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Effect of the Nettle Essential Oil (*Urtica dioica* L.) on the Performance and Carcass Quality Traits in Broiler Chickens

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ABSTRACT: As a result of the worldwide ban on antibiotic growth promoters in food animals, essential oils have gained considerable importance. Animal meat and carcass quality traits have also been reported to be influenced by essential oils. This study investigated the effects of common nettle essential oil (*Urtica dioica* L.) in broiler chicken nutrition on performance and carcass quality characteristics. For this experiment which has lasted a 42-days, a total of 648 one-day-old broilers hybrid Cobb 500 were used. A total of 12 replicates were used per dietary treatment, leading to a total of 18 broilers per replicate, of an average initial body weight 34±0.58 g. The three dietary treatments comprised a control diet (C), a control diet + 0.5% of nettle essential oil (EO1), and a control diet + 1.0% of nettle essential oil (EO2). Despite the carcass and breast yields being higher in the essential oil treatments (EO1 and EO2) compared to the control treatment (C), the thigh yields were unaffected by diet (P>0.05). Shank yield was higher in the EO1 and EO2 treatments as compared with the control (P<0.05) treatment. The weight of the viscera decreased (P<0.05) in the dietary treatments with the addition of essential oils. The EO2 treatment had a lower meat pH compared to the other two treatments. Based on the obtained results, it is concluded that the EO2 treatment was equally effective as the EO1, regarding the carcass traits, and therefore can serve as an alternative to the banned antibiotic growth promoters in broiler chickens.However further *in vivo* studies are required to assess the effect of the nettle essential oil on the gut health, immunity, and welfare of birds.

Keywords: broilers; chickens; essential oil; nettle, nutrition.

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INTRODUCTION

Global meat production has steadily increased over the past decades as a result of steady growth in the human population (Barrett, 2021; Puvača et al., 2013). This trend is expected to continue shortly (Guiné et al., 2021). Growing demand for animal protein and stricter regulations regarding animal welfare (de Jonge & van Trijp, 2013) and environmental protection on the one hand (Ilea, 2009), and the increasing demand for animal protein and regulations, on the other hand, make subsequent adjustments to the production process essential (Montossi et al., 2013). As a result of years and years of rising bans against antibiotic growth promoters in food animals and the tendency to reduce their use in poultry production, the poultry industry and scientific community are under a great deal of pressure to find alternative antibiotic growth promoters (Kostadinović et al., 2016; Puvača et al., 2013). As alternatives to antibiotic growth promoters in animal nutrition, phytogenic feed additives - plant-based ingredients such as herbs, spices, essential oils, and extracts from plants - have therefore gained considerable interest (Iqbal et al., 2022; Popović et al., 2016, 2018; Sevim et al., 2020). It is due to their ability to maintain a healthy gut environment that they can maintain improved performance. Ideally, an alternative to antibiotic growth promoters would have the same benefits when added to animal feed as an antibiotic growth promoter (Redondo et al., 2014). Numerous scientific studies have emphasized the importance of essential oils in supporting health and enhancing the performance of broilers (Allen et al., 2013; Bernardeau & Vernoux, 2013; Brown et al., 2017; Demir et al., 2003). It has been suggested that the essential oils from the plant can help improve poultry gut health, nutrient digestibility, and growth performance (Aćimović & Puvača, 2020; Giannenas, Bonos, Christaki, et al., 2018; Giannenas, Bonos, Skoufos, et al., 2018; Puvača, 2018). Bioactive molecules found in essential oils, like thymol, carvacrol, cineole, and capsaicin, are responsible for their many beneficial effects (Abo Ghanima et al., 2020; Lika et al., 2021; Puvača et al., 2020). Furthermore, poultry carcasses and meat quality characteristics are reported to be positively affected by essential oils when used in daily broiler nutrition (Abd El-Hack et al., 2022; Abo Ghanima et al., 2021; Goliomytis et al., 2015; Kiyma et al., 2017; Suliman et al., 2021).

Nettle (*Urtica dioica* L.) belongs to the family of *Urticaceae* and is rich in steroids, terpenoids, phenylpropanoids, coumarins, lectins, and flavanol glycosides which express positive effects when used in the poultry diet (Mirsaiidi Farahani & Hosseinian, 2022). Additionally, flavonoid glycosides have been found to have immune-stimulating, anticarcinogenic, anti-inflammatory, antioxidant, and antiallergenic properties (Mehrabi & Firouzbakhsh, 2020; Şandru et al., 2016). The essential oil of nettle has been used in animal nutrition because of its anti-oxidative and growth-stimulating effects (Moula et al., 2019). Additionally, the metabolism of protein and lipids may also be affected by the usage of nettle essential oil in broiler nutrition improving their productive performances. Normally, nettles are used as a dietary supplement for humans due to their low cost, non-toxic chemical composition, and easy availability (Abdel-Aziz et al., 2016). Since nettles contain active compounds such as tannins, formic acid, salicylic acid, carvacrol, and thymol, they could be used in veterinary medicine as an alternative to antibiotic drugs as well as in industrial broiler chickens' production (Abdelli et al., 2021).

