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Effect of neem leaves (*Azadirachta indica)* on immunity of commercial broilers against new castle disease and infectious bursal disease

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Most of the commercial poultry grower use antibiotics as growth promoters and to reduce the chance of occurrence of infectious diseases, which usually result in higher costs of production and ultimately lower net returns. Some plants and their extracts improve feed intake and their enzymatic activity may have antimicrobial, coccidiostatic or anthelmintic effects. Some researchers have investigated that neem when used in poultry feed; increases antibody titer against infectious bursal and Newcastle disease (ND). A total of 144 day old commercial broiler chicks were used in this study. The present study showed that mean antibody titer values against ND on day 42 in the groups A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3, C4 were 2048, 1024, 512, 128, 2048, 1024, 512, 64, 2048, 1024, 1024, 64 respectively. The mean antibody titer against infectious bursal diseases (IBD) at day (42) in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 3413, 3368, 3181, 2813, 3552, 3334, 3190, 2884, 3611, 3458, 3319 and 2814 respectively. During this study it was concluded that addition of neem leaves in broiler feed has better effects on antibody production against new castle and infectious bursal disease viruses.

Key words: Newcastle disease, antibody titer, infectious bursal disease, neem leaves.

INTRODUCTION

Pakistan is amongst those countries that are in the race of development and facing serious animal protein shortage (Maqbool et al., 2005). Poultry industry has made enormous progress in boosting animal protein in the country. It is one of the most efficient and economical converter of vegetable food into animal protein and provides a quick and rapid outcome as compared to production of other proteins of animal origin. Decreased weight gain, management problems and infectious diseases are major constraints in the poultry sector. Several antibiotics have been in use as growth promoters of farm animals ever since. Most of the commercial poultry growers use antibiotics as growth promoters and to reduce the chance of occurrence of infectious

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diseases, which usually result in higher costs of production and ultimately lower net returns. The average growth improvement has been estimated to be between 4 and 8%, and feed utilization improved by 2 to 5% (Patrick et al., 2003). Concerns have been raised that the use of antibiotics as therapeutics and for growth promotion could lead to a problem of increasing resistance in bacteria of human and animal origin, particularly regarding resistance in gram - negative bacteria (*Salmonella* spp. and *Escherichia coli*). It is necessary that antibiotic residues in meat would not impair human health. Specifically, it has been recommended that the use of penicillins, tetracyclines, tylosin, and sulfonamides as growth promoters be discontinued (Patrick et al., 2003). That is why these days poultry scientists are pondering, how poultry farmers can rear birds without using antibiotics and other drugs. They are giving more attention to the indigenous medicines. Some plants and their extracts improve feed intake and their enzymatic activity may have antimicrobial, coccidiostatic or anthelmintic effects. Pakistan has cultivated medicinal plants over a large area in different climatic conditions. All these plants have substances which can be used in poultry in one or the other way. One of these plants is Neem (*Azadirachta indica*) which is commonly called 'Indian Lilac' or 'Margosa', belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae (Girish and Shankara 2008). In Pakistan, it is cultivated throughout Sindh, lower Balochistan, Southern Punjab and Southern NWFP (Durrani et al., 2008). Neem (*A. indica)* is among one of those trees in the world which are currently under extensive research.

Various parts of neem tree have been reported to contain chemicals like azadiractin, nimbin, nimbindin, quercetin among others (Makeri et al*.,* 2007; Gandhi et al*.,* 1998; Blaney et al*.,* 1990) which have antimicrobial, antihelminth, antioxidant, antifungal, insecticidal, antiprotozoa and spermicidal activities (Elangovan et al., 2000) properties (Bonsu et al., 2012). *A. indica* is a fast growing evergreen tree which has a potential to provide medicinal and nutritive value to broilers (Schmutterer, 1990). Broilers given neem leaf extract in water show improved nutrient conversation efficiency and weight gain (Chakravarty and Prasad, 1991).

