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IMPACT OF DIETARY CHITOSAN ON GROWTH PERFORMANCE AND BLOOD BIOCHEMICAL PROFILE OF BROILERS

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Abstract
In Pakistan, the poultry sector is playing an important role in bridging the gap between the supply and demand for protein. Feed additives are mainly used for improving poultry health and production worldwide. Chitosan is used as a feed additive due to its biological properties such as biocompatibility, antimicrobial, anti-inflammatory activities. The objective of this experiment aimed to examine the effects of dietary chitosan in broiler on growth performance and hematological parameters. One-day old unsexed broiler chicks (Ross-308) with an average initial weight (average 45 ±0.4g body weight) was allotted into three groups. The first group was fed the basal diet and served as the control; the experimental groups 1 and 2 were fed on basal diet supplemented with chitosan at 0.75 and 1.5g/kg diet respectively. The growth performance of broilers was measured in terms of feed intake, body weight gain and feed conversion ratio calculated by pen basis on day 21, 42, and 1-42. The results showed that experimental group 2 supplemented with 1.5g/kg dietary chitosan significantly ($P \leq 0.001$) improved the feed conversion ratio. The blood samples for serological and hematological profiles were collected on day 42 and the hematological analysis revealed significant ($P \leq 0.001$) increase in the total RBC, hemoglobin, the platelet, HCT, MCH, MCHC and WBC count in experimental groups as compared to control group. The analysis of lipid profile also determined that there is a significant ($P \leq 0.001$) lowering of cholesterol, triglycerides and LDL occurs in experimental groups relative to control group. The levels of bilirubin, ALT, Alkaline Phosphate, Urea, Creatinine, Sodium, and Potassium remain unchanged ($P \geq 0.05$). It is concluded that chitosan may be used as feed additive as it improves the growth performance and hematological indices of broilers.

Introduction
The production of broilers has emerged as the most important source of supplemental protein for human diets all over the world. It is widely acknowledged that the expansion of poultry farming is one of the most important factors in ensuring the nation's continued access to a sufficient food supply. Additionally, the poultry industry plays an unrivaled role in supplying the general population with poultry meat and egg products that are high in the nutrients proteins and vitamins. The climate, which is dry and warm, is ideal for the keeping and rising of poultry (Holbayevich, 2023).

In order to reduce toxicity and dangerous effects, poultry breeders and researchers are working to develop feed formulations that have potential antibiotic alternatives or at the very least, adopt an integrated approach (Janardhana *et al.,* 2009; Nazir *et al.,* 2020). Due to their benefits for both growth and health, amino acids and prebiotics have been widely adopted as alternatives to antibiotics in poultry diets for this purpose (Shi *et al.,* 2005).

The poultry industry's technological advances in the application of modern nutrition programs and the use of genetic improvement programs allow some lines to express their full genetic potential. In addition to this, it has resulted in a generation that has rapid growth and low disease resistance which has led to an excessive use of antibiotics and medicinal drugs in an effort to lower the rate of disease and mortality (Swiatkiewicz *et al*., 2014). In the majority of the countries of the European Union and the USA, the use of antibiotics as growth promoters in poultry diets has been outlawed causing the use of alternative methods to improve immunity and lessen bacterial and fungal disease infection (Soltan *et al*., 2008).

It has been explored that adding chitosan to poultry diets as a non-food additive, which is a substance derived from chitin and is not harmful to human or animal health, and which makes up the majority of the external skeleton of marine organisms like shrimp and crabs, has a positive impact on broiler chicken productivity performance (Qin *et al*., 2006; Jasim *et al*., 2021).

Prebiotics are required for probiotics to survive in the gut more effectively. With the aid of prebiotics, probiotics can thrive in the digestive system because they are better able to withstand anaerobic conditions like low pH low temperature, and low oxygen. The probiotics that function as symbiotic organisms in the lower gut use the prebiotics as substrates for their survival and growth (Nyamagonda *et al*., 2011).Chitosan is derived from chitin, a polysaccharide found in marine diatoms, insects, fungi, crustaceans and algae by deacetylation, demineralization, deproteinization, and discoloration (Keser *et al.,* 2012).

Chitosan is a biopolymer derived from the alkaline deacetylation of chitin from shrimp wastes and fungal biomass (Darwesh *et al*., 2018). Chitosan is a secure linear polysaccharide made up of N-acetyl-D-glucosamine and -1-4 linked D-glucosamine units. Chitosan, also known as chitin, the second-most common natural polysaccharide after cellulose, degrades into COS (Zhou *et al*., 2009; Lan *et al*., 2020).

Chitosan is useful in a variety of situations because it is nontoxic, biodegradable, and antibacterial. These situations include biomedical research, agriculture, genetic engineering, the food industry, pollution control and water treatment (Cheba, 2011). COS (chitosan oligomers, or chito-oligomers), which are polymer chains with an average molecular weight (MW) of not more than 3.9 kDa, contain about 20 monomer units per chain(Lodhi *et al*., 2014). In comparison to other precursor species, COS's favourable molecular weight, better solubility, and lower viscosity significantly increase interest, which ultimately has a positive impact on commercial production (Guan *et al*., 2019).

However, according to a different study chitosan has a detrimental effect on growth performance (Zhang *et al*. 2008). There is still debate regarding how dietary chitosan affects grill chicken growth performance. Chitosan has been viewed as an ingredient with multiple uses, such as acting as an antimicrobial agent against food borne pathogens (Kong *et al*. 2010). Actually, the broilers fed chitosan grew faster than the controls. Because of increased nitrogen utilization and amino acid digestibility, dietary chitosan at low concentrations of 0.5-1 g/kg tended to improve growth rate (Shi *et al*., 2005).

Materials and Methods

3.1. Study Area

The present study was conducted within the animal house located in the Department of Zoology at Lahore College for Women University, Lahore, following to internationally accepted standards for the well-being and ethical treatment of bird species, particularly chickens. The experimental protocol followed to relevant guidelines and regulations regarding the utilization of animals for studies. The research conducted in this study received ethical approval from the Research Board/Committee at Lahore College for Women University in Lahore.

3.2. Birds Housing and Environment

The experimental birds were kept in three-tiered separate cages with 0.7 m^2 of floor space each, measuring 10 ft. long, 3 ft. wide, and 3 ft. in height, equipped with chicken feeders and drinkers, in a well-ventilated solid-walled poultry facility with a lighting schedule of 20 hr. light and 4 hr. darkness, in accordance with standard poultry practices. Throughout the investigation, the birds were physically examined and vaccinated as required.

3.3. Rearing management

The rearing system of broilers is a crucial factor influencing their growth, health and efficiency. Therefore, it is essential that the system is appropriate and encounters the necessary standards to achieve the desired outcomes. A group of newly hatched broiler chickens (Ross 308), aged one day, and exhibiting comparable body weight across both sexes, were purchased from Olympia Hatchery located in Sheikhupura. These birds were weighed individually, with an average body weight of 45 + 0.4g, and then randomly assigned to the three treatment groups A, B, and C using a completely randomized methodology. Each treatment group consisted of 15 chicks, with one replicate per group. The chicks in each replicate were housed in separate pens before the experiment began. The houses went through an extensive disinfection and fumigation process before arrival of chicks. Fumigation was carried out applying potassium permanganate ($KMnO_4$) and formaldehyde (HCHO).

A layer of sawdust measuring 3 to 4 inches in thickness was employed as litter with each pen and it was regularly stirred to maintain dryness and ventilation. Feed and water resources were made available in an ad libitum manner.

The chicks were vaccinated against Newcastle disease, infectious bronchitis, infectious bursal disease, and Hydro pericardium syndrome according to the prescribed schedule in Pakistan. All the prescribed protocols for the rearing of broilers were diligently adhered to during the entire duration of the experiment. Meteorological data, that includes the highest and lowest daily ambient temperature, morning and afternoon relative humidity, during the experimental timeframe. The conditions of the neutral thermal temperature and optimal relative humidity were carefully maintained. Regular cleaning and maintenance of proper sanitation protocols were also implemented to lessen the risk of disease outbreaks and optimize the attainment of maximum weight gain. The study was carried out during the months of February and March, spanning duration of 42 days. The experiment was conducted under conditions of an average temperature of 31± 2ºC and a relative humidity of 66.8.