The present study was aimed to investigate the effects of common nettle essential oil (*Urtica dioica* L.) in broiler chicken nutrition on productive performance and carcass traits.

MATERIALS AND METHODS

The animal study protocol was approved by the Ethics Committee of University BA in Novi Sad, Serbia (EC-26/2/21, date: 01.04.2021).

Broiler chickens housing

A total of 648 day-old Cobb 500 broilers were used as part of a 42-day experimental trial. Used broilers in the experiment was of mixed sex.Weighed chicks of an average initial body weight 34±0.58 g were randomly assigned to 3 dietary treatments, with 12 replications of each treatment and 18 chicks per replication (216 broilers in each treatment). Chopped straw was used as a bedding for chickens, while the lighting program was 23 h of light for the first 7 days, 20 h until the 15th day, and 18 h afterward, according to broiler producer recommendations. Chickens were vaccinated against Marek‘s disease on hatch day (Nobilis®Rismavac, Intervet International B.V., The Netherlands), Newcastle disease on the 7th day and the 21st day (LaSota Strain, Merial Select, INC., Georgia), and infectious bursal disease on the 14th day of age (BURSINE®-2, Zeotis, United States), respectively. The temperature was maintained at 32 °C during the first 2 weeks and decreased to 27 °C subsequently.

Broiler chickens' nutrition

During the trial period chickens were fed with corn-soybean meal-based diets from 1 to 7 days (starter), 8 to 21 days (grower), and 22 to 42 days (finisher), following the researchers own experimental design. These three dietary treatments comprised of a control diet (C), control diet + 0.5% of common nettle essential oil (EO1) and control diet + 1.0% of common nettle essential oil (EO2) Table 1. The chemical composition of trial diets and concentration of polyphenolic compounds in used nettle essential oil in the experimental diets are shown in Table 2 and Table 3. Commercially available essential oil of common nettle used in this research was porched from "Planet fresh" company in Montenegro.From the beginning till the end of the trial feed and water were provided to chickens *ad libitum*.

Analysis of polyphenolic compounds of nettle essential oil

UHPLC-DAD-HESI-MS/MS analysis was performed using a Thermo Scientific liquid chromatography system (UHPLC) composed of a quaternary pump with a degasser, a thermostated column compartment, an autosampler and a diode array detector connected to LCQ Fleet Ion Trap Mass Spectrometer (Thermo Fisher Scientific, San Jose, California, USA), equipped with heated electrospray ionization (HESI). Xcalibur (2.2 SP1.48) and LCQ Fleet (2.7.0.1103 SP1) software were used for instrument control, data acquisition and data analysis. Separations were performed on a Hypersil gold C18 column (50 × 2.1 mm, 1.9 mm) obtained from Thermo Fisher Scientific. The mobile phase consisted of (A) water + 0.2% formic acid and (B) acetonitrile. A linear gradient program at flow rate of 0.250 mL/min was used 0–2 min from 10 to 20% (B), 2–4.5 min from 20 to 90% (B), 4.5–4.8 min 90% (B), 4.8–4.86 min from 90 to 10% and 4.86–12.0 min 10% (B). The injection volume was 5 mL, and the column temperature was maintained at 25 °C. The mass spectrometer was operated in negative mode. HESI-source parameters were as follows: source voltage 4.5 kV, capillary voltage 29 V, tube lens voltage 70 V, capillary temperature 275 °C, sheath and auxiliary gas flow (N_2) 50 and 8 (arbitrary units), respectively. MS spectra were acquired by full range acquisition covering 150-700 m/z. For fragmentation study data dependent scan was performed by deploying the collision-induced dissociation (CID). The normalized collision energy of the CID cell was set at 25 eV. All compounds were identified according to the corresponding spectral characteristics: mass spectra, accurate mass and characteristic fragmentation.

