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Effects of Azolla on Growth, Carcass and Hematological Characteristics in Japanese Quail

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Abstract:

This study was conducted under the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU) Dinajpur to investigate the effects of Azolla on growth, carcass characteristics and hematologic parameters of Japanese quail. A total of 40 fourteen days old quails were assigned into four groups T0, T1, T2 and T3. Group T0 was considered as control, fed only with commercial quail ration. Groups T1, T2, and T3 were supplemented with commercial quail ration with 3%, 5% and 7% Azolla respectively. Live body weight, body weight gain, feed consumption, feed conversion ratio and carcass characteristics were observed and hematologic parameters were determined at the age of 21 days and 42 days of quail. Live body weights were increased significantly ($p<0.01$) in all treated groups in respect to the control and the highest was found in the group T2 supplement with 5% Azolla .Feed consumption were more or less similar among the control and treatment groups. Feed conversion ratio were increased significantly ($p<0.01$) in all treated groups in respect to the control. Dressing percentage, breast and thigh weights were higher among the treatment groups compared to the control group and differed significantly ($p<0.01$). Liver and gizzard weights were also non significantly increased in treatment groups other than control. No significant ($p>0.05$) differences were observed among the groups for Total Erythrocyte Count, Packed Cell Volume, Hemoglobin and Erythrocyte Sedimentation Rate values. But Total Erythrocyte Count, Packed Cell Volume and Hemoglobin values were determined slightly higher in treatment groups compared to the control. Whereas the Erythrocyte Sedimentation Rate values were slightly higher in the control group.The present study reveals that the supplementation of Azolla up to 5% in the quail ration found to be improved performance of quail.

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INTRODUCTION

Bangladesh is highly populated country and growth of population is increasing very fast in comparison to its land size, as a result huge pressure is created on people's basic need. Our national economy mainly depends on agriculture. Being an agricultural country the government of Bangladesh has shifted policy emphasis on poultry rearing. The quail farming has the unique advantage of tapping the growing market demand for poultry products as a supplement of chicken and duck farming (Sultana *et al.* 2007). Nowadays a large number of quail farms have been established in Bangladesh to supply quail meats in hotels, shops, and household consumption as its demand is increasing day by day. Japanese quail is the smallest avian species farmed for meat production (Vali, 2008). The meat from broiler quail is very delicate and tasty. It is considered as a superior item in different restaurant and homes. The broiler quail attains 140-150 gm body weight within 5 weeks of age and yields 72.5 % carcass for consumption (Das, 2004). Success in poultry farming depends on scientific breeding, feeding, management and disease control of the flocks. There is a relationship of Japanese quails (heavy body weight) line to dietary energy levels and graded essential amino acid levels on growth performance and immune competence (Kaur *et al.* 2008). Recently there is an increased emphasis in the use of aquatic plants in poultry rations because the protein and other nutrient content in them are rich The water fern Azolla, grows in association with the blue-green algae, Anabaena Azollae, is considered to be the most promising because of the easiness of cultivation, high productivity and good nutritive value (Singh and Subudhi, 1978). Azolla is one of the plant resources with high biomass and protein production. Azolla pinnata was used as feedstuff in broiler chicken (Querubin *et al.* 1986 and Parthasarathy *et al.* 2002) and laying hens (Khatun, 1996). In view of the above facts, experiments were undertaken to determine the effect of Azolla on growth performance, carcass characteristics and hematologic parameters of quail.

- To know the live body weight, feed consumption and feed conversion ratio.
- To evaluate the dressing percentage, breast weight, thigh weight, liver weight, gizzard weight and heart weight.
- To determine the values of Total Erythrocyte Count, Packed Cell Volume, Hemoglobin and Erythrocyte Sedimentation Rate.

MATERIALS AND METHODS

The experiment was conducted at the poultry research unit under the department of Physiology and Pharmacology, Hajee Mohammad Danesh Science & Technology University, Dinajpur. The duration of experiment was 28 days.

