Original Article

Potency of Protexin® (Mixed Probiotics) on Hematobiochemical Alteration of Commercial Broiler

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ABSTRACT

This study was carried out to determine the hematological changes (TEC, Hb, PCV) and biochemical changes (AST/SGOT, ALT/SGPT and Ca), in commercial broiler chickens due to the use of Probiotics (protexin). Total 60, day old of vencobb broiler chicks was used for the experiment. The chicks were distributed into four dietary treatments having three replications in each treatment. For hematological examination, 5 ml blood sample was collected from wing vein of each group by and kept in vials containing anticoagulant (sodium-EDTA) and this was done on day of 7, 14, 21, 28 and 35 during experimental period. Biochemical parameters, SGOT and SGPT were determined by the use of the specific test kit and analyzer (3000 evolution) and Ca by R.X Monza analyzer. Probiotic effects were found at 35 days old broilers statistically significant at ($P<0.01$) level of significance for TEC, PCV, Hb, SGOT and Caw here as SGPT was at ($P<0.05$) level of significance. Probiotics perhaps increase the beneficial microorganisms and decreases the pathogenic microorganisms in the gastrointestinal tract.

Keywords: Broiler, Calcium, Hematobiochemical parameters, Protexin®.

INTRODUCTION

Probiotics are viable single or mixed cultures of bacteria, beneficial to the health of the host[20]. They contain naturally occurring microorganisms with a short generation time, rapid colonization ability in the gut that can minimize pathogens by competitive exclusion and are stable at intestinal pH. Moreover, they regulate intestinal microorganisms and improve feed

conversion efficiency. They have also been used as alternative tools for helping to colonize newly-hatched chicks with normal microflora[19]. It has been suggested by many researchers that probiotics are convincing alternatives for antibiotics as therapeutic and growth-promoting agents[6]. Broiler industry is playing a greater role in the agricultural economy. It gives maximum return with reasonable expense. Broiler production is important in Bangladesh to meet up the protein requirement of the people. Poultry meat and eggs contribute approximately 37% of total animal protein in the country[4]. There is a great possibility of growth and expansion of this sector, both in domestic and commercial level. Probiotics-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens[5]. By adding Probiotics in feed or water the intestine is populated with beneficial bacteria, avoiding or decreasing the extent of pathogen colonization. Antibody has a great residual effect in respect of Probiotics, which systemized in poultry body. The efficacy of different Probiotics has been demonstrated in humans, fish and in animals such as poultry. Because antibiotics are being removed from the routine practices of animal husbandry. Probiotics are now being considered as promising tools to fight the pathogens. Probiotics improve the environmental microbiome and resistome of commercial poultry production[18]. Probiotics are specific chemical agents produced by a microorganism containing Lactobacillus, *Lactobacillus casei, Bifidobacterium bifida, Aspergillus oryzae* and *Torulopsis*[17]. Probiotics as a live microbial feed supplement which plays a beneficial role in that improving its intestinal microbial balance[9].

At present, there are many Probiotics available in the market and their indiscriminate use without experimental support is not justified. In assessing the value of Probiotics following characteristics should be taken into consideration. Basically, it should be naturally occurring microorganisms with a short reservation time. It is proved that a multiple species product is better than the single species product. The stability of micro-flora can easily be disturb be by many factors like change in feed, vaccination, intestinal pH, bile salt concentration in the gut and use of antibiotics. Many strains of lactic acid producing bacteria are sensitive to the antibiotics. So, the strains should be resistant to such antibiotics. It must have rapid colonizing abilities and strong foothold in the gut so that it can exclude by stable and have long self-life to withstand in our environmental conditions. One of such products available in our market is Probiotics (protexin®) claimed that has been considered in all these facts. The present study was, therefore, undertaken to know the effects of probiotics (protexin®) on broiler production with the aim to achieve the determination of body weight, TEC, PCV, HB, SGOT, SGPT and Ca.

MATERIALS AND METHODS

The experiment was conducted at the Sahara Poultry Farm, Raynagor, and Sylhet under the supervision of Department of Pharmacology& Toxicology, Sylhet Agricultural University, Sylhet, to study the effects of Probiotics (protexin®) on the hemato--biochemical changes of commercial broiler in Sylhet. The experiment was continued from June 2013 to mid July (35 days).

