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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

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Abstract: A research was carried out to study the effect of feed types in the diet based on corn or sorghum in combination with soybean meal or broken green beans on titre antibody to NDV vaccine of broilers. Three hundreds day old chicks broilers with initial body weight of 46.35±4.66 grams were randomly assigned into four treatment groups i.e. T1 (diet based on corn and soybean meal), T2 (diet based on corn and green-bean), T3 (diet based on sorghum and soybean meal), T4 (diet based on sorghum and green-bean). Isoenergy and isoprotein diet and water were given *ad libitum*. NDV vaccines were given via eyedrop on day 4 and intramuscularly on day 21. Serum antibody titres were measured on day 38, 42 and 48 by Haemaglutination Inhibition Test and expressed as Geometric Mean Titre (log2). Antibody titre to NDV vaccine was already detectable on day 38 and the value was highest in T4, reaching 5.2 and lowest in T3 i.e. 3.6 ($P<0.05$). On day 42 the titre of all groups were increased reaching a value of greater than 5.0 (protective) except in T3 which remains lower than 5.0 (not protected). On day 48 the highest titre was reached in T2 (6.4), while T3 remained low below 5 (4.2). These results showed that the types of feed ingredients in the diet can influence antibody titre against NDV vaccination.

Key words: Sorghum, green beans, antibody titre, NDV vaccine

Introduction

Feed ingredients accounts for approximately 70% of total cost in poultry production. While corn and soybean meals are the most common feed ingredients used in poultry diet, imported soybean meal is a limiting factor due to its steady increase in cost. Alternatives to soybean meal therefore has been sought. One of locally available vegetable protein is broken green beans (BGB) which are abundantly available during the harvest season as a by product of green beans sortation and milling process. Its protein contents varies from 22-25% (Indriani and Murwani, 2005). Other locally available grain is sorghum which has been well studied to substitute for corn and contains a potent antioxidants poliphenolic tannin (Awika *et al*., 2000).

The use of locally available feed grains such as corn, sorghum, or green beans provides not only macro- and micro-nutrients but also other functional phytochemicals such as carotenoids in corn and green beans and poliphenols in sorghum and green beans. The antioxidant and immunomodulating properties of carotenoids and poliphenols are well known and they have been shown to affect immune response by protecting against oxidative stress and lipid peroxidation, improving humoral and cellular immune response indicated by increase in B and T cell proliferation (Bendich, 2004).

Such naturally occuring phytochemicals in feed ingredients in the diet therefore could have the same function as modulator of immune response *in vivo*. However, such studies are very limited since feed ingredients are generally associated only with production. On the other hand disease challenges by microbes are common in poultry production, especially in tropical country like Indonesia. As in-feed antibiotics use to reduce microbial challenges is increasingly banned worldwide, alternatives are being sought and studied. This research was carried out to study the effect of feed types in the diet (based on corn or sorghum in combination with soybean meal or broken green beans) in the absence of in-feed antibiotics on titre antibody to NDV vaccine in broilers. The results of this study could provide information on the benefit of feed types in improving poultry health.

Materials and Methods

Birds and diets: All feed ingredients were obtained from local feed producers except for soybean meal which was obtained from commercial feed producers. Corn, sorghum and broken mungbean were obtained in grain form with moisture content around 10 to 11%. These feed ingredients were ground separately and stored in

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Table 1: Composition and nutrient contents of experimental diets

Ingredients	Diet 1 (T1)	Diet 2 (T2)	Diet 3 (T3)	Diet 4 (T4)
Corn	43.0	12	-	-
Sorghum	-	-	44	11.5
Soybean meal	21.5	-	22	-
Green-beans	-	50	-	50.5
Rice meal	20	20	13.5	19
Fish meal	15.5	18	20	19
Coconut oil	-	-	0.5	-
Nutrient Contents:				
Crude protein[3]	20.10	20.06	20.04	20.04
Crude lipid[3]	4.92	4.07	4.39	3.85
Crude fiber[3]	2.91	3.27	2.81	3.26
Metabolic energy (Kkal/kg)[4]	2980.10	2980.80	2967.90	2966.90

[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 Nicotinic acid, 5 g Ca-D Pantothenate, 750 g Na, Ca, K and Mg electrolite. This mix was used at 1g/l drinking water. [2]Mineral contents per kg mineral mix : 32.5% Ca, 10% P, 6 g Fe, 4 g Mn, 0.075 g I, 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. [3]Based on feed composition table (Hartadi *et al.*, 1986) and verified by proximate analysis. [4]Based on feed composition table (Hartadi *et al.*, 1986).

clean water tight plastic drum until mix. They were also checked for the presence of mycotoxin and no mycotoxin was detected.

