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Effects of high level of fungal phytase on newcastle disease vaccination in broiler chickens

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Abstract

Objectives: An experiment was carried out in broiler chicken fed two doses of commercially produced fungal phytase (Natuphos®) addition to observe their growth performance as health status, and to investigate the changes of hemato-biochemical constituents and immune response.

Materials and Methods: Seventy two male broiler chickens (Cobb) at one-day old were allocated to two treatment (T) groups with six cages comprising three replicates, each cage containing twelve birds. Experimental formulating diets arranged with two levels of 0 and 1500 (FTU/kg^{-1} of diet) Natuphos® as considered as T0 and T1, respectively. At seven days intervals, two birds were randomly selected from each treatment, and were weighted for assessing the growth performance and slaughtered for collection of blood and jejunal fluid throughout the experimental period to quantify specific antibody, IgM and IgG, as well as IgA, respectively. At the age of six week, blood was furthermore collected for determining the complete hemogram and blood biochemical constituents. Specific antibodies against Newcastle disease vaccine (NDV) was detected by ELISA.

Results: Data showed that the inclusion of Natuphos® at the dose rate of 1500 FTU kg^{-1} of diet, the significant ($P<0.05$) differences were observed starting from the age of second week and continued until six week. The main effects data on growth performance revealed that average body weight gains of phytase treated birds were increased significantly ($P<0.05$) at all ages. In terms of humoral immune response, data showed that a specific antibody production against NDV was not improved by Natuphos® addition in a low P diet. However, although non-specific blood IgM and IgG levels were not enhanced, the secretory jejunal IgA content in enzyme treated group was increased throughout the experiments. Conversely, no significant, sequential, and consistent changed effects were observed on complete hemogram and blood biochemical constituents in broiler chickens by Natuphos® treatments.

Conclusion: The results obtained in this study decided that high level of fungal phytase (Natuphos®) addition had no side effect on hematological parameters and biochemical constituents at blood level. Rather it enhances the growth performance and mucosal secretory IgA concentration on ND vaccination.

Keywords: Broiler chicken, Fungal phytase, ND vaccination, Immune responses, Body weight, Hemato- biochemical constituents.

Introduction

Phytate is synthesized in the plant cells in which it is stored. The location of phytate within the seed and its chemical associations with other nutrients influence its availability (Angel *et al.*, 2002). Eighty-two percent of the phytate consumed in poultry diets is recovered in the excreta (Cowieson *et al.*, 2004). Phytase is the only recognized enzyme that is capable of catalyzing the phytate in feeds to release inorganic phosphorus as well as inositol (Nelson, 1967), and also to loosen trace minerals (Ca, P, Mg, Zn, Cu, Co, Mn and Fe), protein, amino acid, and starch that bound with phytin. Thus, using of phytase in diet may directly improve the bio-digestibility not only of phosphorus but also indirectly increase divalent cations, energy and of nitrogen utilization (Selle *et al.*, 2000). Indeed, monogastric animals (poultry, swine, pre-ruminant calves) including human cannot synthesize phytate degrading enzyme "phytase". To meet dietary P requirement of these animals, inorganic P (dicalcium phosphate) or exogenous phytase are commonly added to commercial diet. However, di-calcium phosphate supplementation is not only expensive but also leads to environmental problems by over supplementation. Excess inorganic phosphorus in poultry excreta can create environmental concerns such as soil saturation, soil run-off, and eutrophication (Sharple *et al.*, 1993).

For these mentioned reasons at present phytase is being used randomly in poultry industry to get more production and reduce P pollution. Although nutrients digestibility are important measures of any dietary changes in animal body; growth performance, hemato-biochemical constitutes and immune response are generally more sensitive than nutrients bioavailability for evaluating animal's health status. However, research in these contests is the very limited, and having some contradictions among several researchers. So, more comprehensive studies are needed to elucidate the consequence of phytase in animal body. Therefore, the current experiment was planned with the objective of to assess the effect of commercially produced fungal phytase supplementation on the body weight, investigation on the changes of hemato-biochemical constituents and body immunity on Newcastle disease vaccination in broilers.

Materials and Methods

Fungal Phytase: The current experiment was conducted at faculty of veterinary medicine of University Putra Malaysia, Malaysia. The commercially produced fungal phytase (Natuphos®5000) synthesized from *Aspergillus niger* was pioneered by BASF, The Chemical Company, Germany.

