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Effect of Chlortetracycline Additive in Broilers Fed Local Diets on Antibody Titers to NDV Vaccine

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Abstract: A research was carried out to study the effect of antibiotic chlortetracycline in drinking water on broilers antibody titers to NDV vaccine. Broilers were fed local diet with two different sources of essential amino acid i.e. yeast (in diet 1) or mix lysine, methionine and choline (in diet 2). Two hundred and fifty day old chicks CP 707 were randomly assigned into five treatment diets i.e. 1) commercial diet (Dc), 2) diet 1 without chlortetracycline (D1), (3) diet 2 without chlortetracycline (D2), (4) diet 1 plus chlortetracycline 500 ppm via drinking water (D1+), (5) diet 2 plus chlortetracycline 500ppm via drinking water (D2+). Isoenergy diet and water were given *ad libitum*. NDV vaccine were given simultaneously via eyedrop and subcutaneous on day 4. Serum antibody titers were measured on day 18, 21 and 25 by Haemaglutination Inhibition Test and expressed as Geometric Mean Titer (log 2). Antibody titer to NDV vaccine was already detectable two weeks after vaccination (on day 18). The titer value was highest in D1+ reaching 6.0 which was above protective level and the lowest value was found in D1 i.e. 2.75 ($p<0.05$). On day 21 the highest titer was also found in D1+ which remained protective i.e. 5.5 and the lowest one in Dc. On day 25 the highest titer was found in D2 (6.5) and the lowest in Dc (4.25) ($p<0.05$). These results showed that antibody titers to NDV vaccine in broilers receiving local diet minus antibiotics can match the titers of broilers receiving local diet plus antibiotics. Moreover the broilers receiving local diet without antibiotics produced protective and better titers values at the end of sampling period on day 25 or 3 weeks after vaccination, reaching 5.0 in D1 and 6.5 in D2 respectively.

Key words: Chlortetracycline, sorghum, mungbeans, antibody titer, NDV vaccine

INTRODUCTION

Antibiotics are common feed additives found in broilers commercial feed (Murwani and Bayuardhi, 2007). The action of in feed-antibiotics is largely known to be mediated by a decrease in the number of competitive pathogenic bacteria in the gut and therefore reducing bacterial load in the gut (Dibner and Richards, 2005). Reduced bacterial load would decrease immune stimulation which is associated with impaired performance (Klasing, 1997; Cook, 2000; Dibner and Richards, 2005). In feed-antibiotics therefore are useful in preventing bacterial infection and protect broilers from heat stress in hot and humid climate like Indonesia. In addition, it is a common practice in the management of broiler production to administer antibiotics via drinking water (Murwani and Bayuardhi, 2007). Such practice is helpful in maintaining broilers health, suppress mortality, support maximal growth via improved utilization of nutrients and hence remains profitable.

In attempt to reduce and eliminate antibiotic use in feed and medication program, a previous research had been carried out to evaluate the effect of feed ingredient types in the diet of broilers (Murwani, 2008). The usual notion that feed ingredients are only associated with growth performance is bridged by that study to relate feed ingredients with immune response and to use NDV vaccination as a tool to measure one arm of humoral immune response. That study showed that the use of local mungbean or sorghum at certain level in the diet improved antibody titers to NDV vaccine. The improved titers was thought to be due to naturally occurring phytonutrients such as β-carotene and poliphenol in mungbean and poliphenol in sorghum. Such phytonutrients in pure isolated forms have been shown by other studies to modulate immune response (Chew and Park, 2004; Scalbert *et al*., 2005; Hikosaka *et al*., 2006; Neyestani *et al*., 2008). Based on the study, further research was performed to evaluate the effect of utilizing these local feed ingredients together and combined with or without chlortetracycline via drinking water on the response of antibody to NDV vaccine. The effect of such antibiotic use together with diet composition on antibody titers to NDV vaccine has not been studied elsewhere and the following study was carried out to provide such information. The results of this study may provide useful information for rational use of antibiotic additives in broiler production system.

