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Nutritive Quality of β-Mannanase Treated Copra Meal in Broiler Diets and Effectiveness on Some Fecal Bacteria

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Abstract: The copra meal (CM) was hydrolyzed to prepare the enzymatic treated CM (ETCM) by bacterial β-mannanase. The ETCM showed markedly reduction in crude fiber about 14%, while an ether extract, nitrogen-free extract and metabolizable energy value were increased approximately 10%, 4.7% and 16.5%, respectively, compared to non-enzymatic treated CM (NTCM). The digestibility of ETCM nutrient components were also increased, especially, in case of fiber which was increased from 45.35% to 71.98%. The effect on body weight gain of broiler fed with 15% and 20% (w/w) ETCM was not significantly different ($P>0.05$) in all treatments. Feed consumption decreased ($P<0.05$) when fed the diets containing ETCM and NTCM, but feed conversion ratio (FCR) was improved significantly ($P<0.05$), especially at the level of 20% ETCM which the FCR value 2.06 was obtained compare to 2.12 of NTCM group. In addition, the number of *E. coli* and Salmonellae count in broiler feces were also decreased significantly when fed with ETCM diets.

Key words: β-Mannanase, broilers, copra meal, manno-oligosaccharides, fecal bacteria

Introduction

The utilization of agriculture waste as feedstuff is excessively interested as this is one of strategies to reduce the cost of livestock production. About 84% of the dried copra was found in form of the defatted copra meal (CM), by-product from the coconut cream or coconut oil industry, and it is included in the attractive choice for diets cost reduction. CM can be used as energy and protein sources for pig (O'Doherty and McKeon, 2000; Dung *et al*., 2002), steers (Ramos *et al*., 1998) and poultry. However, the CM was limited to use due to the high content up to 20% of β-mannan, a member of non-starch polysaccharide (NSP) found in plant derived feed ingredient. Highly concentrated β-mannan renders CM was found to be a poor quality ingredient for monogastric animals and could not be used in the high levels for the diets (Mendoza *et al*., 1994).

In recent years, a β-mannanase produced from bacteria and fungi has been shown to improve feed conversion and performance to broilers, turkeys and swine (Jackson *et al*., 1999). The important role of β-mannanase is hydrolyzing β-1, 4-glycosidic linkages in β-mannan (Ooi and Kikuchi, 1995) and can reverse the negative impact caused by copra β-mannan. Teves (1989) reported the increase in the metabolizable energy (ME) and nutrient digestibility improvement of CM treated by β-mannanase. In addition, broiler chicks fed with enzymatic treated CM by commercial enzymes were increased in weight gain, feed conversion efficiency, DM digestibility and nutrient digestibility, while the jejunal content viscosity was decreased (Sandu *et al*., 2006).

Manno-oligosaccharides (MOS), product of gaur gum galactomannan hydrolysis by β-mannanase was found to be a substance which could prevent the colonization of *E. coli* and Salmonellae, leading to an improvement of animal growth performance (Ishihara *et al*., 2000; and Perry, 1995). In this paper, the experiment was designed to investigate for nutrient values of β-mannanase treated CM and the effect on performances and changes in fecal microflora of broilers.

Materials and Methods

Preparation of enzymatic treated CM (ETCM): β-Mannanase was produced by *Bacillus subtilis* 5H using locust bean gum as a carbon source (Khanongnuch *et al*., 1998). The extracellular β-mannanase was harvested after 7 hours of cultivation by centrifugation with 6000 rpm for 15 min at 4°C and the supernatant was used as crude enzyme solution. The expeller pressed coconut cream cake, copra meal residue (CM), used in this study was collected from local market in Chiang Mai, Thailand. The CM was hydrolyzed with the prepared β-mannanase at 37°C for 60 hours using 200 units of enzyme solution per 1 kg of CM in 15 liter distilled water within a sealing system. After completion of the reaction, the product was completely dried by vacuum dryer at 65°C for 60 hours to obtain the ETCM.