Neem also plays an important role in strengthening the immune system of the body. Increase in antibodies against new castle and infectious bursal disease viruses have been observed when neem is incorporated in poultry feeds (Durrani et al., 2008). Water based extract (10%) of neem leaves is reported to have anti-viral properties against, fowl pox, infectious bursal diseases (IBD) and Newcastle disease virus (NDV) and it significantly enhances the antibodies production against the IBD and NDV (Sadekar et al., 1998). The present study was therefore designed to record the effect of *A. indica* on immunity of commercial broilers against New castle and infectious bursal disease.

MATERIALS AND METHODS

Experimental chicks

A total number of 144 commercial broiler day old chicks were obtained from Big Bird (Pvt) Ltd. (Hubbard®) and were reared in the experimental sheds of the Department of Pathology, University of Veterinary and Animal Sciences, Lahore. The birds were fed with balanced commercial feed and water *ad libitum* (Table 1).

Experimental design

The birds were divided into three groups (Group A, B and C) having forty eight chicks per each. Birds of each group were sub divided into four sub groups that is, A1, A2, A3 and A4; B1, B2, B3 and B4 and C1, C2, C3 and C4, respectively. Each of the sub group contained 12 birds. Sub groups A4, B4 and C4 were kept as control group receiving no neem leave treatment. The birds of group A were fed with poultry feed containing dry powder of neem leaves at 2 gm/kg feed. The birds of group B were fed with poultry feed containing dry powder of neem leaves at 4 gm/kg feed. The birds of group C were fed with poultry feed containing dry powder of neem leaves at 6 gm/kg feed. The experimental design is explained in Table 2.

Vaccination schedule

The birds of all groups were vaccinated on day 1st by Newcastle disease (ND) vaccines (Live ND Clone 30 Nobilis® and ND killed; Imopest®). The live ND vaccine was given through eye dropping and killed vaccine was given to each birds @ 0.1ml through subcut route. Infectious bursal disease vaccine (Strain D-78; Nobilis®) was given on 10th day of age through drinking water to all birds. Newcastle disease vaccine (Avinew®) booster shot was given on 21st day of age to birds of all groups.

Preparation of neem leaves powder

The neem leaves were harvested from middle aged green trees and were sun-dried for three days on hygienic cement floors until they became crispy but still retaining the greenish tint. The turning of leaves was carried out on regular intervals to prevent uneven drying and possible decay of leaves. Then the leaves were hammered and converted into grinded form.

Serum collection

For antibody titer detection against ND and IBD on weekly basis, blood samples without anticoagulant were taken from 6 birds of each subgroup, in sterile test tubes. The blood was allowed to clot and placed over night at room temperature in slanting position. The serum samples thus obtained were dispensed in separate labeled eppendorf tubes and stored at -20°C until they were further analyzed.

Estimation of antibody response in broilers against new castle disease

The antibody response against Newcastle disease was determined by Haemagglutination (HA) and Haemagglutination inhibition (HI) tests as described by Alexander and Senne (2008). LaSota strain of

Table 1. Commercial broiler feed.

Nutrient composition of commercial broiler feed	Starter feed (1-10 Days)	Grower feed (11-35 Days)	Finisher feed (36-42 Days)
Met. Energy (Kcal/kg)	2750	2800	2886
Crude protein (%)	19.42	21.86	22
Crude fat (%)	2.99	2.97	2.53
Linoleic acid (%)	1.27	1.29	1.26
Crude fiber	5.50	5.50	4.20
Ash (%)	6.15	6.07	5.97
Calcium (%)	1.00	1.00	1.00
Phosporus total (%)	0.88	0.86	0.83
Phosphorus available (%)	0.45	0.50	0.50
Sodium (%)	0.15	0.15	0.20
Potassium (%)	0.80	0.88	0.86
Chloride (%)	0.18	0.22	0.26
Ferrous (ppm)	30	30	30
Manganese (ppm)	79.80	79.80	79.21
Copper (ppm)	10.00	10.00	10.00
Zinc (ppm)	79.21	79.21	79.21
Sulphur (%)	0.30	0.30	0.29
Choline (mg/kg)	2055	2099	1985
Lysine (%)	1.18	1.18	1.29
Methionine (%)	0.54	0.54	0.59
Argnine (%)	1.18	1.39	1.39
Glucosinolate (mmol/kg)	9.00	6.71	5.00

