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Short Note

Evaluation of seabuckthorn (*Hippophae rhamnoides*) berries supplementation for humoral immune response in White Leghorn chicks

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Production losses in poultry industry are directly associated with the poor immune status of birds. In India and other developing countries, synthetic chemotherapeutic agents are used for the improvement of immune status so as to prevent them from infectious diseases. The usefulness of synthetic chemotherapeutic agents is limited on account of availability and exorbitant cost. On the contrary, herbal immunostimultors are cost effective and have abundant supply and are also eco-friendly.

Seabuckthorn has got tremendous potential for therapeutic use in human and veterinary medicine. Many medicinal properties like anti-inflammatory (Ganju *et al.*, 2005 and Padwad *et al.*, 2006), immunomodulatory (Geetha *et al.*, 2005) and antioxidant (Geetha *et al.*, 2003) activities are associated with seabuckthorn leaves. Ascorbic acid present in seabuckthorn juice accounts for 75 % of its total antioxidant activity (Rosch *et al.*, 2003). Further, alcoholic extract of seabuckthorn reduced the radiation-induced bone marrow micronucleation frequency in a dose-dependent manner (Agrawal and Goel, 2002). Considering the immense potential of seabuckthorn in poultry , this study was conducted in White Leghorn chicks to evaluate antibody mediated immune response in seabuckthorn berries treated chicks.

The ripened berries of seabuckthorn were collected from Lahaul area of Himachal Pradesh in month of September. These berries were shade dried and ground to obtain fine powder. Powder was mixed in standard poultry feed to the levels of 500 and 1000 ppm.

For determination of Haemagglutination Inhibition (HI) titre, birds from all the groups were vaccinate with New Castle Disease (NCD) vaccine (Lasota strain) on day 6 and revaccinated on day 32 of their age.

Analysis of total protein, albumin, globulin and A: G ratio and HI test

Serum samples were collected from randomly selected birds from each group on day 15, 30, 45 and 60 of the experiment. The total protein was estimated by Biuret method (Henry and Winkelman, 1974), whereas, albumin was estimated by BCG method (Doumas *et al.*, 1971) in 10 serum samples from each group. Serum globulin values were calculated by subtracting the value of serum albumin from the corresponding total protein value. A: G ratio was calculated by dividing albumin value by corresponding globulin value.

HI test was performed as per standard method (Hierholzer *et al.*, 1969 and Chauhan and Roy, 2000) using standard (NCD) LASOTA antigen (Ventri Biologicals, Pune, India).

Determination of humoral immune response

First 4-HA Unit (4-hemagglutination) of antigen was determined and this unit was used for HI test.

The plate was then incubated at room temperature for 15 min. Then 50 µl of 1 % chicken RBCs suspension in PBS were added to all the wells. The plates were incubated at room temperature for 30 min. Results were recorded at 30 min and expressed as reciprocal of $\log_2$ of titre.

Statistical analysis: The data were analyzed using GraphPad Instat version 3.00 computer software by applying one way ANOVA and Tukey's multiple comparison tests was applied to determine the levels of significance.

Total protein, albumin, globulin, albumin: globulin (A:G) ratio

Increased levels of total protein and globulin were observed in serum of birds from both SBT fed groups (Table 1). Significantly increased ($p<0.05$) in total serum protein was observed on day 15 in birds fed with 1000 ppm SBT. Significantly higher ($p<0.05$) serum globulin level was observed on day 45 in 500 ppm SBT

Table 1. Total protein, albumin, globulin and A:G ratio in serum of poultry birds after dietary supplementation of seabuckthorn berries

Parameter	Groups	Days (post seabuckthorn feeding)			
		15	30	45	60
Total protein g/dl	Control	3.74±0.13	4.00±0.10	4.23±0.15	4.11±0.21
	SBT 500 ppm	3.85±0.14	4.36±0.17	4.60±0.33	4.33±0.15
	SBT 1000 ppm	4.36±0.22*	4.30±0.17	4.69±0.16	4.20±0.12
Albumin g/dl	Control	1.65±0.06	1.63±0.09	1.55±0.98	1.59±0.22
	SBT 500 ppm	1.55±0.05	1.48±0.10	1.42±0.05	1.54±0.11
	SBT 1000 ppm	1.66±0.12	1.41±0.08	1.41±0.09	1.59±0.07
Globulin g/dl	Control	2.09±0.10	2.48±0.20	2.45±0.12	2.64±0.13
	SBT 500 ppm	2.30±0.16	2.70±0.11	2.88±0.15*	3.06±0.24
	SBT 1000 ppm	2.76±0.19*	2.86±0.16	2.83±0.09	3.14±0.19
A:G ratio	Control	0.79±0.04	0.70±0.09	0.65±0.06	0.64±0.01
	SBT 500 ppm	0.71±0.07	0.56±0.05	0.50±0.03*	0.51±0.03
	SBT 1000 ppm	0.61±0.05	0.53±0.04	0.49±0.03*	0.52±0.05

Values are mean ± S. E. (n= 10); * p< 0.05

group and on day 15 in 1000 ppm SBT group. Both the treated groups revealed significant decrease in A: G ratio on day 45. The increased serum globulin might be a contributory factor to an increase in the total serum protein contents. Increased globulin levels were possibly a nonspecific and general indication of an enhancement of immunity.

Table 2. Haemagglutination inhibition titre (log_2 values) in poultry birds after dietary upplementation of seabuckthorn berries

Groups	Days (post seabuckthorn feeding)			
	15	30	45	60
Control	4.60±0.25	6.00±0.45	6.60±0.25	6.60±0.25
SBT 500 ppm	4.80±0.20	6.60±0.25	7.60±0.25	7.60±0.25*
SBT 1000 ppm	5.40±0.25	7.40±0.25*	7.40±0.40	7.60±0.25*

Values are mean ± S.E. (n=5); * (p<0.05)

Haemagglutination inhibition test

Comparatively higher antibody titre was observed in vaccinated birds throughout the experiment in SBT treated groups than that of control. In case of vaccinated groups, significantly higher ($p<0.05$) antibody titre was observed on day 60 in birds fed with 500 ppm SBT whereas, on day 30 and 60 in birds fed with 1000 ppm SBT. Thus an early response was observed in birds fed higher levels of seabuckthorn berries.

An enhancement of humoral immune response was evidenced by increased HI titre in the seabuckthorn-fed birds at both the levels. Increase in HI antibody titre was further substantiated by the increased serum globulin in the seabuckthorn-fed birds in the present study which might be due to functional activation of lymphocytes. A similar type of induction of humoral immune response was observed in rabbits (Spanu *et al.*, 1994) and mice (Liu *et al.*, 1997).

Seabuckthorn grows under extreme climatic conditions and is a rich source of vitamin A, C, E, K, carotenoids and organic acids (Fushion, 1987 and Chen *et al.*, 1990). In cold dry conditions the plant accumulates many pharmacologically active compounds which possess anti-arrhythmic, anti-artherosclerotic, hepatoprotective, mucosal and skin protective activities (Beveridge, *et al.*, 1999, Liu *et al.*, 1985 and Xiao, 1980). Some of these bimolecules might be responsible for the stimulus for production of more antibodies.

It could be concluded that the dietary supplementation of seabuckthorn berries have immunostimulatory effect due to increased humoral immune response (antibody production) in poultry at both the dose levels. The antibodies could enhance the humoral immune response in immunocompromised poultry birds and can provide protection to poultry birds from various infectious diseases. However, there is need of determining the optimal levels at which the use of seabuckthorn berries can be recommended in poultry ration.

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