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Performance, Serum Biochemical Parameters and Immunity in Broiler Chicks Fed Dietary *Echinacea purpurea* and *Thymus vulgaris* Extracts

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ABSTRACT

The objective of this study was to evaluate the effect of administrating herbal extracts of *Echinacea purpurea* and *Thymus vulgaris* into broilers drinking water on performance, immune response and serum biochemical and Phyto hemagglutinin. 270 day-old Ross chicks were assigned to nine dietary treatments in a randomized manner. Each treatment was given to two replicates of 15 birds. The variables of T. vulgaris extract were 1% and 2% and variables of *E. purpurea* extract were 0%, 1% and 2% in drinking water. Body Weight (BW), Feed Intake (FI) and Feed Conversion Ratio (FCR) were recorded at the end of the experiment. Antibody responses against Newcastle disease viruses were measured after blood sampling at 42 days of age. The plant extracts did not affect BW, FI and FCR ($P>0.05$). Antibody titers against NDV were significantly affected by the administration of *E. purpurea* ($P<0.05$). The highest elevation was for the birds that were administrated with 2% *E. purpurea* from 1 to 42 days ($P<0.05$). Administration of thyme extracts had improved serum biochemical parameters as compared with *Echinacea* and control group. It was concluded that under these research conditions, high levels of *E. purpurea* extracts had increased the broiler chickens' immunity.

Key words: Broiler, *Echinacea purpurea* extract, Immunity, Performance, *Thymus vulgaris* extract.

INTRODUCTION

Antibiotics have been widely used in poultry feed as growth promoters for more than 50 years. In the time that antibiotics were either used for curing or as growth promoters, some parts of the profitable microorganism will become damaged and resistance to diseases such as *Salmonella* and the other pathogens will decrease. Increased interest in curbing antibiotic use to reduce antimicrobial resistance has led to a growing interest in alternative growth promoters. Herbal extracts are being used as feed additives to improve performance, feed intake, secretion of digestive tract juices and immune system of animals especially under the intensive management systems (William and Losa, 2001; Amouzmehr et al., 2012).

The *Echinacea purpurea* is commonly known as an immune stimulating substance. Its palliative use in human medicine has been well established. *E. purpurea* has been shown to have non-specific immuno-stimulatory properties *in vitro* (Bauer and Wagner, 1991), including increased phagocytosis (Stotzem et al., 1992), increased cytokine production (Burger et al., 1997), and natural killer cell activity (See et al., 1997). *Thymus vulgaris* has received more attention due to its antioxidant (Bolukbasi and Erhan, 2007), antibacterial (Dorman and Deans, 2000; Ngouana Tadjong et al., 2017) anti-coccidial (Jamroz et al., 2003) and antifungal properties (Hertrampf, 2001). As, growth performance of animals is influenced strongly by the health and immune status, the objective of this study was to evaluate the effect of the utilization of *Echinacea purpurea* and *Thymus vulgaris* extracts in the feeding of

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broilers on the growth performance and immune responses.

MATERIALS AND METHODS

270 day-old broiler chickens (Ross308) of mixed sex were randomly divided into nine groups (30 birds/group) and housed in pens of identical size in a deep litter system with wood shaving for flooring. Each group has 2 replicates (15 birds/pen). Strict sanitation practices were maintained in the house before and during the course of the experiment. All birds were fed a standard commercial diet based on corn and soybean meal and had free access to feed and water (Table 1). Treatment groups were:

1-Control group (plain water)
2- One mL/L (1%) of *E. purpurea* from 1 to 42 day
3-Two mL/L (2%) of *E. purpurea* from 1 to 42 day
4-One mL/L (1%) of *E. purpurea* from 21 to 42 day
5-Two mL/L (2%) of *E. purpurea* from 21 to 42 day
6-One mL/L (1%) of *T. vulgaris* from 1 to 42 day
7-Two mL/L (2%) of *T. vulgaris* from 1 to 42 day
8-One mL/L (1%) of *T. vulgaris* from 21 to 42 day
9-Two mL/L (2%) of *T. vulgaris* from 21 to 42 day

