Rumex nervosus leaves meal improves body weight gain, duodenal morphology, serum thyroid hormones, and cecal microflora of broiler chickens during the starter period

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ABSTRACT A total of 192 one-day-old Ross 308 broiler chicks were assigned to 4 treatments with 8 replicate cages of 6 chicks (3♀ and 3♂) per cage according to a completely randomized block design. The dietary treatments were a basal diet (control) and a control diet supplemented with 1,000, 3,000, and 5,000 mg/kg *Rumex nervosus* leaves meal (**RN**). Gallic acid and some volatile compounds were detected in the RN extract. On day 10 of age, BW was improved ($P = 0.016$) with supplemental RN (1,000–5,000 mg/kg). On day 14 of age, dietary application of RN up to 3,000 mg/kg increased BWG ($P = 0.003$) compared with control, while a 1,000 mg/kg RN had the best feed conversion ratio ($P = 0.016$). On day 10 of age, samples were taken on a single female bird per replicate. The addition of RN (1,000–5,000 mg/kg) increased ($P < 0.001$) serum albumin and triiodothyronine levels and maximized the relative weight of breast meat ($P = 0.003$). Feeding a diet with 1,000 mg/kg RN resulted in greater duodenal villus height ($P < 0.001$) than control and the diet with 5,000 mg/kg RN. Broilers fed diet supplemented with 1,000 mg/kg RN had the best duodenal villus surface area ($P < 0.001$). Feeding a diet with 1,000 mg/kg RN decreased ($P < 0.001$) cecal *Escherichia coli* count compared with control and the diet with 5,000 mg/kg RN. *Salmonella* spp. count tended to increase with 5,000 mg/kg RN leaves meal ($P = 0.069$, linear $P = 0.026$). In conclusion, *R. nervosus* leaves meal could be considered as a phytogenic feed additive in broiler diets up to a 1,000-mg/kg inclusion rate because of its combined positive effects on BWG, feed conversion ratio, villus height, villus surface area, serum albumin and triiodothyronine hormone, and cecal *E. coli* during the starter period (day 10–14 of age). Further study is required to elucidate its molecular mechanism.

Key words: broiler chicken, *Escherichia coli*, gallic acid, *Rumex nervosus*, *Salmonella*

2020 Poultry Science 99:5572–5581
https://doi.org/10.1016/j.psj.2020.08.023

INTRODUCTION

Reports from World Health Organization (WHO, 2014) resulted in formulation diets containing less pharmaceutical products either by law (ESVAC, 2017) or voluntary removal by meat and egg producers. Antibiotics have been included in broiler feeds with a view to promote body weight gain (**BWG**) and control pathogens (Engberg et al., 2000). However, the application of antibiotics in livestock and poultry has resulted in a greater increase in multidrug-resistant germs (Forgetta et al., 2012). Owing to prohibition of antibiotic application in animal production, researchers are encouraged to find alternate products (Diarra and Malouin, 2014). Phytogenic additive (spices, herbs, plants, and products derived thereof) is one of antibiotics' substitutes (Windisch et al., 2008).

Rumex nervosus is known in the Arabian Peninsula as "Ithrib". *R. nervosus* has been used as a traditional herbal plant medicine in Arab (Saudi Arabia and Yemen) and East African (Ethiopia, Kenya, Tanzania, and Somalia) countries; it is spread abundantly on an extensive range in mountains (Sarawat Mountains), roadsides, overgrazed areas, sandy areas, high-altitude areas, and relatively high rainfall and rocky areas (Al-Aklabi et al., 2016; Al Yahya et al., 2018). *R. nervosus* is a much-branched shrub with a height of 6 feet. The leaves are

Received March 31, 2020.
Accepted August 10, 2020.

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often crowded on short striate lateral branchlets, oblong, or the upper lanceolate. The leaves (2–3 inches; length) are bright green, subacute, glabrous, and long narrow to the base, rather firm in texture (Hepper, 1987). We hypothesized that *R. nervosus* leaves meal may have a value as a phytogenic feed additive to broiler feeds because it has plenty of phytochemicals and vitamins (Al Yahya et al., 2018). In addition, *R. nervosus* has been reported to have antimicrobial properties (Getie et al., 2003; Al-Asmari et al., 2015; Al-Naqeb et al., 2015).

