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ECOFRIENDLY SYNTHESIS OF CALCIUM NANOPARTICLES WITH BIOCOMPATIBLE ROSMARINUS OFFICINALIS EXTRACT ON PHYSIOLOGICAL AND IMMUNOLOGICAL EFFECTS IN BROILER CHICKENS

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ABSTRACT: The point of this examination was to research the impacts of dietary supplementation with rosemary extract 1g/kg diet and synthesized calcium nanoparticles with rosemary extract (0.5 and 1 g/kg) on growth performance, blood profiles and histology in broilers. A total of one hundred twelve1-d-old unsexed broiler chicks were randomly allotted to 4 treatments with 4 replications per treatment and 28 chicks per pen floor. Rosemary extract at 1 g/kg diet, nano rosemary (0.5 and 1.0g/kg) were significant of Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) as compared to control group in the starter period. On the other hand, no significant differences were observed among the control group, rosemary extract and nano-rosemary (0.5 and 1 g/kg diet) groups in feed intake and feed conversion ratio during the whole experimental period. Dietary supplementation with rosemary extract (1 g/kg diet), or rosemary (0.5 and 1 g/kg diet) did not significantly affect the plasma levels of glucose, total protein, albumin and high-density lipoprotein (HDL). But, the supplemented groups with rosemary extract and nano- rosemary 0.5 g/kg had slightly higher concentrations of HDL compared with other groups. However, dietary supplementation with rosemary extract and nano-rosemary led to a significant reduction in plasma low-density lipoprotein (LDL) concentrations compared with the control group. The results related for humoral immune response of broiler chickens showed significant increases in immunoglobulins in the treated groups compared with the control group. The rosemary extract and Nano-rosemary (0.5 and 1.0 g/kg), applied in the present study, significantly increased total antioxidant capacity (TAC) in the treated broiler chickens as compared to the control group. In a similar way, malondialdehyde (MDA) was significantly lower in broiler chickens received the rosemary extract containing diets compared with the control group. The present outcomes show that supplemental nano rosemary of diet has a beneficial effect on lipid profile, immunity, antioxidant status and histological observations of broiler chicken.

Key words: Broilers, Nano Rosemary, Lipids Profile, Immune Response, Antioxidant Status

1. INTRODUCTION

To discover viable substitutes to antibiotic growth promoters, several aromatic plants have been evaluated in poultry industry (Abudabos *et al.,* 2016). These herbs, or their extract, show significant antimicrobial, antifungal, antioxidant, and physiological modulation proprieties (Jang *et al.,* 2007). In addition, natural extracts also can serve as natural antioxidant limiting lipid oxidation in meat and meat products (Shah et al., 2014). Rosemary (Rosmarinus officinalis L.) is a native plant of the Mediterranean countries widely used as aromatic and medicinal plant (Charles, 2013).

Nanotechnology is a field of applied science and technology which aims to develop devices and dosage forms in the range of 1 to 100 nm. The uses of nanotechnology for treatment, finding, checking, and control of biological systems. The nanocarriers have been made of safe materials, including manufactured biodegradable polymers, lipids, and polysaccharides. The movement of herbal medicines relies upon generally function of a variety of active components, as all the constituents provide synergistic action and thus enhance the therapeutic value. Each active constituent plays an important role and they are altogether identified with one another. In phyto-definition look into, creating Nano dose structures (Polymeric Nanoparticles, Liposomes, Proliposomes, Solid Lipid Nanoparticles, Nano emulsion) has large number of advantages for herbal drugs, including improvement of solvency and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation, etc. Thus, the nano-sized drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines (Ansari *et al.,* 2012)

Rostami *et al.* (2015) investigated that the effects of rosemary powder and vitamin E as feed additives on several performance parameters. They found that dietary addition of 0.5% rosemary powder promoted insignificantly higher feed intake and weight gain at 42 days compared with 1.0% rosemary powder however, the obtained values were not different from those obtained in the control group. Gharejanloov *et al.* (2017) found that adding different levels of rosemary essential oil (0, 100, and 200 mg/kg) to the diets of broiler chickens revealed a numerical increased in their feed intake during all experimental periods. but body weight gain, feed conversion ratio, carcass breast and thigh relative weights were not affected as compared to control group.

Polat *et al.* (2011) showed that serum triglyceride and total lipid levels were statistically higher in rosemary oil supplemented group than other groups of broiler chickens. Ashan (2011) showed that feeding rosemary supplemented diet led to a decrease in cholesterol and triglyceride in broilers blood. Belenli *et al.* (2015) found that serum glucose level and growth hormone activity were not affected by dietary addition of volatile oils, but levels of cholesterol and total lipids were significantly reduced in the treated compared with the control group.

The rosemary has been reported to attenuate the increase of lipid peroxidation and enhance the levels of reduced glutathione and antioxidant enzyme activities in the kidney and testis when

compared to aspartame (Hozayen *et al.*, 2014). ELnaggar *et al.* (2016) studied the effect of adding addition of rosemary leaves meal to the broiler diet at the concentrations of 0.25, 0.5, 0.75, and 1.0 %. They observed a significantly improvement in the serum immunoglobulins (IgY, IgM, and IgA), interferon-γ and interleukin-10. Ghozlan *et al.* (2017) found that the dietary supplementation of rosemary leaves meal (0.5, 1.0, and 1.5%) to the broiler chickens significantly increased the serum total protein and globulin, while significantly decreased total cholesterol and triacylglycerol levels. They also reported that added rosemary significantly increased the levels of IgG, IgM, interferon-γ, interleukin-10, and muscle reduced glutathione, and activities of total superoxide dismutase and glutathione S-transferase, muscle malondialdehyde levels were significantly decreased, so rosemary could be considered as a natural antioxidant in broiler diet. The influence of rosemary (2 g/kg rosemary aqueous extract), added either alone or in combination with oregano, on intestinal microbial population was investigated by Franciosini *et al.* (2016). Khazaei et al. (2017) demonstrated that a commercial antioxidant blend and rosemary powder added to diets having 6.0% poultry fat can be advantageous mainly in order to modulate the caecal coliform microflora and improve humoral immunity against infectious Bronchitis in broilers. Thusly, this investigation was expected to evaluate the valuable effect(s) of dietary Nano rosemary on performance, blood biochemical, immune responses and histology of broiler chickens.

2. MATERIALS AND METHODS

The experimental work of the present study was carried out on a private farm in Dakranis, Dakahlia Governorate, Egypt, from March to April 2018. The objective of the present study was to evaluate the effect of the nano-herb plant as a nutritional supplement in the growth performance, carcass yield, some blood metabolites, immunity, lipid peroxidation and histology in broilers. This section includes the following subtitles.

