



EFFECT FED VARYING DIETARY LEVELS OF NEEM POWDER OF BROILERS ON SERUM BIOCHEMICAL AND INTESTINAL MORPHOLOGY

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ABSTRACT

This experiment was conducted to check the effect of varying dietary levels of neem powder of broilers on serum biochemical and intestinal morphology. Ninety six (Ross 308) 1-35 day old chicks were distributed randomly to four treatment groups with four replicates six chicks per replicate. The following treatments were used: T1 (control) the birds were provided basal diet, T2: the birds were provided neem 1g /kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3g /kg to the diet. A complete randomized design (CRD) was used to investigate the effects of the studied treatments on different traits. Result revealed that Significant ($P < 0.05$) differences in the total mean of serum biochemical were observed between treatments; T3 surpassed the other treated groups in glucose and HDL, at values 185.50 and 118.13 respectively. However, T3 and T4 produced the highest of phosphor, which were 9.04 and 9.33. However, T1 produced the highest mean of uric acid and LDL, at value 4.99 and 23.0 respectively. On the other hand, T3 produced the highest mean weight of intestine and a clear increase in the length of the vullus ($P < 0.05$) and the ratio of the crypt depth ($P < 0.05$) within the duodenum, jejunum and ileum, while T3 produced the highest of bursa index. The current results reveal provided of neem powder at 2g / kg had a positive role on the serum biochemical and intestinal morphology.

KEY WORDS: Neem, broiler, biochemical and intestinal morphology.

INTRODUCTION

Neem tree (*Azadirachta indica*) is the mahogany family Meliaceae tree. as one of the most researched is neem tree in the world has attracted world-wide prominence due to its breadth range of medical traits like bactericidal, antiviral, hepatoprotective, antifungal, antiprotozoal and different other traits without side effects^[1]. Neem leaf extract has a good immune stimulant effect that stimulates the cellular immune response and thus creates an enhanced response to any of the challenges posed by the disease. Therefore, the poultry feed Neem extract acts as an effective immune modulator by raising the cellular and humoral immune responses^[2]. Neem tree contain a biologically active ingredients and expansion array of chemically divers^[3]. Neem leave extract at low dose It has a large inhibitory effect of germs and immunomodulator actions that induce cell immune response^[3-5]. Prove that Neem leaf and their components have antiviral properties^[6] declared that these medicinal plants could be used as an alternative to antibiotics as growth promoters, neem leaf powder the potential effectively to improve the growth of broiler^[7]. Neem in poultry, the limited dose of neem leaves was used within the hepatoprotector rate without showing the toxicity effects, and showed that the diet proteins were more effective in the neem-treated chicken^[8]. There are no adverse effects of the dietary meal of neem leaf on the physiological indicators of broiler chicks^[9]. The addition of the neem leaf

meal by 0.05% in the poultry diet improves the performance growth and economic benefit of the birds^[10],^[11] confirmed that the addition of neem successfully improves the immune antibody titre of birds, performance growth and gross return at 50 ml / L in drinking water. Neem tree leaves, melia azadirachta, is a potential intervention against coccidiosis in broiler^[12-14] showed that natural medicinal products originating from herbs have been used animal feed enhanced to increase performance during the improvement of the ingredients of the ration, and improve production. Neem plant is also used for various purposes including pest control, manufacture of shampoos, soaps and tooth paste^[15,16] reported that scientists are focusing more attention on the tree for various reasons including; using it as pesticide, antimicrobial, the use of parts of the plant as fertilizer, animal feed and as treatment of malaria^[17] using medical herbs (Neem leaf) has helped to reduce the concentrations of pathogenic intestinal bacteria in the of broiler intestine under normal or heat stress conditions.

MATERIALS & METHODS

The experiment was conducted in the fields of Animal Resources Department, College of Agriculture, University of Baghdad, for the period from 12/3/2016 to 16/4/2016 (35 days) to study the Impact of fed varying dietary levels of neem powder of broilers on serum biochemical and intestinal morphology. Ninety six (Ross 308) 1-35 day old

chicks were distributed randomly among four treatment groups with four replicates by six chicks for each replicate and purchased from a local hatchery in the holy city of Karbala and the weight of an initial 38 g/chick. The birds were kept in floor pens (1.2×1.8m) in open sided house. The following treatments were used: T1 (control) the birds were provided basal diet, T2: the birds were provided neem 1g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the

diet. Each of them was supplied diet initiator of the chicks from the age of 1-21 days and diet growth from the age of 22-35 days (Table 1) In the diet, the chemical composition of the mixture was calculated according to [18]. The chicks were vaccinated via drinking water with ND (La Sota) at 10 day then booster dose of Newcastle virus vaccine (La Sota) at day (18 and 25) and vaccinated with infectious bursal disease (IBDL strain) (Ceva-Hungary) at day (14).

