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Effect of Fish Oil on Immune Response in Broiler Chicks Vaccinated Against IBD

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Abstract: IBD can only be controlled by proper vaccination. The objective of this study was to determine the efficacy of an intermediate strain of live attenuated IBD vaccine in broiler chicks at 7th day of age, as well as the effect of fish oil on immune response in birds vaccinated with IBD vaccine. One hundred forty, day-old broiler chicks were reared and used for this purpose. The chicks were divided into 7 groups A, B, C, D, E, F and G. Groups A, B and C were vaccinated via drinking water route at 7th day whereas D, E and F were vaccinated at 14th day of age. Groups B, C, E and F were fed 50gm fish oil/kg diet for one week either before or after vaccination. Group G was acted as control. Three chicks from each group were sacrificed by decapitation one week interval from the first day until 28th day of age and bursa weight to body weight (Bursal index) was recorded at these periods. Blood samples were collected for detection IBD antibody titer using ELISA at all these periods from control group for detection MDA, whereas the blood has been collected from the treated groups at a period of seven days after vaccination until the end of the experiment at 28th day of age to detect the effect of time of vaccination and fish oil supplementation on the IBD antibody titer. On necropsy, the gross pathological changes were recorded. The changes were occurred after 7 days postvaccination. The affected bursa was edematous and covered with yellowish transudate, other showed pinpoint mucosal surface necrosis, whereas other undergo atrophy. The Bursal Index (BI) was gradually increased from 1st-28th day of age in the control group, whereas in group A it was increased from the 1st to the 7th day and decreased thereafter. In group D the index was increased from the 1st-14th day and decreased thereafter until the 28th day of age. The MDA of control group was decreased from the 1st to the 28th day. The antibody titer of group A was decreased in a way similar to that of the control group, but a significant (p<0.05) difference was present between them at 14th day and thereafter, whereas in group D it was decreased from the 1st-14th day and then increased at 21st and 28th of age. Fish oil supplemented groups exhibited a slight numerical increment of both BI and antibody titer. Although single dose at the 7th day old vaccination could induce slight increase of IBD antibody in comparison to that of the control, vaccination at the 14th day of age induced high and protective level of IBD antibody titer. These may be due to the ability of vaccine at each time of vaccination to neutralize different levels of MDA. It was concluded that single dose vaccination at the $7^{ ext{th}}$ day in broiler chickens with high MDA was ineffective and could not used in broiler chicks. The effect of fish oil on the immune response to IBD vaccine should be further investigated.

Key words: Fish oil, immune response, IBD, MDA, ELISA

INTRODUCTION

The poultry business may be profitable with proper management in any country. The major problem of this business is the outbreak of infectious diseases, including Infectious Bursal Disease (IBD). Although IBD vaccines are available on the commercial market, the quality of vaccines, transportation, storage, distribution, time interval of vaccinations, presence of maternal antibodies, age of vaccination, stress, immunosuppression, route of vaccination etc. are often causes for vaccination failure (Phatak, 2002).

Day old progeny chicks are being sold by many hatcheries with unknown status of parental immunity against various prevalent infections particularly against IBD. Vaccination schedules followed at almost all farms thus becomes ineffective and therefore, commercial

broilers remain vulnerable to the natural IBD infection due to the neutralization of live virus vaccine by maternal antibodies (Wood *et al.*, 1981).

Numerous vaccines and vaccination programs for IBD have been studied and proposed worldwide. This disease has been considered a defined nosologic entity able to cause economic losses because it induces immunosuppression and because of the appearance of new virus strains (Bolis *et al.*, 2003).

During the last decade, very virulent IBDv has caused outbreaks of disease with high mortality in Europe and some other parts in the world. The current vaccination programs failed to protect chick sufficiently (Skelees *et al.*, 1979; Vanden Berg and Meulemans, 1991).

Several vaccination programs are currently being used, depending on the field situation encountered. A common

commercial practice is to vaccinate chickens with live vaccines during the first 3 weeks of life (Kibenge *et al.*, 1988).

Recent studies are beginning to describe significant health benefits from diets that include marine fish oil supplements. These benefits include immunological properties such as immunomodulation (Jessica, 2001). Consumption of n-3 polyunsaturated fatty acids such as fish oil markedly modulates the immune and inflammatory response (Kehn and Fernanetes, 2001). Research with dietary oils in chickens such as fish oil has demonstrated effects on the inflammatory response addition to improvements in immune system functions and resulted in improved body weight, cellular immunity and antibody production (Prickeet et al., 1982). The timing of vaccination is crucial as the occurrence of IBD may be encountered before 3 weeks of age, therefore; the objective of this study was to determine the efficacy of a single dose of 7th day old vaccination in broiler chicks with high Maternally Derived Antibody (MDA) using "intermediate strain" of live attenuated IBD vaccine, as well as determination of fish oil efficacy in modulating immune response in vaccinated birds.