3.4. Experimental Designs and Dietary treatments

The chickens were provided with a basal diet consisting primarily of corn-soybean meal as a prominent component. Chitosan serves as the experimental dietary component in the current investigation. Chitosan is utilized in a crushed state. The degree of deacetylation of the specimen was estimated as 85%, while its molecular weight was measured to be 500,000 Daltons. Additionally, the particle size of the specimen was observed to be 60 mesh. The experimental design employed was that of complete randomization, wherein newly hatched broiler chickens were allocated at random to three distinct experimental groups, each consisting of 15 individuals, with one replication for each treatment. Each of the experimental groups was provided with basal diets during both the starter and finisher phases, in accordance with the recommended nutrient specifications as shown in **table 1**.

The chickens were divided into two main groups. i.e. control group and experimental group.

3.4.1. Control group

The control group remained untreated throughout the experiment receiving diets without supplementation.

Experimental group is further divided into two subgroups.

3.4.2. Experimental group I
The experimental group I received dietary supplementation of 0.75g chitosan per kg feed.

3.4.3. Experimental group II
The experimental group II received dietary supplementation of 1.5g chitosan per kg feed.

Table 3.1: Basal feed composition of broilers, starter and finisher diets

Ingredients (% diet)	Starter Diet (7-21 day)	Finisher Diet (22-42 day)
Maize	59.00	58.00
Soya bean (Full fat)	-	9.58
Soya bean oil	2.00	2.55
Fish Meal	2.15	-
Wheat bran	-	5.08
Salt (NaCl)	0.15	0.15
Limestone	1.50	1.50
Dicalcium phosphate	1.50	1.50
Lysine	0.13	0.13
Methionine	0.19	0.19
Vitamin & Mineral premix*	0.20	0.20
Total	100	100
Calculated composition		
Protein (%)	21.56	19.31
ME (MJ/Kg)	12.97	13.38
Calcium	1.02	0.94
Phosphorus (%)	0.48	0.42

3.5. Growth Performance and Feed Intake (Body Weight Gain, Feed Conversion Ratio)
The body weights of broilers were measured at the initiation and completion of each phase (10, 24, 42 days) in order to determine the rate of body weight gain (BWG). Feed intake (FI) and feed conversion ratio (FCR) need to be determined on a per-pen basis utilizing data obtained from feed residual and body weight gain (BWG) at the completion of each phase (10, 24, 42 days) and for the entire experimental duration (1-42 days). Each day, the rate of mortality was checked, and any dead birds were weighed to correct the data for the duration of the experiment.

The primary goals of poultry production are to keep broilers healthy, increase productivity, and improve meat quality. In this study, broiler chickens exhibit improved body weight. The initial weight measurement was recorded at the onset of the experiment, followed by subsequent weekly weight measurements. Weight gain was determined by calculating a difference between the initial and final weights of the subjects on a weekly basis. The feed intake of each group was assessed on a weekly basis by comparing the quantity of food remaining at the end of the week with the quantity of food initially provided at the beginning of the week. On a weekly basis, the feed conversion ratio (FCR) was determined by employing the following formula:

Feed conversion ratio = Feed consumed (g) / weight gain (g)

3.6. Hematological evaluation (Blood Samples)
At the age of 42 days, two broiler chicks per experimental group was randomly chosen and subsequently subjected to blood sample collection. This was achieved by utilizing sterile needles to extract blood from either the wing vein or jugular vein. Whole blood samples, with the anticoagulant EDTA, will be subjected to analysis in order to determine the hematological parameters and serum indices. Prior to blood collection, chickens fasted over for 12-hour period. Following the collection

process, a centrifugation step was performed at a relative centrifugal force (RCF) of 1050g for duration of 10 minutes. The blood serum shall be carefully transferred into Eppendorf tubes and subsequently preserved at a temperature of -20 °C until the time of analysis.
The lipid, protein, glucose, and liver enzyme levels in each individual's samples was subjected to analysis. Glucose, total protein (TP), albumin, and globulin were quantified utilizing commercially available kits employing an automated chemistry analyzer. The automatic analyzer will be utilized to analyze the levels of total cholesterol (Tch), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine in the blood serum.

3.7. Serum Parameters

Before the termination of the experiment on day 42, blood samples were collected from the avian subjects. Specifically, four birds per replicate were selected for this procedure. The blood samples were obtained either from the wing vein or the jugular vein, using serum vacutainers that contained gel as a clotting activator. Following the process of coagulation, the serums were isolated through the utilization of centrifugal force at a speed of 4500 revolutions per minute for duration of 15 minutes. The sera samples were carefully divided into duplicate Eppendorf tubes to facilitate subsequent biochemical and serological analyses.

3.8. Slaughtering Procedure

On day 42, the birds were exposed to a 6-hour overnight fast in preparation for slaughter. At the conclusion of the grower phase on day 24 and the finisher phase on day 42, four avian specimens with body weights closely resembling the average weight of the group were randomly chosen for the purpose of slaughter, following the Islamic method. Finally, the carcasses were dissected thoroughly for further sampling and analysis.

3.9. Organ Weight Relative to Body Mass

Following the dissection, the vital organs, namely the liver, heart, gizzard, and spleen, were thoroughly cleansed and subsequently measured to facilitate statistical examination within the control and experimental groups. Because the weight of vital organs also indicates the health and performance of organisms.

3.10. Statistical Analysis

Growth performance of broiler chickens was calculated at each observation period by Mean ± SEM. One-way Analysis of Variance (ANOVA) was performed on the data for statistical analysis of growth performance, hematological parameters and serum indices of broilers. If the P-value achieved was less than 0.05, changes were considered significant. Microsoft Excel and Graphpad Prism Software (version 5) was used for statistical analysis. Bar graphs were drawn on excel for demonstration of data.

Results

4.1. Statistical analysis of growth performance of chickens:

The growth performance of broilers was measured in terms of Average Daily Gain (ADG), Average Daily Feed Intake (AFI) and Feed Conversion Ratio (FCR) on day 1-21, day 21-42 and day 1-42 collectively**.** Data was expressed as Mean ± Standard error of mean and was analyzed by using one-way Analysis Of Variance (ANOVA) and is represented via bar graphs for data interpretation.

Table 4.1: Effects of dietary chitosan on average daily gain, average feed intake and feed conversion ratio fed with experimental diets for 3 weeks (Mean ± SEM).

Day 1-21 (6 Birds/ replicate)				
Sr#	**Parameters**	**Control group (0g chitosan per kg feed)**	**Group 1 (0.75g chitosan per kg feed)**	**Group 2 (1.5g chitosan per kg feed)**
1-	Average daily gain	33.8 ± 0.32	36.2 ± 0.06	37.2 ± 0.1
2-	Average Daily Feed Intake	50.7 ± 0.2	51.5 ± 0.3	52.8 ± 0.08
3-	Feed Conversion Ratio	1.49 ± 0.02	1.42 ± 0.002	1.41 ± 0.008

Mean ± SEM with 6 observations. Means of above given parameters are significantly different (P < 0.05)

4.1.1 Average Daily Gain Day 1-21 (6 birds per replicate)

An intergroup comparison showed that as compared to the control group, a significantly higher average daily gain in experimental group 2 (fed with chitosan 1.5g/kg) was measured while there was non-significant changes in experimental group 1 and 2 (Figure 4.1).

4.1.2. Average Daily Feed Intake Day 1-21 (6 birds per replicate)

Average daily feed intake is significantly increase ($P \leq 0.001$) in experimental group 2 (fed with chitosan 0.5g/kg) as compared to the control group and experimental group (fed with 0.75g/kg chitosan) (Fig 4.2).

4.1.3. Feed Conversion Ratio (FCR) Day 1-21 (7 birds per replicate)

The feed conversion ratio of experimental group 2 (fed with chitosan1.5g/kg) significantly ($P \geq 0.05$) improved relative to control group and experimental group 1 (fed with chitosan0.75g/kg). Improved FCR leads to boost up growth performance of broilers (Figure 4.3).

