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EFFECTS OF SOME DIEATARY SUPPLEMENTATION WITH PHYTONUTRIENTS ON SELECTED BIOCHEMICAL PARAMETERS AND GROWTH PERFORMANCE IN BROILER CHIKENS

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Abstract

Maintaining bird health, regarding diseases or agents acting on the gastrointestinal tract, is crucial in broiler production, since this is the entry route of nutrients for bird development. Also, it is well known that broiler production is impossible without antibiotics, which are used as growth promoters and in order to suppress overgrowth of pathogenic microflora in the gastrointestinal tract. Taking into account this, we investigated the growth promoting and beneficial effects of three phytoadditives (*Ocimum basilicum*, *Thymus vulgaris* and *Pimpinella anisum*) on growth performances (body weight, total feed intake, feed conversion ratio and carcass yield), concentrations of glucose, total proteins, albumin, total bilirubin, triglycerides and activity of creatine kinase enzyme, in broiler chicks at 56 days of age. On the day of hatching 150 broiler chicks, were divided into five equally groups (n=30). The control group (1st group) of broilers received a basal diet (BD) without any feed additive. The second, third and fourth groups of chicks were fed BD enriched with 1% of *Ocimum basilicum* pulverised herba, 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* pulverised herba and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* pulverised fructus, respectively. The fifth group was fed with BD mixed with Salinomycin (60 ppm). The results indicated that feeding the diets enriched with selected herbal supplement failed to affect the growth performance of chickens at 56 days of age. In addition, this supplementation had no influence on glucose metabolism, but we observed beneficial effects on some parameters of protein metabolism. The concentration of total proteins was significantly higher in chicks fed with phytoadditives; 41.71±1.08 vs 35.88±1.04 g/L; $p<0.01$. Albumin concentration was also significantly higher in experimental groups (28.29±1.32 vs 19.73±0.51 g/L; $p<0.001$). Creatine kinase activity and total bilirubin concentration were significantly reduced in broilers fed with phytoadditives; $p<0.05$. *Ocimim basilicum* in combination with *Thymus vulgaris* decreased the concentration of tryglicerides (0.89±0.02 vs 1.23±0.05 mmol/L; $p<0.001$), but this was not the case with other phytoadditives.
It could be concluded that supplementation of the diet with phytoadditives has a potential to improve health status in broilers. Further examinations are needed in order to elucidate the exact mechanism of action.

Key words: *phytonutrients, growth performance, blood chemistry, broiler chickens*

Introduction

Expansion of the poultry industry holds the greatest promise for bridging the animal protein gap in the world. This is mainly due to the short generation interval of poultry, particularly of broilers. However, the rising cost of poultry feed, problems of drugs residues, microbial resistance and diseases have become major problems militating against the industry (Puvača et al., 2013). Maintaining bird health, regarding diseases or agents acting on the gastrointestinal tract, is crucial in

broiler production, since this is the entry route of nutrients for bird development. Also, it is well known that broiler production is impossible without antibiotics and coccidiostats, which are used as growth promoters and in order to suppress overgrowth of pathogenic organisms in the gastrointestinal tract.

The prophylactic use of antibiotics in poultry nutrition in order to cause improvements in growth, feed consumption, feed utilization and decreased mortality from clinical diseases is well documented. But, the growing concern over the transmission and the proliferation of resistant bacteria via the food chain has led to a ban of the feed use of antibiotic growth promoters in livestock within the European Union since 2006 (Brenes and Roura, 2010). As a result, new commercial additives derived from plants including aromatic plant extracts and their purified constituents have been examined as a part of alternative feed strategies for the future. Such products have several advantages over commonly used commercial antibiotics since they are residue free and they are also, generally recognized as safe and commonly used items in the food industry (Lv et al., 2011). These botanicals have received increased attention as possible growth performance enhancers for animals in the last decade. Also, „natural" methods are likely to play an increasing role in the control of the disease since they are well accepted by consumers. Plant products are residue-free, and function by mechanisms other than those of chemotherapeutics, involving new therapeutic molecules to which resistance has not yet developed (Orengo et al., 2011).

