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EFFECT OF ADDING DIFFERENT CONCENTRATIONS OF PHYTOGENIC SUPPLEMENTATION (P.E.P) ON SOME IMMUNE AND PRODUCTIVE ASPECTS IN BROILER

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ABSTRACT
This study was carried out to prepare and evaluate phytogenic supplementation (P.E.P) and immunmodulator levamisole (LMS) in drinking water on immune response post routine vaccination with Newcastle disease (ND), Infectious Bronchitis (IB) and Infectious Bursal Disease (IBD). Also on serum total protein (albumin and globulin), blood picture Heterophil/Lymphocyte ratio (H/L), liver enzyme (AST and ALT), finally on growth performance including feed conversion ratio (FCR) and feed conversion efficiency (FCE). Three hundred Ross broiler chicks, weighted and divided randomly into six groups, 50 birds each group, drinking water and diet offered ad libitum, groups were treated as follows: Group 1,2,3, and 4 received phytogenic supplementation (P.E.P) in drinking water (15, 30, 60, and 120ml/1000 L) respectively throughout the duration of the experiment. Group 5: received levamisole (10µg/kg B.W.) in drinking water from one day to the end of the experiment. Group 6: vaccinated group not treated considered as control group. All groups were vaccinated with attenuated IB vaccine (H120-Intervet®) via spray route at 8 and 16 days old. ND live vaccine (La Sota-Ceva®) via drinking water at (8, 16 and 24) days old and attenuated IBD (IBDL-pfizer ®) at 12 days old via drinking water route. The obtained results referred that supplementation of (P.E.P) in drinking water revealed better performance in broilers like weight gain, feed consumption, efficiency of feed utilization, stabilization of serum metabolites with better immune response and improved some blood biochemistry. Moderate doses of (P.E.P) like (30-60 ml/1000L) gave better results of immunity and production performance and higher significance in total serum protein with low H/L, A/G ratio and AST and ALT. Using high dose of (P.E.P) (120 ml/1000L) gave low results than low dose due to strong taste that might have been unpalatable for the young chicks. Supplementation of (LMS) in drinking water revealed better immune response and serum protein, weight gain, feed consumption, efficiency of feed and blood morphology.

KEYWORDS: immunmodulator levamisole, phytogenic supplementation, feed utilization.

INTRODUCTION

As replacement for antibiotics, most frequently used alternative growth stimulators in broiler production are probiotics, prebiotics, enzymes, acidifiers, antioxidants and phytogenic additives (Perić *et al.*, 2010). Phytogenic additives, as natural substances, have been recognized as a very promising alternative solution, as they meet the requirements of consumers in terms of food safety and solve the problem of bacteria resistance that occurs as a result of using antibiotics as growth promoters (Rahal *et al*., 2014). Phytogenic additives comprise a wide range of plants and spices, as well as their derivatives that, as supplemental to basal broiler diets, positively affect production performances, animal health and quality of products (Windisch *et al*., 2008). Effects of phytogenic additives are mostly related to antimicrobial, antiviral, and antioxidative activities (B_lükbaşi and Erhan, 2007). Some research on broilers indicates that phytogenics have positive effects on production performances (Hashemi and Davoodi, 2010). Positive effects on production performances can be related to effects that phytogenic additives have on digestive enzyme activity (Basmacioğlu Malayoğlu *et al*., 2010). The objective of this research is to investigate effects of phytogenic additives as a broiler feed supplement on production, performances, morphological parameters and immune status after vaccination against (ND, IB and IBD), by using Phytogenic Supplementation (P.E.P) and estimate liver functions test by using total protein and albumin and globulin test, Total aminotransferase (ALT) and Aspartate Aminotransferase (AST) enzyme, and Blood picture Heterophil / Lymphocyte ratio.