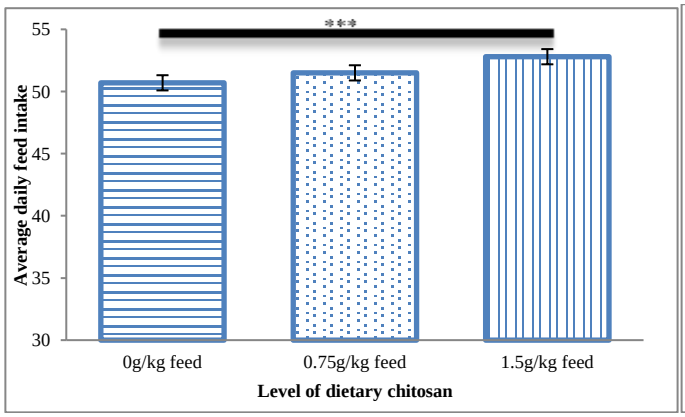


Figure 4.1: Average Daily Gain at day (1-21)

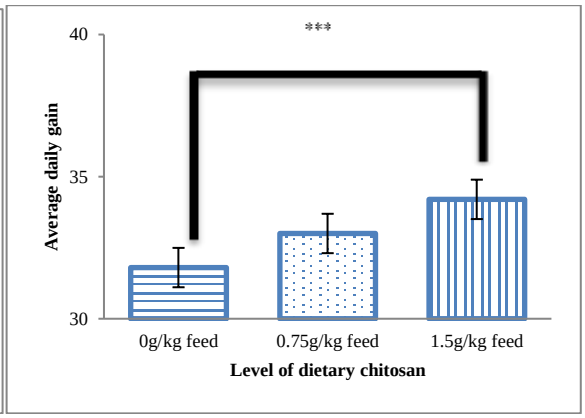


Figure 4.2: Average Daily Feed Intake at day (1-21)

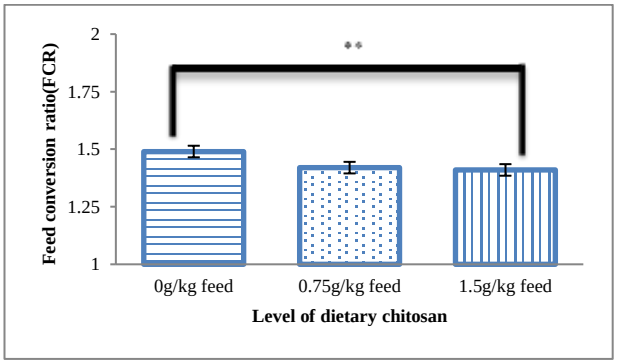


Figure 4.3: Feed Conversion Ratio at day (1-21)

Control group (given no chitosan in feed); Experimental group 1 (given 0.75g chitosan per kg feed); Experimental group 2 (given 1.5g chitosan per kg feed)
*** indicates P ≤ 0.05, **indicates P ≤ 0.01, *** indicates P ≤ 0.001.**

Table 4.2: Effects of dietary chitosan on average daily gain, average feed intake and feed conversion ratio fed with experimental diets for 3 to 6 weeks (Mean ± SEM).

Day 22-42 (5 birds per replicate)				
Sr#	**Parameters**	**Control group (0gram chitosan per kg feed)**	**Group 1 (0.75g chitosan per kg feed**	**Group 2 (1.5g chitosan per kg feed**
1-	Average daily gain	59.02 ± 0.2	62.6 ± 0.17	63.8 ± 0.15
2-	Average Daily Feed Intake	145.7 ± 0.39	149.5 ± 0.63	150.6 ± 0.72
3-	Feed Conversion Ratio	2.46 ± 0.01	2.38 ± 0.01	2.35 ± 0.005

Mean ± SEM with 5 observations. Means of above given parameters are significantly different (P < 0.05).

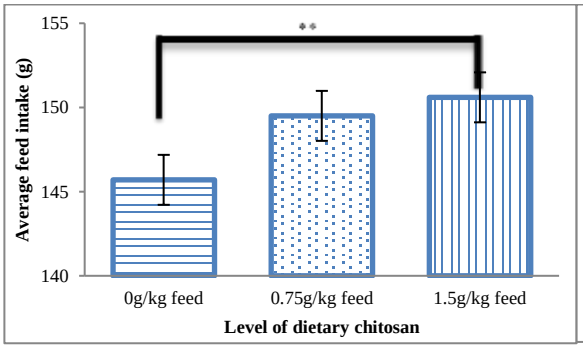


Figure 4.4: Average Daily Gain at day (22-42)

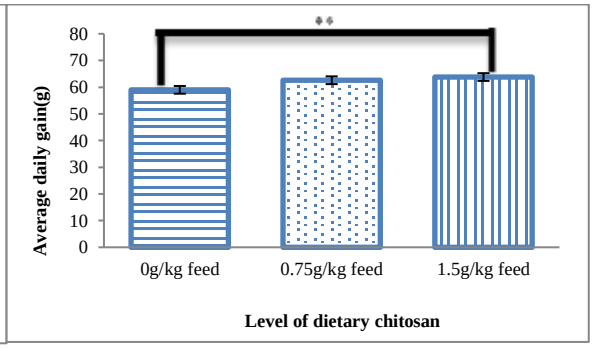


Figure 4.5: Average Feed Intake at day (22-42)

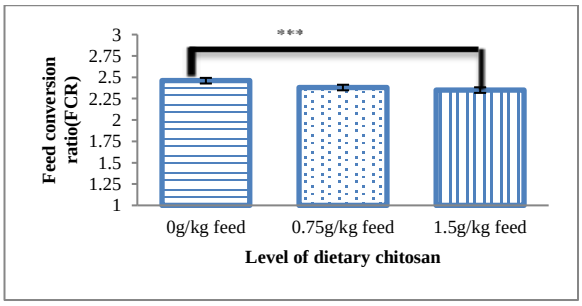


Figure4.6: Feed Conversion Ratio at day (22-42)

4.3. Growth performance of broilers

Effects of dietary chitosan on average daily gain, average feed intake and feed conversion ratio fed with experimental diets for 3 weeks (Mean ± SEM) (Table 4.3).

Table 4.3: Effects of dietary chitosan on average daily gain, average feed intake and feed conversion ratio fed with experimental diets at Day 1-42 (Mean ± SEM).

Day 1-42 (7 birds per replicate)				
Sr#	**Parameters**	**Control group (0g chitosan per kg feed)**	**Group 1 (0.75g chitosan per kg feed)**	**Group 1 (0.75g chitosan per kg feed)**
1-	Average daily weight gain	47.2 ± 0.08	49.6 ± 0.14	50.7 ± 0.11
2-	Average Daily Feed Intake	97.8 ± 0.08	99.7 ± 0.18	100.8 ± 0.11
3-	Feed Conversion Ratio	2.03 ± 0.004	1.99 ± 0.002	1.98 ± 1.72

Mean ± SEM with 6 observations. Means of above given parameters are significantly different (P < 0.05)

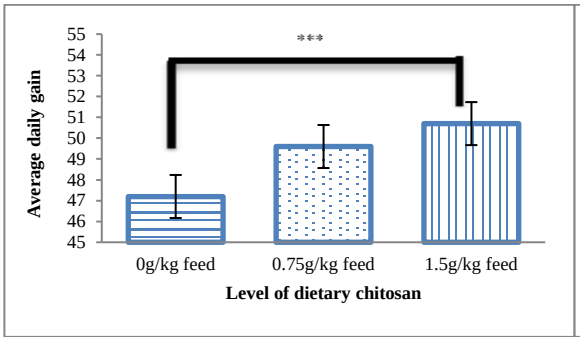


Figure 4.7: Average Daily Gain (ADG) at day (1-42)

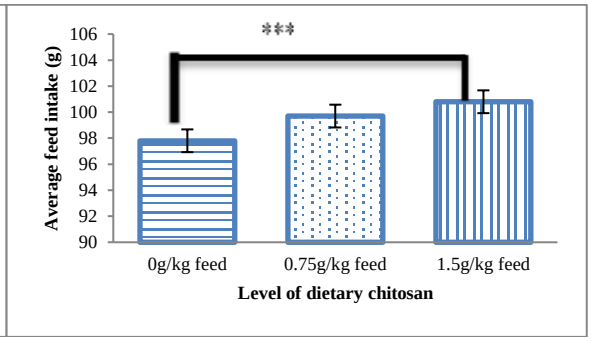


Figure 4.8: Average Feed Intake (AFI) at day (1-42)

