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Effect of MOS, Baker Yeast, or Noni Leaves Extracts on Antibody Titers to NDV and AI Vaccine of Broilers Fed Corn-mungbean Base Diets

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Abstract: A research was carried out to study the effect of MOS, baker yeast, or Noni leave extracts on broilers antibody titers to NDV and AI vaccine. Broilers were fed corn-mungbean base basal diet. Two hundreds 204 Lohman MB-202 day old chicks were randomly assigned into four treatment diets i.e. 1) commercial diet (Dc), 2) basal diet with addition of MOS (D_{MOS}), (3) basal diet with inactivated baker yeast (D_{YEAST}), (4) basal diet with inactivated baker yeast and Noni leaves extracts (D_{Y+L}). Basal diet, commercial diet and water were given *ad libitum*. NDV and AI vaccine were given simultaneously via subcutaneous on day 4. Serum antibody titers were measured on day 18, 21, 25 and 30 by Haemaglutination Inhibition Test and expressed as Geometric Mean Titer (log2). Antibody titers to NDV and AI vaccine were already detectable two weeks after vaccination (on day 18). There were no significant difference of antibody titers to both vaccines among all groups. These results showed that either MOS, or yeast, or combined yeast and noni leaves extracts can be used in corn-mungbean base diet to support broilers production against NDV and AI routine vaccination.

Key words: MOS, baker yeast, noni leaves extracts, mungbean, antibody titer, NDV vaccine, AI vaccine

INTRODUCTION

Increase awareness of consumers to healthy and safe animal products as well as worldwide ban to in feed antibiotics have made natural feed additives being studied and used. Prebiotic and phytobiotic are several alternatives to in feed antibiotic which have been shown to improve broilers performance and health. MOS is a well studied feed additive that can improve gut morphology, providing competitive exclusion against *E. coli* and improve immunity of broilers (Parks *et al*., 2000; Spring *et al*., 2000; Samarasinghe *et al*., 2003; Yang *et al*., 2008). Other study has also shown that yeast can also improve performance and immunity of broiler (Gao *et al*., 2008). Phytobiotic has regained interest as it can improve gut function, provide antimicrobials activity, serve as antioxidant source and promote growth efficiency (Windisch *et al*., 2008; Al-Kassie, 2010). Other attempt to reduce and eliminate antibiotic use in feed and medication program is utilizing feed ingredients that naturally contain phytonutrients which can improve the antioxidant pool of the body and one or several arms of immune system (Chew and Park, 2004; El-Abasy *et al*., 2002; Hikosaka *et al*., 2006). The use of feed grains such as corn, sorghum and mung beans provide not only macro- and micro-nutrients but also other bioactive nutrients such as carotenoids in corn and mung beans and poliphenolic substance in sorghum and mung beans which can improve humoral immune response against vaccination (Awika *et al*., 2000; Murwani, 2008; Murwani and Murtini, 2009). Furthermore, the grains may also contain other plant secondary metabolites in minute amount which might be beneficial to livestock as natural feed. The following research was carried out to study the effect of inclusion of MOS, inactivated baker yeast, or combination of inactivated baker yeast and Noni leaves extracts into corn-mungbean base diet on broilers antibody titres to NDV and AI vaccination.

MATERIALS AND METHODS

Birds and diets: Corn, sorghum and mungbean were obtained locally in grain form with approximately 11-12% moisture content. 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 under UV light and no mycotoxin was detected. Animal protein was obtained from East Java i.e. a by product of sortation and cleaning process from fish egg industry and the protein content by proximate analysis is 56%. Noni (*Morinda citrifolia*) leaves extracts were prepared by mixing Noni fresh leaves and drinking water in a mixer and the resulting extract was filtered and used directly to be mixed with the basal diet. Baker yeast was inactivated by wet heating and then directly mixed with basal diet.

The basal diet was formulated based on corn-mungbean. Feed ingredients i.e. corn, sorghum, mungbean, animal protein, vitamin mix (Medion Indonesia), mineral mix (Medion Indonesia) and Allzyme (Alltech, Indonesia), methionine, lysine and choline were used to compose the basal diet (Table 1). Vitamins and mineral mix were used to provide more than adequate level as recommended by NRC (1994). Lysine, methionine and choline were added after calculating their contents in each feed ingredients. Treatment diets consisted of D_C) commercial broiler diet; D_{MOS}) basal diet + BioMOS (Alltech, Indonesia); D_{YEAST}) basal diet + inactivated baker yeast *S. cerevisiae* (Fermipan); D_{Y+L}) basal diet + inactivated yeast and Noni leaves extracts. Basal diet and each additive were mixed, pelleted and crumbled. The dried crumble diet for each treatment was kept in separated and labeled clean plastic drums for feeding.

