EFFECT OF ANDROGRAPHIS PANNICULATA ON IMMUNITY AND DUODENAL MORPHOLOGY OF PROGENY OF NICOBARI FOWL

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ABSTRACT

The research was carried out to study the inovo effect of Kalmegh herbal feed supplementation in Nicobari fowl on immunity and duodenal morphology of their progeny. At the age of 35 weeks, breeding fowls were assigned to three diets namely, T1 (Control), T2 – diet supplemented with 1 g Kalmegh powder per bird per day and T2 - 3 g of Kalmegh powder. Breeder fowls fed with 3 g and 1 g Kalmegh powder showed significantly higher HA titre against Goat RBC. The progeny of breeders fed with 3 g of kalmegh showed a significantly ($P<0.05$) higher HA titre at 12 and 16 weeks of age and lower crypt depth and higher villi height. It was concluded that 3 g of Kalmegh powder in the feed of breeder fowl could improve immunity and gut health of progeny.

Key Words: Andrographis panniculata, Nicobari fowl, progeny, Immunity, Duodenal Morphology

INTRODUCTION

About sixty one percent of total poultry population of 1165363 (AHVS, 2012) of A&N Islands comprises of desi birds and native indigenous Nicobari fowl. Rural poultry are prime important and of great concern for the resource poor rural farmers and tribal farming community of these Islands as it is the only source of eggs for them. Due to isolated spread of 572 Islands in remote locations, rural farmers are dependant on indigenous knowledge for the treatment of their poultry rather than immediate Animal Husbandry and Veterinary Services. Studies concerning the use of phytogenic feed additives in broiler nutrition are numerous where as in desi birds are scarce. Being hot spot for medicinal plants, A & N Islands where however ethno-veterinary knowledge has had no place in mainstream Veterinary medicine. Hence it was an hour of critical need to exploit medicinal plants for the treatment and control of epidemic and endemic infectious diseases in rural poultry and their progeny. In this arena, Kalmegh (Andrographis panniculata) is a promising medicinal plant commonly used in humans as an immune system booster. Main bioactive compounds are Andrographolide and Diterpenoid lactone. It's immunomodulatory and growth promoting activity has been scientifically validated (Mathivanan and Kalaiarasi, 2007). However, only few studies investigated the effects of Phytogenic feed additives (PFA) on intestinal morphology in Chickens in particular in the progeny of breeding fowl. In view of the above facts, the present investigation was undertaken to study the inovo effect of Kalmegh (Andrographis panniculata) powder as an immune and gut enhancer in progeny.

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MATERIALS AND METHODS

Two hundred seventy breeding Nicobari fowls belonging to same batch were selected at 35 weeks of age. Optimal male female ratio was maintained. Birds were managed under deep litter system and 16 hours light with 3 lux of intensity per sqft. Birds were not subjected to vaccination at the start of experiment. All birds were fed ad libitum feed as per Bureau of Indian Standards (BIS, 2007) recommendation. Thirty six breeders were assigned to each of following dietary treatments in a completely randomized design with three replicates of 30 birds in each replicate; T1: Breeder diet supplemented with Kalmegh powder at the rate of 1g per bird per day; T2: Breeder diet supplemented with Kalmegh powder at the rate of 3g per bird per day. T3: Control diet without Kalmegh supplement. Ad-libitum feeding was done under standard mangemental condition. At 15th day of feeding, humoral immunity of breeders was assessed through haemagglutination test (HA) using goat red blood cells (GRBC) as an antigen as per the method described by Siegel and Gross (1980). The titer was expressed as the log2. The in-vivo cell mediated immune response to PHA-P (Phytohaemagglutinin) was assessed by the method of Miggiano et al.(1976). The PHA-P (0.1 mg/ml in PBS) @ of 0.1 ml was injected inter-digitally between the 3rd and 4th toe of the right foot of the chicken. The left foot served as control and was injected with 0.1 ml of PBS. The skin index was calculated as the difference between the swelling in the right minus left foot, before and 24 hrs after the injection and expressed as millimeter.