The time to reach slaughter age continues to shorten because of genetic improvement in broiler chickens. For example, in the Kingdom of Saudi Arabia, slaughter age is in the range of 28–35 d. This means that female broiler chickens that are marketed at 28 d of age, the starter period of 0–15 d represents over 50% of total fattening period (Leeson, 2012). The main aim of the starter diets (day 1–10) is to provide proper nutrition (ingredients, nutrients, and feed additives) to overcome the possible stresses in the early posthatch period. The first 10–15 d of age is considered a critical time of broiler chick's life. It has been found that thermoregulatory system (Nichelmann and Tzschentke, 2002) and small intestinal mucosal function (Uni et al., 1996, 1998) showed slight decreases in functions and maturation until 7–10 d of age. A significant correlation between the development of thermoregulatory function and the elevated serum triiodothyronine hormone in young broiler chicks has been found, which could enhance their ability to regulate body temperature (Snyder et al., 1991). In addition, a healthy gut microbiota has been reported to have favorable effects on the availability of essential trace elements, which has beneficial effects on thyroid hormone synthesis (Fröhlich and Wahl, 2019; Knezevic et al., 2020). It has been found that antibiotics application on day 1 of age in broiler chicks affects the microbial colonization and gut function negatively over a period of 14 d of age (Schokker et al., 2017). Moreover, initial starter diet is one of the windows to provide the alternatives nonantibiotic products to the birds (Kogut, 2019). Finally, efficiency of protein deposition in the skeletal breast and leg muscles has been reported to be higher by approximately 2.25- to 3-fold in young chickens (day 7–14) compared with older chickens (day 28–42) (Kang et al., 1985).

To our knowledge, there is no study regarding the impacts of *R. nervosus* leaves meal on livestock and poultry. The main aim of this study was to explore the bioactive components of *R. nervosus* leaves meal and evaluate its effect on growth performance, duodenal morphology, serum thyroid hormones, and cecal microflora of broiler chickens during starter period.

MATERIALS AND METHODS

Proximate Analysis of Rumex nervosus *Leaves Meal*

The Fresh mature *Rumex nervosus* leaves (5–6 cm; length) were harvested in July 2018 from the valleys and mountains surrounding the village of Bait Al-Aqra, Kuhlan, Al-Radmah district, Ibb governorate, and the Republic of Yemen at a latitude of 14.234082° North and longitude of 44.508484° East. *R. nervosus* leaves were air-dried at room temperature for 15 d, and the dried leaves were shipped to Animal Production Department, King Saud University. Upon arrival, they were pulverized and ground to a powder form (particle size; 0.25–0.30 mm) using a blender. The powder of *R. nervosus* leaves was directly analyzed and incorporated into the dietary treatments.

Moisture content of dried grounded *R. nervosus* leaves was analyzed by drying in an oven (Binder, Bohemia, NY) (method no. 930.15); total crude protein level was determined by Kjeldahl method (N X 6.25; method no. 990.03) using a 2020 Digester (Foss, Hillerød, Denmark) and a Velp UDK 140 distillation unit (Milano, Italy); crude fat content was analyzed using a Soxhlet extractor (method no. 920.39); crude fiber level was analyzed using a Dosi-Fiber (method no. 978.10); ash was determined by incinerating dried samples at 600°C for 6 h (method no. 942.05); and acid detergent fiber (method no. 973.18) based on (AOAC, 2006). Neutral detergent fiber was analyzed as described by Holst (1973). Gross energy (Kcal/kg) was analyzed by using a bomb calorimeter (6,200 Automatic Isoperibol Calorimeter; Parr instrument company, Moline, IL). All data were expressed based on a dry matter basis.

Bioactive Chemicals Analysis of Rumex nervosus *Leaves Meal*

One hundred gram of *R. nervosus* leaves meal was macerated with methanol at 25°C for 24 h and was filtered by using a filter paper and centrifuged at 4,000 × *g* for 30 min. Methanol was taken away in a rotary evaporator at 40°C, then the methanol extract was obtained by drying the samples in desiccators as described by Al-Fatimi et al. (2007). Ten microliters of filtrate was injected into high-performance liquid chromatography (**HPLC**). The HPLC system consisted of Shimadzu binary pumps LC-10AD combined with a UV-VIS detector SPD-10A (Shimadzu, Japan) and Auto-injector SIL-10A (Shimadzu). The column was a Zorbax RP-18 column (250-cm long × 4 mm i.d. and 5-μm particle diameter; Agilent, Germany) equipped with a guard column (1.0-cm long × 4.6 mm i.d.; Agilent). Gallic acid (phenol), catechin (flavonoid), chlorogenic acid (polyphenol), and caffeine (alkaloid) were used as external standards. Detection was conducted by measurement of ultraviolet absorbance at 280 nm. The mobile phase was composed of water, acetonitrile, methanol, ethyl acetate, and glacial acetic acid (89:6:1:3:1 volume). The mobile phase flow rate was 1 mL/min. All chromatographic analyses were conducted at 25°C ± 2°C.

The *R. nervosus* leaves meal extract was analyzed by gas chromatography-mass spectrometry on an Agilent (Palo Alto, CA) 6890N gas chromatograph equipped with an Agilent HP-5MS column (30 m × 0.25 mm × 0.25 μm

Table 1. Ingredients and nutrients level of the control feed.

