The 3rd ISTAP, Faculty of Animal Science, Gadjah Mada University, 2002 *ISBN : 979 – 97243 – 2 - 5*

EFFECTS OF XYLANASE SUPPLEMENTATION ON *C. PERFRINGENS* AND PERFORMANCE OF BROILER FED A LOW-ME WHEAT AND CHALLENGED WITH COCCIDIA

M. Sinlae[1]

Abstract

An experiment was conducted for five weeks to examine the effects of xylanase supplementation on *C. perfringens* in the ileum and caeca and performance of broilers fed low-ME wheat and challenged with coccidia. 100 day-old broilers were used in the experiment. They were divided into two groups of 50 each and kept separately (group A and group B). Each group was further divided into two groups of 25 birds each (A1, A2 and B1, B2) and were fed low-ME wheat diets, one without xylanase supplementation (T1) and the other with xylanase supplementation (T2). At 9 d of age, all birds in group A (A1 and A2) were orally infected with *Eimeria maxima* at dosage of 2000 oocysts/bird/ml whereas the birds in group B remained unchallenged. At 2 d post-infection (PI), one bird from each group was chosen at random and killed on every other day till week four for *C. perfringens* enumeration. At 21 d of age, the rest of the birds were transferred into slide-in cages with four birds per compartment for diets assessment on feed intake (FI) body weight gains (BWG), feed conversion ratio (FCR), apparent metabolisable energy (AME) and viscosity. There was no *C. perfringens* isolated from either ileum or caeca of birds fed the T2 diet during the experiment. On the contrary, birds fed the T1 diet showed a considerable amount of *C. perfringens* in both the ileum and caeca detected at 10^6/g digest. For birds challenged with coccidia, no *C. perfringens* was cultured from either ileum or caeca of birds fed different diets except on week four, a number of *C. perfringens* was detected at 10^6/g digest in both ileum and caeca of birds fed the T1 diet. Xylanase supplementation had a significant effect ($P<0.05$) on FI, BWG, FCR, AME and viscosity. For birds challenged with coccidia, no significant effects was observed on FI, BWG, FCR and viscosity. However, there was a highly significant effect on AME ($P<0.01$). There were also no significant interaction between diet and challenge ($P>0.05$) for any of the performance parameters measured. It can be concluded that xylanase can be used as an effective means in reducing the growth of *C. perfringens* in the ileum and caeca of broilers challenged with coccidia and thus improve the performance of the birds.

Key words: Xylanase, *Clostridium perfringens*, Low-ME wheat, Coccidia, Broilers

[1] Faculty of Animal science, Nusa Cendana University, Kupang, East Nusa tenggara 85361 Indonesia E-mail : asinlae@yahoo.com

Introduction

Diet composition has been reported to have a marked effect on the flora that develops in the alimentary tract of the chick (Smith, 1965). High levels of fish meal or high dietary fibre such as barley or wheat based diet have been reported to be able to predispose the birds to the outbreaks of necrotic enteritis, a disease caused by *Clostridium perfringens* bacteria (Johnson and Pinedo, 1971; Trusscott and Al-Sheikly, 1977; Branton *et al.*, 1987; Kaldhusal and Hofshagen, 1992). In a subsequent study with the use of several different sources of grains in broiler diet (Riddell and Kong, 1992) found that broilers with diets based on wheat, rye, barley and oat grouts had a higher mortality rate due to necrotic enteritis than those fed diet based on corn.