2.1. Preparation of investigated plant extracts:

Rhizomes of rosemary were purchased from the local market, Mansoura city, Egypt. Final moisture content of raw material was 10±0.4%. Then they were and crushed to fine powder using Braun GmbH Grinding (Model, KSM2; Type, 4041). Ground plants were screened to be fine enough to pass through a sieve size of (75-100 μm).

The extraction of the rosemary was set up as per the technique articulated by Dent *et al.,* (2013). Precisely 5 g of the plant powder was removed independently utilizing 100 mL of Ethanol (30%) and performed at 60°C for 30 minutes on a level water shower shaker (Memmert WB14, Germany). The concentrates were then sifted through Whatman no. 1 channel paper (Whatman International Ltd., Kent, UK) utilizing a Büchner pipe and the filtrates were acclimated to 100 mL in volumetric cups with suitable deionized water. The concentrates were put away at - 18°C till investigation.

2.2. Synthesis of metal nanoparticles:

Calcium nanoparticles were ecofriendly combined utilizing the strategy revealed by Yugandhar and Savithramma (2013) with a slight change. Watery arrangement of calcium chloride dihydrate, (S.D fine

substance constrained, India: 0.05 M) was readied utilizing deionized water and added gradually to a similar volume of the readied plant extricates. The performed blend was mixed at 5000 rpm for 1 hour at room temperature (25±1ºC) for 2-3 days. At that point, they were lyophilized to a fine powder. Phenolic components of rosemary extract used as agent to transform calcium chloride from ionic state to nanoparticles state, which characterized with ultraviolet, TEM and zeta potential.

2.3. Nanoparticles Characteristic *via* ultraviolet-Vis spectroscopy:

The reduction of unadulterated Ca^{++} particles and covering of the subsequent calcium nanoparticles were checked utilizing ATI Unicom UV-Vis Spectrophotometer vision programming V 3.20, by identifying the UV-Vis spectra of the response blend at various wavelengths. The UV-Visible spectra of the integrated metal nanoparticles were recorded around 240-440 nm. The examination was cultivated at 25ºC utilizing quartz cuvettes (1 cm optical).

2.4. Nanoparticles characteristic *via* Transmission Electron Microscopy (TEM):

The size, shape, surface region, precious stone structure and morphological information of the got nanoparticles were portrayed utilizing Transmission Electron Microscopy (TEM), (JEOL TEM-2100) joined to a camera at a quickening voltage of 200 kV. Each example of the blended metal nanoparticles was set up by including a suspension of the example on frameworks of carbon covered with copper and the dissolvable was permitted to be vanished gradually before recording the TEM pictures. The TEM estimations were recorded at "Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt".

2.5. Nanoparticles characteristic *via* zeta potential:

Zeta potential analysis is a technique for determining the surface charge of nanoparticles in suspensions using Malvern Instruments Ltd. Zeta Potential Ver. 2.3 at "Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt".

2.6. Birds, management and experimental Design:

One hundred and twelve one-day-old Cobb500 were divided into four treatment groups, of 28 birds each. Each of which includes four replicates Each of 7 birds (floor of pens). The groups were assigned to four diet treatments (0.0 control group, 1 g / kg of rosemary extract powder, 0.5 g / kg Nano rosemary group and 1 g / kg Nano rosemary group) with four repetitions of 28 birds for 42 days. The birds were raised in floor and long pens, the width of each pen was 70 and 70 cm, respectively. Therefore, the floor area of the pen was 0.49 m^2 (70 × 70 cm). The chickens were raised until 42 days of age and fed with a starter diet of one to 21 days (3199 kcal / kg diet and 23% of CP) and the grower diet of 22 to 42 days (3200 kcal / kg diet 21% CP). That was formulated to cover or exceed recommended requirements or broilers according to NRC, (1994). Pureed food and water were provided freely. The composition and chemical analysis of the experimental items are shown in Table 1.

2.7. Performance of broiler chickens.

Live body weight (LBW), food intake (FI) and body weight gain (BWG) were measured weekly throughout the experimental period, then the feed conversion ratio (FCR) was calculated (g

feed:g gain). The birds were weighed individually to the nearest gram early in the morning before receiving any feed or water at weekly intervals during the experimental period. Live body weights of broilers were recorded at the beginning of the experiment and weekly thereafter. Weekly records on FI and BWG of broilers were also kept in the form of a replicated group. Consequently, the FCR was calculated as the amount of feed consumed per unit of BWG.

2.8. Carcass characteristics:

At the end of study, three chickens for every treatment, whose LBW were around the normal weight of their respective group, were chosen for slaughter test. The chickens were independently weighed, immediately sacrificed and reweighed after complete bleeding. Their carcasses feather was plucked and then eviscerated. Records on weights of carcasses and giblets (including liver, kidney, gizzard and heart) were kept up.

2.9. Blood sampling and biochemical analysis:

Three birds were chosen from each treatment, sacrificed and blood samples were collected in heparinized tubes and then centrifuged at 4000 rpm for 15 minutes. Plasma samples obtained were stored at -20°C until analysis. Plasma samples were analyzed colorimetrically using commercial kits according to the procedures described by the manufacturers, for the determination of glucose (Trinder, 1969), total protein (Doumas *et al*. 1981), albumin (Doumas *et al.* 1971) , total lipids (Frings and Dunn, 1970), cholesterol (Allain *et al*., 1974), triglycerides (Fossati and Prencipe, 1982), high density lipoprotein (HDL) (Myers et al. 1994) and low density lipoprotein (LDL) (Friedewald *et al*. 1972) Total antioxidant capacity (TAC) was determined using the kit available according to Koracevic *et al*. (2001) and malondialdehyde (MDA) determined by kits available according to Mihara and Uchiyama (1978). Immunoglobulins (IgG, IgA and IgM) were determined by the ELISA technique as reported by Engvall and Perlmann (1972)**.**

2.10. Tissues specimens and histological procedures:

Tests of duodenum, bursa of Fabricius and liver tissues were taken during slaughter, quickly fixed in a 10% formalin saline solution and then dehydrated in ascending concentrations of alcohol solutions ranging between 70% and absolute ethanol alcohol. The samples were cleaned in xylene, and then inserted into dissolved paraffin wax, to obtain tissue blocks. They were then segmented and redone with hematoxylin and eosin (Junquerira *et al*. 1971). Sections were inspected with an optical microscope and photographed using a digital camera.

2.11. Statistical analysis:

A completely randomized design was used. Measurable examination for the acquired information was performed by one-way analysis of variance (SAS, 2006). Duncan's new multiple range test was utilized to separate significant differences among means (Duncan, 1955).