Ingredients, kg	Day 1–21	Day 22–34
Yellow corn, 7.8%	532.18	581.40
Soybean meal, 45%	378.50	321.50
Wheat bran	20.00	22.00
Corn gluten meal	14.00	0.00
Rice bran oil	15.00	42.00
Di-calcium phosphate	19.80	16.15
Limestone	9.00	7.90
NaCl	4.00	3.00
DL-methionine	2.92	2.50
L- lysine. HCL	2.10	1.05
Choline chloride	0.50	0.50
Vitamin- mineral premix[1]	2.00	2.00
Rumex nervosus leaves[2]	0.00	0.00
Total	1,000	1,000
Nutrient content (%, based on as-fed basis)[3]		
Crude protein,	22.80	19.70
Digestible lysine	1.26	1.03
Digestible sulfur amino acids	0.91	0.80
Digestible threonine	0.77	0.67
Calcium	0.93	0.79
Nonphytate P	0.45	0.38
Metabolizable energy, kcal/kg	2,900	3,120

[1]Premix per Kg: vitamins (A, 12,000,000 IU; D3, 5,000,000 IU; E, 80,000 IU; K3, 3,200 mg; B1, 3,200 mg; B_2, 8,600 mg; B_3, 65,000 mg; B_5, 20,000 mg; B_6, 4,300 mg; biotin 220 mg; B_9, 2,200 mg; B_{12}, 17 mg); minerals: copper, 16,000 mg; iodine, 1,250 mg; iron, 20,000 mg; Mn, 120,000 mg; Se, 300 mg, and Zn, 110,000 mg.

[2]The basal diet supplemented with 0, 1,000, 3,000, and 5,000 mg/kg *Rumex nervosus* leaves (RN) on top of feed; supplemented levels were recommended based on the Maximum Ingredient level Optimization Workbook (MIOW) (Alhotan et al., 2017).

[3]Calculated according to Rostagno et al. (2011).

film thickness) and 5973N mass selective detector. The oven temperature was ramped from 60°C (2 min) to 320°C (20 min) at a rate of 6°C/min. The identification of the chemical constituents was successfully achieved by comparison of their mass spectrums, retention indices, and chemical compound quality with those of the data base library of the National Institute of Standards and Technology (NIST-based AMDIS software).

Experimental Design and Dietary Treatments

The experiment was carried out based on the guide for the care and use of agricultural animals in research and teaching American society of animal science and poultry science association (2010).

One-day-old Ross 308 broiler chickens (n = 192; 96♀ and 96 ♂) were moved from a commercial hatchery to the research unit of Animal Production Department, King Saud University, after they were vaccinated against Newcastle, infectious bronchitis diseases, and infectious bursal disease virus. Upon arrival, the chickens were weighed individually and assigned as groups to 4 treatments with 8 replicate cages of 6 broiler chickens (3♀ and 3♂) per replicate cage according to a completely randomized block design. The chickens were raised in an environmentally controlled house in a cage sized (58 cm × 50 cm × 35 cm) for length, width, and height, respectively. The temperature and relative humidity were 33°C and 65% up to 5 d of age and were lowered gradually to 24°C and 50% on 21 d of age. A photoperiod of "23-h-on and 1-h-off" was applied.

The treatments were a basal diet (control) and a control diet supplemented with 1,000, 3,000, and 5,000 mg/kg *R. nervosus* leaves meal (**RN**) on top of feed from day 1 to 34 of age. The supplemented levels were recommended in the present study based on the Maximum Ingredient level Optimization Workbook (Alhotan et al., 2017). Experimental diets were met or exceeded the National Research Council (1994) requirements as appropriate. The chickens were fed mash diets with *ad libitum* access to feed and water (nipple drinkers). Ingredients and nutrients levels are shown in Table 1.

Table 2. Proximate analysis of *Rumex nervosus* leaves meal.[1]

Item	%[2]
Dry matter	94.33
Crude protein	13.63
Ether extract	1.54
Crude fiber	8.24
Acid detergent fiber	15.48
Neutral detergent fiber	20.21
Ash	18.01
Gross energy (Kcal/kg)	3,273
Gallic acid (μg/g)[3]	700

[1]Chemical analysis conducted in duplicate.

[2]Data were expressed based on as-fed basis.

[3]Gallic acid in *Rumex nervosus* leaves meal extract was detected by using HPLC.

Growth Performance

Body weight (**BW**) was determined at 1, 7, 10, 14, 21, and 34 d of age per replicate. On day 1 of age (starter phase) and day 22 of age (grower phase), dietary treatments were divided at 32 buckets (1 bucket/replicate) and weighed individually (feed given). Diets were placed at the feeders routinely. On day 14, 21, and 34 of age, the leftover of diets at the feeder and buckets were weighed to calculate feed intake per replicate cage. Feed conversion ratio (**FCR**) was calculated as feed intake divided by the BWG (g/g).

