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EFFECT OF SELENIUM-ENRICHED AZOLLA ON BIOCHEMICAL AND GROWTH PERFORMANCE PARAMETERS IN JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*) BROILERS

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Keywords:

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ABSTRACT: Azolla is a fern plant through conducting 27 % of protein content and involves actively in bioremediation. So, this property of this plant is exploited in the production of chelated minerals. In this study, selenium-enriched azolla is evaluated with Japanese quails on the performance of growth and serological parameters. The present study is designed with 525 quails divided into 7 treatments, each with 3 replicates. Each replicate contains 25 birds. The evaluation was done from day-old chick to 5-week age in a deep litter farming system. The birds in each treatment are distributed in a randomized manner irrespective of gender, later grouped into control, 0.2, 0.3, and 0.4ppm organic selenium treatments, along with 0.2, 0.3, 0.4ppm inorganic selenium treatments sequentially. The body weight gain, feed intake and feed conversion rate were noted every week and significant changes were recorded. In performance trials we observed that 0.2ppm organic selenium shows good performance in body weight gain ($P<0.013$) and feed conversion rate ($P<0.02$) at a significant level. At 0.2ppm concentration of organic selenium, significant levels of increase in albumin ($P<0.017$), phosphorus ($P<0.014$) and protein ($P<0.013$) were observed. After analyzing biochemical parameters, it is concluded that 0.2ppm organic selenium concentration is showing significant growth performance and benefiting certain biochemical parameters in Japanese quails.

INTRODUCTION: Genetic selection for higher growth rate, providing a balanced healthy diet and maintaining optimal health care measures demanded a higher body weight in commercial livestock. However, birds raised in open-sided poultry houses are susceptible to stress caused by constant dynamic changes in the surroundings, which adversely affects their performance [1], meat quality, and immune response [2].

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It is evident from previous studies that heat causes a major stress for chickens due to altered metabolic rate and body temperature [3]. Panda *et al.,* [4] found that high environmental temperatures in tropical regions causes bulk financial losses to the poultry industry due to diminished feed intake and feed

conversion efficiency. Panda *et al.,* [4] also provided a cue that these adversities in the tropics can be minimized by feeding a diet supplemented with organic selenium (Se).

It is well known that selenium is essential to balance and keep good health of animals and humans. Deficiency of Se results in a clinical condition known as exudative diathesis (resulting from deficiency of vitamin E) and pancreatic degeneration [5]. Selenium intake plays an important role in immune function by promoting the production of immunoglobulin's [6]. The majority of biochemical reactions require the participation of trace elements such as selenium, copper and manganese as cofactors to enhance the catalytic activity of enzymes. Among these trace minerals Se has a significant role as cofactor in antioxidant enzymes. Inorganic Se available as a sodium selenite and sodium selenate, another state is organic form, which is absorbed by plants or animals and gets mixed with biomolecules like proteins, RNA *etc*. The molecular structure of selenium is similar to sulphur; hence it occupies the place of sulphur in the proteins. The bioavailability of organic or chelated Se is more when compared with inorganic Se. However, inorganic Se can be toxic to animal tissues, considering its easy binding ability within tissues, but organic or chelated Se cannot bind tightly with tissues hence being involved in metabolic reactions. Nowadays, many poultry industries use organic Se-enriched yeast, but its production cost is high. Researchers also worked on Se enrichment in mushrooms [7] and some of the algal species [8].

Azolla is a fern plant growing on the surface of water with 27% of protein. It is palatable to animals and can reduce the fodder cost in animal husbandry. Azolla plant involves bioremediation with an accumulation of minerals present in lakes, ponds and used in the purification of water bodies. Owing to these factors, Azolla bioremediation capabilities have been explored in the current study to produce the organic or chelated mineral production. Moreover, there is a need to develop alternative ways to reduce the production cost of organic minerals used to supplement animal husbandry feeds. Through this approach, low-income farmers can provide quality feed to their cattle. Considering all the above explanations and reasons proposed in the present study, **Fig. 1** aims to produce low-cost organic selenium and its supplementation to Japanese quails for studying growth performance and biochemical changes.

