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RESEARCH ARTICLE

Effect of Different Oil Supplements on Humoral Immune Response and Lipid Profile in Commercial Broiler

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The experiment was carried out on 600 day old broiler chicks randomly divided into four groups designated as T_0, T_1, T_2 and T_3 with 150 birds in each group for a period of 42 days to find out the effect of different oil supplements on humoral immune response and lipid profile. All groups were maintained on isonitrogenous feed supplemented with soyabean oil (T_1); palm oil (T_2) and fish oil (T_3) @ 2.5%. The birds of group T_0 were maintained as untreated controls without addition of any oil supplement. All birds were vaccinated against Newcastle disease virus (NDV) on 0 and 7th day. Weekly collected serum samples were analyzed for determination of antibody titer against NDV and lipid profile through haemagglutination inhibition (HI) and calorimetric method respectively. Fatty acid profile in abdominal fat was determined through gas chromatographic analysis. On 28th day of age, highest geometric mean antibody titer against NDV was recorded for T_2 (776) group followed by T_1 (256); T_3 (157) and T_0 (39.4). On 42nd day, significantly ($P<0.05$) decreased HDL (18.33±0.67 mg/dl) and triglycerides (93±5.49 mg/dl) were recorded for group T_1 whereas that of LDL (67.67±1.71 mg/dl) was recorded for group T_3. Non-significant ($P>0.05$) difference was recorded between the groups with respect to unsaturated and saturated fatty acids in abdominal fat. It was concluded that palm oil supplement significantly increased the humoral immune response as compared to soybean and fish oil supplements.

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INTRODUCTION

Oils are used as source of extra energy in broiler feed. Oil helps in the digestion and absorption of the fat soluble vitamins. Among the fat soluble vitamins, vitamin A deficiency in human and animals greatly increases susceptibility to infection (Waldenstedt *et al*., 2000; Khan *et al*., 2013). Vitamin A may play a secondary role in systemic immune response. The effect of vitamin A deficiency and infections are synergistic (Yang *et al*., 2011). Addition of small amount of vitamin A enhances both antibody production and T-cell proliferative responses (Sklan *et al*., 1994). The antibody production against fowl pox and Newcastle Disease Virus (NDV) enhances with increased amount of vitamin A in the diet (Sklan *et al.*, 1995). Cellular immunity and antibody responses to NDV were both influenced by diets containing different levels of vitamin A (Lessard *et al.*, 1997). Chicks received supplementation of 200 mg vitamin E/Kg and 0.2 mg selenium/kg produced significantly higher hemagglutina- ting inhibition (HI) antibody. This was associated with an increased serum concentration of total immunoglobulin suggested that vitamin E and selenium have synergistic effects on immune response (Singh *et al*., 2006). Antibody formulation against Newcastle disease was improved by Vitamin E treatment in drinking water (Weber *et al*., 2008).

In Bangladesh Newcastle Disease (ND) is popularly known as Ranikhet disease and it is one of the major killer diseases in backyard poultry causing 40-60% of the total mortality (Bhuyian *et al*., 2003). The disease can be controlled by effective vaccination. In commercial poultry farm maternal antibodies is one of the major interacting

factors for the production of immune response. Red palm oil is the richest food source of carotenoids and vitamin E. Similarly soybean and fish oil are also rich in vitamin A and E. In human, atherosclerosis is linked to high levels of cholesterol in the blood and particularly to high levels of LDL (low density lipoprotein) bound cholesterol. It is negatively correlated with high density lipoprotein (HDL). LDL is composed of triacyl glycerol (TAG), cholesterol and cholesterol esters. Limited works have done to link the relationship between supplemental oil, immune response and lipid profile of birds. Therefore, the present study was undertaken to find out the effect of different oil sources on immune responses of broiler against Newcastle disease vaccination and lipid profile of broilers fed diet supplemented with different types of oils.

MATERIALS AND METHODS

Birds' management: Six hundred one-day-old Hybro-PN broiler chicks were used in a 42-day trial at Chittagong Veterinary and Animal Sciences University (CVASU) Poultry farm. A poultry shed (floor size 914×7621×604 cm^3) was constructed and the floor was divided into 12 pens by wire-net. Floor space for each broiler was 941cm. Fresh and dried rice husk was used as litter material at a depth of 4.50 cm. Old litter materials were removed from the pen and new litter was replaced at every 15 days interval.