MATERIALS & METHODS

Three hundred broilers (Ross-308) were used in the study. The chicks were weighted at hatching 44 gm, and divided randomly into 6 groups, each group contained 50 birds. Blood samples were collected from jugular vein randomly from 10 chicks in 1 day old to measure maternal immunity against Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease (IBD) using ELISA test (Indirect method). Group 1, 2, 3, and 4: received phytogenic supplementation (P.E.P) in drinking water (15, 30, 60, and 120 ml/1000 L) respectively throughout the duration of the experiment. Group 5 received levamisole (10µg/kg B.W.) in drinking water from one day to the end of the experiment. Group 6 considered as control group. All groups were vaccinated with attenuated IB vaccine (H120-Intervet®) via spray route at 8 and 16 days old, and New castle disease live

vaccine (La Sota-Ceva®) via drinking water at (8, 16 and 24) days old and attenuated Infectious Bursal Disease (IBDL-pfizer ®) at 12 days old via drinking water route. Blood samples were collected from jugular vein at 7, 14, 21, 28, 35 and 42 days of age for ELISA test to determine the antibody titer against ND, IBV and IBD. Differential counting of WBC`s method was described by (Campbell, 2004), The total protein in blood serum was measured according to Bayourat (Biuret method) and use it as a commercial kit (RANDOX)® Henry *et al.,* (1974). Measuring the level of albumin in blood serum method was used (Bromocresol Green) to measure the level of albumin in the serum by commercial kit (TC)® and followed by the way pointed out (Doumas and Biggs, 1976). Measuring the level of globulin in blood serum was measured indirectly, after measuring the concentration of total protein and albumin in serum.

Estimate the activity of carrying yeast the amino groups (ALT) and (AST)

Alanine and aspartate amino transferase activities were measured by using enzymatic kit. The glutamic transaminase enzymes, serum glutamic oxaloacete (AST) and serum glutamic pyruvic (ALT) catalyze the transfers of the amino group of glutamic acid to oxalactic acid and pyruvic acid in reversible reactions. The transaminase activity is proportional to the amount of oxalate or pyruvate formed over a definite period of time and measured by reaction with 2, 4-Dinitrophenyythyrozine (DNPH) in alkaline solution. The liquid reagent reacts with certain volume of the sample under defined constant conditions(*e.g.* temperature, pH, time) and produces a change of color that is proportional to the concentration of substances or activity of enzymes. As the indicator dye changes color the reaction is read spectrophotometriclly by light reflectance. This test was done according to instructions of manufactures manual. Component of Aspartate Aminotransferase Kit (AST) and(ALT):Used to measure the activity of these two yeasts commercial kit (RANDOX)® were carried out according to the manufacturer's instruction. Weight was calculated every week by weighing chicks individually. The chicks were weighed individually at days 7, 14, 21, 28, 35 and 42 of age for pen. The average body weight for each treatment was recorded every week and the weight gains calculated depending on the following equation: Weight gain = body weight at every week - body weight at the beginning of other week. P.E.P® is composed of oregano (*Origanumvulgare*), anise (*Pimpinellaanisum*), orange peel (*Citrus sinensis*), and FOS (fructooligosaccharides and prebiotic substances (Biomin® P.E.P essential oils. Austria made as feed additive it is not toxic at medium main ingredients; the essential oil blend contained the phenolic mono terpenes carvacrol, anethol, and flavonoids. Data of all studied groups were analyzed statistically using SPSS program.(version 17).The analysis included chi square test, ANOVA followed by LSD test and $P<0.05$ considered significance .

RESULTS & DISCUSSION

Maternal immunity against (ND, IB and IBD) revealed good immune response, the mean value was (4940.9 ±211.6, 3104.6 ±128.2 and 6204.6 ±182.5) respectively. The current study was conducted to determine the efficient of P.E.P. (commercial feed additive) in different doses, with proper vaccination program against ND, IB and IBD that gave a good immunity against ND in different periods. (Table 1, 2 and3). At the age of 14 day G3 (P.E.P. 60 ml/1000L) and G5 (levamisole) had the highest mean Abs against (ND, IB and IBD) followed by G2, G4, G1 compared with control group (G6). At (21, 28, and 35) day, the mean Abs titers increased significantly ($P<0.05$) in all groups. However G3 and G5 (levamisole) showed the highest mean Abs titer against (ND, IB and IBD). At the age of 42 days, the mean Abs titers further increased significantly ($P<0.05$) in groups G3 and G5 against (ND, IB and IBD) followed by G2 (P.E.P. 30 ml/1000L), G4 (P.E.P. 120 ml/1000L), G1(P.E.P. 15 ml/1000L).