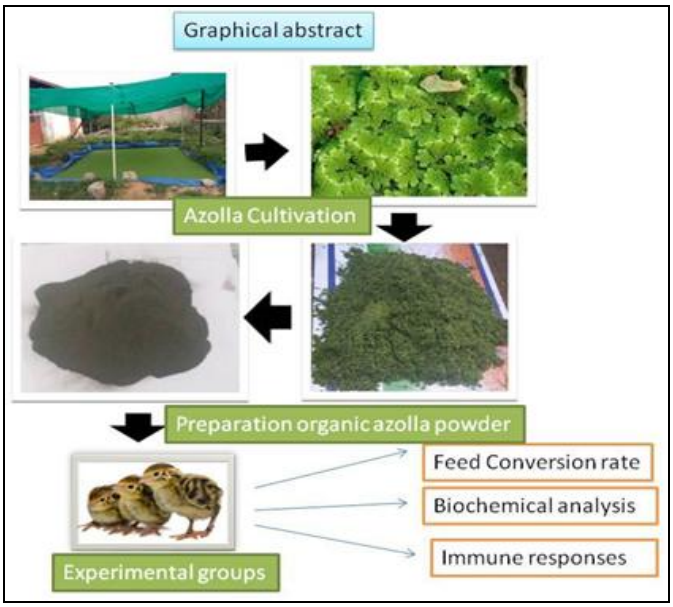

FIG. 1: GRAPHICAL ABSTRACT SHOWING THE PLAN OF WORK

MATERIALS AND METHODS:

Production of Se-Enriched Azolla through Sodium Selenite: The healthy aquatic plant *azolla pinnata* were collected as a gift sample from the college of veterinary Science Rajendra Nagar. Azolla acclimatized for seven days in a greenhouse [9, 10] and cultivated in H40 medium **Table 1**.

TABLE 1: H_{40} MEDIUM USED FOR AZOLLA CULTIVATION

S. no.	Ingredients	1000L Stock (gm)
1	$CaCl_2$.$2H_2O$	5.88
2	$MgSO_4$.7 H_2O	9.86
3	$K2SO_4$	6.97
4	NaH2PO4.H_2O	2.76
5	$CuSO_4$.$5H_2O$	0.0025
6	H3BO3	0.012
7	Na2MoO4.$2H_2O$	0.036
8	$ZnSO_4$7.H_2O	0.0029
9	$CoCl_2$.6 H_2O	0.0024
10	MnCl2.4 H_2O	0.099
11	EDTA Na2.2 H_2O	0.19
12	FeSO4.7 H_2O	0.14

Azolla was exposed to different concentrations of inorganic selenium (0, 1, 2, 3, 4, 5 and 10 ppm) prepared individually along with H40 medium. Each of the azolla culture trays was filled with 1.5 L of H40 medium, including inorganic selenium as per experimental requirement. 5g of azolla culture

was added to the experimental tray and kept for 1 month. Then the yield of azolla wet and dry biomasses were recorded. The azolla was dried in the oven at 45°C for 48 h and Se estimation was carried out in the dried Se-enriched azolla biomass using atomic absorption spectroscopy.

Sample Preparation and Se Estimation: The weighed quantity of Se-enriched azolla produced was digested in the digestion flasks using nitric acid at 85 °C in the digestion system (Kel Plus, Pelican Equipment). Se lamp was used for Se estimation in the azolla biomass and the atomic absorption spectroscopy was calibrated with various concentrations of Se standards. Both the standard and samples were injected through a nebulizer of the spectrometer (AA 400, Perkin Elmer Pvt Ltd). The concentration of Se was retrieved using the software (WinLab32; Perkin ElmerPvt Ltd) [11].

Grouping of Experimental Birds: Experiments were conducted on Japanese quail birds in deep litter farming. For This study, day-old chicks were procured from the Poultry Experimental station, Livestock farm complex, College of the veterinary station, Rajendra Nagar, Hyderabad hatchery and randomly distributed (Ethical committee approval number: 1/2019-1/IAEC/CVSc, Hyd). The day-old quails were randomly distributed. A total of 525 quails were used for experimental purposes. They were divided into 7 groups; each group contains three replicates; each replicate contains 25 birds. They were fed with different concentrations of selenized azolla and inorganic Se. The details of the experimental groups are mentioned in **Table 2.**

TABLE 2: EXPERIMENTAL GROUPS

Treatments	Dosage of Selenium
Treatment 1 (T_1)	Basal diet
Treatment 2 (T_2)	Basal diet + 0.2 ppm Se-AZ
Treatment 3 (T_3)	Basal diet + 0.3 ppm Se-AZ
Treatment 4 (T_4)	Basal diet + 0.4 ppm Se-AZ
Treatment 5 (T_5)	Basal diet + 0.2 ppm inorganic
Treatment 6 (T_6)	Basal diet + 0.3 ppm inorganic
Treatment 7 (T_7)	Basal diet + 0.4 ppm inorganic

Se-AZ- Selenium-enriched Azolla

Feed Management: Table 3 shows the composition of the basal diet, whereas the experimental birds were fed different diets based on their respective group's **Table 2**. They received basal diet (BD, diet I for group T_1), 0.2 ppm organic Se (diet II, group T_2), 0.3 ppm organic Se (diet III, group T_3), 0.4 ppm organic Se (diet IV, group T_4), BD with 0.2 ppm inorganic Se (diet V, group T_5), 0.3 ppm inorganic Se (diet VI, group T_6) and 0.4 ppm inorganic Se (diet VII, group T_7) up to 5 weeks. At the end of the experimental period, serum was collected from each replicates to study changes in the biochemical parameters.