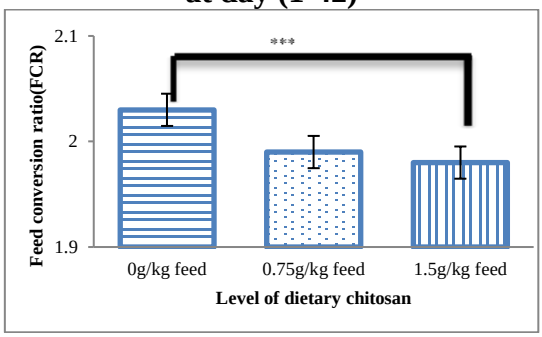


Figure 4.9: Feed Conversion Ratio at day (1-42)

4.4. Relative Weight of Organs

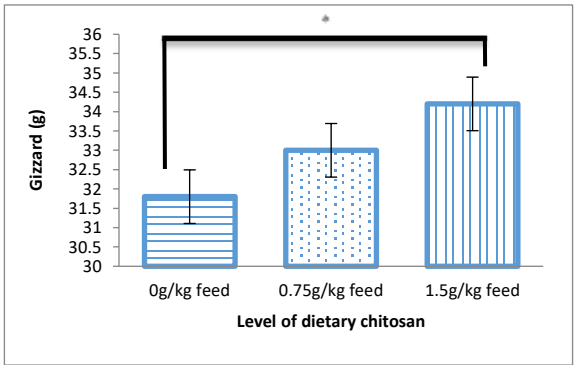
The weight of important internal organs of broiler chickens is weighed after slaughtering.

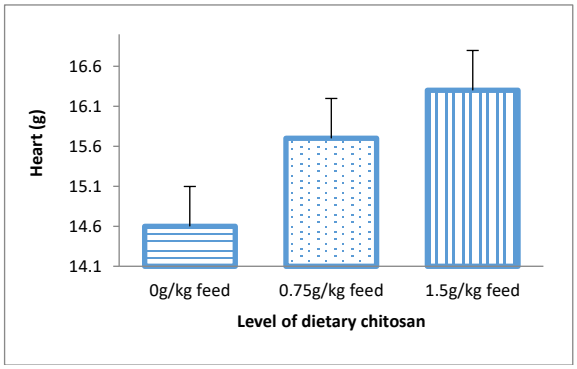
Table 4.4: Data is represented as Mean±SEM for relative organ weight.

Sr. No.	Organ	Control group	Experimental group 1	Experimental group 2
1-	Gizzard(g)	31.8 ± 0.25	33 ± 0.57	34.2 ± 0.67
2-	Heart(g)	14.6 ± 0.31	15.7± 0.35	16.3± 0.20
3-	Liver (g)	49.3 ± 0.76	48.3 ± 0.85	49± 1.52
4-	Spleen(g)	2.26 ± 0.21`	2.53 ± 0.03	2.6± 0.12

Mean ± SEM with 6 observations. Means of above given parameters (heart and gizzard) are significantly different (P < 0.05).

4.4.1Gizzard





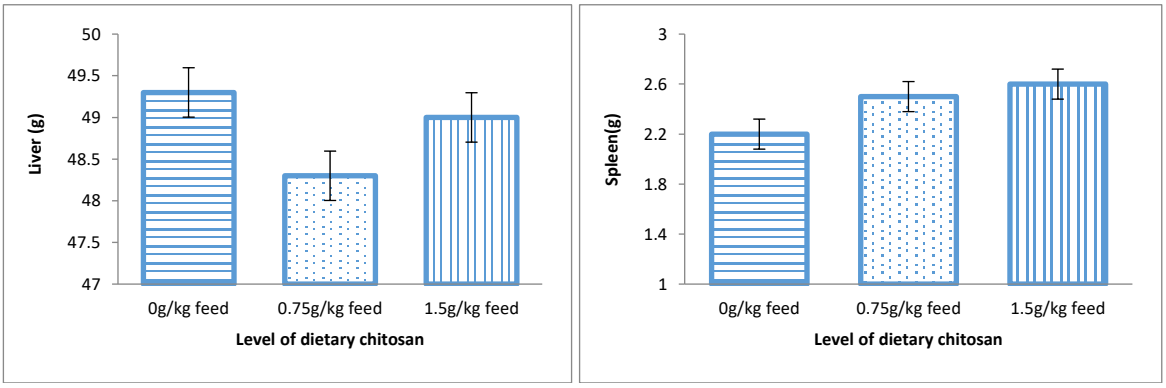


Figure 4.10: Weight of gizzard, Figure 4.11: heart, Figure 4.12: liver, Figure 4.13: spleen in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

4.5. Complete Blood Count (CBC)

The values for the various blood components were obtained after analyzing the blood samples taken from each group. Data is represented as **Mean ± SEM (Table 4.5).**

Sr. No.	Parameter	Control group	Experimental group 1	Experimental group 2
1-	Hemoglobin (g/dl)	9.1 ± 0.05	8.3 ± 0.08	9.6 ± 0.11
2-	Total RBC (x10/l)	2.1 ± 0.05	1.8 ± 0.05	2.5 ± 0.12
3-	Hematocrit (%)	25.3 ± 0.6	23.1 ± 0.4	29.4 ± 0.3
4-	Mean Corpuscular Volume (fL)	122.6 ± 0.70	122.8 ± 0.44	124.5 ± 0.26
5-	MCH (pg)	41.2 ± 0.14	41.2 ± 0.14	41.2 ± 0.14
6-	MCHC (g/dL)	33.3 ± 0.08	33.6 ± 0.09	34.06 ± 0.12
7-	Platelet count (x10/L)	77 ± 1.15	87.1 ± 0.44	59 ± 0.57
8-	WBC Count (x10/L)	10.5 ± 0.12	12.3 ± 0.28	17.03 ± 0.20
9-	Neutrophils (%)	9 ± 0.5	7 ± 1.15	6 ± 1.15
10-	Lymphocytes (%)	90.3 ±0.8	94.3 ± 1.7	97 ± 0.5

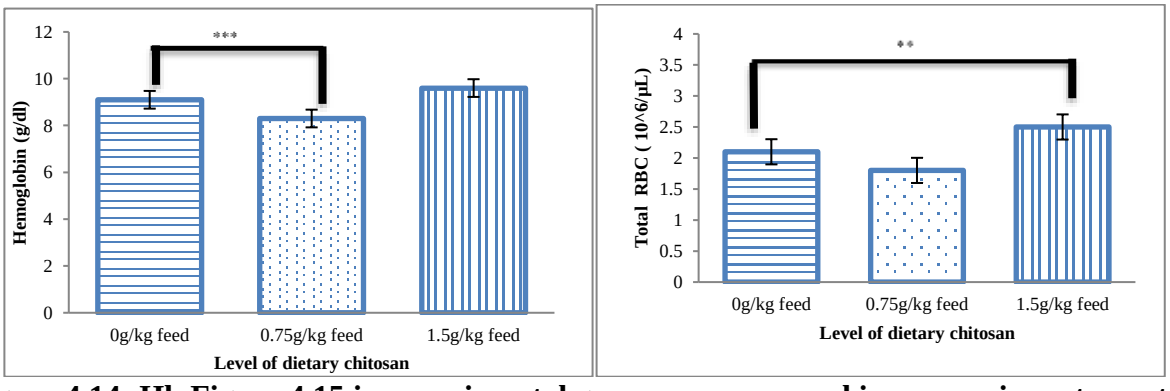


Figure 4.14: Hb Figure 4.15 in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