Collection and Management of Quails

At 14 days of age Japanese quails were collected from A. Hakim quail hatchery and farm Gaibandha. The body weights of assigned quails were taken with digital balance and data were recorded. The finally selected 40 quails were housed under normal husbandry condition and divided into 4 groups T_o, T_1, T_2, and T_3 and reared in cage system. Here Group T_0 were kept control. Group T_1, T_2, T_3 were fed 3%, 5% and 7% Azolla supplementation respectively. All of them were fed with commercial crumbled plus mesh feed and fresh water ad-libitum.

Collection of Azolla

Azolla were collected from few ponds located near the Baserhat, Dinajpur .

Experimental Diets

Azolla were dried in the sun and grinded. The grinded Azolla were added with commercial quail ration and served to different groups.

Growth Performance Parameters

Several parameters were studied to observe the effect of Azolla in growth performance of quail:

Recording of Live Body Weight Gain

The body weights of birds were recorded at 7 days interval and the body weight gains were arrived at for each week. The body weight gain was also calculated on a cumulative basis. Accordingly, the body weight gains in different dietary groups were compared.

Estimation of Feed Consumption

The daily feed offered to all individual groups was accurately recorded and at the end of each week and the residual amount of feed was weight and subtracted from the known weight of feed at the beginning of week. The product was divided by the total number of bird.

Calculation of Feed Conversion Ratio

The feed conversion ratio (FCR) expressed as the ratio of amount of feed consumed to the body weight gained under each group of bird.

Carcass Characteristics

At the end of the experiment (42nd day), three birds from each group, were separated and starved for 12 hours however with a provision of plenty of water. Then immediately after recording their live body weights (pre-slaughter bird weight), the birds were slaughtered and the carcasses were subjected for the study of following parameters:

Dressing Percentage

The slaughtered birds were defeathered, denecked and eviscerated along with two legs beneath the hock joint to observe the effect of various experimental diets on the dressing percentage. The dressing percentage was calculated as the percent of the carcass weight obtained after removing the feathers, neck, legs and internal viscera, to its live body weight.

Organometry

From the sacrificed birds, different organs liver, gizzard and heart and the breast meat portion as well as thigh were carefully separated and weighed to observe the effect of different dietary treatments on growth and development of certain organs.

$$\text{Relative weight (g)} \frac{\text{Organ Weight (g)}}{\text{Live Weight of Bird (g)}} = \times\ 100$$

Hematological Parameters:

Blood samples were collected from wing vein of quail of among the control and treated groups at 21th and 42th days to study the effect of the Azolla on Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Hemoglobin Concentrations (Hb) and Erythrocyte Sedimentation Rate (ESR) of quail.

Determination of Total Erythrocyte Count (TEC)

Total erythrocyte count was done following the method described by Lamberg and Rothstein (1977). Well-mixed blood sample was drawn with red blood cell diluting pipette exactly up to 0.5 marks of the pipette. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The free end of the pipette was wrapped around with the rubber tube stretching to both the ends and held with thumb and middle finger. The content of the pipette was mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. Then the counting chamber was placed with special cover glass under microscope using low power (10 x) objectives. After discarding 2 or 3 drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was spared to allow the cells to settle on the chamber under the cover glass. Taking 5 larger squares (4 in the 4 corners and the central one) of the central large square, the cells were counted from all the 80 small squares (16 x 5) under high power objectives (45 x). After completion of counting, the total number of RBC was calculated as number of cells counted x 10, 000 and the result was expressed in million/µl of blood.

Determination of Packed Cell Volume (PCV)

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette). The tip of the pipette was inserted up to the bottom of a clean, dry Wintrobe hematocrit tube. Then the Wintrobe tube was filled from the bottom by pressing the rubber bulb of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming and the tube was filled exactly to the 10 cm mark. Then the Wintrobe hematocrit tube was placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. Then the hematocrit or PCV was recorded by reading the graduation mark; the percent volume occupied by the hematocrit was calculated by using the following formula as described by Lamberg and Rothstein (1977).

$$\text{PCV\%} = \frac{\text{Height of red cell volume in cm}}{\text{Height of total blood in cm}} \times 100$$

Determination of Hemoglobin Concentrations (Hb)

The N/10 hydrochloric acid (HCl) was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematin mixture in the tube by hemolysing red blood cells by the action of HCl. The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in g %. The above procedure was matched by the Hellige hemometer method as described by Lamberg and Rothstein (1977).