TABLE 3: FEED COMPOSITION FOR 100 KG AS PER NRC 1994

S. no.	Ingredients	100 Kg
1	Maize	54.85
2	Soyabean Meal	40.135
3	Salt	0.4
4	Dicalcium Phosphate	1.076
5	Stone grit	1.226
6	DL-methionine	0.141
7	AB_2D_3EK	0.03
8	B-Complex	0.03
9	Choline Chloride	0.05
10	Toxin binder	0.2
11	Trace mineral	0.15
12	Vegetable Oil	1.776
13	Coccidostat	0.05
14	Antibiotic	0.05

Weekly feed intake and body weight gain were recorded every week throughout the experimental period. At the end of the experimental period, the body weights and data of serological parameters were analyzed through ANOVA analysis.

Blood Biochemical Parameters: The serum samples were analyzed for the estimation of Protein, Albumin, Alanine transaminase, Aspartate aminotransaminase, Calcium, phosphorus, and Cholesterol with commercial kits as per the manufacturer protocol (Erba).

Immune Responses: The effects of selenium supplementation on humoral (ND virus) immune response were conducted as per standard procedure. Blood samples were collected on 35^{th}day of age to study the antibody titers against Newcastle disease vaccine (NDV).

RESULTS AND DISCUSSION:

Production of Organic Selenium: To achieve the primary objective, we cultivated the azolla in H40 medium for 1 month at different concentrations of sodium selenite added to the medium. The concentration of selenium and azolla growth exhibited an inverse relationship. The wet biomass

of Azolla is decreased when the selenite concentration is increased from 1 to 5 ppm, which is like 45.78, 39.88, 37.08, 33.41, 29.29 and 23.84g sequentially **Table 4**. The study groups accumulated between 1.732 and 1.94 mg/g of selenium.

TABLE 4: AZOLLA BIOMASS AND SELENIUM ACCUMULATION

S. no.	Treatment	Sodium selenite Concentration (ppm)	Wet biomass (g)	Dry powder weights (g)	% Of reduction	Selenium accumulated (mg/g)	Total organic selenium for 1.5 lit (g)
1	Treatment 1	0	45.78	2.19	00	0	0
2	Treatment 2	1	39.88	1.9	12.78	1.732	3.29
3	Treatment 3	2	37.08	1.72	19	1.75	3.01
4	Treatment 4	3	33.41	1.6	27	1.8	2.88
5	Treatment 5	4	29.29	1.4	36	1.85	2.59
6	Treatment 6	5	23.84	1.14	47.85	1.94	2.21

A significant disadvantage of inorganic Se consumption is well established. Because inorganic Se cannot be consumed, researchers are exploring the possibility of synthesising organic Se through microalgae, plants, and microorganisms. An organic Se that is safe and effective for animal consumption could be produced using this process. According to studies conducted on algae, low selenium concentrations stimulated moderate growth, but higher concentrations stimulated inverse growth [12, 13]. Additionally, increasing concentrations of selenium resulted in similar growth patterns for azolla **Table 4**. This may be due to selenium antioxidant Property at lower concentrations [14]. However, high selenium concentrations may interfere with growth by altering cell ultrastructure, impairing metabolic reactions, decreasing growth rate, and even causing apoptosis [13, 15, 16].

Effect of selenium Enriched Azolla on Growth Performance of Quails: Selenium supplementation affected body weight gain (P<0.013) and feed conversion rate (P<0.02) in the present study **Table 5.** In contrast, there was no significant change in feed intake values. There was a slight difference in body weight gain between groups treated with 0.2 ppm organic selenium (T2) and control groups, as well as a significant increase in feed conversion rate FCR, refer to **Table 5**. Prakash *et al.* [11] also reported similar results.