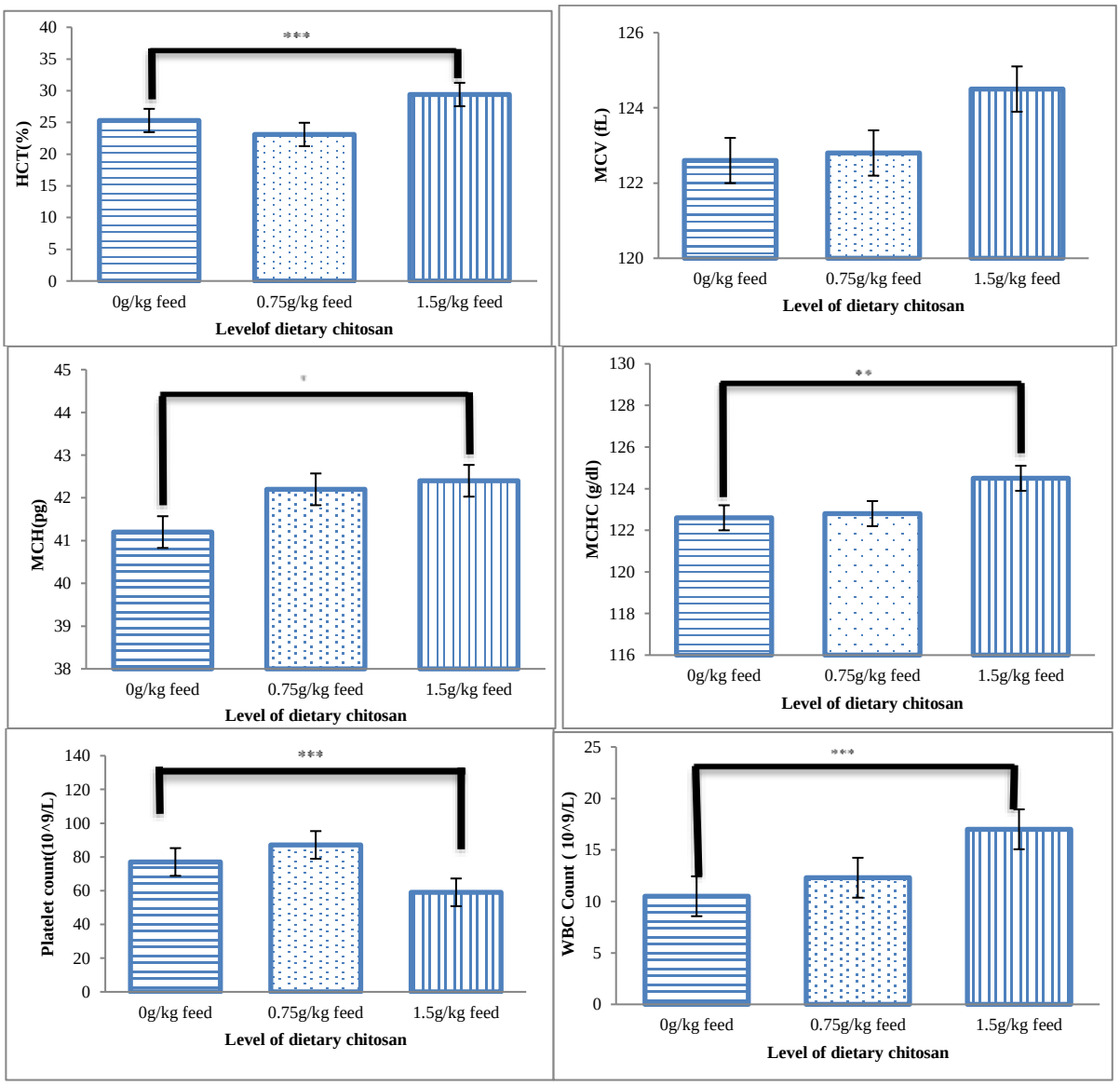


Figure 4.16: HCT %**, Figure 4.17** MCV (ul)**, Figure 4.18** MCH (pg)**, Figure 4.19** MCHC (g/dl)**, Figure 4.20** Platelet count**, Figure 4.21** WBC count in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM. Control group (given no chitosan in feed); Experimental group 1(given 0.75g chitosan per kg feed); Experimental group 2 (given 1.5g chitosan per kg feed)

*** indicates P ≤ 0.05, **indicates P ≤ 0.01, *** indicates P ≤ 0.001.**

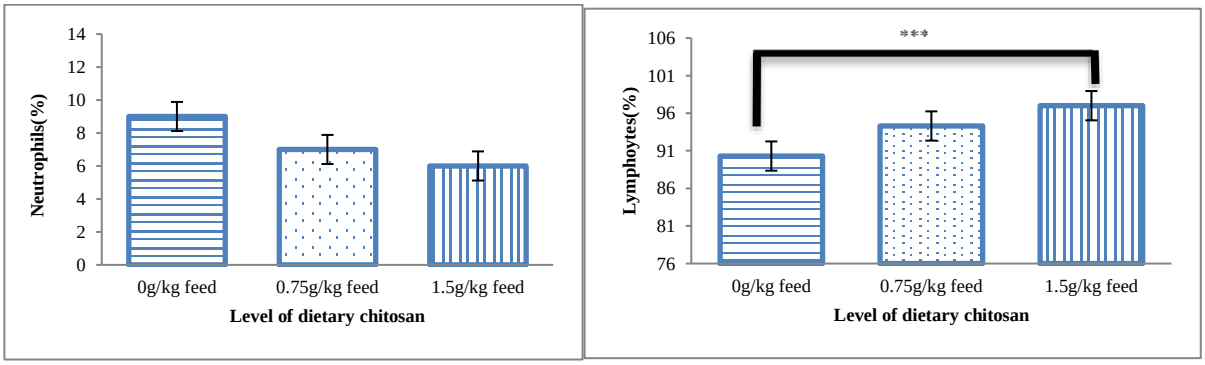


Figure 4.23: Neutrophils, Figure 4.24 Lymphocytes (%) in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

Control group (given no chitosan in feed); **Experimental group 1**(given 0.75g chitosan per kg feed)**; Experimental group 2** (given 1.5g chitosan per kg feed)
*** indicates P ≤ 0.05, **indicates P ≤ 0.01, *** indicates P ≤ 0.001.**

4.6. Lipid Profile Test

The blood samples of chickens are taken which are coagulated then centrifuged and stored the serum at -20°C until lipid profile test is performed.

Table 4.6: Data is represented as Mean±SEM for Lipid Profile Test.

Sr.No.	Parameter	Control group	Experimental group 1	Experimental group 2
1-	Cholesterol (mg/dl)	108.3 ± 0.90	95.6 ± 2.4	61.3 ± 0.88
2-	Triglycerides (mg/dl)	97 ± 1.15	69 ± 0.57	68 ± 0.58
3-	HDL (mg/dl)	20.6 ± 0.9	18 ± 0.6	15.6 ± 0.3
4-	LDL (mg/dl)	35.3 ± 0.9	36.6 ± 2.6	22.3 ± 0.8

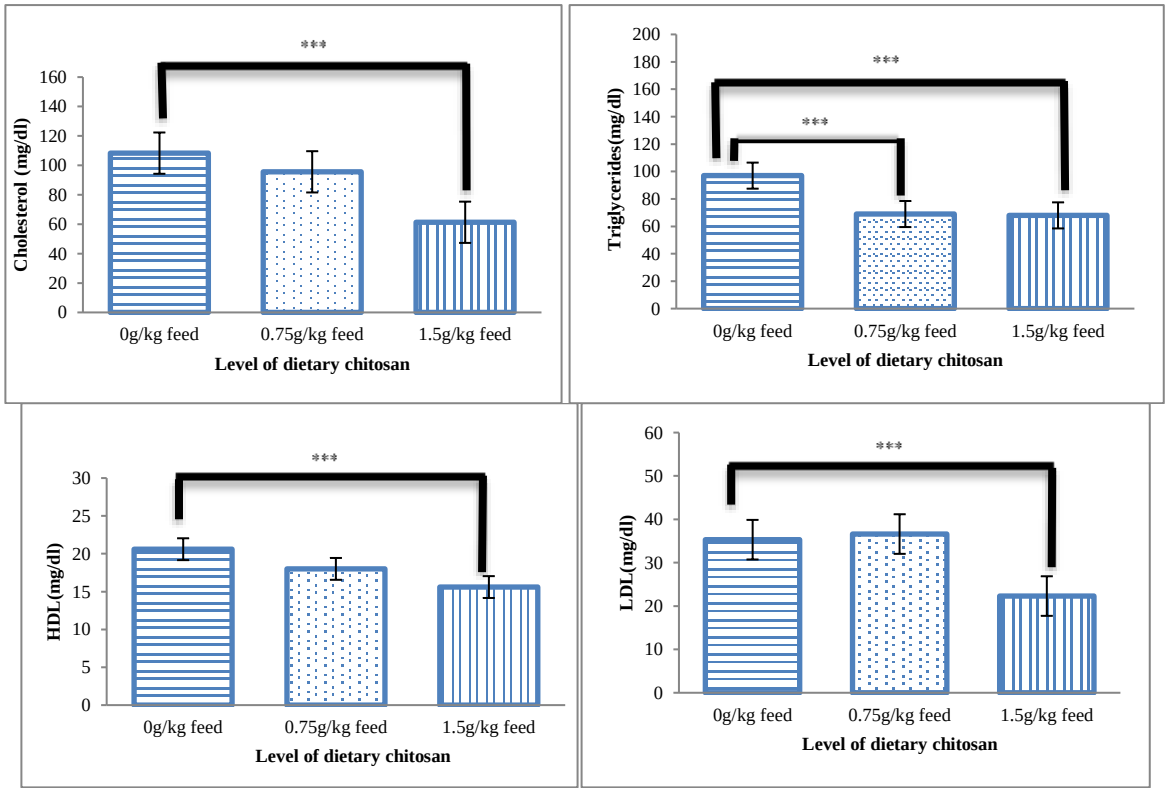


Figure 4.24: Cholesterol (mg/dl), Figure 4.25 Triglycerides(mg/dl), Figure 4.26 HDL (mg/dl),Figure 4.27 LDL(mg/dl) in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

