EFFECT OF CHROMIUM AND ESSENTIAL OILS MIX SUPPLEMENTATION ON GROWTH PERFORMANCE, SERUM METABOLITES, LYMPHOID ORGANS, CECAL MICROFLORA AND IMMUNE RESPONSE OF BROILER CHICKENS

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ABSTRACT

This work was carried out to study the effects of early-life supplementation of chromium picolinate, (CrP), essential oil mix (EOM) (Anise, oregano and garlic oils) or chromium picolinate plus essential oils mix (CrP+EOM) on growth performance, some serum metabolites, cecal microflora, lymphoid organs and immune response against Newcastle disease virus vaccine in broiler chickens. One hundred eighty day-old chicks of commercial meat type (Cobb-500) were randomly divided into four treatment groups. Each treatment group was further sub-divided into three replicates of fifteen chicks each. Chicks were fed isocaloric (3005, 3211 Kcal/kg ME) and isonitrogenous (23 and 20 %, CP) starter and grower-finisher diets supplemented with CrP at level of 1500 ppb, EOM at levels of 200 mg anise oil, 200 mg oregano oil and 100 mg garlic oil /kg diet, or CrP+EOM. Starter diets were provided to chicks from 1-21 days, while grower-finisher diets were provided from 22-42 days of age. Chicks were vaccinated against Newcastle disease virus using Hitchner and Lasota vaccines at 7and 14 days of age, respectively. Body weight and feed intake were measured during each feeding phase and feed conversion ratios were calculated. At 21 and 42 days of age, six chickens from each group were randomly chosen, killed by decapitation and the cecal contents were collected aseptically for bacteriological examination. Thymus, spleen, bursa and ceci were individually weighed and expressed as a percent of live body weight. Blood samples were collected to measure haemagglutinating antibodies using haemagglutination inhibition test. At end of the experiment blood samples were collected to measure glucose, total protein, total cholesterol, high density lipoprotein (HDLP) and low density lipoprotein (LDLP). The obtained results revealed that CrP, EOM or CrP+EOM supplementation had significantly ($P < 0.05$) increased body weight and daily gain compared to the control. Moreover, EOM or CrP+EOM supplementation had significantly increased

feed intake when compared to the control, while Feed conversion ratios were significantly decreased due to CrP or CrP+EOM supplementation. Serum glucose, total cholesterol and LDL significantly (P < 0.05) decreased due to CrP, EOM or CrP+EOM supplementation, while total protein and HDL were significantly increased due to CrP or CrP+EOM supplementation. Supplementation of the diets with CrP, EOM or CrP+EOM had significantly increased weight of the bursa, while weight of thymus, spleen or cecl was not significantly affected. Total bacterial count and pH of the cecal contents in broiler chickens fed the diet supplemented with CrP+EOM mix was significantly decreased, while addition of CrP, EOM or CrP+EOM had significantly reduced Gram –ve bacterial count. Broiler chickens fed diet supplemented with CrP+EOM had a significant higher value of heamagglutinating inhibition antibody titer in their sera. It could be concluded that, supplementation of CrP, EOM or CrP+EOM to broiler diets have beneficial effects as evidenced by improved growth performance parameters and some serum biochemical constituents. Health status and immune response of the birds were enhanced especially due to addition of CrP+EOM.

INTRODUCTION

Chromium is a well-known essential trace element for both human and animals since 1959 **(McDonald et al., 1992)** and widely distributed throughout the body **(Nielsen, 1994)**. Chromium is also involved in lipid, protein and nucleic acid metabolism **(Press et al., 1991, and Lein et al., 1999)** and also most associated with carbohydrate metabolism, being necessary for optimal insulin function and glucose uptake by insulin-sensitive cells **(Anderson, 1985 and Mooradian and Morley, 1987)**. Research on animals has confirmed that chromium from organic complexes such as chromium piclonate, nicotate and high chromium yeast is absorbed more efficiently, about 25-30% more than inorganic compound like chromium chloride, which is poorly absorbed regardless of doses or dietary status **(Underwood and Suttle, 1999; Mowat, 1994)**. National Research Council **(NRC, 1989)** has recommended an intake of 50 to 200 ppb of trivalent chromium for adult humans. However, an appropriate recommendation of the chromium requirement of poultry has not been made **(NRC, 1994)** and most poultry diets are basically composed of plant origin feedstuffs, which are usually low in chromium content **(Giri, 1990)**.

Chromium improves immunity and growth **(Chang and Mowat, 1992)**. In some studies, chromium increased **(Amoikon et al., 1995)** or decreased **(Kitchalong et al., 1995)** serum cholesterol. Improvement in growth rate and feed efficiency with turkey poults were reported when inorganic chromium was supplemented at 20 ppb **(Steele and Rosebrough 1981)**. While **Ward et**

al. (1993) reported that organic chromium supplementation at 200 to 400 ppb did not affect weight gain, feed intake, feed conversion ratio of broilers up to 3 weeks of age. **Sahin et al.** (2001) reported that organic chromium supplementation particularly at 1200 ppb, increased the performance criteria, egg quality and serum insulin concentration of Japanese quail. However, Chromium piclonate supplementation did not affect body weight, feed consumption or feed conversion ratio of broilers during 1-21 day when supplied either at 300 or 400 ppb (**Hossain et al.**, 1998).