TABLE 5: PERFORMANCE OF JAPANESE QUAILS IN FEED INTAKE, BODY WEIGHT GAIN AND FEED CONVERSION RATE ON FIVE WEEKS AGE

Treatment	Supplementation	Feed intake (FI)/25 birds (g)	Body weight gain (BWG)/bird (g)	FCR
1	T1	3973.60	199.82^{c}	2.69^{d}
2	T2	4107.92	203.62^{a}	2.73^{a}
3	T3	4196.11	202.44^{bc}	2.73^{ab}
4	T4	4039.56	201.00^{bc}	2.70^{cd}
5	T5	4086.64	201.50^{abc}	2.71^{bc}
6	T6	4026.69	200.79^{bc}	2.70^{cd}
7	T7	3997.38	200.00^{bc}	2.71^{bcd}

A report showed improved body weight and feed efficiency in chickens raised under heat stress when fed diets containing Se [1, 17]. In broilers chicken fed with Se-enriched yeast [11], feed efficiency and weight gain were also reported to improve.

Changes in Biochemical Parameters: Both protein and albumin levels increased significantly (P<0.013 & P<0.017) in the 0.2ppm treated group **Fig. 2** & **3**. Azolla's protein-rich nature may account for the rise in these levels.

A significant increase in the level of essential minerals such as calcium and phosphorus **Fig. 4** was observed compared to control animals (P<0.014 & 0.178, respectively).

Providing animals with strength and support is well known to be the function of proteins and minerals. So, feeding poultry birds with selenium-enriched azolla can increase vital parameters. As a result, we have noticed significant growth in experimental animals.

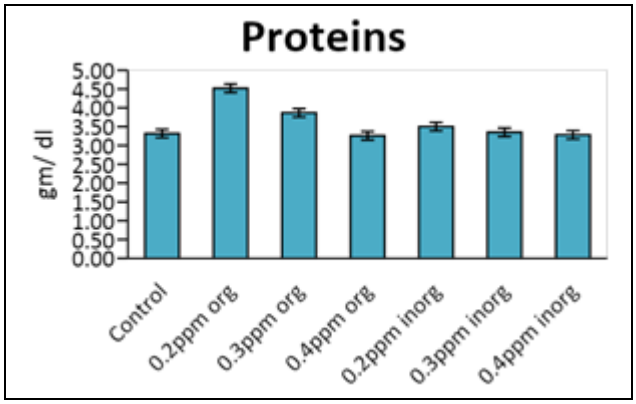

FIG. 2: CHANGES IN THE PROTEIN LEVELS OF STUDIED EXPERIMENTAL GROUPS

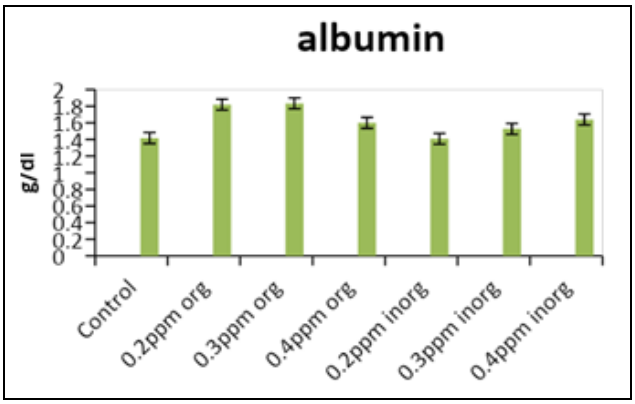

FIG. 3: CHANGES IN THE ALBUMIN LEVELS OF STUDIED EXPERIMENTAL GROUPS

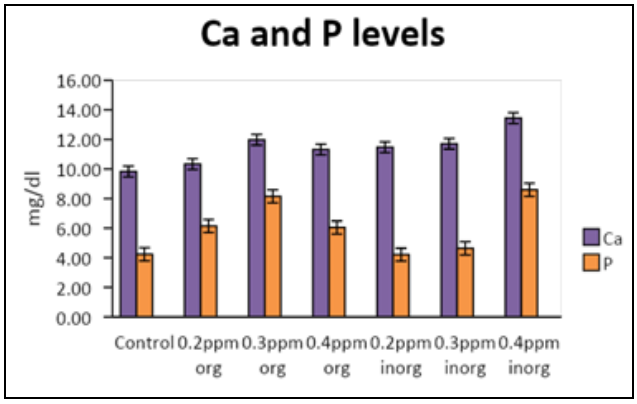

FIG. 4: CHANGES IN THE CALCIUM AND PHOSPHOROUS LEVELS OF STUDIED EXPERIMENTAL GROUPS

A moderate change in the levels of cholesterol was observed in **Fig. 5** throughout all experimental groups. This indicates that organic selenium is not affecting lipid metabolism, which is an advantage. The results obtained for biochemical parameters are in agreement with the results of previous studies of Bhatand Ganai [18] and Edens *et al.*, [19], Chaitra *et al.*, [20].