The live body weight of birds of all groups at 42 days of age was taken. Feed intake and their Feed Conversion Ratio (FCR) were calculated. Sera samples for subjecting to Haemagglutination Inhibition (HI) tests were obtained from all birds of each group following collection of 3mL blood (from wing vein) at 42 days of age to determine the antibody titers of Newcastle disease vaccines. Vaccination program is presented in table 2. To determine some of the serum factors (HDL, LDL, TG, Cholesterol, Albumin, Total Protein, and Glucose), blood samples 42 days of age were collected. At 42 days of age, four birds per replicate were randomly chosen, slaughtered and their lymphoid organs (Bursa of Fabricius, spleen and Thymus) were collected, weighed and calculated as a percentage of live body weight. At d 42 of age, eight chickens per treatment were injected in the right wing with a Phytohemagglutinin (PHA) (Sigma-Aldrich, St. Louis, MO) solution (100 µg in 100 µl PBS (phosphate-buffered saline) and immediately in the left wing with 100 µl PBS. The thickness of the right and left wing web was measured prior to the injection and at 6th, 12th, 24th, 48th, and 72nd hours post PHA injection using a digital caliper; then, the chickens were killed by cervical dislocation. The wing web swelling (WWS) response was expressed as a swelling index, calculated as follows: Swelling index = [(thickness of right wing web post PHA injection − initial thickness of right wing web) − (thickness of left wing web post PBS injection − initial thickness of left wing web)] (Konieczka et al., 2017). The data were analyzed using computerized statistical program (SPSS version 15.0) to determine the Mean ±SD of antibody titer and body weight. Significance differences were denoted by $P < 0.05$.

Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanly handled in respect of the ethical standards laid down in 1964 Declaration of Helsinki and its later amendments.

Table 1. Nutritional composition of the diet

Diet composition	Starter (0-10d)	Grower (11-28d)	Finisher (29-42d)
Metabolizable energy, kcal/kg	2930	3050	3150
Crude protein %	23	19.5	18.5
Digestible lysine %	1.32	1.18	1
Methionine %	0.55	0.5	0.45
met + cys %	0.98	0.88	0.82
Threonine %	0.88	0.78	0.7
Tryptophan %	0.23	0.19	0.18
Arginine %	1.5	1.25	1.13
Calcium %	0.97	0.94	0.9
Available phosphorus %	0.48	0.45	0.42
Sodium %	0.16	0.16	0.16
Chlorine %	0.18	0.18	0.18

Table 2. Vaccination Program

Age	Day 1	Day 8	Day 8	Day 16	Day 26
Vaccine	Infectious Bronchitis-H120	Newcastle Disease-B1	Newcastle-Influenza	Infectious Bursal Disease-D78	**Infectious Bursal Disease-D78**
Route	Corse spray	Drinking water	Injection	Drinking water	**Drinking water**

RESULTS

The effects of herbal extracts on chicken's body weight gain, total feed intake, and FCR have been presented in table 3. Results have shown that the administration of *T. vulgaris* from 21 to 42 days had affected growth performance more (body weight gain and feed consumption ratio) but these two parameters weren't statistically different (P>0.05). As shown in table 3, antibody titers against NDV were significantly affected by administration of *E. purpurea* (P<0.05). The highest elevation was for the birds that administrated 2% *E. purpurea* from 1 to 42 days (P<0.05).

Table 4 summarizes the data obtained on the effect of experimental treatments on serum hematological parameters. No significant influence of experimental diets on albumin was observed (P>0.05). The serum total protein, Triglyceride and HDL of the birds treated with (2%) of *T. vulgaris* from 1 to 42 days old were better than the others (P<0.05). The feeding of the broilers with (1%) of *T. vulgaris* from 1 to 42 days resulted in a marked (P>0.05) decrease in LDL- cholesterol concentration compared to other treatments. Hemoglobin concentration and Cholesterol in groups treated *T. vulgaris* from 1 to 42 days was respectively higher and lower than the other groups (P<0.05) respectively. Table 5 indicates the effect of treatments on lymphoid organs' weight at 42 day. As it is shown, the Bursa weight in birds treated with *E. purpurea* had increased and the differences in Spleen and Thymus weights were not statistically significant.