Measurement of performance, carcass, and edible organ traits

Each chicken was individually weighed at 7, 21, and 42 days of trial. These points correspond to the starter period, grower period, and finisher period. For the entire period, body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were calculated. Data was adjusted according to the mortality recorded each day. After the trial was complete, 12 birds with body weights (BW)closest to the average were chosen from each dietary treatment. A cervical dislocation, followed by exsanguination, resulted in the chickens being weighed and sacrificed. The weight of the eviscerated hot carcass, breast, drumsticks, thighs, and organs (viscera, liver, gizzard, and heart) was measured after the feathers, viscera, shanks, and neck were removed. Based on live weight, carcass yields and yields of breasts, thighs, and drumsticks were measured. Approximately 200 g of chicken breast fillets were stored at 4 °C for 24 h to the determination

Table 1. Ingredient composition of trial diets, %

Ingredients	Starter (1-7 days)	Grower (8-21 days)	Finisher (22-42 days)
Corn flour	54.1	57.0	58.3
Soybean cake	35.6	32.5	30.6
Monocalcium phosphate	2.9	2.9	3.0
Lysine	0.3	0.2	0.2
Methionine	0.6	0.9	1.4
Sodium bi carbonate	2.0	2.0	2.0
Salt	2.0	2.0	2.0
Premix	2.0	2.0	2.0
Toxin binder	0.5	0.5	0.5
Total*	100.0	100.0	100.0

* - Nettle essential oil was added *on top* of basal diets (C) in concentrations of 0.5% (EO1), and 1.0% (EO2).

Table 2. Chemical composition of trial diets, %

Nutrients	Starter (1-7 days)	Grower (8-21 days)	Finisher (22-42 days)
Crude protein	22.0	21.0	19.0
Crude fat	5.3	6.0	6.5
Crude fiber	3.8	3.6	3.5
Calcium	0.96	0.95	0.94
Phosphorus $_{\text{(available)}}$	0.32	0.32	0.31
Lysine	1.10	1.00	0.90
Methionine+cysteine	0.69	0.67	0.67
ME, MJ/kg	11.90	12.10	12.60

Table 3. Concentration of polyphenolic compounds in used nettle essential oil.

Compound	Formula	m/z $[M-H]^-$	MS/MS
Quercetin	$C_{15}H_{10}O_7$	310	151
Gallic acid	$C_7H_6O_5$	169	125
Vanillic acid	$C_8H_8O_4$	166	108
Naringenin	$C_{15}H_{12}O_5$	271	151
Ferulic acid	$C_{10}H_{10}O_4$	193	134
Caffeic acid	$C_9H_8O_4$	179	135
Chlorogenic acid	$C_{16}H_{18}O_9$	353	191
Sinapinic acid	$C_{11}H_{12}O_5$	223	193

m/z $[M-H]^-$ - The conformation of the compounds presence in the analyzed sample was performed comparing the m/z values of the parent product ions with obtained values from analytical standards and available literature. MS/MS - Tandem mass spectrometry.

of pH, drip loss, and moisture contents, according to Puvača et al. (2016). Pieces of breast fillets of approximately 50 g were selected and further stored at 4 °C for 48 h to determine the loss of weight. A pH meter (HI 99163, METTLER TOLEDO, Zagreb) was used to measure the pH of chicken breast meat at different locations across the sample surface.

Statistical analysis

Statistical analyses were conducted using the statistical software program Statistica 10 for Windows (StatSoft, Inc., Tulsa, OK, USA), to determine whether variables differed among treatments. Significant effects were further evaluated using ANOVA, and standard errors of least-square means (SE_{LSM}). Fisher's l.s.d. post-hoc multiple-range test was used to ascertain differences among treatments. A significance level of P = 0.05 was used.

RESULTS AND DISCUSSION

Based on the results shown in Table 4, it can be noticed that the addition of 1.0% of dietary nettle essential oil (EO2; 2414.1 g) expressed the best results regarding the chicken's body weight gain (BWG) with statistically significant differences (P<0.05) when compared with control treatment (C; 2265.4 g). Statistically significant differences can also be noticed between dietary treatment EO1 and C (P<0.05), respectively. Nevertheless, different concentrations of nettle essential oil did not lead to statistically significant differences between these two experimental treatments (P>0.05). The same tendency without any statistically significant differences (P>0.05) can be observed in feed intake (FI) for both experimental treatments (EO1 and EO2), as well for the control treatment (C). The addition of higher concentrations of common nettle essential oil (EO2) has led to the lowest feed conversion ratio (FCR) of 1.65 kg/kg, with statistically significant differences (P<0.05) when compared to the control treatment of chickens (1.87 kg/kg). However, the addition of 0.5% of essential oil in treatment EO1 didn't show any statistically significant differences (P>0.05) in FCR when compared to both control and EO2 dietary treatment, respectively. Similar results were achieved in numerous studies with dietary usage of different essential oils (Giannenas et al., 2013; Yang et al., 2018), medicinal plants (Khaligh et al., 2011; Popović et al., 2018), herbs (Kadam et al., 2009; Puvača et al., 2015), or spices (Marić et al., 2021; Tashla et al., 2019). For the experimental trial, chickens were healthy without statistically significant mortality in the control, EO1, and EO2 treatments (P>0.05), respectively.Besides, Milosevic et al. (2021) in their comprehensive review

