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EFFECTS OF DIETARY SUPPLEMENTATION OF *CATHARANTHUS ROSEUS* ON GROWTH PERFORMANCE AND INTESTINAL MICROARCHITECTURE IN BROILER CHICKENS

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ABSTRACT

The objective of the present study was to examine the effects of *Catharanthus roseus* (*C. roseus*) extract (leaves and roots) on growth performance and microarchitecture of the small intestine in Cobb 500 broilers. One hundred and seventy-five, 1-day-old chicks were randomly divided into seven treatment groups (n = 25/group) with five replicates (n = 5/replicate). Birds in group 1 served as a control and were offered a corn soybean-based basal diet only. The birds in groups 2, 3, and 4 were fed the same diet supplemented with 0.05%, 0.1%, and 0.2% aqueous leaf extracts of *C. roseus*. Whereas, groups 5, 6, and 7 were provided the diet supplemented with 0.05%, 0.1%, and 0.2% aqueous root extracts of *C. roseus*, respectively, for 35 days. Supplementation of *C. roseus* extracts did not improve body weights, weight gain, feed intake, and feed conversion ratio. Relative weights of viscera remained unchanged except that of gizzard and pancreas which were lower ($P < 0.05$) in 0.1% ALE supplemented birds. The microarchitectural attributes including villus height, width, surface area, crypt depth, and the ratio of villus height to crypt depth were higher ($P < 0.05$) in the 0.05% ALE supplemented group. In conclusion supplementation of *C. roseus* extracts did not improve growth performance but improved gut histomorphometric parameters of the small intestine in broilers.

Keywords: Phytobiotics, Poultry, Feed additive, Growth Performance, Intestinal Morphometry

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INTRODUCTION

The status of the intestinal tract, harboring a number of bacteria, is one of the key factors essential for the health and performance of birds (Rehman *et al.,* 2008). Previously, poultry feed was supplemented with sub-therapeutic antibiotics with an aim to modify the intestinal microbiota. However, the European Union (EU) banned the use of antibiotics as growth promoters (AGPs) in poultry and livestock in 2006 due to the transmission and proliferation of resistant bacteria. The ban in the EU and other countries prompted the search for alternatives to AGPs that must be natural and safe for animal production (Amad *et al.,* 2011). The potential candidates include prebiotics, probiotics, and organic acids. A body of evidence demonstrates that phytogenic feed additives may also be included as a new member in the class of non-antibiotic growth promoters (Windisch *et al.,* 2009). Phytobiotics are defined as compounds derived from plants and incorporated into diets that are claimed to increase the production performance of animals. The term "phytobiotic compounds" may refer either to their utilized parts and/or to their respective plant extracts. Dietary plant extract supplementation improves the performance of broilers (Windisch *et al.,* 2009). On the contrary, others have reported no effect on broiler performance (Hernandez *et al.,* 2004). It was reported that phytobiotic feed additives stimulate secretion of intestinal mucus in broilers thus impairing the adhesion of the pathogens to the intestinal epithelium (Vidanarachchi *et al., 2005)*. Phytobiotics are also believed to exhibit strong antimicrobial properties and antioxidant activities (Hazzit *et al.,* 2006).

Various aromatic plants, spices, and fruits constitute possible phytogenic sources. *Catharanthus roseus* (*C. roseus*), an ornamental sub-shrub also recognized as *Vinca rosea*, Sadabahar, (family *Apocynaceae)* is one possible source. The phytochemical analysis showed that *C. roseus* contains flavonoids, alkaloids, terpenoids, and saponins. *C. roseus* exhibits several therapeutic properties, like antifungal (Jaleel *et al.,* 2007), antibacterial, antihyperlipidemic, and antihyperglycemic (Renjini *et al*., 2017). The antioxidant property of *C. roseus* (Zheng and Wong, 2001) has been reported to be due to anthocyanins (flavonoids) which prevent lipid peroxidation by scavenging the reactive oxygen species (ROS). Various researchers have reported the antimicrobial and immunomodulatory effects of leaf

extracts of *C. roseus* against a wide range of pathogenic microorganisms due to the presence of indole alkaloids and some phenolic compounds (Renjini *et al*., 2017). On the basis of the abovementioned properties of the *C. roseus*, it is hypothesized that aqueous extracts of the leaf and root may be an alternative to the AGPs and be used to enhance the production potential of broilers. The present study was thus planned to evaluate the potential role of using aqueous extracts of leaf and roots of *C. roseus* as an alternative to the AGPs on the production performance, organs development, and gut micro-architecture in broilers.