3. RESULTS AND DISCUSSIONS

Some chemical and biological studies were carried out on rosemary (*Rosemarinus Officinalis*), belonging to family *Lamiaceae*. The study included the preparations of nanoparticles from rosemary using the aqueous and ethanoic (30%) extracts via reducing mineral ion to form zero valence. Zero valence of metals aggregated around bioactive compounds

was used to form nanoparticles of the investigated plants.

3.1. Nanoparticles characteristic *via* UV-Vis spectroscopy:

The preparation of calcium nanoparticles has been clarified by examining the UV–Vis spectra. As appeared in Fig. 1, the most extreme ingestion top that recorded at 280 nm is because of the trademark surface Plasmon reverberation of the created metal nanoparticles. The readied calcium nanoparticles were seen as truly stable because of the conceivable nearness of polyphenolic components in the rosemary remove that counteract collection. The polyphenolic components are considered as a cell reinforcement operator with explicit concoction structure and have a basic job in the decrease procedure for amalgamation of metal nanoparticles. The properties of handled nanoparticles were inspected as an element of UV illumination. The utilization of UV–Vis spectroscopic investigation is a compelling technique for exhibiting the nearness of metal nano-structures (Sun *et al.,* 2002; and Darroudi *et al.,* 2011). The UV light job was affirmed to portray the advancement of calcium salt decrease within the sight of rosemary separate at encompassing temperature.

3.2. Nanoparticles characteristic *via* transmission electron microscope (TEM):

Calcium nanoparticles were prepared utilizing rosemary extract. They were portrayed by TEM estimations to affirm the nearness of Ca nanoparticles to appraise the shape, accumulation and particles size of incorporated nanoparticles as indicated by Yugandhar and Savithramma (2013). As appeared in Fig. 2, TEM was performed for the integrated nanoparticles at 100 nm amplification esteem. The size of the particles extended between 23.70 to 38.45 nm. The state of particles was circular with square collection and less numbers were tetragonal. Littler particles causing increasingly surface zone which were motivation to progressively viable reactions.

3.3. Nanoparticles characteristic *via* zeta potential:

Zeta potential is a significant device for understanding the condition of the nanoparticle surface and anticipating the long-haul solidness of the nanoparticle. Nanoparticles have a surface charge that draws in a meager layer of particles of inverse charge to the nanoparticle surface, Zeta potential method was utilized to decide the nanoparticles surface charge. Nanoparticles have a twofold layer of particles that movements as it diffuses all through the arrangement, the electric potential at the limit of the twofold layer is known as the Zeta capability of the particles and had values that commonly went from +100 to -100 milli volt (mV) mean worth. Fig. 3 indicated that the orchestrated calcium nanoparticles utilizing rosemary remove had a Zeta Potential estimation of 2.24 mV which had a high strength because nanoparticles with Zeta potential qualities lesser than +25 mV or greater than -25 mV were accounted for to have high degrees of dependability (Honary and Zahir, 2013).

3.4. Growth performance of rosemary-fed broilers:

The effects of dietary rosemary extract and nano-rosemary supplementation on broiler chicken's performance from one to 42 days of age are presented in Table 2. The LBW, BWG, FI and FCR of broilers fed the basal

diet, rosemary extract at 1 g/kg, nano-rosemary (0.5g/kg) and Nano rosemary (1.0 g/kg) were significant of LBW, BWG, FI and FCR as compared to control group in the starter period. On the other hand, no significant differences were observed among the control group, rosemary extract and nano-rosemary (0.5 and 1 g/kg) groups in feed intake and feed conversion ratio during the whole experimental period but, there were erratic differences among the groups fed rosemary extract and Nano - rosemary (0.5 and 1 g/kg) in live body weight and body weight gain as compared to the control group in the whole experimental period.

These results are in line with those of Al-Kassie. (2008) that feeding diets containing 0.5 and 1% rosemary herb supplementation in the diet clearly improved broiler growth performance at 42 days of age. compared with the control treatment. Ghazalah and Ali (2008) also found that broiler chickens fed 0.5% rosemary herb supplementation in their diet led to better growth response than the control treatment at 49 days of age. Sarica *et al.* (2005) reported that dietary inclusion of herbal natural additives such as thyme, rosemary, garlic and cinnamon significantly increased broiler performance. In agreement with the current result, Petričević *et al.* (2018) found that the use of rosemary during the starter and grower periods had no significant effect on feed intake, average daily gain, or feed conversion ratio of broiler chickens. However, in the finishing period, rosemary addition in their diets improved the feed conversion ratio. On the other hand, Yesilbag *et al*. (2011) observed that no significant effects of dietary rosemary supplementation on broilers live weight gain. Similarly, Gharejanloov *et al.* (2017) found that dietary levels of rosemary essential oil (0, 100, and 200 mg/kg) supplementation to broilers caused a slight increase in their feed intake during all experimental periods. but, supplementing the rosemary essential oil to the diets had no effect on BWG and FCR of the chicks.

3.5. Carcass yield of rosemary- fed broilers:

Table 3 summarizes the effect of dietary rosemary extract and nano-rosemary supplementation on carcass characteristics at 42 days of age. Compared with the control group, weights of carcass, heart, spleen and abdominal fat of broiler chickens were not significantly influenced by the dietary supplementation with rosemary extract and nano-rosemary at the end of the experiment; however, the weights of liver, giblets and bursa were significantly higher in chickens of the control group than other groups. But the gizzard was significantly heavier in chickens of nano-rosemary (0.5g/kg) group than that of chicks received 1.0 g/kg of nano-rosemary.

These outcomes are in concurrence with those of Gharejanloov *et al.* (2017), who found that carcass, breast and thigh relative weights of broilers were not statistically influenced by the supplementation levels of rosemary essential oil (100, and 200 mg/kg). Also, Rostami *et al*. (2017) discovered that the relative weights of carcass and several carcass parts were similar in all treatment groups, proposing that the 0.5 and 1.0% of rosemary supplementation with or without vitamin E had no adverse effect on carcass biometric characteristics. On the other hand, Hernández *et al.* (2004) found that no differences in weights of gizzard and liver of broiler chickens fed on wheat–

soybean meal-based diets supplemented with two plant extracts (an essential oil extract from oregano, cinnamon and pepper and a Labiatae extract from sage, thyme and rosemary)**.**