The starter basal diet was formulated to meet nutrient requirement of broilers with protein level of approximately 21% and calculated ME of 2900 Kcal/kg (day 1 to day 21). ME of basal diet was calculated from ME value of each feed ingredient from local feed composition data (Hartadi *et al.*, 1986) or proximate analyses (when published data is not available) or the combined data. From day 22 to day 30, birds were given the same treatment diets. The difference is that for finisher the protein content is reduced to 19-20% by reducing the percentage of mungbean in the basal diet.

A total of 204 Lohman MB-202 one day old unsexed broilers with average initial body weight of 43-44 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 4 large groups. The unselected chicks were raised separately until market weight. The chicks were kept in a warm brooder and given the above described treatment diets (Table 1). Chicks were given *ad libitum* access to the diet and drinking water.

On day 7, each large groups were allocated into two large brooders to give more space to the growing birds. On day 11, the birds from each large groups were further allocated randomly into 5 replicates with 9 chickens in each replicate. Birds were vaccinated with commercial combined ND La Sota and AI vaccine (PT. Medion Indonesia) on day-4 via eye drop and subcutaneous simultaneously.

Vaccination was carried out by Animal Health Technical Officer who routinely vaccinating chickens in the region. 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-AI vaccine. Such combined vaccination on day 4 has been routinely applied in commercial setting for small scale broiler producers. Vaccination on day 4 coincides with the disappearance of maternal antibody after four days post hatch (Nitsan *et al.*, 1991). The treatment diets were performed up to 30 days to follow the appearance of antibody against ND-AI vaccination. Experiments were conducted in an open broiler-house at the Faculty facilities with similar condition as that found in most small scale broiler chicken producers (Murwani and Bayuardhi, 2007).

Table 1: Composition and nutrient contents of experimental diets

Feed ingredients (%)	D_C* (feed label)	D_{MOS}	D_{YEAST}	D_{Y+L}
Corn	NA	32.45	32.45	32.95
Sorghum	NA	3.48	3.28	3.28
Mungbean	NA	39.5	39.5	39.5
Rice meal	NA	5	5	5
Fish protein	NA	11.2	11	11
MOS	NA	0.1	-	-
Baker yeast	NA	-	0.5	0.5
Noni leave extracts	NA	-	-	water extract
Vitamins, minerals and allzyme	NA	7.7	7.7	7.7
Lysine, methionine and choline	NA	0.57	0.57	0.57
Total	NA	100	100	100
Nutritive values				
[a]ME (Kcal/kg)	3071	2939	2936	2937
[b]Crude protein (%)	22.7*	21.4	21.5	21.4
[b]Crude lipid (%)	3.7*	1	1.66	1
[b]Crude fiber (%)	2.8*	2.3	2.4	2.3
[c]Total methionine (%)	NA	0.5	0.5	0.5
[c]Total lysine (%)	NA	1.1	1.1	1.1
[c]Total choline (%)	NA	0.14	0.14	0.14
[d]Total Ca (%)	1.4	1.3	1.2	1.0
[d]Total P (%)	0.9	0.7	0.7	0.7

*Dc is composed of Corn, soybean meal, MBM, CGM, Palm Olein, Essential Amino Acids, Essential Minerals, Premix and Vitamins. It contains antibiotic and anticoccidiostat. NA: the value of each ingredients or the diet is Not Available. Lipid and Crude fibre of commercilal diet were analysed by proximate analyses.

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 calculated and used accordingly to provide more than adequate level of vitamins (NRC, 1994).

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 calculated and used accordingly to provide more than adequate level of minerals (NRC, 1994).

[a]Based on calculated ME.

[b]Calculated based on local feed composition table (Hartadi *et al.*, 1986) or proximate analysis.

[c]Calculated based on local feed composition table and known supplemented lysine, methione and choline.