Four diets designated as T_0 (without oil supplementation), T_1 (2.5% soyabean oil), T_2 (2.5% Palm oil) and T_3 (2.5% fish oil) were formulated using locally available ingredients. All diets were isonitrogenous and iso-caloric (Table 1). The birds were given dry mash feed throughout the whole experimental period. Feed and water were supplied ad-libitum to the broilers. The broilers were brooded in respective pens at a temperature of 32-35ºC. The broilers were exposed to a continuous 24 hours lighting.

Birds were vaccinated against NDV at day 0 by baby chick Ranikhet disease vaccine (BCRDV) and at day 7 by Ranikhet disease vaccine (RDV). Both vaccines were prepared by the Department of Livestock Services, Bangladesh.

Sample collection and analysis: Sera were collected from four flocks after one week interval of NDV (Newcastle Disease Vaccination) from three birds of each replication in four groups (T_0, T_1, T_2) and T_3 and stored at -86°C (New Brunswick Freezer, USA) till analysis at CVASU Animal Nutrition Laboratory, Chittagong. NDV antigen was La Sota vaccine strain (Avinew, Merial, France) and NDV antiserum was collected from Veterinary Laboratory Agency, UK. HI test was carried out by following the recommendations of OIE (2012).

At 3, 4, 5 and 6 weeks of age, one broiler was randomly selected from each replicate of treatments and 3 blood samples from each treatment were collected, serum was separated and stored at -5ºC until cholesterol analysis. Serum samples were analyzed for lipid profile according to the colorimetric method (Prasad *et al*., 2009) at BCSIR (Bangladesh Council of Scientific and Industrial Research) Laboratory, Chittagong, Bangladesh. From abdominal fat of broiler lipid extraction was carried out following the procedure described by Prasad *et al*. (2009).

For the determination of fatty acid profile in abdominal fat of broiler, Shimadzu (Japanese) Gas chromatographic and flame ionization detector were used with initial temperature of 100ºC. Injector and detector temperatures were 280ºC and 290ºC, respectively. Column: BP-50 (Polar). Integrator: LKB 2220. This work was followed by the rapid preparation of fatty acid esters for Gas Chromatography analysis method. The analysis was carried out in Applied Chemistry Laboratory at University of Dhaka, Bangladesh.

Statistical analysis: Data related to total serum cholesterol, HDL, LDL, triglyceride, Unsaturated and Saturated fatty acid profile of birds were analyzed by one way ANOVA using Stata 11C and SPSS version 16. Means showing significant differences were compared by Duncan's New Multiple Range Test. Statistical significance was accepted at 5% ($P<0.05$).

RESULTS

The results on $\log_2$ HI titers of NDV in blood sera of broilers belonged to four treatment groups are shown in Table 2. At day 7, the GMT of HI titer in blood sera to NDV were recorded almost same in all of the four treatment groups. No significant difference was observed among the four treatment groups. On day 14, the GMT of the broilers of all the treatment groups was increased reaching a value of 32 except T_0 where the GMT level was below the protective threshold. The protective titer was decreased again in birds of all the treatment groups with the exception of the birds of T_3 at 21 days of age. The highest GMT was recorded in birds of T_2 group, which was 776±1.20 and the lowest GMT was found in T_0 i.e. 39.54±0.33, while the mean HI titer of birds of T_1 and T_3 were found more than 150 on 28 days of age. At 7, 14 and 21 days of age, the oils had no significant effects on the mean antibody level. Improved immunological response was observed at 28 days of age. At the end of the experiment the HI titer in the chicks of the T_1, T_2 and T_3 groups were significantly higher than that of the T_0 group.

It has been observed that maximum levels of HI antibodies were seen after 21 days of age. Antibody response started to appear at a value below 30 in the birds of T_0 during first week and increased to a protective level at 28 days. In T_1 and T_2, mean antibody titer was increased from 7 days reaching a protective value at 14 days. In T_3 the titer was found protective at 14 days after which it was remained stable and reached maximal at 28 days.

Serum cholesterol: The data of serum cholesterol level of broilers at different stages of growth fed on different types of oil supplemented diets are presented in Table 3. There was no significant variations among mean values of total serum cholesterol level of birds fed diets supplemented with or without oils at 3, 5 and 6 weeks of age, except at 4 weeks of age of the broilers. At this age, the level of cholesterol of palm oil group showed the lowest value and different significantly from that of control ($P<0.05$). The second lowest value was found in fish oil group, whereas soybean oil group showed the highest value among the oil supplemented groups, similar to without oil.

