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Effect of Thyme Leaf (*Thymus Schimperi R.)* as Feed Additive on Selected Hematological and Serum Biochemical Profiles of White Leghorn Chicken

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The feeding trial was conducted to evaluate the effect of thyme leaf as an additive on selected hematological and serum biochemical profiles of white leghorn layers at Haramaya university poultry farm for 11weeks. One hundred twenty white leghorn (WLH) layers at the age of 26 weeks with average initial body weight of 1216.1±6.62 gram (mean±SD) were randomly allotted to four dietary treatments. The treatments were T1 (Layers ration (LR) with 0% Thyme leaf meal (TLM), T2 (layer's ration with 1% TLM), T3 (layer's ration with 2% TLM) and T4 (layer's ration with 3% TLM). Each treatment group was replicated three times with ten laying hens and two cocks per replicate in completely randomized design (CRD). Samples of feed ingredients, thyme and formulated diets were subjected to chemical analysis before the actual experiment began. The result indicated that red blood cells (RBC), white blood cells (WBC), hemoglobin and hematocrit percentage of birds did not show significant deference among treatment groups. Serum triglycerides, total cholesterol and LDL-cholesterol were significantly ($p<0.05$) lower in birds fed a diet containing 2% and 3% than birds fed a control diet. Therefore, the study showed that thyme leaf significantly affects serum metabolites but did not blood hematology.

Key words: Hematology, layers, Ration, Serum, Thyme leaf

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INTRODUCTION

The main goal of any animal production system is to raise healthy animals, improve animal productivity and produce quality animal products, which are stable and appropriate for further processing. Many studies describe that the possibility of production of quality and healthy animal product by feeding animals feed from organic sources instead of inorganic or synthetic products. Since the use of antibiotic growth promoters was banned by the European Union (Neu, 1992), demand for using alternatives to antibiotics has increased from time to time. Nowadays, researchers are focusing on the use of additives that have not negative effects on health and the environment. Therefore, the use of natural feed additives in animal nutrition has gained attention in many countries across the world. These feed additives are derived from herbs, spices, or aromatic plants (Windisch *et al.,* 2008) that have gained considerable attention in recent years in the feed industry (Abd El-Hack and Alagawany *et al.,* 2015). Plant-derived products are considered safe to be used as ingredients in the food industry as well as in animal diets as an ideal growth promoter (Li *et al.*, 2016). Phytogenic feed

additives (PFA) are attaining great importance in poultry production, as well as in health care systems, because of their beneficial effects on promoting growth and production, immune enhancement and health protection (Alagawany *et al*., 2015a, b).Herbs and phytobiotics are incorporated into poultry diets for effective nutrient utilization, which may subsequently result in a more rapid gain in body weight and improved feed conversion efficiency. Many reports indicate the positive effects of herbs with anti- coccidian, anti-oxidant and anti-fungi properties (Farag *et al.,* 2016).

Thyme herb in the Lamiaceae family is cultivated worldwide for culinary, cosmetic perennial and medical purposes. In Ethiopia, there are two indigenous species of thyme namely *Thymus serrulatus* and *Thymus schimperi* (Jaafari *et al.,* 2007). These species are endemic to Ethiopian highlands and are restricted to the Afromontane and Afro Alpine zones of the country (IBC, 2008). *Thymus schimperi* is a wild-growing perennial herb that is rich in medicinally important metabolites. It grows in open grasslands, between bare rocks, on slopes and tops of mountains between 2250-4000 m.a.s.l (Nigist and Sebsebie, 2009). It is well known in Central, Eastern and Northern highlands of Ethiopia and is harvested and dried by people living close to the towns of Dinsho (Bale Zone) and Menz (North Showa Zone) and sold at local markets (Sebsibe, 1993). *Thymus serrulatus* was found in Jimma (Parvez and Yadav, 2008) and Mahbere Silassie, Alamata, and Ofla (Atey, 2008). Furthermore, it was widely distributed in the northern part of Ethiopia (Nigist and Sebsebe, 2009).