A total of 300 one day old unsexed broilers (Arbor Acress CP-707) with initial body weight 46.35 ± 4.66 g were used in this experiment. They were given free access to sugar containing water at their arrival. The experimental chicks were then selected randomly and assigned into four large groups (in a warm brooder) with 60 birds and equal average body weight in each group. The groups were given the following treatment diets: T1 (diet based on corn and soybean meal), T2 (diet based on corn and green-bean), T3 (diet based on sorghum and soybean meal), T4 (diet based on sorghum and green-bean). Green-beans were used to replace soybean meal (Table 1). Local fish meal and rice meal were added to complete and formulate the diets so it meets nutrient requirement for broilers and provides equal energy (approximately 2980 Kkal/kg) and protein (20%). The remaining chicks served as control group to provide sample birds to detect the appearance of antibody formation to NDV vaccine. Chicks were given *ad libitum* access to the diet and drinking water. Antibiotic-free vitamin and mineral mixture were given through drinking water and diet respectively to meet micronutrients needs. On day 7, the birds from each large group were further allocated randomly into 5 replicates with 12 chickens in each replicate. Birds were vaccinated with commercial NDV La Sota vaccine (Medion) on day-4 via eye drop and on day-21 intramuscularly (inactive vaccine). The dose and vehicle of vaccine was used according to instruction sheet. Intramuscular route was given by technical personnel from Local Veterinary Health Office with automatic injector so that each bird received the same amount of NDV vaccine. The experiments were performed in an open broiler-house at the Faculty facilities with similar condition as that used by most small to medium scale broiler chicken producers in the region (Murwani and Bayuardhi, 2007).

Sample collection: One bird of control group was sampled randomly each week to collect blood sample to detect antibody against NDV vaccine appearance in serum. After the antibody appearance was detected, two chickens of each replicate group were sampled randomly for blood collection for determination of serum antibody titres to NDV vaccine. Blood was collected on day 38, 42 and 48. Blood samples were then centrifuged to obtain serum samples which was stored frozen until analyses.

Determination of antibody titres to NDV vaccine: Antibody titres were measured using Haemaglutinin Inhibition method. Fifty microlitres standard viral suspension (4 Haemaglutination Unit) was suspended into 12 wells of 96 well plates. The titres 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 sample 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 minutes at room temperature. The end point was observed when erythrocyte in the control well was agglutinated. The antibody of serum samples was read as the highest dilution that can inhibit agglutination (Indonesian Directorate of Veterinary Society, 1999). The titre is expressed as Geometric Mean Titre (log2) to simplify numerical writing.

Statistical analysis: A completely randomized design with 4 treatments and 5 replicates was employed. All data were analyzed using ANOVA and Duncan's multiple range test was used when means were significantly difference ($p<0.05$).

Results

Antibody titre to NDV vaccine was already detectable on day 38 (Table 1). The titre value was highest in T4, reaching 5.2 (Log 2 GMT) and lowest in T3 i.e. 3.6 ($P<0.05$), while T1 and T2 were the same. On day 42 the titre of all groups were increased reaching a value of greater than 5.0 (protective) except in T3 which remained lower than 5.0 (not protected) i.e. 4.2. On day 48 the highest titre was reached in T2 (6.4), while T1 was not different than T2 and T4 was not different than T1. T3 again remained low below 5 (4.2).

Observing the titre value for each group at different

Table 2: Antibody titres to NDV vaccine at different broilers age as affected by the types of feed ingredients in the diet

Age of experimental broilers	Treatments				CV
	T1	T2	T3	T4	
Log2 GMT					
38 days	4.40±0.55^{b}	4.80±0.45^{ab}	3.60±0.55^{c}	5.20±0.45^{a}	11.11
42 days	5.80±0.45	5.00±1.00	4.60±0.55	5.40±0.89	14.58
48 days	5.80±0.45^{ab}	6.40±0.55^{a}	4.20±0.45^{c}	5.40±0.55^{b}	9,17

Means within row with no common superscript differ significantly ($p<0.05$). T1 (corn and soybean meal based diet), T2 (corn and green-bean based diet). T3 (sorghum and soybean meal based diet), T4 (sorghum and green-bean based diet). Each treatment group consisted of 5 replicates with 12 chickens in each replicates. Serum was sampled from 2 chickens from each replicates, with a total of 10 chickens per treatment on each sampling age.

broilers age, it can be seen in Fig. 1 that in T1 antibody response start to appear at a value below 5 and increase significantly to a protective value (5.80) at 42 days and remained stable at 48 days old. In T2, antibody titre was increased steadily from 38 days to 48 days reaching a protective value at 42 days. In T4 the titre is already protective at 38 days and reached maximal at 42 days, after which it remained stable. Comparing this antibody response among treatments, T2 and T4 in which green beans was used in combination with corn and sorghum respectively, are comparable to T1 in their ability to response to NDV vaccine. T1 as the common diet based on corn and soybean meal therefore can be replaced with diet based on corn-green bean or sorghum-green bean base diet.