MATERIALS AND METHODS

One hundred forty, one-day old broiler chicks were used in this study. The chicks were randomly divided into 7 equal groups and treated as depicted in (Table 1) which summarized the Experimental Design of the study.

Intermediate strain of live attenuated IBD vaccine (Cevac Gumbo L, Ceva phylaxia, Hungary) via drinking water route in both period of vaccination (7th or 14th day) was used for all groups except G which served as control. Groups C, D, E and F were fed diet containing 50 gm fish oil (Shanghai-China)/kg. Fish oil has been supplemented for 7 consecutive days either before or after vaccination with IBD vaccine after thorough mixing with ration. Feed and water were given ad libitum. Three chicks at each time from each group were sacrificed by decapitation at different ages as shown in Table 1 to estimate bursa of Fabricius weight to body weight ratio [Bursal Index (BI)]. Blood samples of about 1-4 ml (according to age) from each were collected from these birds at the same ages to detect IBD antibody titer using Enzyme Linked Immunosorbent Assay (ELISA). The control group was used for detection of Maternally Derived Antibody (MDA) from the 1st until the end of the experiment at 28th day of age. On necropsy, the gross pathological changes were also recorded for all sacrificed birds (Hair-Bejo et al., 2004).

The ELISA technique was carried out according to the method described by Synbiotic Laboratories Incorporation, USA. Briefly, the antigen coated plates and ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted five hundred folds (1:500) with sample diluent prior to the

assay. A 100 µl of diluted sample was then placed into each well of the plate. This was followed by 100 µl of undiluted negative control into well A1 and A2 and 100 µl of undiluted positive control into well A3 and A4. The plate was incubated for 30 min at room temperature. Each well was then washed with approximately 300 of distilled water for 3 times. Horseradish peroxidase conjugated anti-chicken IgG (100 µI) was dispensed into each well. The plate was incubated at room temperature for 30 min, followed by washing each well with 300 distilled water for 3 times. A 100 µl substrate solution was dispensed into each well. The plate was then incubated at room temperature for 15 min. Finally 100 μl of stop solution were dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm by ELISA reader (Alam et al., 2002).

The data were subjected to analysis of variance and the significance differences at (p<0.05) were determined by ANOVA (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The Chicks of all 7 groups did not exhibit any abnormal clinical signs throughout the experiment. No gross lesions were recorded in the chicks of the control and 7th day vaccinated groups throughout the experiment. Gross pathological changes were observed and confined in a few bursas of Fabricius of chicks of groups which were vaccinated at 14th day. These changes were occurred at 21st day of age or 7 day postvaccination. The affected bursas were edematous and covered with yellowish transudate on the mucosal and serosal surfaces of the organ. Other bursas also had mild pinpoint necrosis on mucosal surface and others were elongated and atrophied. These results were in agreement with that of Hair-Bejo et al. (2004) who reported that clinical signs and gross pathological changes were not recorded in the control and day old vaccinated chicks, but lesions were observed in bursa Fabricius of chicks which were vaccinated at 14th day of age and the lesions were noticed 7 days postvaccination. The result of the present study indicated that vaccine failed to establish and proliferate in the tissue of bursa Fabricius when given at 7th day old because of MDAs interfere or react and neutralize the live virus vaccine (Alam et al., 2002).

Table 2 displayed the effect of timing of vaccination on Bursal Index (BI). Data presented in this table indicated that bursa to body weight ratios (BI) were gradually increased from the 1st day (1.22 \pm 0.05) to the 28th day (3.99 \pm 0.08) in the control group. The index in group A which was vaccinated at the 7th day of age increased from the 1st day (1.24 \pm 0.07) to the 7th day (2.33 \pm 0.13) and decreased thereafter. Whereas in group B the ratio was increased from the 1st day (1.23 \pm 0.10) to the 14th day (3.29 \pm 0.27) and then decreased thereafter until the end of the experiment at the 28th day.

