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EFFECT OF USING THYME (THYMUS VULGARIS L) AND CITRIC ACID FOR IMPROVING THE UTILIZATION OF LOW PROTEIN LOW ENERGY BROILER DIETS

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ABSTRACT: An experiment was conducted to evaluate the effect of using thyme (Thymus vulgaris L; TH) or citric acid;(CA) either alone or in combination (at level 0.2 % of each) to improve the utilization of the low protein low energy broiler diets (LPLE), containing 4% lower protein and 200 Kcal /Kg lower energy than those of control diets at starter, grower and finisher periods. Control diets contained 21, 19 and 18% CP and 2988, 3083 and 3176 kcal ME/kg diet at starter, grower and finisher periods, respectively. It was supplied with required nutrients to satisfy the recommended requirements of Cobb broilers.

A total number of 180, seven days-old of unsexed Cobb broiler chicks were distributed into 6 treatments of 30 chicks each in three replicates.

Main results obtained can be summarized as follows:

-There were significant differences between values of weight gain in different growth periods while there were insignificant differences between values of feed conversion ratio except for total growth period (7-42d.).

-All over growth period, the LPLE diet decreased weight gain and degraded feed conversion ratio by 23.24 and 19.47% compared to control diet. In general, it seemed that addition of TH or CA either alone or in combination to the LPLE diet improved weight gain compared to LPLE diet and recorded the better feed conversion ratio compared to low protein (LP) or LPLE diet. The combination of such feed additives improved weight gain and feed conversion ratio by 10.46 and 10.13%, respectively compared to LPLE diet.

-There were insignificant differences between treatments for carcass characteristics and values of ash and nitrogen retention percentage. The LPLE diet decreased daily nitrogen excretion by 25.92% compared to control diet.

- The LPLE diet increased plasma globulin level by 32.75 % compared to control diet.

In conclusion, the addition of TH or CA either alone or in combination to LPLE broiler diet improved weight gain and feed conversion ratio, decreased plasma globulin and increased total plasma antioxidants capacity compared to LPLE broiler diet.

Key words: Thyme, citric acid, low protein and energy and broiler.

INTRODUCTION

High levels of production and efficient feed conversion are the need of the modern poultry industry, which to a certain extent could be achieved by the use of specific feed additives. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora, improve the general performances and prevent some specific intestinal pathology (Hassan et al. 2010). However, increased pressure on livestock industry to phase out the use of prophylactic dosages of antibacterial growth promoters (AGP) in the European Union and other parts of world due to microbial resistance in animals has stimulated increased interest in alternative natural growth promoters (Fature and Matanmi, 2008).Several alternatives to antibiotic growth promoters have been proposed such as phytogenic feed additives (Galik and Rolinec, 2011),organic acids (Kral et al., 2011), probiotics (Corcionivoschi et al., 2010), products of bees (Petruska et al., 2012) and enzymes (Benţea et al., 2010).

Thyme (Thymus vulgaris L.) is a popular medicinal plant belonged to Lamiaceae sp. and mostly grown in Mediterranean regions (Chevallier, 1996). This herb has been paid more attention due to its antioxidant (Bolukbasi and Erhan, 2007), antibacterial (Dorman and Deans, 2000) anticoccidial (Jamroz et al., 2003), and antifungal properties (Hertrampf, 2001). Volatile oils from thyme were assessed as inhibitors of microbial growth (Toghyani et al., 2010). The major derived components of thyme plant are thymol and carvacrol, the phenolic compounds which have shown antioxidant and antibacterial activities (Demir et al., 2008). These compounds exhibit beneficial effects in poultry health and production (Mitsch et al., 2004).

Organic acids have growth-promoting properties and can be used as alternatives to antibiotics (Fascina et al., 2012). Dietary supplementation of organic acids in the diet of broilers enhanced nutrient utilization, growth, and feed efficiency (Denil et al., 2003). Recently, Hashemi et al. (2014) added an acidifier mixture (formic, phosphoric, lactic, tartaric, citric and malic acids) in the broiler diet at the rate of 0.15%. An increase in body weight gain was observed in the organic acid group compared to the control group at the end of 42 day of experiment.

The use of citric acid creates an acidic environment (pH 3.5 to 4.0) in the gut that favors the development of lactobacilli and inhibits the replication of Escherichia coli, Salmonella, and other gram-negative bacteria (Chowdhury et al., 2009).Several studies support the statement that the addition of citric acid to broiler rations improved weight gain (Afsharmanesh and Pourreza 2005), increased feed consumption (Moghadam et al., 2006) and improved feed efficiency (Abdel-Fattah et al. 2008). It also decreased pH of cecal digesta, crop and gizzard (Andrys et al., 2003) and intestine (Denil et al., 2003). Furthermore, Chowdhury et al. (2009) showed that dietary supplementation of citric acid at the level of 5 g/kg had positive effects on growth, feed intake, feed efficiency, carcass yield, bone ash, and immune status of broiler chickens. Shabaan (2012) found that chicks fed the low energy diets (200 Kcal /Kg diet lower energy than those of control diets) supplemented with a mixture of 0.15% thyme and 0.15% cumin gave an equal growth performance and higher economical efficiency as compared to the control group and suggested that the positive effects of these additives may be attributed to the biological function or pharmacological activities of these extract components like thymol, carvacrol….etc.