It has been stated that dietary antibiotics had played a fundamental role in broiler industry as growth and health promoters. However, due to the rapid and continuous development of microbial drug resistance, higher cost of medication, consumer needs for poultry products free of drug residues, and the ban of most of antibiotics as feed additives in several countries (**Gunal et al., 2006 and Oviedo-Rondon et al., 2006**), there is an increasing interest in the search for alternate products that can aid in growth promotion, improved feed utilization and maintenance of gut health. Herbs have been used as food and for medicinal purposes for centuries. The world-Health Organization estimated that 80% of the earth's inhabitants rely on herbs and plant extracts or their active components for their primary health care needs (**Ciftci et al., 2005**). In this view, aromatic plants and essential oils extracted from them are being more important due to their antimicrobial properties and the stimulating effects on animal digestive system (**Lee et al., 2004**). Essential oils are natural vegetable products extracted by steam and/or water distillation. Most essential oils consists of mixture of hydrocarbons, alcohol, esters, aldehydes, ketones and small percentage of non volatile residues such as paraffins and waxes (**Zhang, et al.**, 2005). In addition to their antimicrobial activities (**Singh et al., 2002**), there is evidence to suggest that essential oils possess biological activities such as that of antioxidants (**Botsoglou et al., 2002 and Miura et al., 2002**) and as hypocholesterolemics (**Craig, 1999**) and stimulate effect on animal digestive enzymes (**Williams and Losa, 2001 and Hernandez et al., 2004**). Therefore, the present experiment was planned to study the impact of early life supplementation of chromium or a mixture of essential oil (anise, oregano and garlic oil) in early life on growth performance, some serum metabolites, cecal microflora, lymphoid organs and immune response against New castle disease virus in broiler chickens.

MATERIALS AND METHODS

Birds, management and diets:

One hundred eighty one-day-old chick of a commercial meat type (Cobb 500) obtained from a local commercial hatchery were used in this study. On arrival, chicks were weighed individually

and randomly allocated to four treatment groups. Each treatment group was further sub-divided into three replicates of fifteen chicks each. Chicks were reared in naturally ventilated open house with wood shavings as litter. Isocaloric and isonitrogenous starter and grower-finisher diets were formulated to meet the nutrient requirements of broiler chickens according to **NRC (1994)** as shown in Table 1. The dietary treatments consisted of one control diet and the other three experimental diets were supplemented with chromium Piclonate (CrP) at level of 1500 ppb, essential oil mix (EOM) at level of 200 mg anise oil, 200 mg oregano oil and 100 mg garlic oil /kg diet, or chromium piclonate plus essential oil mix (CrP+EOM). Feed and water were available at all times. Starter diets were provided to chicks from 1-21 days, while grower-finisher diets were provided from 22-42 days of age. Chicks were vaccinated against Newcastle disease virus using Hitchner and Lasota vaccines at 7and 14 days of age, respectively.

Growth performance and serum biochemical parameters :

Body weight and feed intake were measured during each feeding phase and feed conversion ratios were calculated. At end of the experiment, blood samples were collected (through wing vein puncture) from six birds in each treatment group (two bird/replicate), centrifuged and the clear sera were used for determination of glucose **(Trinder, 1969)**, total protein **(Cornel et al., 1949)**, total cholesterol **(Melattini, 1978)**, high density lipoprotein **(Clark et al., 1983)** and low density lipoprotein **(Friedwald et al., 1973)** using the available commercial kits.

Bacteriological examination :

Collection of the samples :

At 21 and 42 days of age, six chickens from each group were randomly chosen, killed by decapitation. Thymus, spleen, bursa and ceci were removed, individually weighed and expressed as a percent of live body weight, while pH of the cecal content was measured using a pH meter and part of the cecal contents was aseptically collected for bacteriological examination. Also, blood samples were collected at 21 and 42 days of age and clear sera were obtained determine haemagglutinating antibodies titer using haemagglutination inhibition test.

Determination of total bacterial count, Gram -ve and lactobacillus bacteria :

About 0.1 to 0.5 g of cecal contents from each killed chicken (six chicken from each group) was weighted and placed in a sterilized glass tube. The initial dilution was made by adding 9 volumes of sterile saline (NaCl solution 0.9%). Further serial 10 fold dilutions using sterile saline

solution were made. Following dilution, 0.01 ml of the suspension from the dilutions, including the initial dilution, were taken and spread on Nutrient agar for determination of aerobic total bacterial counts and MacConkey,s agar for determination of Gram negative bacterial counts. The plates were aerobically incubated for 24 hr at 37°C, and the number of cfu per gram of cecal content was determined. On other hand, C. perfringens was isolated on sheep blood agar which is supplemented with Neomycin 200 µg/ml and incubated anaerobically at 37°C for 48 hours. Mann Rogosa Sharpe (MRS) agar, from Oxoid, was used for isolation of Lactobacillus. The plates were anaerobically incubated for 48 hr at 37°C, and the number of cfu per gram of cecal content was determined (**Elsayed, 2002**).

Haemagglutination Inhibition (HI) test :

Antigen of NDV was diluted in HI buffer to contain 10 haemagglutinating unites (HA) in 50 µl. One hundred µl of antigen was deposited in all wells. Twenty five µl of serum was deposited in the first well using 25 µl microliter diluter. Fifty µl from each well was serially passed from each well using a microliter transfer diluter. The plates were incubated for 20-30 minutes at room temperature and 50 µl of chicken red blood cells (CRBC) 0.5% was added to all wells. The plates were agitated gently and let stand for 45 minutes at room temperature. The HI titer was determined according to **Villegas (1991)**.