Control group (given no chitosan in feed); **Experimental group 1**(given 0.75g chitosan per kg feed)**; Experimental group 2** (given 1.5g chitosan per kg feed)
*** indicates P ≤ 0.05, **indicates P ≤ 0.01, *** indicates P ≤ 0.001.**

4.7. Liver Function Test

The values for the various hepatological components were obtained after analyzing the blood samples taken from each group. Data is represented as **Mean±SEM.**

Sr.No.	Parameter	Control group	Experimental group 1	Experimental group 2
1.	Bilirubin (mg/dl)	0.183 ± 0.008	0.18 ± 0.011	0.183 ± 0.012
2.	SGPT(ALT)	20.3 ± 1.4	17 ± 1.5	15 ± 1.1
3.	SGOT(AST)	256.3 ± 8.5	381.3 ± 18.4	478.3 ± 21.6
4.	Alkaline Phosphate	2337.6 ± 21.5	3570.6 ± 35.5	4500.3 ± 58.2
5.	Total Protein	3.8 ± 0.08	2.7 ± 0.06	3 ± 0.05
6.	Albumin	1.3 ± 0.06	1.2 ± 0.03	1.1 ± 0.03
7.	Globulin	2.3 ± 0.05	1.73 ± 0.08	1.87 ± 0.03
8.	A/G Ratio	0.53 ± 0.003	0.69 ± 0.005	0.51 ± 0.003

Table 4.7: Mean ± SEM for different hepatological parameters.

4.7.1. Bilirubin (mg/dl)

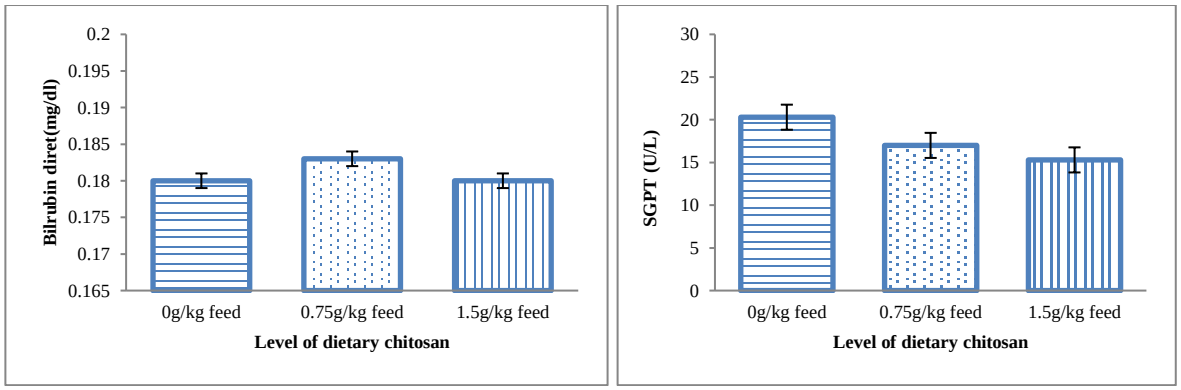
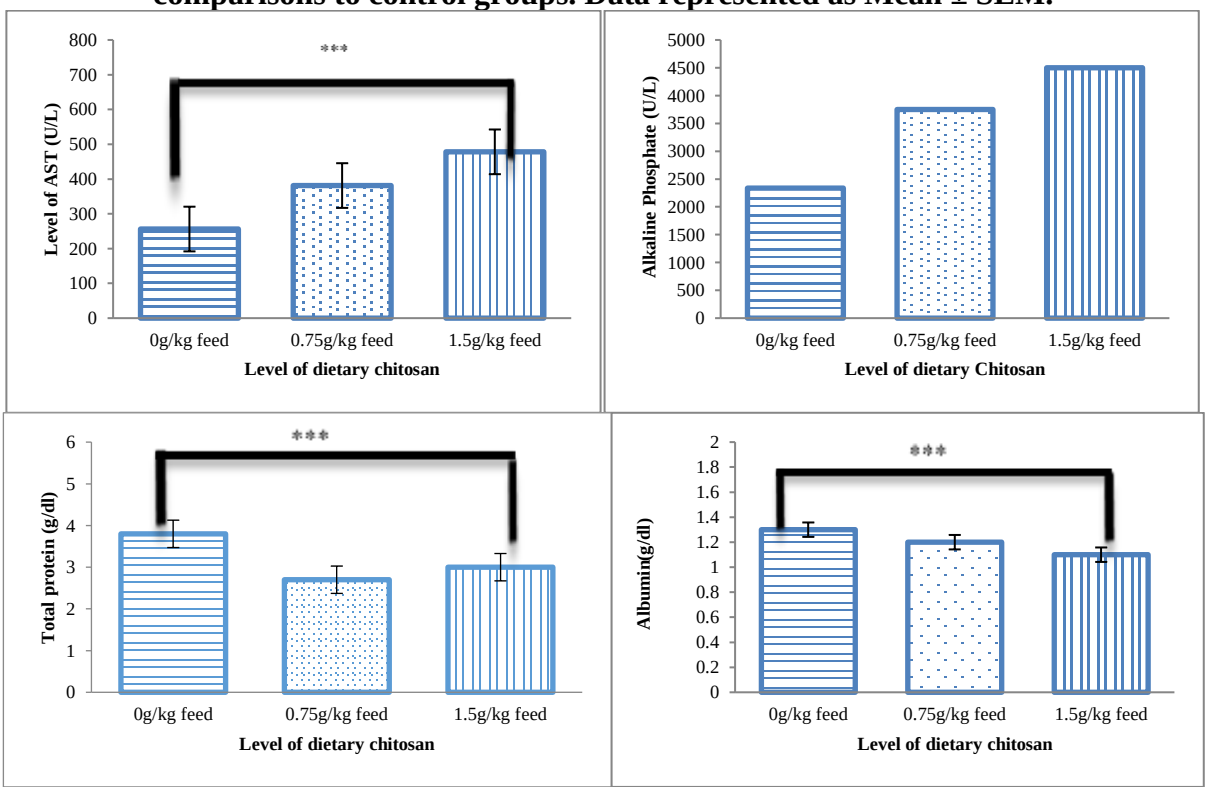


Figure 4.28 Bilirubin(mg/dl), Figure 4.29 SGPT(U/L) in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.