Note: This nutrient composition of commercial broiler feed was obtained from local feed producer on special request.

Table 2. Experimental design.

Days of treatment (days)	Dose rate of neem powder (grams/kg) feed			Control groups*
	2 gm/kg	4 gm/kg	6 gm/kg	
0-42	A1	B1	C1	A4
14-42	A2	B2	C2	B4
28-42	A3	B3	C3	C4

*Birds in groups (A4, B4 and C4) were exposed to same vaccination protocol as other treatment groups, However they did not receive any neem leave powder treatment and were regarded as control groups.

NDV was used as antigen in both HA and HI tests. The results of HA tests were interpreted as follows:

Positive: The bottom of the well covered by a thin layer of finely clumped RBCs.
Negative: A small sharply outlined button of RBCs (bead formation) at bottom of the well.

The results of HI were recorded as following:

Positive: Clear button formation at the bottom of wells.
Negative: Formation of uniform thin layer of finely clumped RBCs.

Geometric mean HI titer of birds from all groups was calculated as described by Villegas and Purchase (1989).

Estimation of antibody response of broilers against infectious bursal disease virus

The assay on commercial ELISA (IDEXX, USA) kit was performed according to the manufacturer's instructions. Test samples were diluted five hundred fold (1:500) with sample diluents prior to being assayed. According to the protocol the positive and negative control sera were not diluted and used as such. ELISA titer of 1000 and above was considered as protective in day old chicks against IBD virus. Titer of 3000 and above was considered as protective in adult birds against IBD virus.

RESULTS

The following results were recorded to see the effects of

neem leaves on immunity of commercial broilers against New castle and infectious bursal diseases. The results have been summarized in Figures 1 to 6.

Antibody titers at day 0

The geometric mean haemagglutijnation inhibition (GMHI) titer at day 0 was found 64 in group A1, A2, A3, A4, B2, C1 and C4. The GMHI titer was 32 in group B1, B4, C3 whereas the titer was 16 in groups B3 and C2. The mean antibody titer against IBD at day 0 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 2223, 2225, 2300, 2228, 2260, 2273, 2228, 2223, 2235,2241, 2263 and 2213 respectively.

Antibody titers at day 7

The geometric mean haemagglutijnation inhibition (GMHI) titer at day 7 was found 128 in group A1, A2, A3, A4, C1,C3 and C4. The GMHI titer was 64 in group B1, B2,B4 whereas the titer was 32 in groups B3, C2. The mean antibody titer against IBD at day 7 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 1560, 1390, 1234, 1160, 1583, 1476, 1390, 1042, 1667, 1450, 1410 and 1032 respectively.

Antibody titers at day 14

The GMHI titer at day 14 was found 1024 in group C1. The GMHI titer was found 512 in group A1, B1. The titer was 256 in groups A2, A3, A4, B2 and B3, C3 whereas the titer was 128 in groups B4, C2 and C4. The mean antibody titer against IBD at day 14 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 1320, 1160, 1034, 920, 1318, 1224, 1128, 896, 1420, 1310, 1195 and 854 respectively.

Antibody titers at day 21

The GMHI titer at day 21 was found 256 in group A1. The GMHI titer was 128 in groups B1 and C1. The titer was 64 in groups A2, A3, B2, B3, C232 in group A4 and C3 and titer was 16 in groups B4 and C4. The mean antibody titer against IBD at day 21 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 3240, 3123, 3097, 2956, 3420, 3260, 3143, 2946, 3555, 3364, 3264 and 3007 respectively.