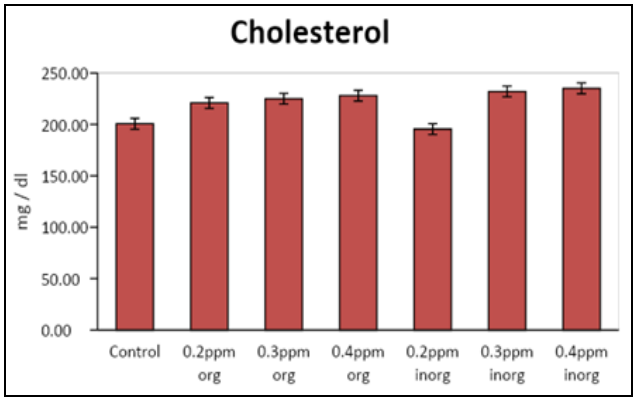

FIG. 5: CHANGES IN THE CHOLESTEROL LEVELS OF STUDIED EXPERIMENTAL GROUPS

Since, any adverse change in the diet or medicine will affect the liver's health status, we assessed the effect of organic selenium on this vital organ by estimating the changes in the levels of marker enzymes like aspartate transaminase (AST) and alanine transaminase (ALT). These results suggest that selenium-enriched azolla is not causing any adverse toxic effects on the metabolism of liver as both the marker enzymes remain unaffected **Fig. 6.**

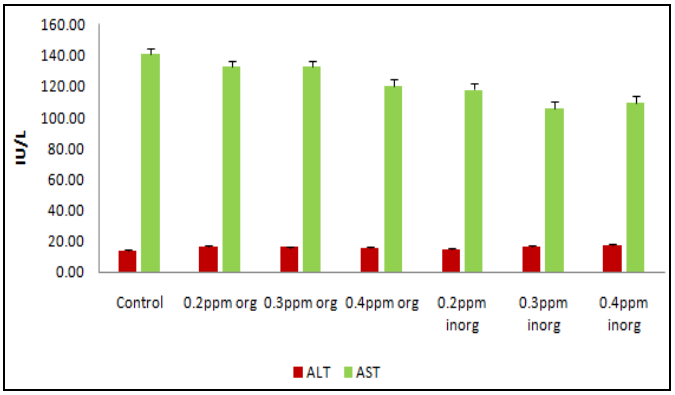

FIG. 6: CHANGES IN THE MARKER ENZYMES LEVELS OF STUDIED EXPERIMENTAL GROUPS

Changes in Immune Response: Since, selenium is important for optimal immune response, it affects both innate and acquired immunity. **Table 6** shows the effect of dietary selenium concentrations on ND vaccine antibodies in commercial broilers. By feeding the diet containing varying concentrations of Se, antibody titres against ND vaccine were not

affected (p>0.05), which may be in part due to the ideal ambient temperature during finisher phase. In order to show their maximum efficiency, antioxidant enzymes need selenium as a cofactor. The gradual improvement of endogenous antioxidant status may be promoted by feeding birds with organic selenium. The results are in consistent with domestic animals [21], humans [22] and broilers [23, 24] fed diets with Se supplementation. Similarly, Zhang *et al.* [25] Zampiga *et al.* [26] Ruff *et al.* [27] Sun *et al.* [28] and Shojadoost *et al.* [29] reported that the selenium supplementation in the chicken diets improved the immunological parameters.

TABLE 6: HUMORAL IMMUNE RESPONSE BY ND TITRE TEST

S. no.	Treatment	ND titre log_2 values
1	Control	4.666^{b}
2	0.2ppm org	6.000^{a}
3	0.3ppm org	5.833^{a}
4	0.4ppm org	5.666^{a}
5	0.2ppm inorg	5.666^{a}
6	0.3ppm inorg	5.500^{a}
7	0.4ppm inorg	5.500^{a}

CONCLUSIONS: Owing to the observations and the results obtained in this study, we conclude that supplementation of azolla-enriched selenium influences the growth, performance and biochemical parameters in a positive way. In performance trials, we observed that 0.2ppm organic selenium significantly impacts body weight gain (P<0.013) and feed conversion rate (P<0.02). Biochemical parameters like albumin (P<0.017), phosphorous (P<0.014) and protein (P<0.013) levels were increased at significant levels. The growth and performance of poultry birds could benefit from Increase in these crucial biochemical parameters. As a result, the present study suggests 0.2ppm organic selenium concentration as a possible supplement to improve Japanese quail growth performance and biochemical parameters.

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CONFLICTS OF INTEREST: The author(s) declares no potential conflict of interest.

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