TABLE 1: Antibody titer (ELISA, mean ± SE) against Newcastle disease of different groups in different days

Group	7 days	14 days	21 days	28 days	35 days	42 days
G1	1924.9±44.1 BC	2106.1±46.1 BC	2543.4±73.3 C	2807.4±65.6 C	3377.7±105 C	4058±99.1 C
G2	2031±72.7 AB	2228.6±53.3 B	2765.9±92.2 BC	3109.4±86.2 B	3676.2±109.3 B	4522.4±97 B
G3	2181.9±81.2 A	2469.9±85.5 A	3133.1±85.4 A	3576.7±116.7 A	4279.2±104.9 A	5116.2±109.6 A
G4	1945.6±61.5 BC	2060.3±49.1 D	2563.7±90.9 C	2942.2±46.9 B	3507.1±115.5 B	4274±105 B
G5	2166.3±76.5 A	2453.5±59.3 A	2910.2±96.2 AB	3401.4±95.3 A	4132.8±57.3 A	4863.2±131.9 A
G6	1813.6±73 C	1918.6±41 D	2127.1±77.1 D	2546.4±69 D	3196.9±102.4 D	3619.9±122.6 D
LSD	196.36	164.25	244.49	235.61	280.12	316.66

Means having different capital letters (in columns) are significant difference * ($P<0.05$).

Antibody titers against NDV, IBV and IBDV at 21 and 42 days of age in broiler administrated P.E.P supplemented components from (oregano, anis and citrus peel, and FOS Fructo oligo saccharides) in drinking water were higher than in control broiler chickens ($p<0.05$). Elevated antibody titers in broilers administrated 30 or 60 ml/1000L P.E.P. these results agree with (Ruberto *et al*., 2002; Rahim *et al*., 2011) who mentioned citrus oil could increase the production of Ab improving indirectly the immune system by their antivirus and antibacterial effect

and stimulating the immune system through the increase of IgG and IgM antibody production and, stimulating macrophages increase cytokines production especially the amounts of immune-modulating effects enhance phagocytosis and increase production of interleukins, IFNy**,** TNF factor secretary metabolism of macrophages, antigen presenting cells and antioxidant . Supplementation of anis oil led to a higher Ab titer against IBDV (Al-beitaw *et al.*, 2009). The result agree with (Lee *et al*., 2003, Lagu, . and Kayanja, 2010) whom reported that antibody titers against IBV was improved in broiler chickens fed phytogenic supplemented P.E.P. Phytogenic supplementation P.E.P has a significant enhancing effect on the cellular and humoral immune function (Castillo *et al.*, 2006).

TABLE- 2: Antibody titer (ELISA, mean ± SE) against infectious bronchitis disease of different groups in different days.

Group	7 days	14 days	21 days	28 days	35 days	42 days
G1	924.5±44.8 B	1539.4±94.8 C	2193.4±74.5 C	2357.4±84.6 C	3067.7±52.5 CD	3758±111.2 C
G2	1026.3±34.7 B	1856.4±59.2 B	2435.9±103,4 B	2709.4±118.3 B	3406.2±152.8 B	4022.4±99.1 B
G3	1286.8±82.6 A	2239.9±61.6 A	2845±71.3 A	3206.7±65.3 A	3869.2±96.4 A	4613.9±97.4 A
G4	884.3±43.9 B	1688.5±83.2 BC	2246.9±65.8 BC	2691.2±111.2 B	3107.1±117.5 C	3874±102.7 BC
G5	1321.8±68.7 A	2193.5±91.7 A	2773.8±84.6 A	3101.4±99.3 A	3731.8±83.6 A	4463.2±100.4 A
G6	651.6±66.7 C	1204±40.1 D	1928.2±96.7 D	2116.4±40.2 D	2796.9±87.9 D	3219.9±105 D
LSD	168.31	210.92	237.68	257.85	289.55	296.75

Means having different capital letters (in columns) are significant difference. * (P<0.05).

TABLE 3: Antibody titer (ELISA, mean ± SE) against infectious bursal disease of different groups in different days.

Group	7 days	14 days	21 days	28 days	35 days	42 days
G1	1924.5±44.8 C	939.4±53.4 B	1553.4±101.4 C	2877.4±83 C	3457.7±102.7 C	4388±156.9 C
G2	2046.3±38.5 C	1096.4±124.5 B	1815.9±73.8 B	3009.4±86.7 C	3876.2±84.1 B	4622.4±90 BC
G3	2916.8±100 A	1439.9±87.3 A	2215±191.3 A	3696.7±106.1 A	4239.2±85.3 A	5236.2±151.8 A
G4	1984.3±87 C	988.5±59.4 B	1546.9±92.5 C	2992.2±73 C	3437.1±115 C	4454±141.8 C
G5	2621.8±165.9 B	1393.5±114.7 A	2193.8±74.2 A	3401.4±130.1 B	4182.8±74.5 A	4873.2±116.1 B
G6	1631.6±57.4 D	684±64.9 C	1158.2±66.5 D	2366.4±86.5 D	3036.9±80.8 D	3949.9±63.6 D
LSD	263.59	250.61	225.25	272.41	259.31	353.64