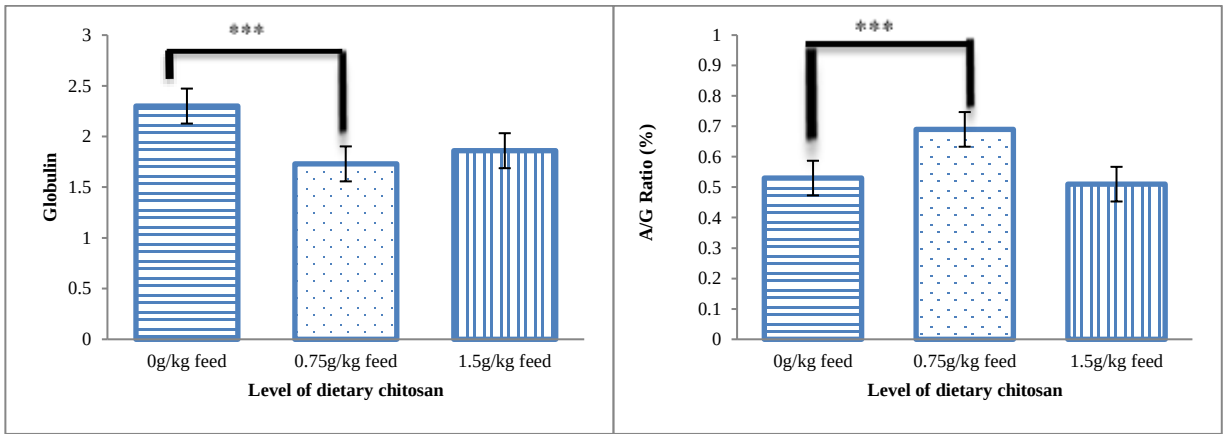


Figure 4.29: AST(U/L), Figure 4.30: Alkaline Phosphate, Figure 4.31 Total protein, Figure 4.32 Albumin, Figure 4.33 Globulin, Figure 4.34: A/G ratio in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

Control group (given no chitosan in feed); **Experimental group 1** (given 0.75g chitosan per kg feed)**; Experimental group 2** (given 1.5g chitosan per kg feed)
*** indicates P ≤ 0.05, **indicates P ≤ 0.01, *** indicates P ≤ 0.001.**

4.8. Renal Function Test

The values for the various renal parameters were obtained after analyzing the blood samples taken from each group. Data is represented as **Mean±SEM.**

Sr.No.	Parameter	Control group	Experimental group 1	Experimental group 2
1-	Urea (mg/dl)	4.3 ± 0.3	5 ± 0.5	4.3 ± 0.3
2-	Creatinine(mg/dl)	0.16 ± 0.06	0.2 ± 0.05	0.13 ± 0.03
3-	Uric acid (mg/dl)	3.5 ± 0.05	2.6 ± 0.32	3.5 ± 0.05

Table 4.8: Mean ± SEM values for renal parameters

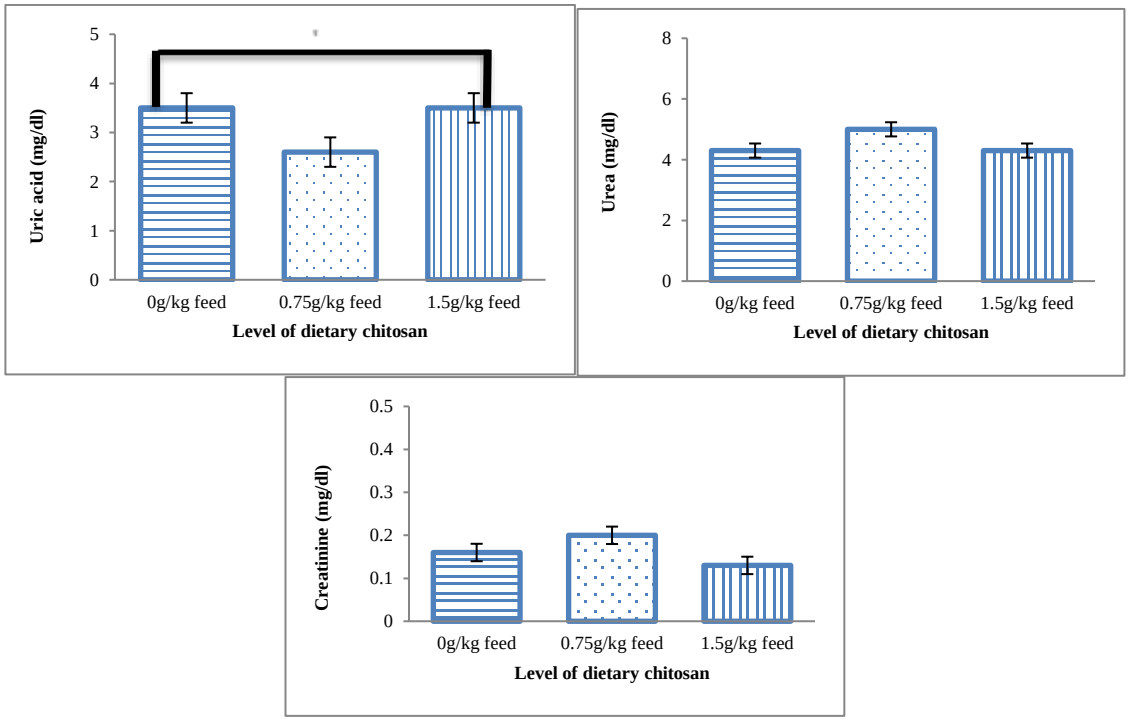


Figure 4.35: AST(U/L), Figure 4.36: Alkaline Phosphate, Figure 4.37 Total protein, Figure 4.38 Albumin, Figure 4.39 Globulin, Figure 4.40: A/G ratio in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

4.9 Serum Electrolyte test

The values for the various serum parameters were obtained after analyzing the serum samples from each group. Data is represented as **Mean±SEM.**

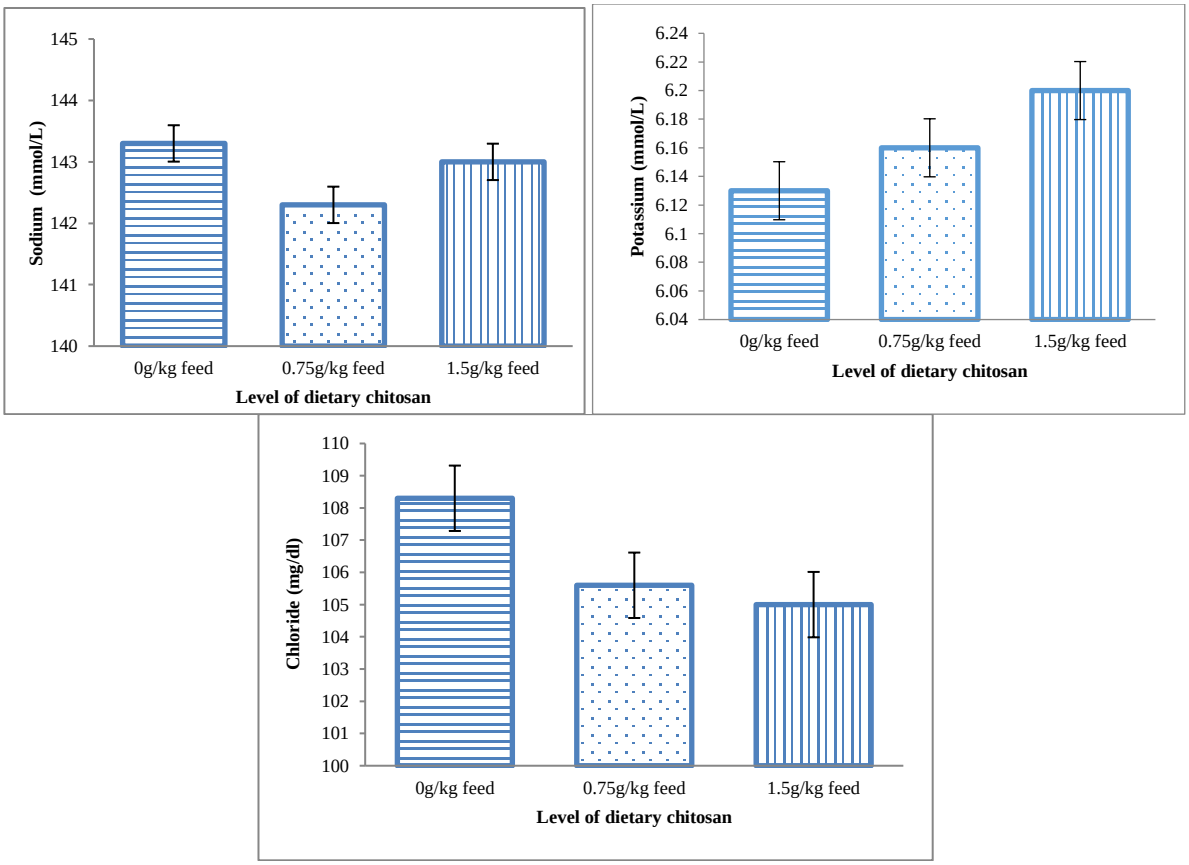


Figure 4.41: Sodium, Figure 4.42 Potassium, Figure 4.43 Chloride in experimental groups as compared in comparisons to control groups. Data represented as Mean ± SEM.