Chicks were hatched out from experimental birds. The duodenal morphology of progeny from experimental birds was measured at the age of eight weeks and their immunity was assessed at 12 and 16 weeks of age. Chicks were slaughtered by humane method at the age of 8 weeks. Duodenal tissue samples were carefully cleansed and fixed in 4% buffered formalin for two days. Tissue was processed through dehydration in Phosphate Buffer Saline and graded ethanol solutions, clearing with xylene and embedding in paraffin. Tissue samples were deparaffinised, rehydrated and stained with hematoxyline-eosine according to the method described by Bancroft and Marilyn (2008). Sections of 5 µm were prepared and placed on glass slides.The villi heights and crypt depth of duodenum was examined by photomicroscope. A total of 12 intact well-oriented villus-crypt units were randomly selected at each tissue sample. Villus height was measured from the tip of the villus to the villus-crypt junction and crypt depth was measured as the extent of invagination between two villi.

Statistical analysis of measurements was carried as per Snedecor and Cochran (1994). The significance of the difference among the groups was determined by Duncan's multiple range tests (Petrie and Watson, 1991).

RESULTS AND DISCUSSION

The results (Table 1) of the humoral immune response of breeding fowl revealed that the antibody titer values in breeders fed with diet containing 3 g of Kalmegh powder was found to be significantly ($P< 0.05$) higher (1.81) than control group (1.2) at 1 week post inoculation (PI) and its peak antibody titer was maintained till 2nd week post inoculation; whereas the humoral response of Nicobari fowls fed with 1 g of kalmegh and control groups started reducing from 2nd week post inoculation onwards and were found statistically ($P< 0.05$) on par with each other. Similarly, Cell mediated immune response in terms of foot pad thickness was found to be significantly ($P< 0.05$) higher with Kalmegh supplementation @ 3 g/bird/day. The analgesic, antipyretic, and anti inflammatory activities of andrographolide and its derivatives has been described (Suebsasana et al.,2009); these pharmacological activities can be of relevance during production of immunity against antigen. Reports in poultry (Mathivanan

and Kalaiarasi, 2007) have found the improvement in the immune status of broilers with inclusion of A.panniculata. The finding of this work was also justified by the report of the positive influence of Morinda citrifolia juice on immune response in poultry by Sunder et al. (2011). All these reports substantiate the higher immunity elicited in Nicobari fowl fed with Kalmegh.

The progeny from breeders fed with 3 g of kalmegh powder produced significantly ($P< 0.05$) higher antibody titer (Table 2) at both 12 and 16 weeks of 0.55 and 0.38 respectively as compared to control group at 1st week post inoculation. The significant ($P<0.05$) humoral response was present till 3rd week of post inoculation in progeny from breeders fed with kalmegh where as antibody titer was nil in control groups. The basis by which breeder herbal feed supplementation could improve immune responses at 16 weeks of post hatch period is not known. However, the result of this study is supported by the report of Koutous et al. (2006) that the in-ovo carotenoid exposure provides the foundation for carotenoid-mediated immunomodulation through tissue carotenoid deposition. The other studies also have demonstrated that the manipulation of egg carotenoids can affect post-hatch chick immune responses, and inoculating barn swallow eggs with lutein resulted in increased wing web swelling in response to phyto-hemagglutinin (Saino et al., 2003). The mechanism for embryonic carotenoid effects later in the chick's life may be related to developmental effects of embryonic carotenoids or due to the persistence of altered carotenoid concentrations in critical tissues in the post-hatch chick. Since the liver is critically involved in the acute-phase component of the inflammatory immune response, it may be that embryonic exposure to kalemegh rich in immunomodulating active principles might have affected the liver concentration of immune active components, thus affected the hepatic component of the inflammatory immune response. Alternately, it might be that the enrichment of immune cells with immune active components affected the immune response. Thus, enrichment of immune cells with herbal immune active principles from dietary or embryonic origin might have affected the inflammatory immune response.