Birds, Feeding and Management: In this research, a total number of 72, one-day-old male broiler chicks (Cobb strain) of nearly similar live body weight were obtained from a commercial hatchery. The birds were housed in an environmentally controlled automatic climatic chamber for seven weeks with continuous lighting and controlled ventilation. Temperature was maintained at 30-32^{0}C for the first week and then gradually reduced according to normal management practices until a temperature of at around 25^{0}C which was maintained during

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remaining period. The chicks were randomly assigned to one control and one experimental groups comprising three replicates of twelve birds each, and were placed in separate cages. There were fed a basal diet grouped T0 (control) and the basal diet supplemented with 1500 FTU $kg^{-1}$of diet grouped as T1. The phytase enzyme (Natuphos®5000) was added just prior to give feed to chicks at a day. The basal diet was formulated to cover nutrient requirements of broiler chicks as recommended by NRC (1994). The ingredients and calculated analysis of the experimental basal diets are shown in Table 1. Feed in a dry mash form and fresh water were offered ad libitum basis consumption throughout the experimental period. Vaccines of ND 'V4 HR' for ND was obtained from Malaysian Vaccines and Pharmaceuticals Sdn. Bhd (Co. No. 82381-X), Kuala Lumpur, Malaysia, and vaccination schedules were followed according to manufacturer's instructions.

Sampling and Measurement: Initial body weight of each chick was recorded on replicate basis at arrival. At every week interval, before giving feed two birds from each treatment per replicate were picked up randomly and were then tagged numerically on their convenient place (legs &/or wings). For evaluation of growth performance at weekly, lives body weight gains were weighted by digital balance and recorded from the selecting birds. The birds were then slaughtered to obtain blood for serum preparation to determine specific antibody (Ab), IgM, as well as IgG and mucosal fluid to quantify IgA. Roughly, 3 ml of blood was directly collected from each slaughtering bird into Vacationer tube without anticoagulant and serum was prepared according to method described by Bush (1975). Jejunal mucosa was used to prepare mucosal fluid, immediately after blood collection from the slaughtering birds. The procedures were followed according to the method described by Liu *et al*., 2008. At the end of experiment when the birds were of six week old, blood samples were collected for measurement of complete hemogram, and blood biochemical constituents. Usually around 4 ml of blood was directly aliquoted from the selecting birds into collecting tubes with lithium heparin as an anticoagulant. Each sample was put in its own-labeled tube. After estimation of complete hemogram, the remaining blood was then centrifuged at 5000 rpm for 10 minutes. The plasma was later separated into microtube and stored at -20^{0}C until measurement of biochemical constituents.

IDEXX FlockChek* NDV (IDEXX Laboratories, USA) was used for the detection of Ab to NDV in chicken serum, and the procedures were followed according to manufacturer's instructions.Chicken IgM ELISA Quantitation Set, Chicken IgA ELISA Quantitation Set, and Chicken IgG ELISA Quantitation Set (Bethyl Laboratories, Inc., USA) were used to determine the non-specific IgM, IgA, and IgG in chicken serum, respectively.The values of red blood cell (RBC), white blood cell (WBC), platelet (PLT) count, hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular Hb concentration (MCHC) were determined by an automated hematology analyzer (Abbott CELL-DYN 3700 Hematology Analyzer, GMI) using commercial reagents and other values (packed cell volume, heterophil, eosinophil, basophil, lymphocyte, monocyte, icterus index and total plasma protein) were determined manually.Using a chemistry analyzer (HITACHI 902, Japan), the technical procedures of test methods were described to measure blood biochemical constituents in broiler chickens. The constituents, such as albumin (Alb), total protein (TP), alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), cholesterol (Chol), triglyceride (Trig), Glucose (Glu), calcium (Ca), phosphorus, urea, creatinine (Creat), and uric acid (UA) were determined by colorimetry (absorbance measurement) method using available commercial kits. The parameters of sodium (Na), potassium (K), and Chloride (Cl) were determined by ion selective electrode method.

