

USING OF DRIED BETONY AND PEPPERMINT IN BROILER DIETS ON PERFORMANCE, HEMATOLOGICAL AND IMMUNITY RESPONSE.

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ABSTRACT

This experiment was aimed to investigate the effect of using different levels of betony and peppermint alone or together on body performance, hematological traits and immunological response in Ross-308 broiler male chickens, in this experiment three hundred and sixty (360) day old sexed male chicks (Ross-380) were randomly divided into 12 replicates, 30 chicks per replicate (three replicates per treatment). The four treatments in this experiment are: T0=control (standard ration), T1 (2% dried betony), T2 (2% dried peppermint) and T3 (1% betony+ 1% peppermint).

The effect of betony and peppermint added to broilers diet is presented showed significant increases ($P<0.05$) in body weight in T2 at 25 days, relative weight of heart, liver, gizzard, proventriculus and fabricius, also showed significant increases ($P<0.01$) in body weight at 42 days, body weight gain at 25 and 42 days in T1, T2 and T3 also in eviscerated dressing, the relative weight of (breast, thigh, drumstick, wings and spleen), but showed that FCR differ significantly ($P<0.01$) among treatments, better feed conversion was found in T1, T2 and T3 at ages 25 and 42 days. While, the results shows significant induces ($P<0.01$) in mortality and relative weight of (abdominal fat, neck and back), but there were no significant differences among the treatments with control in Feed consumption at ages 25 and 42 days. Thus the results showed significant increases ($P<0.01$) in total count of red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), total protein concentration and high density lipoprotein (HDL), While, the results shows significant induces ($P<0.01$) in H/L ratio, cholesterol concentration and low density lipoprotein (LDL) in T2, T3 and T1 as compared with T0, but there were no significant differences among the treatments with control in total count of white blood cells (WBCs). Also the results showed significant increases ($P<0.01$) in

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antibodies titer of Newcastle Disease (HI), Gumboro Disease (IBD) and Infectious Bronchitis Viral (IBV) in T1, T2 and T3. In all traits T2 achieved more improving as compared with the other treatment of this experiment.

Keywords: betony; peppermint; broiler; performance; hematological; immunity

INTRODUCTION

Betony and peppermint are plant species being abundantly available, is extensively used for the treatment of several diseases, scientific evidences suggest that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties and promote the growth of beneficial bacteria and inhibit the growth of unfavorable bacteria in the gut (Wenk, 2000; Kamel, 2001). Betony (*Stachys floridanum*) is related to mint family (Ginger *et al.*, 2009), it contains various alkaloids, saponins, tannins, glycosides and volatile oil as active constituents that make the herb rather effective herbal remedy in case of diarrhea, bronchitis, asthma, and even kidney stones, is an effective remedy for headaches and nervousness, due to glycoside constituent, that is believed to lower blood pressure. It relaxes the whole body (especially the head area) and gives calm and peace, stimulates digestive systems, purifies blood system, and even gives protection both for good health and good fortune, its cousins may all contribute to its activity, it used as antistress (Singleton, 2010 and Vogl *et al.*, 2013).

Peppermint (*Mentha piperita*), is aromatic, almost exclusively perennial, rarely annual, herbs, that are widely distributed and can be found in many environments (Brickell and Cole, 2002). Mint essential oil and menthol are extensively used as flavorings, antiseptic. The active of the mint depend on the abundant volatile oil, which has been found to contain a hydrocarbon, thymol and higher oxygenated compounds (Grieve, 1981; Chopra *et al.*, 1992). Analgesic (topical), anti-inflammatory, calms and strengthens nerves, calms an upset stomach, digestive aid, dissolves gallstones, eliminates heartburn, improves solubility of bile, inhibits and kills micro-organisms (*Influenza A viruses*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus*), inhibits constipation, inhibits diarrhea, normalizes gastrointestinal activity, prevents congestion of blood to the brain, reduces bile cholesterol levels, stimulates circulation, stimulates contractile activity and bile secretion in the gallbladder (Blumenthal, 1998).