Studies have described the biological and protective properties of thyme, including antispasmodic, expectorant, antiseptic, antioxidant, antibacterial, anti-inflammatory, immunomodulatory and health-promoting activities of birds (Dhama *et al.,* 2015). Thyme was also reported that it enhance the digestive activity of enzymes like protease, amylase, and lipase, which results in improved digestibility of nutrients (Abdel-Wareth *et al.,* 2012). The study of Mansoub (2011) investigated the effect of different levels of thyme inclusion on the performance of laying hens; and reported best feed conversion ratio, high egg weight and percent of egg production at 2 % inclusion of thyme on layer diet. The finding of Abdel-Wareth *et al.* (2013) reported that egg weight, egg mass and hen-day-egg production were improved in response to the inclusion of 20 mg/kg of thyme and oregano in the layer diet. Inclusion of 0.2% of thyme and garlic in the diet of laying hens improves the yolk index and increase the lymphocyte in the blood (Ghasemi *et al.,* 2010). However, in Ethiopia there is insufficient information on the use and inclusion level of thyme as a natural feed additive in the poultry diet.

Therefore, the preset study is designed to evaluate selected hematological and serum biochemical profiles of white leghorn chicken fed a diet containing dried thyme leaf.

MATERIALSAND METHODS

Nutrient Composition Analysis

The representative samples of feed ingredients were randomly taken from top, middle and bottom of the sack. The samples from the same ingredient were mixed together and divided in to four on the plate to take two representative samples. The two representative samples of approximately 500 g each: one was submitted for analysis and the second one was retained as a backup (Herrman, 2001).The representative samples were analyzed by duplicating (two times) for chemical composition. The result of the analysis was used to formulate the treatment rations. Samples were also taken from each treatment ration and analyzed for composition. The feed samples were analyzed for dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF) and ash at Haramaya university animal nutrition laboratory following the procedure of Weende or proximate analysis method (AOAC, 1990). Nitrogen (N) was analyzed by Kjeildhal procedure and CP was determined by multiplying the N value with 6.25. Calcium (Ca) was determined by Atomic Absorption Spectrophotometer method and total Phosphorus in the feed samples was determined by wet digestion procedure, at soil chemistry and central laboratories of Haramaya University. The metabolizable energy (ME) of feed ingredients and experimental diet were determined by indirect method according to Wiseman and lessire (1987) as follows

$$\mathrm{ME}\left(\frac{\mathrm{Kcal}}{\mathrm{kg}}\right) = 3951 + 54.4\mathrm{EE} - 88.7\mathrm{CF} - 40.8\mathrm{ASH}$$

Where;

EE=Ether extract, CF=Crude fiber

Experimental Diet Preparation

After conducting the laboratory analysis, necessary feed ingredients were ground at 5 mm sieve size and mixed at Haramaya University feed processing unit. The experimental diets were a ration without the addition of thyme leaf and ration containing different levels of thyme leaf. The ME and CP levels of the diet wasiso- caloric and iso-nitrogenous within the ranges of the recommended levels of 2800-2900 kcal/kg and 16-18% respectively (NRC, 1994). After formulating the experimental diet, the representative samples were randomly sampled and prepared for laboratory analysis to check up the required nutrient level of the diet.

Fresh thyme was collected from Debresina where it is locally available. The leaf part of the thyme was manually collected and air-dried under shade. The dried thyme leaf was prepared and sampled for laboratory analysis. Thereafter thyme was ground and prepared to mix according to the required proportion in the treatment.

Experimental Design and Treatments

A Completely Randomized Design (CRD) with four treatments and three replications was used in the study. A total of one hundred twenty white leghorn layers at the age of twenty-six weeks randomly selected and distributed with 10 layers. The treatments were layers ration with 0% thyme leaf meal (control) and ration containing 1%, 2% and 3% of thyme leaf meal designated as T_1, T_2, T_3, and T_4, respectively.

Management of Experimental Birds

The experiment was conducted for 11 week including one week for acclimatization in deep litter housing. Before instigation the actual experiment, the house was cleaned, disinfected, maintained and partitioned into experimental pens. Each pen was equipped with watering and feeding troughs, which were thoroughly cleaned and disinfected. Before the experiment started, the birds were dewormed against internal parasites. During the experiment, layers were fed on an *ad-libitum* basis at 8:00 and 14:00 hours and had free access to water

Measurements

Hematological blood Profiles

At the end of the experiment blood samples were drawn from the neck (jagular vein) of 3 birds randomly selected from each replicate. After 12 hrs of fasting, 4ml of blood samples were collected from each bird by using a 5 ml sterile syringe of a 22-gauge needle. Half of the blood (2 ml) from the syringe was transferred into the tube containing anti-coagulant Ethylene Diamine Tetra Acetate (EDTA) for hematological assay. The second half was transferred into tubes without EDTA for the serological test.