Determination of proximate composition and ME values of ETCM: The proximate composition of ETCM and non-enzymatic treated CM (NTCM) were determined, according to A.O.A.C. (1984) methods. The ME value was determined by the modified force feeding method as described by Sibbald (1977ab). The birds were adjusted to adapt on metabolic cage for one week (preliminary period). Each type of CM was ground to

Table 1: Proximate compositions and ME values of NTCM and ETCM

Nutrient composition	Materials	
	NTCM	ETCM
Moisture, %	7	5
Organic matter, % DM	96.57	97.89
Crude protein, % DM	5.84	6.01
Ether extract, % DM	18.75	29.25
Crude fiber, % DM	34.53	20.53
Ash, % DM	3.43	2.11
Nitrogen-free extract, % DM	37.45	42.1
Metabolizable energy (kcal/g)[1/]	3.22	3.75

Note :NTCM = Non-enzymatic treated CM , ETCM = Enzymatic treated CM. [1]As fed basis.

pass through a 1.0 mm screen to ensure a fine particle size. About 30 g of CM was force-fed into the chicken's crop through a 35-cm-long, 8-mm inside diameter plastic tube, which connected to the 60-ml plastic syringe, after the 24 hr period without feed (experiment period). Exactly 24 hr after force feeding, the voided excreta of each bird were collected quantitatively (collection period), dried immediately and weighed. Samples of ground feed and excreta were analyzed for gross energy using an adiabatic bomb calorimeter from duplicate samples.

Determination of digestibility of NTCM and ETCM: Nutrients digestibility of each CM was determined in the colostomized chicks. Each type of CM was ground and mixed before sampling for analysis or used as the diets for digestion studies. Force feeding technique was used to feed into chicks. Separation to collection of urine and feces collection were performed using plastic bag for clinging to skin around cloaca and artificial anus for urine and feces, respectively. The dried CM and feces were analyzed for proximate composition to calculate the coefficient of nutrient digestibility.

Investigation the effect of ETCM in the diets on broiler performances: Three hundred broilers (Arbre Acre 707), males and females mixed, seven days old, were used to investigate broiler performances with completely randomized design. The total of birds was adjusted as five treatments consisted of three replicates and were randomly assigned to litter floor pens providing 0.12 m^2/bird. The experiment diets formulated on the basis that the ETCM and NTCM were supplied 15 and 20% (w/w), and non-copra diet served as a control. All treatments were exactly balanced with the same ME level at 3200 kcal/kg. Broiler feeding and water were provided *ad libitum*. The broiler growth performance was determined.

Determination of microbial population in broiler feces: Six birds from each treatment group were selected randomly and collected the feces weekly during 1-5 weeks of age to determine the fecal microflora of *Escherichia coli,* Salmonellae, which was examined after ingestion of the experiment diets at day 7. One gram of freshly collected feces was mixed with 9 ml of saline solution (0.85%NaCl) and homogenized and diluted with ten-fold serial dilution. For each dilution, 0.1 ml was spread separately onto selective agar plate. The medium used were: Eosin Methylene Blue (EMB) agar for *E. coli* and Salmonella-Shigella (SS) agar for Salonellae and MRS agar was used for lactic acid bacteria. Total numbers of bacterial colonies were counted at the end of each incubation period.

Statistical analyses: Experiment data were subjected to analysis of variance using the ANOVA of SPSS Programming. Duncan's multiple range tests was used to identify significant differences between means of treatments.

Results and Discussion

Proximate composition and ME values of ETCM and NTCM: There was evidently difference of proximate composition between ETCM and NTCM, especially an ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE) composition as presented in Table 1. The ETCM showed a markedly reduction in CF from 34.53% to 20.53%. In contrast, the EE and NFE composition were increased from 18.75% to 29.25% and 37.45% to 42.10%, respectively. The effects of bacterial β-mannanase treatment also significantly improved in the ME value of CM from 3.218 to 3.751 kcal ME/g. This was corresponding with Teves *et al.* (1989), who reported that the energy could be increased about 18%, presumably due to the increasing proportion of β-mannan degradation. From these results, it could be suggested that an improvement of the nutrients and ME values of ETCM due to hydrolysis of β-mannan, a hemicellulose component in CM cell wall structure. That lead to the reduction of CF and simultaneously released of NFE and oil globules, the high energy component, from the endosperm cell.

