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RESEARCH ARTICLE

Effect of Azolla inclusion on immunological profile of White Pekin broiler ducks

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Abstract

Azolla is a free-floating freshwater fern that is renowned for its rapid growth and rich in proteins, essential amino acids, vitamins, growth promoter intermediaries, and minerals. So this feeding trial was conducted to measure the effect of inclusion of Azolla on immunological profile of white pekin broilers. Unconventional nutrient Azolla was included in the ration of white pekin broilers at 5 % and 10% level and immunological profile of birds examined through haemagglutination test (HA test) and cutaneous basophillc hyper sensitivity (CBH) test. No significant ($P \geq 0.05$) differences between the groups were recorded for antibody titre or CBH response. So it was suggesting that feeding of Azolla did not adversely affect the immunity status of ducks and can be included in the feed to reduce the cost of production.

INTRODUCTION

The planktonic algae of both marine and fresh water origin are considered to be potential sources of alternative feed items as a substitute for cereal grains in poultry ration (Shamna et al., 2013). Azolla is a free-floating freshwater fern that is renowned for its rapid vegetative spread and invasive biology, being one of the world's fastest growing aquatic macrophytes (Collinson et al., 2010). Azolla, abundantly available in stagnant water in tropical and subtropical regions of the world, can be used as a suitable feed substitute, as it is very rich in proteins, essential amino acids, vitamins, growth promoter intermediaries, and minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese etc., probiotics and biopolymers (Pillai et al., 2002).

Very few works have been taken up on the effect of Azolla on the immunological performance of poultry birds. In an experiment by Dhumal et al (2009) on commercial Vencobb broiler chicks for 6 weeks, fed with Azolla meal at 0, 2.5 or 5% level as partial replacement of soya bean meal in their diet, it was concluded that feeding Azolla at these levels had no significant bearing on body weight gain, weekly feed consumption and weekly feed conversion ratio and improved the immunity status. They analyzed antibody titre of birds against New Castle Disease Virus at 35 days of age, and the results thus obtained revealed improved Ab titre values in 2.5% or 5% Azolla fed groups as compared to the control group. Seth et al. (2013) studied the effect of dietary fresh Azolla, as a protein substitute on vanaraja chicken and she found that immunity status was not differ significantly ($p \geq 0.05$) between groups as measured by CBH and SRBC response and weights of lymphoid organs. Sujatha et al. (2013) examined the prospects of supplementing commercial feed with raw Azolla (Azolla pinnata) on the production performance of Nicobari fowl where she reported that the mean HI titre in Azolla group was higher than the control

group. The cell mediated immune (CMI) response expressed as Foot index (FI) in the Azolla group was not different from the control.

Therefore, the proposed study **"**Effect of Azolla inclusion on immunological profile of White Pekin broiler ducks " has been envisaged to find out the potential effect of fresh Azolla on immunocompetence of broiler ducks in the agro- climatic conditions of coastal Odisha.

MATERIALS AND METHODS

Location and period of experiment

The experiment was conducted in the department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar.

Experimental Programme

Experimental Design

One hundred eight, day-old White-Pekin ducklings of either sex were purchased from Central Avian Research Institute (CARI), Bhubaneswar. All ducklings were weighed and randomly distributed into three treatment groups with three replicates each, and each replicate having twelve ducklings, maintaining uniformity in body weight. Ducklings were grown in deep litter system of rearing and the experimental diets were provided as per BIS specification.

The experimental groups were subjected to dietary treatment on completion of two weeks of age. The experimental groups were as under;

Group 1: Basal diet (**G_1**)

Group 2: Basal diet + 5% Fresh Azolla of Basal diet (**G_2**)

Group 3: Basal diet + 10% Fresh Azolla of Basal diet (**G_3**)

The diets were made isocaloric and isonitrogenous as per BIS standards.

Immunological analysis

Measure of humoral immunity

The measure of humoral immunity was carried out as per the method described by Abdallah et al. (2009). Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyze specific antibody response as measure of humoral immunity. At 35th day of age, one bird from each replicate in each dietary treatment were immunized intravenously via a wing vein with 0.07 ml packed RBC mixed with 0.93 ml physiological saline (0.9% NaCl) for measurement of primary response. Seven days following the antigen challenge, blood samples were collected and serum samples were used to measure humoral immunity. Antibody production to SRBC was measured using microtitration haemagglutination technique with microtiter plate U shape of 96 wells (8 rows X 12 column) according to Bachman and Mashaly (1986) and Kai et al. (1988). All SRBC antibodies were expressed as $\log_2$ of the reciprocal of the highest serum dilution causing agglutination of SRBC.