Layout of the experiment

Total 60, day old of Vencobb commercial broiler chickens was used for the experiment. The chicks were distributed into 4 (four) dietary treatments having 3

(three) replication in each treatment. The layout of the experiment is shown in table 01. The chicks were randomly distributed in a separate pen of battery brooder as per experimental design. The chicks were distributed to 04 dietary treatments having 03 replications in each treatment.

T_0= Control group, i.e. without Probiotics (protexin®) in drinking water.

T_1= 1.0 gm Probiotics (protexin®) per 05 liters drinking water.

T_2= 2.0 gm Probiotics (protexin®) per 05 liters drinking water.

T_3= 4.0 gm Probiotics (protexin®) per 05 liters drinking water.

Hematological examination

Using sterile syringe and needle maintaining aseptic condition, 5 ml blood sample was collected from wing vein of each group by and kept in vial containing anticoagulant (sodium-EDTA) and this was done on day of 7,14,21,28 and 35during experimental period. Hematological studies performed following the methods described by[11,7] and for the Total Erythrocyte Count and Hemoglobin and by[16] for Packed Cell Volume.

Biochemical examination

Biochemical parameters such as SGOT or AST and SGPT or ALT were determined by the use of the specific test kit and analyzer (3000 evolution).

Determination of Ca (Calcium)

For the quantitative *in vitro* determinate of calcium in serum. This product in suitable for manual use and the R.X Monza analyzer.

Statistical analysis of experimental data

Data obtained from the experiment were calculated and expressed as Mean ± SE on hematological parameters (e.g. TEC, Hb concentration, PCV and biochemical parameters (e.g. SGOT or AST, SGPT or ALT and Ca were analyzed statistically using students paired T-test following the standard methods by Khan[14]. Statistical analysis of data was performed using the MS-STAT statistical software[3] and DMRT were also done for ranging[10].

RESULTS AND DISCUSSION

The experiment was carried out to evaluate the hemato--biochemical changes following the administration of Probiotics (protexin®) as a different does as in commercial broiler chickens. To observe the effects on PCV, HB, TEC, AST/SGOT, ALT/SGPT and Calcium parameters. **:** Probiotics effects were found at 35 days old broiler statistically significant at ($P<0.01$) level of significant for TEC, PCV, Hb, SGOT and Ca where as SGPT was at ($P<0.05$) level of significant.

Effects of Probiotics on Total Erythrocytes Count (TEC) (Million/mm^3)

Effects of daily feeding Probiotics (Protexin®) in different doses on Total Erythrocytes Count (TEC) of broiler chicken which are present in table 02.

Figure with a similar superscripts mean, did not differ significantly among respective groups. A figure with dissimilar subscript men differed significantly among the respective group as per DMRT.

In the 14 days of the experiment, it was observed that the Total Erythrocytes Count of broiler chickens was statistically also insignificant ($p>0.05$). In the 21 days of the experiment the total erythrocyte count was statistically significant at ($p<0.05$) level of significance. After the age of 28 days of experiment of the Total Erythrocytes Count was statistically significant at ($p<0.01$) level of significance.

In the control group T_0 was 3.86 ± 0.057 million/mm^3 and treated group

T_1 was 4.12±0.057million/mm^3, in group T_2 was 3.8±0.058 million/mm^3.Finally at 35 days of experiment in control group T_0 was recorded 3.93±0.057 million/mm^3 and treated group T_1 was recorded 4.26±0.058 million/mm^3. The Total Erythrocytes Count was statistically significant at ($p<0.01$) level of significance.

Effects of Probiotics on Packed Cell Volume (%)

Effects of daily feeding Probiotics (Protexin®) in different doses on Packed Cell Volume (PCV) of broiler chicken which are presented in table 03.

Figure with a similar superscripts mean, did not differ significantly among respective groups. A figure with dissimilar subscript men differed significantly among the respective group as per DMRT.