Table 1: Experimental Design

Groups	No. of Birds	Fish oil supple	mentation			
		Age/Day	Quantity Gm/Kg Diet	Duration/day	Age of *Vaccination/ Day	Time of Sacrificing/Day
A	20	-	-	-	7 th	**1st, 7th, 14th, 21st, 28th
В	20	1st 7th	50	7 Before Vaccination	7 th	1st, 7th, 14th, 21st, 28th
С	20	7 th - 14 th	50	7 After Vaccination	7 th	1st, 7th, 14th, 21st, 28th
D	20	-	-	-	14 th	1st, 7th, 14th, 21st, 28th
E	20	7 th - 14 th	50	7 Before Vaccination	14 th	1 st , 7 th , 14 th , 21 st , 28 th
F	20	14 th - 21 st	50	7 After Vaccination	14 th	1 st , 7 th , 14 th , 21 st , 28 th
G	20		Control			1 st , 7 th , 14 th , 21 st , 28 th

^{*}Intermediate live IBD vaccine was used in all vaccinated groups. **Three chicks have been sacrificed at each time to estimate individual Bursal Index (BI) and to collect about 1-4 ml (according to age) of blood samples from each

Table 2: Bursal Index (BI) after different time of vaccination and fish oil supplementation

	Bursa to body weight ratio (BI) from day 1 st -28 th					
Groups and type of treatment	1 st	7 th	14 th	21 st	28 th	
A, only 7 th day ∨accination	*1.24±0.07	2.33±0.13	2.15±0.09°	1.90±0.06°	1.61±0.18°	
B, fish oil before 7 th day ∨accination	1.25±0.06	2.35±0.14	2.18±0.08	1.94±0.07	1.63±0.17	
C, fish oil after 7 th day ∨accination	1.24±0.07	2.34±0.13	3.20±0.07	1.96±0.08	1.65±0.16	
D, only 14 th day ∨accination	1.23±0.10	2.34±0.20	3.29±0.27b	2.82±0.12b	1.01±0.02b	
E, fish oil before 14th day vaccination	1.24±0.90	2.36±0.21	3.30±0.26	2.84±0.11	1.10±0.03	
F, fish oil after 14 th day ∨accination	1.25±0.10	2.36±0.22	3.31±0.25	2.86±0.10	1.18±0.02	
G, control	1.23±0.05	2.35±0.09	3.27±0.17 ^b	3.87±0.23ab	3.99±0.08ªb	

a.b.abMean figures with different superscripts in the vertical column were significantly differed at (p<0.05).

The result of the control group of the present study was in disagreement with that of Hiar-Bejo *et al.* (2004) who stated that BI was gradually increased from the 1st day to the 14th day in the control unvaccinated group and decreased on the following days. According to Glick (1956) the largest mean bursa weight for the Leghorn occurred at 4 weeks of age and a significant positive correlation between bursa and body weight was found up to 43 days of age.

Bolis *et al.* (2003) reported that evaluation of bursa weight and determination of bursa weight to body weight ratio is the most used model to estimate protection rate given by vaccines against IBD and also demonstrated that BI is gradually increased simultaneously with age of the bird. In the present study, the two methods of vaccination (7th and 14th day) induced discrete reduction in bursa weight after administration of the vaccine. Such discrete reduction in bursa weight, more evident in group D which was vaccinated at the 14th day of age.

The results of groups A and D in the present study were in agreement with those of Hair-Bejo *et al.* (2004) who mentioned that BI was decreased after about 1-2 weeks postvaccination in birds vaccinated at 1st or 14th day of age. Table 2 also showed that there was a significant increase (p<0.05) of BI of control group at the 14th day of age than that of group A which was vaccinated at the 7th day. A significant differences (p<0.05) also occurred at the 21st and 28th of age among all groups. These differences may by due to the interference of MDAs which react with live virus vaccine and become neutralized (Wood *et al.*, 1981; Alam *et al.*, 2002). The

table also exhibited that fish oil supplementation for seven consecutive days either before or after each time of vaccination in groups B, C, E and F resulted in a very slight numerical increment in the BI of the treated chicks especially in those supplemented after vaccination. This enhancement as explained by Weng and Denbow (2000) may be due to fish oil which considered to be a substrates for the generation of prostaglandin and leukotrein. The later two substances are known to be immunomodulators.

Omega-3 polyunsaturated fatty acids had very strong immunomodulatory activities and among omega-3 polyunsaturated fatty acids, those obtained from fish oil. Fish oil is the best single sources of omega-3 fatty acids (Richell, 2001). Omega-3 fatty acids improved immune status and lessen inflammatory conditions (Klasing, 1998).

