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Technical note

Chitosan supplementation in the diet of Rhode Island Red pullets and its effect on productive behavior and hematological variables

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Abstract:

Chitosan is a polymer obtained from crustacean byproducts, which has antioxidant, antimicrobial and immunostimulant properties that can be used to promote growth in poultry without altering their health status. The objective of the study was to evaluate the effect of dietary inclusion of chitosan (0.55 % and 0.65 %) on productive behavior and hematological

parameters in Rhode Island Red pullets. Forty-five pullets with an average weight of 36 ± 7.8 g were used, which were housed in cages of 1.0 m^2 (5 animals/cage). The pullets were fed for 21 days with diets with different inclusions of chitosan: 1) control diet (commercial type), 2) control diet + 0.55 % chitosan (CH55), 3) control diet + 0.65 % chitosan (CH65). At the end of the feeding trial, growth, feed conversion ratio (FCR), blood biometry and blood biochemistry were analyzed. Dietary supplementation with chitosan increased the final weight of the pullets and, by up to 27 %, the daily weight gain (P<0.05), compared to the control treatment. The FCR showed no significant changes with chitosan supplementation. Blood glucose concentration increased with the addition of chitosan to the diet (P<0.05). The variables of total cholesterol, HDL cholesterol, VLDL cholesterol, triglycerides, total protein and blood biometry showed no significant changes (P>0.05). It is concluded that the addition of chitosan in both doses favors growth without altering the health status of the pullets.

Keywords: Pullets, Chitosan, Productive performance, Hematological parameters.

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Poultry production is one of the most important activities within the agrifood sector, both economically and in terms of food security. In Mexico, a production of 3 million 578 thousand tonnes of chicken meat has been reported for the year 2020[1]. Poultry production is characterized by implementing two main production phases, egg production phase, and meat production phase; this has significantly encouraged the development of small, medium and large producers based on greater sources of employment and profitability of companies[1,2]. In this regard, the poultry industry is constantly looking for alternatives to reduce feed costs (diets) based on animal productivity. This has led to an increase in the use of compounds of natural origin in poultry diets, in order to promote their growth through the improvement of energy and protein utilization, and of their health status[2].

Chitosan is a natural polymer formed by units of N-acetyl D-glucosamine, derived from the alkaline deacetylation of chitin, which is available in the exoskeleton of crustaceans, algae and up to one third of the total cell wall in fungi[3]. This polymer is soluble in water, exhibits bioactive properties, such as antimicrobial, immunomodulatory and antioxidant, and is also recognized as a natural and harmless compound[4,5], which is why it is appropriate for use in the food industry[6].

The use of chitosan as a feed supplement could have a positive effect on farm animals, since, due to its bioactive properties, it can interact with the intestinal microbiota, favor the use of

energy, and consequently the productive variables in the pen, such as weight gain and feed conversion ratio[7,8,9]. Additionally, it is worth mentioning that the process of extracting and obtaining chitosan represents a low cost, which can favor the profitability of poultry farms[10].

Recent studies in animal production have shown positive effects of chitosan on productive performance[11,12,13]. However, high concentrations (more than 10 g/d) of this compound in the diet can cause negative effects on productive performance[14,15,16]. Therefore, the effects of chitosan on productive variables are still inconsistent, and its evaluation in physiological aspects is necessary. The objective of this study was to evaluate the inclusion of chitosan in the diet of Rhode Island Red pullets and its effect on productive behavior and hematological parameters.

The present study was conducted in the spring of 2021 at the feed workshop of the Technological Institute of Mazatlán, in Mazatlán, Sinaloa, Mexico. The climatic conditions of this region are tropical, defined by a hot and humid climate, with maximum temperatures in summer of 34 °C and minimum temperatures in winter of 16 °C; the average annual rainfall is 722 mm with an average relative humidity of 62 %.

Forty-five (45) dual-purpose pullets of the Rhode Island Red breed with an average weight of 36 ± 7.8 g were used, which were distributed under a randomized complete design of three treatments with 15 pullets each, and they were assigned as follows: 1) Control diet (commercial diet), 2) CH55= control diet + 5.5 g chitosan/kg diet (0.55 %) and 3) CH65= control diet + 6.5 g chitosan/kg diet (0.65 %).

A control diet (Industrias Melder, Mexico) that meets the nutritional requirements of the Rhode Island Red breed for the initial stage was used and it was composed of corn, soybean meal, rapeseed meal, distillers dried grains, beef meal, amino acids: L-lysine, D-methionine, vitamins: A, D_3, E, K_3, B_1, B_2, B_3, B_4, B_5, B_6, B_7, B_9, B_{12}, minerals: sodium bicarbonate, calcium carbonate, sodium chloride, manganese, iron, copper, selenium, iodine, zinc, betaine, antioxidants ETQ, BHT, BHA, Nicarbazin; with a proximate composition of 12 % moisture, 21.5 % crude protein, 3 % fat, 6 % crude fiber, 5 % ash and 52.5 Kcal/kg of net energy. The control diet (Industrias Melder, Mexico) with inclusions of chitosan of 5.5 g and 6.5 g per kg of diet was provided in the experimental treatments.