In addition, coccidia are believed to have a significant role in the occurrence of necrotic enteritis in chickens. In an early study Balauca (1976) reported that chicken infected with a combination of *C. perfringens* type A and *Eimeria acervulina*, *Eimeria necratrix* or mites oocysts resulted in a significant phatologic-anatomic changes being haemorrhagic, ulcerative and necrotic intestinal inflammations, primarily in the small intestine. Al-Sheikly and Al-Saige (1980) also demonstrated an experiment to determine the relationship between coccidia infection and the outbreaks of NE in chickens by producing NE using *C. perfringens* type A, *E. acervulina* and *E. necatrix*. They found that mortality due to the NE was highest (53 %) in birds infected with *E. acervulina* before infection with clostridia. In a subsequent study Braunius and Litjens (1984) examined a total of 1,485 broiler breeders for the presence of coccidiosis and revealed that coccidiosis may be associated with NE due to *C. perfringens* being particularly associated with *E. necratrix* and *E. Maxima*.

This experiment was designed to further demonstrate the relationship between coccidiosis and the status of *C. perfringens* bacteria in the intestine of broiler chickens by means of infected the birds with *E. maxima* and fed low-AME wheat diets. The experiment was also designed to determine whether xylanase supplementation in the diet could prevent the growth of *C. perfringens* in the ileum and caeca of the birds under such conditions.

Material and Methods

The experiment was designed to measure the feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), viscosity, apparent metabolisable energy (AME) and *C.perfringens* in the ileum and caeca of broilers fed a low–ME wheat diet and challenged with coccidia.

Birds and Management

100 day-old broilers were distributed at 1 d of age into 2 groups of 50 each and kept separately (group A and group B). Each group was further divided into 2 groups of 25 each (A1, A2 and B1, B2) and fed diets of low – ME wheat, one without xylanase supplementation (T1) and the other with xylanase supplementation (T2). At 9 d of age, all birds in group A were orally infected with *Eimeria maxima* at dosage of 2000 oocysts/bird/ml, whereas the birds in group B remained unchallenged. At 2 d post-infection (PI), one bird from each group was chosen at random and killed on every other day till week four for *C. perfringens* enumeration. At 21 d of age, the rest of the birds were weighed and transferred into slide-in cages with four birds per compartment for diets assessment on FI, BWG, FCR, AME and viscosity. Both diets and water were provided ad libitum throughout the entire experiment. The diets were formulated using 'Feedmania' package (Maniaa Software Pty. Ltd., A. B. R. I., and the University of New England). The composition of both starter and finisher diets are shown in Table 1 and Table 2, respectively.

Table 1. The composition of starter diet

Ingredient	Wheat Control (T1)	Wheat Enzyme (T2)
Wheat (12% CP)	34.85kg	34.85kg
Soybean (48 %)	7.4kg	7.4kg
Meat meal (50 %)	3.5kg	3.5kg
Canola meal (36 %)	2.5kg	2.5kg
Sunflower oil	0.5kg	0.5kg
Limestone	400 g	400 g
Dicalcium phosphate	300 g	300 g
Lysine	175 g	175 g
DL Methionine	150 g	150 g
Salt	125 g	125 g
Vitamin	100 g	100 g
Choline	10 g	10 g
Xylanase	NO	YES

Table 2. The composition of finisher diet

Ingredient	Wheat Control (T1)	Wheat Enzyme (T2)
Wheat (12% CP)	64.329kg	64.329kg
Soybean (48 %)	8.64kg	8.64kg
Meat meal (50 %)	4.0kg	4.0kg
Canola meal (36 %)	0.8kg	0.8kg
Limestone	1.280kg	1.280kg
Dicalcium phosphate	1.120kg	1.120kg
Lysine	288 g	288 g
DL Methionine	240 g	240 g
Salt	264 g	264 g
Vitamin	160 g	160 g
Choline	16 g	16 g
Xylanase	NO	NO

C. perfringens Enumeration

To enumerate the *C. perfringens* in the ileum and caeca of birds, *a Perfringens* agar (OPSP) media was used Table 1.