The effects of the dietary *Echinacea purpurea* and *Thymus vulgaris* extracts on PHA challenge on the WWS response are shown in Table 6. PHA challenge led to a higher increase in wing web thickness in chickens fed with *E. purpurea* diets than in those fed *T. vulgaris* diets (P<0.05). WWS was the highest at 24 h post PHA injection in group 3, post PHA injection (P<0.05).

DISCUSSION

There are no appetizing effects with both *Echinacea* and *Thymus*. The feed intake and FCR of the broilers didn't show a varying influence by administration with different dosages of *Echinacea* and *Thymus* in comparison with control group. Other authors have reported also a missing effect of various supplementations of mixed herbs on the feed intake of broilers and layers (Roth-Maier et al., 2005; Nasir and Grashorn, 2010). Toghyani et al. (2010) reported that the low dosage of Thyme has had a significant effect on broiler body weight and their feed conversion ratio. But Tekeli et al. (2006) and Demir et al. (2008) had reported opposite results; they found that thyme had no influence on broilers performance. Ngouana Tadjong et al. (2017) reported that feed intake, live body weight, weight gain and feed conversion ratio are affected by using thyme and oregano essential oil in broiler diets.

Inability to improve feed conversion ratio with *E. purpurea* is in agreement with the findings of Habibian et al. (2011) who had also reported that *E. purpurea* supplementation as a feed additive didn't improvefeed conversion ratio. Absence of positive effect of some herbal extracts in some experiments may be due to using a smaller dose which was insufficient to produce its effect on poultry. Improving immunity in poultry production is very important to prevent common important diseases. There was a significant effect on the immunity of treated chicks in the present study. All chicks that were administrated *E. purpurea* had significantly higher antibody titer against *T. vulgaris* (P<0.05). Among them, the highest (8.9) increasing was belong to 2% *E. purpurea* extract from day old to 42 days (P<0.05).

Our findings indicated that the dietary *E. purpurea* could not only affect the PHA-induced swelling response, but also increased the weight of Bursa. The PHA-induced WWS is a good indicator of acquired immunity and allows the assessment of leukocyte interactions during the immune response (Konieczka et al., 2017). Increasing in antibody titer of the birds administrated *E. purpurea* are consonant with the increasing of the weight of bursa. The studies of the immune system have shown that some herbs such as coneflower (*Echinacea purpurea*) were most effective in achieving immune system improvement, because this herb had increased the stimulation of non-specific immune system. It is though that immune enhancement of *Echinacea* is provided by certain polysaccharides, flavonoids and *isobutylamides* (Rehman et al., 1999). Also, 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 (Manach et al., 1996).

Obtained data from biochemical parameters showed that supplementing broiler diet with *thyme* extracts had insignificant better effect on cholesterol, triglyceride, LDL, total protein, albumin and hemoglobin ratio against supplementing *Echinacea* In agreement with our finding, Ali (2014) and Toghyani et al. (2010) reported supplementing diets with thyme leaves powder had no significant effect (P≥0.05) on serum parameters. Thyme supplementation in broiler diets significantly increased glucose level compared to those of the control group. The possible reason for increasing serum glucose may be due to the abdominal lipids catabolism of gluconeogenesis process as feeding inclusion thyme by birds, since crushed thyme consumption in broiler chickens has been reported to increase the serum glucose as mentioned by El-Ghousein and Al-Beitawi (2009).