Means having different capital letters (in columns) are significant difference. * (P<0.05)

Radwan *et al.* (2008) stated that P.E.P is immune organizations that have a significant role in the cellular immune reaction by binding protein receptors on the surface of phagocytes or lymphocytes cells and work to stimulate and generate an effective immune response by cooperation with cytokines to activate the non-specific immune response to broiler against viral infections. The higher significant (P<0.05) in antibody titre against ND, IB, IBD virus in fifth group that parallel to antibody titre in third group that return to role of levamisole (LMS) to induce higher immunity against (ND, IB, IBD) vaccination Chawak *et al.* (1993) confirmed that the use of (LMS) after immunization works as a multifunctional modulator to mediate cell-mediated T-cell response, also promote activated B cells to produce antibodies. LMS helps in the production and proliferation of lymphoid cells in chickens and mice (Yin *et al.*, 2006.) At 21 days there was a significant difference at level (P<0.05) among all groups, the highest means total protein level was given by G3 which was (4.669) followed by G5, G2, G4 and G1, which were (4.482, 4.370, 4.338 and 4.217) respectively, as compared to the G6 which was (4.071) (Tab. 4). At the age of 42 days, the mean value total protein further increased significantly (P<0.05) in group G3 which was (4.977) followed by G5, G2, G4 and G1, which were (4.796, 4.660, 4.557 and 4.441) respectively, as compared to the G6 which was (4.284) (Table 5). The albumin concentration reflected a significant differences (P<0.05) among all groups at day 21, the highest concentration recorded in G3 (2.534) followed by G5, G2, G4 and G1, (2.502, 2.442, 2.434 and 2.383) respectively, as compared to the G6 which was (2.335) (Table 4). At the age of 42 days, the mean value total protein further increased significantly (P<0.05) in groups G3 (2.614) followed by G5, G2, G4 and G1, (2.528, 2.464, 2.428 and 2.374) respectively, as compared to the G6 which was (2.30) (Table 5).