Birds were killed by cervical dislocation on every other day starting from d 2 post-infection of coccidia till week four once the birds were killed, 1 g of ileal and of caecal contents were transferred immediately into tubes containing glass beats. To each of sample, 9.0 ml of anaerobic dilution solution (ADS) were added before being mixed thoroughly on a vortex mixer. Strained samples were serially diluted ten-fold in ADS to a final dilution of 10^8. Three dilutions (10^5, 10^6 and 10^7) were then used to inoculate the selective media roll tubes in triplicate with 0.2 ml for each dilution. The tubes were immediately rolled horizontally on ice before incubating them at 39°C for 3 days in a culture bath. At d 3, the tubes were taken out for bacteria enumeration

Viscosity measurement. At the end of the experiment (d 35), all birds were killed by cervical dislocation and the contents of the ileum (from meckle's diverticulum to 4 cm above the ileo-caecal junction) of birds from every cage were collected. Approximately 10 g of fresh ileal contents were taken and centrifuged (Beckman Model, J2-21M, USA) at 10,000 g for 15 minutes at 20°C. The supernatant was then separated from the residue and taken for viscosity determination using a Brookfield DV-III Model Viscometer at 25°C with CP 40 cone and a shear rate of 2-500 S^{-1}

Statistical Analysis

Data on FI, BWG, FCE, AME, and Viscosity were analysed using fully factorial analysis of variance. StatView (SAS Institute Inc. USA, 1998) was used to perform the analyses. Bacterial data were log transformed before statistical analysis.

Results

Table 3 shows all the performance data. The xylanase supplementation had a significantly effects (P<0.05) on FI, BWG, FCE, AME and viscosity. Birds fed T2 diet shown an increase of 6.24 percent in FI, 12.78 percent in BWG, an improved of 6.95 percent in FCE, an increased of 7.44 percent in AME and a marked decreased of 90.09 percent in the viscosity.

Table 4 shows the effect of coccidia challenged on the performance of the birds. There was no significant effect (P>0.05) found on FI, BWG, FCE, and viscosity except on the AME (P<0.01) due to coccidia challenged. There were no significant interaction between diet and challenged (P>0.05) for any of the performance parameters measured.

Table 3. The effects of diet on F1, BWG, FCE, AME and viscosity

Measures	Wheat Control		Wheat Enzyme		
	Mean	SE	Mean	SE	P-Value
FI (g/bird)	1867.09	37.94	1991.48	23.87	0.0163
BWG (g/bird)	949.26	21.08	1088.41	21.42	0.0008
FCE	1.97	0.03	1.83	0.04	0.0137
AME (MJ/Kcal)	12.93	0.19	13.97	0.12	<0.0001
Viscosity (mPa.s.)	43.69	4.66	4.33	0.25	<0.0001

Table 4. The effects of coccidia challenged on FI, BWG, AME and viscosity

Measures	Wheat Control		Wheat Enzyme		
	Mean	SE	Mean	SE	P-Value
FI (g/bird)	1867.09	37.94	1991.48	23.87	0.0163
BWG (g/bird)	949.26	21.08	1088.41	21.42	0.0008
FCE	1.97	0.03	1.83	0.04	0.0137
AME (MJ/Kcal)	12.93	0.19	13.97	0.12	<0.0001
Viscosity (mPa.s.)	43.69	4.66	4.33	0.25	<0.0001

No *C. perfringens* were cultured from either ileum or caeca of birds challenged with coccidia for the firs three week. On the week four (the last samples) a number of *C. perfringens* were found in both ileum and caeca of the challenged group (Figure 2 and Figure 3). *C. perfringens* in the ileum and caeca of the birds without coccidia challenged *were* cultured only from the birds fed T1 diet. Figure 1 shows the number of *C. perfringens* in the ileum of broilers fed wheat with or without a xylanase.