MATERIALS AND METHODS

The experiment was conducted in Murtka/Qushtapa broiler field- Erbil, with 360 one day old broiler sexed male chicks (Ross-380) reared for a period of 42 days from 21/3/-2/5/2013. Chicks initial weight (42.4 g) were randomly distributed into 4 treatments, each treatment contain 3 replicates (30 chicks). T0=control (standard ration), T1 (added 2% dried betony), T2 (added 2% dried peppermint) and T3 (added 1% betony+ 1% peppermint). Feed and water were

supplied *ad libitum*, the feed prepared from Kosar company that content (3003, 2968 , 2985 kcal/kg) metabolized energy, (21, 19.75, 20.35%) crude protein in (starter, grower and finisher) diet respectively. Throughout this experiment body weight, feed intake, feed conversion ratio, body weight and body gain were recorded in 25 and 42 days, also mortality , eviscerated dressing percentage , edible & non edible internal organs, , abdominal fat , carcass cuts (breast , thigh , drumstick , back , wings and neck) were measured at 42 days.

In this study blood was collected in EDTA tubes from the brachial vein of 12 birds from each treatment, and used fresh blood for determination of hematological indexes. The number of total RBC ($10^6/\text{mm}^3$) and total WBC ($10^3/\text{mm}^3$) were determined using Natt-Herrick staining solution (Natt and Herrick, 1952) in a hemocytometer chamber. Differential leukocyte count (heterophil and lymphocytel) made on slides stained with Wright-Giemsa and observed in an optical microscope (100x) to determined H/L ratio.

Hemoglobin level (g/100 mL) was measured by the cyanmethemoglobin method and hematocrit (PCV) (%) was determined using a micro-hematocrit capillary, blood centrifuged for biochemical analyzer, thus serum collected for studying the concentration of: cholesterol, HDL, LDL and total protein by using Kits, also the same chicks serum used for measuring antibody titer of Newcastle disease (ND) Gumboro (IBD) and IBV.

All data were analyzed by using CRD (Complete Randomize Design) by SAS (Statistical Analysis System, 2002), as per variance, significant differences among treatment means were determined by Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

The effect of betony and peppermint added to broilers diets are presented in Table (1) shows significant increases ($P<0.05$) in body weight in T2 (2% peppermint) at 25 days, also shows significant increases ($P<0.01$) in body weight at 42 days, body weight gain at 25 and 42 days when added 2% betony in T1, 2% peppermint in T2 and T3 (1% betony+1% peppermint) as compared with the control (T0), at the same table showed that FCR differ significantly ($P<0.01$) among treatments, better feed conversion was found in T2, T3 and T1 respectively as compared with T0 at ages 25 and 42 days. While, the results shows significant induces ($P<0.01$) in mortality at the end of the experiment in T2, T3 and T1 as compared with T0, but there were no significant differences among the treatments and control in Feed consumption at ages 25 and 42 days. Herbal planet could stimulate the digestion system in poultry, improve the function of liver and increase the pancreatic digestive enzymes. Enhancement of the metabolism of herbal plant carbohydrates and proteins in the major organs would increase growth rate of these organs (Mellor, 2000). The high content of

peppermint in menthol, potassium, manganese, calcium, phosphorus, niacin, foliate, sugar, carbohydrates, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids, energy and (omega -3) (USDA, 2012), thereby improving the consumption of feed, feed conversion efficiency, body weight and body gain also herbals because it content high percentage of fibers led to reduce the speed of the passage of food into the gastrointestinal tract and thereby increase the rate of digestion and absorption of feed materials (Kwropatcin, 1982; Naji and Kabro, 1999).

Table 1. *Effect of betony and peppermint added on broiler performance.*

| treatments | T0 | T1 | T2 | T3 |
|-----------------------------------|------------------------|------------------------|------------------------|-------------------------|
| Body weight 25 day (g) | 1079±57 ^b | 1137±55 ^{ab} | 1240±41 ^a | 1192±39 ^{ab} |
| Body wt. gains 1-25 Day (g) | 1036±53 ^c | 1094±50 ^b | 1197±35 ^a | 1149±36 ^{ab} |
| Feed consumed 1-25 day (g) | 1637±79 ^a | 1619±65 ^a | 1652±58 ^a | 1631±60 ^a |
| Feed conversion efficiency 25 day | 1.58±0.11 ^a | 1.48±0.09 ^b | 1.38±0.05 ^c | 1.42±0.07 ^{bc} |
| Body weight 42 day (g) | 2720±108 ^c | 2805±98 ^b | 2911±83 ^a | 2865±88 ^{ab} |
| Body wt. gains 1-42 Day (g) | 2677±104 ^c | 2762±96 ^b | 2868±82 ^a | 2822±85 ^{ab} |
| Feed consumed 1-42 day (g) | 4979±153 ^a | 5027±140 ^a | 4933±110 ^a | 4995±126 ^a |
| Feed conversion efficiency 42 day | 1.86±0.13 ^a | 1.82±0.10 ^a | 1.72±0.08 ^b | 1.77±0.07 ^b |
| Mortality % | 6.56±1.45 ^a | 4.33±1.11 ^b | 2.66±0.25 ^c | 3.33±0.30 ^c |