3.6. Blood profile of rosemary-fed broilers:

The effects of dietary supplementation with rosemary extract (1 g/kg diet) and nano-rosemary on blood plasma level of glucose, total protein, albumin, total lipids, triglyceride, cholesterol, HDL and LDL of broiler chickens are shown in Table 4. Dietary supplementation with rosemary extract (1 g/kg diet), or rosemary (0.5 and 1 g/kg diet) did not significantly affect the plasma levels of glucose, total protein, albumin and HDL. But, the supplemented groups with rosemary extract and nano- rosemary 0.5 g/kg had slightly higher concentrations of HDL compared with other groups. However, dietary supplementation with rosemary extract and nano-rosemary (0.5 and 1 g/kg) led to a significant reduction in plasma LDL concentrations compared with the control group. This result agrees with that illustrated by Belenli *et al.* (2015) who observed no significant differences in serum glucose levels of broiler chicks supplemented with various volatile oils. They also noticed no significant changes in serum total cholesterol levels among the experimental groups fed the different dietary treatments. Ghazalah and Ali (2008) found that dietary levels of rosemary leaf meal caused no significant differences in blood albumin, however the albumin / globulin ratio was reduced in broilers fed diet supplemented with 0.5% rosemary leaf meal compared with the control and other treatment groups (0.5, 1.0 and 2.0% of rosemary leaf meal). They suggested that this reflects the ability of chicks to store reserve protein even after the body has reached its maximum capacity for depositing protein to tissues. In this regard, Ashan (2011) showed that feeding –supplemented diets resulted in reductions in levels of cholesterol and triglyceride in broilers blood. Additionally, Ghozlan *et al*. (2017) stated that the rosemary oil supplemented group displayed the highest levels of serum total protein as compared to other groups. Also, he found that the dietary supplementation of rosemary leaves meal (0.5, 1.0 and 1.5%) significantly increased the serum total protein and globulin, while significantly decreased total cholesterol and triacylglycerol levels. On the other hand, Polat *et al*. (2011) found that serum triglyceride and total lipid levels were statistically higher in rosemary oil - supplemented broiler chickens than other groups.

3.7. Immune response and antioxidant status of rosemary-fed broilers:

The effects of dietary supplementation with rosemary extract (1 g/kg diet), nano-rosemary (0.5g/kg diet) and nano-rosemary (1g/kg) on immunoglobins (IgG, IgA and IgM), total antioxidant capacity (TAC) and malondialdehyde (MDA) of broiler chickens are shown in Table 5. The results related for humoral immune response of broiler chickens, reported here, showed significant increases in immunoglobulins in the treated groups (rosemary extract, nano- rosemary 0.5g/kg and nano-rosemary, 1 g/kg) compared with the control group. This result agreed with that of Abd El-Latif *et al.* (2013) who found that dietary rosemary and garlic oils supplementation (100 or 200 mg rosemary oil /kg, produced a significant increase in

phagocytic activity and phagocytic index in all treated groups but all dietary treatments failed to induce any significant effect on antibody titers at any age. Harmony with the present results, ELnaggar *et al*. (2016) reported that the addition of rosemary to the broiler diet at the concentrations of 0.25, 0.5, 0.75, and 1.0 % led to a significant improvement in the serum immunoglobulins (IgY, IgM, and IgA), interferon-γ and interleukin-10. Also, Ghozlan *et al*. (2017) found that dietary supplementation of rosemary leaves meal (0.5, 1.0, and 1.5%) significantly increased the IgG, IgM, interferon-γ and interleukin-10. significantly increased muscle reduced glutathione levels and activities of total superoxide dismutase and glutathione S-transferase compared with the control group. Whereas, muscle malondialdehyde levels were significantly decreased, so rosemary could be considered as a natural antioxidant in broiler diet. Working with growing rabbits, El-Gogary *et al*. (2018) observed no significant influence of dietary supplementation with rosemary essential oil (0.25, 0.5 and 0.75 g/kg) on IgG and IgM but, rabbits received 0.5 g/kg rosemary essential oil in their ration had significantly higher level of IgA than the other groups.

It is of incredible interest to take note of that additional dietary rosemary extract and nano- rosemary (0.5 and 1.0 g/kg), applied in the present study, significantly increased TAC in the treated broiler chickens as compared to the control group. In a similar way, MDA was significantly lower in broiler chickens received the rosemary extract containing diets compared with the control group. These beneficial effects of added dietary rosemary essential oil may be attributable to its antioxidant properties. Our outcomes are additionally in concurrence with Bakırel *et al.* (2008), who observed that rosemary extract level (200 mg/kg) fundamentally diminished the degree of serum MDA in rabbits. It is commonly acknowledged that nature of animal products is commonly improved because of dietary supplementation of rosemary basic oils for animals. The rosemary fundamental oil are rich sources of natural antioxidants, for example, the phenolic mixes, and because of their high redox properties and synthetic structure they have and their capacity to neutralize the free radicals produced (Zheng and Wang, 2001). Indeed, MDA is a result of lipid peroxidation and it is utilized as a pointer of cell membrane injury. It is ordinarily recognized that an opposite connection exists between concentration of MDA and HDL-C cholesterol.

3.8. Histological observations of rosemary- fed broilers:

3.8.1. Duodenal histology:

Histological examination of the duodenal sections from birds fed rosemary-supplemented diets is illustrated in Fig 4-7. It is clear from these sections that the villi size and shape are greatly influenced by different treatments. The number of villi/microscopic filed was more abundant in the control section (Fig. 4) than in the other treatments.

The histological appearance of duodenal sections from chicks fed rosemary-supplemented diets showed an interesting result. The villi height groups fed rosemary extract was greater in nano-rosemary (0.5 g/kg) Fig (5 and 6, respectively). This increase was accompanied by a very well-developed crypt with different numbers and sizes, along with many goblet cells in the surface

epithelium of the crypts and in the covering epithelial lining of the villi.

It is accepted from the past histological perceptions that the expanded villus tallness is paralleled by an expansion in the stomach related chemicals action and the absorptive capacity of the small digestive tract sections because of expanding the retention surface region. In this respect, Hodges (1974) claimed that the crypts of Lieberkùhn are the main source of epithelial cells lining the villi which contain numerous goblet cells, endocrine cells, lymphocytes and undifferentiated cells. It is well known that the crypts had the ability to secrete fluids containing different vital substances essential for enhancing the internal micro-environment of the gastrointestinal tract (GIT) segments. These fluids are almost pure extracellular fluids with a neutral pH in the range of 6.5 to 7.5 which act as in a watery vehicle supply for improving nutrients absorption, elaboration and production of antibodies and lymphocytes along with an increase in goblet cells that function as mucous secreting glands and (or) intestinal hormones, especially secretin (Denbow, 2015).