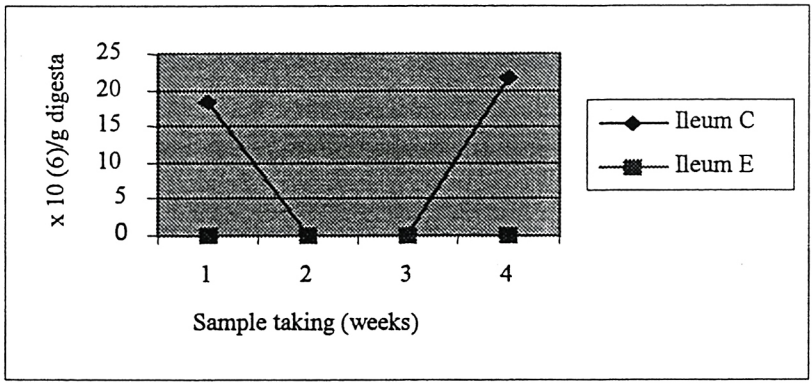

Figure 1. The number of *C. perfringens* in the ileum of broilers fed control (C) or enzyme diet (E)

Figure 2 shows the number of *C. perfringens* in the caeca of broilers influenced by the test diets. No *C. perfringens* were found in the caeca of birds fed wheat-based diet with a xylanase.

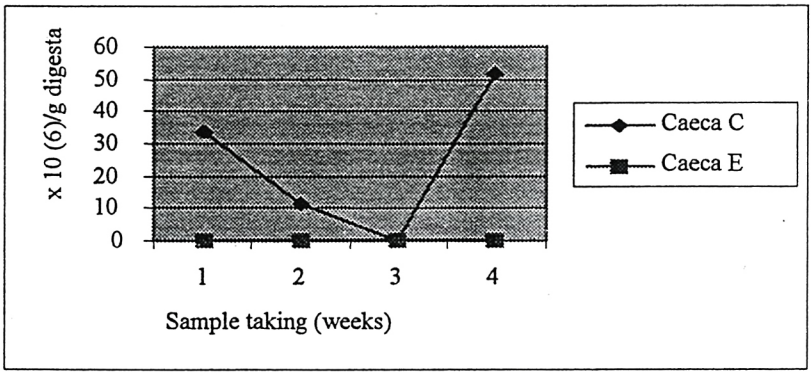

Figure 2. The number of *C. perfringens* in the caeca of broilers fed control (C) or enzyme diet (E) with coccidia and control

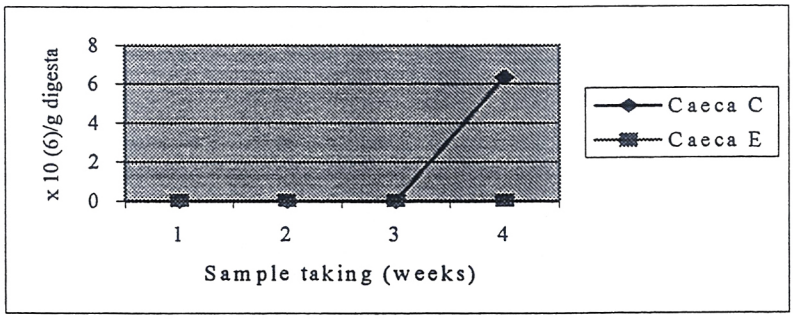

Figure 3. The number of *C. Perfringens* in the ileum of broilers challenged with coccidian and control (C) or enzyme diet (E)

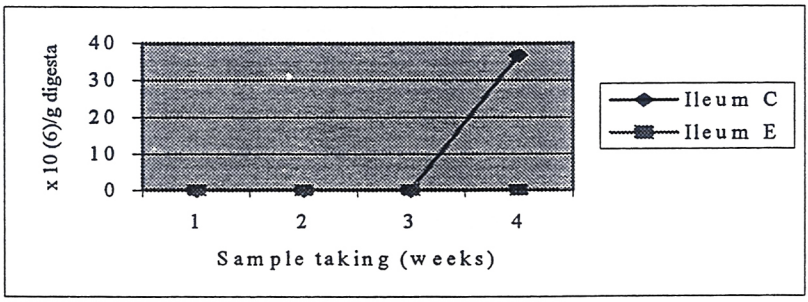

Figure 4. The number of *C. perfringens* in the caeca of broilers challenged with coccidia and control (C) or enzyme diet (E)