T0= control T1=2% betony T2= 2% peppermint T3=1% betony +1% peppermint
^{a-c} Means within rows with different superscripts differ significantly at (P≤ 0.05) and (P≤ 0.01)
 Non-Significant differences within rows same letters.

Table (2) shows significant increases (P<0.01) at 42 days in the percentage of heart, liver and proventriculus in T2 (2% peppermint), also shows significant increases (P<0.01) in Gizzard % in T2 and T3, thus shows significant increases (P<0.01) in the percentage of eviscerated dressing, breast, thigh, drumstick and wings in T1, T2 and T3 as compared with the control (T0). While, the results shows significant induces (P<0.01) in the percentage of abdominal fat, neck and back in T2 and T3 as compared with T1 and T0. The high body weight in table (1) led to high dressing, internal organs and carcass portion percentages.

Table 2. *Effect of betony and peppermint added on broiler eviscerated dressing, internal organs and carcass portion percentages at 42 days.*

| treatments | T0 | T1 | T2 | T3 |
|---------------------------------------|--------------------------------|---------------------------------|--------------------------------|---------------------------------|
| Eviscerated dressing percentage % | 71.44±2.8 ^c | 72.00±1.7 ^c | 73.69±1.0 ^a | 72.88±1.3 ^b |
| Internal organs%/ Heart % | 0.497±0.033^b | 0.537±0.030^{ab} | 0.552±0.025^a | 0.530±0.030^{ab} |
| Liver % | 2.395±0.18 ^b | 2.482±0.16 ^{ab} | 2.643±0.09 ^a | 2.504±0.08 ^{ab} |
| Gizzard % | 1.419±0.13 ^b | 1.442±0.11 ^b | 1.509±0.10 ^a | 1.528±0.07 ^a |
| Proventriculus % | 0.414±0.025 ^b | 0.428±0.030 ^b | 0.482±0.018 ^a | 0.439±0.020 ^b |
| Abdominal fat % | 0.856±0.032 ^a | 0.793±0.029 ^{ab} | 0.656±0.020 ^b | 0.728±0.020 ^b |
| Carcass portion%/ Breast % | 25.28±2.4^c | 26.19±1.9^b | 28.19±1.3^a | 26.66±1.7^b |
| Thigh % | 17.29±1.6 ^c | 18.64±1.5 ^b | 19.20±0.9 ^a | 18.40±1.2 ^b |
| Drumstick % | 13.67±0.83 ^b | 14.09±0.67 ^{ab} | 14.33±0.49 ^a | 14.36±0.38 ^a |
| Wings % | 12.20±0.65 ^a | 11.92±0.34 ^a | 10.04±0.28 ^b | 10.60±0.33 ^b |
| Neck % | 4.66±0.17 ^b | 3.95±0.13 ^{ab} | 3.18±0.11 ^b | 3.34±0.13 ^b |
| Back % | 19.93±0.45 ^a | 19.19±0.28 ^{ab} | 18.12±0.31 ^b | 18.61±0.29 ^b |

T0= control T1=2% betony T2= 2% peppermint T3=1% betony +1% peppermint

^{a-c} Means within rows with different superscripts differ significantly at (P< 0.01)

The results in Table (3) shows a significant increase ($p<0.01$) in total count of red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), total protein concentration and high density lipoprotein (HDL) in blood serum in the treatments T1, T2 and T3 as compared with control (T0).

While, shows significantly induces ($p<0.01$) in T2, H/L ratio, cholesterol concentration and low density lipoprotein (LDL) in blood serum in the treatments T1, T2 and T3 as compared with T0. But there were non significant among all treatments in total count of white blood cells (WBCs).