Control group (given no chitosan in feed); **Experimental group 1** (given 0.75g chitosan per kg feed); **Experimental group 2** (given 1.5g chitosan per kg feed)
*** indicates P ≤ 0.05, **indicates P ≤ 0.01, *** indicates P ≤ 0.001.**

Discussion

Dietary chitosan improves broiler growth performance by increasing their Feed Conversion Ratio (FCR) and improving their hematological parameters. The current study examined the effects of chitosan supplementation in broiler chicks and found noteworthy results. Among the experimental groups, Group 2, which received 1.5g/kg chitosan, showed a significant increase in Average Daily Gain (ADG) and FCR, contributing to improved overall growth performance. While the differences between Group 1 (0.75g/kg chitosan) and Group 2 (1.5g/kg chitosan) was not statistically significant ($P > 0.05$), there was a significant difference between the control and treatment groups. These findings demonstrate the potential of chitosan as a feed addition to improve broiler growth, which is consistent with previous studies in the sector.

Previous research has also supported the beneficial effects of introducing chitosan into broiler diets. Incorporating 0.125% chitosan into the diet appeared to produce higher FCR. Similarly, the introduction of 0.5% or 0.625% chitosan in broiler diets resulted in increased growth and feeding efficiency. This impact is due to chitosan's potential role in controlling intestinal microflora, which therefore improves protein digestion and absorption. Research by Shi et al. (2005) provides further credibility to this perspective.

However, the literature provides a nuanced picture. For example, male Marshall Chunky broilers treated with 0.6g/kg chitosan over a 7-week period showed improvements in body weight gain

(BWG) and feed intake but no significant alterations in feed efficiency. This study by Khambualai et al. (2009) highlights the complicated relationship between chitosan dosage and broiler performance. It is critical to recognize that differences in growth performance outcomes among research may be linked to chitosan's various properties, such as concentration, degree of acetylation, and molecular weight. Goy et al. (2009) and Khambualai et al. (2009) highlighted the importance of these aspects in determining the effectiveness of chitosan as a growth enhancer in broiler chickens. These variances emphasize the importance of doing thorough research and optimizing chitosan properties in order to fully capitalize on its potential advantages.

The primary goals of chicken production include keeping broilers healthy, increasing productivity, and improving meat quality. Within the context of this study, the use of dietary supplements such as Chitosan and Xylo-oligosaccharides produced positive results in these areas. Notably, the incorporation of dietary chitosan has resulted to significant improvements in broiler body weight, which agrees with our own findings (Li et al., 2017).

Furthermore, dietary supplementation with chitosan has been shown to improve several aspects of broiler health. This includes an increase in body weight gain, consistent with our findings, while also improving intestinal barrier functioning, antioxidant capacities, and immunological response. A noteworthy result of this supplementation strategy has been a decrease in feed consumption, which may imply a novel method of action. Parallel findings have been obtained from research involving oxidative challenged piglets, where dietary chitosan supplementation resulted in enlarged antioxidant enzyme activities and the modulation of cytokines (Li et al., 2019).

Feed intake, efficiency, and antioxidant capacity are all important factors to consider while enhancing growth performance. Notably, Chitosan and Xylo-oligosaccharides have been linked to good impacts in these domains, which could explain the observed positive influence on growth performance in our study (Xu et al., 2018). These findings highlight the possibility of nutritional supplementation with Chitosan and Xylo-oligosaccharides to boost the growth performance of broilers while also imparting additional health benefits.

In recent years, the importance of public health has become a major concern. As a result, a rising number of customers are paying close attention to the nutritional value and safety of their protein sources. This trend has resulted in an increased preference for low-fat chicken products, which have become smoothly incorporated into people's everyday dietary habits. Intriguingly, our study investigated the effects of dietary chitosan supplementation, which revealed a significant hypocholesterolemic effect. Notably, within this study, experimental group 2, which received a chitosan dosage of 1.5g per kilogram of feed, exhibited a statistically significant ($P \leq 0.001$) reduction in cholesterol, triglycerides, and low-density lipids (LDL) when compared to both experimental group 1 (administered 0.75g chitosan/kg feed) and the control group (fed solely on a basal diet).

Our findings are consistent with those of Osman et al. (2010) and Tufan and Arslan (2020). The various dietary treatments had observable effects on the serum biochemistry of broilers, notably those given COS 262 g/ton supplementation. This treatment resulted in a considerable reduction in serum levels of total cholesterol, triglycerides, LDL cholesterol, and VLDL cholesterol. In contrast, after administering COS at the same dosage, HDL-cholesterol concentrations increased. These findings are consistent with previous studies, including those by Tang et al. (2005), Li et al. (2007), and Zhou et al. (2009), which emphasized the cholesterol-lowering properties of chitosan and COS.

However, differences have emerged across research. For example, Keser et al. (2012) reported that adding chitosan to broiler feeds had no significant effect on serum HDL cholesterol levels. Similarly, Khambualai et al. (2008) reported that providing dietary chitosan at a concentration of 0.06% did not cause any detectable changes in blood lipid profiles. Leblebicier and Aydogan (2018) observed that chitosan, COS, and MOS had no detectable impact on blood cholesterol and triglyceride levels in broilers, which contradicted previous findings.

The dosage of COS is identified as a critical element impacting its effect on blood lipid markers. According to Khambualai et al. (2008), the observed dip in blood lipid concentrations may be due to an increase in digesta viscosity inside the gastrointestinal system, which is accompanied by a decrease in lipid absorption. Chitosan's interaction with bile acids can inhibit the activity of the lipase enzyme

in the small intestine, allowing cholesterol to be eliminated from the body via waste disposal processes (Chiang et al., 2000; Zheng and Zhu, 2003).

The significance of these findings becomes clear when considering poultry health. The lowering in serum triglyceride levels has consequences for preventing hepatic fat accumulation and the resulting fatty liver syndrome. Similarly, lower blood LDL concentrations indicate better health conditions for broiler birds and, by extension, consumers. This delicate interplay of dietary COS supplementation and blood cholesterol regulation highlights the multifaceted impact of such treatments in boosting poultry health and hence changing the consumer experience.

The current investigation found that adding dietary chitosan had no noticeable effects for broiler protein metabolism. This was demonstrated by a decrease in serum concentrations of total protein, albumin, and globulin across all treatment groups relative to the control group. This is consistent with previous studies, which found that adding chitosan to broiler diets at varied rates did not affect blood protein levels (Zhou et al., 2009; Keser et al., 2012). Similar investigations demonstrated that chitosan supplementation had no effect on serum albumin and total protein concentrations (Leblebicier and Aydogan, 2018).

Serum creatinine concentration is a well-established indication of kidney health. Interestingly, the current study found a significant fall in Creatinine content, which remained within the normal range. The therapy groups showed typically normal levels of alanine aminotransferase (ALT), a key enzyme involved in liver function. In contrast, aspartate aminotransferase (AST) levels rose in parallel with increased chitosan content in the basal diet. Except for experimental group 2 (given 0.5 g/kg chitosan), the AST rise was only slightly above the usual threshold; however, this minor increase may not be of immediate concern. The literature confirms the occurrence of inflammatory liver alterations in broilers throughout their lifespan, and enzymatic liver examination provides useful insights into liver function. Higher AST levels may indicate inadequate feed ingredient utilization or growth rates, which could contribute to general liver diseases. Notably, high AST/ALT levels in quickly growing broilers can cause chronic liver injury, which has been associated to the development of sudden death syndrome (SDS) (Shi *et al*., 2005).

In accordance with the current study, the effect of COS meal on feed consumption resulted in a considerable increase in AST levels that hovered near the threshold level. Broilers' ALT levels remained within the usual range, indicating that the birds were in good health. This complex interplay of food variables and enzyme levels highlights the broader consequences for broiler health and well-being.

Conclusion

Poultry is a valuable protein source and the industry has been adopting modern technologies and practices to improve production efficiency, disease control, and product quality. The poultry industry in Pakistan primarily focuses on the production of broiler chickens for meat as chicken meat is a major source of animal protein. Chitosan can be used as a feed additive for poultry because of its Immunomodulatory, anti-oxidative, antibacterial and hypocholesterolemic properties. Chitosan can be used as feed additive as it is natural and safe alternatives in poultry production to lessen the risk of resistance transfer to humans via the food chain. Thus, it can be concluded that chitosan supplementation (0.75-1.5 g/kg) is one of the natural feed supplements expected to enhance the overall health and productivity of broiler chickens.

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