MATERIALS AND METHODS

Birds and diets: All feed ingredients were obtained from local feed producers. Corn, sorghum and mungbean were obtained in grain form with moisture content around 11%. These feed ingredients were ground separately and stored in clean water tight plastic drum until mix. They were also checked for the presence of mycotoxin and no mycotoxin was detected.

Two different diets were composed utilizing local feed ingredients (Table 1). Corn, sorghum, mungbean, protein mix, vitamin mix and mineral mix were used to compose the basal diet. Diet 1 was added with inactivated yeast *S. cerevisiae* (Fermipan) and diet 2 with commercial lysine, methionine and choline. Protein mix consisted of locally produced milk powder and egg white powder. Egg white powder was prepared by drying egg white from side product of local bakery producers and grounding the dried product. The protein mix was also intended to replace meat bone meal which remains uncertain in term of producing "Halal" poultry product for consumers.

A total of 250 Ross CP 707 one day old unsexed broilers with initial body weight 37±5 g were used in this experiment. They were given free access to sugar containing water at their arrival. The experimental chicks were then selected for uniform body weight from the available population and randomly assigned into 5 large groups (in a warm brooder) and given the following treatment diets: 1) commercial diet (Dc), 2) diet 1 without chlortetracycline (D1), (3) diet 2 without chlortetracycline (D2), (4) diet 1 plus chlortetracycline 500 ppm via drinking water (D1+), (5) diet 2 plus chlortetracycline 500ppm via drinking water (D2+) (Table 1).

The dose of antibiotic used was 500 ppm (500 mg/L water). The diet was formulated to meet nutrient requirement of broilers with protein level of 22,7% in diet 1 and 21,7% in diet 2. Both diet was made with approximately equal Metabolic Energy (ME) i.e. 3700 Kkal/kg, while commercial diet had ME of 3600 Kkal/kg. ME was calculated from ME value of each feed ingredients from published data as well as direct *in vivo* measurement. We also found in our field tests that ME in broiler commercial feed ranged from >3400 up to 4000 Kkal/kg (Murwani, unpublished data). Therefore we intentionally made high energy diet with addition of coconut oil. Chicks were given *ad libitum* access to the diet and drinking water.

Antibiotic-free vitamin and mineral mixture was given through drinking water and diet respectively. On day 7, the birds from each large groups were further allocated randomly into 5 replicates with 10 chickens in each replicate. Birds were vaccinated with commercial ND La Sota (PT. Medion Indonesia) on day-4 via eye drop and subcutaneous (inactive vaccine) simultaneously. The dose and vehicle of vaccine was used according to instruction sheet. Subcutaneous route was given with automatic injector so that each bird received the same amount of NDV vaccine. Such vaccination on day 4 is routinely applied in commercial setting aside from the usual vaccination on day 4 and 21. Vaccination on day 4 coincides with the disappearance of maternal antibody after four days post hatch (Nitsan *et al.*, 1991). The treatments were performed up to four days after the end of starter period (25 days), in an open broiler-house at the Faculty facilities with similar condition as that found in most small to medium scale broiler chicken producers (Murwani and Bayuardhi, 2007).

Blood sample collection: Blood was sampled starting two weeks after NDV vaccination or on day 18. Subcutaneous NDV vaccination on day 4 usually produces antibody titers two weeks after vaccination. The antibody titers were then followed every four successive days i.e. on day 21 and 25. At the end of sampling period on day 25 the treatments were terminated. One bird of each replicates was sampled randomly for this blood collection. There were 5 blood samples from each treatment. Serum was separated by centrifugation and kept frozen until analyses.

