

«Research Note»

Chitinolytic Activity of Mucosal Enzymes in the Different Parts of the Digestive Tract in Broilers

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The present study was performed to measure activities of mucosal chitinase and *N*-acetyl- β -D-glucosaminidase (NAGase) in the proventriculus, duodenum jejunum and ileum of broilers given standard and shrimp meal (SM) diets. Activities of both enzymes were extremely high ($P < 0.05$) in the proventriculus, and low or negligible in other parts, and in the proventriculus, activity of chitinase was much greater than that of NAGase. Dietary SM containing chitin had little or decreasing effect on activities of both enzymes. The result obtained here suggests that in broilers, chitin digestion by mucosal enzymes occurs mainly in the proventriculus, and the enzyme activities are not stimulated by dietary chitin.

Key words: activity, broiler, chitinase, digestive tract, *N*-acetyl- β -D-glucosaminidase

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Introduction

Shrimp meal (SM) can be used as a protein source of poultry diets (Fanimo *et al.*, 1996; Rosenfeld *et al.*, 1997; Gernat, 2001; Oduguwa *et al.*, 2004; Khempaka *et al.*, 2006a, b), but in most case, replacing the greater part of protein source by SM lead to decreased performance. One of the reason of the decreased performance may be decreased digestibility by chitin, an indigestible polysaccharide, contained in SM. Although apparent digestibility of chitin in chicken is reported to be as low as 18–24% (Khempaka *et al.*, 2006b), information about chitinolytic activity in the chicken digestive tract is quite limited (Jeuniaux and Cornelius, 1978; Suzuki *et al.*, 2002).

In digestion and assimilation of chitin, at least two enzymes, such as chitinase (E.C. 3.2.1.14) and *N*-acetyl- β -D-glucosaminidase (NAGase) (E.C. 3.2.1.30) are needed. The former hydrolyses chitin into trimers and dimers of *N*-acetyl- β -D-glucosamine (NAG) and the latter further hydrolyses these into monomers (Gooday, 1990). Therefore, research about these two enzyme activities in the digestive tract of chickens may promote a better understanding of chitin digestion in chicken, and hence practical use of SM.

The aim of the present study was to measure activities of mucosal chitinase and NAGase in the different parts of the digestive tract of broilers given standard and SM diets, and to

discuss 1) what part of digestive tract digest is responsible for chitin digestion and 2) whether dietary SM can stimulate the activities of these enzymes.

Materials and Methods

This research was conducted in accordance with guidelines for regulation of animal experimentation of Shinshu University, Japan.

Diets, Birds and Sampling

SM containing 30.4% of chitin was made from headless shells of black tiger shrimp (*Penaeus monodon*). Details about SM were given in our previous report (Khempaka *et al.*, 2006a). Composition of the experimental diets was given in Table 1. SM was added at 40 g/kg of diet at the expense of soybean meal and maize. Chitin was measured by the method of Hornung and Stevenson (1971). Chitin nitrogen was ignored when CP content was calculated, because of no evidence for utilization of chitin nitrogen in birds. A total of 12 male broilers (15 d of age) were divided into 2 groups of 6 birds having similar body weight, and allowed free access to diets and water. At 25 d of age, they were sacrificed by exsanguination under ether anaesthesia, and proventriculus, duodenum, jejunum and ileum were collected.

Extraction of the Crude Enzymes

In order to decide pH of extraction buffers, pH of water-homogenised mucosa of each part of digestive tract was measured prior to enzyme extraction. For extraction of the crude enzyme, mucosa of each part was collected and homogenised in each extraction buffer having desired pH adjusted with citric acid and Na₂HPO₄. After centrifugation of the homogenate at 1,500 g for 20 min, the supernatant was

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Table 1. Composition of the experimental diets

Ingredients and analysis	Control Diet	Shrimp meal diet
Ingredients		
Commercial diet (g/kg) ^a	550.0	550.0
Soybean meal (g/kg)	104.7	78.5
Corn (g/kg)	328.7	303.5
Shrimp meal (g/kg)	None	40.0
Corn oil (g/kg)	None	11.4
Vitamin and mineral mix (g/kg)	16.6	16.6
Analyses		
Chitin (%)	0	1.2
CP (%), calculated	23.6	23.5
ME (kcal/kg, calculated)	3183.0	3183.0

^a Broiler starter diet (CP ≥ 23.5%, ME ≥ 3050 kcal/kg, Nippon Formula Feed Mfg, Kanagawa, Japan).

Table 2. Chitinase and NAGase activities in mucosa of different parts of the digestive tract in broilers

Mucosa	Chitinase activity (μ mol of NAG/h/g of protein)		NAG activity (μ mol of <i>p</i> -nitrophenol/h/g of protein)	
	Control	SM	Control	SM
Proventriculus	1702 ^a	1398 ^a	165 ^a	133 ^{a*}
Duodenum	18 ^b	9 ^{b*}	21 ^b	21 ^b
Jejunum	3 ^b	2 ^b	6 ^c	1 ^{c*}
Ileum	3 ^b	5 ^b	5 ^c	7 ^c

Values are means of 6 observations. Means with different super scripts within the same column are significantly different ($P < 0.05$). Means with asterisk within the same row are significantly different from the control value ($P < 0.05$).

precipitated with a 20% saturation with $(\text{NH}_4)_2\text{SO}_4$ and then centrifuged at 16,000 g for 20 min. The resultant supernatant was precipitated again with an 80% saturation with $(\text{NH}_4)_2\text{SO}_4$ and then centrifuged at 16,000 g for 20 min. The resultant precipitate was recovered and dialysed against the extraction buffer. After dialysis, the insoluble matter was removed by centrifugation at 16,000 g for 20 min and then the crude enzyme fraction was obtained (Hirano, 1991). All procedure was performed at 4°C. Protein content of the crude enzyme fraction was measured by CBB dye-binding methods (Bradford, 1976).