Statistical analysis :

The data were statistically analyzed by analysis of variance using general linear model procedure (GLM) in a window-based statistical package program, **SAS (1985)**.

RESULTS AND DISCUSSION

Growth performance :

The effects of chromium and essential oil mix on growth performance parameters of broiler chickens are shown in Table2. Treatments had significant ($P < 0.05$) effects on body weight and weight gain during the starter period. Broiler chickens fed diets supplemented with either EOM or CrP+EOM had s significant higher values of body weight (843.33 g and 868.33 g, respectively) and weight gain (38 43 and39.54 g/day, respectively) as compared to that of broiler chickens fed the control or CrP supplemented diets. The dietary treatments had no significant ($P > 0.05$) effects on either feed consumption or feed conversion ratio during the starter period. Throughout the grower-finisher period growth performance parameters were significantly ($P< 0.05$) affected

by the dietary treatments as shown in Table 2. Broiler chickens fed diets supplemented with either CrP or EOM had significant higher values of body weight, body gain and daily gain as compared to that of chickens fed the control diet. Moreover, supplementation of the diet with CrP+EOM had significantly ($P < 0.05$) increased body weight, body gain and daily gain as compared to the control or CrP supplemented diets. Supplementation of grower-finisher diets with either CrP or EOM had no significant effect on feed consumption as compared to the control diet; while supplementation of the diets with CrP+EOM had significantly ($P < 0.05$) increased feed consumption of broiler chickens as compared to the control. Feed conversion ratios differed significantly among the dietary treatments. Broiler chickens fed diets supplemented with CrP, EOM or CrP+EOM had significant ($P < 0.05$) lower feed conversion ratios (2.13, 2.14 and 1.99, respectively) as compared to the control one (2.3).

An effect of dietary treatments on allover growth performance parameters of broiler chickens throughout the experimental period is shown in Table 2. Broiler chickens fed diets supplemented with either CrP or EOM have significant ($P < 0.05$) higher values of final body weight (2020 and 2103.33 g, respectively) and daily gain (47.24 and 49.21 g/day, respectively) as compared to that of the control (1843 g and 42.78g/day), while broiler chickens fed diets supplemented with CrP+EOM have significant higher values of body weight (2239 g) and daily gain (52.40 g/day) when compared to broiler chickens fed the other diets. Addition of EOM oil or CrP+EOM had significantly ($P < 0.05$) increased average feed consumption of broiler chickens (91.06 and 92.51 g, respectively) compared to the control (86.09 g). Broiler chickens fed diet supplemented with CrP, EOM or CrP+EOM mix have significant lower values of feed conversion ratios (1.89, 1.85 and 1.76, respectively) when compared to that of the control (2.01). Moreover, feed conversion ratios of the chickens group fed diet supplemented with CrP+EOM was significantly lower than that of the group fed the CrP supplemented diet, while no significant difference was detected between group fed the CrP or EOM supplemented diets.

The improved growth performance parameters for broiler chicken fed the CrP supplemented diet is in accordance with the results of **Sahin et al. (2002)** who found that supplementation of broiler diets with either 200, 400, 800 or 1200 ppb CrP had resulted in significant ($P < 0.05$) linear increase in body weight, feed intake and feed efficiency. Also, increasing supplemental CrP up to 1200 ppb was found to significantly ($P < 0.05$) increase body weight, feed intake and feed efficiency in laying Japanese quail **(Sahin et al., 2001)**, broiler Japanese quail **(Ezzat et al., 2006)** and broiler chickens **(Mohamed and El-Sayed, 2001)**. The improved growth performance parameters due to chromium supplementation confirm its beneficial effects on physiological functions required for growth performance.

The primary role of Cr in metabolism is to potentiate the action of insulin through its pres-

ence in an organometallic molecule, the glucose tolerance factor (**Anderson, 1994 and Sahin et al., 2001 and 2002**). Insulin metabolism influences lipid peroxidation (**Gallaher, 1993**); and chromium, as an insulin potentiator, is therefore postulated to function as an antioxidant (**Preuss et al., 1997**). Moreover, Cr is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids (**Linder et al., 1991**). Cr deficiency can disrupt carbohydrate and protein metabolism, reduce insulin sensitivity in peripheral tissues and impair growth rate (**Pagan et al., 1995**).

The improved feed utilization in the group fed EOM supplemented diet could be due to the active compounds such as thymole and carvacrol in oregano oil and anothole in anise oil which have digestive stimulating effects (**Cabuk et al., 2003**). Besides, these active ingredients have been reported to possesa antimicrobial (**Burt and Reinders, 2003 and Tabanco, 2003**). In addition, studies had shown that essential oils increased digestion of protein, cellulose and fat (**Jamroz and Kamel, 2002**), improved apparent whole tract and ileal digestibility of the nutrients (**Hernandez et al., 2004**) and increased effects of pancreatic lipase, and amylase (**Ramakrashna et al., 2003**). A number of studies have reported the positive effects of essential oils on food digestion are due to increased bile secretion (**Bhat and Chandrasekhara, 1987 and Sambaiah and Srinivasan, 1991**) and/or stimulate digestive enzyme activities of intestinal mucosa and of pancreas (**Platel and Srinivasan, 1996, and 2000**). In addition, thymole (Active ingredient in oregano oil) at level of 100 ppm was found to stimulate secretion of pancreatic digestive enzymes, i.e. amylase, lipase, trypsin and chymotrypsin in female broiler chickens (**Lee et al., 2003**).