MATERIALS AND METHODS

Plant material: *Catharanthus roseus* plants were collected from the University of Veterinary and Animal Sciences (UVAS), Lahore-Pakistan. For authentication, a sample of plant specimen was submitted to the Government College University, Lahore-Pakistan, and the catalog number obtained was GC-Herb-Bot-3415.

Preparation of plant extracts

Aqueous extract preparation: The leaves and roots of the plant were collected, washed, and then shade-dried. The dried plant material was weighed using an electronic weighing balance and ground into fine powder form. Aqueous extracts of leaves and roots were prepared by using a Soxhlet apparatus (50/42 PYREX QUICKFIT® UK EX5/83 B.S.2071 24/29) by following the standard protocol (Vogel, 1978) with little modifications. Fifty grams of the dried powdered sample of roots or leaves were taken in the inner tube of the soxhlet apparatus and 500 ml of water was placed in the round bottom flask of the apparatus. The apparatus was run for 24 hours. The extract obtained in the flask was filtered by using filter paper (No. 1) and the filtrate was concentrated by using a rotary evaporator (Eyela® rotary vacuum evaporator), and placed in a hot air oven at 40°C to obtain a water-free residual extract of *C. roseus*.

Experimental protocol

Animals, housings, and diets: All the study procedures were approved by the Ethical Committee of UVAS, Lahore-Pakistan, with vide letter no. DR/1944 dated 4/12/2018.

Table 1. Ingredient (%) and nutrient composition of the basal diet.

Ingredients	Units	Starter Feed	Grower Feed
Maize	%	56.49	59.29
Soybean meal (48%)	%	33.80	29.80
Soy oil	%	3.70	5.00
Sunflower oil	%	1.50	1.50
Calcium carbonate	%	1.48	1.48
Monocalcium phosphate	%	1.42	1.34
Premix[1]	%	1.20	1.20
Dl-Methionine	%	0.26	0.26
L-Lysine-HCL	%	0.13	0.12
L-Threonine	%	0.02	0.02
l-Tryptophan	%	-	0.02
Nutrient composition			
ME_N	**MJ/kg**	12.62	13.05
Crude protein	g/kg	22.20	20.50
Lysine	g/kg	1.30	1.17
Methionine	g/kg	0.59	0.57
Methionine+cysteine	g/kg	0.96	0.90
Threonine	g/kg	0.88	0.81
Tryptophan	g/kg	0.24	0.22
Crude fiber	g/kg	2.35	2.29
Crude fat	g/kg	9.71	9.87
Calcium	g/kg	0.90	0.88
Phosphorus	g/kg	0.70	0.66
Sodium	g/kg	1.60	1.60

[1]Contents per kg Premix: 400000 U Vit. A; 40000 U Vit. B_1; 250 mg Vit. B_2; 2500 mg Vit. B_6; 2000 µg Vit. B_{12}; 25000 µg Vit. D_3; 8000 mg Vit. E (α-Tocopherole acetate); 300 mg Vit. K_3; 250 mg Nicotinic acid; 400 mg Biotin; 1000 mg calcium pantothenate acid; 100 mg Choline chloride; 5000 mg Folic acid; 80000 mg Zn (Zinc oxide); 2000 mg Mn (Manganese oxide); 1200 mg Fe (Iron carbonate); 6000 mg Cu (Copper sulfate-pentahydrate); 45 mg Co (Cobalt- (II)-sulfate-heptahydrate); 35 mg J (Calcium jodate); 30 mg Se (Sodium selenite); 130 g Mg (Magnesium oxide) Na (Sodium chloride); 55 g.