Samplings

Birds were weighed individually before being slaughtered on day 10 of age. All the samples were taken from a single female bird per replicate (n = 8; one bird per replicate). Blood samples were collected from the brachial vein and allowed to clot, and then the serum was separated by centrifugation (3,000 × *g*, 10 min). The serum was collected and stored in Eppendorf tubes at −20°C. Birds were humanely slaughtered by using a sharp knife through a cut to the jugular vein, carotid artery, and windpipe. The empty weight of the proventriculus and gizzard and whole heart, spleen, liver, pancreas, thymus, and bursa of Fabricius were recorded. In addition, entire skinless breast meat with keel bone and whole legs were weighed and expressed as g/100 g of live BW. Moreover, 1 g from the cecal contents was

Table 3. Main volatile compounds of the *Rumex nervosus* leave meal extract.[1]

Retention time (min)	Main volatile compounds	Quality	Molecular weight (amu)	Molecular formula
4.211	Oxime-, methoxy-phenyl-_	74	151.063	$C_8H_9NO_2$
7.026	Cyclotrisiloxane, hexamethyl-	72	222.056	$C_6H_{18}O_3Si_3$
7.146	5-Methyl-2-phenylindolizine	59	207.105	$C_{15}H_{13}$ N
7.146	1,2-Bis(trimethylsilyl) benzene	50	222.126	$C_{12}H_{22}Si_2$
25.41	Hexadecanoic acid, methyl ester	98	270.256	$C_{17}H_{30}O_2$
24.41	Tridecanoic acid, methyl ester	86	228.209	$C_{14}H_{28}O_2$
24.41	Nonadecanoic acid, methyl ester	53	312.303	$C_{20}H_{40}O_2$
28.054	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	99	294.256	$C_{19}H_{34}O_2$
28.054	9,17-Octadecadienal, (Z)-	90	264.245	C18H32O
28.123	10,13-Octadecadienoic acid, methyl ester	99	294.256	C19H34O2
28.123	7-Pentadecyne	96	208.219	C15H28
28.50	Octadecanoic acid, methyl ester	98	298.287	C19H38O2

[1]The volatile compounds were identified by using a gas chromatography-mass spectrometry.

collected and stored at −80°C until processed. Last, 1 cm from the medial portions of duodenum was taken and then washed in physiological saline solution and fixed in 10% buffered formalin.

Serum Biochemical Analysis and Thyroid Hormones

Serum total protein, albumin, uric acid, aspartate aminotransferase, and alanine aminotransferase were analyzed by using Randox kits (RANDOX Laboratories Ltd., Crumlin, United Kingdom) using a microplate reader (MR-96A; Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Triiodothyronine (T3) and thyroxine (T4) were determined through analyzed serum total T3 and T4 hormones using ELISA Kit (CTK Biotech, San Diego, CA).

Duodenal Morphology

Duodenal tissues were embedded in paraffin, and 20 cross-sections 2-μm thick of each sample were cut. Each of 5 semi-serial cuts were placed on one microscopic slide and stained with Alcian blue and Periodic Acid-Schiff reagent. Ten well-oriented villi were selected to measure duodenal morphology. Villus height was measured from the tip to the villus-crypt junction of each villus. Crypt depth was calculated based on the distance from the junction to the basement membrane of the epithelial cells at the bottom of each crypt. Then, the ratio between villus height and crypt depth was calculated. The villus width was at the base area of each villus. The villus surface area was calculated based on the formula [$2\pi \times (W/2) \times L$], where W = villus width and L = villus length as described by Sakamoto et al. (2000) using a Nikon microscope (Nikon Corp, Japan) and Olympus digital video camera (DP72, Aartselaar, Belgium) with cellSens software. Goblet cells along 10 oriented villi were counted visually under the microscope using 200x magnifications.

Cecal Microflora

The cecal contents were diluted in 9 mL (1 gm/1,000) Buffered Peptone Water (Scharlau, Spain), then the tube was 10-fold serially diluted. From each tube, 0.1 mL was transferred to each different selective media. The *Lactobacillus* spp. was enumerated on the MRS agar. The media was incubated at 37°C with 5% CO_2 using anaerobic incubator (Thermo fisher Heracell150, Golden Valley, MN) for 72 h. *Escherichia coli and Salmonella* spp. were enumerated using MacConkey agar and *Salmonella* Shigella agar at 37°C for 24 h, respectively. All data were expressed in colony-forming units (**cfu**) (Log_{10} CFU/g of tissue).

Statistical Analysis

The experimental units for growth performance indices were replicate cages (8 cages per treatment). For samples, one female broiler chicken was slaughtered per replicate. Growth performance and physiological indices were analyzed statistically by a one-way ANOVA (SPSS 16; SPSS Inc., Chicago, IL). Orthogonal polynomial contrasts (linear and quadratic) were used to test the effects of graded levels of RN (0, 1,000, 3,000, and 5,000 mg/kg). Dunnett's test was used to compare means, and the differences between means were considered to be significant at $P < 0.05$.

RESULTS

The Proximate Analysis and Bioactive Components of Rumex nervosus Leaves Meal

The proximate analysis of RN leaves meal is presented in Table 2. The data reveal that RN contained appreciable amounts of dry matter (94.33%), crude fiber (8.24%), CP (13.63%), crude fat (1.54%), ash (18.01%), and gross energy (3,273 kcal/kg). The gallic acid was detected by using HPLC, and its concentration was 700 μg/g with a 3.830-min retention time, 554,308 area, and 38,945 height (Table 2). The main volatile compounds of RN extract are presented in Table 3.