Nutrients digestibility of NTCM and ETCM: The digestibility coefficient of dry matter and other nutrients was listed in Table 2. The ETCM showed higher improvement than NTCM, this might be due to the action of β-mannanase for polysaccharide fiber degradation. Dry matter, CP, EE and CF digestibility, were increased in birds fed with ETCM. Digestible CF of ETCM diet showed the highest improvement from of 45.35% to 71.98% of digestibility. This can be suggested that the enzyme hydrolyzed and converted the CM β-mannan to either manno-oligosaccharides or more simple sugar molecules which represents in the soluble carbohydrates. Increasing of CP and EE digestible may be attributed to the release of proteins and fats from the

Table 2: Coefficients of digestibility of dry matter and other nutrients of ETCM and NTCM

Variable	Materials	
	NTCM	ETCM
Dry matter, %	58.18	66.67
Crude protein, % DM	55.56	68.98
Ether extract, % DM	96.98	98.03
Crude fiber, % DM	45.35	71.98
Nitrogen-free extract, % DM	51.08	45.23

Note: NTCM = Non-enzymatic treated CM ETCM = Enzymatic treated CM

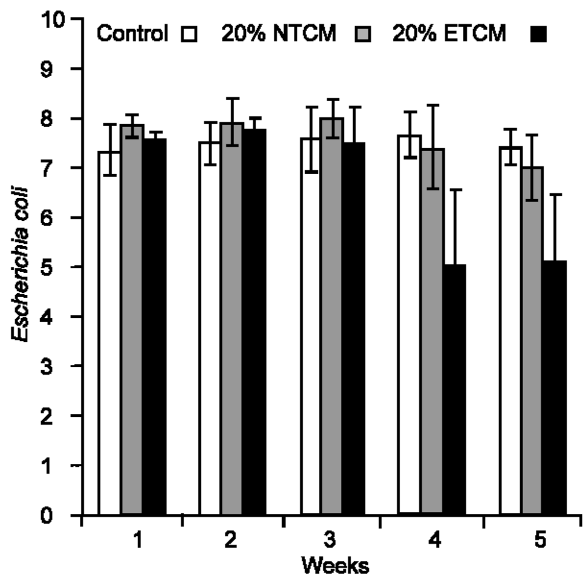

Fig. 1: Effect of ETCM diet on the changes of *E. coli* population in broiler feces during of feeding Control, 20% NTCM and 20% ETCM

lipid-protein-polysaccharide complex of the CM, which is similar to the observation made by Teves *et al.* (1989). On the other hand, only the NFE, ETCM showed lesser digestibility than those of NTCM. This could be suggested that although the properties of soluble fiber or oligosaccharides derived from ETCM could be improved the NFE composition, but could not be furthermore digested and absorbed by the GI system of birds. Thus, it could be found the high value of NFE content in feces.

Growth performance evaluation of ETCM fed for broiler: The effects of feeding with the enzymatic digested CM diets in various levels were shown in Table 3. The body weight gain (BWG) of broilers decreased ($P<0.05$) when fed the diets containing ETCM and NTCM in starter period (2 to 3 weeks) compared with that of control. However, there were no significant differences in BWG, in fed diets containing ETCM and NTCM at all levels and control diet at finisher period ($P>0.05$) which was corresponded with the Panigrahi's report that the high lipid CM residue could be used up to 40% in the broiler diet by without any adverse remark (Panigrahi, 1991). Those broilers on the CM diets group consumed significantly less feed intake at all feeding period compare to that of control ($P<0.05$). This may be due to the lower density and more swelling (high CF) of both NTCM and ETCM, consequently reduced feed intake in the broiler (Panigrahi *et al.*, 1987). The ETCM substituted diets showed a positive effect upon FCR at finishing phase compare to control diet and the trend toward FCR improvement at 20% ETCM was observed, but the statistic differences were not significant to 20% NTCM diet (2.06 vs 2.12). This non-significant difference may be due to excessive energy content in feed that is lowering the enhancing effect of enzyme. Several experiments demonstrated that β-mannanase improved BWG and FCR with 3% approximately and it could predict that feed conversion improvement would be more increased, if the ME value in the diets was decreased (McNaughton et al., 1998).

Furthermore, the broilers fed with ETCM diet exhibited higher productivity and positive effect to percent of mortality of broilers in the finisher period, which was observed at 2.5, 8.3 and 15.0% in broiler fed with ETCM, NTCM and control diet, respectively. It can be suggested that, the oligosaccharides released by enzymatic hydrolysis may be contribute to the maintenance of a healthy gut flora which lead to an improvement the host health (Graham and Balnave, 1995).