Table 1. Ingredient Compositions (g) and Calculated Values of the Negative Control (Low in P) Experimental Basal Diets

Items	Amount					
Ingredient Compositions	Diet (Local Phytase)				Diet (Commercial Phytase)	
	0 FTU	500 FTU	1000 FTU	1500 FTU	0 FTU	1500 FTU
Corn grain	522.5	522.5	522.5	522.5	522.5	522.5
Rice bran	150	150	150	150	150	150
SBM	280	280	280	280	280	280
Corn oil	11	11	11	11	11	11
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
NaCl	3.2	3.2	3.2	3.2	3.2	3
Ca carbonate	27	27	27	27	27	26.9
Ca phosphate	3	3	3	3	3	3
Vitamins†	2	2	2	2	2	2
Trace mineral‡	1	1	1	1	1	1
Local Phytase	Volume Depended on Enzyme Activity				0	0
Natuphos®5000	0				0	0.3
Total (gm)	1000				1000	
Calculated Nutritive Values						
ME (Kcal/kg)	3000.6				3000.6	3000.6
CP (%)	1.998				1.998	1.998
Ca (%)	1.189				1.189	1.185
Total P (%)	0.609				0.609	0.609
nPP (%)	0.193				0.193	0.193
K (%)	0.973				0.973	0.973
Cl (%)	0.237				0.237	0.225
Mg (%)	0.292				0.292	0.292
Na (%)	0.152				0.152	0.144
Gly&Ser (%)	0.91				0.91	0.91
Leu (%)	1.7				1.7	1.7
Met &Cys (%)	0.34				0.34	0.34
Thr (%)	0.75	0.75	0.75			

Statistical Analysis: All the experiments were conducted using completely randomized design (CRD) with three replications. The data were subjected to analysis of variance (ANOVA) and tested for significance using the least significant difference (LSD) by PC-SAS software (SAS Institute, 2009). Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Growth performance: To evaluate the growth performance, the effects of Natuphos® on average body weight gains (BWG) over the ages of one, two, three, four, five and six week in ND vaccinated broiler chickens are presented in Table 2 and Figure 1

Table 2. Body weight gains of broiler chickens at weekly fed on a low P basal diet supplemented with Natuphos® and vaccinated with a ND vaccine

Treatments	Body weight gain (mean)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
T0 (0 FTU kg-1)	107.17a	280.00b	539.50b	931.17b	1501.83b	1804.00b
T1 (1500 FTU kg-1)	129.50a	372.33a	691.17a	1241.00a	1753.83a	2000.50a
$LSD_{0.05}$	41.39	36.99	71.68	66.67	85.26	88.49

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability

Treatment 0 (T0) = Basal diet (Negative control) without phytase
Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

The results show that at all ages, the average BWGs of broiler chicks were consistently and significantly (P<0.05) increased from the ages of two to six week between treated groups. The average weight gains at phytase treated birds (T1) are always higher than the birds without phytase (T0). In a current study, the results were almost similar to those obtained by Khin, 2011; Rakibul *et al*., 2011; Nasrollah, 2010; Ahmed *et al*., 2004; Shirley and Edwards, 2003; Ahmad *et al*., 2000; Viveros *et al.* 2002.

Hematological parameters: Over the age of six week, the effects of Natuphos® on hematological parameters in ND vaccinated broiler chickens are summarized in Table 3. The results show that no significant (P>0.05) as well consistent treatment effects on hematological parameters were found between treated groups. Only a few studies have investigated the influence of dietary microbial phytase on hematological parameters (El-Badry*et al.,* 2008; Anna Czech and Eugeniusz, 2004) in animals. El-Badry*et al.,* (2008) reported that WBC, RBC, and PCV were not affected, but blood Hb concentration was increased by dietary phytase treatments. The exogenous microbial phytase inclusion to a low P swine diets did not influence PCV, BRC, but diminished WBC number and increased Hb content in blood (Anna Czech and Eugeniusz, 2004).

Table 3. Hematological parameters of broiler chickens fed on a low P basal diet supplemented with Natuphos® and vaccinated with a ND vaccine

Parameters	Units	Treatments		$LSD_{0.05}$
		T0 (0 FTU kg-1)	T1 (1500 FTU kg-1)	
TEC	$\times 10^{12}$/L	2.50a	2.59a	0.35
Hb	g/L	125.17a	130.33a	15.72
PCV	L/L	0.29a	0.30a	0.03
MCV	fL	117.83a	116.17a	5.93
MCHC	g/L	427.17a	434.17a	18.65
TLC	$\times 10^{9}$/L	17.40a	12.95a	20.02
Heterophil	%	25.50a	30.33a	9.60
Eosinophil	%	0.50a	0.50a	0.80
Basophil	%	12.83a	6.00a	5.69
Lymphocyte	%	61.83a	60.33a	11.74
Monocyte	%	2.17a	2.83a	3.07
Thrombocyte	$\times 10^{9}$/L	7.94a	9.11a	8.90
Ic. Index	Unit	2.00a	2.00a	0.00
Pl. Protein	g/L	29.83a	30.17a	3.27

Values within a row with no common superscript differ significantly (p ≤ 0.05)

Treatment 0 (T0) = Basal diet (Negative control) without phytase
Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

Blood Biochemical Constitutes: The effects of Natuphos® on blood biochemical constituents of ND vaccinated broiler chickens are presented in Table 4. The results indicate that no significant (P>0.05) and consistent treatment effects were observed between treated birds. The findings of these parameters are accorded with those reported by Shehab *et al*., 2012; Aureli *et al*., 2011; Danek *et al*., 2007; Al-Harthi, 2006; Eisa *et al.,* 2003; Attia, 2003; Qota *et al*., 2002; Viveros *et al*., 2002; Huff *et al*., 1998.