3.8.2. Bursa of Fabricius histology:

The bursa of Fabricius is a primary lymphoid organ in birds. In general, it is composed of about 15-20 plicae (folds) each of them contains several follicles (B). These follicles have two distinct areas, cortex and medulla (C, M) enclosed in a pseudostratified columnar epithelial layer (e) as clearly illustrated in Fig. 8 (1 g/kg rosemary extract). The cortex is more deeply-stained than the medulla, since it is composed of many small lymphocytes. The medulla is composed of undifferentiated epithelial cells and lymphoblasts which appeared as a pale-stained area. This structure was clearly observed in the control section; however, the bursa follicles were enlarged with relatively fine connective tissue septa between follicles and many small lymphoblasts in the medullary area (Fig. 9).

The observed improvement of the bursa structure in birds fed rosemary extract (1g/kg) -supplemented diet (Fig. 10) and those fed nano rosemary (0.5 g/kg) diet (Fig. 11) which was the best section of all studied chicks.

It is likely that both forms and levels of rosemary supplementation to broiler diets could stimulate bursal follicles to produce many lymphocytes that help improving the immune response of birds. This hyperactivity is associated with the presence of many lymphocytes in the medullary area with many phagocytes and macrophages in the luminal areas in between bursal plicae. There is also many plasma cells and dendritic secretory cells within the lumina of bursal follicles. These cells are responsible for phagocytosis and for maintaining B-cells production as reported by Glick (1983). The birds fed different nano rosemary-supplemented diets had better immunity in terms of higher plasma levels of immunoglobulins.

3.8.3. Liver histology:

Histological examination of liver section showed normal hepatic structure of liver from the control treatment (Fig. 12), in which the hepatocytes were well-arranged with normal bile duct lined by columnar epithelium. However, the control vein was dilated and some necrotic areas with numerous Küpffer cells could be seen. This may indicate hyperactivity of liver cells, as liver is considered the main metabolic organ in the body.

It could be noticed that, the protective effect of rosemary treatments on liver structure, where the hepatocytes were deeply stained with normal-sized central vein especially in rosemary extract treatment (Fig. 13). This enhancement was also noticed in nano- rosemary treatments (Fig. 14 and 15), although the central vein showed long axis, due to the press of section during culling and mounting on slides.

From the previous observations, it could be concluded rosemary forms can protect liver cells from damage during the growing period Hepatocyte have multiple functions in detoxifying, conjugation, elaboration of some vital substances and storage of lipids and glycogen. This role was confirmed by the presence of many Küpffer cells in all sections, which are known to have phagocytic activity, since they are capable to protect liver tissue and the systemic blood circulation from bacteria and other debris.

CONCLUSION

Considering the present outcomes, it tends to be inferred that dietary supplementation with rosemary extract and rosemary calcium nanoparticles can improve the effectiveness of feed usage, and immune status of broiler chicken. Likewise, supplemental Nano rosemary can actuate an accommodating effect on the lipid profile and oxidative status of broiler chicken. The rosemary calcium nanoparticles can securely be utilized in diets of growing broiler chicken, since it has no unfavorable consequences for growth performance or carcass characteristics.

Table1: Composition and chemical analysis of the basal Diets.

Ingredients (Kg/ton)	**Starter**	**Grower**	**Chemical Analysis%**	**Starter**	**Grower**
Yellow corn	628.3	691.8	ME, kcal/kg	3199	3200
Soybean meal 44	130	95.8	CP, %	23	21
Corn gluten meal 60.2	185.4	167.5	Crude Fiber, %	2.53	2.41
Dicalcium phosphate	18.2	13.5	Ether extract, %	2.95	3.12
Limestone	14.6	15	Calcium, %	1	0.91
DL-methionine	0.5	1.2	Av-Phosphorus, %	0.45	0.357
L-Lysine	4	4	Methionine, %	0.52	0.553
Sodium chloride	3	3	Meth, +Cys, (TSAA, %)	0.92	0.925
Vit+Min Premix[1]	3	3	Lysine, %	1.1	1.1
Soybean oil	13	5.2			

[1] Premix provided the following per kilogram of diet: VA, 2654 µg; VD3, 125 µg; VE, 9.9 mg; VK3, 1.7 mg; VB1, 1.6 mg; VB12, 16.7 µg; riboflavin, 5.3 mg; niacinamide, 36 mg; calcium pantothenate, 13 mg; folic acid, 0.8 mg; d-biotin, 0.1 mg; choline chloride, 270; BHT, 5.8; Fe, 50 mg; Cu, 12 mg; I, 0.9 mg; Zn, 50 mg; Mn, 60 mg; Se, 0.2 mg; Co, 0.2 mg".

Table (2): Effect of dietary Rosemary extract and Nano-rosemary supplementation on performance of broiler chickens at different ages

Criteria	Age (day)	Control	Rosemary extract (1g/kg)	Nano Rosemary (0.5g/kg)	Nano Rosemary (1g/kg)	Pooled SEM	Sig.
Live body gain, g/bird	1	40.62	40.52	40.32	40.55	0.183	NS
	21	652.8^{b}	673.40^{ab}	666.80^{ab}	690.52^{a}	10.905	*
	42	1970^{b}	1988.57^{ab}	2033.35^{a}	2028.72^{ab}	18.274	*
Body weight gain, g/bird	1-21	612.2^{b}	632.87^{ab}	626.50^{ab}	649.97^{a}	10.910	*
	21-42	1929^{b}	1948.05^{ab}	1993.02^{a}	1988.17^{ab}	18.262	*
Feed intake, g/bird	21	875.0^{a}	825.75^{b}	826.50^{b}	809.50^{b}	12.712	*
	42	3774	3731.75	3837.75	3848.75	35.822	NS
Feed conversion ratio	21	1.43	1.30^{bc}	1.32^{b}	1.24^{c}	0.020	*
	42	1.95	1.92	1.92	1.93	0.020	NS

"Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Table (3): Impact of dietary rosemary extract and Nano-rosemary supplementation on carcass traits of broiler chickens at marketing age.

carcass traits	Control	Rosemary extract (1g/kg)	Nano rosemary (0.5g/kg)	Nano rosemary (1g/kg)	Pooled SEM	Sig.
Live body gain (Kg)	2.131^{a}	2.058^{ab}	2.176^{a}	2.045^{b}	0.037	*
Carcass (kg)	1.513	1.433	1.591	1.538	0.046	NS
Heart (g)	11.40	9.433	10.03	10.1	1.067	NS
Gizzard (g)	35.06^{ab}	31.33^{b}	37.66^{a}	33.00^{ab}	1.778	*
Liver (g)	64.93^{a}	40.90^{b}	55.16^{ab}	49.46^{b}	4.514	*
Spleen (g)	3.100	2.133	2.833	3.200	0.498	NS
Abdominal fat (g)	50.86	45.933	39.60	47.86	5.903	NS
Giblets (g)	114.50^{a}	83.80^{b}	105.70^{ab}	95.76^{ab}	6.924	*
Bursa (g)	1.733^{a}	1.166^{b}	1.033^{b}	0.866^{b}	0.166	*

"Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Table (4): Influence of dietary rosemary extract and Nano- rosemary supplementation on plasma glucose, total protein, albumin, total lipids, triglycerides, cholesterol, HDL and LDL in 6-week-old broiler chickens

Blood plasma constituents	Control	Rosemary extract (1g/kg)	Nano-rosemary (0.5g/kg)	Nano-rosemary (1g/kg)	Pooled SEM	Sig.
Glucose(mg/dl)	123.00	126.66	119.00	140.00	6.333	NS
Tp (g/dl)	3.96	4.08	4.12	3.97	0.158	NS
Alb (g/dl)	2.34	2.40	2.21	2.24	0.076	NS
TL (mg/dl)	626.90^{a}	550.96^{b}	572.16^{ab}	571.23^{ab}	16.901	*
Tri g(mg/dl)	123.16^{a}	101.56^{b}	82.36^{c}	99.10b^{bc}	5.342	*
Chol (mg/dl)	175.90^{a}	147.23^{b}	134.03^{bc}	129.93^{c}	4.337	*
HDL (mg/dl)	56.83	58.66	59.63	58.00	4.907	NS
LDL (mg/dl)	94.43^{a}	68.26^{b}	57.90^{b}	52.10^{b}	5.736	*

"Means in the same row with different superscripts differ significantly (P≤ 0.05)".
(TP)total protein, (Alb) albumin ,(TL) total lipids, (Chol) cholesterol, (HDL) high-density lipoprotein, (LDL) low-density lipoprotein,

Table (5): Influence of dietary rosemary extract and Nano-rosemary supplementation on immune response and antioxidant status in 6-week-old broiler chickens

Measurements	Control	Rosemary extract (1g/kg)	Nano-rosemary (0.5g/kg)	Nano - rosemary (1g/kg)	Pooled SEM	Sig
IgG (ug/ml)	475.03^{b}	672.80^{a}	691.63^{a}	626.10^{a}	24.438	*
IgM(ug/ml)	114.16^{b}	149.33^{a}	152.20^{a}	136.30^{ab}	7.013	*
IgA (ug/ml)	125.86^{b}	152.83^{a}	156.90^{a}	147.13^{ab}	7.211	*
MDA(Nmol/dl)	21.23^{a}	15.06^{b}	17.96^{ab}	18.03^{ab}	1.224	*
TAC (nmol/dl)	1.22^{b}	1.49^{a}	1.46^{a}	1.49^{a}	0.072	*

"Means in the same row with different superscripts differ significantly (P≤ 0.05)".
Immunoglobulins (IgG, IgA and IgM), (TAC) total antioxidant capacity, (MDA) malondialdehyde

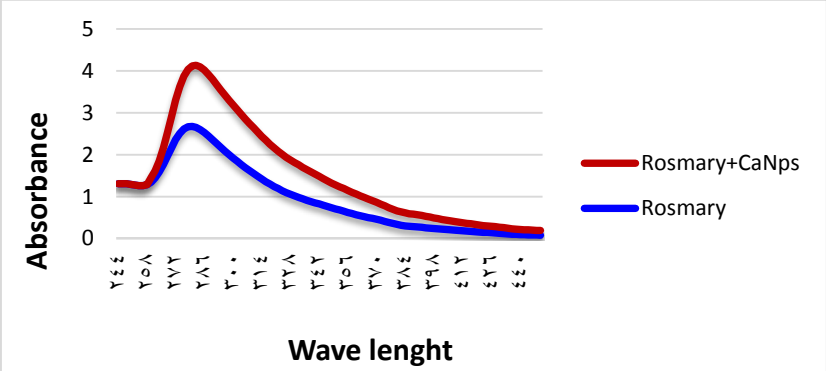


Fig (1): UV-Vis spectroscopic measurements of rosemary and its calcium nanoparticles.

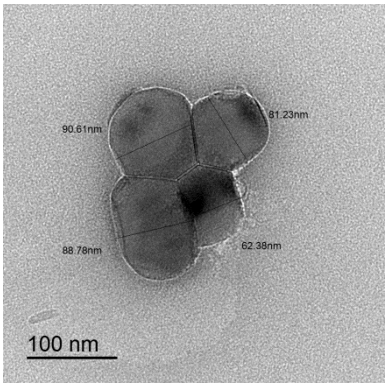


Fig (2): TEM micrographs and size distributions for calcium nanoparticles synthesized by rosemary extract at 100 nm magnification value.

System

Temperature (°C):	25.1	**Zeta Runs:**	29
Count Rate (kcps):	91.9	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	7

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	2.24	**Peak 1:**	2.24	100.0	4.49
Zeta Deviation (mV):	4.49	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.864	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				

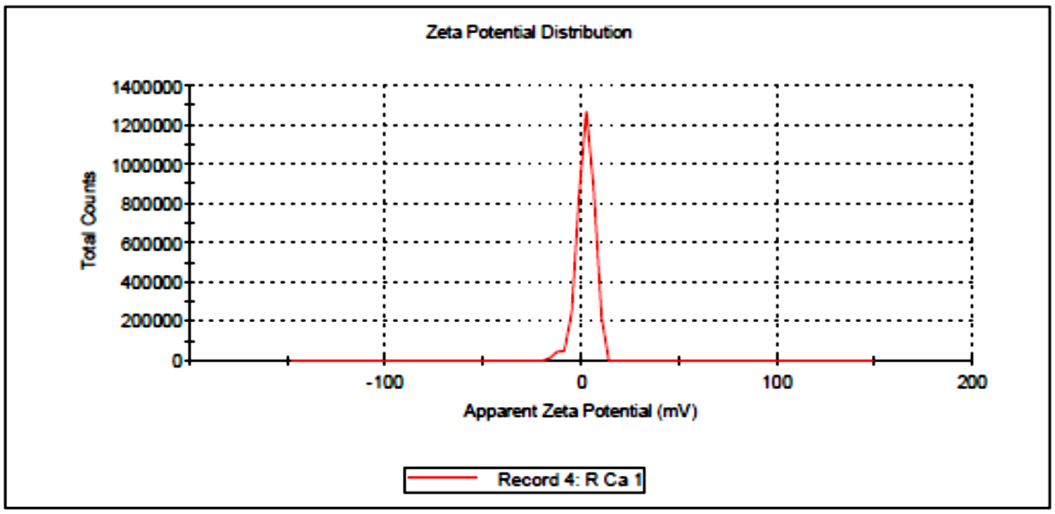


Fig (3): Zeta potential distribution for calcium nanoparticles synthesized by rosemary extract.