Day-old chicks (n=175) (Cobb 500) were obtained from a hatchery (Big-birds Pvt. Ltd., Lahore) and placed in a controlled shed at the UVAS-Pattoki campus. Wood shaved litter was used as bedding material for birds. The birds were weighed on day 1 and randomly allocated into seven experimental groups (n = 25), with five replicates in each group and five birds (n = 5) per replicate. The temperature was gradually reduced from 35°C during the first week, i.e., brooding period and reduced to 3°C each week to approximately 26°C from the 4th week till the end of the experiment while relative humidity was maintained at 65 ± 5%, according to standard management practice. Chicks in group 1 were supplemented by antimicrobials and coccidiostats-free corn-soybean-based basal diet as shown in Table 1 (Yousaf *et al.,* 2016). The birds in groups 2, 3, and 4 were provided diets supplemented with the three different levels of aqueous leaf extracts **(ALE)** of *C. roseus,* i.e., 0.05%, 0.1%, and 0.2%, respectively, in the basal diet. Similarly, groups 5, 6, and 7 were offered basal diets supplemented with 0.05%, 0.1%, and 0.2% of aqueous root extracts **(ARE)** of the *C. roseus*, respectively. *Ad libitum* feed and water were available for all chicks for 35 days experimental period.

Growth performance attributes: The average body weight (BW) and weight gain (BWG) of birds in each replicate were taken on day one and then weekly till the end of the experimental period. Average feed consumption (FC) and mortality of each replicate were noted weekly and also on a daily basis. The feed conversion ratio (FCR) was calculated by using BW and FC on a weekly basis.

Viscera development: On day 35, 10 birds from each group (2 birds/replicate) were selected on a random basis, weighed, and slaughtered through exsanguination. After slaughtering, the bird's abdominal cavity was opened for the collection of viscera. The weights of the viscera including liver, gizzard, proventriculus, heart, spleen, pancreas, bursa, small intestine, and caeca were taken and the length of the ceca and small intestine were measured. Relative weights of viscera were calculated by the respective absolute body weight of the bird.

Intestinal morphometric attributes: After slaughtering, the whole intestinal tract was removed and before removal of the content, different parts of the small intestine including the duodenum (the segment encompassing the duodenal loop), ileum (distal part of the small intestine present before the ileo-caecal junction equal to the length of the caecum) and Jejunum (part of the small intestine between the duodenum and ileum) were separated (n = 10 birds/group). To study the morphology of the small intestine, 2-cm fragments from the duodenum, jejunum, and ileum were collected. The ingesta was washed with normal saline (0.9%) and the tissue samples were fixed in 10% neutral buffered formalin solution for 24 hours. The paraffin embedding technique was used to process the tissues for light microscopy and 4-5µm tissue sections were obtained. The sections were then processed and stained with Hematoxylin and Eosin (H & E) staining technique for morphometrical studies. For histomorphometry, the software "Prog Res®2.1.1 Capture Prog Camera Contro" was used. Five villi per slide were taken from the tissue based on intact lamina propria to measure gastrointestinal morphometric variables including villus height (VH), villus width (VW), crypt depth (CD), villus height to crypt depth ratio (VH:CD), villi surface area (VSA), lamina propria thickness (LPT), muscularis mucosae (MMT), and muscularis externa thickness (MET) using a microscope (LABOMED® USA). The VH (µm) was calculated from the top of each villus to LP and VW was taken from its center. CD was measured from the depth of the invagination between neighboring villi. The thickness of lamina propria was measured from the villus base to muscularis mucosa. MM and ME thickness were measured across the villus width. The formula used to calculate the villus surface area (VSA) was as follows:

$$VSA = (2\pi) \times (VW/2) \times (VH).$$

Statistical analysis: The data were analyzed through one-way analysis of variance (ANOVA), using SPSS software version 20.0 (SPSS Inc. Chicago, IL, USA). The normality of data was checked by the Shapiro-Wilk test. Differences in means were considered significant at $P < 0.05$. Group differences were compared by Tukey's posthoc test in cases of significant F-values and results were presented as Means ± S.E.M.

RESULTS

Growth performance attributes: All the birds were healthy during the entire experimental period. The performance data for broiler chickens are summarized in tables 2-5. The supplementation of aqueous leaf and root extracts of *C. roseus* had non-significant ($P > 0.05$) effects on the BW, BWG, FC, and FCR among all treatment groups when compared to the control birds.

Viscera development: The relative organ weights (ROW) of chicks have been presented in Table 6. ROW of gizzard and pancreas were lower ($P < 0.05$) in the 0.1% ALE supplemented group compared with the control birds. The relative length of the small intestine was lower in the 0.05% ALE and 0.05% ARE supplemented groups compared with the control birds. However, the ROW of the liver, proventriculus, heart, spleen, bursa, ceca, and small intestine and relative lengths of caecum remained unchanged.

Table 2. Effect of *C. roseus* aqueous extracts (leaf and root) on mean body weight (g) in broilers (n=25/group).