Determination of antibody Titer to NDV vaccine: Antibody titers were measured using Haemaglutinin Inhibition method. Fifty microlitres standard viral suspension (4 Haemaglutination Unit) was suspended into 12 wells of 96 well plates. The titer of this viral suspension was determined as described by Villegas (1987). Fifty microlitres serum from each samples was then added into the first well. For controls, the serum samples were replaced with phosphate buffered saline. The mixture in the first well was mixed well and serially diluted 2 folds. The plate was then incubated for 15 minutes at room temperature. Fifty microlitres of 0.5% chicken erythrocyte was then added into the mixture, mixed well and incubated further for 30 min at room temperature. The end point was observed when erythrocyte in the control well was agglutinated. The antibody titer of serum samples was read as the highest dilution that can inhibit agglutination (Indonesian Directorate of Veterinary Society, 1999). The titer is expressed as Geometric Mean Titer (log 2) to simplify numerical writing.

Statistical analysis: A completely randomized design with 5 treatments and 5 replicates was employed. All data were analyzed using ANOVA and Duncan's multiple range test was used when means were significantly different ($p<0.05$). A split plot analyses were also performed on titer values to compare means of different days within each treatment.

RESULTS

Antibody titer to NDV vaccine was already detectable two weeks after vaccination as predicted (Table 2). The titer value was highest in D1+ reaching 6 which was above protective level and lowest in D1 i.e. 2.75 ($p<0.05$). On day 21 the highest titer was also found in D1+ which

Table 1: Composition and nutrient contents of experimental diets

Ingredients	DC	D1	D2	D1+	D2+
(%)					
Corn	NA	39	38	39	38
Sorghum	NA	3	3	3	3
Mungbean	NA	31	33	31	33
Protein mix	NA	21	23	21	23
Coconut oil	NA	3	3	3	3
S. cereviciae	NA	3	-	3	-
Total	NA	100	100	100	100
DL-Methionine (%)	NA	-	0.5	-	0.5
Lysine (%)	NA	-	1.1	-	1.1
Choline (mg/kg)	NA	-	1300	-	1300
Vitamin[1] and Mineral[2] Mix	NA	+	+	+	+
Antibiotic in drinking water	-	-	-	500 ppm	500 ppm
Nutrient Contents:					
Crude protein[3]	23.7	22.7	21.7	22.7	21.7
Crude lipid[4]	8.4[3]	1	1.66	1	1.66
Crude fiber[4]	3.0[3]	2.3	2.4	2.3	2.4
Metabolic energy (Kkal/kg)[5]	3600	3775	3764	3775	3764

[1]Vitamin contents per kg vitamin mix: 6000000 IU vitamin A, 1200000 IU vitamin D3, 2.5 IU vitamin E, 3 g vitamin K, 2 g vitamin B1, 3 g vitamin B2, 1 g vitamin B6, 2 mg vitamin B12, 20 g vitamin C, 15 g Nicotinate acid, 5 g Ca-D Panto-thenate, 750 g Na, Ca, K and Mg electrolite. This mix was used at 1 g/l drinking water according to manufacturers instruction. [2]Mineral contents per kg mineral mix: 32.5% Ca, 10% P, 6 g Fe, 4 g Mn, 0.075 g , 0.3 g Cu, 3.75 g Zn, 0.5 g vitamin B12, 50000 IU vitamin D3. This mix was used at 20 g/kg diet according to manufacturers instruction. [3]Based on proximate analysis. [4]Based on local feed composition table (Hartadi *et al*., 1986). [5]Based on *in vivo* measurement of broilers at 21 days old, NA: Not Available

Table 2: Antibody titres to NDV vaccine

Age of experimental broilers	Treatments					
	DC	D1	D2	D1+	D2+	CV
Log 2 GMT						
18 days	4.50 ± 1.29^{abA}	2.75 ± 1.26^{cA}	3.00 ± 0.82^{bcA}	6.00 ± 1.41^{aA}	3.25 ± 0.50^{bA}	15.47
21 days	3.25 ± 0.50^{cA}	5.00 ± 0.82^{abB}	5.00 ± 1.41^{abB}	5.50 ± 0.58^{aAB}	4.00 ± 0.82^{bcAB}	19.45
25 days	4.25 ± 0.50^{bA}	5.00 ± 0.00^{bB}	6.50 ± 1.00^{aC}	4.50 ± 1.00^{bB}	5.00 ± 0.00^{bB}	13.28