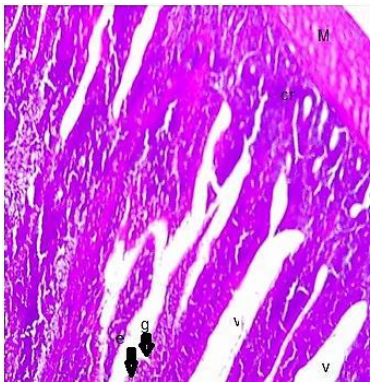


Fig (4): T. S. of duodenum control group of broilers (H&Ex100). Key: M= muscularis mucosa, cr= crypts of lieberkùhn, V= villi, g = goblet cells, e= epithelial lining.

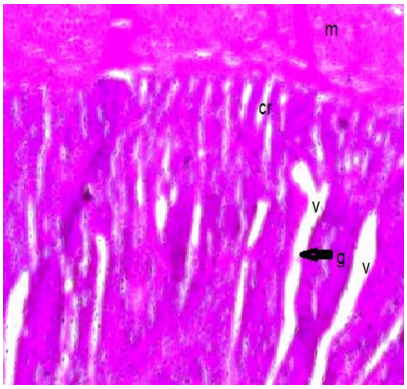


Fig (5): T. S. of duodenum rosemary extract 1g/kg group of broilers (H&Ex100). Key: M=muscularis mucosa, cr= crypts of lieberkùhn, V= villi, g = goblet cells, e= epithelial lining.

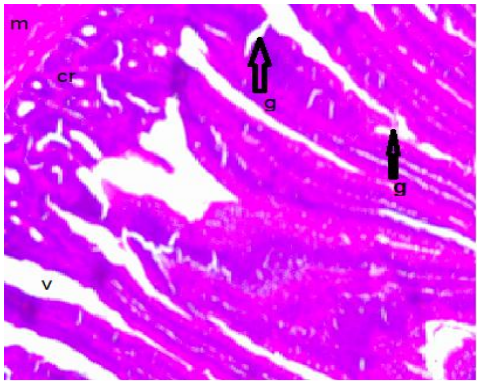


Fig (6): T.S. of duodenum Nano rosemary 0.5 g/kg group of broilers (H&Ex100). Key: M= muscularis mucosa, cr= crypts of lieberkùhn, V= villi, g = goblet cells, e= epithelial lining.

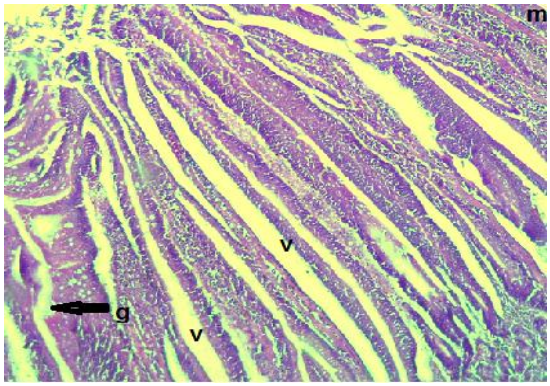
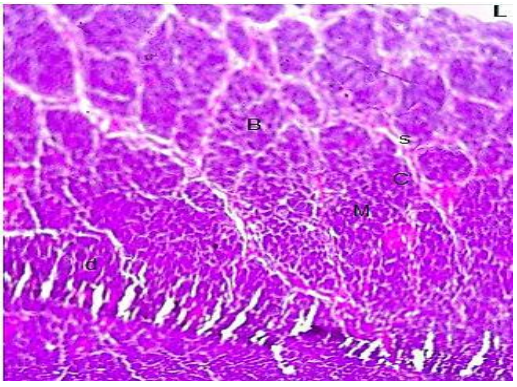
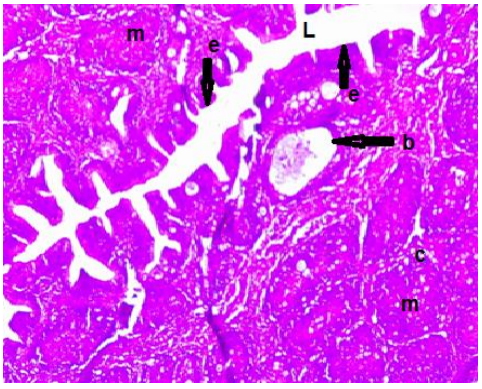


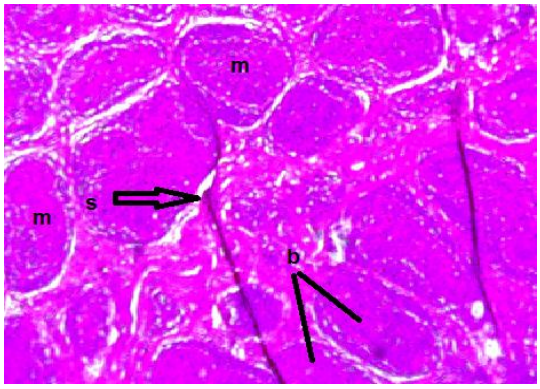
Fig (7): T.S. of duodenum Nano rosemary 1.0 g/kg group of broilers (H&Ex100). Key: M= muscularis mucosa, cr= crypts of lieberkùhn, V= villi, g = goblet cells, e= epithelial lining.





Fig(8): T.S. of bursa of Fabricius control group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen.

Fig (9): T.S. of bursa of Fabricius rosemary extract 1.0 g/kg of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen.



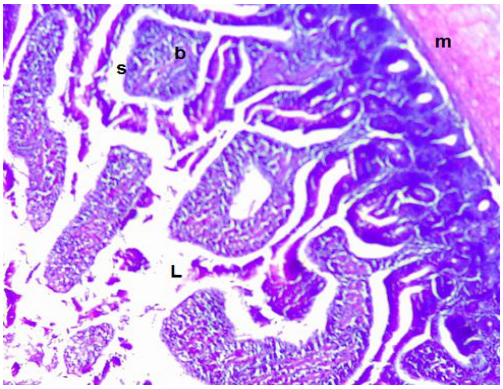


Fig (10):T.S. of bursa of Fabricius Nano rosemary 0.5 g/kg group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen.

Fig (11): T.S. of bursa of Fabricius Nano rosemary 1.0 g/kg group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen.