Table 1: Composition of experimental diets

Feed Ingredient (Kg/100 kg)	T_0		T_1		T_2		T_3	
	Starter (0-21 d)	Finisher (22-35 d)	Starter (0-21 d)	Finisher (22-35 d)	Starter (0-21d)	Finisher (22-35 d)	Starter (0-21 d)	Finisher (22-35 d)
Maize	61.00	68.25	58.00	61.91	61.01	65.75	58.00	62.50
Rice Polish	4.67	0.67	4.67	3.66	2.36	0.67	4.67	3.17
Soybean Oil	0	0	2.50	2.50	0	0	0	0
Palm Oil	0	0	0	0	2.50	2.50	0	0
Fish Oil	0	0	0	0	0	0	2.50	2.50
Soybean Meal	28.50	22.80	29.00	25.80	27.00	22.80	29.00	25.50
Meat and Bone meat	0	2.10	0	1.50	0	2.10	0	1.50
Protein Concentrate	3.50	4.00	3.50	2.30	4.80	4.00	3.50	2.50
Limestone	1.10	0.95	1.10	1.10	1.10	0.95	1.10	1.10
Dicalcium Phosphate	0.50	0.50	0.50	0.50	0.50	0.5	0.50	0.50
L-Lysine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Vit-min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Antioxidant	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total (Kg)	100	100	100	100	100	100	100	100
				Calculated values				
ME (Kcal/Kg)	2895.00	2952.00	3027.00	3051.00	3023.00	3052.00	3022.00	3051.00
CP (g/100g)	21.09	20.15	21.04	19.93	21.04	19.92	21.04	19.91
EE (g/100g)	3.25	2.99	5.69	5.63	5.51	5.42	5.68	5.58
CF (g/100g)	3.41	2.94	3.38	3.18	3.17	2.90	3.37	3.14
Ca (g/100g)	0.89	0.94	0.89	0.88	0.89	0.94	0.89	0.89
Total Phosphorus	0.67	0.65	0.65	0.63	0.66	0.65	0.66	0.63
DM (g/100g)	89.89	89.89	89.90	89.90	89.90	89.90	89.91	89.91
Total (Kg)	3014.2	3069.56	3148.55	3171.15	3144.17	3171.73	3143.55	3171.06

T_0=Diet without oil; T_1=Diet containing 2.5% soybean oil; T_2=Diet containing 2.5% palm oil; T_3=Diet containing 2.5% soybean oil

Table 2: HI titers to NDV of broilers at different ages fed on diets supplemented with different types of oil

Age (days)	Dietary oil group (25g/kg)				Sig.
	T_0	T_1	T_2	T_3	
7	26±0.3	32±0.6	26±1.2	26±0.67	NS
14	19.7±0.3	39.4±0.6	32±0.6	39.4±0.3	NS
21	$6.5±0.3^{d}$	$13±0.7^{c}$	$19.7±0.3^{b}$	$52±0.8^{a}$	**
28	$39.4±0.3^{d}$	$256±1.2^{b}$	$776±1.2^{a}$	$157±0.3^{c}$	*

*(P<0.05); **(P<0.01); [abcd]Means with different superscripts in the same row differ significantly; NS: Non Significant

The average HDL cholesterol content of broilers at different ages fed on diets supplemented with soybean oil, palm oil and fish oil has been presented in Table 3. Data in the table show that HDL cholesterol level of blood varied significantly (P<0.05) between the treatments at 3, 5 and 6 weeks of the age of broilers but there was no significant variation at 4 weeks. At 3 week HDL cholesterol of the broilers of palm oil group was significantly (P<0.05) higher than those of the other groups of broilers. At 5 weeks of age, HDL cholesterol of the broilers of marine fish oil group was (P<0.05) higher than soybean oil groups of broilers. At the age of 6 Week, HDL–cholesterol level of the broilers of palm oil group was (P<0.05) lower those of the other groups.

The values for average LDL-cholesterol of broilers fed on diets supplemented with different types of oil are presented in Table 3. Dietary supplementation of oils had (P<0.05) effect on LDL-cholesterol level of blood of broilers at 4 and 6 weeks of age. However, there was no significant effect of oil supplementation on the blood LDL-cholesterol of the broilers at 3 and 5 weeks of age.

Triglycerides: Table 3 shows the effect of supplementation of different oils in diets on triglyceride contents of broilers. At weeks 3 and 4, there were no marked variation among the values of blood triglyceride. However, at the age of 5 and 6 weeks, there were significant (P<0.05) effect of dietary supplementation with different types of oil. At 5 weeks of broilers age, triglyceride level in blood decreased significantly due to supplementation of oil with diets. At 6 weeks, there was no significant decrease in comparison with without oil.