Tolba and Hassan (2003) found that garlic as a natural feed additive, improved broiler growth, feed conversion ratio and decreased mortality rate. Garlic oil in essential oil mix might have a role in improvement of growth performance indirectly through its effect on microflora, minimizing gastrointestinal bacteria. It had been hypothesized that gut microflora decrease nutrient absorption by increasing thickness of gastrointestinal tract, the rate of digestive passage, and also increase nutrient requirements of the host by increasing turnover of the gut mucosa and competing with the host for the dietary energy and protein (**Apajalathi et al., 2004**). **Adibmoradi et al. (2006)** reported that garlic meal as a feed additive significantly increased villus height and crypt depth and decreased epithelial thickness and goblet cell numbers in duodenum, jejunum and ileum of broiler chickens. Thinner intestinal epithelium enhance nutrient absorption and decrease the metabolic demands of the gastrointestinal system (**Visek, 1978**), this in turn, increase net energy committed to maintaining the luminal tissue comes out at the expense of more productive purposes such as muscle accretion (**Bedford, 2000**).

Serum biochemical parameters :

The effect of dietary treatments on serum biochemical parameters of broiler chickens is shown in Table 4. The results showed that broiler fed diets supplemented with CrP, EOM or CrP+EOM have significant (P 0.05) lower values of serum glucose (137.16, 167.45 and 136.33 mg/dl, respectively) compared to that of the control group (191.25 mg/dl). Moreover, supplementation of diets with CrP+EOM had significantly decreased serum glucose level compared to supplementation with EOM only and this might indicate a synergistic effect. These results are in agreement with other reports **(Mohamed and El-Sayed, 2001; Sahin et al., 2002; and Ibrahim 2004)** where dietary supplementation with CrP at concentration ranged from 200 ppb up to 3200 ppb had significantly decreased glucose concentration in serum of broilers chickens. Similarly, glucose concentration in serum of broiler Japanese quails was significantly reduced when their diets were supplemented with CrP at levels ranged from 200 up to 1200 ppb **(Ezzat et al., 2006)**. It has been suggested that Cr stimulate the biological activity of insulin by increasing insulin sensitive cell receptors **(Ward et al., 1994)**. In addition, Cr is a component of glucose tolerance factor that work with insulin to move glucose into cells for energy generation **(Sahin et al., 2002)**. Also increased insulin secretion due to Cr supplementation is associated with decreased corticosterone secretion **(Sahin et al., 2001)**. It is well known that cortisole promotes gluconeogenesis and reduces glucose utilization **(Cupo and Donaldson, 1987)**. **Rosebrough and Steele (1981)** reported that turkey fed diets supplemented with Cr had greater liver glycogen levels as a result of increased activity of the enzyme glycogen synthetase. The significant decrease of glucose in the group fed diet supplemented with EOM could be due to the anti-hyperglycemic activity of oregano oil, where **Sahelian (2004)** found that oral administration of aqueous extract of wild oregano produced a significant decrease on blood glucose level in diabetic rats.

Supplementation of diets with CrP or CrP+EOM had significantly ($P < 0.05$) increased serum total protein (7.66 and 7.53 g/dl, respectively) when compared to the control or EOM supplemented diets. Enrichment of the diets with CrP, EOM or Cr+EOM had significantly ($P < 0.05$) decreased total cholesterol (91.38, 86.0, and 86.33 mg/dl, respectively) and LDL (18.1, 17.3 and 15.23 mg/dl, respectively) as compared to the control ones (105.33 and 34.12 mg/dl, respectively). Broiler chickens fed the CrP-supplemented diet have significant ($P < 0.05$) high values of HDLP in their sera as compared to that of chicken fed the other dietary treatments. These findings assure the role of Cr in decreasing incidence of coronary heart disease in human. **Howard et al. (1993)** indicated that insulin increased liver LDL receptors and consequently reducing the LDL level in the serum. **Lin et al. (1999)** found that the supplementation of CrP had effectively decreased serum total cholesterol and increased HDL in serum of laying hens. Dietary supple-

mentation of broiler diets with CrP at 1200 or 3200 ppb had significantly decreased total cholesterol and LDL and increased HDL **(Lein et al., 1999 and Mohamed and El-Sayed, 2001)**. In addition, the study of **Ezzat et al. (2006)** had showed that supplementation of broiler Japanese quail with CrP at levels up to 1200 ppb had significantly decreased total cholesterol LDL while increased HDL. It had been hypothesized that Cr act as activator of the biological activity of insulin which depresses adipocyte lipolysis by reducing the activity of cAMP and hormone sensitive lipase **(Lambert and Jacquemin, 1979)** and increases glucose utilization for glycogen formation **(Anderson et al., 1991)**.