TABLE 1: Composition of experiment's diets prepared in this study

Constituents	Percentages of ingredients in starter diet	Percentages of ingredients in final diet
Plant Protein (40 % protein)	5 %	5 %
Soybean meal (48 % protein)	25 %	24 %
Yellow corn	39 %	45 %
Wheat	28 %	22 %
Sun flower oil	1 %	2 %
Dicalcium phosphate	1 %	1 %
Minerals and Vitamin mixture	1 %	1 %
Chemical composition		
Crude protein (%)	21.94	20.07
Metabolized energy (kcal / kg)	2921.9	3038.2
Calcium (%)	0.84	0.84
Available phosphorus (%)	0.42	0.42
Lysine (%)	1.20	1.02
Methionine +Cysteine (%)	0.82	0.78

Provided per kg of diet: vitamin A: 22000IU, D3:60, E: 60mg, B1: 60mg, B2: 140mg, B6: 80mg, B12: 700mcg, Biotin: 2.00mcg, Folic acid: 20mg, Vitamin E K3: 5mg, Choline chloride: 7.5mg, Cu: 200mg, Mn: 1.6mg, Zn: 1.2mg, Fe: 1.0mg, I: 20mg, Se: 5mg, Calculated composition of experimental diets according to [18].

In vivo study

Blood samples were collected from (5 samples) of blood from each treatments of any (one bird) of each replicate in the treatments and collected blood samples at the age of 21 and 35 days randomly for each replicate by puncturing wings of the brachial vein zone. The blood was collected in test tubes contained anti-clotting K2EDTA, and placed in a centrifuge of speed 300 r/min for 5 min to separate plasma, then the plasma was transferred to other tubes which were sealed and frozen under (-15 – -20) degrees until testing. Traits concentration of glucose, ca, po4, high density lipoprotein (HDL) and low density lipoprotein (LDL) were measured.

Production parameters

Weekly feed conversion ratio, were recorded. At the end of the feeding trial, five birds per treatment were selected at random and slaughtered for sampling [19]. The intestinal parts were separated carefully; duodenal, jejunum and ilium. The cecum was also separated their length and weight were calculated take parts of the intestines for the purpose of histological study. And the samples were gently flushed

twice with physiological saline (1% NaCl) to remove the intestinal content and placed in 10% Formalin for fixation. The sample was processed for 24 h in a tissue processor with ethanol for dehydration and the samples were in paraffin embedded. (5 µm) section were made from the tissue and stained with eosin and haematoxylin. Slides of Crypt dept, and villi height were measured using light microscopy The measurement of the length of villi conducted mediated scale kind delicate Oculometer be installed on the microscope and then be matched with the slide installed on the theater stage and records the value of the constant and flying, and then replace the glass slide containing tissue and read the measurement of the length of villus and depth of crypts units (Micron) reportedly for [20] and then hits the gross fixed value and be output for Micron Extracting fixed following equation cited by the researchers [20].

$$\text{Fixed} = \frac{\text{Eyepiece scale} \times \text{lens power}}{\text{Oculometer}}$$

Fabricia gland weight and the relative weight

5 glands Fabricia separated from the carcasses of birds each transaction experimental treatments after cutting the connective tissue around the gland and weighed by a sensitive balance. It calculated the relative weight of the gland [19] according to the following equation:

$$\text{The relative weight of the gland Fabricia} = \frac{\text{Gland weight (g)}}{\text{Live body weight}} \times 100$$

Bursa Index

$$\text{Bursa of Fabricia guide} = \frac{\text{The relative weight of the bursa treatment}}{\text{The relative weight of the bursa in control}}$$

Statistical analysis

Complete Randomized Design (CRD) was used to investigate the effect of the studied treatments on different traits. Polynomial (21) was used to compare between means, using [22] program.

RESULTS

Table 2 illustrates the significant differences in the serum levels of uric acid (P < 0.05) between the treated groups during the first period of the experiment. The first treatment

surpassed the other treatments, significant differences in the glucose and phosphor levels were not observed between the treated groups. During the second period of the experiment, significant differences in the uric acid levels were observed between the treated groups, whereas significant differences in the glucose and phosphor levels were observed between the treated groups and Third treatment was superior to the other treatments. The overall mean shows significant differences between the third treatment and the other treatments.