Superscripts with lower case are results of Duncan Test. Means within row with no common superscript (lower case) differ significantly ($p<0.05$). Superscripts with upper case are results of Split Plot Test. Means within the same column with no common superscript (upper case) differ significantly ($p<0.05$)

Table 3: Feed conversion ratio

Treatments					
DC	D1	D2	D1+	D2+	CV
1.43±0.11cd	1.67 ± 0.16^{c}	2.35 ± 0.21^{a}	1.35 ± 0.14^{d}	1.94 ± 0.26^{b}	10.35

Means within row with no common superscript (lower case) differ significantly ($p<0.05$)

remained protective i.e. 5.5., where the lowest one was found in Dc i.e. 3.25. On day 25 the highest titer was found in D2 (6) and lowest in Dc (4.25) ($p<0.05$).

Observing the titer value for each treatment at different broilers age, it can be seen in Fig. 1 and Table 2 that in broilers fed commercial diet antibody titers started to appear on day 18 at a value below protective level i.e. 4.5 (<5), decreased insignificantly to 3.25 on day 21 and return to 4.25 on day 25 ($p>0.05$). In broilers fed Diet 1 or Diet 2 (without antibiotic), antibody titer increased steadily from day 18 to day 25. In broilers fed Diet 1, the titer value was 2.75 on day 18 and increased significantly to a value of 5 on day 21 and remained the same on day 25. In broilers fed Diet 2, the titer value was 3.0 on day 18 and increased significantly to a value of 5.0 on day 21 and to 6.5 on day 25.

In broilers fed Diet 1 plus antibiotic, the titer value already reached 6 on day 18 and decreased insignificantly to 5.5 on day 21 and significantly to 4.5 on day 25. However, in broilers fet Diet 2 plus chlortetracycline via drinking water, the titer value started to appear at 3.25, increased insignificantly to 4.0 on day 21 and significantly to 5.0 on day 25. Comparing this antibody titers among treatments, broilers fed Diet 1, Diet 2, or Diet 2 plus chlortetracycline via drinking water showed a similar increasing trend, where Diet 2 having the highest titer on day 25 i.e. 6.5. On the other hand the titer values of broilers fed Diet 1 plus chlortetracycline showed a decreasing trend, while in broilers fed commercial diet showed a flat trend.

The data for feed conversion showed administration of antibiotics via drinking water could improve feed

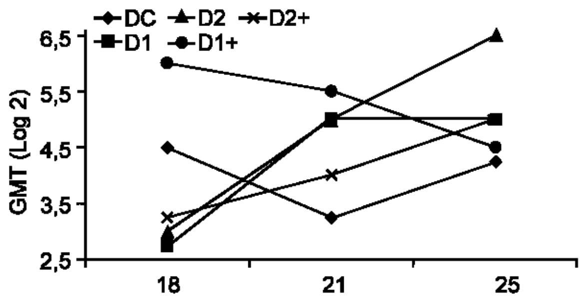

Fig. 1: Antibody titers to NDV vaccine at different broilers age as affected by treatment diets. Each treatment groups consisted of 5 replicates with 10 chickens in each replicates. Serum was sampled from one chicken from each replicates, with a total of 5 chickens per treatment on each sampling day. Dc: commercial diet, D1: diet 1, D2: diet 2, D1+: diet 1 plus chlortetracycline 500 ppm, D2+: diet 2 plus chlortetracycline 500 ppm

conversion in broilers fed either Diet 1 or Diet 2. However the only broilers that could match the feed conversion of commercial diet were broilers fed local Diet 1 plus chlortetracycline (Table 3).

