Journal of Animal and Veterinary Advances 9 (3): 610-616, 2010
ISSN: 1680-5593

Tryptophan Stimulates Immune Response in Broiler Chickens Challenged with Infectious Bursal Disease Vaccine

[1]Mozhdeh Emadi, [2]Fatemeh Jahanshiri, [3]Kamran Kaveh,
[4]Mohd Hair-Bejo, [3]Aini Ideris and [1]Razak Alimon
[1]Department of Animal Science, Faculty of Agriculture,
[2]Department of Microbiology, Faculty of Biotechnology,
University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[3]Department of Clinical Studies,
[4]Department of Pathology-Microbiology, Faculty of Veterinary Medicine,
University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract: Infectious bursal disease is still a challenging issue by posing a serious threat to the commercial poultry industry especially due to the emergence of highly Infectious Bursal Disease Virus (IBDV). In the present study, we evaluated the immunomodulatory effects of Tryptophan (Trp) on innate, humoral and cellular immune responses in chickens challenged by oral administration of intermediate plus strain of IBD virus at 28 days of age. A corn-soybean meal based diet containing different levels of Trp (0, 0.10 and 0.20) for the starter, (0, 0.07 and 0.15) for the grower and (0, 0.05 and 0.13) for the finisher has been utilized. In a completely randomized design with three treatments of five replicates each and 10 chickens per replicate, 150 Cobb 500 male broiler chickens from 0-49 days of age were subjected to Trp diet. To measure the innate, cellular and humoral immunity indicators (interferon-α, interferon-γ, immunoglobulin G, respectively) at 27, 35, 42 and 49 days of age, serum samples from each replicate of treatments were collected and subjected to ELISA. The result showed that Trp supplementation in the chickens basal diets significantly increased the serum levels of interferon-α at 35, 42 and 49 days of age ($p<0.05$), interferon-γ at 27, 35 and 49 days of age ($p<0.05$) and immunoglobulin G at 27, 35, 42 and 49 days of age ($p<0.05$). These results strongly suggest that tryptophan plays a vital role in modulation of protective immune response against IBDV.

Key words: Tryptophan, infectious bursal disease virus, immune response, immunity indicators, broiler chicks

INTRODUCTION

Infectious Bursal Disease (IBD) is an acute and contagious progressive disease of chickens that induces high morbidity and mortality in chickens at 3-6 weeks of age. The disease in younger chickens is usually sub-clinical and results in immunosuppression with subsequent poor immune response to different infections and vaccines. Therefore, the disease has a significant economic impact on poultray industry (Lukert and Saif, 2003).

Tryptophan, an essential amino acid has emerged as a regulator of many immunological and physiological processes. Its plasma concentration declines in animals suffering from different illnesses and inflammations (induced or natural), suggesting an increased utilization of the amino acid in such instances (Le Floc`h *et al*., 2004). IBDV primarily impairs the humoral immune response which is followed by sever immunosupression due to downregulation of T cells and macrophages. These two cells are the main cytokines producers which in turn regulate innate and cell mediated immunities. Cytokines are low-molecular-weight proteins or glycoproteins and sub-divided into various classes, one of the most significant of which is interferon family. Similar to mammalian interferons (IFN), there are two types of chicken IFNs; types I and II.

Type I IFN with antiviral activity, including IFNα produced by monocytes and macrophages and IFNβ produced by fibroblast and epithelial cells. IFNγ, a type II IFN, is produced by activated Natural Kill cells (NK) as well as T cells and considered to be a TH1-biased cytokine. Chicken IFNγ has many biological properties in common with mammalian IFNγ (Lowenthal *et al*., 2001). It activates macrophages to boost their antimicrobicidal activities, up-regulates the expression of Major Histocompatibility Complex (MHC) I molecules to increase antigen presentation and induces secretion of other

Corresponding Author: Razak Alimon, Department of Animal Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

cytokines such as interleukin-2 (IL-2) and IL-12 to develop Cell Mediated Immunity (CMI). IFNγ secreted by TH1 cells can also switch on the antibody classes to support phagocytosis and fixation of complement (such as IgG2a in mouse) (Tuting *et al*., 1999).