Plants and their extracts have been used for many centuries as treatments for ailment of many pathological conditions, yet only in the past 20-30 years have scientists seriously begun to determine whether plant-derived traditional remedies are effective, and, if so, their mode of action. Less than 10% of approximately 250000 of the world's flowering plant species have been investigated scientifically for their pharmacological properties but almost 25% of active medical compounds currently prescribed in the USA and UK were isolated from higher plants. Plants are an important source for drug discovery – particularly for parasites because of the long association between the coexistence of parasites, humans and herbal remedies (Anthony et al., 2005). Recently, a number of plants and their extracts have been used in poultry nutrition. Their functional substances (such as flavonoids, polyphenols and terpenoids) are mainly secondary metabolites synthesized by plants to deter herbivorous predators, repel competitors and attract pollinators (Petrović et al., 2011).

Taking into account this, we investigated the growth promoting and beneficial effects of three phytoadditives (*Ocimum basilicum*, *Thymus vulgaris* and *Pimpinella anisum*) grown and harvested in Serbia, on growth performances, concentration of glucose, total proteins, albumin, total bilirubin, triglycerides and activity of creatine kinase enzyme, in broiler chicks at 56 days of age.

Material and methods

Plant materials

The herba of basil (*Ocimum basilicum*) and thyme (*Thymus vulgaris*) and fructus of *Pimpinella anisum* were collected in Vojvodina (North Province of Serbia), dried at room temperature away from sunlight. The dried leaves and fructus were pulverised and kept at 8 ^{o}C before mixing with broiler diets.

Experimental animals and diets

On the day of hatching 150 broiler chicks, were randomly divided into five equally groups (n=30). The broilers were kept in large pens on wood shavings. On the day of hatching, the room temperature was kept at 32 ^{o}C and then was gradually decreased by 3 ^{o}C per week to a final temperature of 23 ^{o}C on the day 21, which was then maintained constantly. Continuous lighting

regimen (24 h of light per day) was kept throughout the fattening period. Relative humidity in the room was maintained at 70%.

All birds were fed *ad libitum* with the commercial standard diets for broilers (Veterinarski Zavod-Zemun, Serbia): „Starter“ from day 1 to 14; „Grower“ from day 15 to 35 and „Finisher“ from day 36 to 56. The control group (1st group) of broilers received a basal diet (BD) without any feed additive. The second, third and fourth groups of chicks were fed BD enriched with 1% of *Ocimum basilicum* pulverised herba, 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* pulverised herba and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* pulverised fructus, respectively. The fifth group was fed with BD mixed with Salinomycin (60 ppm). The broilers had free access to feed and water.

All experiments were performed according to our institutional guidelines for animal research and principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (Official Daily N. L 358/1–358/6, 18, December 1986).

Sample collection

None of broiler chickens in any group died during the trial. The body weight of broilers, feed conversion ratio and carcass yield were among the growth parameters studied and recorded on the day 56 of life. On day 56 of age all broilers were slaughtered by decapitation. Blood was taken without anticoagulants' presence, enabling the separation of blood serum. Sera were obtained after a spontaneous blood coagulation attained by centrifugation lasting 10 min at 3000 rpm. Thus obtained blood sera were frozen at -20 ^{o}C until further analysis.

Biochemical assaying

The kinetic method was used to determine creatine kinase (CK) enzyme activity, while triglycerides concentration was determined after enzymatic hydrolysis with lipases and quinoneimine as indicator. The biuret method was used to determine total protein concentration, while bromocresol green was used to determine albumin concentration. Total bilirubin (the sum of conjugated and unconjugated bilirubin) was determined in the reaction with diazonium ion of sulphanilic acid. All of the above mentioned biochemical parameters were determined using commercial kits (Bayer Diagnostics, Germany). Spectrophotometric measurements were performed with Cecil CE 2021UV/VIS spectrophotometer. Blood glucose concentration was determined by using Precision-Xtra plus test strips.