Also globulin concentration recorded significant differences (P<0.05) among all groups at day 21, the highest concentration recorded in G3 (2.134) followed by G5, G2, G4 and G1, (1.979, 1.928, 1.904 and 1.833) respectively, as compared to the G6 which was (1.735) (Tab.4). At the age of 42 days, the mean value total protein increased significantly (P<0.05) in group G3 (2.362) followed by G5, G2, G4 and G1, which were (2.268, 2.197, 2.129 and 2.069) respectively, as compared to the G6 which was (1.984) (Table 5). Therefore, the results of Albumin/Globulin ratio explained the presence of significant differences at level (P<0.05) among all groups at age 21 and 42 day, the lowest ratio was registered in the G3 which was 1.184 at 21 day and 1.1 at 42 day followed by G5, G2, G4 and G1 which were 1.26, 1.262, 1.272, 1.296 respectively at 21 day compared to the G6 which was 1.34. And 1.11, 1.12, 1.13, 1.14 compared with G6 which was 1.15 respectively at day 42. In the treatment groups, in the earlier stages total proteins and globulins in the serum of broilers increased significantly (P≤0.05) and at all stages the A/G ratio significantly decreased (P≤0.05). This shows that P.E.P can improve protein metabolism and immune function in broilers. This effect can be probably explained by two reasons. Firstly, P.E.P can promote protein deposition in broilers *in vivo*, keep the colloid osmotic pressure stable, improve the transportation of metabolic products *in vivo*, (Sheng Y *et al*. 2014), improve feed conversion rate and promote growth. Mathlouthi *et al*. (2012), Cho *et al*. (2014), proved that P.E.P can improve feed conversion rate and promote growth. Similar results were obtained in the present study, where higher contents of total protein and albumin in the blood serum especially in third group (60 ml/1000L) from P.E.P may also indicate enhanced nutrient supply and transport the increase in the plasma total protein. At age 21 day the results of H/L ratio revealed that G3 (P.E.P. 60 ml/1000L) and G5 (levamisole) have the lowest (P<0.05) than G2 (P.E.P. 30 ml/1000L), G1 (P.E.P. 15 ml/1000L) and G4 (P.E.P. 120 ml/1000L) (0.45, 0.46, 0.52, 0.54 and 0.56) respectively compared to control group (G6) which was (0.64). Also the results of H/L ratio at day 42 registered further decreased significantly (P<0.05) in groups G3 which was (0.33) followed by G5, G2, G4 and G1which were (0.36, 0.40, 0.45 and 0.47) respectively compared to the control group (G6) which was (0.68). The increase in lymphocyte counts and decreases in heterophil to lymphocyte ratios by P.E.P supplementation in the present study may be attributed to decreased glucocorticoid secretion (Cabrera *et al*., 2008). Soltan *et al*. (2008) found that the supplementation of poultry feed with anise grains, improved blood parameters and increased the phagocytic activity and lymphocyte counts. The decreased of H/L ratio in fifth group return to role of levamisole that can enhance lymphocyte proliferation and it acts as a multifunctional promote post vaccination to stimulate the cell-mediated activation B- cells to produce antibody these results agree with Montero-Rocha *et al*.(2006) and Yin *et al*. (2006). Levels of AST at 21 and 42 days showed a low significant difference (P<0.05) between the 6 groups at 21 days, G3 showed the lowest level of AST (6.1) as compared with G2, G1, G4 and G5 (6.74, 7.28, 8.22 and 8.46)respectively, and G6 had high level value which was (9.04). At 42 day the same trends were recorded in the 6 groups with significantly lower (P<0.05) levels of AST within and between the 6 groups. However, G3 (P.E.P. 60 ml/1000L) rank in the first place followed by G2, G1, G4 and G5in the second, third and fourth rank respectively compared to control group (G6). Presence of a significant difference at level (P<0.05) among all groups in (ALT) level at (21 and 42) days old chicks, at day 21 the (ALT) showed significant decrease (P<0.05) between the 6 groups, G3 showed the lowest level of AST (3.84) as compared with G2, G1 and G4 which were (4.98, 5.28 and 5.70) respectively, but no significant difference recorded between G5 and G6 that have height level value.(6.32 and 6.64)respectively. At 42 days the same trends were recorded in the 6 groups with significantly lower (P<0.05) levels of ALT within and between the 6 groups. However, G3 (P.E.P. 60 ml/1000L) rank in the first place in the lower followed by G2, G1, G4 and G5 in the second, third and fourth rank respectively which were 5.94, 6.42, 6.46 and 7.22compared to control group (G6) which was 7.36. AST, ALT and ALP) data recorded in Table (7 and 8), at 21 and 42 days clearly indicated the role of administration of P.E.P to maintain the normal level of liver enzyme (AST and ALT) activities. Aspartate transaminase is mainly distributed in myocardial cell, liver tissue, *etc*. Serum ALT and AST are very low *(*Sertel *et al.*, 2011*)*.

TABLE 4: Weight gain and final weight (gram) of different groups in different periods (Mean ± SE) of the first experiment

Groups	7 days	14 days	21 days	28 days	35 days	42 days	Final weigh
G1	100.4±1.59 B	235.8±1.4 C	315.8±1.27 C	400.6±0.17 C	618.4±1.95 C	658±3.01 C	2373±2.47 C
G2	105.2±1.29 A	244±1.41 B	323.8±1.14 B	422.4±0.17 B	633.6±1.9 B	689.4±2.922 B	2462.4±4.03 B
G3	109.2±0.75 A	257.2±1.17 A	336±1.68 A	422.6±0.17 A	650.6±3.06 A	712.8±2.58 A	2552.4±2.39 A
G4	87.6±1.39 D	209±1.5 E	292.6±1.35 D	338.8±0.14 E	545±2.83 E	603±2.53 DE	2119.6±4.012 F
G5	94.6±0.17 C	217.8±1.69 D	293.6±1.59 D	374.4±0.17 D	582.2±3.65 D	608.6±2.99 D	2215.2±2.61 D
G6	88.6±0.17 D	217.8±1.78 D	293.8±2.18 D	371±3.9 D	576.2±2.72 D	594.8±1.96 E	2186.2±8.15 E
LSD	4.381	6.221	6.509	6.61	11.39	11.11	18.257

Means having different capital letters in columns are significant difference * (P<0.05).