Feeding low protein diets has been reported to affect growth performance and carcass yield of broiler chickens (Bregendahl et al., 2002). However, there is negative correlation between body weight and immune responsiveness (Siegel and Gross, 1980), which is of concern to both breeders and health specialists. Selection for increased growth has resulted in conflict with maturation of the immune system and the magnitude of the immune response (Maatman et al., 1993). This may be because of the limited resources available for birds to accommodate all the physiological demands imposed upon them (Siegel et al., 1982; Dunnington, 1990).

Others, however, observed a reduced growth and enhanced fat deposition when animals were fed a reduced energy feed; these effects were even more pronounced when protein levels were reduced (Lippens et al., 2002).

Schutte et al. (1993) reported that for each percentage of nitrogen decrease in the feed, nitrogen excretion is reduced by 10%. In response, dietary means to decrease nitrogen excretion by feeding low Crude Protein (CP) diets to poultry has been reported (Parsons, 1995; Aletor et al., 2000). Grune et al. (1997) showed that the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack. Abd El-Hakim et al. (2009) have been used Curcuma longa plus thyme as natural antioxidants in broiler diet to decrease the damage of protein by free radical and decrease the lost of protein in feces. They obtained significantly increasing in nitrogen retention by 13.25% compared to control diet and indicated that natural antioxidants may decrease the damage of protein by free radical. They also found that addition of thyme alone or in combination with Curcuma longa increased the apparent metabolizable energy by 3.89 and 3.56%, respectively compared to basal diet.

Spais et al. (2002) found that a commercial feed additive containing herb extracts and organic acids exerts a growth promoting effect comparable to that of flavomycin. Lippens et al. (2005) showed that organic acids and plant extracts work differently while, the combination of both groups of products could give an additive effect. Moreover, Phytogenic additives and organic acids improve nutrient metabolizability in broilers at the initial growth stages. The use of organic acids, alone or associated with phytogenic additives in broiler diets, is an alternative, given the ban on antibiotic growth promoters (Fascina et al., 2012).Recently, Naela et al. (2016) concluded that using of thyme or formic acid in broiler feeds have significant positive effect on performance and immunity parameters. Also, addition of thyme plus formic acid is more effective than thyme or formic acid alone.

The present study was conducted to investigate the effect of supplementing thyme (Thymus vulgaris L; TH) or citric acid (CA) either alone or in combination (at level 0.2 % of each) to improve the utilization of the low protein low energy broiler diet.

MATERIALS AND METHODS

The experimental work was carried out at El-Takamoly Poultry Project, Research

Unit, Fayoum, Egypt .The laboratory work was done at Poultry Nutrition Department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. One hundred and eighty, one day-old, unsexed Cobb commercial broiler chicks were used in this experiment. Chicks were fed the control diet (Table 1) for the first week of age and then they were wing-banded, individually weighed and distributed into 6 groups each in three replicates of 10 chicks each in caged battery brooders. Experimental diets and water were offered ad-libitum over the experimental period. Chicks in all treatments were kept under similar conditions of management. Artificial lighting was provided 24 h daily during the whole experimental period. Thyme was purchased from local market, Cairo, Egypt. The control diets were supplied with required nutrients to satisfy the recommended requirement of Cobb broilers (Table 1). Chicks were allotted on the following dietary treatments:

1- Control diet.

2- Low protein diet (LP diet) contained 4% lower protein than control diet.

3- Low protein and energy diet (LPLE diet) contained 4% lower protein and 200 Kcal /Kg lower energy than control diet.

4- Low protein and energy diet + 0.2 % thyme

5- Low protein and energy diet + 0.2 % citric acid

6- Low protein and energy diet + 0.2 % thyme + 0.2 % citric acid

The LP and LPLE diets were provided by level of lysine and methionine to achieve their corresponding levels of the control diet. The body weight (BW) and feed intake (FI) were weekly recorded while feed conversion ratio (FCR) and body weight gain (BWG) values were calculated. At the end of experimental period (42 days), three birds were taken randomly from each treatment and slaughtered and the edible organs included heart, empty gizzard and liver were weighed. Carcass and organs weights percentage were calculated on the basis of live body weight. Individual blood samples were taken from 3 birds within each treatment, and collected into dry clean centrifuge tubes containing drops of heparin and centrifuged for 20 minutes on (3000 rpm) for obtaining plasma . Antioxidant capacity in plasma was determined using commercial kit produced by Biodiagnostic Company. Plasma cholesterol, total protein, albumin, uric acid and zinc were determined by suitable commercial kits. Globulin concentration of each assayed sample was calculated by subtracting the albumin value from its total protein concentration. The ash nitrogen retention, nitrogen intake g/day and nitrogen excreted g/day of the tested diets were determined at the end of experiment (42 days) using 3 birds from each treatment throughout digestion trials for 3 days. Feed and the finely ground excreta were analyzed for moisture, ash and nitrogen content (N) according to official methods (A.O.A.C., 1990). Nitrogen retention (NR %) was calculated from the following formula. NR= (N content of dry feed-N content of dried excreta) X 100/ N content of dry feed. The obtained data were statistically analyzed using one – way analysis of variance procedure (SAS, 2000) computer program using the following fixed model: $Y_i=\mu + T_i + e_i$

Where: Y_i = The observation; μ = Overall mean; T_i = Effect of treatments (i = 1, 2, 3, 4, 5 and 6); e_i= Random error component assumed to be normally distributed. Differences among treatment means were tested using Duncan's Multiple Range Test (Duncan, 1955).All data are presented as least square means.