The role of herbs and their essential oils as to their cholesterol lowering properties has been reviewed **(Craig, 1999)**. The pure components of essential oils were found to inhibit 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis **(Crowel, 1999)**, as a result, a hypocholesterolemic effect of essential oils is expected. **Goldstein and Brown (1990)** proposed that the complete inhibition of cholesterol synthesis requires two regulators, i.e. cholesterol derived from low density lipoprotein and a non-sterol products derived from mevalonate, both of which modulate HMG-CoA reductase activity. Thymole and carvacrol in essential oil mix might had induced a regulatory non-sterol product as proposed by **Case et al. (1995) and Elson (1996)**. Garlic oil was found to decrease the incorporation of acetate into cholesterol and increase fecal bile acid and neutral sterol excretion **(Chang and Johnson, 1980)**. **Qureshi et al. (1983)** studied the effect of garlic oil on lipid metabolism in White Leghorn pullet and found a significant decrease in hepatic HMG-CoA reductase (79-83%), cholesterol 7-_-hydroxylase (43-51%) and fatty acid synthetase (17-29%), as a result, a significant decrease in serum total cholesterol by 20-25%, low density lipoprotein cholesterol by 28-45% and triglycerides by 10-28% was reported. Allin and allicin, highly polar and odorous compounds in garlic oil were reported to have hypocholesterolemic properties **(Augusti and Mathew, 1974 and Vanderhock, 1980)**.

Lymphoid organs and ceci :

The effect of dietary treatments on weight of lymphoid organs and ceci is shown in Table 4. During the starter period, treatments had no significant ($P > 0.05$) on either absolute weight of lymphoid organs and ceci or the weight as a percentage of the live body weight. However, at the end of grower-finisher period, broiler chickens fed diet supplemented with CrP, EOM or CrP+EOM have a significant ($P < 0.05$) higher weight of lymphoid organs compared to the control.

Microflora, cecal pH and immune response :

The effect of supplementing broiler diets with CrP, EOM or CrP+EOM on microflora population of the ceci, cecal pH and immune response against Newcastle disease virus is displayed in Table 5. The treatments had no significant effect on either, total bacterial count, gram negative or lactobacillus at the end of the starter period. However, at the end of the grower-finisher period, total bacterial count as well as Gram -ve bacteria were significantly ($P < 0.05$) reduced in the ceci of chickens fed the diet supplemented with CrP+EOM as compared to the ceci of chickens fed the other diets. On the other hand, addition of CrP, EOM or CrP+EOM had significantly ($P < 0.05$) decreased the colony forming unit of gram negative bacteria as compared to the control.

The intestinal microflora plays a role in acquired mucosal immunity and enteritis (**Cebra, 1999; Kelly and Conway, 2005**). Normal gut microflora in healthy birds inhibits the pathogenicty of Clostridia perferenges (**Fukata et al., 1991**), and modulates the immune responses against coccidia (**Dalloul et al., 2003**). The results reported herein are consistent with the findings of **Ross et al. (2001)** who indicated that garlic oil possesses a substantial and broad-spectrum antibacterial activity against Gram positive and Gram negative bacteria. The antimicrobial properties of garlic oil was attributed to its content of allicin. These properties were confirmed against E. coli and Staphylococcus aureus (**Hughes and Lawson, 1991**). In addition, garlic oil has been shown to inhibit E. coli and Salmonella typhimurium in vitro (**Johnson and Vaughn, 1969**). **Mitsch et al. (2004)** found that a specific blend of essential oil components can control Clostridium perferinges colonization and proliferation in the gut of broiler and therefore may be of help to prevent problems with necrotic enteritis. Dietary addition of essential oil blend showed a significant decrease in colony forming units of E. coli in ileo-cecal digesta. Eugenol, component of the essential oil from cloves, has been shown to inhibit S. typhimurium and E. coli in vitro (**Karapinar and Aktug, 1987; Helander et al., 1998**). Similarly, carvacrol, a major component of essential oil oregano has been shown to be a strong inhibitor of E.coli (**Friedman et al., 2002**). **Jang et al. (2007)** found that dietary addition of essential oil blend showed a significant decrease in colony forming unit of E. coli in ileo-cecal digesta. No significant differences in pH values of cecal content were detected due to the dietary treatments, although diets supplemented with EOM or CrP+EOM tended to have lower values compared to control.

The effect of dietary treatments on the immune system response of broiler to Newcastle disease virus live vaccine is displayed in Table 5. At the end of the starter phase, the results showed that supplementation of the diet with CrP+EOM had significantly ($P < 0.05$) increased haemagglutination inhibition antibody titer against Newcastle disease virus when compared to that of the control. Furthermore, the antibody titer in broiler chickens fed the diet supplemented with CrP+EOM sustained high during the grower-finisher phase and recorded a significant high-

er value as compared to that in broiler chickens fed the other dietary tratements. CrP was found to increase total globulins, immunoglobulin M **(Chang and Mowat, 1992)** and immunoglobulin G1 **(Moonesi-shageer and Mowat, 1993)**. Improvement in immune response may be due to a reduced cortisol production associated with CrP supplementation. It had been reported that cortisol has immunosuppressive effect and inhibits production and activities of cytokines and antibodies **(Kegly and Spears, 1995)**. In addition, CrP supplementation in stressed animals can prevent urinary losses of zinc, iron, copper, manganese and selenium **(Anderson et al., 1988)**. The deficiency of such minerals had been reported to lower resistance to diseases **(Bull, 1990)**. The surpass immune response in the group fed the diet supplemented with CrP+EOM may be due to a synergistic effect between the two additives. Herbs may have other beneficial effects such as immune and/or endocrine stimulants. **Chang et al. (1998)** found that garlic oil inhibited _6 desturase enzyme activity and changed the membrane arachidonic acid content of liver cells in rat, both of which had immunomodulatory potential. **Al-Ankari et al. (2004)** related the improved immune response of broiler chicken because of essential oil to: increased total bile secretion, improved hepatic antioxidant status, by decreasing lipid peroxidation, increased hepatic glutathione and superoxide dismutase activity.