Growth Performance

The effects of *R. nervosus* leaves meal (RN) on growth performance are shown in Table 4. On 10 d of age, BWG was improved ($P = 0.016$, linear trend, $P = 0.004$) with supplemental RN (1,000–5,000 mg/kg), while supplemental RN up to 3,000 mg/kg diet

Table 4. Effect of *Rumex nervosus* leaves meal (RN) on growth performance of broiler chickens from day 1 to 34 of age.[1]

	Rumex nervosus leaves meal, mg/kg					*P* value		
Item	0	1,000	3,000	5,000	SEM	RN	Linear	Quadratic
Day 1–10								
Body weight (BW)								
BW, g (Day 1)	45.67	45.73	45.63	45.77	0.08	0.344	0.433	0.597
BW, g (Day 7)	140^{b}	151^{a}	149^{a,b}	149^{a,b}	3.78	0.020	0.013	0.069
BW, g (Day 10)	224^{b}	239^{a}	240^{a}	240^{a}	5.57	0.016	0.004	0.147
Body weight gain (BWG)								
BWG, g (Day 1–7)	94^{b}	106^{a}	103^{a,b}	104^{a,b}	3.80	0.022	0.014	0.068
BWG, g (Day 1–10)	178^{b}	194^{a}	194^{a}	195^{a}	5.57	0.016	0.004	0.145
Day 1–14								
BW, g (Day 14)	372^{b}	419^{a}	411^{a}	392^{a,b}	12.20	0.003	0.055	0.001
BWG, g	327^{b}	373^{a}	366^{a}	346^{a,b}	12.23	0.003	0.056	0.001
Feed intake, g	362	357	360	359	12.59	0.982	0.813	0.781
FCR, g/g	1.11^{a}	0.96^{b}	0.98^{a,b}	1.04^{a,b}	0.04	0.016	0.088	0.006
Day 1–21								
BW, g (Day 21)	777	837	837	802	29.10	0.135	0.229	0.044
BWG, g	731	791	791	757	29.11	0.136	0.230	0.043
Feed intake, g	940	964	971	943	33.01	0.738	0.739	0.322
FCR, g/g	1.29	1.22	1.23	1.25	0.06	0.669	0.441	0.336
Day 1–34								
BW, g (Day 34)	1,839	1,900	1,861	1,861	83.11	0.903	0.826	0.566
BWG, g	1,793	1,855	1,815	1,815	83.09	0.903	0.827	0.565
Feed intake, g	2,612	2,743	2,719	2,743	61.69	0.132	0.042	0.309
FCR, g/g	1.46	1.48	1.50	1.51	0.07	0.869	0.419	0.849
Mortality, %	0	0	0	0	-	-	-	-

The superscript lowercase letters indicate significant differences ($P < 0.05$) when compared each RN level to the non-supplemented control diet.

Abbreviation: FCR, feed conversion ratio.

[1]Data are means of 8 replications with 6 chicks per replicate cage (3 male and 3 female birds per replicate cage).

increased BWG ($P = 0.003$, quadratic trend, $P = 0.001$) on 14 d of age compared with control. On day 14 of age, feeding 1,000 mg/kg RN decreased FCR compared with control (1.11 vs. 0.96, $P = 0.016$, quadratic trend $P = 0.006$). Growth performance was not changed ($P > 0.05$) by RN application on day 21 and 34 of age. All chickens were in healthy status without recording mortality.

Serum Biochemical Indices

The effects of *R. nervosus* leaves meal (RN) on serum biochemical indices are presented in Table 5. On day 10 of age, the levels of serum albumin were increased ($P < 0.001$, linear trend $P < 0.001$, quadratic trend $P = 0.017$) with supplemental RN (1,000–5,000 mg/kg). However, total protein, uric acid, aspartate aminotransferase, and alanine aminotransferase were not differed among dietary treatments.

The effects of RN leaves meal on serum thyroid hormones are presented in Figure 1. The addition of graded levels of RN leaves meal (1,000–5,000 mg/kg) increased triiodothyronine level ($P < 0.001$, linear $P = 0.001$, and quadratic $P < 0.001$).

Organs Development

The effects of RN leaves meal on organs' relative weight are presented in Table 6. The addition of RN leaves meal with 1,000, 3,000, and 5,000 mg/kg resulted

Table 5. Effect of *Rumex nervosus* leaves meal (RN) on serum biochemical indices of female birds on day 10 of age.[1]

	Rumex nervosus leaves, mg/kg					*P* value		
Items	0	1,000	3,000	5,000	SEM	RN	Linear	Quadratic
Total protein, g/dl	4.36	4.29	4.20	4.55	0.728	0.969	0.838	0.690
Albumin, g/dl	1.44^{b}	1.82^{a}	2.31^{a}	2.33^{a}	0.096	<0.001	<0.001	0.017
Uric acid, mg/dl	2.17	2.82	2.33	2.08	0.413	0.311	0.569	0.139
AST, U/L	197	222	212	204	35.97	0.909	0.947	0.523
ALT, U/L	11.4	11.4	12.8	12.5	1.138	0.496	0.200	0.854

[a-b]It indicates significant differences ($P < 0.05$) when compared each RN level to the non-supplemented control diet.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[1]Data are means of 8 chicks per treatment (1 bird/replicate).