RESULTS AND DISCUSSION

Growth performance:

The effect of dietary treatments on body weight gain (BWG) and feed conversion ratio (FCR) is shown in Table (2). There were significant differences between values of weight gain in different growth periods while there were insignificant differences between values of feed conversion ratio except for total growth period. In starter period, LP or LPLE diet degraded feed conversion. Feed additives used in this study improved feed conversion while LPLE diet recorded the worst value (1.9). All over growth period, the LPLE diet decreased weight gain and degraded feed conversion ratio by 23.24 and 19.47% compared to control diet. These results agree with those obtained by Aletor et al. (2000) who found that the increased feed consumption in the low-protein diets led to a corresponding inferior feed conversion efficiency.

In general, it seemed that addition of TH or CA either alone or in combination to the LPLE diet improved weight gain compared to LPLE diet and recorded the better feed conversion ratio compared to low protein (LP) or LPLE diet. The combination of such feed additives improved weight gain and feed conversion ratio by 10.46 and 10.13%, respectively compared to LPLE diet. These results agree with those obtained by Ali et al. (2011) who found that in starter period, addition of cumin plus citric acid to LPLE improved feed conversion not only comparable to LPLE but also compared to control diet. Recently, Naela et al. (2016) concluded that using of thyme or formic acid in broiler feeds have significant positive effect on performance and immunity parameters. Also, addition of thyme plus formic acid is more effective than thyme or formic acid alone.

The beneficial effect of thyme can be explained on the basis that stimulatory effect of herbal products on growth and digestion is the main reason for these results, so essential oils and related components derived from thyme have been widely used for the appetizing and stimulating effect on digestion. Such materials have traditionally been used to stimulate the production of endogenous secretions in the small intestine mucosa, pancreas and liver, and thus aid digestion (Cross et al., 2007). Langhout (2000), as well as Williams and Losa (2001) discovered that essential oils of thyme have a stimulating effect on the animal digestive system, due to the increase of digestive enzymes and improve nutrients utilization through the enhanced liver function (Safa and Al-Beitawi, 2009). Volatile oils from thyme were assessed as inhibitors of microbial growth (Toghyani et al., 2010). Moreover, thymol has been shown to reduce the number of coliforms within the digsta of chickens (Cross et al., 2004). Herb derivatives may have an effect through an increase in production of lactic acid bacteria, thus increasing the population of beneficial bacteria and reducing the presence of gram negative bacteria (Savage et al., 1996).

Herbs like thyme (Thymus vulgaris) that are rich in active compounds such as flavonoids extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Cook and Samman, 1996 ; Manach et al., 1996). In this respect, Abd El-Hakim et al. (2009)

used Curcuma longa plus thyme as natural antioxidants and obtained significantly increasing in nitrogen retention by 13.25% compared to control diet. Using herbs as natural antioxidants saving protein by decreasing free radical production which degrade the protein (Ali et al., 2011).

Recently,(Fardos and Ashraf, 2017) found that supplementation of thyme down regulated the pro-inflammatory mediators and increased the expression of mucin2 mRNA in the jejunum of chickens and concluded that thyme supplementation in the diet of broilers at 5 g/kg has the potential to favorably influence productive performance via an improvement in the immune status of the broiler.

In different growth periods we can observed, addition CA to the LPLE diet improved feed conversion ratio compared to LP or LPLE diet. However, chicks fed LPLE diets supplemented with citric acid improved feed conversion ratio by 21.05, 11.01and 5.19 %, at starter, grower and finisher period, respectively compared to LPLE diet.

The beneficial effect of citric acid can be explained on the basis that, dietary supplementation of organic acids increased the body weight and improved feed conversion ratio (FCR) in broiler chicken. Adil et al. (2010, 2011b) found that the highest weight gains were achieved in the birds fed 3% fumaric acid as compared to the group fed diet supplemented with 3% lactic acid. Chicks fed the diet supplemented with organic acids showed a significant ($P < .05$) improvement in the FCR as against the chicks fed the control diet. The improvement in the FCR could be possibly due to better utilization of nutrients resulting in increased body weight gain in the birds fed organic acids in the diet.

The above workers also conducted another trial, in which broilers were given basal diet supplemented with 2–3% each of butyric acid, fumaric acid and lactic acid (Adil et al. 2011a). The improvement in FCR in this study, could be possibly due to lower amount of feed intake as a result of better utilization of nutrients in the birds fed organic acids in the diet.

In allover growth period (7-42 d), there were significant differences between values of BWG and FCR. However, chicks fed LPLE diets supplemented with citric acid increased weight gain and improved feed conversion ratio by 7.68 and 8.81%, respectively compared to LPLE diet.

Recently, Hashemi et al. (2014) added an acidifier mixture (formic, phosphoric, lactic, tartaric, citric and malic acids) in the broiler diet at the rate of 0.15%. An increase in body weight gain was observed in the organic acid group (2402 g) compared to the control group (2276 g) at the end of 42 day of experiment. In present study, we use citric acid alone and further studies are needed in the future with mixed organic acid. Such a positive impact of dietary acidifiers on growth performance might be attributed to a reduction of pH values in the feed and digestive tract, serving as a barrier against pathogenic organisms which are sensitive to low pH; the direct antimicrobial effect; the reduction in buffering capacity in conjunction with improving nutrient digestibility (Ghazala et al., 2011).

Some studies also showed no performance difference, in comparison with the negative control and/or the birds fed antibiotics (Gunal et al. 2006; Abdel-Fattah et al. 2008; Vieira et al.2008; Kopecky et al. 2012). There are conflicting results regarding the use of acidifiers in poultry and, according to Hernandez et al. (2006), these effects depend on the chemical form of the acid, pKa values, bacterial species, animal species and the site of action of acids. Moreover, most of the studies that used organic acids as additives in broilers diets were conducted in low health challenge environments which could explain the inconsistent results, because the growth-enhancing effects of antimicrobial additives become apparent when chickens are subjected to suboptimal conditions, such as a less digestible diet or a less clean environment. This inconsistency would be related to the source, the amount of organic acids used and the composition of the diets.