Kirk (1998) developed a model for challenging birds in the absence of medication. His research showed that following a challenge with salmonella, food intake and immune response of bird were superior in the group which received fish oil in their diet compared with others fed corn oil.

Sakr (2007) demonstrated that fish oil considered to be immune modulator and recommended to be given after vaccination against Newcastle disease.

On the other hand, fish oil also have been shown to be immunosuppressive at high concentrations and immunostimulatory at lower concentrations when have been used in chicken diet (Wang *et al.*, 2000).

^{*}Figures are mean values ± SE for 3 birds in each group

Table 3: Antibody titer against IBD after vaccination and fish oil supplementation

		Mean Ab titer ± SE throughout the experiment					
Groups	Day of vaccination and treatment	1st	7 th	14 th	21 st	28 th	
A	7th, Only vaccination	*4795.96±36.45	2898.99±34.28	842.17±30.15°	788.75±20.61°	697.96±18.64°	
В	7th +Fish oil before vaccination	4793.85±35.46	2897.96±35.29	848.18±29.14	791.87±21.15	701.71±19.61	
С	7th + Fish oil after vaccination	4794.61±25.37	2896.99±31.25	850.95±21.11	794.96±19.18	705.89±18.69	
D	14 th ,Only vaccination	4796.17±35.39	2900.78±28.26	745.83±18.71	1027.91±28.02b	1271.89±20.40b	
E	14th + Fish oil before vaccination	4795.91±34.29	2899.91±26.24	744.98±17.69	1036.95±20.12	1290.95±21.39	
F	14th + Fish oil after vaccination	4796.08±29.40	2898.89±41.29	745.65±18.20	1049.89±31.35	1311.89±11.13	
G	Control	4800.17±38.44	2910.88±24.27	746.63±19.70 ^b	529.94±21.66 ^{ab}	234.27±19.67ab	

a.b.ab Mean figures with different superscripts in the vertical column were significantly differed at (p<0.05).

Table 3 showed that the antibody titer of the control unvaccinated group G which represent MDA was decreased from the first day (4800.17±38.44) to the 21st day (529.94±21.66) and remained low thereafter as presented in the table. This result was in agreement with that of Cao *et al.* (1995) who found that MDA levels were high at the 1st day and also with that of Maly Mitra *et al.* (1988) who reported that MDA levels were significantly lower at 21st day of life than at of the 1st day old.

Table 3 was also exhibited that MDA titer gradually declined below positive level 21st day after hatching. Azab *et al.* (1991) carried out an investigation on determination of MDA against IBD in broiler chicks and found that the antibody titer lasted 14 days after hatching. Wisniewska and Stosik (1999) mention that MDA may persist until 19 days and sometimes 23 days posthatching. Iordanides *et al.* (1991) stated that MDAs persist up to 30 days after hatching. These variations may be due to the type of vaccine, strain of chickens, method of vaccination parental immunity and other conditions of the studies.

A perusal of the data presented in Table 3 indicated that the antibody titer of group A which vaccinated at 7th day was decayed from the 1st to the 21st day and remained low at the 28th day similar to that of the control group G although there was a signification increment (p<0.05) of IBD antibody titer of group A than that of G at the 14th day and thereafter. This suggested that the virus vaccine was being partially neutralized MDA and thus slightly multiplied and proliferated in the bursa of Fabricius and induced moderate level of specific IBD antibody titer. This result was in agreement with that of Hair-Bejo et al. (2004) who mentioned that vaccination at 14th day of age has shown to be effective and able to induce high and protective level of IBD antibody titer up to 42nd day of age, because the ability of the virus vaccine to fully neutralized or overcome MDA could lead the virus to replicate in the bursa of Fabricius and become able to induce high IBD antibody.

Lindal (2002) recommended continuous monitoring of the vaccination responses and the level of MDAs to determine the best day for the first vaccination. Voss and Vielitz (1994) observed that chickens with MDA showed no response to vaccination with live vaccine, because timing of vaccination of chickens depend on the degree of MDA. Sahar *et al.* (2004) advised to vaccinate chickens with intermediate strain of IBD at 2 weeks old. Ahemd *et al.* (2003) mentioned that immune response against different vaccines varied in accordance with the vaccine schedule and level of MDA against IBD in chicks, and vaccination of day old chicks with high level of MDA against infections bursitis virus failed to produce primary immune response.

The result of the present study was in discrepancy with those of (Wyeth and Chettle, 1990; Giambrone and Clay, 1986; Whitfill *et al.*, 1995; Haddad *et al.*, 1997) who advised early vaccination of broiler chicks.