Table 4. Blood biochemical constituents of broiler chickens fed on a low P basal diet supplemented with Natuphos® and vaccinated with a ND vaccine

Parameters	Units	Treatments		$LSD_{0.05}$
		T0 (0 FTU kg-1)	T1 (1500 FTU kg-1)	
Albumin	g/L	11.63a	11.83a	4.90
T. Protein	g/L	28.47a	30.18a	3.93
ALT	U/L	3.87a	5.13a	3.31
ALP	U/L	1896.7a	1443.8a	1219.4
AST	U/L	230.75a	252.63a	28.43
GGT	U/L	18.33a	20.33a	5.53
LDH	U/L	2205.3a	2514.8a	1644.1
Cholesterol	mmol/L	2.78a	2.88a	0.62
Triglyceride	mmol/L	0.68a	0.53a	0.37
Glucose	mmol/L	12.00a	12.80a	1.07
Ca	mmol/L	2.39a	2.26a	0.85
P	mmol/L	1.58a	1.81a	0.32
Na	mmol/L	134.90a	143.30a	25.56
K	mmol/L	4.10a	3.57a	0.89
Cl	mmol/L	95.33a	100.33a	15.75
Urea	mmol/L	2.32a	2.00a	0.62
Creatinine	umol/L	22.33a	25.83a	10.71
Uric Acid	umol/L	101.87a	79.57a	43.77

Means in a row with no common superscript differ significantly (p ≤ 0.05)

Treatment 0 (T0) = Basal diet (Negative control) without phytase

Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

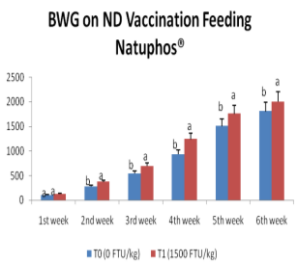

Fig. 1. Body weight gains of broiler chickens (weekly intervals) fed on a low P diet supplemented with Natuphos® and vaccinated with a ND vaccine

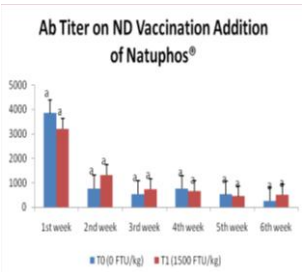

Fig. 2. Effect of Natuphos® (weekly) on Ab production to NDV in broiler chickens fed on a P deficient basal diet and vaccinated with a ND vaccine

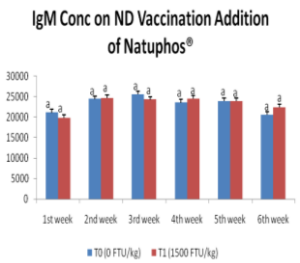

Fig. 3. Effects of Natuphos® (weekly intervals) on IgM concentrations of broiler chickens fed on a P deficient basal diet and vaccinated with a ND vaccine

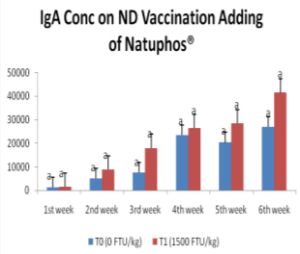

Fig. 4. Effects of Natuphos® on mucosal IgA contents (weekly intervals) of the ND vaccinated broiler chickens fed on a P deficient basal diet

Humoral Immune Responses

The effects of Natuphos® on specific Ab production to NDV in broiler chickens at weekly interval are presented in Table 5 and Figure 2.The results show that the blood Ab titers of NDV in broiler chickens over the ages of two, three, four, five, and six week were very low compared to one week and no significant treatment effects were found. Furthermore, the Ab titers of all treatments were not consistent to their respective doses at all ages. The effects of Natuphos® on blood IgM concentrations of broiler chickens on ND vaccination at weekly interval are shown in Table 6 and Figure 3.The results indicate that the blood IgM contents of broilers at all ages were almost similar and stationary, but no significant and consistent treatment effects were observed between different groups.