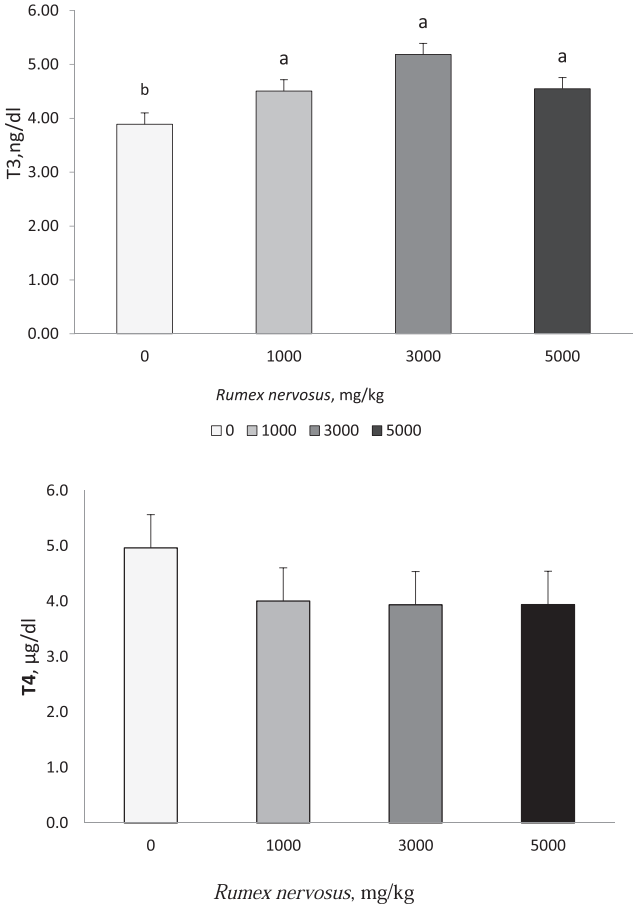

Figure 1. Effect of *Rumex nervosus* leaves meal (RN) on serum levels of triiodothyronine (T3) and thyroxine (T4) hormones of female birds on day 10 of age. [a,b]Means with different indicates significant differences ($P < 0.05$) observed in serum Triiodothyronine hormone (T3) when compared each RN level to the non-supplemented control diet.

in increased relative weight of breast meat ($P = 0.003$, linear trend $P = 0.001$, quadratic trend $P = 0.044$). Proventriculus, heart, gizzard, liver, pancreas, thymus, spleen, and bursa of fabricius were not differed among dietary treatments.

Duodenal Morphology

The effects of *R. nervosus* leaves meal (RN) on duodenal morphology are shown in Table 7. Feeding a diet with 1,000 mg/kg RN resulted in greater villus height value ($P < 0.001$, quadratic trend $P < 0.001$) than control and the diet with 5,000 mg/kg RN. Broilers fed diet supplemented with 1,000 mg/kg RN had the best ($P < 0.001$, linear trend $P = 0.003$, quadratic $P < 0.001$) duodenal villus surface area. Feeding a diet with 1,000–3,000 mg/kg RN increased ($P = 0.003$, quadratic trend $P = 0.001$) ratio of villus height to crypt depth compared with the diet with 5,000 mg/kg RN. The response of crypt depth to supplemental RN was significant ($P = 0.001$, quadratic trend $P < 0.001$) at an increasing rate, while feeding a diet with 3,000–5,000 mg/kg resulted in decreased villus width ($P < 0.001$, linear and quadratic $P < 0.001$). The addition of RN (1,000–5,000 mg/kg) resulted in increased goblet cells count ($P = 0.001$, linear and quadratic $P = 0.001$).

Cecal Microflora

The effects of *R. nervosus* leaves meal (RN) on cecal microflora are shown in Table 8. Feeding a diet with 1,000 mg/kg RN decreased ($P < 0.001$, linear and quadratic $P < 0.001$) cecal *E. coli* count compared with control and the diet with 5,000 mg/kg RN. *Salmonella* spp. count tended to increase with 5,000 mg/kg RN leaves meal ($P = 0.069$, linear $P = 0.026$). However, *Lactobacillus* spp. count was not changed among dietary treatments.

DISCUSSION

Phytogenic feed additives have been reported to have great impacts on thyroid function and goblet cells density in female mice (Panda and Kar, 1999, 2005) and female broiler chicken (Humer et al., 2015). Thus, both genders were reared to evaluate the growth performance to mimic the commercial chain, while the samples were taken from female birds.