At initial 7 day all data on Packed Cell Volume (PCV) were also not statistically significant ($p>0.05$). At 14 day all data of Packed Cell Volume (PCV) were also not statistically significant ($p>0.05$). After 21 days of the experiment the Packed Cell Volume (PCV) was statistically significant at ($p<0.05$) level of significance. After the age of 28 days of the experiment of Packed Cell Volume (PCV) were statistically significant at ($p<0.05$) level of significance. In the control group T_0 was 28.66±0.058% and treated group T_1 was 30.66±0.058%. The highest data was recorded T_1 group. Finally, at 35 days of experiment in control group Packed Cell Volume (PCV) T_0 were recorded in the control group T_0 were 30±0.58% and treated group T_1 was recorded 32±1%, in group T_2 was recorded 29.67±0.58% and T_3 was recorded 29.3±0.58%. PCV was statistically significant at ($p<0.01$) level of significance. All the data were statistically significant at ($p<0.01$) level of significant on 35 days. The Packed Cell Volume (PCV) level increased on the 35 days in treating T_1 group. The highest record count in T_1 group.

Similar to present finding, increasing hemato--biochemical parameters by Probiotics has been reported by many authors. TEC, Hb and PCV values were significant ($p<0.01$) increased in Probiotics treated poultry (broiler)[8,15]. Observed significantly ($p<0.01$) increased TEC, Hb& PCV in Probiotics treated broiler chickens[13]. Also reported a significant ($p<0.01$) increasing in TEC, Hb and PCV values and significant ($P<0.01$) was observed in Probiotics treated broiler chickens. The cause of change in hematobiological values might be due to the action of Probiotics on hematopoetic system which is responsible for such alterations in hematological parameters.

Effects of Probiotics on blood hemoglobin of broiler chicken

Effects of daily feeding probiotics (Protexin®) in different doses on blood hemoglobin of broiler chicken which are presented in table 04.

Figure with a similar superscripts mean, did not differ significantly among respective groups. A figure with dissimilar subscript men differed significantly among the respective group as per DMRT.

On the initial 14 days of the experiment, it was observed that the blood hemoglobin of broiler chickens in control group T_0 was 8.5±0gm% and treated group T_1 was 8.66±0.058gm%. All the data were also statistically insignificant ($p>0.05$). In the 21 days of the experiment the hemoglobin was statistically significant at ($p<0.05$) level of significance. After the age of 28 days of experiment of blood

hemoglobin was statistically significant at ($p<0.01$) level of significance. In the control group T_0 was $8.867^{\pm}0.058$gm% and treated group T_1 was $9.6^{\pm}1$gm%. Finally, at 35 days of experiment in control group T_0 was recorded $9.2^{\pm}0.57$gm% and treated group T_1 was recorded $9.86^{\pm}0.58$gm%, in group T_2 was recorded $9.5^{\pm}0$gm%. Blood hemoglobin was statistically significant at ($p<0.01$) level of significance. All the data were statistically significant at ($p<0.01$) level of significance. The hemoglobin level increased in the 35 days in treating T_1 group the height record count in T_1 group.

Effects of Probiotics on SGOT/ AST (IU/L)

Effects of daily feeding Probiotics (Protexin®) in different doses on SGOT (IU/L) /AST of broiler chicken which are presented in table 05.

Figure with a similar superscripts mean, did not differ significantly among respective groups. A figure with dissimilar subscript men differed significantly among the respective group as per DMRT.

At initial 7 days and 14 days of the experiment all data of SGOT/AST were not statistically also significant ($p>0.05$). After 21 days of the experiment the SGOT/AST was statistically significant at ($p<0.01$) level of significant. In the control group T_0 was $29.46^{\pm}1.5$ IU/Land treated group T_1 was $27.7^{\pm 0.01}$ IU/L. After the age of 28 days of the experiment of SGOT/AST were statistically significant at ($p<0.05$) level of significant. In the control group T_0 was $30.3^{\pm}0.1$ IU/L and treated group T_1 was $26.57^{\pm}0.32$IU/L. The SGOT/AST lowest (26.57 IU/L) decreased in treating group T_1 due to 1gm Probiotic supplementation with 5 liter water. Finally, at 35 days of experiment in control group SGOT/AST T_0 were recorded in the control group T_0 were $31.4^{\pm}0.1$ IU/L and treated group T_1 was recorded $24.27^{\pm 0.057}$ IU/L. AST/SGOT was statistically significant at ($p<0.01$) level of significance. All the data were statistically significant at ($p<0.01$) level of significant on 35 days. The SGOT/AST level decreased on the 35 days in treating T_1 group. The lowest data record in T_1 group. The SGOT/AST lowest (24.27 IU/L) decreased in treating group T_1 due to 1gm Probiotic supplementation with 5 liter water.