At weekly, the effects of Natuphos® on mucosal IgA concentrations in ND vaccinated broiler chickens are mentioned in Table 7 and Figure 4.The results show that although no significant (P>0.05) treatment effects were observed, from the age of 1 to 6 wks, the mucosal IgA concentrations of treated groups were consistently increased to the relative doses and the IgA levels of phytase treated group (T1) were always higher than the birds without phytase (T0).

The effects of Natuphos® on blood IgG concentration in ND vaccinated broiler chickens at weekly are shown in Table 8 and Figure 5.The results show that at all ages, the blood IgG contents of broilers chickens were almost similar and stationary for all treatments, but at all ages, the concentrations were inconsistent to their corresponding doses.

In the current study, overall, the specific Ab production of broiler chickens to NDV was not affected by phytase supplementation in a P deficient diet. This is partly confirmed by Liu *et al*. (2008) that although phytase (Phyzyme®, *Escherichia coli*-derived phytase) addition in high phytate (0.44%) diet improved the anti-NDV antibodies significantly (P<0.05) at the ages of three and four week, low phytate (0.22%) diet did not influence the serum Ab production at the ages of two, three and four week birds on ND vaccination. Phytase addition, after all, increases the non-specific mucosal secretory IgA concentration of broiler chickens, and similar to results previously reported by Liu *et al*., 2008.To our knowledge, there are no reports of the IgM and IgG concentration due to dietary administration of phytase for broiler chickens.

Table 5. Ab titers of NDV in broiler chickens (weekly intervals) fed on a low P basal diet supplemented with Natuphos® and vaccinated with a ND vaccine

Treatm-ents	Antibody titer (mean)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
T0 (0 FTU kg-1)	3840a	759.5a	527.5a	746.8a	516.0a	262.8a
T1 (1500 FTU kg-1)	3197a	1300.3a	740.8a	658.5a	442.3a	509.3a
$LSD_{0.05}$	3160.2	872.6	873.26	2083	538.99	631.62

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability

Treatment 0 (T0) = Basal diet (Negative control) without phytase

Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

Table 6. Effects of Natuphos® on blood IgM concentrations at weekly in broiler chickens fed on a low P basal diet and vaccinated with a ND vaccine

Treatments	IgM Concentration (mean)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
T0 (0 FTU kg-1)	21054a	24361.0a	25515.0a	23474.7a	23864a	20490a
T1 (1500 FTU kg-1)	19814a	24611.3a	24234.0a	24481.7a	23824a	22345a
$LSD_{0.05}$	7311.6	1054.6	1790.7	2273	2984.4	13513

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability

Treatment 0 (T0) = Basal diet (Negative control) without phytase

Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

Table 7. Effects of Natuphos® (weekly intervals) on mucosal IgA contents in broiler chickens fed on a low P basal diet and vaccinated with a ND vaccine

Treatment	IgA Concentration (mean)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
T0 (0 FTU kg-1)	1310.3a	5061a	7528a	23348a	20232a	26894a
T1 (1500 FTU kg-1)	1449.3a	8869a	17941a	26381a	28366a	41408a
LSD0.05	514.12	11870	26872	26029	11826	33798

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability

Treatment 0 (T0) = Basal diet (Negative control) without phytase

Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

Table 8. Effects of Natuphos® on blood IgG contents in broiler chickens (weekly intervals) fed on a low P basal diet and vaccinated with a ND vaccine

Treatments	IgG Concentration (mean)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
T0 (0 FTU kg-1)	19695a	11808.3a	15222a	14763a	15594a	16706a
T1 (1500 FTU kg-1)	13846a	11915.0a	13838a	15348a	15048a	16510a
LSD0.05	6261.2	2706.1	4159.3	3824.5	2906.2	6928.3

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability

Treatment 0 (T0) = Basal diet (Negative control) without phytase

Treatment 1 (T1) = Basal diet with phytase (1500 FTU/kg of diet)

Conclusion

The results obtained in this study decided that high level of fungal phytase (Natuphos®) addition had no side effect on hematological parameters and biochemical constituents at blood

level. This suggests that diet supplementation with Natuphos® did not affect the health of broiler chickens. Rather it enhances the growth performance and mucosal secretory IgA concentration on ND vaccination. It would be necessary for further research to evaluate interaction with dietary mineral content, biochemical constituents including inositol and steroid hormones, and the rate of phytase supplementation, so that the optimal dose could be used in gaining the most benefit from phytase supplementation.

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