Gallic acid (phenol), catechin (flavonoid), chlorogenic acid (polyphenol), and caffeine (alkaloid) were used as external standards in the present study. These standards are reputable as antioxidants, antimicrobials, and anti-inflammatory (Lo and Chung, 1999; Ibrahim et al., 2006; Thompson and Collins, 2013; You et al., 2014;

Table 6. Effect of *Rumex nervosus* leaves meal (RN) on relative weight of internal organs and breast and leg meat of female birds on day 10 of age.[1]

	Rumex nervosus leaves, mg/kg					*P* value		
Items (g/100 g of live BW)	0	1,000	3,000	5,000	SEM	RN	Linear	Quadratic
Proventriculus	0.91	0.94	0.80	0.90	0.06	0.169	0.443	0.510
Heart	0.70	0.71	0.73	0.67	0.03	0.372	0.405	0.177
Gizzard	3.23	3.10	3.05	3.06	0.09	0.215	0.066	0.323
Liver	2.85	3.01	2.99	3.09	0.13	0.412	0.135	0.769
Pancreas	0.45	0.47	0.41	0.43	0.04	0.602	0.504	0.943
Thymus	0.35	0.58	0.42	0.59	0.11	0.149	0.152	0.746
Spleen	0.07	0.09	0.10	0.06	0.01	0.121	0.654	0.026
Bursa of Fabricius	0.21	0.27	0.25	0.24	0.33	0.477	0.720	0.208
Breast meat	15.92[b]	18.06[a]	18.51[a]	18.51[a]	0.71	0.003	0.001	0.044
Whole legs	16.28	16.09	16.81	16.28	0.55	0.607	0.696	0.669

The superscript lowercase letters indicate significant differences ($P < 0.05$) when compared each RN level to the nonsupplemented control diet.

[1]Data are means of 8 chicks per treatment (1 bird/replicate).

Table 7. Effect of *Rumex nervosus* leaves meal (RN) on duodenal morphology of female birds on day 10 of age.[1]

Items	*Rumex nervosus* leaves, mg/kg				SEM	*P* value		
	0	1,000	3,000	5,000		RN	Linear	Quadratic
Villus height (μm)	552[b]	721[a]	646[a,b]	594[b]	35.9	<0.001	0.184	<0.001
Villus width (μm)	111[a]	110[a]	93[b]	79[b]	5.5	<0.001	<0.001	<0.001
Villus surface area, mm^2	0.19[b]	0.25[a]	0.19[b]	0.15[c]	0.013	<0.001	0.003	<0.001
Crypt depth (μm)	58.52[b]	71.70[a]	67.9[a]	72.5[a]	3.320	0.001	<0.001	0.13
Villus height/crypt depth	9.25[a,b]	10.16[a]	9.57[a]	8.13[b]	0.503	0.003	0.074	0.001
Goblet cell numbers	64.80[b]	105[a]	104[a]	100[a]	4.49	0.001	0.001	0.001

The superscript lowercase letters indicate significant differences ($P < 0.05$) when compared each RN level to the nonsupplemented control diet.

[1]Data are means of 10 well-oriented villi.

Wang et al., 2015). In the present study, only gallic acid was detected in the RN extract. This finding was supported by Desta et al. (2015) who also detected gallic acid in Ethiopian RN flowers extract. In addition, polyphenol compounds such as luteolin, acacetin, catechin, chlorogenic acid, caffeic acid, hesperetin, and quercetin have been detected in Ethiopian RN leaf and stem extracts (Desta et al., 2016). It has been pointed out that environmental factors affect the active ingredients of same species of a plant (Peñuelas and Llusià, 1997; Florou-Paneri et al., 2019).

A significant linear correlation between development of thermoregulatory function and elevated serum triiodothyronine hormone has been reported in broiler chickens (Snyder et al., 1991). In addition, a linear correlation has been observed between relative weights of the breast muscle and BW of broilers chickens and the serum triiodothyronine level (Xiao et al., 2017). In addition, they concluded that higher levels of triiodothyronine in the serum accelerate growth in chickens.

Gallic acid and other active components in the leaves of RN may have positive effects on serum albumin and triiodothyronine in the present study. For example, vitamin E has been detected in RN leaf extract (Al Yahya et al., 2018), and it is considered one of the antioxidants as gallic acid. Gallic acid can increase free triiodothyronine by modulating antioxidant status in rats (Mohamed and Abd El-Twab, 2016). In the present study, the level of serum albumin response to supplemental *R. nervosus* leaves meal was linear. Other phytogenic feed additive such as thyme has been reported to increase serum albumin (Sadek et al., 2014). One of the active compounds that have been detected in thyme extract was a gallic acid (Roby et al., 2013; Köksal et al., 2017). The binding site of gallic acid with human serum albumin and bovine serum albumin has been reported (Chanphai and Tajmir-Riahi, 2020).