Significant microscopical changes have taken place in crypt depth and villi height at the level of duodenum in layers of the intestinal wall of progeny from breeders fed with kalmegh feed additive (Table 3 and plate A & B). The villi height and crypt depth varied significantly ($P<0.05$) in the progeny from breeders fed with kalmegh (Plate A) than the progeny from control group (Plate B). The control group mucosa contained villi with a height of approximately 269.28 ± 18.48 µm that was statistically ($P<0.05$) lower than the dietary inclusion levels of 1 g (323.11 ± 16.48) and 3 g (365.06 ± 16.0). The crypt depth of progeny from 3 g (54.42 ± 4.81 µm) and 1 g (58.15 ± 3.42 µm) were significantly ($P<0.05$) lower than the progeny of control group (63.79 ± 1.72 µm).

Intestinal glands attached to the villi of control chicks consisted of a small lumen and epithelium as well as a small number of leukocytes. Lax connective tissue from the villi and interglandular corrion connect both the lymphatic and capillary network and was loaded with many infiltrate cells. The capillary network showed evidence of hyperplasia and hypertrophy; where as the chicks from kalmegh fed breeders had a villi with intestinal glands of the duodenum having a large lumen and were surrounded by thin interglandular spaces, with the interglandular villi containing collagen fibres, fibroblasts and leukocytes infiltrate. The interior muscular layer, made up of circular muscle fibres in the endomysium and perimysium capillary ectasia and leukocytes infiltrates were recorded. These images through the duodenum suggest that the angiogenesis process has been stimulated judging by the presence of the capillary ectasia in the main

villi and interglandular villi. The capillary network underwent both hyperplasia and hypertrophy and seen to be powerfully stimulated by the lymphoid infiltrate.

Structure of intestinal mucosa can reflect the health condition of intestine (Xu et al., 2003). The finding of this work is agreed by the report of Lavinia et al.(2009) who provided the data regarding the changes in the microscopic structure of chicken duodenum and immune response as a consequence of aromatic plant extracted essential oils present in their feed. The findings of the present study were consistent to Adibmoradi et al.(2006) and Abdulkarim et al.(2013) who reported that jejunal villus height was increased leading to increased villus height: crypt depth ratio in birds fed with phytogenic feed additive and garlic meal. Similarly, in the present study, active constituents deposited in the yolk might have stimulated development of digestive system with higher villi height contributing to increased surface area for more nutritional absorption and lower crypt depth caused the favorable microbial environment of intestine in the progeny of herb supplemented breeders. The increased villus height/crypt depth ratio obtained with kalmegh supplementation also indicated the improved disease resistance (Xu et al., 2003). It has been suggested that longer villi would result in an increased surface area and higher absorption of available nutrients (Yasar and Forbes, 1999). The histological changes that were brought in the progeny of breeding fowl fed with kalmegh has provided indepth base information of kalmegh for its growth promoting and immune enhancing property that can be utlilised as an alternative to antibiotic growth promoters and to produce quality chicks. Further, this study has provided the base to strengthen the yolk sac at inovo stage itself since yolk sac is source of critical nutrition for the immunity development and gut function in post hatch period. From this investigation, it is concluded that Kalmegh feed additive in Nicobari fowl improves the immunity and gut health of their progeny.