Generally, the results of the present study indicated that supplementation of CrP, EOM or CrP+EOM broiler diets had beneficial effects as evidenced by improved growth performance parameters and some serum metabolites. Health status and immune response of the birds were enhanced especially due to CrP+EOM supplementation.

Table 1. Ingredients and calculated composition of the experimental diets[1].

Ingredients	Experimental diets	
	Starter	Grower-finisher
Yellow corn, ground	56.00	62.50
Soybean meal, 44%	31.10	22.00
Corn gluten meal, 60%	4.96	5.50
Fish meal, 65%	3.00	3.00
Soybean oil	1.70	3.60
Lysine·HCl, 78%	0.16	0.20
DL-Methionine, 99%	0.13	0.16
Limestone	1.15	1.15
Dicalcium phosphate	1.20	1.29
Vitamin and mineral premix[2]	0.30	0.30
NaCl	0.30	0.30
Calculated composition[3]		
ME, Kcal/kg	3005	3211
CP, %	23.0	20.0
EE, %	4.41	6.38
CF, %	3.42	2.99
Ca, %	1.10	1.10
Total P, %	0.65	0.65
Lysine, %	1.41	1.19
Methionine, %	0.58	0.55

[1] starter and grower finisher diets were supplemented with CrP (1500 ppb), EOM (200 mg anise oil, 200 mg oregano oil and 100 mg garlic oil /kg diet) or CrP+EOM.

[2] Provide per kg diet: vitamin A (palmitate), 12,000 IU; vitamin D (cholecalciferol, 2,500 IU; vitamin E (L-tocopherol) 12 mg; vitamin K_3 (menadione), 2.5 mg; vitamin B_1, 1.2 mg; vitamin B2, 6 mg; pantothenic acid, 12 mg; folic acid, 1.2 mg; niacin, 36 mg; pyridoxine, 2 mg; vitamin B_{12}, 0.01 mg; biotin, 0.06 mg; , Choline, 500 mg; iron, 36 mg; copper, 5 mg; manganese, 72 mg; zinc, 60 mg, iodine, 0.45 mg,; selenium, 0.12 mg.

[3] Calculated according to feed composition tables NRC (1994).

Table (2) : Effect of chromium and essential oil mix supplementation on growth performance of broiler chickens (Means ± SE).

	Dietary treatments			
	Control-diet	CrP-diet	EOM-diet	CrP+EOM-diet
Starter period (0-3 weeks)				
Initial Body weight, g	36.0 ±1.52	35.66 ±1.45	36.33 ±2.6	38.0 ±2.08
Average body weight, g	740 ±18.9^{b}	776.66 ±16.4^{b}	843.33 ±14.81^{a}	868.33 ±23.1^{a}
Average weight gain, g/day	33.52 ±0.90^{b}	35.28 ±0.81^{b}	38.43 ±0.74^{a}	39.54 ±1.1^{a}
Average feed consumption, g/day	51.19 ±1.74	53.01 ±2.22	54.16 ±1.79	54.56 ±1.52
Average feed conversion ratio	1.53 ±0.046	1.5 ±0.054	1.41 ±0.055	1.38 ±0.058
Grower-finisher Period (3-6 weeks)				
Average body weight, g	1843 ±30.8^{c}	2020 ± 30.1^{b}	2103 33 ±27.7^{b}	2239 ±29.1^{a}
Average weight gain, g	1103 ±11.9^{c}	1243.33 ±23.33^{b}	1260 ±40.4^{b}	1370.67 ±7.44^{a}
Average daily gain, g/day	52.52 ±0.6^{c}	59.2 ±1.1^{b}	60 ±1.9^{b}	65.26 ±0.4^{a}
Average feed consumption, g/day	121.01 ±2.4^{b}	126.06 ±2.7^{ab}	127.98 ±1.6^{ab}	130.47 ±2.9^{a}
Average feed conversion ratio	2.3 ±0.064^{a}	2.13 ±0.040^{b}	2.14 ±0.041^{b}	1.99 ±0.033^{b}
Allover performance				
Average final body weight, g	1843 ±30.8^{c}	2020 ±30.1^{b}	2103.33 ±27.74^{b}	2239 ±29.1^{a}
Average daily gain, g/day	42.78 ±0.60^{c}	47.24 ±0.75^{b}	49.21 ±0.70^{b}	52.40 ±0.67^{a}
Average total feed consumption, g	86.09 ±0.40^{b}	89.53 ±1.9^{ab}	91.06 ±0.96^{a}	92.51 ±1.32^{a}
Average feed conversion ratio	2.01 ±0.033^{a}	1.89 ±0.035^{b}	1.850 ±0.033^{bc}	1.76 ±0.029^{c}

^{abc} Means in the same row with different superscripts are significantly different ($P < 0.05$)

Table (3) : Effect of chromium and essential oils mix supplementation on serum metabolites of broiler chickens at end of the experiment[1].

