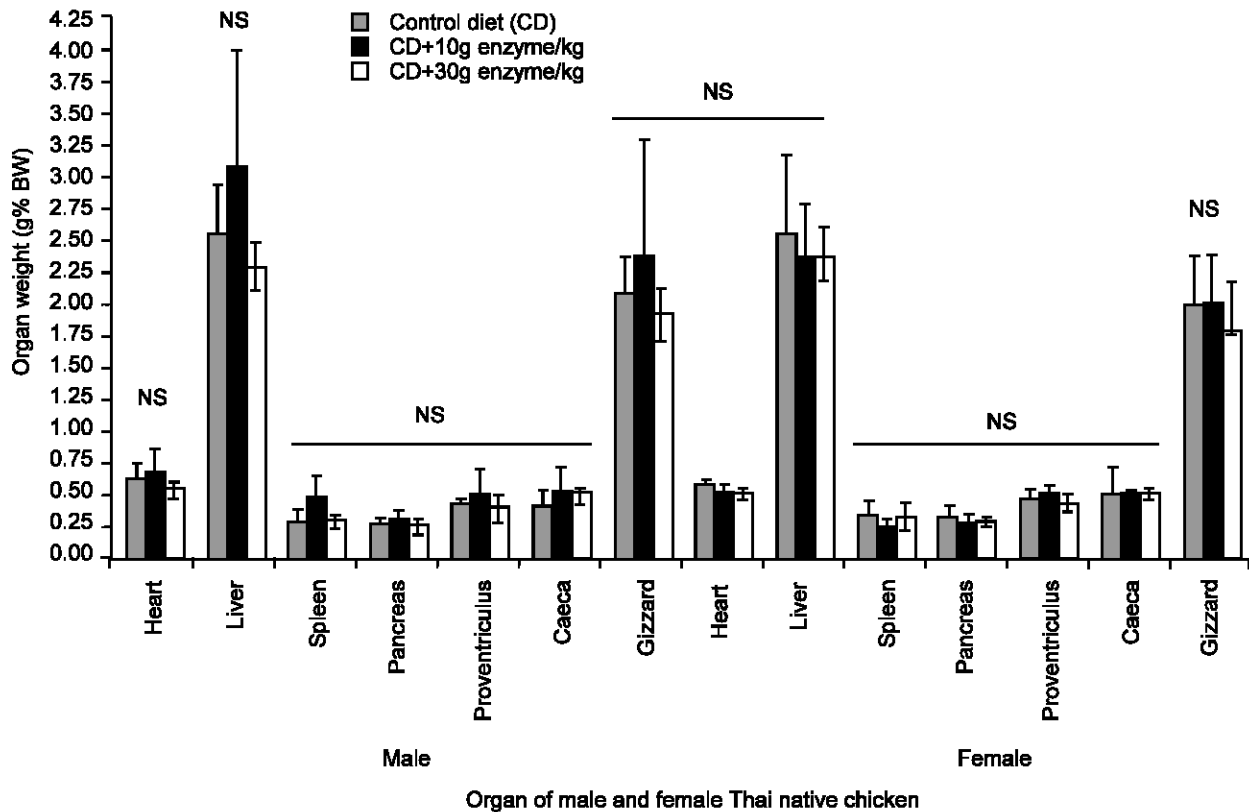


Fig. 2: Internal organs weight of the Thai native chicken treated with CX form mixed diet NS Mean in top bearing different superscripts are not significantly different (* $P \leq 0.05$).

The BUN and crea levels were analyzed to determine toxic kidney injuries. This study revealed that crude xylanase produced from *T. aurantiacus* SL16W had no toxic effects to cause the dysfunction of the kidney in transforming ammonia to urea usually caused by some toxic substances. This result is supported by our previous result in the toxicological test in albino rat using enzyme prepared from *T. aurantiacus* SL16W (Table 1). Nevertheless, in xylanase supplementation group, 30g/kg feed group was lower than all other groups but not significantly different to control diet group. This result can be explained due to the elevated BUN level indicating that xylanase supplemented diet no toxic effect on kidney function. Also, crea levels in treated groups are not significantly different from the control group was confirmed. Hepler (1988) demonstrated that increasing of BUN and crea levels indicated impairment of the kidney function because of excretion of crea and uric acid which could be attributed to cell necrosis or changes in cell membrane permeability or urinary obstruction.

Also, the significant increase of SGPT level in both xylanase supplemented diets is unclear due to being slightly increased compared to control diet. Moreover, the increasing of the SGPT level in xylanase may lead

to hepatocyte dysfunction or liver injury, as suggested by Lohleka (1990). This will be further investigated by histological analysis as part of our further research works.

Effect on internal organs weight: Internal organ such as the heart, liver, spleen, pancreas, proventriculus, caeca and gizzard in both sexes of chicken were controlled for any primary toxic effects. The relative organ weights were calculated by g/% kg bw of each bird (Fig. 2). The result showed that the xylanase supplemented diet had no effect on the relative weights of all organs. This is in accordance to our previous results about body weight gain, feed intake, FCE and primary toxicity test of blood and blood biochemistry analysis because these results did not show any toxic effect. Therefore, it can be concluded that xylanase supplement in diet has no effect on internal organ weights. This is in agreement with Alam *et al.* (2003) who reported that exogenous dietary enzyme supplementation had no effect on gizzard, head, heart and feather weight in broiler chickens. Similarly, Wu *et al.* (2003) showed that xylanase supplementation had no effects on the relative weight of crop, proventriculus, gizzard, pancreas, liver and heart of broiler chickens. However, this study disagree with the

finding of Hetland *et al.* (2002) that relative gizzard weight in of broilers that were fed diets containing whole wheat at 24 and 38 days of age increased by 56-86 and 36-100%, respectively while, Taylor and Jones (2001) reported relative gizzard weights of birds that were administered diets containing 200 g/kg whole wheat were 7.8-10.7% higher than those of birds fed diets containing ground wheat.

The exact mechanism by which whole grain feeding improves bird performance is unclear. An increase in gizzard size will not only increase the grinding action but also increase the incidence of gastric reflexes that serve to re-expose the digesta to pepsin in the proventriculus, enhance the mixing of digesta with enzymes, improve digestion and also discourage microbial proliferation which may cause disease or compete for nutrients (Gabriel *et al.*, 2003). Improved ileal starch digestibility (Svihus and Hetland, 2001; Hetland *et al.*, 2002; Perston *et al.*, 2000).

Conclusion: Xylanase supplementation improved performance of Thai native chicken because of an increase in body weight and decreased FCE. The latter seems to be dependent on the level of xylanase.

Xylanase supplementation in diet had no effect on BUN, the positive effect can be decreased BUN seemed to be dependent on the high level, 30 g/kg feed. But xylanase supplementation resulted in a little increased SGPT level.

Xylanase supplementation in diet did not cause any weight change of the internal organs. Hence, xylanase supplements in chicken diet, demonstrated here could improved further the efficiency of the Thai native chicken production.

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