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Immunomodulatory Potential of *Cajanus indicus* Leaves Powder on Dietary Supplementation in Broiler Birds

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ABSTRACT

The present study was conducted to evaluate the immuno-modulatory potential of *Cajanus indicus* leaves powder on dietary supplementation in broiler birds. Day-old broiler chicks of Vencobb strain (n = 36) were randomly assigned into 3 treatments with 3 replicates each, 4 chicks (2 each for cell mediated and humoral immune response) in each replicate. The dietary treatments composed of the basal diet in control group, 0.05% Bacitracin Methylene Disalicylate added to the basal diet in standard group and 1.5% *Cajanus indicus* leaves powder added to the basal diet in test group. The HA titre (as measured against sheep RBC) was significantly ($p<0.01$) higher in birds of test group as compared to birds of control and standard group. There was significant ($p<0.01$) elevation of cell mediated immunity (CMI) in broiler birds of test group as compared to control and standard group when evaluated by DNFB skin contact sensitization test. The result indicated that ration supplemented with *Cajanus indicus* leaves powder @1.5% significantly improved cellular and humoral immune responses in broiler birds.

Keywords: Broiler bird, *Cajanus indicus*, immune response

Immunomodulators are agents which stimulate or suppress the components of immune system including both innate and adaptive immune responses (Agarwal and Singh, 1999). Herbs or medicinal plants that modulate immune system has always been a subject of scientific investigation worldwide now a days. One such plant, *Cajanus indicus*, commonly called 'Pigeon pea' is considered to be excellent for inducing improvement of health.

In addition to being an agricultural and food resource, many folk lore uses pigeon pea leaves for anti-bacterial, anti-inflammatory, anti-oxidant, anti-cancer, hepato-protective and antihelmintic activities. Extracts of pigeon pea leaves are good for jaundice, diarrhea, sores, cough, bronchitis, bladder stones and diabetes (Pal *et al*., 2011). Poultry farming is always prone to a heavy risk of increased disease incidences leading to high mortality even if scheduled mass vaccination programs are implemented. In the present study, immuno-modulatory potential of *Cajanus indicus* leaves powder (CLP) in feed was evaluated by monitoring their effects on humoral and cellular immune response in broiler birds.

MATERIALS AND METHODS

The study was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh, India. A total of 36, day-old broiler chicks were randomly divided into 3 dietary treatment groups and each group divided into 3 replicates of 2 birds each.

For cell mediated immunity study, 18 chicks were divided into 3 treatment groups of 6 chicks in each. Similarly, for humoral immunity study, 18 chicks were divided into 3 treatment groups of 6 chicks in each. All the groups were maintained as per the following treatment schedule for 6 weeks.

◊ Control Group (T1): Basal diet

◊ Standard Group (T2): Basal diet supplemented with BMD

◊ Test Group (T3): Basal diet supplemented with 1.5% CLP.

Composition of broiler starter, grower and finisher diet is presented in table1.

Table 1: Composition of broiler starter, grower and finisher diet (on % DM basis)

Ingredient	Starter	Grower	Finisher
Yellow maize	54.80	55.00	55.32
Deoiled soybean meal	37.00	33.40	28.47
Rice polish	2.60	7.00	10.00
Soybean oil	2.00	2.00	2.50
Dicalcium phosphate (DCP)	1.60	1.60	1.60
Limestone powder (LSP)	0.70	0.70	0.70
L- methionine	0.28	0.26	0.24
Lysine	0.04	0.02	0.17
Sodium bi carbonate	0.14	0.15	0.16
Common salt	0.28	0.29	0.26
Mineral mixture	0.56	0.58	0.58
Total	100.00	100.00	100.00
C.P. (%)	23	22	20
ME (kcal/kg)	2978	3141	3200

All the birds were provided with feed and water *ad lib* throughout the experiment period. Each bird of different groups was individually identified by using leg bands.

Cell mediated immunity

Cell mediated immune response was measured by 2, 4-Dinitro-fluorobenzene (DNFB) test as described by Tamang *et al.* (1988). Featherless area was marked on both sides of abdomen, cleaned thoroughly with acetone and air dried. Right lateral side of abdomen was used for DNFB application whereas left side served as control. 2000 µg of DNFB in 0.1 ml of acetone and olive oil (4:1) was applied on the right marked area on the abdomen using a plastic ring to avoid spillage. The sensitized birds were challenged with 50µg of DNFB in 0.1 ml of acetone and olive oil (4:1) on the same area on day 14th after initial sensitization. The response to DNFB was assessed by measuring the skin thickness using engineer's micrometer on 0, 24 and 48 hours post challenge with three readings each and the overall mean skin thickness was calculated.

Humoral immunity

Humoral immune response was assessed by micro haemagglutination test according to the method of Thaxton *et al.* (1974) with minor modification. Sheep blood was collected in equal volume of Alsever's solution and allowed to stabilize for one week. SRBCs obtained after centrifugation was washed thrice in normal saline solution (NSS) and finally a 7% suspension of SRBC was prepared. For immunization, 1ml of SRBC suspension was injected intravenously in six birds from each group and the birds were bled on day 10th following injection. The blood was allowed to clot at room temperature for 2-4 hours and serum was harvested. Serum was heated in a boiling water bath to inactivate the complement fraction of the serum and antibody production in response to the immunization was assessed by micro-haemagglutination test. The reciprocal of the highest dilution of serum that caused complete haemagglutination was recorded as HA titre and expressed as $\log_2$ values.