Enzyme Assay

Chitinase activity was measured as follows (Hirano, 1991): the reaction mixture composed of 2.0 ml of buffer containing 20 mg of N-acetylchitosan and 1.0 ml of crude enzyme extract was incubated at 37°C for 60 min, and then the reaction was stopped by adding of 10% tungstate and 2/3N sulphuric acid (0.5 ml each). This reaction mixture was centrifuged and the reducing sugar in the supernatant, which was recognised as NAG, assayed according to the modified Schales method (Imoto and Yagishita, 1971). Chitinase activity was expressed as the micromoles of NAG liberated per 60 min per g of protein. NAGase activity was measured as follows (Ohishi *et al.*, 1993): the reaction mixture composed of 0.3 ml of crude enzyme extract, 0.5 ml of extraction

buffer and 0.2 ml of 5 mM *p*-nitrophenyl- β -*N*-acetylglucosaminide was incubated at 37°C for 10 min, and then reaction was stopped by adding 0.2 ml of 0.25 M sodium carbonate. *p*-nitrophenol liberated from *p*-nitrophenyl- β -*N*-acetylglucosaminide was determined by measuring absorbance at 405 nm. Activity was expressed as the micromoles of *p*-nitrophenol liberated per 60 min per g of protein.

Statistical Analysis

Statistical significance between intestinal parts was evaluated using one-way analysis of variance followed by Tukey-Kramer test and that between dietary groups was done using Student's *t*-test.

Results and Discussion

In the present study, the mucosal chitinase activity in the control group was extremely high ($P < 0.05$) in the proventriculus, low in the duodenum and negligible in jejunum and ileum (Table 2). This findings are supported by the previous reports: mucosal chitinase was found in the proventriculus of 8 bird species including chickens, which were more or less insectivorous (Jeuniaux and Cornelius, 1978), and more recently, gut chitinase mRNA in White Leghorn hens was expressed in the proventriculus abundantly (Suzuki *et al.*, 2002). When chitin in chitinous diets such as cuticles of insects and crustaceans is digested, their

protein digestibilities are probably improved, because proteolytic enzymes can access easily to protein in crustacean cuticles composed predominantly of chitin cross-linked with proteins (Stankiewicz *et al.*, 1998). In this context, increased chitinase activity is desirable in consideration of practical use of SM. Unfortunately, mucosal chitinase activity decreased in duodenum and remain unchanged in other parts, when SM diet was given (Table 2), suggesting that dietary chitin has no stimulating effect on mucosal chitinase activity. Our previous study (Koh *et al.*, 2008) supports this observation, which showed that chitin digestibility in chickens did not increase by elongated chitin feeding period. In order to improve the nutritional value of SM, studies on enhancement of chitinolytic activity in the digestive tract in chicken are needed.

When degradation products of chitin by chitinase, trimers and dimers of NAG, are hydrolysed into absorbable monomers, NAGase is required. So far, limited information about NAGase activity in chickens has been reported: Jeuniaux and Cornelius (1978) found that NAGase activity was absent or very low in the chicken digestive tract with the exception of ceecal contents. In the present study, mucosal NAGase activity in the control group was high in the proventriculus, low in the duodenum and negligible in jejunum and ileum (Table 2). This trend is similar to that observed in mucosal chitinase activity, but in the proventriculus, NAGase activity was much lower than chitinase activity. Consequently, it is unlikely that trimers and dimers of NAG are degraded to absorbable NAG monomers actively in the digestive tract. In addition, it is uncertain whether the NAG monomers are assimilated to chickens actively, because absorption efficiency of NAG monomers is reported to be much less than that of glucose at least in seabirds, Leach's Storm-Petrels (Jackson *et al.*, 1992). When SM diet was given, mucosal NAGase activity decreased in the proventriculus and jejunum, and not in the duodenum and ileum (Table 2). Because of little information on mucosal NAGase in birds, it is difficult to discuss why SM decreased NAGase activity in the proventriculus and jejunum.

Interestingly, chitinolytic enzymes exists not only in the mucosa of chicken digestive tract but also plants, such as maize, barley (Collinge *et al.*, 1993) and soybeans (Hirano *et al.*, 1988). Because these plants are generally used as poultry diet ingredients, it may be possible that the chitinolytic enzymes derived from diets can function with the mucosal ones in the digestive tract of chickens. This hypothesis remains to be verified.

In conclusion, 1) chitin digestion may occur mainly in the proventriculus by mucosal enzymes, but it is uncertain whether degradation product of chitin is assimilated, because of low activity of NAGase, and 2) dietary chitin may not stimulate the chitinolytic enzyme activities in mucosa.

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