The pullets were housed in a natural environment room, in galvanized metal cages with dimensions of 100 x 100 x 50 cm, where five pullets were housed per cage for 21 d, with 7 d of adaptation to the control diet. The temperature was measured daily with a digital thermometer (Wenmeice, Xi´an Lonn M&E Equipment, China), which ranged between 30 and 33 °C. A photoperiod of 16 h of artificial light and 8 h of darkness was controlled. Feed and water were provided *ad libitum*. Uneaten feed was collected and weighed daily to record its daily intake. Excreta were removed daily to prevent contamination and diseases.

Weekly biometric tests were performed during the experimental period. All pullets were weighed individually. The initial weight, final weight, feed consumed and number of dead pullets were recorded. The variables of survival (S), weight gained (WG), feed conversion ratio (FCR), specific growth rate (SGR) and daily weight gain (DWG) were calculated with the following formulas: S (%): (final number of animals / initial number of animals)* 100); WG (g): [final average weight (g) – initial average weight (g)]; FCR: [feed consumed (g) / weight gained (g)]; DWG (g/day): average weight gained / days; measurements were made on an ACCULAB balance (model ACL-210.4).

At the end of the test, the blood parameters (glucose, total cholesterol, high- and low-density lipoproteins and total protein) and blood biometry of 6 pullets (24 h fasting) per treatment (2 pullets per cage) were evaluated. The blood sample was collected by puncture in the wing vein with a 1 ml syringe (Venosafe, Terumo), and placed in Eppendorf (1 ml) and microtainer tubes with heparin. The samples were centrifuged at 3,500 xg for 15 min at 10 °C. Plasma was separated into vials to determine glucose, total cholesterol, serum cholesterol, high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), triglycerides, and total protein using blood chemistry equipment (Model DT-60, Johnson Co.; High Wycombe, UK) and blood biometry using an auto hematology analyzer (Auto Hematology Analyzer, Mindray, BC-2800 Vet; Shenzhen, China).

Data normality was determined by the Kolmogorov-Smirnov test, and homogeneity by the Levene test at a significance value greater than 5 %. The productive and blood variables were analyzed with a one-way ANOVA and the Tukey-Kramer multiple comparison test was used to determine differences between treatments. Prior to the statistical analysis, survival values (%) were normalized using arcsine, but results were reported as a percentage. The differences were considered at a significance level of 0.05. The software NCSS version 2007 (Kayville, UT, USA) was used for the statistical analyses.

The effects of chitosan inclusion are shown in Table 1. On d 14, the treatment CH55 showed an increase in the weight gained (16.5 %) with respect to the control diet ($P<0.05$). The treatment CH65 did not show significant differences compared to the treatment CH55 and the control ($P>0.05$). On d 21, the treatments CH55 and CH65 increased pullet weight by 17.9 % and 15.1 % respectively, compared to the control group ($P<0.05$). DWG increased 27 % with chitosan supplementation compared to animals fed the control diet ($P<0.05$). Additionally, the treatment CH55 showed 100 % survival, unlike the control group where 80 % ($P<0.05$) was obtained. The values of feed intake and FCR of the control group were not different from the treatments including chitosan ($P>0.05$). Similar results were reported by Suk[17] when finding an increase in live weight and DWG in pullets from day 21 to 35, when supplementing 10.5 mg of chitosan/kg of body weight/d, in addition, the FCR increased from d 15 of supplementation. Another study[18] reported a linear increase and a quadratic effect on live weight from d 1 to 22, when the diet of fattening pullets was supplemented

with a chitosan oligosaccharide (0.5 to 2.5 g/kg). In a study conducted by Razdan *et al*[19], it was reported that dietary supplementation of 30 g of chitosan oligosaccharide/kg of diet (approximately five times more the inclusion of chitosan compared to the present study) reduced live weight gain and FCR in fattening pullets, compared to the control treatment. While it is true that the mechanism by which chitosan improves the growth performance of fattening pullets is not fully elucidated, it has been suggested that this compound stimulates pepsin activity and helps protect the mucous membrane of the stomach, which improves digestion and absorption of nutrients such as proteins[13,15,20,21], stimulates the proliferation of beneficial microorganisms and inhibits the proliferation of pathogenic microorganisms, thus regulating the intestinal microbiota[22,23].