TABLE 2: effect of fed varying dietary levels of neem powder on serum biochemical (mg/100 ml) ± the standard error

Treatments	First period			Seconded period		
	Glucose	Uric acid	Po4	Glucose	Uric acid	Po4
T1	272.70 ± 25.42	11.45± 2.54 a	2.67 ± 1.16	142.45 ±8.78 b	4.99 ± 0.59 a	6.66 ±0.99 b
T2	337.70 ± 55.6	8.30± 1.14 ab	4.11 ± 0.38	167.68 ± 4.62 ab	3.60 ± 0.21 b	8.27 ± 0.94 ab
T3	374.66 ± 69.51	5.98 ± 0.20 b	4.47 ± 0.60	185.50 ± 9.86 a	3.43 ± 0.09 ab	9.04 ± 0.63 a
T4	304.80 ± 67.66	6.26 ± 0.09 b	4.35 ± 0.26	167.67 ± 10.84 ab	4.26 ± 0.23 ab	9.33 ± 0.45 a
Significant	NS	*	NS	*	*	*

* Different letters in the same column indicate significant differences between treatments at P < 0.05. N.S.: no significant difference between treatments. T1(control) the birds were provided basal diet, T2: the birds were provided neem 1 g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the diet.

The results presented in table 3 show significant differences in the blood lipid profile levels (P < 0.05) between the treated groups during the first period of the experiment. The first treatment produced higher levels of LDL, as for the concentration of HDL treatment third was superior significantly (P <0.05) contrast of other groups in the first

period, results refer to significant differences (P <0.05) of HDL between third treatment and treatment was superior to the others in the second period and compared with the other treatments T1 and T2 and T4. whereas no significant differences were observed in the LDL levels between the treated groups.

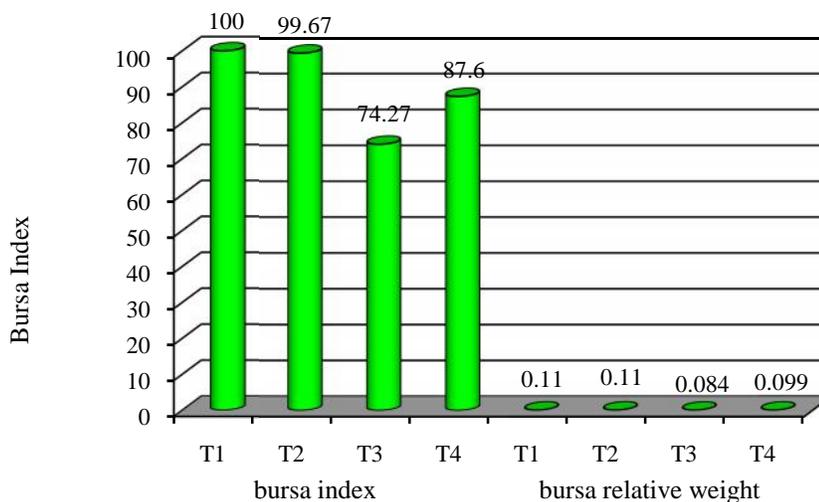


FIGURE 1: impact of fed varying dietary level with neem on bursa index and bursa relative weight

TABLE 3. effect of fed varying dietary levels of neem powder on lipid profile (mg/100 ml) \pm the standard error.

Treatments	First period		Seconded period	
	LDL	HDL	LDL	HDL
T1	23.0 \pm 2.64 a	99.91 \pm 4.99 b	24.75 \pm 8.51	83.33 \pm 9.20 b
T2	14.0 \pm 0.57 b	107.97 \pm 6.59 ab	14.25 \pm 3.32	107.36 \pm 8.94 ab
T3	13.0 \pm 0.57 b	122.53 \pm 5.89 a	12.25 \pm 3.32	118.13 \pm 9.30 a
T4	16.0 \pm 3.21 b	104.57 \pm 4.73 ab	11.0 \pm 1.63	84.97 \pm 5.09 b
Significant	*	*	NS	*

* Different letters in the same column indicate significant differences between treatments at $P < 0.05$. N.S.: no significant difference between treatments. T1 (control) the birds were provided basal diet, T2: the birds were provided neem 1g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the diet.

The results of the statistical analysis, there were significant differences in the relative weight of the Bursa of Fabricia gland. Results refer to significant differences ($P < 0.05$) of the relative weight of the Fabricia gland in third treatment compared with the other treatment. As for the guide Bursa of Fabricia gland is noticed a significant differences ($P < 0.05$) of third treatment compared with the other treatment.