Statistical analysis

Statistical significance of differences of all examined parameters were determined by means of the ANOVA, followed by the Tukey test. Data were expressed as means ± standard error. Significance level was set at $p < 0.05$. Statistical analysis was performed using the Graph Pad Prism 5.0 Software, CA, USA.

Results

The effect of diet supplementation with 1% of *Ocimum basilicum*, 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum*, in experimental groups, as well as in control groups (C_1 – fed with BD; and C_2 – fed BD plus Salinomycin) on the body weight, total feed intake, feed conversion ratio and carcass yield are shown in Table 1.

Our results showed no statistically significant differences ($p > 0.05$) in the growth performance indices (such as body weight, total feed intake, feed conversion ratio and carcass yield), between groups of chicken.

Table 1. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); Control groups (C_1 – fed with BD and C_2 – BD plus Salinomycin) on growth performance of broilers at the age of 56 days

	C_1	I group	II group	III group	C_2
Body weight	2602±64.99	2654±70.48	2573±75.04	2375±55.25	2694±91.49
Total feed intake (g)	8405	7214	6985	7365	8210
Feed conversion ratio	2.15	1.99	1.87	1.89	2.05
Carcass yield	1605	1794	1768	1601	1715

Concentrations of total proteins (Fig. 1) were statistically significant higher in groups of chickens supplemented with phytoadditives; the higher concentrations were recorded in I (41.02±0.76 g/L; $p < 0.01$) and III groups (41.71±1.08 g/L; $p < 0.001$) compared to C_2 group (35.26±1.06 g/L).

The albumin concentrations (Fig. 2) were also statistically significant higher in broilers fed with phytoadditives. Also, in this case the higher albumin concentrations were determined in I (28.29±1.32 g/L; $p < 0.001$) and III groups (26.50±0.89 g/L; $p < 0.001$) compared to C1 (19.73±0.51 g/L) and C_2 (19.31±0.53 g/L) groups. But it is also worth to mention that we determined higher values of albumin in I group (fed BD plus 0.5% basil) compared to II group (24.39±0.85 g/L; $p < 0.05$).

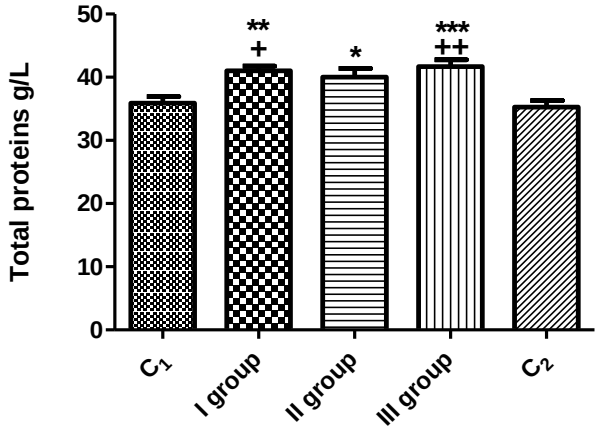

Fig. 1. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); C_1 – control group fed with BD; C_2 – control group fed with BD plus Salinomycin) on concentration of total proteins in broilers at the age of 56 days; $^{+}$ $p<0.05$; $^{++}$ $p<0.01$ vs. C_1 control group; ****** $p<0.01$; ******* $p<0.001$ vs. C_2 control group

Creatine kinase activity (Fig. 3) is another biomarker which can be used for evaluation, not only of functional status of hepatocytes, but also for skeletal muscle integrity. In this experimental design we determined the lowest activity in group of broilers fed BD with 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (403.4±30.55 U/L; $p < 0.01$) and broilers fed BD with 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (470.1±41.06 U/L; $p < 0.05$), compared to C2 group (695.8±74.5 U/L). There were no differences between C1 group (BD only) and I group of broilers (BD plus basil), $p > 0.05$.