Antibody titers at day 28

The geometric mean haemagglutijnation inhibition (GMHI) titer at day 28 was found 2048 in groups A1, B1 and C1. The GMHI titer was 1024 in groups A2 and B2. The titer was 512 in groups A3, C2, C3 and titer was found 256 in groups A4, B3.The GMHI titer was 128 in group C4 and titer was 64 in group B4. The mean antibody titer against IBD at day 28 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 3413, 3381, 3166, 3044, 3560, 3342, 3209, 3014, 3649, 3468, 3356 and 3061 respectively.

Antibody titers at day 35

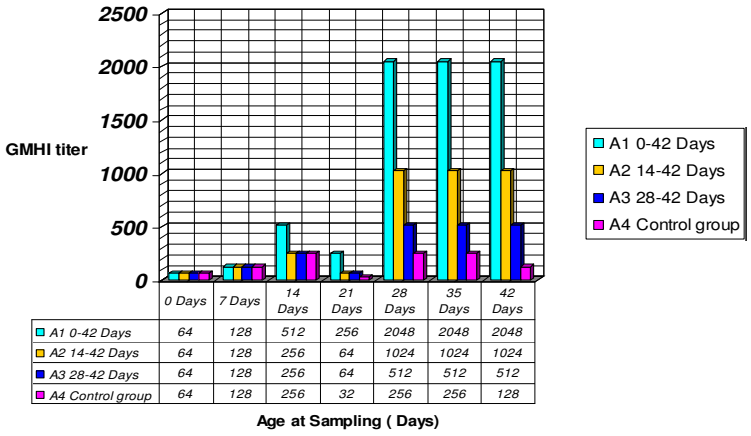
The geometric mean haemagglutijnation inhibition (GMHI) titer at day 35 was found 2048 in groups A1, B1, C1 and C2. The titer was 1024 in groups A2 and B2. The GMHI titer was 512 in groups A3, C3. The titer was 256 in groups A4, B3, C4 and titer was 128 in group B4. The mean antibody titer against IBD at day 35 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 3411, 3378, 3172, 2947, 3569, 3332, 3210, 2941, 3665, 3473, 3343 and 2918 respectively.

Antibody titers at day 42

The geometric mean haemagglutijnation inhibition (GMHI) titer at day 42 was found 2048 in groups A1, B1 and C1. The GMHI titer was 1024 in groups A2, B2, C2 and C3. The GMHI titer was 512 in groups A3 and B3. The GMHI titer was 128 in group A4 and titer was 64 in group B4 and C4. The mean antibody titer against IBD at day 42 in group A1, A2, A3, A4, B1, B2, B3, B4, C1, C2, C3 and C4 was found to be 3413, 3368, 3181, 2813, 3552, 3334, 3190, 2884, 3611, 3458, 3319 and 2814, respectively.