The impact of fed varying dietary level with neem on feed conversion(FC) is present in table 4 In 2nd and 3rd week the FC no significant differences were observed between the treated groups .third and fourth treatment was significantly ($P < 0.05$) better than other treatment in 4th and 5th weeks.

TABLE 4. effect of fed varying dietary levels of neem powder on feed conversion \pm the standard error

Weeks	Treatments				Significant
	T1	T2	T3	T4	
1	1.83 \pm 0.37	1.69 \pm 0.20	1.61 \pm 0.15	2.02 \pm 0.16	N.S
2	1.51 \pm 0.22	1.62 \pm 0.10	1.59 \pm 0.03	1.55 \pm 0.08	N.S
3	1.34 \pm 0.06	1.19 \pm 0.10	1.33 \pm 0.05	1.44 \pm 0.09	N.S
4	1.20 \pm 0.14 b	1.34 \pm 0.07 ab	1.63 \pm 0.01 a	1.44 \pm 0.07 ab	*
5	1.63 \pm 0.09 b	1.71 \pm 0.10 b	2.15 \pm 0.12 a	2.17 \pm 0.17 a	*

* Different letters in the same column indicate significant differences between treatments at $P < 0.05$. N.S.: no significant difference between treatments. T1 (control) the birds were provided basal diet, T2: the birds were provided neem 1 g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the diet.

Morphological measurements of the duodenum, jejunum, ileum and cecum are shown in Table 5, 6 and 7. Treatment fed on neem had higher ($P < 0.05$) weight and length of duodenum, jejunum, ileum and cecum in third treatment when compared with other treatment. whereas significant differences ($P < 0.05$) in the length of the villi and crypts

depth and the ratio between them in the duodenum, jejunum and ileum Noting that there is a superior highly significant ($P < 0.05$) in the length of the villi (villi length) and the depth of the crypts (crypts depth) and the ratio between (Height villi to the crypt ratio of depth) in favor of the Third treatment was superior to the compared with the other treatment.

TABLE 5. effect of fed varying dietary levels of neem powder on length of duodenum, jejunum, ileum and cecum \pm the standard error

Treatments	Length of duodenal	length of jejunum	Length of ileum	length of cecal	length of large intestines
T1	27.31 \pm 0.48 c	77.12 \pm 1.14 c	64.54 \pm 0.27 d	28.33 \pm 5.78	7.32 \pm 0.01 b
T2	29.83 \pm 0.06 b	81.16 \pm 0.55 bc	75.30 \pm 0.19 c	32.33 \pm 5.78	7.32 \pm 0.01 b
T3	33.68 \pm 0.55 a	88.43 \pm 1.49 a	84.00 \pm 0.50 a	37.67 \pm 1.86	8.09 \pm 0.05 a
T4	28.83 \pm 0.88 bc	83.57 \pm 1.68 b	79.30 \pm 0.44 b	27.00 \pm 6.03	7.71 \pm 0.33 ab
Significant	*	*	*	N.S	*

* Different letters in the same column indicate significant differences between treatments at $P < 0.05$. N.S.: no significant difference between treatments. T1 (control) the birds were provided basal diet, T2: the birds were provided neem 1g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the diet.

TABLE 6.effect of fed varying dietary levels of neem powder on weight of duodenum, jejunum, ileum and cecum \pm the standard error

Treatments	Weight of duodenal	Weight of jejunum	Weight of ileum	Weight of cecal	Weight of large intestines
T1	1.14 \pm 0.01d	1.74 \pm 0.10 c	1.72 \pm 0.04 c	0.66 \pm 0.11b	0.22 \pm 0.02
T2	1.35 \pm 0.01 c	2.08 \pm 0.12 b	1.99 \pm 0.01 b	0.75 \pm 0.05b	0.25 \pm 0.04
T3	2.01 \pm 0.005 a	2.58 \pm 0.01a	2.42 \pm 0.08 a	1.17 \pm 0.05a	0.25 \pm 0.01
T4	1.55 \pm 0.07 b	2.01 \pm 0.07c b	2.03 \pm 0.10 b	1.05 \pm 0.01a	0.26 \pm 0.01
Significant	*	*	*	*	NS

* Different letters in the same column indicate significant differences between treatments at $P < 0.05$.

N.S.: no significant difference between treatments. T1(control) the birds were provided basal diet, T2: the birds were provided neem 1 g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the diet.