Groups	1st Week	2nd Week	3rd Week	4th Week	5th Week
Control	110±3	303±18	594±23	1035±33	1542±36
0.05% ALE	115±3	315± 27	622±25	1119±28	1669±38
0.1% ALE	109±1	303±14	607±6	1105±22	1677±29
0.2% ALE	106.±2	284±25	588±29	1093±56	1646±89
0.05% ARE	109±4	297±20	578±25	1073±41	1603±44
0.1% ARE	116±2	307±21	592±7	1056±19	1585±31
0.2% ARE	114±2	305±9	583±20	1068±21	1581±44

Values are shown as mean±SEM.

Table 3. Effect of *C. roseus* aqueous extracts (leaf and root) on mean body weight gain (g) in broilers (n=25/group).

Groups	1st Week	2nd Week	3rd Week	4th Week	5th Week
Control	76 ±4	193 ±18	291 ±17	441 ±12	508 ±23
0.05% ALE	82 ±3	200 ±24	307 ±6	497 ±10	550 ±13
0.1% ALE	76 ±1	194 ±14	304 ±18	497 ±23	572 ±14
0.2% ALE	73 ±2	179 ±25	303 ±25	505 ±36	554 ±39
0.05% ARE	76 ±3	189 ±20	281 ±28	495 ±21	530 ±11
0.1% ARE	82 ±2	191 ±21	285 ±26	464 ±15	529 ±15
0.2% ARE	82 ±2	191 ±70	278 ±21	486 ±20	512 ±25

Values are shown as mean±SEM.

Table 4. Effect of *C. roseus* aqueous extracts (leaf and root) on feed consumption (g/bird) in broilers (n=25/group).

Groups	1st Week	2nd Week	3rd Week	4th Week	5th Week
Control	126 ±2	371 ±15	499 ±10	729 ±13	914 ±44
0.05% ALE	127 ±3	357 ±11	492 ±14	744 ±20	957 ±42
0.1% ALE	132 ±2	357 ±15	475 ±18	736 ±5	977 ±16
0.2% ALE	124 ±1	375 ±21	487 ±14	755 ±27	966 ±56
0.05% ARE	131±3	386 ±16	479 ±3	740 ±16	959 ±18
0.1% ARE	127±1	367 ±16	489 ±13	745 ±22	934 ±12
0.2% ARE	127±1	399 ±16	477 ±13	708 ±11	950 ±24

Values are shown as mean±SEM.

Table 5. Effect of *C. roseus* aqueous extracts (leaf and root) on feed conversion ratio (FCR) in broilers (n=25/group).

Groups	1st Week	2nd Week	3rd Week	4th Week	5th Week
Control	1.66 ±0.10	1.96 ±0.17	1.73 ± 0.11	1.65 ± 0.03	1.80 ±0.06
0.05% ALE	1.55 ± 0.06	1.84 ±0.15	1.60 ±0.06	1.50 ± 0.05	1.73 ±0.06
0.1% ALE	1.73 ± 0.02	1.87 ±0.15	1.59 ± 0.15	1.48 ±0.06	1.71 ±0.05
0.2% ALE	1.71 ± 0.05	2.17 ±0.17	1.63 ±0.12	1.50 ±0.05	1.74 ±0.03
0.05% ARE	1.73 ±0.07	2.09 ±0.17	1.76 ±0.19	1.50 ±0.07	1.81 ±0.02
0.1% ARE	1.54 ±0.05	1.96 ±0.13	1.78 ±0.24	1.60 ±0.06	1.76 ±0.03
0.2% ARE	1.55 ±0.03	2.09 ±0.05	1.74 ±0.11	1.46 ±0.05	1.86 ±0.06

Values are shown as mean±SEM.

Histomorphometry of small intestine

Duodenum: In the duodenum VH was higher ($P < 0.05$) in 0.05% ALE, 0.1% ARE, and 0.2% ARE supplemented groups compared with the control animals. VW was non-significantly higher in the 0.05% ALE and 0.1% ARE groups but significantly lower ($P < 0.05$) in the 0.2% ALE supplemented group when compared with the control group. The CD was deeper ($P < 0.05$) in the 0.05% ALE group than the control birds. The VH:CD was higher ($P < 0.05$) in all treated groups compared to the control birds. The highest VH:CD was observed in

the 0.1% ALE group. The highest VSA ($P < 0.05$) was observed in the 0.05% ALE group compared with all treated groups. Lamina propria thickness (LPT) was lower in the 0.2% ALE supplemented group compared with the control group. MMT was lower ($P < 0.05$) in groups supplemented with 0.1% ALE, 0.2% ALE, 0.05% ARE, and 0.2% ARE compared with the control group. Similarly, the MET was higher in groups supplemented with 0.05% ALE but lower in all other groups compared to the control. The findings are presented in table 7.