Table 4. The effect of nettle essential oil on the performance of broiler chickens

	C	EO1	EO2	SE_{LSM}	P value
BWG, g	2265.4[b]	2381.3[a]	2414.1[a]	25.41	0.021
FI, g	3961.8[a]	3732.2[a]	3660.3[a]	17.25	0.082
FCR, kg/kg	1.87[a]	1.71[ab]	1.65[b]	0.72	0.014
Mortality rate, %	3.51[a]	2.11[a]	1.52[a]	0.31	0.037

Means within a row followed by the different letters are significantly different (P<0.05)

provides evidence of nettle bioactive components, and its stimulative effects on growth and feed utilization, metabolic processes, and support in immune system of broilers chickens.

Hosseini Mansoub (2011) investigated the effects of nettle (1.5%) and probiotic (1.0%) on performance and serum composition of broiler chickens. According to the results, the amount of total cholesterol and triglyceride in the serum showed significant differences, but HDL was not significantly different among groups, while overall performances of broiler fed with the addition of nettle were improved. Besides its good combination with probiotics, it has been noticed that nettle can be usefully combined with other natural nutrients such as pumpkin seed oil. Tabari et al. (2016) evaluated the effect of nettle root and pumpkin seed on production traits and intestinal microflora of Ross 308 mail broiler chickens. Nettle root and pumpkin oil addition improved overall feed conversion ratio. Effects of dietary treatments on carcass weight, portion yield and the relative weights of the heart, spleen, liver and gizzard were insignificant except for thigh, drumstick and bursa.Loetscher et al. (2013) have investigated the oxidative stability of the meat of broilers supplemented with dietary nettle and various botanicals, and their effects on performance and meat quality. Nettle did not improve oxidative stability of meat, although tocopherol content was elevated. Nettle treatment strongly intensified skin yellowness (b^*) compared with the control treatment.Puvača et al. (2022) showed that the addition of nettle essential oil has influence on the expression of Ki-67 marker protein in the liver with the recorded expression of moderate to strong intensity, which indicates a positive effect of supplemented essential oil on increased hepatocyte activity. The addition of a common nettle essential oil to the chicken's diet led to a significant (P<0.05) increase in the height of the intestinal villi, a decrease in the depth of the crypts, and an increase in the villocryptal ratio(Puvača et al., 2022).

The following results in Table 5 indicate that with the reduction in viscera weight achieved through the addition of both concertation of nettle essential oil, less energy is required for maintaining the gut, which leaves more energy to be used for optimizing productivity, resulting in increased BWG and decreased FCR (Table 4). Supplementation with essential oils further supports these findings since BWG and FCR were improved by dietary additions of essential oils. There is evidence that plant extracts and essential oils can improve broiler performance (Kiyma et al., 2017; Moula et al., 2019; Şandru et al., 2016; Yang et al., 2018), which is following our findings.

Compared with the control treatment (139.4 g/

Table 5. The effect of nettle essential oil on the carcass traits and relative organ weight, (g/kg of live weight)

	C	EO1	EO2	SE_{LSM}	P value
Carcass cuts yield					
Carcass yield	728.9[a]	734.2[a]	742.6[a]	7.54	0.128
Breast	183.9[a]	187.2[a]	191.4[a]	0.63	0.072
Drumstick	139.4[b]	152.8[a]	156.9[a]	0.98	0.002
Thigh	74.1[a]	78.8[a]	76.2[a]	0.12	0.061
Frame	251.2[a]	233.4[a]	243.0[a]	2.45	0.922
Organ weight					
Viscera	115.6[a]	83.7[b]	82.4[b]	1.11	0.012
Liver	24.4[a]	23.2[a]	22.7[a]	0.03	0.563
Gizzard	49.3[a]	50.5[a]	44.3[a]	0.04	0.094
Heart	6.7[a]	8.0[a]	7.8[a]	0.13	0.326