Table 1: Productive variables in pullets fed different diets supplemented with chitosan

	Treatments					
Variables	**Control**	**CH 0.55%**	**CH 0.65%**	**SEM**	***P*-value**	**K-S, *P*-value**
Live weight, g						
Day 0	52.1	50.7	50.9	1.2	0.75	0.70
Day 7	88.7	105.5	102.5	6.7	0.70	0.20
Day 14	151.9^{a}	176.9^{b}	171.1^{ab}	4.5	0.70	<0.05
Day 21	164.1^{a}	193.5^{b}	188.9^{b}	4.6	0.49	<0.01
Feed intake, g/d	18.3	22.4	20.9	1.4	0.66	0.13
Daily weight gain, g/d	5.3^{a}	6.8^{b}	6.7^{b}	0.2	0.77	<0.01
FCR	3.4	3.4	3.3	0.2	0.92	0.89
Survival, %	80	100	93	3.8	<0.05	0.14

SEM= standard error of the mean. FCR= Feed conversion ratio.
K-S= probability value of the Kolmogorov-Smirnov normality test (P>0.05).
^{ab} Averages with different literals in the same row indicate a significant difference (P<0.05).
Survival. Chi-square (value of X^2= 3.841, P>0.05, 2 df).

Although the percentage of mortality in the control group was not significant, it is consistent with what was reported by Nuengjamnong and Angkanaporn[21]. This is possibly due to the increase in environmental temperature from the early afternoon, since the main cause of mortality in chickens is sudden death due to high temperatures, which is a challenge for poultry[24]. On the other hand, the lower reduction in mortality observed in treatments including chitosan can be attributed to the antioxidant activity of this compound, and to its effectiveness in counteracting oxidative stress caused by environmental factors (temperature increase)[25,26].

The results of the hematological tests are shown in Table 2. The treatments CH55 and CH65 showed a significant increase in glucose concentration 190.06 and 193.10 mg/dL,

respectively, compared to the control group (P<0.05). The levels of cholesterol, HDL, VLDL, triglycerides, total protein and those of the blood biometry were not significantly affected by the treatments (P>0.05). Other authors report that supplementation of chitosan and chitosan isomers decreases triglyceride and cholesterol levels in animals fed diets supplemented with 100 mg of chitosan/kg of diet[27,28]. Significant changes in glucose levels may suggest that the weight gain improved by chitosan is not due to a hormonal effect, but probably to a higher intake of feed including chitosan, although not significant[29]. The blood parameters evaluated suggest that pullets fed diets supplemented with chitosan maintained their osmotic pressure and acid-base balance in the body, without showing signs of any stress[30].

Table 2: Serum concentrations of metabolites and hematological components in pullets supplemented with chitosan

	Treatments					
Variables	**Control**	**CH 0.55%**	**CH 0.65%**	**SEM**	**K-S, *P*-value**	***P*-Value**
Glucose, mg/dL	172.76[a]	190.06[b]	193.10[b]	8.11	0.34	<0.05
Cholesterol, mg/dL	133.63	154.6	142.53	11.57	0.55	0.48
HDL, mg/dL	97.16	105.13	99.96	9.04	0.62	0.82
VLDL, mg/dL	5.61	6.0	6.4	0.57	0.44	0.64
Triglycerides, mg/dL	28.16	30.16	32.16	2.82	0.23	0.62
Total protein, mg/dL	3.61	3.65	4.05	0.45	0.88	0.76
Blood biometry:						
Hematocrit, %	34.13	33.73	33.36	0.59	0.71	0.67
Hemoglobin, g/dL	11.3	10.83	11.13	0.27	0.79	0.50
Erythrocytes, x 10^6 mm^3	3.1	3.13	2.9	0.21	0.82	0.73
MCV, x 10^{15} L	105.7	106.9	108.66	6.11	0.61	0.94
MCH, Pg	33.1	32.1	33.4	0.64	0.90	0.39
MCHC, g/dL	37.83	34.53	38.36	2.92	0.53	0.62

HDL= high-density lipoproteins; VLDL= very low-density lipoproteins; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin h; MCHC= mean corpuscular hemoglobin concentration.

[ab] Averages with different literal in the same row differ (P<0.05).

K-S= probability value of the Kolmogorov-Smirnov normality test (P>0.05).

In general, an improvement was observed in the productive variables of live weight at d 14 and 21 of feeding and in daily weight gain, with both chitosan treatments. The treatment CH55 reduced mortality and increased blood glucose concentration. However, the other variables of hematology and blood biochemistry were found unchanged by the experimental treatments. These results suggest that dual-purpose pullets did not modify their cellular homeostasis, in addition to the fact that their metabolic and physiological states were not compromised by the effect of the consumption of chitosan in the diet. It is concluded that chitosan can be an alternative to economize the growth in pullets. However, further studies and analyses of mechanisms of action of chitosan in pullets are suggested to demonstrate its positive effect and bioactivity.

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Conflict of interest

The authors establish that there is no conflict of interest in relation to the preparation, review and publication of this work.

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