Various approaches are available to boost the immune response. An efficient one is manipulation of the basal diet by adding different nutrient. Studies have shown that the sever immunosupression and pathological symptoms caused by IBDV infection, could be reduced by supplementation of chicken's basal diet with immunostimulator nutrients such as Trp. Therefore, the present study was aimed to investigate the effects of Trp on the systemic immune response. We assessed the serum levels of IFNα, IFNγ and total IgG as the indicators of innate, CMI and humoral immune responses, respectively.

MATERIALS AND METHODS

Three different dietary levels (A, B and C) of Trp for three age group periods (starter, grower and finisher) of broiler chickens were used. There were (Table 1) three levels of Trp for the starter (S) 0-21 days of age (S-A, 0-control; S-B, 0.10 and S-C, 0.20), three levels for the grower (G) 21-42 days of age (G-A, 0-control; G-B, 0.07 and G-C, 0.15) and three for the Finisher (F) 42-49 days of age (F-A, 0-control; F-B, 0.05 and F-C, 0.13). All diets met the National Research Council (1994) recommendations for broilers. One-day-old Cobb500 male broiler chickens (150) were utilized in the experiment consisting of 3 treatments with 5 replicates and 10 chickens per replicate each. Birds were housed randomly in pen, so that initially each bird occupied approximately 0.11 m^2 of floor space.

The pens were floor pens with wood litter. Birds were maintained under continuous light and the environmental temperature in the barn was initially set at 31°C and was gradually reduced to 20°C by week 7. Feed and water were provided *ad libitum* throughout the experiment. Chickens were challenged by oral administration of intermediate plus strain of IBD virus (10-fold greater than normal vaccination doses) at 28 days of age. This strain was obtained from the poultry vaccine testing laboratory, Malaysian Vaccines and Pharmaceuticals. At 27 (Day Before Challenge: DBC), 35, 42 and 49 (Days After Challenge: DAC) days of age, two chicken was randomly selected from each replicate in each treatment and blood samples were collected from wing vein by Terumo Syringe with needle (0.7×32 mm). Blood samples (ten samples for each treatment) were centrifuged, serum was separated and stored at -20°C until use and analyzed in triplicate to assure that calculated and analyzed values were in agreement. Analysis of the immune response indicators including interferon α (INF α), interferon γ (INF γ) and total Immunoglobulin G (IgG)) were determined using appropriate Enzyme linked immunosorbent assay (ELIZA) (USCN, USA). INFα, INFγ and total IgG were evaluated as indicators of the effects of orally administered

Table 1: Composition of experimental diets of broiler chickens during 0-49 days of age

	Starter (S)			Grower (G)			Finisher (F)		
Ingredients	S-A	S-B	S-C	G-A	G-B	G-C	F-A	F-B	F-C
Corn grain	54.4	54.4	54.4	67.9	67.9	67.9	71.10	71.10	71.10
Soybean meal	35.3	35.3	35.3	22.6	22.6	22.6	20.03	20.03	20.03
Fish meal	1.09	1.09	1.09	4.3	4.30	4.30	2.50	2.50	2.50
Dicalcium phosphate	1.35	1.35	1.35	0.54	0.54	0.54	0.55	0.55	0.55
Limestone	1.17	1.17	1.17	1.19	1.19	1.19	1.12	1.12	1.12
Vitamin-mineral mix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vegetable oil	2.80	2.80	2.80	-	-	-	-	-	-
Salt	0.40	0.40	0.40	0.240	0.24	0.24	0.20	0.2	0.2
DL-Methionine	0.14	0.14	0.14	-	-	-	-	-	-
Tryptophan	0	0.10	0.20	0	0.07	0.15	0	0.05	0.13
Wheat bran	2.80	2.69	2.59	2.80	2.53	2.47	2.80	2.59	2.48
Calculated analysis	2900	2900	2900	2900	2900	2900	2900	2900	2900
ME[2] (kcal kg^{-1})									
Crude protein (%)	20.8	20.8	20.8	18.2	18.2	18.2	16.3	16.3	16.3
Crude fiber (%)	3.70	3.70	3.70	3.20	3.20	3.20	3.05	3.05	3.05
Linoleic (%)	2.20	2.20	2.20	1.60	1.60	1.60	1.70	1.70	1.70
Ca (%)	0.91	0.91	0.91	0.82	0.82	0.82	0.72	0.72	0.72
Available. P (%)	0.41	0.41	0.41	0.32	0.32	0.32	0.27	0.27	0.27
Na (%)	0.18	0.18	0.18	0.14	0.14	0.14	0.11	0.11	0.11
Arginin (%)	1.44	1.44	1.44	1.20	1.20	1.20	1.00	1.00	1.00
Lysine (%)	1.15	1.15	1.15	1.00	1.00	1.00	0.85	0.85	0.85
Methionine+cystine (%)	0.82	0.82	0.82	0.65	0.65	0.65	0.58	0.58	0.58
Tryptophan (%)	0.23	0.34	0.46	0.18	0.27	0.36	0.17	0.25	0.34