Discussion

The results of this experiment indicated that xylanase had a marked influence on the performance of broiler chickens. Chickens fed wheat with a xylanase grew more rapidly and utilized more energy (higher AME) than those fed wheat control. The viscosity was significantly reduced by 90.09 per cent from 43.69 mPa.s in chickens with wheat control to 4.33 mPa.s in chickens with supplemented diet. Given this tremendous difference in the viscosity, it is undoubted that the different in the performance between birds of different diets was due to the significant different in the viscosity. Increasing viscosity due to increased content of soluble arabinoxylan leads to lower AME content of wheat and poorer FCR (Classen *et al.*, 1995; Choct *et al.*, 1996; Jeroch *et al.*, 1997; Barrier-Guillot, *et al.*, 1997).

It is well established that the soluble non-starch polysaccharides of wheat elicit anti-nutritive activities, depressing nutrient digestion and absorption (Annison, 1991). In this particular experiment the birds were on the test diet since day old. Consequently, the influence of wheat NSPs in birds fed low-AME wheat was significant, resulted in high viscosity, poor BWG, poor FCR, and poor AME. On the other hand, for birds fed low-AME wheat with xylanase supplementation, the significant influence of the activity of xylanase had resulted in improving the nutritive value of the diet by reducing the viscosity, improving FI, AME, FCE and the BWG. The xylanase has been regarded an effective means of reducing viscosity by depolymerising the soluble NSPs of wheat (Choct and Annison, 1992; Bedford, 1994; Smits and Annison, 1996).

Given the birds on the test diets since day one also resulted in a significant difference ($P<0.01$) in the number of *C. perfringens* in both the ileum and caeca between the birds fed control diet (low-AME wheat) and those with xylanase supplemented diet. In the previous experiment (Sinlae and Choct, 2000), it was found that started from day five of receiving the test diets, no *C. perfringens* were cultured from the caecal content of birds fed xylanase supplemented diet. In this current experiment, on the other hand, as the birds were on the test diets since day old, no *C. perfringens* were cultured from either ileum or caeca of birds fed xylanase supplemented diet during the whole sample taking periods. This findings strongly suggest that xylanase is able to acting in a manner as antibiotic in preventing the increased population of *C. perfringens* in the ileum and caeca of broilers due to the effect of high viscous grain such as wheat.

No *C. perfringens* were cultured from birds challenged with coccidia until week four, suggesting that the oocysts of *E. maxima* were slow in mediating the growth of *C. perfringens*, even acting in such a way that prevent the growth of *C. perfringens*. This can be seen from the absent of *C. perfringens* bacteria in the ileum and caeca of birds fed low-AME wheat plus coccidia challenged during the first three sampling. Again no *C. perfringens* were cultured from either ileum or caeca of birds challenged with coccidia and fed xylanase-supplemented diet. This is new evidence that xylanase alone can be an effective means in restricting the growth of *C. perfringens* even in coccidia infected birds.

The post mortem examination, in general, shown that there was no severe lesion due to either coccidia infection or necrotic enteritis disease on the internal organs such as liver and the gastrointestinal tract of the birds. However, there appeared that there was a slightly changes along the intestinal lumen, having several red tiny spots along the gut of most infected birds. Some changes in liver texture and colour were also observed. A longer experimental period, however, is needed in order to further observe the relationship between the coccidiosis and the occurrence of necrotic enteritis in broiler chickens fed wheat based diet, as well as to further determine the efficacious of xylanase in preventing the occurrence of the diseases.

References

Al-Sheikhly F. and A. Al.Saige 1980. Role of coccidia in the occurrence of necrotic enteritis of chickens. *Avian Dis.* 24 (2) : 324 – 33.