The addition of TH or CA both alone or in combination to LPLE improved body weight gain and feed conversion ratio compared to LP or LPLE diet. At the end of grower period, the addition of TH + CA improved weight gain and feed conversion ratio by 10.58 and 11.86%, respectively compared to LPLE. Regarding to the finisher period, the addition of TH + CA to the LPLE diet increased weight gain and improved feed conversion ratio by 11.38 and 6.92%, respectively compared to LPLE diet. The increase in BWG and improved in FCR with TH + CA may be due to a synergistic effect (Ali et al., 2008). This may be explained based on the use of citric acid creates an acidic environment (pH 3.5 to 4.0) in the gut that favors the development of lactobacilli and inhibits the replication of Escherichia coli, Salmonella, and other gram-negative bacteria (Chowdhury et al., 2009). At a low pH the essential oils (Eos) increase the hydrophobicity, enabling them to more easily dissolve in the lipids of the cell membrane of target bacteria (Juven et al., 1994).

The beneficial effect of thyme plus citric acid in this study are agree with those obtained by Spais et al. (2002) who found that a commercial feed additive containing herb extracts and organic acids exerts a growth promoting effect comparable to that of flavomycin. Also, Lippens et al. (2005) showed that organic acids and plant extracts work differently while, the combination of both groups of products could give an additive effect. Putting in mind, that in this study we use the combination of two additives in low protein low energy diet and the response of this combination may be different in case of only low protein or low energy diet. Further studies are needed with this combination with different level of protein or metabolizable energy.

Carcass characteristics:

The effect of dietary treatments on carcass, liver, heart, gizzard, total edible parts and abdominal fat percentage are shown in Table (3). No significant differences were found between chicks fed the control diet and all treatments in all carcass characteristics. However, the numerically increase of abdominal fat percentage observed in groups fed LP or LPLE can be explained on the basis that low protein diet promote lipid synthesis. Donaldson (1985) showed that low-protein diets promote higher rates of de novo hepatic lipid synthesis in chickens than high protein diets. The addition of TH or CA either alone or in combination to the LPLE diet numerically decreased the abdominal fat percentage compared to LPLE diet indicating that these additives may save the protein and consequently decrease fat deposition.

These results agreed with those obtained by Abd El-Hakim et al. (2009) who reported that no differences were noticed for carcass, liver, gizzard, abdominal fat, total edible parts and giblets in broilers fed a 18% crude protein diet supplemented with Thymus vulgaris, Curcuma longa, citric acid or their combinations. Moreover, Hernandez et al. (2004) reported that no effects of plant extracts on organ weights in broilers. These results agree with those obtained by Denli et al. (2003) who reported that organic acid (mixture of propionic and formic acid) had no effect ($P > .05$) on the carcass yield, abdominal fat pad, abdominal fat percentage and liver weight compared with control. Similar results were obtained by Skinner et al. (1991), who compared the effects of dietary fumaric acid supplementation at 0.125%, 0.25% and 0.50% on broiler performance from 0 to 49 days. Similarly, Garcia et al. (2007) reported that the carcass, right breast and right thigh yields of broilers at 49 days of age were unaffected by supplementation of formic acid (0.5% or 1.0%).

Islam et al. (2008) noted that carcass characteristics were not affected by supplementation of organic acids. Also, Kopecky et al, (2012) concluded that were no statistically significant differences ($P>0.05$) between groups on carcass yield of broiler chickens. Differences in breasts and thighs of chicken from control and experimental groups with addition of organic acids were not statistically significant ($P>0.05$). Recently, Brzoska et al. (2013) reported that organic acid (0.3–0.9%) had no significant influence on carcass yield or proportion of individual carcass parts.

On the other hand, several studies noted that carcass characteristics were affected by supplementation of herbal feed additives, herbal plant extracts or organic acids (Lee et al.,2004; AL-Kassie, 2009). For example, Shabaan (2012) showed that carcass percentage and total edible parts percentage were significantly by supplementing mixture of 0.15% thyme plus 0.15% cumin to low energy broiler diet compared to low energy diet or control group. This may be due to the two herbs have synergetic effect. Moreover, Tollba et al. (2010) found that in comparison to control diet, significant increases ($P<0.05$) in dressing, breast, giblets and edible relative weights were observed due to the addition of aromatic herbal extract. This may be explained based on herbal plants that have stimulatory effects on pancreatic secretions such as digestive enzymes which help to digest and absorb more amino acids from the digestive tract and thereby improve carcass traits as reported by Mansoub (2011) and Fascina et al. (2012) who reported that the use of an organic acids mixture (comprising 30.0% lactic acid, 25.5% benzoic acid, 7% formic acid, 8% citric acid and 6.5% acetic acid) in broiler diets provided better carcass characteristics. Also, these feed additives may decrease the protein degradation by free radical and save the protein for growth (Ali et al., 2011).

Ash and nitrogen retention:

The data in Table (4) showed that there were insignificant differences between values of ash and nitrogen retention percentage recorded by different treatments. The LPLE diet decreased daily nitrogen excretion by 25.92% compared to control diet.

These results disagree with those obtained by Abd El-Hakim et al. (2009) who found that significantly increasing in nitrogen retention by 13.25% compared to control diet and indicated that natural antioxidants may decrease the damage of protein by free radical and decrease the lost of protein in feces.