[1]Supplied per kg of diet: vitamin A, 10000 IU; vitamin D_3, 9790 IU; vitamin E, 121 IU; B_{12}, 20 μg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamine, 4 mg; zinc sulphate, 60 mg; manganese oxide, 60 mg. [2]Metabolizable energy

intermediate plus strain of IBDV with or without Trp on the systemic innate, cell-mediated and humoral immune responses, respectively. ELISA was performed according to the manufacturer's instructions. Briefly, equal amounts (100 mL) of standard, negative controls and serum samples were added to the previously marked wells and the plates were incubated for 30 min at room temperature. After 3 washings, 100 mL of conjugate provided with the kit was added to each well and incubated for 30 min at room temperature. The plates were again washed and 100 mL of substrate solution was added and incubated for 15 min. Later, stop solution was added and plates were read at ELISA plate reader set at 450 nm.

At 49 days of age bursas and spleens were removed, sections of bursas and spleens tissues from the inoculated and control chickens were fixed in 10% formalin solution and stained with Hematoxylin and Eosin (H and E). Sections of bursas and spleens were examined microscopically and photographed by digital camera equipment (LEICA DMIRB, Leica Microsystems Wetzlar, Germany).

A completely randomized experimental design was used. All data were statistically analyzed using the General Linear Models (GLM). Procedure of SAS software (SAS Institute, 1996) was employed for the analysis of variance. To determine the differences among treatment means Duncan (1955)'s multiple range test was used. Means were considered different at $p<0.05$.

RESULTS AND DISCUSSION

Effects of different levels of Trp on the immune system indicators in broiler chickens are shown in Table 2-4. As the results indicate, tryptophan supplementation into the basal diets significantly increased interferon-γ, at 35, 42 and 49 DAC days of age ($p<0.05$) (Table 2), interferon-γ at 27 DBC, 35 and 49 DAC days of age ($p<0.05$) (Table 3) and immunoglobulin G at 27 DBC, 35, 42 and 49 DAC days of age of chickens ($p<0.05$) (Table 4). The different levels of Trp at 27 DBC days of age did not significantly affect interferon-γ. The different levels of Trp at 42 DAC days of age did not significantly affect interferon γ.

All the unimmunized control birds succumbed to infection by challenge virus. These chickens showed typical signs of IBD, as such some of them had gross bursal lesions characterized by pale color edema, point bleeding and significantly lower bursa to body weight ratio ($p<0.05$) compared to other chickens.

Spleen enlargement was also significantly higher in control group than the other groups. The bursal lesion scores are shown in Table 5 and 6 and histopathological lesion scores are shown in Fig. 1. The spleen lesion scores are shown in Table 7 and 8 and histological lesion scores are shown in Fig. 2.