Annison, G. 1991. Relationship between the levels of soluble non-starch polysaccharides and the apparent metabolisable energy of wheat assayed in broiler chickens. *J. Agric. Food Chem.* 39: 1252 – 6.

Balauca, N. 1976. Experimental reproduction of necrotic enteritis in the chicken.1. Mono- and polyinfections with *Clostridium perfringens* and coccidia with reference to cage management. *Arch Exp Veterinarmed.* 30 (6): 903 – 12.

Barrier-Guillot, M. Bedford, M. R., Metayer, J. P. and F. Gatel. 1997. Nutritional value of wheat in meat type chickens: the effects of variety, place of cultivation and addition of enzymes. *Deuxiemes Journees de la Recherche Avicole,* 8 – 10 April.

Bedford, M. R. 1994. Xylanase source, activity and influence on the response of broilers when fed wheat based diets. In:. *Aust. Poult. Sci. Symp. Vol. 6.* University of Sydney, Australia. pp. 79 – 82.

Branton, S. L., Reece, F. N. and W. M. Hagler. Jr. 1987. Influence of a wheat diet on mortality of broiler chickens associated with necrotic enteritis. *Poult. Sci.* 66: 1326 – 30.

Braunius, W. W. and J. B. Litjens. 1984. Coccidiosis during the rearing of broiler breeding stock in Limburg. *Tijdschr Diergeneeskd.* 109 (10): 394 – 9.

Choct, M and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: role of viscosity and gut microflora. *Br. Poult. Sci.* 33: 821 – 34.

Choct, M., Hughes, R. J., Wang, J., Bedford, M. R., Morgan, A. J. and G. Annison. 1996. Increased small intestine fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37: 633 – 45.

Classen, H. L., Scott, T. A., Irish, G. G., Hucl, P., Swift, M. and M. R. Bedford. 1995. The relationship of chemical and physical measurements to the apparent metabolizable energy (AME) of wheat when fed to broiler chickens with and without enzyme source. *Proc. 2nd Europ. Symp. Feed Enzymes,* Noordwijkerhout, Netherland, pp. 65-77.

Jeroch, H., Danicke, S., Dusel, G. and H. Kluge. 1997. New breeds and varieties of wheat, barley and oats. *Proc. 11th Europ. Symp. Poult. Nutr.*, Faaborg, Denmark. pp. 88-102.

Johnson, D. C., and C. Pinedo. 1971. Gizzard erosion and ulceration in Peru broilers. *Avian Dis.* 15: 835 – 7.

Kaldhusal, M. and M. Hofshagen. 1992. Barley inclusion and avoparcin supplementation in broilers diets. 2. Clinical, pathological and bacteriological findings in a mild form of necrotic enteritis. *Poult. Sci.* 71 (7): 1145-53.

Riddell, C. and X. M. Kong, 1992. The influence of diet on necrotic enteritis in broiler chickens. *Avian Dis.* 36: 499 – 503.

SAS Institute Inc. 1998. StatView, Second Edition , USA.

Sinlae, M. and M. Choct. 2000. Xylanase supplementation affects the caecal microflora of broilers. In: *Aust. Poult. Sci. Symp. Vol. 12.* University of Sydney, Australia, p. 209.

Smith, H. W. 1965. The development of the flora of the alimentary tract in young chickens. *J. Pathol. Bacteriol.* 90 : 495 – 513.

Smits, C. H. M. and G. Annison. 1996. Non-starch polysaccharides in broiler nutrition towards a physiologically approach to their determination. *World's Poult. Sci. J.* 52: 203 – 221.

Trusscott, R. B. and F. Al-Sheikhly. 1977. Reproduction and treatment of necrotic enteritis in broilers. *Am. J. Vet. Res.* 38: 857 – 61.

Untawale, G. G. and J. McGinnins. 1979. Effect of rye and levels of raw and autoclved beans (*Phaseolus vulgaris*) on adhesion of microflora to the intestinal mucosa. *Poult. Sci.* 58: 928-933.