In this study, the addition of TH decreased nitrogen excreted (g/day) by 22.5% compared to LPLE diet. These results agree with those obtained by Schiavone et al.(2008) who reported that inclusion of chestnut wood extract in broiler diets reduced total nitrogen in the litter. The decrease in fecal nitrogen in TH may be due to the effect of thyme on microbes which improved feed conversion and reduced nitrogen content in feces thereby increasing nitrogen retention. Bacteria compete with host for uptake of amino acids, thereby reducing nitrogen utilization (March et al., 1978; Furuse and Yokota, 1985). In addition, thyme have antioxidant properties that may decrease the reactive oxygen species and consequently decrease the protein damage thereby increasing nitrogen lost in feces.

The acidic anion has been shown to complex with Ca, P, magnesium and zinc, which results in an improved digestibility of these minerals (Edwards and Baker 1999). The results of this study disagree with those obtained by Smulikowska et al. (2009) who reported that fat-coated organic acid preparations increased nitrogen retention in comparison with the un-supplemented control diet. The increase in nitrogen retention can be connected with greater epithelial cell proliferation in the gastrointestinal tract.

Non-protected organic acids added into poultry feed are readily digested (Sugiharto, 2014), while the fat-coated preparation prevented dissociation of organic acids in the stomach and helped to address their bioactivity towards distal parts of the intestine and effectively modulate the intestinal microflora and mucosal morphology in chickens (Hu and Guo 2007). In this study we used citric acid without coating and further studies are needed with coated organic acid.

Blood plasma parameters:

The data in Table (5) showed that there were insignificant differences between plasma cholesterol values recorded by different dietary treatments. In the same way, Vasileios et al. (2017) reported that increasing phytogenic feed additive level linearly did not affect triglycerides, and cholesterol concentration in the plasma of 42-d-old broiler chickens. However, some studies showed reduces in the plasma total cholesterol by dietary supplementation of 0.25% thyme plant in laying hen (Ali et al., 2007), in broiler chickens by dietary levels of 0.5, 1.0, 1.5 and 2.0% crushed thyme (El- Ghousein and Al-Beitawi, 2009). Also, Rahim et al. (2011) who conclude that thyme extract supplementation in drinking water decreases the plasma total cholesterol and triglyceride in broiler chickens. Nevertheless, higher plasma levels of triglyceride, HDL-cholesterol and LDL-cholesterol were reported in broilers by dietary supplementation of 100 and 200 ppm thyme essential oil (Bolukbasi et al., 2006).

There were significant differences among treatments in values of plasma total protein and globulin while, there were insignificant differences between albumin values. It was surprise that LPLE diet recorded the highest value of plasma total protein (3.02 g/dl) and globulin (2.31g/dl). However, there is negative correlation between body weight and immune responsiveness (Siegel and Gross, 1980), which is of concern to both breeders and health specialists. Selection for increased growth has resulted in conflict with maturation of the immune system and the magnitude of the immune response (Maatman et al., 1993). This may be because of the limited resources available for birds to accommodate all the physiological demands imposed upon them (Siegel et al., 1982 ; Dunnington, 1990).

The addition of TH plus CA significantly decreased plasma globulin by 74.8 % compared to LPLE diet. The reduction in plasma globulin in birds fed LPLE plus CA have been observed before by Ali et al. (2008) who found with rabbit that the addition of CA plus Turmeric decreased both the harmful microorganisms in the caecum and values of plasma globulin. Richards et al. (2005) showed that microflora–specific immunoglobulin A and immunoglobulin G secretion alone can cost the animal several hundred grams of protein over a lifetime that is not directed towards growth. Le Floc'h et al. (2004) showed that stimulation of the immune system disturbs normal body processes and in turn is able to induce specific amino acid requirements. The difference between LP and LPLE diet in globulin values can be explained on the basis that LP diet contained soy bean oil which plays a role in protection of the feed passage in digestive tract from bacteria attack since fatty acids have been identified as bactericidal factors (Canas-Roderiquez and Smith, 1966). Recently, Naela et al. (2016) concluded that using of thyme or formic acid in broiler feeds have significant positive effect on immunity parameters. Also, addition of thyme plus formic acid is more effective than thyme or formic acid alone. Based on results observed in this study, we can indicate that these feed additives may save the protein by protecting the protein from free radical and/or decrease protein consumed in immune globulin synthesis (immune cost). These results disagree with those obtained with Tollba et al. (2010) who suggested that the addition of aromatic herbal extract to the diets increased ($P<0.05$) total protein as well as albumin and globulin compared to un-supplemented control group under cold environmental temperature.

There were significant differences among plasma uric acid levels due to different dietary treatments. The birds fed LPLE diet recorded value lowered plasma uric acid by 34.45% compared to control diet while, the birds fed LPLE diet + TH + CA recorded the highest value (2.25 mg/dl) Table (5). The reduction in plasma uric acid of birds fed LPLE agree with those of Corzo et al. (2005) who found that control diet (22% CP) significantly increased uric acid concentration compared to low protein diet (18% CP) and suggested that this increase means that an excess of nitrogen compounds are needed to eliminate. The of addition of TH or CA either alone or in combination to the LPLE diet increase plasma uric acid and these results indicated that these birds have an excess of nitrogen compounds. We have shown previously that, the addition of TH or CA either alone or in combination to the LPLE diet may act synergy to inhibit bacteria population and consequently decrease immune cost and save the protein and/or protect protein from free radical attack and this saving in protein occur also in daily nitrogen excretion. However, Naela et al. (2016) found that no significant effect on serum uric acid when using of thyme or formic acid alone or thyme plus formic acid in broiler feeds.