May return to contain peppermint powder high amounts of iron 5.08 mg / 100gm (USDA, 2012), which enters in the composition of blood hemoglobin and then in the manufacture of blood red cells (RBC) and consequently an increase in the size of blood cells volume (PCV), also peppermint contain B-complex vitamins, participated in manufacturing erythrocytes processes in bone marrow (Sturike, 1986). The reason for the high volume of blood cells related to contain peppermint powder on vitamin A and E, which has an important role in preventing the decomposition red blood cells (Erythrolysis) through their work and protection plasma membranes from the damage of anti-oxidant that occurs of oxidation stress (Coles, 1986), The high level of fibers in peppermint 21.2/100gm can increase the excretion of bile and this can decrease the cholesterol level of blood (Al-Kassie, 2009). The reason for the high concentration of protein in the treatments fed peppermint powder to the role of vitamin A and carotenes as provide protection against catabolism reactions in the body through its physiological due as antioxidant (Burton, 1989), also

vitamin A equality free radicals and inhibiting crash of DNA and proteins in the body (Surai *et al.*, 2000).

The low concentration of cholesterol in the blood plasma in the T1, T2 and T3 as compared with T0, which have due to the high fiber in peppermint and not contain cholesterol (0%) (USDA, 2012) which leads to reduce the absorption of diet cholesterol in the gastrointestinal tract (GI) (Nakaue *et al.*, 1980). Peppermint leaf positively supports digestion by helping to breakdown fats and reduce bad cholesterol levels (LDL) and reduce the work load required from the liver (Gray, 1984).

Table 3 . Effect of mint and betony on some whole blood and blood biochemical parameters at 42 days.

| treatments | T0 | T1 | T2 | T3 |
|---|-------------------------|-------------------------|-------------------------|--------------------------|
| Total RBC (10 ⁶ cells/mm ³) | 2.96±0.23 ^c | 3.21±0.25 ^b | 3.62±0.17 ^a | 3.25±0.20 ^b |
| Total WBC (10 ³ cells/mm ³) | 23.32±1.9 ^a | 22.81±1.5 ^a | 22.75±0.9 ^a | 22.77±0.8 ^a |
| Hb (gm/100ml) | 9.44±0.57 ^c | 10.65±0.60 ^b | 12.35±0.33 ^a | 10.98±0.40 ^b |
| PCV % | 36.2±2.45 ^c | 37.80±2.33 ^b | 39.85±2.0 ^a | 38.15±1.8 ^b |
| H/L ratio | 0.34±0.018 ^a | 0.31±0.016 ^b | 0.29±0.013 ^c | 0.31±0.017 ^b |
| Total protein (g/dL) | 2.84±0.034 ^c | 3.38±0.022 ^b | 3.98±0.022 ^a | 3.64±0.025 ^{ab} |
| Cholesterol (mg/dL) | 127±3.95 ^a | 116±3.50 ^b | 101±2.88 ^c | 108±3.04 ^{bc} |
| HDL (mg/dL) | 52.8±2.08 ^d | 57.6±2.10 ^c | 63.7±1.72 ^a | 59.5±1.66 ^b |
| LDL(mg/dL) | 67.1±2.00 ^a | 41.5±1.3 ^b | 27.6±0.67 ^d | 35.8±0.80 ^c |

T0= control T1=2% betony T2= 2% peppermint T3=1% betony +1% peppermint

^{a-c} Means within rows with different superscripts differ significantly at (P≤ 0.01)

Non-Significant differences within rows same letters.

The results in table (4) shows significantly increases (p<0.01) in the antibodies titer of Newcastle Disease (HI), Gumboro Disease (IBD) and Infectious Bronchitis Viral (IBV) in the treatments T1, T2 and T3, also there were significantly increases (p<0.01) in the percentage of fabricius in T2 and spleen relative weight in the treatments T2 and T3 as compared with the control T0 at the age 42. Reported that herbal plants had anti stress, immune modulator properties betony and peppermint is the most medicinally valuable of all mints, with great cooling properties due to its high content of menthol is used to treat gastric and digestive disorders and nervous complaints such as tension and insomnia (Richmond and Mackley, 2000). The evidence of improving the immunity showed from the table (1) that dried peppermint and its oil treatments reduced the mortality because improving in body health. Betony and peppermint constituent have also been shown to have significant antiviral, antibacterial, antifungal and antiplasmid activity and to potentiate the antibiotics effect (Schelz, 2006).