[d]Total calcium of all diets were analyzed by AAS and total P by Spectrophotometer (AOAC, 1984)

Blood sample collection: Blood was sampled starting two weeks after NDV vaccination or on day 18. The antibody titers were then followed every on day 21, 25 and 30. At the end of sampling period on day 30 the treatments were terminated. One bird of each replicates was sampled randomly for this blood collection. However, on day 18 two birds of each replicates were sampled to give enough serum samples. Serum was separated by centrifugation and kept frozen until analyses.

Determination of antibody titer to NDV and AI vaccine: Antibody titers were measured using Haemaglutinin Inhibition method as describe in Murwani (2008). 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 (log2) 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

Two weeks after vaccination antibody titers to NDV and AI vaccines were already detectable (Table 2 and 3). Statistical analyses for each sampling period on day 18, 21, 25 and 30 days showed that there was no difference in antibody titres to both NDV and AI vaccines among treatments ($p<0.05$).

DISCUSSION

The antibody titers to NDV vaccines in all groups showed a protective value from day 18 to day 30 i.e. ≥ 4 (Medion). As all titres are statistically not significantly different, the present results showed that broiler fed local corn and mungbean based diet either with MOS, or yeast, or yeast and Noni leaves extracts produced the same titer values as that of broiler fed commercial diet. ND vaccination is routinely administered to prevent and successfully preventing ND disease among broilers. Our previous study on the effect of feed ingredient types on antibody titres against ND vaccination has shown that mungbean in high percentage and sorghum in low percentage in the diet can improve antibody titres (Murwani, 2008). Further study also showed that addition of methionine and lysine in the diet to complete the requirement of essential amino acids improves humoral response against ND vaccination (Murwani and Murtini, 2009). Our present study which used diet based on corn and mungbean with addition of methionine and lysine and feed additives of MOS, or yeast, or yeast and Noni leaves extracts, without any antibiotics in feed or in drinking water can protect experiment broilers from day 18 up to day 30 against NDV is consistent with previous results. As present modern broilers can be harvested as early as 28 days (market weight), such protective antibody against NDV up to 30 days is beneficial in broilers production.

The antibody titers to AI vaccines in all groups showed unprotective value (<4) ranging from the lowest 1 to the highest of 4.2. A protective value was found in group with

Table 2: Antibody titres to NDV vaccine

Age of experimental broilers	Treatments D_C	D_{MOS}	D_{YEAST}	D_{Y+L}	CV Transformation
18 days	6.00±0.71	5.20±1.10	5.00±0.71	5.60±1.14	17.16
21 days	5.80±2.05	5.00±0.71	5.00±1.41	5.40±0.89	11.51
25 days	4.80±1.79	4.20±0.84	5.40±1.14	4.40±0.55	10.68
30 days	5.60±1.82	5.00±0.71	4.40±1.52	4.80±1.30	13.28

Table 3: Antibody titres to AI vaccine

Age of experimental broilers	Treatments D_C	D_{MOS}	D_{YEAST}	D_{Y+L}	CV Transformation
18 days	1.20±1.30	2.20±0.84	2.80±1.30	1.00±1.00	11.15
21 days	1.60±1.52	1.80±2.39	0.40±0.55	1.80±1.30	13.32
25 days	3.60±1.14	4.00±1.22	3.40±1.14	3.60±1.14	14.68
30 days	4.20±1.48	3.60±1.52	2.60±1.52	2.00±1.58	9.83

commercial diet on day 30 and in group DMOS on day 25. However, as all titres are statistically not significantly different, the present results indicated that broiler fed local corn and mungbean based diet either with inclusion of MOS, or yeast, or yeast and Noni leaves extracts produced the same titer values against AI vaccine as that of broiler fed commercial diet. The humoral immune response to AI vaccine takes place slowly and the protective values were found much later than ND vaccine. AI is also found more frequently in layers than broilers as layers life span and production time is longer than broilers. Therefore, even with commercial diet the titer values against AI vaccine remains low at the end of blood sampling on day 30. However, such titres have been shown in practical setting to provide protection during their short production time. Therefore simultaneous NDV-AI vaccination to broilers at 4 days of age as one of routine vaccination methods to broilers is beneficial and provide one time effective vaccination. Furthermore the present result added evidence that corn-mungbean base diet is beneficial to humoral immune response to NDV-AI vaccination.