Determination of Erythrocyte Sedimentation Rate (ESR)

The fresh anticoagulant blood was taken into the Wintrobe hematocrit tube by using special loading pipette exactly up to 0 marks. Excess blood above the mark was wiped away by sterile cotton. The filled tube was placed vertically undisturbed on the wooden rack for one hour. After one hour the ESR was recorded from the top of the pipette. The result was expressed in mm/in 1st hour.

Statistical Analysis The data were analyzed by analysis of variance using complete randomized design with factorial arrangement of time and treatments (Steel and Torrie, 1986). All analyses were performed by SPSS Program Version 22.

RESULTS AND DISCUSSION

The present investigation was undertaken with a view to study the effect of Azolla supplementation in growth, carcass charactaristics and hematologic parameters of quails. After doing the analysis, the findings of the present investigation was presented and discussed in this chapter under the following headings

Effect of Azolla Supplementation on Growth Performance

Live Body Weight

The observations for live body weight (g) means of T_0, T_1, T_2 and T_3 groups after six weeks of the experimental period were 116.20 ±1.43 g, 123.20 ±1.16 g, 128.20±1.46 g and 119.00 ±1.00 g respectively (Table 1). So quails of Group T_2 got the maximum weight ($p<0.01$) followed by Group T_1 and group T_3 among all of the experimental groups and the control group T_0 got the lowest body weight. The improvement in body weight is similar to the finding of Basak *et al.* (2002) who observed significant ($p<0.01$) improvement in live weight of broiler chicks than control diet when they were fed with 5 percent Azolla meal. Similarly, Naghshi *et al.* (2014) reported chickens fed diets containing 5% Azolla powder significantly ($p<0.01$) improved daily weight gain compared to control .Further, Balaji *et al.* (2009) reported that dietary inclusion of dried Azolla up to 4.5% level did not have any adverse effect on production performance of broiler chicken.

Feed Consumption

The average feed consumption of quails from 14 to 42 days of age in different groups were presented in(Table 1) In the present study average feed consumption were 438 g , 440 g , 440 g and 439 g among the experimental groups T_0, T_1, T_2 and T_3 respectively. The quails of treatment groups T_1, T_2 and T_3 consumed slightly higher than control group T_0. Similarly higher results were obtained by Dhumal *et al.* (2009) who observed feed consumption for groups treated with azolla meal was slightly higher than control (A-983.79, B-1045 and C-1009 g).

Feed Conversion Ratio

The feed conversion ratio of T_0, T_1, T_2 and T_3 groups at 42 days of the experimental period were 4.88 ± 0.10g, 4.56±0.09g, 4.33±0.05 and 4.75±0.08g respectively (Table 1). So quails of Group T_2 got the maximum weight ($p<0.01$) followed by Group T_1 and group T_3 among all of the experimental groups and the control group T_0 got the lowest body weight.This finding was similar to the finding of Basak *et al.* (2002) feed conversion ratio and energy efficiency were significantly ($p< 0.01$) improved in diet with 5% Azolla meal than control diet. Sujatha *et al.* (2013) also reported chickens fed diets containing 5% Azolla powder significantly ($p<0.05$) improved feed conversion ratio compared to control

Carcass Characteristics

Dressing Percentage

The dressing percentage of of T_0, T_1, T_2 and T_3 groups at 42 days age of the experimental quails were 62.78 ± 0.25g, 63.39 ±0.30g, 64.26 ± 0.19g, and 62.95 ± 0.24g respectively (Table 2). So dressing percentage was significantly higher ($p<0.01$) in T_2 compared to the other two treatment groups and control group got the lowest dressing percentage. Similarly Basak *et al.* (2002) and Parthasarathy *et al.* (2002) reported highest dressing percentage in the birds fed with 5 pecent level of Azolla than control.