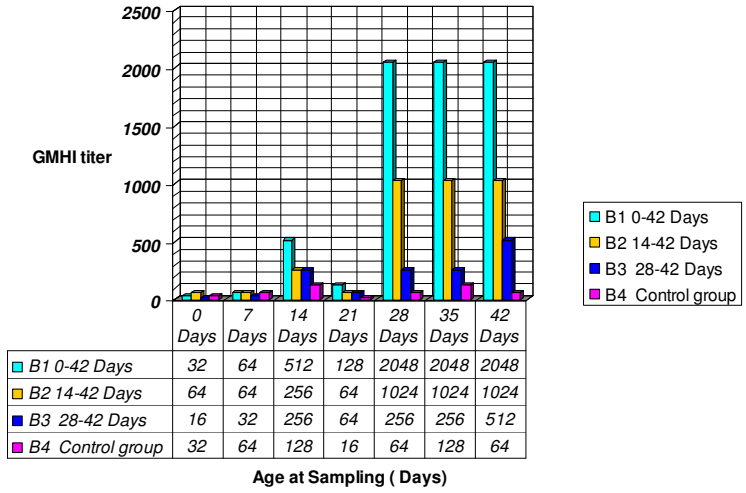
DISCUSSION

In Pakistan the poultry sector has developed rapidly in the last two decades. The poultry production as practiced today is specialized one and concentrating more on the use of high performance birds. The major factors for successful poultry production are high genetic potential, balanced nutrition and health maintenance (Nayaka et al., 2012). Utilization of immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious diseases. Immunostimulation comprises a prophylactic and therapeutic concept aimed at stimulation of the non-specific and specific immune response (Hyde and Patnode, 2001). Most of the commercial poultry growers use antibiotics as growth promoters and to reduce the chance of occurrence of infectious diseases, which usually result in higher costs of production and ultimately lower net returns. There are great concerns about the use of antibiotics as therapeutic immunomodulators and growth promoters as it has given



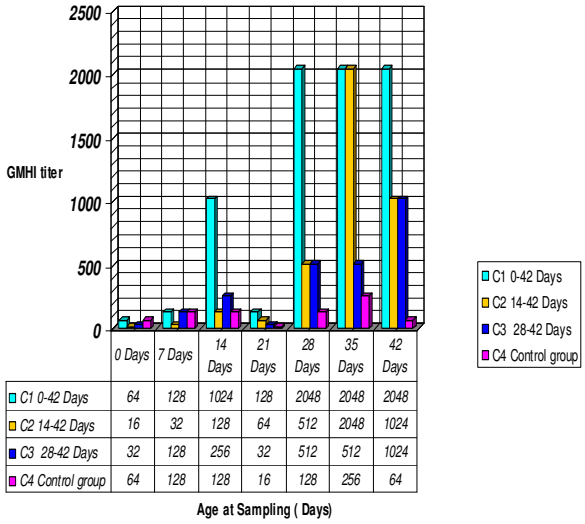
	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
A1 0-42 Days	64	128	512	256	2048	2048	2048
A2 14-42 Days	64	128	256	64	1024	1024	1024
A3 28-42 Days	64	128	256	64	512	512	512
A4 Control group	64	128	256	32	256	256	128

Figure 1. HI titer for ND recorded in birds of group A.



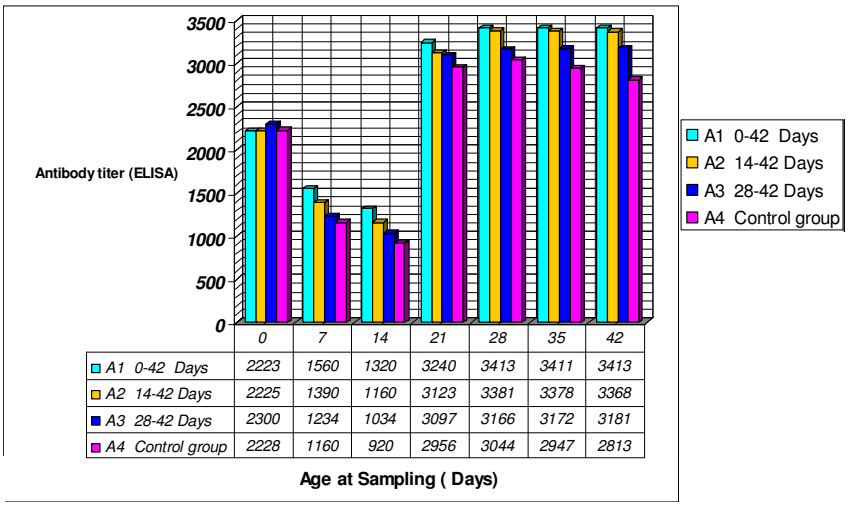
	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
B1 0-42 Days	32	64	512	128	2048	2048	2048
B2 14-42 Days	64	64	256	64	1024	1024	1024
B3 28-42 Days	16	32	256	64	256	256	512
B4 Control group	32	64	128	16	64	128	64

Figure 2. HI titer for ND recorded in birds of group B.