Effects of Probiotics on SGPT/ALT (IU/L)

Effects of daily feeding Probiotics (Protexin®) in different doses on SGPT/ALT (IU/L) of broiler chicken which are presented in table 06.

Figure with a similar superscripts mean, did not differ significantly among respective groups. A figure with dissimilar subscript men differed significantly among the respective group as per DMRT.

The SGPT/ALT were statistically significant at ($p<0.01$) level of significant on 21 days of age. After the age of 28 days of the experiment of SGPT/ALT were statistically significant at ($p<0.01$) level of significance. In the control group T_0 was $25.47^{\pm}0.208$ IU/L and treated group T_1 was $22^{\pm}0.17$ IU/L. The lowest SGPT/ALT was recorded in the treated group. The SGPT/ALT lowest (22 IU/L) decreased in treating group T_1 due to 1gm Probiotic supplementation with 5 liter water. Finally, at 35 days of experiment in control group SGPT/ALT T_0 were recorded in the control group T_0 were $28.31^{\pm}0.057$ IU/L and treated group T_1 was recorded $20.86^{\pm}0.208$ IU/L, in group T_2 was recorded $22.13^{\pm}0.057$ IU/L. ALT/SGPT was statistically significant at ($p<0.01$) level of significance. Similar to present findings,

reduction of biochemical parameters by Probiotics has been reported by many authors[9]. Found that SGPT/ALT & SGOT/AST values were significant ($p<0.01$) reduced in Probiotics treated broiler chickens[2]. Observed significantly ($p<0.01$) decreased SGPT/ALT & SGOT/AST in Probiotics treated broiler chickens. The cause of the change in biochemical values might be due to the action of Probiotics in increasing liver functioning of broiler chickens.

Effects of Probiotics on Calcium (Ca) (mg/dL)

Effects of daily feeding Probiotics (Protexin®) in different doses of Calcium (Ca) (mg/dL) of broiler chicken which are present in table07.

Figure with a similar superscripts mean, did not differ significantly among respective groups. A figure with dissimilar subscript men differed significantly among the respective group as per DMRT.

At initial 7 days and 14 days of the experiment all data of Ca (Calcium) were also not statistically significant ($p>0.05$).The Ca (Calcium) were statistically significant at ($p<0.05$) level of significant on 21 days of age. After the age of 28 days of the experiment of a (Calcium) were statistically significant at ($p<0.01$) level of significance. In the control group T_0 was $8.63^{\pm}0.058$mg/dL and treated group T_1 was $9.266^{\pm}0.058$ mg/dL. Finally, at 35 days of experiment in control group Ca (Calcium) T_0 were recorded in the control group T_0 were $8.86^{\pm}0.0575$ mg/dL and treated group T_1 was recorded $10.03^{\pm}0.1147$ mg/dL. Ca (Calcium) gradually increases due to T_1 due to 1gm Probiotic supplementation with 5 liter water. The average Ca (Calcium) of all treated groups was statistically significant ($p<0.01$) than the control group. The highest Ca (Calcium) was recorded in group T_1 (10.03mg/dL). Data cataloged on 7,14,21,28 and 35 days of age showed that Ca (Calcium) increased in the treated group T_1. The highest Ca (Calcium) was recorded in the treated group T_1 due to 1 GM Probiotic supplementation with 5 liter water.

Similar to present finding, increase of calcium (Ca) parameters by Probiotics has been reported by many authors[12]. Observed significantly ($p<0.01$) increased Calcium (Ca) in broiler chickens[1]. Observed that a significant increase of Calcium (Ca) in Probiotics treated broiler chickens.

CONCLUSION

It may be summarized from the present study that Probiotics can play a positive role in improving the blood function as well as liver function following administration of Probiotics (protexin). The present research work was conducted to find out the mechanism of action of Probiotics that have beneficial effects to the commercial broiler birds. The results provided that Probiotics (Protexin) significantly influence PCV, HB, TEC, SGOT, SGPT and Calcium of commercial broiler chickens. Probiotics perhaps increase the beneficial microorganisms and decreases the pathogenic microorganisms in the gastrointestinal tract. On the basis of the results it may be summarized that Probiotics (Protexin) supplementation with drinking water significantly PCV, HB, TEC, SGOT, SGPT and Calcium of commercial broiler chickens.