Weight of lymphoid organ

Weight of bursa of fabricius, spleen and thymus were taken at the end of study from the sacrificed birds of each group to calculate the organ weight factor as per the following formula,

$$\text{Organ weight factor} = \left(\frac{Organ\ weight}{Whole\ body\ weight}\right) * 100$$

Data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) by applying completely randomized design (CRD) single factor analysis.

RESULTS AND DISCUSSION

Cell mediated immune response was evaluated by measuring the skin thickness of reactive skin lesion after application of 2,4-Dinitro-fluorobenzene (DNFB) at 24 and 48 hrs. The skin thickness in the birds of T3 (3.59 ±

Table 2: Effect of *Cajanus indicus* on DNFB response (mean skin thickness in mm) in broiler birds (Left side served as vehicle control and right side treated with the DNFB) (n=6)

Skin thickness (in mm)	Abdominal side	Group			Level of significance
		Control (T1)	Standard (T2)	Test (T3)	
Before sensitization	Left	0.55 ± 0.01	0.57 ± 0.01	0.55 ± 0.01	NS
	Right	0.57 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	NS
24 hrs after sensitization	Left	0.56 ± 0.01	0.6 ± 0.02	0.59 ± 0.02	NS
	Right	2.32 ± 0.06^{c}	2.51 ± 0.05^{b}	3.59 ± 0.03^{a}	*
48 hrs after sensitization	Left	0.56 ± 0.02	0.57 ± 0.13	0.56 ± 0.01	NS
	Right	2.24 ± 0.05^{b}	2.36 ± 0.06^{b}	3.52 ± 0.02^{a}	*

Mean values with dissimilar superscripts within row vary significantly

NS-Non significance difference (*P<0.01)

0.03 mm) was highest among all the treatments followed by T2 (2.51 ± 0.05 mm) and T1 (2.32 ± 0.06 mm) at 24 hrs post sensitization as shown in table 2. The skin thickness in T3 (3.52 ± 0.02 mm) was highest among all the treatments followed by T2 (2.36 ± 0.06 mm) and T3 (2.24 ± 0.05 mm) at 48 hrs post sensitization. The results of the present study indicated that the highest CMI response among the different groups was better on supplementation of *Cajanus indicus* leaves powder in feed. Similar result was also reported by Datta *et al.* (1999) by foot pad thickness test in Swiss albino mice. *Cajanus indicus* treatment probably caused a significant activation of macrophages, infiltration of polymorph nuclear cells, increased vascular permeability and oedema, thereby it probably induces T cell mediated response (Datta *et al*., 1999).

Table 3: Effect of *Cajanus indicus* on humoral immune response against SRBC in broiler birds (n=6)

Parameter	Group			Level of significance
	Control (T1)	Standard (T2)	Test (T3)	
HA Titre (log_2 values)	3.17 ± 0.31^{b}	4.00 ± 0.52^{b}	5.33 ± 0.33^{a}	*

Mean values with dissimilar superscripts within row vary significantly

NS-Non significance difference (*P<0.01)

Humoral immune response was judged by estimating haemagglutination (HA) titers of birds against sheep RBC at the 6th week. The HA titers was significantly (P < 0.01) higher in T3 (5.33 ± 0.33) as compared to T2 (4.00 ± 0.52) and T1 (3.17 ± 0.31) as showed in table 3. Similarly, Datta *et al.* (1999) also demonstrated significant increase in HA titer in herbal cajanus protein CI-1 treated immunized mice as compared to control immunized mice. Cajanus protein is itself an immunogenic agent which causes an increase in the synthesis of class of antibodies IgG and IgM (Datta *et al.*, 1999).

The organ weight factor for spleen among different treatment groups (T1, T2 and T3) were recorded as 2.08 ± 0.29, 1.43 ± 0.31 and 1.26 ± 0.39 (Table 4). The organ weight factor for bursa among different treatment groups (T1, T2 and T3) were recorded as 0.86 ± 0.17, 0.64 ± 0.05 and 0.79 ± 0.19. The organ weight factor for thymus gland among different treatment groups (T1, T2 and T3) were recorded as 1.64 ± 0.29, 1.35 ± 0.23 and 2.52 ± 0.33. Non significant increase in organ weight factor of bursa and thymus has been observed. The difference in weight of thymus, bursa and spleen obtained in this study is similar to those reported by Soltan *et al.* (2008).

Table 4: Effect of *Cajanus indicus* on organ weight factor in broiler birds (n = 6)

Organ	Group			Level of significance
	Control (T1)	Standard (T2)	Test (T3)	
Spleen	2.08±0.29	1.43±0.31	1.26±0.39	NS
Bursa	0.86±0.30	0.93±0.20	1.00±0.12	NS
Thymus	1.64±0.29	1.35±0.23	2.52±0.33	NS

NS-Non significance difference (*P<0.01)

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