Table 6. Effect of *C. roseus* aqueous extracts (leaf and root) on organ characteristics (ratio ± SE) in broilers (n=25/group).

ORGANS	TREATMENT GROUPS						
	Control	0.05%ALE	0.1%ALE	0.2%ALE	0.05%ARE	0.1%ARE	0.2%ARE
	RELATIVE ORGAN WEIGHTS (%)						
Liver	2.35± 0.11	2.27±0.05	2.30±0.11	2.40±0.13	3.37±0.07	2.29±0.07	2.28±0.05
Gizzard	1.25±0.05^{a}	1.23±0.06^{a}	0.99±0.03^{b}	1.26±0.07^{a}	1.13±0.06^{ab}	1.10±0.06^{ab}	1.14±0.05^{ab}
Proventriculus	0.35±0.01^{ab}	0.33±0.01^{ab}	0.30±0.01^{b}	0.38±0.02^{a}	0.39±0.02^{a}	0.34±0.02^{ab}	0.38±0.01^{a}
Heart	0.45±0.02	0.46±0.02	0.42±0.02	0.43±0.02	0.47±0.02	0.41±0.02	0.44±0.01
Spleen	0.10±0.01	0.13±0.01	0.10±0.01	0.10±0.01	0.12±0.01	0.09±0.01	0.12±0.00
Pancreas	0.22±0.01^{a}	0.20±0.01^{ab}	0.18±0.01^{b}	0.22±0.01^{ab}	0.21±0.01^{ab}	0.24±0.01^{a}	0.20±0.01^{ab}
Bursa	0.13±0.02	0.13±0.00	0.13±0.01	0.12±0.01	0.15±0.01	0.14±0.01	0.13±0.02
Small Intestine	2.16±0.11	2.05±0.06	2.18±0.11	2.06±0.09	2.17±0.14	2.03±0.11	2.20±0.19
Caeca	0.28±0.02	0.26±0.02	0.25±0.02	0.26±0.01	0.25±0.01	0.24±0.01	0.30±0.02
	LENGTH						
Small Intestine	4.07 ± 0.14^{a}	3.51 ± 0.11^{b}	3.58 ± 0.12^{ab}	3.56 ±0.08^{ab}	3.47 ±0.12^{b}	3.77 ±0.16^{ab}	3.59 ±0.08^{ab}
Caeca	0.78 ±0.03	0.71 ±0.04	0.70 ± 0.03	0.71 ±0.02	0.75 ± 0.03	0.72 ±0.04	0.69 ±0.03

$^{a–b}$within the same row, means with different superscripts are significantly different ($P < 0.05$).

Table 7. Effect of *C. roseus* aqueous extracts (leaf and root) on histomorphometry of duodenum in 35-day old broilers.

Parameters	TREATMENT GROUPS						
	Control	0.05%ALE	0.1%ALE	0.2%ALE	0.05%ARE	0.1%ARE	0.2%ARE
VH (µm)	1277±103^{b}	2003±76^{a}	1426±45^{b}	1007±32^{c}	1242±36^{bc}	1401±60^{a}	1326±12^{a}
VW (µm)	159 ± 17^{ab}	182 ±18^{a}	136 ± 5.8^{abc}	101±6.3^{c}	119±9.1^{bc}	174±16.4^{a}	110± 3.9^{bc}
CD (µm)	238±10^{b}	342±22^{a}	157±5^{cd}	151±8^{d}	163±5^{cd}	204±13^{bc}	162±1^{cd}
VH:CD	5.31±0.34^{d}	5.99±0.29^{cd}	9.14±0.32^{a}	6.77±0.3^{bc}	7.67±0.36^{b}	7.1±0.49^{bc}	8.15±0.14^{ab}
VSA (mm^2)	664 ± 108^{b}	1162 ± 137^{a}	611± 38^{bc}	324 ± 33^{c}	468 ± 43^{bc}	775± 88^{b}	458± 15^{bc}
LPT (µm)	126 ± 15^{ab}	152±18^{a}	101±5^{bc}	76±5^{c}	92±9^{bc}	126± 13^{ab}	83± 4^{bc}
MMT (µm)	27±2^{a}	24 ±1^{a}	19±1^{b}	18 ±1^{b}	17 ±1^{b}	24±1^{a}	16 ±1^{b}
MET (µm)	145±8^{b}	187±17^{a}	93±4^{c}	98±4^{c}	93±5^{c}	115± 11^{bc}	90±3^{c}

$^{a–d}$within the same row, means with different superscripts are significantly different ($P<0.05$). Values are shown as mean±SEM.