Table 2: Effects of tryptophan on the serum level of interferon-α in broiler chickens (pg mL^{-1})

Trp groups	27	35	42	49
	----------days----------			
A	845.34	903.80^{c}	803.64^{c}	702.67^{b}
B	850.60	1035.78^{b}	910.78^{b}	827.51^{a}
C	855.62	1198.54^{a}	948.54^{a}	834.49^{a}
SE	43.52	25.200	10.97	24.47
p-value	00.80	0.0001	0.0001	0.006
R^2	0.078	0.880	0.9100	0.670

Table 3: Effects of tryptophan on the serum level of interferon-γ in broiler chickens (pg mL^{-1})

Trp groups	27	35	42	49
	----------days----------			
A	913.40^{c}	1148.3^{b}	1232.90	1332.84^{b}
B	1105.63^{b}	1244.7^{ab}	1296.89	1604.55^{a}
C	1279.39^{a}	1400.8^{a}	1424.06	1648.63^{a}
SE	43.00	76.46	46.61	70.32
p-value	0.0007	0.046	0.060	0.013
R^2	0.8000	0.490	0.460	0.610

Table 4: Effects of tryptophan on the serum level of immunoglobulin G in broiler chickens (ug mL^{-1})

Trp groups	27	35	42	49
	----------days----------			
A	23.06^{b}	25.64^{c}	36.91^{c}	36.38^{c}
B	26.07^{ab}	37.39^{b}	47.16^{b}	54.16^{b}
C	29.58^{a}	43.94^{a}	64.07^{a}	69.66^{a}
SE	1.270	1.470	1.620	2.620
p-value	0.017	0.0001	0.0001	0.0001
R^2	0.590	0.8900	0.9400	0.8900

$^{a-c}$Means in each column with different superscripts are significantly different ($p<0.05$)

Table 5: Effects of tryptophan on the bursa lesion scores in broiler chickens

Trp groups	49 days
A	5
B	3
C	2

As these data confirmed sever lesions were observed in group A whose diet was not supplemented with Trp and with the increase of Trp the lesions' severity decreased (Fig. 1 and 2).

The results revealed that with an increase in Trp level, INFα, INFγ and IgG were enhanced linearly. This linear enhancement was continuous up to group C. As a whole, the highest level of all immunity indicators, were induced on DAC and DBC of age that is fitted in group C. Although, no statically responses were observed for INFα at 27 DBC and INFγ at 42 DAC, trends of their improvement were observed as Trp level was increased in diet. IBD is an acute, highly infectious and immunosuppressive disease caused by IBDV, a member of *Birnaviridae* family affecting mainly young chickens (Lukert and Saif, 1997). The primary target of the virus is

Table 6: Lesion scoring for Bursa of fabricius

Score	Grade	Lesions
0	Normal	Normal or non pathognomic lesions
1	Mild	Slightly degenerative changes at the lining epithelium and the medullary region of lymphoid follicles
2	Mild to Moderate	Mild to moderate degenerative and or necrotic events of lymphoid cells in the medullas of some lymphoid follicles-infiltration of some acute- sub acute inflammatory in the oedematous connective tissue
3	Moderate	Moderate necrosis of lymphoid follicles involving both medullas (mostly) and cortex (occasionally). Pyknosis as early nuclear change was observed and the interstitial connective tissue is invade by hetrophils, macrophages and plasma cell together with few escaped erythrocytes
4	Moderate to sever	Karryorrhexis and karryolysis as advance nuclear changes is seen in some necrotic cells within lymphoid follicles together with moderate depletion of lymphoid cells in these follicles. Bursa with infectious bursists presenting cycts and lymphoid follicle hypotrophy. The interstitial connective tissue spaces were infiltrated with sub acute-chronic inflammatory cells (lymphocytes, plasma cells, macrophages, some few fibroblasta). The intra and extra follicular area might be hyperaemic and/or hemorrhagic. The epithelial lining, were thickened and some times appear corrugated together with increased number of goblet cells
5	Sever	Frank severs necrosis involving both cortex and medulla. Fibrinous exudates and necrotic cheesy depress were frequently observed. Oedematous connective tissue was obvious that infiltrated by sub acute-chronic inflammatory cells especially macrophages, lymphocytes, plasma cells and fibroblasts. The epithelial lining of the burses was thickend and vaculat of due to the prominence of goblet cells