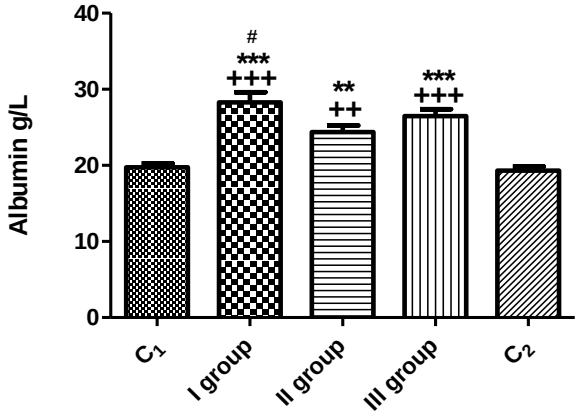

Fig. 2. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); C_1 – control group fed with BD; C_2 – control group fed with BD plus Salinomycin) on albumin concentration in broilers at the age of 56 days; $^{++}$ $p<0.01$; $^{+++}$ $p<0.001$ vs. C_1 control group; ****** $p<0.01$; ******* $p<0.001$ vs. C_2 control group; $^{\#}$ $p<0.05$ I group vs. II group

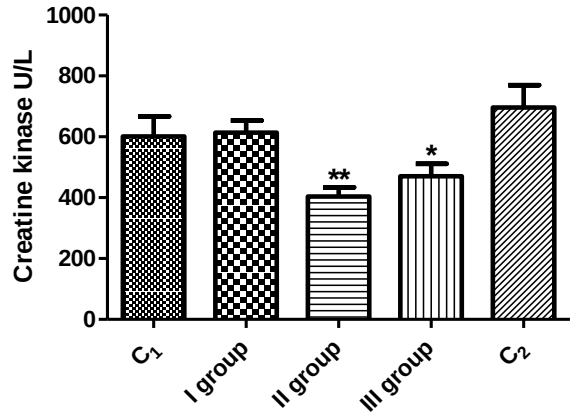

Fig. 3. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); C_1 – control group fed with BD; C_2 – control group fed with BD plus Salinomycin) on creatine kinase activity in broilers at the age of 56 days; ***** $p<0.05$; ****** $p<0.01$ vs. C_2 control group

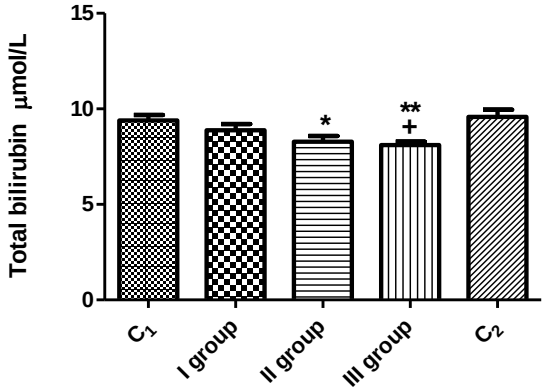

Fig. 4. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); C_1 – control group fed with BD; C_2 – control group fed with BD plus Salinomycin) on concentration of total bilirubin in broilers at the age of 56 days; ***** $p<0.05$; ****** $p<0.01$ vs. C_2 control group

Concentrations of total bilirubin (Fig. 4) were the lowest in III group of broilers (8.11±0.16 µmol/L; $p < 0.01$) and in II group (8.27±0.31 µmol/L; $p < 0.05$) compared to C_2 group. It is of interest to note that we did not find statistically significant difference between C_1 (BD only) and C_2 groups (BD plus Salinomycin); $p > 0.05$.