Changes in *E. coli* and Salmonella population in broiler feces during feeding: The number of *E. coli* population in the broiler feces fed diets with the ETCM compare to NTCM and control group, were presented in Fig. 1. The 20% ETCM showed the most decreasing of *E. coli* population form 10^7 to 10^5 cfu/g at 4 weeks onwards in broiler feeding. Similarity to Salmonellae population, Fig. 2, which was decreased since 2 weeks onwards of feeding and the decrease level was more different between the ETCM and other groups after 4 weeks feeding. These results agree with the finding of Ishihara *et al.* (2000) which reported that administration of feed supplemented with partially hydrolyzed guar gum, the galactomannan containing polysaccharides, can prevent the colonization of Salmonellae in chicken. The reduction of *E. coli* and the pathogenic Salmonellae number found in this study was assumed to be caused by the action of manno-oligosaccharides (MOS) containing in the ETCM diets. These oligosaccharides were unable to degrade by digestive enzymes and passed through the GI tract and believed to benefit the host animal by improving intestinal microflora balance. Some carbohydrates derived from yeast cell wall as the α-mannan, which is also the mannose containing

Table 3: Broiler performances during 2-7 weeks of feeding with various levels NTCM and ETCM diets

Variables	Age (week)	Treatments				
		Control diet	NTCM levels (%)		ETCM levels (%)	
		0	15	20	15	20
Body weight (kg/bird)	2-3	0.66[a]±0.008	0.61[b]±0.01	0.63[b]±0.007	0.62[b]±0.006	0.60[b]±0.003
	2-6	1.87±0.04	1.79±0.01	1.79±0.02	1.83±0.04	1.78±0.03
	2-7	2.18±0.06	2.13±0.01	2.12±0.03	2.15±0.06	2.11±0.04
Feed intake (kg/bird)	2-3	0.95[a]±0.01	0.88[b]±0.005	0.88[b]±0.006	0.86[b]±0.01	0.86[b]±0.008
	2-6	3.77[a]±0.10	3.50[b]±0.01	3.50[b]±0.03	3.48[b]±0.04	3.46[b]±0.04
	2-7	4.80[a]±0.14	4.45[b]±0.02	4.49[b]±0.05	4.42[b]±0.09	4.39[b]±0.07
FCR	2-3	1.44[a]±0.003	1.43[ab]±0.02	1.41[ab]±0.008	1.38[b]±0.01	1.42[ab]±0.01
	2-6	2.01[a]±0.05	1.95[ab]±0.02	1.95[ab]±0.03	1.90[b]±0.02	1.94[ab]±0.01
	2-7	2.20[a]±0.03	2.09[b]±0.01	2.12[ab]±0.04	2.08[b]±0.02	2.06[b]±0.02

Means in the same rows with different letters are significantly different at P<0.05.

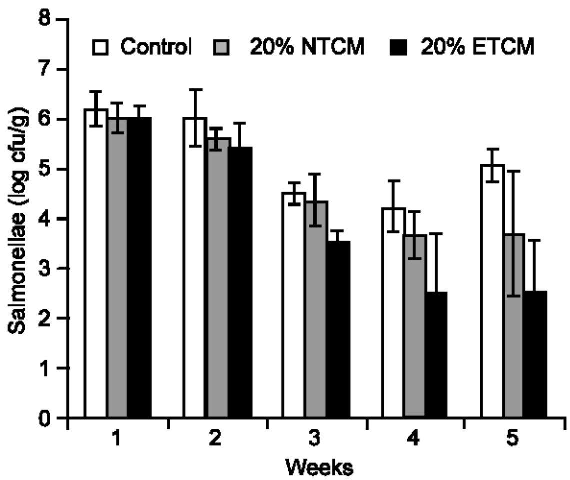

Fig. 2: Effect of ETCM diet on the changes of Salmonellae population in broiler feces during of feeding Control, 20% NTCM and 20% ETCM

oligosaccharides the same as those from ETCM, was effective on resistance to Salmonellae and *E. coli* colonization in chicks (Gibson, 1998; Perry, 1995). The resistance action was suggested to cause by the specifically increased in growth enhancing of lactic bacteria in gut and resulting in growth reduction of some pathogenic bacteria such as *Salmonella* sp.

From our result, it clearly showed the possibility of feeding with ETCM diets in broiler chickens in protection of *E. coli* and Salmonellae. However, the further more result of our study clearly exhibited that the beneficial effect of MOS derived from ETCM did not concern with the specific increasing of lactic acid bacteria (data not shown). The mechanism of prevention for colonization of pathogenic bacteria by MOS derived from ETCM need to be furthermore investigated.

Conclusion: The effect of β-mannanase hydrolysis CM was significantly improved the nutrition, metabolizable energy and digestibility values. The feeding of ETCM diets showed the highest positive result to FCR and also reduced the percent of mortality in finisher period. Moreover, the ingestion of feed containing ETCM is also positively affected on the fecal microbial population by reduction of *E. coli* and Salmonellae number.

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