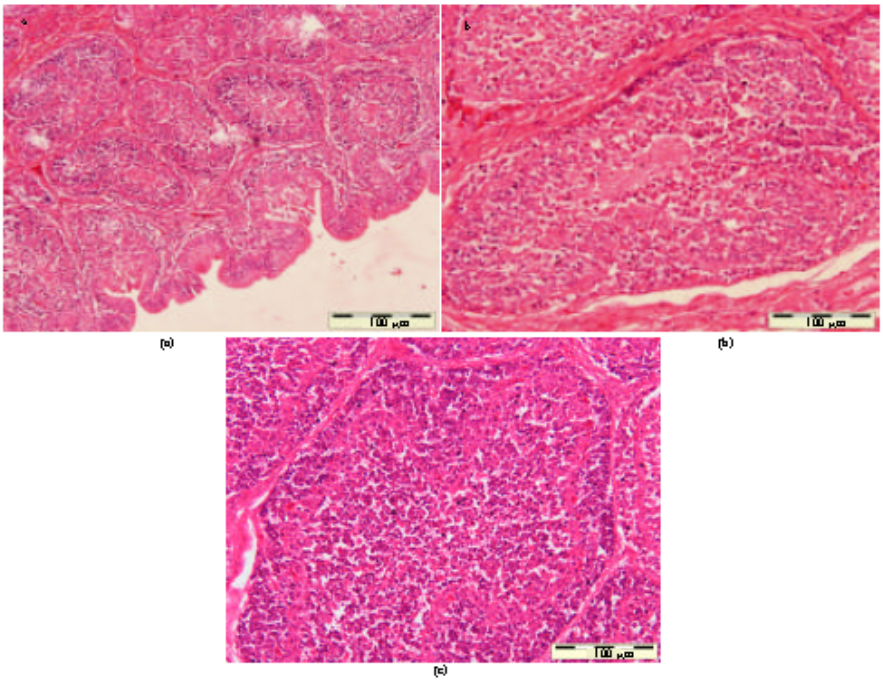

Fig. 1: Histopathology slides of bursa in different groups

actively dividing B cells in bursa of fabricius ultimately causing destruction of these lymphocytes, which leads to transient immunosuppression in affected chickens (Tayada *et al*., 2006). The main objective of the study was to evaluate the immunostimulatory effects of high levels of Trp on the systemic immune response.

It is has been shown that Trp can reduce the immunosuppressive effects of live hot virus and amplify specific protective immune response against herpes simplex virus (Adams *et al*., 2004). In agreement with this finding, the results showed that dietary Trp indeed increased the serum levels of INFα, INFγ and IgG. The enhancement of immune response was further confirmed by histopathological examination of bursa and spleen. Innate immunity is anatomically positioned to serve as the first line of defense against variety of pathogens such as viral infections. One of the elements of innate immunity is IFNα, which is quickly upregulated after infection with viruses (Kawai and Akira, 2006). Mo *et al*. (2001) showed that recombinant IFNα suppressed IBDV and Newcastle disease infection in chickens. This finding is in line with the result in which the clinical signs of IBDV were reduced by increasing of IFNα in Trp fed group challenged with intermediate plus IBDV. Besides, Adams *et al*. (2004) demonstrated an enhancement of IFNα, IFNβ in chickens excessed with Trp and challenged