Chemical Reagents

Alsever's solution and phosphate buffer saline (PBS), prepared as per the composition given below.

Alsever's solution

Tri sodium citrate dihydrate	–	0.80 g
Sodium chloride	–	0.42 g
Dextrose	–	2.05 g
Citric acid	–	0.055 g
Distilled water	–	100 ml

The above ingredients were taken in a 200 ml conical flask and the pH of the solution was adjusted to 6.5 by addition of citric acid. The solution was stored at 4^0C for use.

Phosphate buffer solution (PBS)

Sodium chloride (NaCl)	–	8 g
Potassium chloride (KCl)	–	0.2 g
Potassium dihydrogen phosphate (KH_2PO_4)	–	0.2 g
Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$)	–	1.44 g
Distilled water	–	1000 ml

The above ingredients were taken in a conical flask and the pH of the solution was adjusted to 7.2 with the help of a litmus paper. Then the solution was sterilized at 15 lb pressure for 15 min. The solution was stored at 4^0C for use.

Preparation of SRBC suspension

Blood from the jugular vein of a healthy sheep was collected in Alsever's solution. The blood was centrifuged at 2500 rpm for about 10 min. The supernatant was discarded and the red blood cells were washed three times in PBS solution. Suspension of SRBC (1% v/v) in PBS was prepared and stored at 4^0C for use.

Haemagglutination test (HA test)

Procedure

With the help of an auto-pipette 50 µl of PBS was distributed in each well of the round bottom micro titre plate having 96(12x8) wells. 50 µl of test serum was added in the first well. Two-fold serial dilutions were made up to row 11 and row 12 was kept control. Volumes of 50 µl of 1% SRBC were added in each well and mixed by gentle tapping. The plate was kept at 37^0C for 1 hour incubation. Then the plates were read under bright light. The reciprocal of highest dilution showing clear agglutination was the end titre. The titres were expressed as $\log_2$.

Measure of cellular immunity

At 28th day of age, two birds from each replicate in each dietary treatment were injected intra-dermally in the foot pad with 100 micro gram of Phytohaemaglutinin-P (PHAP) in 0.1 ml of normal saline to measure the cellular immune response by Cutaneous Basophillc Hyper Sensitivity (CBH) test (Edelman et al., 1986). The normal thickness of the comb and thickness 12hrs, 24 hrs, 36 hrs of post injection were measured by using a digital slide caliper (Mitityo, Japan).

The CBH response was calculated using the formula:

$$\text{CBH response} = \frac{\text{Post injection skin thickness}}{\text{Pre-injection thickness}} \text{ X } 100$$

Statistical analysis

Data obtained from the experiment were subjected to statistical analysis wherever required. The statistical analysis of the data was done according to the method described by Snedecor and Cochran (1998).

RESULT AND DISCUSSION

SRBC and CBH response

The antibody titres ($\log_2$) against SRBC and CBH responses, for the three groups of ducks are presented in Table 1.

Table 1 SRBC and CBH response of ducks at 6 weeks of age

Parameters	Treatments			P value
	G_1	G_2	G_3	
Ab titre ($\log_2$)	7.46±0.06	7.84±0.13	7.71±0.09	0.09
CBH response	167.00±0.68	167.84±0.14	167.18±0.56	0.52

Values bearing different superscripts in a row differ significantly (P≤0.05)

The antibody titres ($\log_2$) ranged from 7.46±0.06 to 7.84±0.13 and CBH responses from 167.00±0.68 to 167.84±0.14. No significant (P≥0.05) differences between the groups were recorded for antibody titre or CBH responses. Dhumal et al. (2009) reported increased antibody titre values by feeding of Azolla. Seth et al. (2013) also reported numerically higher antibody titre as well as CBH responses upon feeding of Azolla to Vanaraja chicken. Chichilichi et al. (2012) found that commercial broilers fed of Azolla had higher (P≤0.05) antibody titre, with similar CBH responses, compared to the birds not fed Azolla. However, in this study, slightly higher values were recorded for either of the Azolla- fed groups as compared to the control in respect of both the parameters suggesting that feeding of Azolla did not adversely affect the immunity status of ducks.

CONCLUSION

The antibody titres and CBH responses of broiler ducks suggesting that feeding of Azolla did not adversely affect the immunity status of ducks and can be included in the ration for reducing cost of production.

Conflict of interest: **Authors declare no conflict of interest.**

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