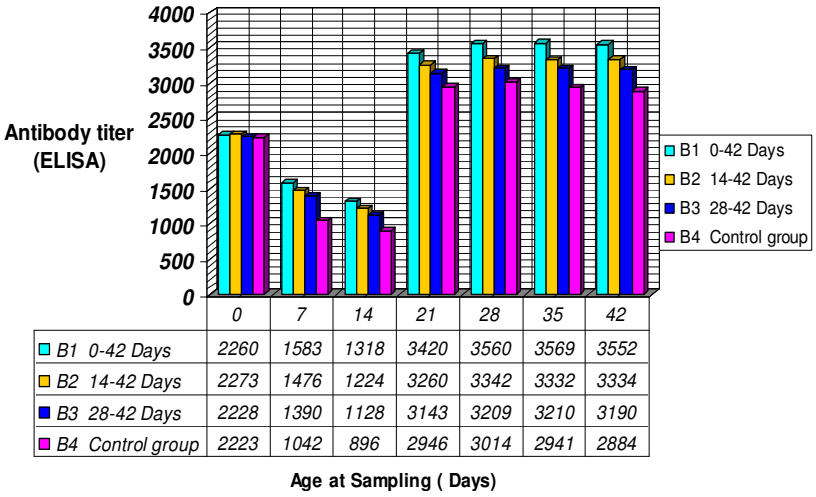
	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
C1 0-42 Days	64	128	1024	128	2048	2048	2048
C2 14-42 Days	16	32	128	64	512	2048	1024
C3 28-42 Days	32	128	256	32	512	512	1024
C4 Control group	64	128	128	16	128	256	64

Figure 3. HI titer for ND recorded in birds of group C.



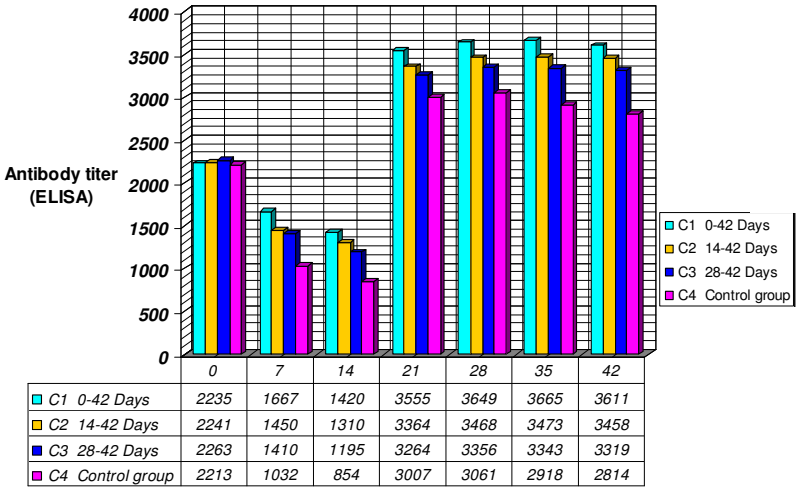
	0	7	14	21	28	35	42
A1 0-42 Days	2223	1560	1320	3240	3413	3411	3413
A2 14-42 Days	2225	1390	1160	3123	3381	3378	3368
A3 28-42 Days	2300	1234	1034	3097	3166	3172	3181
A4 Control group	2228	1160	920	2956	3044	2947	2813

Figure 4. Antibody titer (ELISA) for IBD recorded in birds of group A.