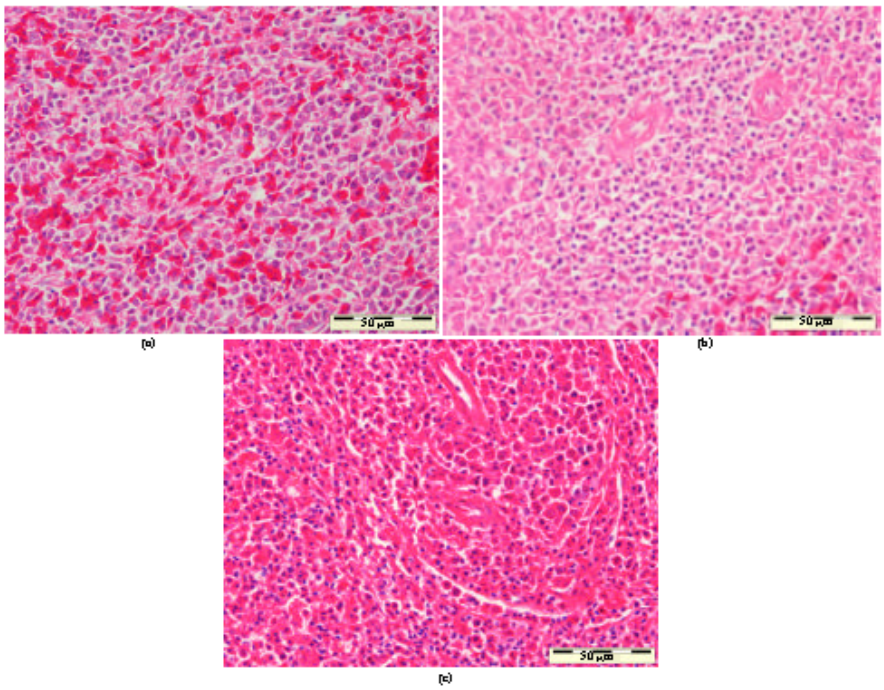

Fig. 2: Histological lesion of spleen in different groups

Table 7: Effects of tryptophan on the spleen lesion scores in broiler chickens

Trp groups	49 days
A	4
B	3
C	2

by herpes simplex virus. As a result, virus replication was restricted. In another study, the oral administration of Trp to rats enhanced phagocytosis by macrophages and could boost the innate immune response as well (Esteban *et al*., 2004).

IFNγ is associated with adaptive immune responses and as such would be expected to be upregulated later in the immune response (Takaoka and Kani, 2006). IFNγ is a pleiotropic cytokine secreted by Natural Killer (NK) cells and many T cells. This cytokine has the ability to direct the balance of TH cells to TH1 and induce cellular immune responses which leads to the lysis of virus-infected or tumor cells (Urban *et al*., 1996). Cellular immune response is centrally involved in the pathogenesis of various diseases. Within the immunological cascades of cellular immunity, interferon γ, among other cytokines, is critically involved (Winder *et al*., 2000). It has been reported that chicken IFNγ level is increased before the appearance of the first clinical signs. During the acute phase of IBD. The rise in antibody titres at 35 DAC indicates the booster effect of challenge virus on immune system; where in clonal proliferation and increased numbers of plasma cells lead to enhanced antibody production (Tizard, 2004) an increase in the levels of circulating cytokines like chicken IFNγ and TNFα occurs as demonstrated by the capture ELISA and cytotoxic bioassay, respectively (Saif, 1984). Co-administration of either IFNγ protein or plasmid DNA encoding IFNγ with DNA vaccine showed that the immune response was enhanced in felines (Flynn *et al*., 2000), ducks (Long *et al*., 2005) and chickens (Min *et al*., 2001) against challenge to feline immunodeficiency virus, hepatitis B virus and *Eimeria acervulina*, respectively. In another study, the increase of IFNγ in chickens whose diet enriched with Trp restricted the replication of herpes simplex virus (Adams *et al*., 2004). Consistent with these findings, the results showed that Trp supplementation into the basal diets significantly increased interferon γ, at 27 DBC and at 35 and 49 DAC days of age ($p<0.05$). In spite of the fact, the different levels of Trp at 42 DAC day of age did not significantly affect interferon γ. Available evidence suggests that Trp catabolism plays a role in immune responses by producing a local immunosuppressive environment that is able to control T-cell homeostasis and self-tolerance during inflammation (Platen *et al*., 2005).