Breast Weight

The breast weight (g) means of T_0, T_1, T_2 and T_3 groups at 42 days of the experimental period were 19.60 ± 0.45, 22.13 ± 0.26, 25.36 ± 0.94 and 20.89±0.49g respectively (Table 2). So, quails of group T_2 got the highest breast weight ($p<0.01$) followed by group T_1 and group T_3 among all of the experimental groups and the control group T_0 got the lowest body weight.

Thigh Weight

The thigh weight (g) means of T_0, T_1, T_2 and T_3 groups at 42 days of the experimental period were 6.23±0.28 g, 7.37 ±0.40 g, 8.28±0.37g and 6.69±0.02 g respectively (Table 2). So quails of group T_2 got the maximum thigh weight ($p<0.01$) followed by group T_1 and group T_3 among all of the experimental groups and the control group T_0 got the lowest thigh weight. Similarly, Naghshi *et al.* (2014) reported supplementation of 5%. Azolla powder significantly increased ($p<0.05$) thigh weight.

Liver Weight

The liver weight (g) means of T_0, T_1, T_2 and T_3 groups at 42 days of the experimental period were 2.27 ±0.28 g, 2.76±0.23 g, 2.82±0.26 g and 2.51±0.01g respectively (Table 2). So, quails of group T_2 got the maximum liver weight followed by group T_1 and group T_3 among all of the experimental groups and the control group T_0 got the lowest liver weight. But not significantly ($p >0.05$) differed among the groups. Similarly Naghshi *et at.* (2014) reported supplementation of 5%. Azolla powder were not significantly ($p>0.05$) differed liver weight.

Gizzard Weight

The gizzard weight (g) means of T_0, T_1, T_2 and T_3 groups at 42 days of the experimental period were 1.70 ±0.01 g, 1.64 ±0.02 g , 1.81±0.23 g and 1.67±0.00 g respectively (Table 2). So, quails of group T_2 got the highest gizzard weight among all of the experimental groups. But no significant ($p > 0.05$) difference among the groups. Similarly Naghshi *et al*. (2014) reported supplementation of 5%. Azolla powder not significantly ($p>0.05$) differed gizzard weight.

Heart Weight

The heart weight (g) means of T_0, T_1, T_2 and T_3 groups at 42 days of the experimental period were 0.86 g, 0.82 ±0.01 g, 0.78±0.01 g and 0.83 g respectively (Table 2). So, quails of control group T_0 got the highest heart weight and significantly ($p<0.01$) higher among the groups.

Hematologic Parameters

Total Erythrocyte Count (million/ mm^3)

Total erythrocyte count of of T_0, T_1, T_2 and T_3 groups at 21 days were 2.20 ± 0.15, 2.27 ± 0.15, 2.20 ± 0.06 and 2.23 ±0.23 respectively (Table 3) and at 42 days were 2.30 ± 0.10, 2.43 ± 0.25, 2.57 ± 0.40 and 2.40 ± 0.26 respectively (Table 4). The values of TEC in all treated groups and control group were more or less similar and the values were within the normal range. The highest TEC was recorded in group T_2 and lowest in Group T_0 at 42 days of age. Although these values show a little fluctuation they were not statistically significant ($p>0.05$). Similarly Alagbe, (2017) and Alalade *et al.* (2007) reported that the Total Erythrocyte Count were not significantly ($p>0.05$) differed by the dietary inclusion of Azolla in birds but the highest TEC was recorded in treatment groups compared to control group.

Packed Cell Volume (%)

Packed Cell Volume of T_0, T_1, T_2 and T_3 groups at 21 days were 35.10 ± 0.10, 35.13 ± 0.59, 35.33 ± 0.33 and 35.03 ±1.73 respectively (Table 3) and at 42 days were 35.40 ± 0.31, 35.77 ± 0.62, 35.87 ± 0.13 and 35.70 ± 0.30 respectively (Table 4). The values of PCV in all treated groups and control group were more or less similar and the values were within the normal range. The highest PCV was recorded n group T_2 followed by T_1, T_3, and control group T_0 was lowest at 42 days of age. Although these values show a little fluctuation they were not

statistically significant (p>0.05). Similarly Alagbe (2017) and Alalade *et al.* (2007) reported that the Pack Cell Volume (PCV) values were not significantly (P>0.05) differed by the dietary inclusion of Azolla in birds but the highest PCV was recorded in treatment groups compared to control group.