TABLE 7.effect of fed varying dietary levels of neem powder on Intestinal morphological of duodenum, jejunum, ileum and cecum \pm the standard error

Intestinal morphological	Treatments				Significant
	T1	T2	T3	T4	
Duodenum					
Length of villi	92.16 \pm 1.41 b	97.44 \pm 2.20 b	122.57 \pm 3.87 a	115.40 \pm 1.51 a	*
crypt width	27.61 \pm 0.83 c	33.48 \pm 1.60 b	41.80 \pm 3.18 a	37.76 \pm 0.75 b	*
The ratio of villus height to crypt width	3.16 \pm 0.32 b	3.68 \pm 0.25 ab	4.44 \pm 0.39 a	4.82 \pm 0.31 ab	*
Jejunum					
Length of villi	85.92 \pm 3.53 b	89.14 \pm 1.93 ab	96.24 \pm 1.94 a	92.84 \pm 2.33 ab	*
crypt width	21.90 \pm 1.07 c	24.82 \pm 1.70 bc	31.98 \pm 1.92 a	28.38 \pm 0.70 ab	*
The ratio of villus height to crypt width	2.49 \pm 0.43 b	2.86 \pm 0.32 ab	3.92 \pm 0.29 a	2.96 \pm 0.30 ab	*
Ileum					
Length of villi	39.55 \pm 1.15 d	46.04 \pm 0.70 c	61.46 \pm 0.82 a	58.02 \pm 0.77 b	*
crypt width	8.56 \pm 0.33 c	9.21 \pm 0.86 c	12.08 \pm 0.54 a	10.30 \pm 0.25 b	*
The ratio of villus height to crypt width	1.63 \pm 0.04 d	1.82 \pm 0.02 c	2.87 \pm 0.03 a	2.12 \pm 0.007 b	*

* Different letters in the same column indicate significant differences between treatments at $P < 0.05$.

N.S.: no significant difference between treatments. T1(control) the birds were provided basal diet, T2: the birds were provided neem 1 g / kg to the diet, T3: the birds were provided neem 2 g / kg to the diet and T4: the birds were provided neem 3 g / kg to the diet.

DISCUSSION

The results of the study of the serum traits were consistent with the results reported by [23] the use of neem leaves in a diet chicken meat led to the increase in productivity traits and blood traits. While results were treated neem (T3) matching with the findings of the [24]. Add neem improve increase the weight and the rate of feed conversion efficiency and impact on blood traits [9] suggested that the addition of neem leaves to chicken meat diet has an effect on blood traits and blood serum and he found a increase in blood sugar is significant differences while the observed decrease in the level of cholesterol is significant differences ($p < 0.05$) also mention that increasing the concentration of leaf Neem lead to a reduction in liver enzymes [17] who observed the use of neem leaves in a diet chicken meat has increased the efficiency of feed conversion and feed consumption and increase the weight and the rate of Mortality and carcass traits in addition to the lymph organs (Bursa of Fabricia, Thymus gland) and an increase in total

protein, albumin , globulin and triglycerides compared with cholesterol and fat College was low [6], who reported the use of neem leaves led to an increase in the rate of body weight and feed conversion. Increasing Bursa of fabricia gland weight guide for the existence of causes of unsatisfactory and that the lack of the bursa weight and guide standard of the health status of birds [25]. The increase in weight of intestine observed among the birds fed the experimental diets could be attributed to the inflammatory response to the neem toxins [16, 26] explain that increased weights of the proventriculus and intestine of all the broilers fed on *Napoleona imperialis* seed meal.

Increasing the height of the villus to the depth of Crypt is associated with the massive numbers of bacteria acid Lactobacillus and Bifidobacterium in the gastrointestinal tract, which produces a range of fatty acids short-chain, which is a source of energy for the intestinal cells (Enterocytes) for the purpose of sustaining and continuously updated to perform vital functions [27-29] showed that the

increase villus height relative to the depth of crypt results from the intestinal formation, which is more digestible, with improved absorption and protein analysis, in addition to a few requirements of the food components of the intestinal maintenance^[30] proved that the length of the villi has to do with improving the intestine healthy as well as increase in the length and height of both duodenum and jejunum^[31] showed that the increase in the elevation of the villus of the small intestine is due to the role of intestinal epithelium, which acts as a natural barrier against the pathogenic bacteria and toxins found in the intestinal lumen. Acknowledgement to the College of Veterinary Medicine and the College of Agriculture / University of Baghdad.

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