Munn and Mellor (2002) showed that high affinity Trp transporter could be used to stimulate T cell mediated immune responses and increase T cell activation. Moffett and Namboodiri (2003) suggested that Trp can increase leukocyte in the spleen and immune reactivity. The levels of neutralizing antibodies determine the

Table 8: Lesion scoring for spleen

Score	Grade	Lesions
0	Normal	Normal histologic architecture without pathognomic lesion
1	Mild	Few splenic corpuscles revealed scattered lymphoid degeneration and necrosis in the white pulp, the germinal center and peripheral zones were indistinct with slight congestion of parenchymatous blood vessels
2	Moderate	Moderate lymphoid depletion due to (necrosis and apoptosis) in some splenic corpuscles and lymphatic cords of red pulp with moderate congestion and few petechial hemorrhagic area in the intstitial connective tissue together slight hamosiderosis
3	Moderate to sever	Moderate to sever lymphoid depletion in most splenic corpuscles (atrophy) and some lymphatic cords of red pulp with frank petechial hemorrhages and hemosiderin deposition
4	Sever	Frank loss or atrophy of splenic corpuscles with deposition of necrotic depress nests and petechial hemorrhage with infiltration of macrophages plasma cells and ectopic lymphocytes

protection against IBDV in chickens (Van den Berg, 2000). Binding of these antibodies with virus forms immune complexes, which are removed by complement-mediated lysis (Rautenschlein *et al*., 2002). Similar to other findings (Tayada *et al*., 2006), we observed a strong antibody response in all groups; however antibody titers were significantly higher in challenged groups which received Trp. The elevated antibody response and protection are attributed to the various immunoregulatory functions of Trp on the immune system. Antibodies specific to IBDV might be involved in neutralizing virus and the resultant immune complexes will be removed by complement-mediated lysis. The results of the study revealed that as well as adaptive immunity, innate immunity also plays an important role in the control of IBD infection (Rautenschlein *et al*., 2002).

CONCLUSION

The results demonstrate that full protection was achieved against intermediate plus IBDV in chickens supplemented with tryptophan. Tryptophan as a strong immunoregulator enhanced the innate immunity and the IBDV-specific antibody response. It also reduced the immunosuppressive effects of challenge virus on cell mediated immunity by increasing of IFNγ.

To the knowledge this is the first comprehensive study in which the stimulatory effects of tryptophan on innate, humoral and cellular immune responses of chickens challenged with live intermediate plus strain of IBDV have been investigated.

As the results revealed two times of NRC level of Trp supplementation in feed was safe and free from any side effects as well as having the optimum effects on all mentioned parameters which led to the protection of chickens against the intermediate plus strain of IBDV. We suggest that tryptophan as a safe, inexpensive, easy to administer in feed and valuable immunomodulator could be used not only for IBD but also for other viral infections of poultry.

REFERENCES

Adams, O., K. Besken, C. Oberdorfer, C.R. MacKenzie, O. Takikawa and W. Daubener, 2004. Role of indoleamine-2,3-dioxygenase in alpha/beta and gamma interferon mediated antiviral effects against herps simplex virus infections. J. Virol., 78: 2632-2636.

Duncan, D.B., 1955. Multiple range and F-tests. Biometrics, 11: 1-42.

Esteban, S., C. Nicolaus, A. Garmundi and R.E. Rial, 2004. Effect of orally administration tryptophan on serotonin, melatonin and the innate immune response in the rat. Mol. Cell. Biochem., 267: 39-46.

Flynn, J.N., M.J. Hosie, M.A. Rigby, N. Mackay, C.A. Canon and T. Dunsford, 2000. Factors influencing cellular immune responses to feline immunodeficiency virus induced by DNA vaccination. Vaccine, 18: 1118-1132.

Kawai, T. and S. Akira, 2006. Innate immune recognition of viral infection. Nat. Immunol., 7: 131-137.

Le Floc`h, N., D. Melchior and C. Obled, 2004. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livestock Prod. Sci., 87: 37-45.