Fatty acids: Unsaturated fatty acid content of the abdominal fat of broilers at different ages fed on diets supplemented with different oils is presented in Table 4. No marked differences were observed among the mean values of unsaturated fatty acid content among different dietary groups.

Similar to unsaturated fatty acid, saturated fatty acid content of fat of broiler were not influenced by supplementation of diet with soybean oil, palm oil and fish oil at different age of broilers. Feeding diets supplementing with different levels of oils had no significant effect on saturated fatty acid content of the abdominal fat of broilers (Table 4). It was also observed that the values of the parameter had tendency to decrease as the age proceeded up to 5 weeks. However, further advancement of age resulted in decrease the saturated fatty acid content. During the experimental period, it was observed that supplementation of palm oil had inconsistently variable effects on saturated fatty acid content of the abdominal fat of broiler.

DISCUSSION

Comparing this antibody response among the birds belonged to four treatments, T_1 to T_3 where different types of oil were supplemented with normal diets comparable to T_0 where no oil was supplemented, in their ability to evoke immunological response to NDV. There are several factors that influence the immune response of birds. Nutritional condition is one of them. Several studies have shown better immune-competence as a result of diet supplementation including the effects of oil. In the present

Table 3: Total serum cholesterol, HDL Cholesterol and triglyceride (mg/dl) of broiler at different ages fed on diets supplemented with different types of oils

	Age	Dietary oil				SEM	Sig.
	(Wk.)	To	T_1	T_2	T_3		
Cholesterol	3	111.33	103.00	112.33	110.67	1.54	NS
	4	118.67^{a}	118.67^{a}	104.67^{b}	110.33^{ab}	3.89	*
	5	109.67	105.00	108.33	102.33	5.22	NS
	6	112.33	113.33	116.33	110.33	1.02	NS
HDL	3	15.00^{a}	14.33^{b}	17.67^{a}	14.33^{b}	0.70	*
	4	14.67	14.00	14.33	15.00	0.54	NS
	5	19.33^{a}	17.67^{b}	19.33^{a}	20.00^{a}	1.02	*
	6	20.00^{a}	18.33^{b}	17.33^{b}	20.00^{a}	0.67	*
LDL	3	72.67	67.67	71.00	72.33	1.68	NS
	4	83.00^{a}	80.00^{a}	70.33^{b}	72.00^{b}	3.06	*
	5	58.33	67.33	65.67	63.00	4.56	NS
	6	67.67^{b}	76.00^{a}	79.67^{a}	67.67^{b}	1.71	*
Triglyceride	3	117.33	103.00	116.00	121.67	1.44	NS
	4	103.33	104.33	98.67	114.67	5.11	NS
	5	157.33^{a}	97.33^{bc}	113.67^{b}	94.67^{bc}	8.42	*
	6	123.33^{a}	93.00^{b}	95.00^{b}	111.00^{ab}	5.49	*

*(P<0.05); **(P<0.01); ^{abc}Means with different superscripts in the same row differ significantly; NS: Non Significant

Table 4: Unsaturated and Saturated fatty acid found in broilers abdominal fat at different ages fed on diets supplemented with different types of oil (%)

	Age	Dietary oil				SEM
	(Wk.)	To	T_1	T_2	T_3	
Unsaturated fatty acid	4	66.81	67.28	61.98	67.52	1.59
	5	59.07	65.96	60.28	66.94	3.89
	6	67.90	69.04	73.26	66.55	1.75
Saturated fatty acid	4	32.87	32.46	38.01	32.43	1.55
	5	40.90	34.00	39.70	33 .04	3.88
	6	32.94	30.94	26.39	33.43	1.85

All the values in row differ non-significantly (P>0.05)

experiment the mean antibody titers against NDV were assessed in the sera of broilers at 7, 14, 21 and 28 days of age. The immune status was measured based on the level of HI titers to NDV circulated in blood sera of the experimental birds. The HI titer of 32 was considered as the threshold level of antibodies against ND. The results of the study showed that use of different types of oil supplemented with normal diet affect immune response. All the three types of oil used in this experiment showed a similar protective immune response to NDV in which antibody titers were achieved maximally on day 28. The antibodies detected at 7 days were probably maternal antibodies that were still present in chicken serum, which was not enough to confer protection on broilers.