Hemoglobin (g/dl)

Hemoglobin count of T_0, T_1, T_2 and T_3 groups at 21 days were 8.07 ±0.58, 8.10 ± 0.10, 35.33 ± 0.33 and 8.03 ± 0.29 respectively (Table 3) and at 42 days were 8.37 ± 0.32, 8.43 ± 0.09, 8.60 ± 0.25 and 8.07 ± 0.12 respectively (Table 2) . The values of Hb in all treated groups and control group were more or less similar and the values were within the normal range. The highest Hb was recorded in group T_2 than control group T_0 at 42 days of age. All the data were statistically

not significant (p>0.05). Similarly Alagbe, (2017) and Alalade *et al.* (2007) reported that the Hb counts were not significantly (p>0.05) differed by the dietary inclusion of Azolla in birds but the highest Hb was recorded in treatment groups compared to control group.

Erythrocyte Sedimentation Rate (mm/1st hour)

Erythrocyte sedimentation rate of T_0, T_1, T_2 and T_3 groups at 21 days were 10.84 ± 0.65, 10.70 ± 0.30, 10.83 ± 0.60 and 10.67 ± 0.67 respectively (Table 3) and at 42 days were 9.70 ± 0.35, 9.50 ± 0.36, 9.40 ± 0.31 and 9.63 ± 0.35 respectively (Table 4). The values of ESR in all treated groups and control group were more or less similar and the values were within the normal range. The highest ESR was recorded in control group T_0 and lowest in group T_2 at 42 days of age. Although these values show a little fluctuation they were not statistically significant (p>0.05).

CONCLUSION

The present study indicates that Azolla may be used as a growth promoter for Japanese quail up to 5% level. The highest body weight was obtained in T_2 followed by T_0 which differ significantly (p<0.01) from each other .The feed consumption was similar for all treatments all over the experimental period. Cumulative feed consumption for T_0, T_1, T_2, and T_3 were 438g, 440g, 440g and 439g respectively.FCR improved significantly for T_2 and T_1 which were 4.33±0.05 and 4.56±0.09 respectively. FCR for T_3 and T_0 were poorer i.e. 4.75±0.08 and

4.88±0.10. The highest dressing percentage, breast, thigh liver and gizzard were observed for treatment groups than control group. However, further research using large number of birds with similar objectives is needed before giving final recommendation to use Azolla meal as a feed ingredient in quail ration.

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List of tables

Table 1: Live Body Weight of Experimental Quails at 7 Days Interval and Weight Gain, Feed Consumption and Feed Conversion Ratio of Quail from 14 to 42 Days of Age.

Days	T_0	T_1	T_2	T_3	P-value	Level of significance
Day 14 (g)	26.40^{a} ±0.68	26.60^{a} ± 0.68	26.60^{a}± 0.24	26.40^{a} ± 0.87	0.992	**NS**
Day 21 (g)	45.60^{a} ± 1.03	46.80^{a} ± 0.58	47.60^{a} ± 0.75	45.80^{a} ± 0.49	0.236	**NS**
Day 28 (g)	65.60^{a} ± 1.44	68.40^{ab} ± 1.21	71.80^{b} ± 1.46	66.20^{a} ± 1.28	0.021	*
Day 35 (g)	91.80^{a} ± 1.39	96.20^{b} ± 0.86	101.80^{c} ± 1.28	93.00^{ab} ± 1.14	0.000	**
Day 42 (g)	116.20^{a} ± 1.43	123.20^{b} ± 1.16	128.20^{c} ± 1.46	119.00^{a} ± 1.00	0.000	**
Weight gain (g)	89.80^{a} ± 1.83	96.60^{a} ±1.81	101.60^{a} ±1.29	92.60^{a} ± 1.57	.001	**
Feed consumption (g)	438^{a}	440^{a}	440^{a}	439^{a}		
Feed conversion ratio (FCR) g feed consumed/g weight gain	4.88^{a} ± 0.10	4.56^{ab} ± 0.09	4.33^{b} ± 0.05	4.75^{c}± 0.08	.001	**