Data presented in Table 3 also indicated that the effect of fish oil on ELISA antibody titer in chicks of groups B, C, E and F which have been fed fish oil either before or after vaccination with intermediate strain of live attenuated IBD vaccine at 7th or 14th day, resulted in a numerical elevation of antibody titer as compared with that of the unsupplemented groups. This increment was not statistically significant. This result was in line with that of Sakr (2007) who proved that broiler chicks vaccinated with Hitchner B1 and LaSata vaccines at 7th and 21st day of age respectively and supplemented with 50 gm fish oil/kg diet for 14 days before or after each time of vaccination were exhibited significant increase of HI antibody titers. The result of the present study was in agreement with that of Michel (2002) who found that quail injected with sheep red blood cells and fed 50 gm of fish oil/kg of diet, for one week was exhibited an increase antibody titer in comparison with that of other groups which fed the same amount of soybean or chicken fat.

On the other hand, this result was in disagreement with that of Parmentier *et al.* (1997) who reported that antibody production is decreased against bovine serum albumin injection in chickens fed fish oil, as well as the latter did not modulate prostaglandins production which has an important role in the enhancement of humoral immunity.

This diversity in the results of these researches may be due to the origin of fish oil, type of birds, duration of supplementation, type of antigen used to elicit antibody production and other conditions of the trials.

^{*}Data were mean of 3 samples for each time of testing

Conclusion: It was concluded that 7th day old vaccination of broiler chicks with high MDA was ineffective to induce suitable level of IBD antibody. Vaccination at 14th day of age was shown to be able to induce high level of IBD antibody up to 28th day of age. Further investigation should be carried out to explore the real effect of fish oil as immunostimulator simultaneously with other type of vaccines.

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REFERENCES

- Ahemd, Z., S. Inayat, K. Naeem and S.A. Malik, 2003. Comparative Immune Response Pattern of commercial infectious bursal disease vaccines against field isolates in Pakistan. Int. J. Poult. Sci., 2: 449-453.
- Alam, J., M.M. Rahman, B.K. Sil, M.S.B. Khan and M.S.K. Sarker, 2002. Effect of maternally derived antibody of vaccination against infectious bursal disease (Gumboro) with live vaccine in Broiler. Int. J. Poult. Sci., 1: 98-101.
- Azab, A., S.M. Hassan and T.S. Qubih, 1991. Determinate titer after the first immunization of broiler chicks. Assiut Vet. Med. J., 25: 211-215.
- Bolis, D.A., F.J. Paganini, V.A. Simon, M.F. Zuanaze, N.H. Scanavini, A.R. Correa and N.M. Ito, 2003. Evaluation of serological and anatomopathological responses in vaccinated broiler chickens challenged with very virulent virus strain. Int. J. Poult. Sci., 5: 137-146.
- Cao, Y.C., B.I. Ying zuo and J. Zhu, 1995. Application of enzyme-linked immuo-sorbent assay for evaluation of immunological efficacy of chicks against IBD. Chinese J. Vet. Med., 21: 9-10.
- Giambrone, J.J. and R.P. Clay, 1986. Vaccination of day old broiler chicks against Newcastle disease and Infectious Bursal disease using commercial live and/or inactivated vaccines. Avian Dis., 30: 557-561.
- Glick, B., 1956. Normal growth of bursa of Fabricius in chickens. Poult. Sci., 35: 843-851.
- Haddad, E.E., C.E. Whitfill, A.P. Avakian, C.A. Ricks, P.D. Andrews, J.A. Thoma and P.S. Wakenell, 1997. Efficacy of novel IBD virus immune Complex vaccine in broiler chickens. Avian Dis., 41: 882-889.
- Hair-Bejo, M., M.K. Ng and H.Y. Ng, 2004. Day old vaccination against infectious bursal disease in broiler chickens. Int. J. Poult. Sci., 3: 124-128.
- Iordanides, P., M. Koumpate and E. Artopoise, 1991. Role of maternal antibodies in preventing IBD in chicks in the first week of life. Cited by Alam *et al.* (2002). Int. J. Poult. Sci., 4: 98-101.