	0	7	14	21	28	35	42
B1 0-42 Days	2260	1583	1318	3420	3560	3569	3552
B2 14-42 Days	2273	1476	1224	3260	3342	3332	3334
B3 28-42 Days	2228	1390	1128	3143	3209	3210	3190
B4 Control group	2223	1042	896	2946	3014	2941	2884

Figure 5. Antibody titer (ELISA) for IBD recorded in birds of group B.



	0	7	14	21	28	35	42
C1 0-42 Days	2235	1667	1420	3555	3649	3665	3611
C2 14-42 Days	2241	1450	1310	3364	3468	3473	3458
C3 28-42 Days	2263	1410	1195	3264	3356	3343	3319
C4 Control group	2213	1032	854	3007	3061	2918	2814

Figure 6. Antibody titer (ELISA) for IBD recorded in birds of group C.

rise to antibiotic resistant bacteria. In this modern world of science, the researchers are once again intending towards exploring the potential benefits of the conventional medicines. Neem (*A. indica*) is a herbal plant which is known to exhibit various beneficialpharmacological properties including immunomodulatory effect in broilers (Upadhyay et al., 1992). Ray et al. (1996) observed neem modulations also in mice on their humoral and cell mediated immune responses when those were treated with neem meal (100 mg neem leaf extract/kg diet). The mice showed higher levels of IgM and IgG along with increased anti-ovalbumin antibody titer. The results of present study revealed that neem leaf meal had good immunomodulatory effects against ND and IBD as indicated by the serum antibody titers. The groups fed with neem leaf meal showed higher mean antibody titer values against NDV as compared to the negative control group. In groups A1, B1 and C1 (fed with neem leaves from day zero of life), the titers were produced not only earlier but these also remained higher as compared to the other groups. There was no difference in GM antibody titers against NDV between groups A1, B1 and C1. Mean antibody titers against IBDV were higher in groups fed with neem leaves meal as compared to the control groups. In groups A1, B1 and C1 antibody titer against IBDV were highest as compared to those groups which were fed with the meal from day 14 (groups A2, B2 and C2) and 28 (groups A3, B3 and C3). Mean antibody titer against IBDV was highest in groups fed with 6 g of neem leaves while the groups fed with 4 and 2 g of neem leaves showed second and third highest titers against the virus, respectively.

The results of present study were in-line with the results obtained by Sadekar et al. (1998) who fed powder dry leaves of *A. indica* to broiler chicks (2 gm/kg) which significantly enhanced the antibody titer against Newcastle disease virus and IBDV. The author concluded that neem increased both humoral and cell mediated immune responses and at the same time it killed or slowed down the growth of many organisms such as bacteria, virus and fungus.

The findings of the present study were also in alignment with the work of Durrani et al. (2008) who gave neem leaves infusion at 30, 40 and 50 ml/ltr in fresh drinking water to the broiler chicken. They concluded that 4% neem leaves *(A. indica)* infusion at 50 ml/ltr of fresh drinking water successfully improved antibody titer against IBD, growth performance and lowered the mortality. The findings of current study were also in-line with the work of Ahsan et al. (1999) who used dried Garlic powder and neem leaves in the broilers feeds to check the antibody titers against Newcastle and infectious bursal diseases.

The authors concluded that neem leaf meal increased the antibody titers against ND and IBD. The results of the present study were also in accordance to the outcome of a work done by Landy et al. (2011) who conducted the experiment to see the effects of different levels of neem in combination with an antibiotic (Flavofosfolipol) on humoral immune response of broiler chicks. Neem at 7gm/kg in diet led to the highest antibody titers against Newcastle disease virus. During the present study conducted on effect of neem leaves on immunity of commercial broilers against new castle and infectious bursal diseases it was concluded that neem plays an important role in trigerring a better humoral immune response against new castle and infectious bursal disease viruses, which defines the worth of neem leaves as immune modulator in commercial broilers, however, the overall judgement about the performance of neem as immune modulator needs more experiments which include the B and T lymphocyte assays accompanied by virus challenging. These modulations were beyond the scope of this study.

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