Long, J.E., L.N. Huang, Z.Q. Qin, W.Y. Wang and D. Qu, 2005. IFN-gamma increases efficiency of DNA vaccine in protecting ducks against infection. World J. Gastroenterol., 11: 4967-4973.

Lowenthal, J.W., P. Staeheli, U. Schultz, M.J. Sekellic and P.I. Marcus, 2001. Nomenclature of avian interferon proteins. J. Cytokine Interferon Res., 21: 547-549.

Lukert, P.D. and S.M. Saif, 2003. Infectious Bursal Disease. In: Diseases of Poultry, Saif, Y.M. (Ed.). 11th Edn., Iowa State University Press, Ames, IA., USA., pp: 161-180.

Lukert, P.D. and Y.M. Saif, 1997. Infectious Bursal Disease: Diseases of Poultry. Iowa State University Press, Ames, IA., USA., pp: 721-738.

Min, W., H.S. Lillehoj, J. Burnside, K.C. Weining, P. Staeheli and J.J. Zhu, 2001. Adjuvant effects of IL-1beta, IL-2, IL-8, IL-15, IFN-alpha, IFN-gamma TGF-beta4 and lymphotactin on DNA vaccination against *Eimeria acervulina*. Vaccine, 20: 267-274.

Mo, C.W., Y.C. Cao and B.L. Lim, 2001. The *in vitro* and *in vivo* effects of chicken interferon alpha on infectious bursal disease virus and Newcastle disease virus infection. Avian Dis., 45: 389-399.

Moffett, J. and M.A. Namboodiri, 2003. Tryptophan and the immune response. Immunol. Cell Biol., 81: 247-265.

Munn, D. and A. Mellor, 2002. Regulation of T cell mediated immunity by tryptophan. Pharm. Biotechnol., 19: 70-75.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., National Academy of Sciences, USA., pp: 19-34.

Platen, M., P.P. Ho, S. Youseff, P. Fontoura and H. Garren *et al*., 2005. Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. Science, 310: 850-855.

Rautenschlein, S., H.Y. Yeh and J.M. Sharma, 2002. The role of T cells in protection by an inactivated infectious bursal disease virus vaccine. Vet. Immunol. Immunopathol., 89: 159-167.

SAS Institute, 1996. SAS User's Guide: Statistics. Version 7.0, SAS Inst. Inc., Cary, NC., USA.

Saif, Y.M., 1984. Infectious bursal disease virus. Proceedings of the 19th National Meeting of Poultry Health Condemnations, (NMPHC'84), Ocean City, MD., pp: 105-107.

Takaoka, A. and H. Kani, 2006. Interferon signaling network in innate defence. Cell Microbiol., 8: 907-922.

Tayada, C., T.N. Jaiswal, S.C. Mishra and M. Koti, 2006. L-argininine stimulates immune response in chickens imunized with intermediate plus strain of infectious bursal disease virus. Vaccine, 24: 5473-5480.

Tizard, I.R., 2004. Veterinary Immunology an Introduction. W.B. Saunders Co., Philadelphia.

Tuting, T., A. Gambotto, P.D. Robbins, W.J. Storkus and A.B. Deleo, 1999. Codelivery of T helper 1-biasing cytokine genes enhances the efficacy of gene gun immunization of mice: Studies with the model tumor antigen beta-galactosidase and the BALB/c Meth A p53 tumor-specific antigen. Gene Ther., 6: 629-636.

Urban, J.R., J.F. Fayer, R. Sullivan, C. Goldhill and J. Shea-Donohue *et al*., 1996. Local TH1 and TH2 responses to parasitic infection in the intestine: Regulation by IFN-gamma and IL-4. Vet. Immunol. Immunopathol., 54: 337-344.

Van den Berg, T.P., 2000. Review article, acute infectious bursal disease in poultry: A review. Avian Pathol., 29: 175-194.

Winder, B., B. Wirleitner, G. Baier-Bitterlich, G. Weiss and D. Fuchs, 2000. Cellular immune activation, neopterin production, tryptophan degradation and the development of immunodeficiency. Arch. Immunol. Ther. Exp., 48: 251-258.