Table 3. Effects of *E. purpurea* and *T. vulgaris* on growth performance and antibody titer (log2 HI titer) against Newcastle virus (M ± SE)

Treatments	Body weight (g)	Feed intake (g)	Feed conversion (g/g)	Antibody titer (log2 HI titer)
Control	2477±226.1	4260	1.72	6.5^{a}±0.15
***E. p* (1%) 1 to 42d**	2412±268.3	4200	1.74	8.2^{b}±.024
***E. p* (2%) 1 to 42d**	2409±277.6	4170	1.73	8.9^{c}±0.21
***E. p* (1%) 21 to 42d**	2410±318.8	4170	1.73	7.4^{d}±1
***E. p* (2%) 21 to 42d**	2526±230	4320	1.71	8.1^{b}±0.18
***T. v* (1%) 1 to 42d**	2389±300.5	4325	1.81	6.5^{a}±0.14
***T. v* (2%) 1 to 42d**	2434±252.7	4260	1.75	6.6^{a}±0.18
***T. v* (1%) 21 to 42d**	2567±232.8	4340	1.69	6.4^{a} ±0.13
***T. v* (2%) 21 to 42d**	2460±272.1	4260	1.73	6.3^{a}±0.19

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

Table 4. Effect of experimental diets on serum biochemical parameters of broilers at d 42.

Factor(mg/dl)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Blood Glucose	$127±4.08^{ac}$	$117.5±8.34^{a}$	$123.5±7.18^{ac}$	$129.25±10.5^{bc}$	$116.5±4.79^{a}$	$118.5±9.67^{a}$	$122.75±9.97^{ac}$	$131.25±12.97^{bc}$	$124.5±10.5^{ac}$
Cholesterol	$95.5±23.33^{ab}$	$97.25±17.11^{ab}$	$96±23.93^{ab}$	$102.75±16.35^{b}$	$95.5±19^{ab}$	$72.75±7.5^{a}$	$71.25±7.67^{a}$	$85.25±11.58^{ab}$	$84.25±11.35^{ab}$
Triglyceride	$58±6.16^{b}$	$53.50±9.84^{b}$	$56±8.52^{b}$	$51.25±12.12^{bc}$	$54.50±4.65^{b}$	$44.50±11.95^{ac}$	$38.50±13.69^{a}$	$41.25±19.5^{a}$	$42.5±11.81^{a}$
LDL	$47±11.9^{c}$	$43.25±8.9^{bc}$	$45.5±7.32^{c}$	$46.75±6.39^{c}$	$36.75±8.65^{abc}$	$27.25±4.11^{a}$	$31±9.09^{ab}$	$30±9.2^{ab}$	$29.25±9.28^{a}$
HDL	$52.25±7.5^{a}$	$61.25±11.5^{abc}$	$60.25±4.92^{ab}$	$64.75±4.11^{bc}$	$59.75±8.05^{ab}$	$72.50±4.79^{cd}$	$76.25±5.90^{d}$	$63.75±6.70^{bc}$	$68±7.11^{bcd}$
Total protein	$3.80±0.59^{a}$	$3.97±0.22^{ab}$	$4.09±0.11^{abc}$	$4.22±0.11^{bc}$	$3.83±0.09^{a}$	$4.32±0.07^{bc}$	$4.41±0.07^{c}$	$4.18±0.06^{abc}$	$4.05±0.19^{abc}$
Albumin	1.42±0.09a	1.47±0.03a	1.41±0.06a	1.39±0.04a	1.38±0.06a	1.46±0.03a	1.44±0.03a	1.39±0.1a	1.47±0.04a
Hemoglobin	$7.85±0/32^{a}$	$8.08±0.39^{ab}$	$8.31±0.06^{bcd}$	$7.97±0.24^{ab}$	$8.25±0.09^{bc}$	$9.40±0.11^{e}$	$9.06±0.29^{e}$	$8.49±0.3^{cd}$	$8.69±0.16^{d}$

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

Table 5. Effect of experimental diets on weights of lymphoid organs at day 42**.**