This experiment shows that P.E.P can significantly decrease the ALT level, but it has no significant effect on AST. Therefore, the addition of P.E.P will not damage liver cells, probably because it is derived from natural herbaceous plant; therefore it is beneficial to animals. Our study demonstrated that, as a result of addition of P.E.P, ALP levels in the serum of broilers tended to show a statistically significant increase (P≥0.05) on day 21.Consequently, it can be inferred that P.E.P can improve bone metabolism in broilers and therefore their growth. However, Sharma and Gangwar (1986) suggested that, as broilers grow and age, the ALP activity tends to decrease, as shown in our study. In particular, ALP in the serum of broilers in the treatment groups tended to decrease significantly (P≥0.05) on day 42 compared with the control group. Plasma ALT and AST recorded for individuals fed diets supplemented with phyto additive indicate that the used treatment did not negatively alter liver enzyme activity but also had a non-toxic effect on the kidneys and liver (Biswas *et al*., 2011). Furthermore, no increase in serum concentration of ALT and AST may provide evidence to protect of liver against hepato cellular degeneration. The present study confirmed previous results (Habibi *et al*., 2014) on the decreasing effect of phyto additives on the activities of plasma ALT and AST in animals. Meanwhile, Abd El-Ghang and Ismail (2013) who used oregano essential oil in broiler feed, observed increase in the activity of ALT and AST. The progress in weight gain has been evident in treated groups in significant differences at level (P<0.05), specially the G3 and G2 that received moderate doses of P.E.P. recorded progress senior from the other groups (G1, G4 and G5) during days 7, 14, 21,28and 35, the weight gain of all groups especially in G3 at 42 days at slaughter age followed by other groups (G1, G4 and G5) respectively, in comparison with control group (G6) (Tab. 4). Essential oils components increase BW gain by their ability to destroy pathogen microorganisms in the digestive system and consequently increasing the production of digestive enzymes which improve utilization of digestive products (Khattak *et al*., 2014). These results are in agreement with the finding of (Khattak *et al*., 2014) they reported that supplementation of broiler diet with essential oil through diet or drinking water had a positive significant effect on the broiler`s body weight gain. The higher body weight was noted in the broilers administrated P.E.P in drinking water may be due to the beneficial effects of these herbs in birds nutrition which includes improvement of endogenous digestive enzyme secretion and antibacterial, antiviral, antioxidant and anthelmintic actions. All these actions cause improvement in health, growth and performance of broiler (Rahimi *et al*., 2011).

The present study indicated that Levamisole hydrochloride could enhance body weight gain even in healthy broilers. However, the drug's influence on cumulative body weight gain at the 8th week of age was not as significant as that of the fourth week and sixth week (Keles *et al.*, 1995). And registered a significant difference at level (P<0.05) in feed conversion ratio among all groups, so G3, G2, G1, G4, G5 and G6 gave (1.5, 1.7, 1.74, 1.76, 1.93, and 2.05) respectively, on the contrary, the results of feed conversion efficiency recorded matching results achieved to the feed conversion ratio and explained a significant difference at level (P<0.05) in feed conversion efficiency among all groups, where G3, G2, G1, G4, G5 and G6 groups were (0.65, 0.58, 0.56, 0.55, 0.5, and 0.48) respectively, (Tab. 5).

Administration P.E.P in drinking water from 0-6 weeks of age especially in third group (60 ml/1000L) achieved the best feed conversion ratio compared with that of the control diet. The results of feed conversion are in agreement with the findings of (Al-Jugifi, 2009; Al-Mashhadani *et al*., 2011; Khattak *et al*., 2014) they found that supplementation broiler diet with P.E.P had a significant positive effect on the feed conversion ratio compared to the control diet which is due to Volatile oil from (Citrus oil and Oregano oil) which was assessed for antibacterial and antiviral activity as inhibitors of microbial growth (Dorman and Deans, 2000). The fourth group (120 ml/1000L) had less significant effect on feed intake which could be due to increase of elements or components in P.E.P that affect the bird appetite and then feed intake (Ansari et al., 2012). Levamisole reduced the cumulative feed intake and resulted in a better feed efficiency in 14-42 age of the fifth group. Levamisole enhances the immune response and the important changes resulting from this reducing the appetite and voluntary feed intake (Klasing and Johnstone (1991). Concluded that supplementation of (P.E.P) in drinking water revealed better performance in broilers like weight gain, feed consumption, efficiency of feed utilization, stabilization of serum metabolites with better immune response and improved some blood biochemistry.

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