The mean HI titer was found higher in birds fed on diet supplemented with palm oil. Although the titers of the birds provided with fish oil were not as high as in other groups but the protective titer value appeared earlier than the other two groups. A striking difference in mean antibody titer occurred in birds of T0 where no oil was used. The mean titers in this group could not achieve a protective value until 28 days indicates a lower immune response of birds. The findings of the experiment provide evidence that supplementation of oil with normal diet has positive effect on humoral immune response and caused a significant increase in HI titre to NDV. Because Palm oil, Soybean oil, and fish oil are rich source of vitamin A and E (Krinsky, 1992; Hendrich *et al*., 1994). The results coincided with findings of Singh *et al*. (2006). They suggested that vitamin E and selenium have synergistic effects on immune response. The results also agreed with Weber *et al*. (2008) they reported that antibody formation against Newcastle disease was improved by vitamin E treatment in drinking water. Sklan *et al*. (1994) found that addition of small amounts of vitamin A enhanced both antibody production and proliferative responses. Vitamin E increase immune response even in heat stress (Niu *et al*., 2009: Rashidi *et al*., 2010).

Lower levels of serum cholesterol of the broilers at 4 week of age fed palm oil and fish oil supplemented diets, respectively indicates that supplementation of these oils has beneficial effects on broiler health. Although there was no significant differences observed among the groups of birds at 5 weeks the parameter showed the lower values in the broilers of oil supplemented group compared to that of the un-supplemented. This also indicates that fish oil supplementation keeps the serum cholesterol at lower level.

Although there were significant variation in the HDL-cholesterol content in the broilers of treated and untreated groups and also within treated groups, there were no consistencies in the variation for example, treatment T_2 containing palm oil showed higher content than other at 3 week where as it showed the lowest value at 6 weeks. However, the trend of variation in HDL-cholesterol of the broilers of soybean and fish oil groups compared to others consistently increased as the age of the broilers advanced. The result also indicates that at 4, 5 and 6 weeks of age of broilers blood HDL-cholesterol levels were the highest both for fish oil supplemented as well as un-supplemented groups.

Variation in LDL-cholesterol level in the blood of broilers fed without or with different oil supplemented diets indicates that fish oil and palm oil supplementation resulted in consistently lower level as the age of broilers increased up to 5 weeks. The un-supplemented diet resulted in inconsistency in the variation in the level of LDL-cholesterol. It is also indicated from the results that LDL-cholesterol level of blood of the broilers, irrespective of age, were the lowest among the values of other treatments.

Significantly (P<0.05) lower triglyceride without oil in blood of broilers at 5 and 6 weeks of age indicates that supplementation of soybean palm and fish oil in the decreases triglyceride level of the broilers. At the age of 5 weeks fish oil supplementation resulted in the lowest level of triglycerides indicating its best beneficial effect on broilers. The trend of variation in the parameter also indicated that the supplementation of oil resulted in progressive decrease in the triglyceride level in the blood of broilers.

Supplementation of diets with soybean oil, palm oil and fish oil did not change the content of unsaturated fatty acids in the abdominal fat of broilers. It was also true in case of age of the broilers, as there was no much change observed in the parameter due to age of the birds from 4-6 weeks. There was slight increase at week 6 in case of palm oil supplemented group of broilers which might have for high standard error in the observation. Crespo and Garcia (2002) suggested that differences in fat deposition between broilers fed diets having different dietary fatty acid profiles are more related to different rates of lipid oxidation than lipid synthesis. This idea is in good agreement with data from various authors who reported significant changes in the composition of the

abdominal fat after feeding with different fat sources (Hrdinka *et al.*, 1996; Sanz *et al.*, 2000; Pesti *et al.*, 2002).

NDV outbreaks occurred in broiler despite vaccination against NDV. There are many reasons of vaccination failure like immune suppressed birds, lack of cool chain and presence of maternal antibody. We found that humoral immune response against NDV can be ameliorated by oil supplementation in feed. Supplementation of oil did not have any effect on lipid and fatty acid profile of broilers. It was also evident that supplementation of different oil had no significant variation of lipid and fatty acid profile among the different groups of birds.

Conclusion: Oil supplementation in feed ameliorated the humoral immune response of broilers against NDV without significantly affecting their lipid and fatty acid profiles. The mean HI titer against NDV was although highest in palm oil supplemented group but the required protective titer values were also achieved by soybean and fish oil supplemented groups. Failure of control group to attain the required protective titer value until 28th day of age further intensifies the importance of oil supplementation for humoral immunity.

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