Triglycerides concentration (Fig. 5) was the lowest in II group of broilers fed with addition of 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (0.89±0.02 mmol/L; $p < 0.001$), compared to C_1 (1.23±0.05 mmol/L) and C_2 (1.14±0.03 mmol/L). The same statistical differences ($p < 0.001$) were noticed between I (1.20±0.02 mmol/L) and II group (1.25±0.06 mmol/L) of broilers.

We did not find any significant differences ($p > 0.05$) between broiler groups regarding concentration of glucose (Fig. 6)

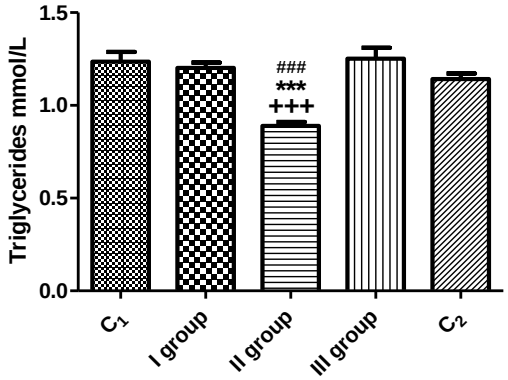

Fig. 5. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); C_1 – control group fed with BD; C_2 – control group fed with BD plus Salinomycin) on concentration of triglycerides in broilers at the age of 56 days; $^{+++}$ $p<0.001$ vs. C_1 control group; ******* $p<0.01$ vs. C_2 control group; $^{###}$ $p<0.001$ I group vs. II group

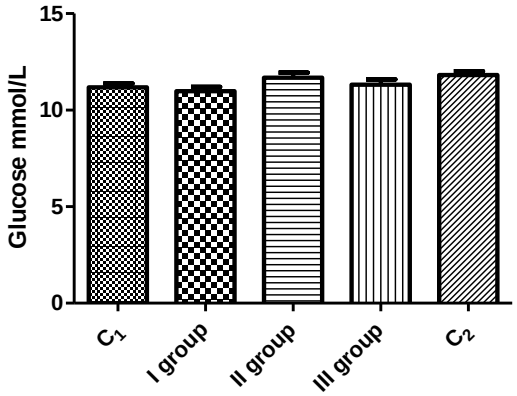

Fig. 6. The effects of diet supplementation with 1% of *Ocimum basilicum* (I group), 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* (II group) and 0.5% of *Thymus vulgaris* herba and 2% of *Pimpinella anisum* (III group); C_1 – control group fed with BD; C_2 – control group fed with BD plus Salinomycin) on concentration of glucose in broilers at the age of 56 days

Discussion & Conclusion

The practice of feeding livestock with subtherapeutic levels of antibiotics has been in use for over fifty years. But, usage of antibiotics has negative effects on animal's health and production

(Marković et al., 2009). Furthermore, now days consumers request poultry products that are free from residual chemotherapeutics. The use of natural products as an alternative to drugs may be the best solution to this consumer demand (Harper and Makatouni, 2002).

The herbal products are complex mixtures of ingredients, where the relative concentrations of each phytomolecule may vary considerably (Orengo et al., 2012). Instead of testing each and every one, our study focuses on three plants used alone or in combination. The aim of this research was to find what plant and effective doses of herbal additives could have a beneficial impact on the growth performance and health status of broilers.

Plants and their extracts (called essential oils) possess antibacterial, antifungal and antiviral properties and have been screened worldwide as potential sources of novel antimicrobial compounds, alternatives to treat infectious diseases and agents promoting food preservation (Solorzano-Santos and Miranda-Novales, 2011). Weight gain is one of the most sensitive and informative measure of efficacy of certain additives (Conway et al., 1999). From productive point of view, it is of interest to mention that our results showed that phytoadditives can improve growth performance in broilers, although there were no statistically significant differences between control and experimental groups (Table 1). But, it is obvious from results showed in Table 1, that broilers received BD with 0.5% of *Ocimum basilicum* and 0.5% of *Thymus vulgaris* had a better feed conversion ratio and carcass yield compared to other groups of broilers. Our trial was performed under highly hygienic conditions, which could have alleviated the animals' response. Environmental conditions, such as density and stress status of the animals, are important for detecting performance responses to plants used as feed additives (Catala-Gregori et al., 2008). According to Ertas et al. (2005), the combination of some plants extracts could present better effect on the growth performance in poultry in comparison with their individual supplementation. Synergism among some herbal constituents was highlighted in the *in vitro* studies performed by Montes-Belmont and Carvajal (1998). Moreover, Burt (2004) reported that an antagonistic effect has been expected as well. We presumed that potential synergistic effects between basil and thyme could result in beneficial effect on both the growth performance of broilers and their hepatoprotective effects.