Means within a row followed by the different letters are significantly different (P<0.05) kg of live weight), most carcass traits were not statistically significant (P>0.05), except for the drumstick yield. The drumstick yield was higher in EO2 (156.9 g/kg of live weight) and EO1 (152.8 g/kg of live weight), with statistically significant differences (P<0.05) when compared to the control treatment. There was no significant change in relative organ weight with the addition of essential oils to the diet (P>0.05); however, visceral weight decreased in the dietary treatments that had nettle essential oils added (P<0.05). As compared with the control, the EO1 and EO2 treatments increased the heart's weight, but only numerically. Keshavarz et al. (2014)show no significant differences in broiler performance among the treatments fed dietary addition of nettle essential oil and nettle powder. In contrast, some of the carcass internal organs such as liver, bile sac, gizzard, proventriculus, and lungs weight were affected by the different level of powder and essential oil (P<0.05), which led to a conclusion that inclusion of 10g/kg nettle essential oil in diet probably can induce a potential toward improve internal organs.Nasiri et al. (2011) have investigated the effects of different levels of nettle in starter and grower feeds on carcass traits of broilers. The results showed that the use of different levels of nettle in starter and grower feeds had significant effects on carcass traits of broilers (P<0.05). The overall results showed that the use of 1.5% of nettle in starter and grower feeds without having any significant effects on performance and blood biochemical and immunity parameters, showed positive effects on carcass traits of broilers.

No significant effect of the diets was observed on drip loss (Figure 1) and pH of meat (Figure 2) although EO2 treatment tended to have a lower meat pH compared to the other 2 treatments (P>0.05).

Since 2006, the use of antibiotic growth promoters as feed additives in the European Union has been completely banned (Mark B. Ampode & C. Mendoza, 2021) due to the risk of generating microbiota with increased resistance to antibiotics used to treat humans, food animals, as well as pet animals (Puvača & de Llanos Frutos, 2021). Essential oils are becoming increasingly popular as an alternative feeding strategy to replace antibiotic growth promoters (Callaway et al., 2021; Seidavi et al., 2021; Zhang et al., 2021). Furthermore, animal carcasses and meat quality characteristics are reported to be positively affected by dietary essential oils added to daily ratios (Smeti et al., 2021). Based on our results, both treatments with nettle essential oils led to a higher drumstick yield compared to the control. Despite supplementing nettle essential oil to the diet, the relative organ weights did not change significantly. However, the weight of the viscera decreased in the dietary treatments with nettle essential oil supplementation.

CONCLUSION

From reported findings, it can be concluded that the dietary addition of nettle essential oil to the broiler diet resulted in an improvement in broiler productive results and carcass quality traits. Based on the carcass and performance traits, the nettle essential oil concentrated at 1.0% was just as effective as the nettle essential oil concentrated at 0.5%. Thus, nettle essential oil acted as an effective natural instrument, and could usefully serve as an alternative to antibiotics growth promoters; however, further investigation of its bene-

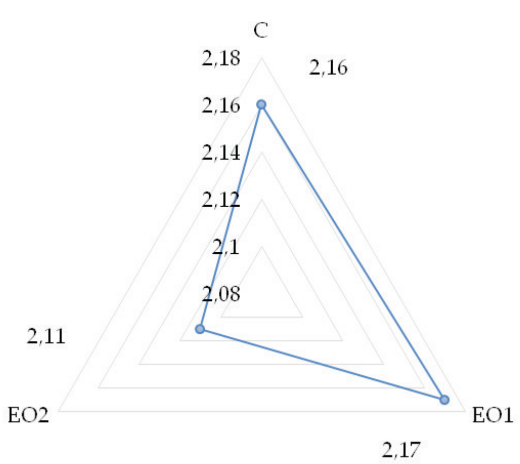

Figure 1. Drip loss measured after 24h of storage at 4 °C. The diagram illustrates the effect of the nettle essential oil on the meat drip lossof broiler chickens

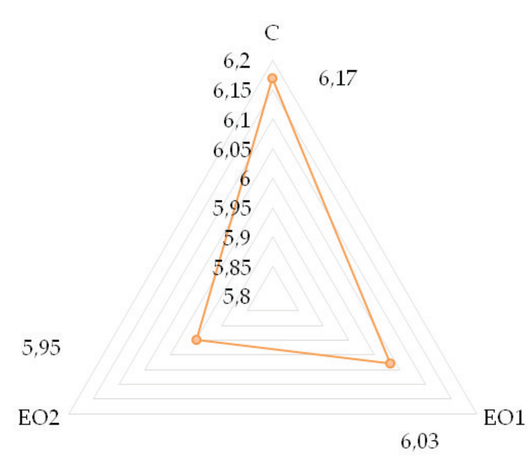

Figure 2. Meat pH values measured after 24h of storage at 4 °C. The diagram illustrates the effect of the nettle essential oil on the pH of breast fillets in broiler chickens

ficial mechanisms is still necessary.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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