Analysis of blood hematological and chemistry part was conducted at Haramaya University veterinary laboratory and higher clinic respectively. The hematological test was carried out to determine RBC, WBC, hemoglobin count and hematocrit percentage of collected blood samples. Samples of non-coagulated blood were tested shortly after collection for the estimation of hematocrit percentage, which was determined by the microhaematocrit method (Schalm *et al.,* 1975).Hemoglobin (Hb) concentration was measured by Sahli's method (Sahli, 1905).The total RBC and WBC counts were estimated by using improved neubauerhemocytometer chamber (Bernard *et al*., 2000).

Serum Biochemical profiles

Samples of blood without anti-coagulant were kept in the refrigerator at about 4°C for about 3 hours pending sedimentation. Thereafter the samples were transferred and spun in a centrifuge at 3,000 rpm for 15 minutes to separate serum. The serum was stored and frozen at -20°C for further analysis. Individual serum samples were analyzed for total protein, urea, triglycerides, total cholesterol, HDL and LDL by using automated chemistry analyzer (Douglas *et al*., 2010).

Statistical Analysis and Models

The data collected during the study period was subjected to analysis by using Statistical Analysis System (SAS) computer software version 9.3 (SAS, 2012). All data analysis except for yolk color has followed the procedure of one-way analysis of variance. When the analysis of variance indicated the existence of a significant difference between treatment means, the difference between treatments was separated using the Turkey test. Significant differences were declared at $p<0.05$. The model used for statistical analysis was;

$Y_{ij}= \mu + T_i + e_{ij}$
Where;
Y_{ij} = individual observation
μ = over all mean
T_i = treatment effect
e_{ij}= random error

RESULTS AND DISCUSSION

Blood Hematology

The addition of thyme in the layers diet did not show significant ($p>0.05$) effect on RBC, WBC, Hemoglobin and Hematocrit percentage of blood (Table 1). A similar result by Toghyani *et al*. (2010) affirmed that supplementation of broilers diet with thyme was not shown any detrimental impact on RBC and WBC counts, hemoglobin content and hematocrit percentage. It has also been reported that supplementation of broiler diet with active components of thyme (thymol and carvacrol) did not show a significant effect on WBC and RBC count, hemoglobin concentration and hematocrit percentage (Hashemipour *et al.* 2013). Nonetheless, a study by Al-Kassie (2009) indicated supplementation of feeding diets with oil extract derived from thyme and cinnamon significantly increased RBC, HCT, Hb and WBC values in the broiler chickens.

Table 1. Hematological blood profiles of white leghorn chicken fed rations containing different levels of thyme leaf as an additive

	Treatments				SEM	SL
	T1	T2	T3	T4		
RBC(10^6/µl)	3.30	3.43	3.53	3.56	0.07	NS
WBC(10^4 /µl)	6.00	6.26	6.40	6.83	0.18	NS
Hb(g/dL)	10.56	10.60	10.96	10.86	0.13	NS
Hematocrit %	32.16	32.30	31.86	32.23	0.77	NS

NS=Non- significant. RBC=Red Blood Cells, Hb= Hemoglobin, WBC= White Blood Cells, HDL= High Density Lipoprotein l, LDL= Low Density Lipoproteinl, LR=Layer Ration, TLM=thyme leaf meal, T1 = ration containing 0 % TLM/100kg LR, T2 = ration containing 1% TLM/100kg LR,T3 = ration containing 2% TLM /100kg LR, T4 = ration containing 3% TLM/100kg LR.

Serum Biochemical Profiles

Among serum metabolites total protein and urea were not significantly ($p>0.05$) affected with the addition of thyme in to the layers ration. Similar to the present study, it has been reported that feed supplemented with thyme essential oil did not affect serum urea (Saleh *et al.*, 2014) and albumin levels (Safa and Al-Beitawi, 2009; Toghyani *et al.*, 2010; Saleh *et al.,* 2014).Fallah and Mirzaei (2016) also suggested that no significant ($p>0.05$) differences were observed in blood glucose, total protein, and uric acid concentrations of different treatment groups that receive thyme as additive. However, the finding of the current experiment disagree with Safa and AL-Beitawi(2009) who reported that the addition of thyme to the layers ration significantly increased serum levels of glucose, total protein and globulin.