	Dietary treatments			
	Control-diet	CrP-diet	EOM-diet	CrP+EOM-diet
Glucose (mg/dl)	191.25 ±3.78[a]	137.16 ±4.35[c]	167.45 ±4.72[b]	136.33 ±4.9[c]
Total protein (g/dl)	5.20 ±0.34[b]	7.66 ±0.82[a]	5.46 ±0.51[b]	7.53 ±0.71[a]
Total Cholesterol (mg/dl)	105.33 ±2.40[a]	91.38 ±1.76[b]	86.0 ±4.16[b]	88.66 ±1.76[b]
High density lipoprotein (mg/dl)	66.33 ±1.45[b]	76.26 ±0.94[a]	70.73 ±3.62[ab]	72.96 ±1.02[ab]
Low density lipoprotein (mg/dl)	34.12 ±3.05[a]	18.1 ±2.08[b]	17.30 ±2.33[b]	15.23 ±0.96[b]

[1] Means ± SE

[ab] Means in the same row with different superscripts are significantly different (P < 0.05)

Table (4) : Effect of chromium and essential oils mix supplementation on weight of lymphoid organs and ceci at end of starter (21 day) and grower-finisher (42 day) periods (Means ± SE).

	Dietary treatment							
	Control-diet		CrP-diet		EOM-diet		CrP+EOM-diet	
	Weight, g	% of BW	Weight, g	% of BW	Weight, g	% of BW	Weight, g	% of BW
Starter								
Thymus	3.05 ±0.40	0.45 ±0.05	3.63 ±0.35	0.57 ±0.06	3.10 ±0.46	0.47 ±0.05	4.09 ±0.17	0.57 ±0.05
Spleen	0.71 ±0.13	0.10 ±0.02	0.73 ±0.08	0.11 ±0.01	0.66 ±0.13	0.09 ±0.01	0.78 ±0.25	0.1 ±0.02
Bursa	1.13 ±0.23	0.16 ±0.02	1.02 ±0.30	0.17 ±0.03	0.84 ±0.14	0.13 ±0.02	0.93 ± 0.14	0.13 ±0.02
Ceci	5.97 ±0.74	0.9 ±0.08	6.3 ±0.62	1.0 ±0.14	5.42 ±0.90	0.85 ±0.17	6.75 ±0.64	0.93 ±0.06
Grower-finisher								
Thymus	6.62 ±1.03	0.33 ±0.04	7.78 ±1.74	0.41 ± 0.08	6.19 ±1.26	0.35 ±0.07	8.19 ±1.65	0.41 ±0.08
Spleen	1.0 ±0.06	0.05 ±0.004	1.45 ±0.30	0.07 ±0.01	0.94 ±0.01	0.05 ±0.003	1.2 ±0.22	0.06 ±0.01
Bursa	1.54 ±0.15^{B}	0.08 ±0.005^{b}	1.91 ±0.10^{A}	0.09 ±0.003^{b}	2.15 ±0.06^{A}	0.11 ±0.002^{a}	2.26 ±0.11^{A}	0.11 ±0.003^{a}
Ceci	19.43 ±2.9	0.99 ±0.16	16.39±1.3	0.85 ±0.08	15.35 ±0.68	0.87 ±0.06	13.83 ±1.34	0.70 ±0.09

^{ab} Means in the same row with different capital or small superscripts are significantly different ($P < 0.05$)

Table (5) : Effect of chromium and essential oils mix supplementation on microflora population, cecal pH and Log GM of HI titer against Newcastle virus at end of starter (21 day) and grower-finisher (42 day) periods (Means ± SE).

	Dietary treatments			
	Control-diet	**CrP-diet**	**EOM-diet**	**CrP+EOM-diet**
Starter				
Total bacterial count log/g	8.69 ±0.96	7.79 ±0.72	8.65 ±0.93	8.46 ±0.71
Gram –ve, log/g	6.0 ±1.30	4.19 ±0.68	5.87 ±1.35	5.84 ±1.12
Lactobacillus, log/g	9.52 ±0.09	9.34 ±0.48	8.52 ±0.88	8.72 ±0.45
PH	6.75 ±0.64	6.30 ±0.73	5.97 ±0.62	5.42 ±0.92
HI*	1.30 ±1.05 ^{b}	2.02 ±1.6 ^{ab}	2.02 ±1.6 ^{ab}	2.42 ±2.1^{a}
Grower-finisher				
Total bacterial count, log/g	10.12 ±0.07^{a}	9.72 ± 0.25^{ab}	9.67 ±0.30^{ab}	9.30 ±0.13^{b}
Gram –ve, log/g	8.07 ±0.32^{a}	6.01 ±0.79^{b}	5.84 ±0.23^{b}	5.80 ±0.12^{b}
Lactobacillus, log/g	9.0 ±0.51	9.25 ±0.75	9.47 ±0.25	9.50 ±0.38
pH	6.62 ±0.06^{a}	6.55 ±0.03^{a}	6.52 ±0.12^{a}	6.27 ±0.06^{b}
HI*	1.22 ±0.75 ^{b}	1.12 ±0.75 ^{b}	1.12 ±0.75 ^{b}	2.38 ±1.9^{a}

* Log geometric mean of antibody titer ± SD.