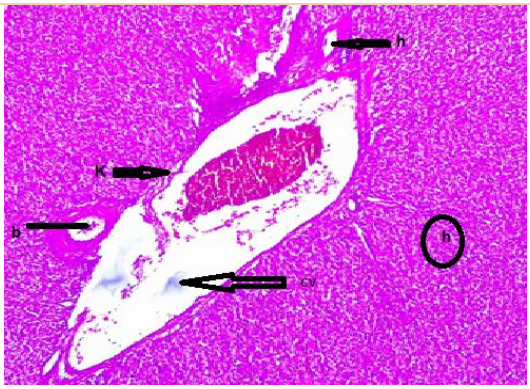


Fig 12. T.S. of liver control group of broilers (H&Ex100) Key: cv= central vein, h= hepatocytes, b= bile duct, k= küpffer cells, n= necrotic area, s= blood sinusoid, f= infilterable fluids.

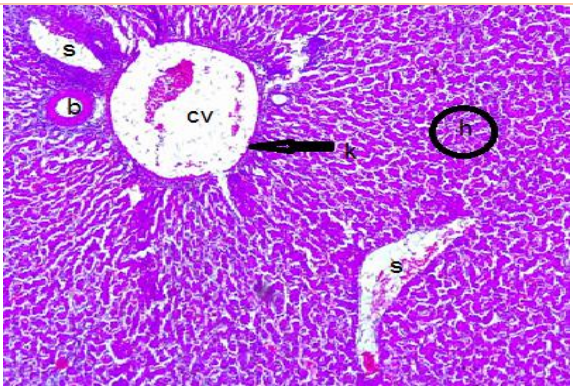


Fig 13. T.S. of liver rosemary extract group of broilers (H&Ex100) Key: cv= central vein, h= hepatocytes, b= bile duct, k= küpffer cells, n= necrotic area, s= blood sinusoid, f= infilterable fluids.

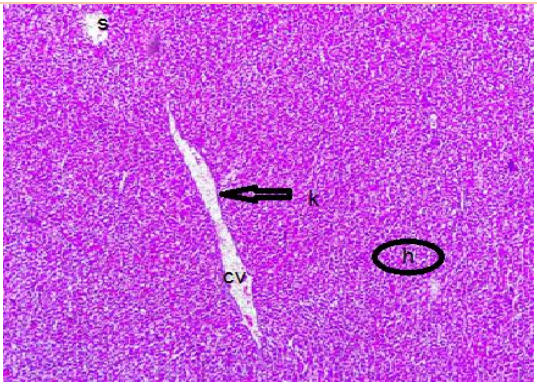


Fig 14. T.S. of liver nano rosemary 0.5g/kg group of broilers (H&Ex100) Key: cv= central vein, h= hepatocytes, b= bile duct, k= küpffer cells, n= necrotic area, s= blood sinusoid, f= infilterable fluids.

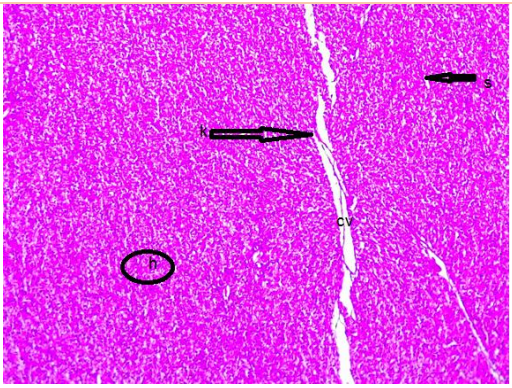


Fig 15. T.S. of liver nano rosemary 1.0 g/kg group of broilers (H&Ex100) Key: cv= central vein, h= hepatocytes, b= bile duct, k= küpffer cells, n= necrotic area, s= blood sinusoid, f= infilterable fluids.

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الملخص العربى

تأثير استخدام جزيئات الكالسيوم النانوية مع مستخلص أكليل الجبل الحيوي على كلا من الأداء الإنتاجي والآثار الفسيولوجية والمناعية لدجاج التسمين

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[1] قسم إنتاج الدواجن، كلية الزراعة، جامعة المنصورة، 35516، مصر

[2] قسم الكيمياء الزراعية، كلية الزراعة، جامعة المنصورة، المنصورة 35516، مصر

كان الهدف من هذه الدراسة هو بحث تأثيرات المكملات الغذائية مع مستخلص إكليل الجبل 1 جم / كجم وجسيمات نانوية من الكالسيوم مع مستخلص إكليل الجبل (0.5 و1 جم / كجم) على أداء النمو وبعض قياسات الدم والأنسجة في دجاج التسمين. حيث أجريت هذه التجربة على عدد 112 كتكوت تسمين عمر يوم من سلاله الهبرد حيث تم توزيعها عشوائيا إلى أربع معاملات وتم تقسيم كل معاملة إلى أربع مكررات بكل مكرره 7 كتاكيت بحيث تحتوي كل معاملة على 28 كتكوت تم تربيتها على الأرض وتم تقديم الغذاء والماء بصورة حرة حتى انتهاء التجربة. وجد أن هناك تأثير معنوي عند أضافة مستخلص أكليل الجبل وجزيئات النانو لأكليل الجبل عند مستوي (0.5 و1.0 جرام/ كجم عليقة) على وزن الجسم ومعدل الزيادة في وزن الجسم واستهلاك العلف ومعدل التحويل الغذائي مقارنة بمجموعة الكنترول خلال فترة البادي ومن ناحية أخرى وجد أن هناك اختلاف معنوي في استهلاك العلف ومعدل التحويل الغذائي بين مجموعة الكنترول والمجموعات التجريبية لأكليل الجبل عند نهاية التجربة. وجد أن عند أضافة مستخلص أكليل الجبل وجزيئات النانو الخاصة به عند مستوي (0.5 و1.0 جرام/ كجم عليقة) لم يظهر اختلافات معنوية لكلا من مستوي جلوكوز البلازما والبروتين الكلي والالبيومين والكولسترول مرتفع الكثافة ولكن وجد أن تركيز الكولسترول منخفض الكثافة يكون منخفض في المجموعات التجريبية مقارنة بمجموعة الكنترول. أظهرت النتائج المتعلقة بالاستجابة المناعية الخلطية لكتاكيت التسمين، زيادات ملحوظة في الجلوبولين المناعي في المجموعات التجريبية مقارنة بمجموعة الكنترول. وبالمثل وجد أن هناك زيادة ملحوظة من أجمالي القدرة المضادة للأكسدة (TAC) في المجموعات التجريبية مقارنتا بمجموعة الكنترول، ومن ناحية أخري كان هناك انخفاض في MDA بالمجموعات التجريبية مقارنتا بمجموعة الكنترول. وتخلص النتائج الحالية أن أضافة مستخلص أكليل الجبل أو جزيئات النانو الخاصة به لها تأثير مفيد علي الأداء الإنتاجي والفسيولوجي والمناعي لكتاكيت التسمين.