Except for HDL cholesterol content, the promising effect of thyme was shown on serum triglyceride, total cholesterol and LDL-cholesterol of white leghorn layers (Table 2). Serum triglyceride, total cholesterol and LDL-cholesterol were

significantly ($p<0.05$) lower in a group of birds fed a diet containing 1,2 and 3% thyme compairing to birds fed the control diet. The possible explanation for decreased serum cholesterol may be related to, thymol and carvacrol, the active component of thyme. These components reduce the activity of the liver enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCo Areductase), which is a key enzyme in cholesterol synthesis (Abdulkarimi *et al*., 2011). Bloedon and Szapary (2004) reported that a 5% reduction in HMG-CoA reductase activity consequences a 2% reduction in poultry serum cholesterol. In agreement with the present finding, it has been reported that thyme significantly decreases cholesterol and triglyceride levels in broiler chickens (Safa and Al-Beitawi, 2009), laying hens (Ali *et al.,* 2007; Abd El-Hack and Alagawany, 2015) and quails (Khaksar *et al.,* 2012). Also the report of Lee (2003) found that the active component of thyme, carvacrol, reduces plasma triglycerides and phospholipids. Similarly, Abdolkarimi *et al*. (2011) reported that thyme extract added in the drinking water of broilers chicken significantly decreased LDL cholesterol, total cholesterol and also triglyceride. In contrast, Saleh *et al*.(2014) reported that the addition 0.3% of thyme in the poultry diet significantly increase total cholesterol and LDL. On the other hand, Toghyani *et al*.(2010) indicated that the incorporation of 0.5 and 1%/ thyme powder into broiler rations did not affect serum levels of triglyceride, cholesterol or LDL.

Table 2. Serum biochemical of white leghorn chicken fed rations containing different levels of thyme leaf as an additive

Blood profiles	Treatments				SEM	SL
	T1	T2	T3	T4		
Total protein (g/dL)	3.33	3.46	3.53	3.50	0.07	NS
Urea (g/dL)	9.33	8.03	8.43	8.10	0.47	NS
Lipid profiles (mg/dL)						
Triglycerides	60.23^{a}	52.30^{b}	51.53^{b}	51.70^{b}	1.70	*
Total cholesterol	167.83^{a}	158.10^{ab}	152.73^{b}	147.13^{b}	3.22	*
HDL-cholesterol	52.96	54.73	54.50	59.26	1.57	NS
LDL-cholesterol	125.40^{a}	116.27^{ab}	104.37^{bc}	100.37^{c}	2.34	**

$^{a,b \text{ and } c}$ =Means with in a row with different superscripts are significantly different; *=Significant at ($p<0.05$); **= Significant at ($p < 0.01$); NS=Non- significant. HDL= High Density Lipoprotein, LDL= Low Density Lipoproteinl, LR=Layer Ration, TLM=thyme leaf meal, T1 = ration containing 0% TLM/100kg LR, T2 = ration containing 1% TLM/100kg LR, T3 = ration containing 2% TLM /100kg LR, T4 = ration containing 3% TLM/100kg LR.

Inclusion of thyme in the layers ration did not significantly ($p>0.05$) affect HDL cholesterol of birds. Similar to the present study, it has been reported that serum HDL-cholesterol of birds was not exhibited a significant difference due to diet supplemented with thyme (Abd El-Hack and Alagawany, 2015). However, according to Rahimi *et al.* (2011) HDL-cholesterol level was significantly elevated by dietary supplementation of thyme to the chickens' diet.

CONCLUSIONS

The result indicated that the RBC, WBC, hemoglobin and hematocrit percentage of birds did not show significant ($p>0.05$) difference among treatment groups. The group of birds in T_2, T_3 and T_4 showed shat significantly low serum triglycerides, total cholesterol and LDL-cholesterol comparing to control groups.

Further research should be needed to determine the optimal dietary inclusion above this level of thyme on blood profiles of chicken.

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