The analysis of variance indicated that there were insignificant differences between plasma zinc values. LPLE diet recorded the lowest value (674.1μg/dl) of plasma zinc. The addition of CA to LPLE diet numerically increased plasma zinc value by 5.05% compared to LPLE diet. In this respect, Boling et al. (2001) reported in chickens that the Zn utilization increased by the addition of CA to diet. Also, Sugiura et al. (1998) observed an increase in the apparent availability of calcium, phosphorus, magnesium, manganese, and iron in rainbow trout fed fish meal based diets supplemented with CA.

There were significant differences between plasma antioxidants capacity values. LP diet recorded significantly higher value than control diet. LP diets contain much oil compared to others which may increase the availability of antioxidants compounds soluble in lipid and consequently increased plasma antioxidants capacity. The addition of TH or CA either alone or their combination to LPLE diet increased numerically total plasma antioxidants capacity compared to LPLE diet or control diet. The addition of TH plus CA to LPLE diet increased plasma antioxidants capacity by 12.12% compared to LPLE diet. These results are in good agreement with other studies in which the supplementation phytogenic feed additive resulted in improvements on the antioxidant status of broilers (Demir et al., 2008; Hoffman-Pennesi and Wu, 2010; Polat et al. 2011; Shabaan 2012). The improvement of plasma antioxidant capacity by dietary phytogenics could be due to the active components and their phenolic group constituents which exhibit a strong antioxidant effect (Polat et al., 2011). Antioxidant status improvements may also result from the induction of antioxidant enzyme activities (Ciftci et al., 2010). However, Ali et al. (2011) found that addition of cumin either alone or with CA to LPLE diet increased total plasma antioxidants capacity compared to LPLE diet. In present study, improvement in plasma antioxidants capacity when the addition of TH plus CA to LPLE may be due to synergy effect.

CONCLUSION

Using LPLE diet decreased broiler weight gain and impaired feed conversion by 23.24 and 19.47%, respectively compared to control diet. Also, LPLE diet decreased daily nitrogen excretion by 25.92% while, increased plasma globulin by 32.75 % compared to control diet. Addition of TH or CA either alone or in combination to LPLE diet improved weight gain and feed conversion ratio. Addition of TH numerically decreased nitrogen percentage in feces by 22.5% compared with the LPLE diet. Further studies are needed to explain the role of plant extracts, organic acids and their combination under suboptimal commercial conditions.

Table (1): Composition and calculated analysis of the experimental diets.

Item	Control			Low protein (LP)			Low protein and energy (LPLE)		
	Starter 7-14d%	**Grower 15-28d%**	**Finisher 29-42d%**	**Starter 7-14d%**	**Grower 15-28d%**	**Finisher 29-42d%**	**Starter 7-14 d%**	**Grower 15-28d%**	**Finisher 29-42d%**
Yellow corn	58.19	61.96	64.00	65.70	73.09	73.57	63.82	70.64	76.10
Soybean meal (44%)	30.12	27.00	23.85	25.60	20.09	17.25	23.70	18.45	16.00
Corn gluten meal (60%)	5.00	3.40	3.68	-	-	-	-	-	-
Soy oil	2.05	3.20	4.19	2.05	1.90	3.40	-	-	-
Lime stone	1.71	1.65	1.55	1.74	1.67	1.58	1.75	1.69	1.58
Mono calcium phosphate	1.70	1.64	1.54	1.75	1.69	1.59	1.68	1.65	1.57
Salt (NaCl)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin & Min. Mix.*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL- Methionine	0.15	0.18	0.18	0.32	0.33	0.34	0.32	0.32	0.33
L- Lysine HCl	0.16	0.17	0.21	0.36	0.43	0.47	0.39	0.45	0.49
Sodium bicarbonate	0.27	0.15	0.15	0.27	0.15	0.15	0.27	0.15	0.15
Wheat bran	-	-	-	1.56	-	1.00	7.42	6.00	3.13
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated analysis**									
CP, %	21.06	19.02	18.00	17.07	15.06	14.04	17.00	15.06	14.01
ME (Kcal /kg)	2988	3083	3176	2988	3083	3176	2788	2883	2976
Crude fiber, %	3.63	3.46	3.28	3.55	3.14	3.06	4.01	3.63	3.26
Crude fat, %	4.82	6.03	7.05	4.99	4.97	6.49	3.07	3.20	3.25
Calcium, %	1.00	0.96	0.90	1.01	0.96	0.90	1.00	0.96	0.90
Available phosphorus, %	0.50	0.48	0.45	0.506	0.48	0.45	0.50	0.48	0.45
Lysine, %	1.20	1.11	1.05	1.204	1.10	1.05	1.20	1.10	1.05
Methionine, %	0.54	0.53	0.52	0.613	0.59	0.59	0.612	0.58	0.58
Methionine + Cystine, %	0.89	0.84	0.82	0.894	0.84	0.82	0.899	0.84	0.82
Sodium, %	0.22	0.19	0.19	0.22	0.19	0.19	0.22	0.19	0.19

***** Premix contain per 3 kg Vit. A 12 000 000, Vit. D3 4000 000 IU, Vit. E 40 000mg, Vit. K3 7000mg , Vit. B1 5500mg, Vit. B2 7700 mg, Vit. B6 6600 mg, Vit. B12 30 mg, Pantothenic acid 18000 mg, Niacin 80000 mg, Biotin 250 mg, Folic acid 1800 mg, Choline 600 g, Selenium 350 mg, Copper 15000 mg, Iron 110000 mg, Manganese 100000 mg, Zinc 80000 mg, Iodine 1500 mg, Cobalt 250 mg and $CaCO_3$ up to 3000g ******Analysis of ingredients calculated according to NRC (1994)

Table (2): Effect of dietary treatment on growth performance.