Discussion

The results of the present study showed an additional new evidence that corn or sorghum base diet in combination either with soybean meal or green beans can affect immune response. Corn-soybean meal and corn-green bean base diet showed a similar protective immune response to NDV vaccine in which antibody titres were achieved maximally on day 48. Corn-green bean base diet which showed higher titres than corn-soybean meal base diet indicates that combination of these grains can stimulate a better response to NDV vaccine. While corn and sorghum provide the same function as source of energy, green beans can provide not only vegetable protein to substitute for soybean meal but also source of energy. As the diet has been formulated to have an equal level of energy, protein and micronutrients, there is a possible role of phytochemicals content in these grains to immune response. While corn contains carotenoid as xanthophyll, α- and β carotene, green beans contains β-carotene dan poliphenol (Murwani, 2008). The use of corn and green beans together in the diet T2 therefore could increase total phytochemicals content in the diet and could have synergistic effect in upregulating antibody titres. On the other hand the use of corn-soybean meal base diet which contain less

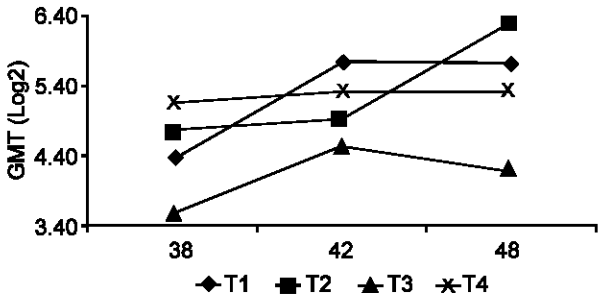

Fig. 1: Antibody titres of broilers at different broilers age as affected by treatment diets. T1 (corn and soybean meal based diet), T2 (corn and green-bean based diet), T3 (sorghum and soybean meal based diet), T4 (sorghum and green-bean based diet). ach treatment group consisted of 5 replicates with 12 chickens in each replicates. Serum was sampled from 2 chickens from each replicates, with a total of 10 chickens per treatment on each sampling age.

phytochemicals (as soybean meal contains only trace amount of carotenoids after oil extraction) produced lower antibody titres than corn-green bean base diet. The immunomodulating properties of pure carotenoids and poliphenols are well studied and they have been shown to affect immune response by improving humoral and cellular immune response indicated by increase in B and T cell proliferation (Bendich, 2004). The present results indicate that the kind and amount of naturally occurring carotenoids and poliphenol in broilers diet can affect antibody formation.

Sorghum-green bean base diet can also give a protective antibody titres. Although the titres are not as high as in groups with corn base diets, the protective value appears earlier than the corn base diets. Early protective titres in sorghum-green bean base diet may indicate the possible roles of phytochemicals containing sorghum-green bean diet in modulating antibody response to NDV vaccine. Sorghum and green beans contain phenolic compounds (one of which is tannin) and β-carotene respectively (Awika *et al*., 2003; Awika and Rooney, 2004; Murwani, 2007). Tannin contents in this group are 0.13% and β-carotene contents are similar to that green beans in corn-green bean base diet (approximately 500 g/kg) but lack of xanthophyl and β-carotene from corn. The combination of low phenolic tannin and β-carotene might be responsible for the early appearance of protective antibody titres in this group. The diet formulation in this group to achieve proper protein and energy content has made the amount of sorghum use (115g/kg) considerably less than that in sorghum-soybean meal diet so that the tannin content in this sorghum-green bean diet is low (0.13%). The positive effect of tannin as poliphenolic compounds is

well known. It can stimulate glucose transport, inhibit adipocyte differentiation, lower blood lipid, act as an antioxidant and antiinflammatory agents, prevent LDL oxidation and produce higher antibody response to red blood cells (Yang *et al*., 1998; Liu *et al*., 2005; Scalbert *et al*., 2005; Hikosaka *et al*., 2006). This results showed that low amount of sorghum in broilers diet (115g/kg) in which naturally occurring tannin content is also low (0.13%) exerts a positive effect by upregulating antibody formation against NDV vaccine. This finding provides important further evidence that raw sorghum containing diet can improve humoral response in broilers only when the tannin level is low.

A striking difference in antibody titres occurs in groups base on sorghum-soybean meal diet. The titres in this group could not achieve a protective value until the end of sampling period on day 48 (4.2 which is less than 5 as the protective value). The low titres in this group might be due to tannin contents in the diet (0.43%) which might be too high for broilers to adapt. Tannin sorghum has been shown to reduce feed consumption and reduce body weight gain due to its interaction with salivary and enzymatic proteins (Bennick, 2002). Tannin sorghum in the diet at 1% or 2.5% equivalent to tannic acid resulted in reduced body weight gain, reduced liver protein synthesis and significant increase in liver proteolytic activity (Fuller *et al*., 1967; Badawy *et al*., 1969; Marzo *et al*., 2002). On the other hand raw red sorghum can be used up to 33% (0.475% tannin) in the diet replacing 50% corn with no significant difference in body weight gain and feed intake (Mandall *et al*., 2005, Kumar *et al*., 2007). The findings of lowest antibody titres in this group is a new evidence that tannin containing sorghum can negatively affect not only broiler chickens performance as found by others but can also lower immune response in this study. Low immune response is possibly due to reduced protein availability for liver protein synthesis associated with immune response or antibody production. This finding also provides important further evidence that raw sorghum containing diet can have negative effect on humoral response in broilers when the tannin level is high.

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