Lymphoid organ weight	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Bursa/BW (gr)	$0.144±0.007^{a}$	$0.168±0.005^{c}$	$0.18±0.011^{d}$	$0.177±0.011^{d}$	$0.155±0.009^{abc}$	$0.148±0.004^{ab}$	$0.15±0.007^{ab}$	$0.16±0.007^{bc}$	$0.16±0.007^{bc}$
Spleen/BW (gr)	$0.113±0.002^{abc}$	$0.11±0.002^{abc}$	$0.12±0.002^{d}$	$0.114±0.002^{abc}$	$0.118±0.005^{cd}$	$0113±0.002^{abc}$	$0.117±0.005^{abc}$	$0.111±0.001^{a}$	$0.113±0.002^{ab}$
Thymus/BW(gr)	$0.195±0.03^{a}$	$0.26±0.04^{b}$	$0.247±0.03^{ab}$	$0.24±0.05^{ab}$	$0.242±0.03^{ab}$	$0.212±0.03^{ab}$	$0.212±0.04^{ab}$	$0.22±0.04^{ab}$	$0.227±0.04^{ab}$

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

Table 6. Phytohemagglutinin (PHA) injection time on the wing web swelling response in chickens challenged with PHA

Wing web reaction (PHA-P)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Stimulation index after 24 h (mm)	$1.22±0.04^{ab}$	$1.55±0.08^{f}$	$1.76±0.1^{g}$	$1.48±0.07^{f}$	$1.52±0.06^{f}$	$1.35±0.05^{cd}$	$1.3±0.02^{bc}$	$1.15±0.05^{a}$	$1.37±0.03^{cde}$
Stimulation index after 48 h (mm)	$1.31±0.04^{bc}$	$1.69±0.06^{g}$	$1.71±0.05^{g}$	$1.71±0.1^{g}$	$1.47±0.04^{ef}$	$1.36±0.04^{cd}$	$1.53±0.04^{f}$	$1.28±0.03^{b}$	$1.44±0.01d^{ef}$

The different superscripts on the same line are significantly different (P < 0.05); *Mean ± standard deviation

CONCLUSION

Both herbal extracts (*Echinacea* and *thyme*) used in drinking water did not improve the performance of broilers. However, *Echinacea* extracts had significantly improved the immune response of birds to the NDV vaccine, therefore, more studies with different growth facility need to be performed to analyze the effect of herb extracts on the performance, carcass yield, hematology and immune response of poultry.

Competing interests

The authors have no competing interests to declare.

Authors' contributions

The present study was funded by H Habibi and S Firouzi. Habibi and Firouzi were also involved in the collection of data, statistical analysis and drafting of the manuscript. Both authors read and approved the final manuscript.

REFERENCES

Ali AHH (2014). Productive performance and immune response of broiler chicks as affected by dietary thyme leaves powder. Egyptian Poultry Science Journal, 34: 71–84.

Amouzmehr A, Dastar B, Ghassemi Nejad J, Il Sung K, Lohakare J and Forghani F (2012). Effects of Garlic and Thyme Extracts on Growth Performance and Carcass Characteristics of Broiler Chicks. Journal of Animal Science and Technology, 54: 185-190. Doi: 10.5187/JAST.2012.54.3.185.

Bauer R and Wagner H (1991). Echinacea species as potential immunostimulatory drugs. In: Economic and medicinal plant research, Wagner H, (Eds.), Academic Press Limited, London, U.K. pp. 243-321.

Bolukbasi SC and Erhan MK (2007). Effect of dietary thyme (*Thymus Vulgaris*) on laying hen's performance and Escherichia coli (E.coli) concentration in feces. International Journal of Natural and Engineering Sciences, 1: 55-58.

Burger RA, Torres AR, Warren RP, Caldwell VD and Hughes BG (1997). Echinacea-induced cytokine production by human macrophages. International Journal of Immunopharmacology, 19(7): 371-379. Doi:10.1016/S0192-0561(97)00061-1.

Demir E, Kilinc K, Yildirim Y, Dincer F and Eseceli H (2008). Comparative effects of mint, sage, thyme and flavomycin in wheat-Based broiler diets. Archiva Zootechnica, 11: 54-63.