Although total protein concentrations were also affected by phytoadditives in BD (Fig. 1), the most remarkable are results in albumin concentrations (Fig. 2). Our results showed that 1% of basil had the best stimulatory effect on hepatocytes to synthesized albumin, since the liver is the only site for its synthesis. It is possible that eugenol and carvacrol, two major ingredients of basil (Lv et al., 2011), are responsible for such liver response. Total proteins, also were much higher in herbal supplemented broilers; the most higher values were recorded in group supplemented with mixture of 0.5% basil and 0.5% of thyme (Fig. 1). If one takes into account that globulins are the second protein fraction, very important as a part of defensive systems of an organism (and that they are elevated in this case), the contribution of this herbal combination should not be neglected in stimulation of broilers immune system.

Dietary supplementation of our plants combination to chickens for 8 weeks did not cause a significantly lower blood plasma glucose concentration, and in this respect our results are contradictory. Namely, a number of spices and herbs have a long history of traditional use in treating elevated blood sugar levels. For example Jarvill-Taylor et al. (2001) reported that cinnamon stimulated glucose uptake, glycogen synthesis, and activated glycogen synthase in 3T3-L1 adipocytes. Later studies of Bakirel et al. (2008) showed that ethanolic extracts of rosemary leaves lowered blood glucose in normoglycemic and glucose-hyperglycemic rabbits.

In the present experiment, there was a significant decrease in total bilirubin concentration in birds fed diets supplemented with 0.5% basil and 0.5% thyme (II group) and 0.5% basil and 2% anis (III group). A decrease in total bilirubin level in the blood sera in the current study could be explained by stimulation of uridine diphosphate glucuronyltransferase enzyme (UDP-glucuronyltransferase),

or by protective effects of essential oils presents in our combination of plants on wall of liver cells (Figs. 3 and 4). There are several reports concerning an inhibition or activation of hepatic UDP glucuronyltransferase by certain plant constituents. Siraki et al. (2005) found out that borneol inhibited glucuronidation of non steroidal antiinflammatory drugs in isolated rat hepatocytes. This could be also applied to activity of creatine kinase activity. Namely, we found significantly decrease of its activity in the blood sera of broilers fed with combinations of basil and thyme (II group) and basil and anis (III group). Undoubtedly, these findings coupled with total bilirubin concentration, suggest that wall of liver cells was strengthened in some way (Bakkali et al., 2008).

Little is known about the effects of herbs on lipid metabolism of broilers. The effect of some functional herb substances (cyclic terpenes) on serum cholesterol and total lipids in poultry were reported by Faxova et al. (2009). On the other hand, no changes in serum cholesterol and plasma lipids because of dietary supplementation of herb extracts (some monoterpens and essential oils) to broilers were observed. Najafi and Torki (2010) found that total cholesterol, triglycerides and high-density lipoproteins in the blood of broilers did not respond to the dietary supplementation of clove extract. Our results are in line with these findings, except for the combination of 0.5% basil and 0.5% thyme (Fig. 5), where this combination of phytoadditives significantly decreased the concentration of triglycerides in the blood of broilers.

It could be concluded that supplementation of the diet with phytoadditives has a potential to improve health status in broilers. Further examinations are needed in order to elucidate the exact mechanism of action.

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