Conclusion: Corn and mungbean base diet with inclusion of MOS, or inactivated baker yeast, or combined inactivated yeast and Noni leaves extracts can be used comparably to commercial corn-soybean meal base diet to support broilers health to ND and AI vaccination.

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REFERENCES

AOAC, 1984. Official Methods of Analyses. Association of Official Analytical Chemists. Inc. Arlington. USA.

Al-Kassie, G.A.M., 2010. The effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks. Int. J. Poult. Sci., 9: 495-498.

Awika, J.M., L.W. Rooney and R.D. Waniska, 2000. Sorghum brans high in anthocyanins and tannins had higher antioxidant powers than blueberries and garlic. American Association of Cereal Chemists (AACC) Annual Meeting. Kansas City.

Chew, B.P. and J.S. Park, 2004. Carotenoid action on the immue response. J. Nutr., 134: 257S-261S.

El-Abasy, M., M. Motobu, K. Shimura, K.J. Na, C.B. Kang, K. Koge, T. Onodera and Y. Hirota, 2002. Immunostimulating and growth-promoting effects of Sugar Cane Extract (SCE) in chickens. J. Vet. Med. Sci., 64: 1061-1063.

Gao, J., H.J. Zhang, S.H. Yu, S.G. Wu, I. Yoon, J. Quigley, Y.P. Gao and G.H. Qi, 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult. Sci., 87: 1377-1384.

Hartadi, H., S. Reksohadiprodjo and A.D. Tillman, 1986. Feed Composition Table for Indonesia. Gadjah Mada University Press, Yogyakarta.

Hikosaka, K., M. El-Abasy, Y. Koayama, M. Motobu, K. Koge, T. Isobe, C. Kang, H. Hayshidani, T. Onodera, P. Wang, M. Matsumura and Y. Hirota, 2006. Immunostimulating effects of the polyphenol-rich fraction of sugar cane (*Saccharum officinarum L.*) extract in chickens. Phytother. Res., 21: 120-125.

Indonesian Directorate of Veterinary Society, 1999. Guide to Control of Infectious Disease. Directorate of Veterinary Society. Directorate General of Animal Husbandry. Indonesian Agriculture Department. Jakarta.

Murwani, R. and B. Bayuardhi, 2007. Broilers serum cholesterol and glutamic oxaloacetic transaminase and their relation to antibiotic in feed and medication programs in four broiler producers in Semarang Region-Central Java, Indonesia. Int. J. Poult. Sci., 6: 266-270.

Murwani, R., 2008. Effect of corn or sorghum in combination with soybean meal or mungbean as feed ingredients on the serum antibody titres to NDV vaccine in broiler chickens. Int. J. Poult. Sci., 7: 497-501.

Murwani, R. and S. Murtini, 2009. Effect of Chlortetracyclin additive in broilers fed local diets on antibody titers to NDV vaccine. Int. J. Poult. Sci., 8: 755-759.

Nitsan, Z., G. Ben-Avraham, Z. Zoref and I. Nir, 1991. Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. Br. Poult. Sci., 32: 515-523.

NRC, 1994. National research Council, Nutrient Requirements of poultry. 9th Rev. Edn., National Academy Press, Washington, DC.

Parks, C.W., J.L. Grimes, P.R. Ferket and A.S. Fairchild, 2000. The case for mannanoligosaccharides in poultry diets. An alternative to growth promotant antibiotics? Proceedings of Alltech's Sixteenth Annual Symposium. Edited by TP Lyons and KA Jacques. Nottingham University Press, pp: 45-60.

Samarasinghe, K., C. Wenk, K.F.S.T. Silva and J.M.D.M. Gunasekera, 2003. Turmeric (*Curcuma longa*) root powder and mannanoligosaccharides as alternatives to antibiotics in broiler chicken diets. Asian-Aust. J. Anim. Sci., 16: 1495.

Spring, P., C. Wenk, K.A. Dawson and K.E. Newman, 2000. The effect of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella* challenged broiler chicks. Poult. Sci., 79: 205-211.

Villegas, P., 1987. Laboratory Manual Avian Virus Disease (AM 805). College of Veterinary Medicine. University of Georgia. Athens, Georgia.

Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr, 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci., 86(E. Suppl.): E140-E148.

Yang, Y., P.A. Iji, A. Kocher, E. Thomson, L.L. Mikkelsen and M. Choct, 2008. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. Br. Poult. Sci., 49: 186-194.