Dorman HJD and Deans SG (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of Application Microscopy, 88: 308-316. Doi: 10.1046/j.1365-2672.2000.00969.

Habibian Dehkordi S, Fallah V and Habibian Dehkordi S (2011). Enhancement of broiler performance and immune response by *Echinacea purpurea* supplemented in diet. African Journal of Biotechnology, 10 (54): 11280-11286. Doi: 10.5897/AJB11.1484.

Hertrampf JW (2001). Alternative antibacterial performance promoters. Poultry International, 40: 50-52.

Jamroz D, Orda J, Kamel C, Wiliczkiewicz A, Wertelecki T and Skorupinska J (2003). The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and maicrobial status in broiler chickens. Journal of Animal and Feed Sciences, 12: 583-596. Doi: 10.22358/jafs/67752.

Konieczka P, Barszcz M, Chmielewska N, Cieślak M, Szlis M and Smulikowska S (2017). Interactive effects of dietary lipids and vitamin E level on performance, blood eicosanoids, and response to mitogen stimulation in broiler chickens of different ages. Poultry Sciences, 96(2): 359–369. doi: 10.3382/ps/pew219.

Manach F, Regerat F and Texier O (1996). Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutrition Research, 16: 517-544.

Najafi P and Torki M (2010). Performance, Blood Metabolites and Immunocompetence of Broiler Chicks Fed Diets Included Essentioal Oils of Medicinal Herbs. Journal of Animal and Veterinary Advances, 9: 1164-1168. Doi: 10.3923/javaa.2010.1164.1168.

Nasir Z and Grashorn MA (2010). Effects of *Echinacea purpurea* and *Nigella sativa* supplementation on broiler performance, carcass and meat quality. Journal of Animal and Feed Sciences, 19: 94–104.

Ngouana Tadjong R, Kana JR, Tsafack Necdem B, Yemdjie Mane DD, Mube Kuietche H, Kuiede S, Teguia A and Meimandipour A (2017). Performances of Broiler Chickens Fed on Diet Supplemented with Thyme and Oregano Essential Oils Stabilized in a Plant Charcoal Matrix. Journal of worlds Poultry Research, 7 (2): 79-87.

Rehman J, Dillow JM, Carter SM, Chou J, Le B and Maisel AS (1999). Increased production of antigen-specific immunoglobulins G and M following in vivo treatment with the medicinal plants *Echinacea angustifolia* and *Hydrastiscana densis*. Immunol Lett, 68(2-3): 391- 395.

Roth-Maier D, Barbara A, Böhmer M, Nicole M, Damme K and Brigitte PR (2005). Efficiency of *Echinacea*

purpurea on performance of broilers and layers.Arch. Geflügelk, 69 (3): 123–127.

See DM, Broumand N, Sahl L and Tilles JG (1997). In vitro effects of Echinacea and Ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. Immunopharmacology, 35(3): 229-235. Doi: 10.1016/S0162-3109(96)00125-7.

Stotzem CD, Hungerland U and Mengs U (1992). Influence of *Echinacea purpurea* on the phagocytosis of human granulocytes. Medical Science Research, 20: 719-720.

Tekeli A, Çelik L, Kutlu HR and Gorgulu M (2006). Effect of Dietary Supplemental Plant Extracts on Performance, Carcass Characteristics, Digestive System Development, Intestinal Microflora and Some Blood Parameters of Broiler Chicks. XII European Poultry Conference, Verona, 10-14 September 2006.

Toghyani M, Tohidi M, Gheisari AA and Tabeidian SA (2010). Performance, Immunity, Serum Biochemical and Hematological Parameters in Broiler Chicks Fed Dietary Thyme as Alternative for an Antibiotic Growth Promoter. African Journal of Biotechnology, 9: 6819-6825. Doi: 10.5897/AJB09.

William P and Losa R (2001). The Use of Essential Oils and Their Compounds in Poultry Nutrition. World Poultry, 17: 14-15.