^{ab} Means in the same row with different superscripts are significantly different (P < 0.05)

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الملخص العربى

تأثير إضافة بيكلونات الكروم ومخلوط الزيوت الأساسية على معدلات النمو، مكونات مصل الدم، بكتيريا الأعور، الأعضاء الليمفاوية والاستجابة المناعية فى دجاج التسمين

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تم إجراء هذا البحث لدراسة تأثير إضافة كل من بيكلونات الكروم ومخلوط من الزيوت الأساسية على النمو، بعض مكونات مصل الدم، النشاط البكتيرى فى الأعور، الأعضاء الليمفاوية والاستجابة المناعية ضد لقاح فيروس مرض النيوكاسل فى دجاج التسمين. أستخدم عدد مائة وثمانون كتكوت عمر يوم من سلالة Coob-500 قسمت عشوائياً إلى أربعة مجموعات متساوية فى كل منها 3 مكررات (كل مكررة بها 15 كتكوت). تم تغذية الكتاكيت على علائق (بادى لمدة 3 أسابيع، ونامى - ناهى لمدة 3 أسابيع أخرى) متساوية فى محتوى الطاقة (3000 كيلو كالورى عليقة بادى و 3200 كيلو كالورى عليقة ناهى) والبروتين (23% عليقة بادى و 20% بروتين عليقة ناهى). كانت المعاملات الغذائية كالتالى : 1- عليقة ضابطة، 2- عليقة أضيف إليها بيكلونات الكروم (1500 جزء فى البليون) 3- عليقة أضيف إليها مخلوط من الزيوت الأساسية (200 مجم من زيت الينسون + 200 مجم من زيت البردقوش + 100 مجم من زيت الثوم / كجم من العليقة)، 4- عليقة أضيف إليها كل من بيكلونات الكروم ومخلوط الزيوت الأساسية بنفس التركيزات السابقة. تم تحصين الطيور ضد فيروس مرض النيوكاسل باستخدام لقاحى Hitchner و Lasota عند عمر 7 أيام و 14 يوماً على التوالى، تم وزن الكتاكيت مرة كل 3 أسابيع وحساب معدل إستهلاك العلف ومعامل التحويل، عند عمر 21 يوماً، 42 يوماً تم إختيار 6 طيور عشوائياً من كل مجموعة وذبحها للحصول على الدم لإجراء إختبار منع التلازن وقياس معدل إنتاج الأجسام المناعية ضد لقاح فيروس مرض النيوكاسل، تم وزن كل من الغدة التيموثية، الطحال وغدة فابريشيوس والأعور لتلك الطيور. كم تم أخذ محتويات الأعور لقياس الـ PH والنشاط البكتيرى (العدد البكتيرى الكلى، عدد البكتريا سالبة الجرام والبكتريا المحبة للبن)، فى نهاية التجربة تم أخذ عينات من الدم لقياس كل من الجلوكوز، البروتينات الكلية، الكولبستيرول الكلى، البروتينات الدهنية عالية الكثافة والبروتينات الدهنية منخفض الكثافة.

ويمكن إيجاز أهم النتائج فيما يلى :

- إضافة كل من بيكلونات الكروم، مخلوط الزيوت الأساسية أو بيكلونات الكروم مع مخلوط الزيوت الأساسية أدى إلى زيادة معنوية فى وزن الجسم ومعدل إستهلاك العلف، بينما تحسنت قيم معامل التحويل الغذائى لتلك المعاملات الغذائية معنوياً عند مقارنتها بالمجموعة الضابطة.
- إنخفض مستوى من الجلوكوز، الكولبستيرول الكلى والبروتينات الدهنية منخفضة الكثافة معنوياً عند إضافة كل من بيكلونات الكروم، مخلوط الزيوت الأساسية أو بيكلونات الكروم مع مخلوط الزيوت الأساسية، بينما إرتفع مستوى كل من البروتينات الكلية والبروتينات الدهنية عالية الكثافة.
- إضافة كل من بيكلونات الكروم، مخلوط الزيوت الأساسية أو بيكلونات الكروم مع مخلوط الزيوت الأساسية كان له تأثير معنوى على وزن غدة فبريشى عند المقارنة بالمجموعة الضابطة، بينما لم يتأثر وزن أى من الغدة التيموثية، الطحال أو غدة فابريشيوس معنوياً نتيجة لتلك الإضافات.
- إضافة بيكلونات الكروم مع مخلوط الزيوت الأساسية أدى إلى إنخفاض معنوى فى العدد البكتيرى الكلى وتركيز الأس الهيدروجينى pH، بينما إنخفض معنوياً عدد البكتريا سالبة الجرام عند إضافة كل من بيكلونات الكروم، مخلوط الزيوت الأساسية أو بيكلونات الكروم مع مخلوط الزيوت الأساسية، لم يتأثر العدد الكلى للعصويات المحبة للبن معنوياً نتيجة لأى من المعاملات الغذائية.
- إضافة بيكلونات الكروم مع مخلوط الأساسية كان له تأثير معنوى على زيادة المعيار الكمى للأجسام المناعية ضد لقاح فيروس مرض النيوكاسل.

أظهرت نتائج هذه الدراسة أن إضافة بيكلونات الكروم (1500 جزء فى البليون)، ومخلوط الزيوت الأساسية (بمعدل 200 مجم زيت الينسون + 200 مجم زيت البردقوش + 100 مجم زيت الثوم) أو بيكلونات الكروم مع مخلوط الزيوت الأساسية كان له تأثير معنوى إيجابى على قياسات النمو واعتدال بعض قياسات التمثيل الغذائى فى مصل الدم، إضافة بيكلونات الكروم مع مخلوط الزيوت الأساسية له تأثير معنوى مرغوب على النشاط البكتيرى للأعور وتأثير محفز للجهاز المناعى.