Treatments	Starter gain (g) 7-14d	Starter feed conversion (g feed/g gain) 7-14d	Grower gain(g) 15-28d	Grower Feed conversion (g feed/g gain) 15-28d	Finisher gain(g) 29-42d	Finisher feed conversion (g feed/g gain) 29-42d	Total gain (g) 7-42d	Total Feed Conversion (g feed/g gain) 7-42d
Control	246.3^{a}	1.48	667.0^{a}	1.83	1109.0^{a}	2.04	2022.3^{a}	1.90^{c}
LP	227.3^{ab}	1.72	586.3^{b}	2.09	906.8^{bc}	2.41	1720.4 ^{b}	2.21^{ab}
LPLE	214.9^{b}	1.90	485.6^{c}	2.36	851.8^{c}	2.31	1552.3^{c}	2.27^{a}
LPLE+ TH	218.9^{b}	1.51	501.4^{c}	2.14	939.2^{bc}	2.20	1663.9^{bc}	2.09^{abc}
LPLE+ CA	226.1^{ab}	1.50	533.8^{bc}	2.10	904.2^{bc}	2.19	1671.6^{bc}	2.07^{abc}
LPLE+TH+CA	228.9^{ab}	1.49	537.0^{bc}	2.08	948.8^{b}	2.15	1714.7^{b}	2.04^{bc}
Pooled MSE	±3.15	±0.053	±9.06	±0.056	±13.6	±0.04	±22.1	±0.036
P value	0.0438	0.0802	0.0001	0.1814	0.0001	0.0978	0.0001	0.0202

a, b,..etc.: Means in the same column with different letters, differ significantly (P<0.05). LP= Low protein, LPLE= Low protein and energy, TH= Thyme and CA=Citric acid.

Table (3): Effect of dietary treatment on carcass characteristics.

Treatments	Carcass%	Liver%	Gizzard%	Heart %	Total edible part%	Abdominal fat%
Control	72.14	2.19	1.58	0.62	76.55	1.75
LP	69.55	2.38	1.47	0.62	74.03	3.17
LPLE	71.72	2.83	1.71	0.48	76.75	2.45
LPLE+ TH	71.08	2.32	1.56	0.63	75.61	1.89
LPLE+ CA	72.25	2.14	1.73	0.53	76.66	2.00
LPLE +TH+CA	71.03	2.35	1.70	0.55	75.57	1.74
Pooled MSE	±0.321	±0.077	±0.049	±0.021	±0.323	±0.184
P value	0.1290	0.1028	0.6653	0.0698	0.0926	0.1667

LP= Low protein, LPLE= Low protein and energy, TH= Thyme and CA=Citric acid.

Table (4): Effect of dietary treatment on ash and nitrogen retention at 42 days.

Treatments	Ash retention %	Nitrogen intake g/day	Nitrogen excreted g/day	Nitrogen retention %
Control	41.27	3.20	1.08	64.70
LP	43.49	2.57	1.005	60.69
LPLE	57.39	2.32	0.80	66.01
LPLE+ TH	45.87	1.79	0.62	65.48
LPLE+ CA	38.43	2.32	0.86	63.01
LPLE +TH+CA	46.92	2.40	0.83	63.53
Pooled MSE	±2.03	±0.202	±0.067	±1.31
P value	0.0633	0.5354	0.5007	0.9380

LP= Low protein, LPLE= Low protein and energy, TH= Thyme and CA=Citric acid.

Table (5): Effect of dietary treatments on some plasma parameters.

Treatments	Total cholesterol mg/dl	Total protein g/dl	Albumin g/dl	Globulin g/dl	Uric acid mg/dl	Zinc µg/dl	Antioxidants capacity mmol/L
Control	95.85	2.77^{a}	1.03	1.74^{a}	1.19^{bc}	703.2	0.56^{b}
LP	108.08	1.63^{b}	0.96	0.67^{b}	0.97^{bc}	705.2	0.94^{a}
LPLE	108.08	3.02^{a}	0.71	2.31^{a}	0.78^{c}	674.1	0.66^{b}
LPLE+ TH	111.24	2.55^{a}	0.80	1.75^{a}	1.85^{ab}	683.9	0.72^{b}
LPLE+ CA	102.16	1.43^{b}	0.81	0.62^{b}	1.39^{abc}	708.2	0.68^{b}
LPLE +TH+CA	105.45	1.49^{b}	0.91	0.58^{b}	2.25^{a}	689.8	0.74^{b}
Pooled MSE	±3.53	±0.183	±0.063	±0.201	±0.153	±5.49	±0.038
P value	0.8922	0.0031	0.5464	0.0026	0.0187	0.4427	0.0091

a, b,..etc.: Means in the same column with different letters, differ significantly ($P<0.05$).

LP= Low protein, LPLE= Low protein and energy, TH= Thyme and CA=Citric acid.