Table 4 . Effect of mint and betony in some immunological parameters at 42 days.

| treatments | T0 | T1 | T2 | T3 |
|-----------------------------|--------------------------|---------------------------|--------------------------|---------------------------|
| HI Log 2 ⁿ | 4.5±0.55 ^c | 6.0±0.60 ^b | 7.5±0.35 ^a | 6.5±0.50 ^b |
| IBD Log 2 ⁿ | 3.00±0.25 ^c | 4.0±0.15 ^b | 5.3±0.15 ^a | 4.5±0.20 ^b |
| IBV Log 2 ⁿ | 2.33±0.10 ^c | 2.75±0.15 ^b | 3.66±0.10 ^a | 3.33±0.15 ^{ab} |
| fabricius relative weight % | 0.189±0.025 ^b | 0.192±0.013 ^{ab} | 0.205±0.010 ^a | 0.195±0.009 ^{ab} |
| spleen relative weight % | 0.122±0.014 ^b | 0.119±0.010 ^b | 0.149±0.012 ^a | 0.140±0.010 ^a |

T0= control T1=2% betony T2= 2% peppermint T3=1% betony +1% peppermint
^{a-c} Means within rows with different superscripts differ significantly at (P≤ 0.01)

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تأثير استخدام البطنج والنعناع في علائق فروج اللحم على الأداء الإنتاجي، صفات الدم والأستجابة المناعية.

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المستخلص

درس في هذه التجربة تأثير استخدام البطنج والنعناع في علائق فروج اللحم على الأداء الإنتاجي والفحوصات الدموية والأستجابة المناعية باستخدام ٣٦٠ فرخ فروج اللحم من سلالة Ross-308 بعمر يوم واحد ووزعت عشوائيا الى ٤ معاملات و٣ مكررات. معاملة السيطرة T₀ (عليقة قياسية بدون اية إضافة)، وأضيفت الى المعاملات T1 (٢ % مسحوق البطنج المجفف) و T2 (٢ % مسحوق النعناع المجفف) و T3 (١ % مسحوق البطنج المجفف+١ % مسحوق النعناع المجفف) على التوالي. أن تأثير إضافة البطنج والنعناع في علائق فروج اللحم أظهرت النتائج إرتفاعا معنويا ($p<0.05$) في وزن الجسم والزيادة الوزنية بعمر ٢٥ يوما في المعاملة T2 والوزن النسبي للقلب، الكبد، الفانصة، المعدة الغدية وجراب فابريشا، كذلك وجد ارتفاع معنوي ($p<0.01$) في وزن الجسم بعمر 42 يوما في المعاملات T1، T2 و T3 أيضا في نسبة التصافي بدون الأحشاء الداخلية، والوزن النسبي لـ (الصدر، الفخذ، الوصلة الفخذية، الأجنحة والطحال)، لكن وجد تحسن في كفاءة التحويل الغذائي في المعاملات T1، T2 و T3 بعمر ٢٥ و ٤٢ يوما. بينما وجد انخفاض معنوي ($p<0.01$) في النسبة المئوية للهلاكات، الوزن النسبي لـ (دهن البطن، الرقبة والظهر)، نسبة H/L. ووجد ارتفاع معنوي ($p<0.01$) في العد الكلي لخلايا الدم الحمراء (RBC)، الهيموغلوبين (اليحمور) Hb ، حجم خلايا الدم المرصوصة (PCV) ، البروتين الكلي، الكولسترول عالي الكثافة HDL. بينما وجد انخفاض معنوي ($p<0.01$) في نسبة H/L، الكولسترول المنخفض الكثافة (LDL) في المعاملات T1، T2 و T3 مقارنة بمعاملة السيطرة T₀. في حين لم توجد أي فروق معنوية بين جميع المعاملات في العد الكلي لخلايا الدم البيضاء (WBC). وأظهرت النتائج ارتفاعا معنويا ($p<0.01$) في المعيار الحجمي لاختبار التلازن الدموي لحمى النيوكاسل (HI)، مرض التهاب كيس الجراب (الكمبورو) (IBD) و مرض التهاب الشعب المعدي (IBV) في المعاملات T1، T2 و T3. في جميع الصفات أظهرت المعاملة T2 تفوقا على بقية معاملات التجربة.

الكلمات المفتاحية: البطنج، النعناع، فروج اللحم، الصفات الإنتاجية، الفسلجية، المناعة .