ACKNOWLEDGEMENT

The author is highly grateful to the personnel of the Sahara poultry farm and Department of Pharmacology & Toxicology for their valuable assisting in conducting such survey.

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Table 1. Layout of the experiment

Dietary treatment	Age of bird (day)	No. of chicks in each replication			Total number of birds on each treatment
		R_1	R_2	R_3	
T_0	7	5	5	5	15
T_1	7	5	5	5	15
T_2	7	5	5	5	15
T_3	7	5	5	5	15

Table 2. Effects of daily feeding Probiotics (Protexin®) in different doses on Total Erythrocytes Count (TEC)

Treatment	TEC (Million/mm^3)				
	Initial 7 days	After 14 days	After 21 days	After 28 days	After 35 days
T_0 (Control)	$3^{\pm}0.1$	$3.5^{\pm}0.1$	$3.6^{a\pm}0.1$	$3.86^{a\pm}.057$	$3.933^{ab\pm}0.057$
T_1 (1gm/5L water)	$3^{\pm}0.1$	$3.6^{\pm}0.1$	$3.8^{b\pm}0.1$	$4.12^{b\pm}.057$	$4.26^{a\pm}0.058$
T_2 (2gm/5L water)	$3^{\pm}0.1$	$3.5^{\pm}0$	$3.63^{ab\pm}.0578$	$3.8^{a\pm}0.058$	$3.86^{ab\pm}0.058$
T_3 (4gm/5L water)	$3^{\pm}0.1$	$3.33^{\pm}0.058$	$3.5^{a\pm}.057$	$3.6^{ab\pm}.01$	$3.72^{a\pm}0.058$
Level of significance	NS	NS	*	**	**

The values are expressed as meant $^{\pm}$ SE of 5 chickens in each group.
** Significant at (P<0.01). * Significant at (P<0.05). NS= Non significant.

Table 3. Effects of daily feeding Probiotics (Protexin®) in different doses on Packed Cell Volume (PCV)

Treatment	PCV (%)				
	Initial 7 days	After 14 days	After 21 days	After 28 days	After 35 days
T_0 (Control)	$25.66^{\pm}0.5735$	$27^{\pm}0$	$28.33^{b\pm}0.57$	$28.66^{ab\pm}0.58$	$30^{ab\pm}0.58$
T_1 (1gm/5L water)	$26.0^{\pm}0$	$27.66^{\pm}.58$	$29.0^{a\pm}0.58$	$30.66^{a\pm}0.58$	$32^{b\pm}1$
T_2 (2gm/5L water)	$25.66^{\pm}0.57$	$26.66^{\pm}0.58$	$28^{b\pm}1$	$28^{ab\pm}1$	$29.67^{a\pm}0.58$
T_3 (4gm/5L water)	$25.66^{\pm}.55$	$26^{\pm}1$	$28^{b\pm}1$	$28.33^{ab\pm}0.58$	$29.3^{ab\pm}.58$
Level of significance	NS	NS	**	*	**

The values are expressed as meant $^{\pm}$ SE of 5 chickens in each group.
** Significant at (P<0.01). * Significant at (P<0.05). NS= Non significant.

Table 4. Effects of daily feeding Probiotics (Protexin®) in different doses on blood hemoglobin of broiler chicken

Treatment	Hemoglobin (gm %)				
	Initial 7 days	After 14 days	After 21 days	After 28 days	After 35 days
T_0 (Control)	$8.467^{\pm}.057$	$8.5^{\pm}0$	$8.76^{ab\pm}.058$	$8.867^{ab\pm}0.058$	$9.2^{ab\pm}0.57$
T_1 (1gm/5L water)	$8.6^{\pm}0$	$8.66^{\pm}0.058$	$8.86^{b\pm}0.057$	$8.96^{a\pm}0.057$	$9.86^{a\pm}0.058$
T_2 (2gm/5L water)	$8.6^{\pm}0.057$	$8.7^{\pm}0$	$8.8^{a\pm}.01$	$9^{b\pm}0.1$	$9.5^{ab\pm}0$
T_3 (4gm/5L water)	$8.6^{\pm}.057$	$8.6^{\pm}0$	$8.8^{a\pm}0.1$	$8.86^{ab\pm}0.577$	$9.13^{a\pm}0.057$
Level of significance	NS	NS	*	**	**

The values are expressed as meant $^{\pm}$ SE of 5 chickens in each group.
** Significant at (P<0.01). * Significant at (P<0.05). NS= Non significant.