The addition of *Moringa oleifera* leaves has been found to increase BW coupled with an increase in the duodenal villus height and villus surface area without affecting feed intake and FCR (Khan et al., 2017), which is a similar finding with chickens fed a diet with 1,000 mg/kg RN. One of the active compounds that have been detected in *M. oleifera* leaf extract was gallic acid (Luqman et al., 2012; El-Hadary and Ramadan, 2019). The addition of 100 mg/kg of gallic acid has been reported to increase the relative breast muscle percentage (Samuel et al., 2017). In the present study, the addition of RN (1,000–5,000 mg/kg) increased the relative weight of breast meat.

The duodenum was selected for morphology here because the villus height in the duodenum reaches a plateau between day 6 and 8 after hatch, while the villus height in the jejunum and ileum increases after day 10 of age (Choct, 2009). In addition, the duodenum is the first part of small intestine encounter microbiota that enters orally (Reynolds et al., 2020). The addition of RN (1,000–5,000 mg/kg) resulted in increased goblet cells counts. In the present study, an obvious increase in crypt depth was detected in all groups. The increase in crypt depth may be favorable because of an increase in the number of proliferating stem cells, which could maximize the count of mucin-producing goblet cells (Hutsko et al., 2016).

Feeding a diet with 5,000 mg/kg RN decreased duodenal villus height, villus width, villus surface area, and villus height-to-crypt depth ratio. This finding may be due to irritation of intestinal tissues

Table 8. Effect of *Rumex nervosus* leaves meal (RN) on cecal microflora of female birds on day 10 of age.[1]

Items	*Rumex nervosus* leaves, mg/kg				SEM	*P* value		
	0	1,000	3,000	5,000		RN	Linear	Quadratic
Lactobacillus Spp.	6.64	5.93	5.97	6.15	0.354	0.244	0.235	0.113
E. coli	7.32[b]	4.96[c]	5.99[b,c]	11.16[a]	0.534	<0.001	<0.001	<0.001
Salmonella Spp.	3.39	3.06	4.21	4.40	0.485	0.069	0.026	0.470

The superscript lowercase letters indicate significant differences ($P < 0.05$) when compared each RN level to the nonsupplemented control diet.

[1]Data are means of 8 chicks per treatment (1 bird/replicate).

(Windisch et al., 2009; adapted from Zeng et al., 2015). In addition, although feeding a diet with 5,000 mg/kg RN increased cecal *E. coli* count, all chickens were in healthy status without recording mortality. Moreover, BW and feed intake were not decreased in chickens fed a diet with 5,000 mg/kg RN. Reduced growth performance is a sign of pathological gut inflammation (Roura et al., 1992; Gaskins, 2008). In addition, it has been pointed out that changes in large intestine microbial populations have less effect on animal growth (Gaskins, 2008) than microbiota in the small intestine because of their competitiveness for nutrients (energy and amino acids) with the host (Hedde and Lindsey, 1986). Moreover, *E. coli* are considered a part of the cecal microbiome and can become a pathogenic factor because of stress, poor welfare, host factors, or a secondary infection (Wigley, 2015). In addition, *E. coli* may function as a reservoir for triiodothyronine by binding it to bacterial thyroid-binding hormone (Salvatore et al., 1963; adapted from; Fröhlich and Wahl, 2019). Here, the addition of RN (1,000–5,000 mg/kg) resulted in elevated serum triiodothyronine levels. Therefore, shorter villus and BW of broiler chickens maybe is not highly correlated during starter phase and vice versa. For example, an improvement of feed intake and villus height has been observed, without affecting BWG in broiler chickens (Santos dos et al., 2019). In addition, it has been reported that villus height tended ($P = 0.073$) to be shorter without affecting feed intake and BWG in piglets fed formic acid (Manzanilla et al., 2004). It seems plausible to measure the concentration of cecal volatile fatty acid in the future study to evaluate the protective effect of RN on *E. coli* infection.

Feeding a diet with 1,000 mg/kg RN resulted in greater duodenal villus height, villus width, and villus surface area and lower count of cecal *salmonella* and *E. coli*. Oxime-, methoxy-phenyl-, and hexadecanoic acid, methyl ester was identified in the RN extract, which has an antimicrobial activity (Hema et al., 2011; Harini et al., 2014). Recently, it has been found that a mixture of *Potentilla anserine*, *Polygonum aviculare*, and *Rumex crispus* extracts decreased the count of cecal *E. coli* (Kupczyński et al., 2019). It has been pointed out that *R. nervosus* may have identical biological activities to other species of *Rumex* (Desta et al.,2015).

CONCLUSIONS

The demand for natural phytogenic feed additives will be greater when antibiotics are completely banned. *R. nervosus* leaves meal could be considered as a phytogenic feed additive up to a 1,000 mg/kg inclusion rate in broiler diets because of its combined positive effects on BWG, FCR, villus height, villus surface area, serum albumin and triiodothyronine hormone, and cecal *E. coli* during the starter period. Further study is required to elucidate its molecular mechanism.

ACKNOWLEDGMENTS

The authors extend their acknowledgment to the Deanship of Scientific Research at King Saud University, Saudi Arabia, for funding this work through research group no. RG-1440-146.

Conflicts of Interest Statement: The authors did not provide a conflict of interest statement.

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