- Jessica, E.R., 2001. Effect of omega-3 fatty acid on immunity, inflammation, cancerous cells. J. Immunol., 70: 115-125.
- Kehn, P. and G. Fernanetes, 2001. The importance of omega-3 fatty acids in the attenuation of immunemediated diseases. J. Clin. Immunol., 21. Abstr.
- Kibenge, F.S., A.S. Dhillon and R.G. Russell, 1988. Biochemistry and immunology of infectious bursal disease virus. J. Gem. Virol., 69: 1757-1775.
- Kirk, C.L., 1998. N-3 fatty acids and infectious disease resistance. Poult. Sci. Cited by Sakr (2007).
- Klasing, K.C., 1998. Nutritional modulation of resistance to infections diseases. Poult. Sci., 77: 1119-1125.
- Lindal, J., 2002. Infectious Bronchitis Virus and Infectious Bursal Disease Virus; a study performed at the University of Costa Rica. Int. Vet. Microbiol., 48: 650-655.
- Maly Mitra, H., M. Bhattacaryya, R. Duttagubta, A.K. Pramanik, G.P. Sen and M. Mitra, 1988. Studies on maternally derived antibody level of different viral diseases in broiler. Indian Vet. J., 75: 495-497.
- Michel, D., 2002. Immunomodulation by dietary lipids: Fish oil, soybean oil, chicken fat and hydrogenated soybean oil. Anim. Sci., 195: 199-218.
- Parmentier, H.K., M.G. Nieuwland, M.W. Barwegen and R.P. Scharma, 1997. Dietary unsaturated fatty acids affect antibody Response and growth of chickens. Poult. Sci., 76 (Abstract).
- Phatak, R.K., 2002. Vaccination failures and their solutions. Cited by Bose *et al.* (2003). Ital. J. Anim. Sci., 2: 157-162.
- Prickeet, J.D., D.R. Robinson and K.J. Dloch, 1982. Enhanced production of IgG and IgE antibodies associated with a diet enriched in eicosapentaenoic asid. Immunol., 46: 819-826.
- Richell, 2001. Fish oil, fish consumption, omega-3 fatty acids and cardiovascular disease. J. Nutr., 106: 2747-2757.
- Sahar, M.O., A.S. Ali and E.A. Rahman, 2004. Residules Pathogenic effects of infectious bursal disease vaccines containing intermediate and hot strains of the virus in broiler chickens. Int. J. Poult. Sci., 3: 415-418.
- Sakr, W.M., 2007. Enhancement of humoral and cellular immunity of broiler chicks vaccinated with Newcastle disease by the dietary supplementation of fish oil. Msc. thesis, Univ. Basrah, Basrah, Iraq.
- Skelees, J.K., P.D. Lukert, E.V. DeBuysscher, O.J. Fletcher and J. Brown, 1979. Infectious bursal disease viral infections. Complement and virus neutralizing antibody response following infection of susceptible chickens. Avian Dis., 23: 95-106.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics. 1st Edn. McGraw-Hill, New York.

- Vanden Berg, T.P. and G. Meulemans, 1991. Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. Avian Patho., 20: 409-421.
- Voss, M. and E. Vielitz, 1994. Gumboro vaccination trials with different vaccines and vaccination programs. Vet. Poult. Assoc., 21: 305-311.
- Wang, Y.W., C.J. Field and J.S. Sim, 2000. Dietary polyunsaturated fatty acids alter lymphocyte subset proportion and proliferation, serum Ig concentration, and immune tissue development in chicks. Poult. Sci., 79 Abstr.
- Weng, B.C. and D. Denbow, 2000. Dietary lipids fish oil, soybean oil and chicken fat affected both cell-mediated immunity and humoral immunity *in vivo* and *vitro* of Bobwhite quail. Anim. Sci., 160: 170-180.

- Whitfill, C.E., E.E. Hadad, C.A. Ricks, J.K. Skeeles, L.A. Newberry, J.N. Beasly, P.D. Andrews, J.A. Thoma and P.S. Wakenell, 1995. Determination of optimum formulation of a novel infectious bursal disease virus vaccine constructed by mixing bursal disease antibody with IBD. Avian Dis., 39: 687-699.
- Wisniewska, J. and M. Stosik, 1999. Serum antibody titer after the first immunization of broiler against IBDV. Medycyna Weterynaryna, 55: 48-51. Cited by Alam *et al.* (2002). Int. J. Poult. Sci., 4: 98-101.
- Wood, G.W., J.C. Muskett and D.H. Thomton, 1981. The interaction of live vaccine and maternal antibody in protection against infectious bursal disease. Avian Patho., 10: 365-373.
- Wyeth, P.J. and N.J. Chettle, 1990. Use of infectious burasl disease vaccines in chick with maternally derived antibodies. Vet. Rec., 126: 577-578.