Different letters in the same row indicate the significant difference

*= Significant at 5% level of probability ($p < 0.05$)

**= Significant at 1% level of probability ($p < 0.01$)

NS= Non-Significant ($P > 0.05$)

Table 2: Dressing Percentage (%), Breast, Thigh, Liver, Gizzard and Heart Weight (g) of quail

Variables	Control	Treatments			P Value	Level of significance
	T_0	T_1	T_2	T_3		
Dressing Percentage (%)	62.78^{a} ±0.25	63.39^{a} ±0.30	64.26^{b} ±0.19	62.95^{a} ± 0.24	0.012	*
Breast Weight(g)	19.60^{a} ±0.45	22.13^{b} ±0.26	25.36^{c} ±0.94	20.89^{ab} ±0.49	0.001	**
Thigh Weight(g)	6.23^{a} ± 0.28	7.37^{b} ± 0.40	8.28^{c}± 0.37	6.69^{ab} ± 0.02	0.007	**
Liver Weight (g)	2.27^{a} ± 0.28	2.76^{a} ± 0.23	2.82^{a}± 0.26	2.51^{a} ± 0.01	0.351	NS
Gizzard Weight(g)	1.70^{a} ± 0.01	1.64^{a} ± 0.02	1.81^{a} ± 0.23	1.67^{a} ± 0.00	0.756	NS
Heart Weight (g)	0.86^{c} ± 0.00	0.82^{b} ± 0.01	0.78^{a} ± 0.01	0.83^{bc} ± 0.00	0.001	**

Different letters in the same row indicate the significant difference

*= Significant at 5% level of probability ($p < 0.05$)

**= Significant at 1% level of probability ($p < 0.01$)

NS= Non-Significant ($P > 0.05$)

Table 3: Hematological Parameters of Quails

Days of post treatment	Treatment		Mean ± SE	Significance value
21st day	TEC (million/mm^3)	T_0	2.20^a ± 0.15	NS
		T_1	2.27^a ± 0.15	
		T_2	2.20^a ± 0.06	
		T_3	2.23^a ±0.23	
	PVC %	T_0	35.10^a ± 0.10	NS
		T_1	35.13^a ± 0.59	
		T_2	35.33^a ± 0.33	
		T_3	35.03^a±1.73	
	Hb(gm/dl)	T_0	8.07^a ±0.58	NS
		T_1	8.10^a ± 0.10	
		T_2	8.07^a ± 0.41	
		T_3	8.03 ^{a}± 0.29	
	ESR (mm in 1st hour)	T_0	10.84^a ± 0.65	NS
		T_1	10.70^a ± 0.30	
		T_2	10.83^a ± 0.60	
		T_3	10.67^a ± 0.67	
42nd day	TEC(million/mm^3)	T_0	2.30^a ± 0.10	NS
		T_1	2.43^a ± 0.25	
		T_2	2.57^a ± 0.40	
		T_3	2.40^a± 0.26	
	PCV %	T_0	35.40^a ± 0.31	NS
		T_1	35.77^a ± 0.62	
		T_2	35.87^a ± 0.13	
		T_3	35.70^a ± 0.30	
	Hb (gm/dl)	T_0	8.37^a ± 0.32	NS
		T_1	8.43^a ± 0.09	
		T_2	8.60^a ± 0.25	
		T_3	8.07^a ± 0.12	
	ESR (mm in 1st hour)	T_0	9.70^a ± 0.35	NS
		T_1	9.50^a ± 0.36	
		T_2	9.40^a ± 0.31	
		T_3	9.63^a ± 0.35	

Same letters in the same column indicate the no significant difference

*= Significant at 5% level of probability ($p < 0.05$)

**= Significant at 1% level of probability ($p < 0.01$)

NS= Non-Significant ($P > 0.05$)

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