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الملخص العربى

تأثير استخدام الزعتر وحمض الستريك لتحسين الإستفادة من عليقة دجاج التسمين المنخفضة في البروتين الخام والطاقة الممثلة

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تهدف هذه الدراسة إلى دراسة تأثير استخدام الزعترأو حمض الستريك كل منهما على حده أو مشتركين معاً (بنسبة 0,2 % لكل منهما)على تحسين الاستفادة من عليقة بدارى التسمين المنخفضة فى البروتين الخام والطاقة الممثلة. حيث احتوت العليقة المنخفضة فى البروتين الخام على نسبة بروتين خام (17 ، 15 ،14%) لعلائق البادئ والنامى والناهى على الترتيب وكانت الطاقة الممثلة تبعا لإحتياجات السلالة (كب) . بينما احتوت العليقة المنخفضة فى نسبة البروتين الخام والطاقة الممثلة معاً على (17% بروتين خام ، 2788 ك ك طاقة ممثلة/ كجم عليقة و15% بروتين خام ، 2883 ك ك طاقة ممثلة / كجم عليقة و 14% بروتين خام ، 2976 ك ك طاقة ممثلة / كجم عليقة) لعلائق البادئ والنامى والناهى على التوالى. وبالنسبة لعليقة المقارنة فإنها كانت تغطى الإحتياجات الغذائية لدجاج التسمين سلالة (كب) حيث احتوت على (21% بروتين خام 2988 ك ك طاقة ممثلة / كجم عليقة و 19% بروتين خام 3083 ك ك طاقة ممثلة / كجم عليقة و 18% بروتين خام ، 3176 ك ك طاقة ممثلة / كجم عليقة) فى مراحل البادئ والنامى والناهى على الترتيب. تم استخدام عدد 180 كتكوت غير مجنس عمر يوم واحد من سلالة كب حيث تم تقسيم هذه الكتاكيت إلى 6 مجاميع بكل مجموعة 3 مكررات وبكل مكرر 10 كتاكيت (6 مجاميع × 3 مكررات × 10 كتاكيت) تم استخدامهم لقياس قدرة الزعترأو حمض الستريك أحدهما أو كلاهما على تحسين العلائق المنخفضة البروتين الخام و الطاقة الممثلة معاً (بروتين خام أقل 4% ، طاقة ممثلة أقل 200 كيلو كالوري/ كجم عليقة مقارنة بعليقة الكنترول).

<u>وكانت أهم النتائج المتحصل عليها كما يلي:-</u>

- كانت هناك اختلافات معنوية بين المعاملات فى معدل الزيادة فى النمو للكتاكيت خلال فترات النمو المختلفة بينما لم تظهراختلافات معنوية بين المعاملات بالنسبة إلى معاملات التحويل الغذائى خلال فترات النمو فيما عدا الفترة الكلية للتجربة (من 7-42 يوم).
- أدى استخدام العليقة منخفضة البروتين الخام والطاقة الممثلة معاً إلى انخفاض فى معدل النمو و تقليل معامل التحويل الغذائى بمقدار 23,24 ، 19,47 % على الترتيب بالمقارنة بالعليقة الكنترول وذلك خلال الفترة الكلية للتجربة. ولكن بوجه عام فقد لوحظ أن إضافة الزعترأو حمض الستريك كل منهما على حده أو مشتركين معاً (بنسبة 0,2 % لكل منهما) أدى إلى زيادة فى معدل نموالكتاكيت بالمقارنة بالعليقة المنخفضة فى نسبة البروتين الخام والطاقة الممثلة معاً. كما أدت الإضافة أيضاً إلى تحسين فى معامل التحويل الغذائى وذلك بالمقارنة بالعليقة المنخفضة فى نسبة البروتين الخام فقط أو بالمقارنة بالعليقة المنخفضة فى نسبة البروتين الخام والطاقة الممثلة معاً. ويمكن القول أن إضافة الزعتر وحمض الستريك معاً (بنسبة 0,2 % لكل منهما) أدى إلى زيادة فى معدل نموالكتاكيت وتحسين فى الكفاءة الغذائية بمقدار 10,46 ، 10,13 % على الترتيب بالمقارنة بالعليقة المنخفضة فى نسبة البروتين الخام والطاقة الممثلة معاً وذلك خلال الفترة الكلية للتجربة.
- كانت هناك اختلافات غير معنوية بين المعاملات بالنسبة لصفات الذبيحة والنسبة المئوية للرماد المحتجز والنسبة المئوية للنيتروجين المحتجز. كما لوحظ أن استخدام العليقة المنخفضة فى نسبة البروتين الخام والطاقة الممثلة معاً أدت إلى تقليل النيتروجين المفرز يومياً من الطيور بمقدار 25,92% بالمقارنة بالعليقة الكنترول .
- كما لوحظ أن الطيور التى تغذت على العليقة المنخفضة فى نسبة البروتين الخام والطاقة الممثلة معاً سجلت أعلى مستوى لكل من البروتينات الكلية والجلوبيولين فى بلازما الدم حيث زاد مستوى الجلوبيولين بمقدار 32,75 % بالمقارنة بالعليقة الكنترول.
- وعليه يمكن القول أنه تحت ظروف هذه التجربة فإن إضافة الزعترأو حمض الستريك كل منهما على حده أو مشتركين معاً (بنسبة 0,2 % لكل منهما) إلى عليقة دجاج التسمين المنخفضة فى نسبة البروتين الخام والطاقة الممثلة أدت إلى زيادة فى معدل النمو وتحسين فى معامل التحويل الغذائى و زيادة فى مستوى مجموع المواد المضادة للأكسدة فى بلازما الدم.
- كما يوصي بمزيد من الدراسات لتفسير دور النباتات الطبية ومستخلصاتها والأحماض العضوية والمستويات المثلى منها كمواد منشطة للنمو مع مستويات مختلفة من البروتين الخام والطاقة الممثلة لتحسين العلائق التحت قياسية .