Table 1

Effect of Kalmegh supplementation on immune response of Nicobari breeding fowl

HA titre various intervals				
Treatments	**0 day**	**7th day**	**14th day**	**21st day**
1 g / bird/day	0.35 ±0.09	1.51^{b}±0.12	0.90^{b}±0.17	0.60^{b}±0.43
3 g/bird/day	0.34 ±0.07	1.81^{a}±0.32	1.81^{a}±0.56	1.20^{a}±0.81
Control	0.36 ±0.06	1.20^{c}±0.54	0.90^{b}±0.85	0.60^{b}±0.92

Cell mediated immune response

Treatments	Foot pad thickness (mm)
1 g / bird/day	0.70^{b}± 1.17
3 g/bird/day	0.91^{a}±2.12
Control	0.17^{c}± 2.15

*- Significant (P<0.05) Mean values having different superscript in same column differ significantly

Table 2

HA titre of progeny of Nicobari breeding fowl fed with Kalmegh supplement

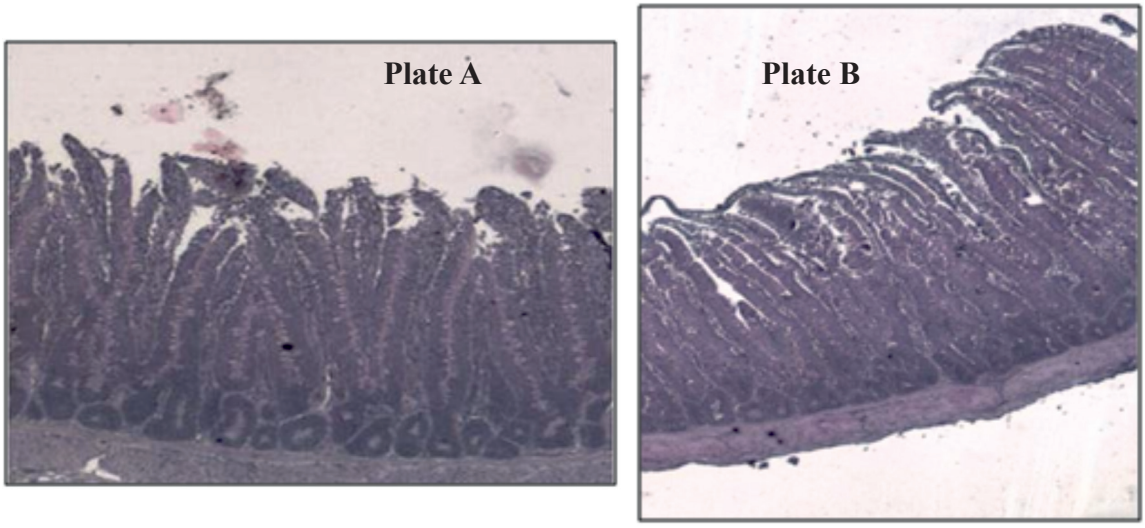
Treatments	At 12 weeks of age				At 16 weeks of age			
	0 day	7th day	14th day	21st day	0 day	7th day	14th day	21st day
3g /bird /day	0.11 ± 1.17	0.55[a]± 1.12	0.20[a]± 1.08	0.23[a]± 0.17	0.10± 1.31	0.38[a]± 1.17	0.26[a]± 0.81	0.25[a]± 1.21
Control	0.13 ± 0.27	0.3[b]± 1.02	0.10[b]± 0.17	0.10[b]± 0.07	0.11[b]± 0.09	0.20[b]± 0.48	0.10[b]± 0.27	0.10[b]± 0.16

*- Significant (P<0.05) Mean values having different superscript in same column differ significantly

Table 3

Duodenal morphometry of progeny of Nicobari breeding fowl fed with Kalmegh supplement

Duodenal parameters	1 g /bird/day	3g /bird /day	Control
Crypt depth (µm)	58.15[b] ± 3.42	54.42[a] ± 4.81	63.79[c] ± 1.72
Villi Length (µm)	323.11[b] ± 16.48	365.06[a] ± 16.0	269.28[c] ± 18.48



Villi height and crypt depth of duodenum of progeny of breeders fed with diet containing Kalmegh [(Plate A @ 3 g and Plate B (Control)]

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