Table 5. Effects of daily feeding probiotics (Protexin®) in different doses on SGOT (IU/L) /AST

	Initial 7 days	After 14 days	After 21 days	After 28 days	After 35 days
T_0 (Control)	$28.27^{\pm}.057$	$28.27^{\pm}0.1$	$29.46^{b\pm}1.5$	$30.3^{b\pm}0.1$	$31.4^{a\pm}0.1$
T_1 (1gm/5L water)	$28.27^{\pm}0.058$	$28.006^{\pm}.0058$	$27.7^{a\pm}0.1$	$26.57^{ab\pm}0.32$	$24.27^{b\pm}0.057$
T_2 (2gm/5L water)	$28.24^{\pm}0.058$	$28.01^{\pm}0$	$28.01^{b\pm}0.1$	$28.866^{b\pm}0.577$	$28.84^{ab\pm}0.0578$
T_3 (4gm/5L water)	$28.24^{\pm}0$	$28.5^{\pm}0$	$28.6^{ab\pm}0.1$	$29.43^{a\pm}0.208$	$29.86^{b\pm}0.058$
Level of significance	NS	NS	**	*	**

The values are expressed as meant $^{\pm}$ SE of 5 chickens in each group.
** Significant at (P<0.01). * Significant at (P<0.05). NS= Non significant.

Table 6. Effects of daily feeding Probiotics (Protexin®) in different doses on SGPT/ALT (IU/L)

Treatment	SGPT/ALT (IU/L)				
	Initial 7 days	After 14 days	After 21 days	After 28 days	After 35 days
T_0 (Control)	$23.6^{\pm}0.1$	$23.74^{\pm}0.58$	$24.57^{a\pm}.0577$	$25.47^{ab\pm}.208$	$28.31^{ab\pm}.057$
T_1 (1gm/5L water)	$23.57^{\pm}.058$	$23.14^{\pm}.058$	$22.24^{b\pm}.053$	$22^{a\pm}0.17$	$20.86^{a\pm}.208$
T_2 (2gm/5L water)	$23.53^{\pm}.057$	$23.26^{\pm}0.11$	$23.3^{a\pm}0.1$	$23.3^{ab\pm}0.2$	$22.13^{a\pm}.057$
T_3 (4gm/5L water)	$23.466^{\pm}.152$	$23.53^{\pm}.208$	$24.54^{a\pm}0.208$	$24.37^{b\pm}0.208$	$26.34^{b\pm}0.057$
Level of significance	NS	NS	**	**	*

The values are expressed as meant $^{\pm}$ SE of 5 chickens in each group.
** Significant at (P<0.01). * Significant at (P<0.05). NS= Non significant.

Table 7. Effects of daily feeding Probiotics (Protexin®) in different doses of Calcium (Ca) (mg/dL)

Treatment	Ca (mg/dL)				
	Initial 7 days	After 14 days	After 21 days	After 28 days	After 35 days
T_0 (Control)	$8.1^{}\pm0$	$8.16^{}\pm0.058$	$8.33^{a}\pm0.056$	$8.63^{a}\pm.058$	$8.866^{a}\pm.0575$
T_1 (1gm/5L water)	$8.1^{}\pm0$	$8.36^{}\pm0.058$	$8.86^{b}\pm0.057$	$9.266^{b}\pm0.58$	$10.03^{a}\pm.1147$
T_2 (2gm/5L water)	$8.1^{}\pm0$	$8.23^{}\pm0.058$	$8.43^{b}\pm0.058$	$8.9^{ab}\pm0.1$	$8.93^{ab}\pm0.057$
T_3 (4gm/5L water)	$8.13^{}\pm0.058$	$8.16^{}\pm0.057$	$8.33^{a}\pm0.056$	$8.4^{ab}\pm0$	$8.56^{a}\pm0.057$
Level of significance	NS	NS	*	**	**

The values are expressed as meant ± SE of 5 chickens in each group.
** Significant at (P<0.01). * Significant at (P<0.05). NS= Non significant.