DISCUSSION

The results of the present study showed that broiler fed local diet based on corn, sorghum and mungbean can match the titer value of broiler fed commercial diet. Administration of chlortetracycline via drinking water can improve antibody titers to NDV vaccine in broilers fed Diet 1 but not Diet 2 (Table 2) on day 18. While Diet 1 or Diet 2 without antibiotics can provide protective antibody starting with low titer and reaching protective value after 3 weeks of vaccination, a similar pattern was also shown in groups receiving Diet 2 with antibiotics. This antibody response of broilers to NDV vaccination indicated that the effect of antibiotics via drinking water with similar feed composition was not significant with Diet 2. However when chlortetracycline was administered to broilers fed Diet 1 which contain *S.cerevisiae,* the titers were already protective on day 18 reaching a value of 6.0. The only difference between Diet 1 and Diet 2 is the presence of yeast in Diet 1. Yeast as a single cell was used in this study to provide natural essential amino acids within its protein. In addition, it also contains non starch polysaccharide such as oligomannan found in the wall of *S.cerevisiae.* Oligomannan has been shown to be able to reduce non beneficial gram negative bacteria such as *E.coli* and *Salmonella*, increasing villus height and mucus thickness of intestinal wall (Spring *et al.*, 2000; Hooge *et al.*, 2003; Ferkett, 2004). It appears that this active substance well known as prebiotic, in combination with antibiotics can improve the response of broilers during starter period to NDV vaccination. This was shown by the protective titer value which already reached 6.0 as early as two weeks after vaccination. It appears that administration of chlortetracycline may suppress bacterial load in the intestine, however when this suppression is not followed by improved development of intestinal lining (which can be induced by oligomannan of *S.cerevisiae*), the effect on the formation of antibody titers are not significant. This was shown by titer value in broilers fed diet 2 plus chlortetracycline (D2+) which only reached 3.25 on day 18. Improved development of intestinal lining was supported by improved feed conversion in broilers fed Diet 1 plus chlortetracycline (D1+) which indicated better absorption of nutrients. Although this may need further confirmation on the morphology of intestinal lining, such mechanism was in line with other findings that Mannanoligosaccharide (MOS) or antibiotics tended to reduce the coliform load at the gut mucosa and hence improved food conversion efficiency in broilers during 3 weeks posthatch (Hooge *et al.*, 2003; Samarasinghe *et al.*, 2003; Yang *et al.*, 2007; Gao *et al.*, 2008; Yang *et al.*, 2008). Our present results added further evidence that inactive yeast-containing diet in combination with chlortetracycline can modulate humoral immune response to NDV vaccination in broilers during starter phase.

The response of broilers fed Diet 1 or Diet 2 without administration of antibiotics to NDV vaccine began with low titer production which increased steadily at the end of sampling period on day 25. This response indicated that the immune system of broilers during starter period (up to 21 days) was slowly developed and became more mature when approaching 21 days of age. In the absence of antibiotics the birds must adapt to microbial stimulation which comes with feed and water and from the surrounding such as litter, excreta and the air. These antigens are important in stimulating the development of chicks immune system (Honjo *et al.*, 1993). As immune system develops along with gut development stimulated by feed, the response to antigenic stimulation such as NDV was also developed. This adaptation was shown by increase formation of antibody titers from day 18 to day 25 in these D1 and D2 treatments.

At the end of sampling period titer values in broilers fed D2 are significantly higher than D1 indicating that addition of essential amino acids in local diet can improve the response of antibody formation to NDV vaccination. This is in line with other studies that show methionine and lysine are important for cellular and humoral immunity (Kidd, 2004). However when broilers fed D2 was administered chlortetracycline, the antibody titers were not improved as shown in D2+ broilers. This may indicate that when adaptive humoral immunity has developed, chlortetracycline does not affect antibody formation to NDV vaccination. This finding add important evidence that chlortetracycline is beneficial to humoral immunity of broilers to NDV vaccination during starter phase. However, it appears that it is no longer needed after starter phase, especially when diet composition such as the use of sorghum, green bean and inactive yeast can provide natural substance with immunomodulating properties.

Conclusion: Chlortetracycline additive administered via drinking water is beneficial to the formation of antibody titers to NDV vaccination in broilers during starter phase when combined with inactive yeast-containing local diet. Four days after starter phase the titers remains protective in the absence of antibiotics.

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