Jejunum: In the jejunum, VH was higher ($P < 0.05$) in the 0.05% ALE, 0.1% ALE, 0.2% ALE, and 0.1% ARE supplemented groups compared with the control group. VW and CD were non-significant in all the supplemented groups compared to the control. The ratio of VH:CD was higher ($P < 0.05$) in the 0.1% ALE, 0.1% ARE, and 0.2% ARE supplemented groups compared to the control birds. VSA and LPT remained unchanged in all the groups. MMT and MET were lower ($P < 0.05$) in groups supplemented with 0.2% ARE and 0.1% ALE compared with the control group (Table 8).

Ileum: In the ileum, VH and CD were higher ($P < 0.05$) in the 0.2% ALE and 0.05% ARE supplemented groups compared with the control group. VW was higher ($P < 0.05$) in the 0.05% ALE supplemented group compared with the control birds. VH:CD ratio was similar in all the groups. The highest VSA ($P < 0.05$) was observed in the 0.05% ARE followed by the 0.2% ALE group. LPT was higher ($P < 0.05$) in the 0.05% ARE group but was lower in the 0.05% ALE supplemented group than the control group. The MMT and MET were non-significant in all the groups. The summary of the results has been presented in Table 9.

DISCUSSION

Phytobiotics are plant-derived products and are well-known for their pharmacological effects. They possess a broad variety of activities in both poultry and livestock nutrition that include the stimulation of feed intake, coccidiostatic, antimicrobial, antihelmintic, and immunostimulating (Grashorn, 2010). Phytobiotics have been gaining interest as natural growth promoters in broiler production for a few years (Mohammadi & Kim, 2018). The purpose of the present study was to evaluate the efficacy of aqueous extracts of leaves and roots of *C. roseus* on performance, organ development, and intestinal microarchitecture in broilers.

The body weights and body weight gains of Cobb 500 broilers during the whole trial were not significantly different between the control and treated groups. These findings are in accordance with those of other researchers (Hernandez *et al.,* 2004; Paguia *et al.,* 2014) who concluded that supplementation of plant extracts had no beneficial effects on the growth performance in broilers. Contrary to our findings, Akhouri *et al.* (2013) and Ayssiwede *et al.* (2011) reported an increase in the body weights of Vencobb broiler chicks supplemented with an aqueous extract of plant (*Moringa oleifera*) leaf. Similarly, El-katcha *et al.* (2016) reported that the addition of garlic extract (0.4 mg/kg) decreased the final body weight of the broilers non-significantly by around 1.13 % when compared with the control broilers.

Table 8. Effect of *C. roseus* aqueous extracts (leaf and root) on histomorphometry of jejunum in 35-day old broilers.

Parameters	TREATMENT GROUPS						
	Control	0.5%ALE	0.1%ALE	0.2%ALE	0.05%ARE	0.1%ARE	0.2%ARE
VH (µm)	540±49^{b}	866±44^{a}	746±33^{a}	806±14^{a}	738±84^{ab}	813±31^{a}	668±36^{ab}
VW (µm)	148±6^{ab}	169±14^{ab}	169±14^{ab}	184±23^{a}	197± 34.28^{a}	103±7.61^{b}	139±6^{ab}
CD (µm)	183±14^{abc}	205±19^{ab}	127±8^{c}	218±9^{a}	176±29^{abc}	161±8^{abc}	145±12^{ab}
VH:CD	2.98±0.21^{c}	4.5±0.44^{bc}	6.18±0.64^{a}	3.75±0.17^{bc}	4.48±0.29^{bc}	5.1±0.19^{ab}	4.8±0.45^{ab}
VSA (mm^2)	255± 29^{a}	465 ± 51^{a}	394 ± 31^{a}	471 ± 65^{a}	525 ± 152^{a}	264 ± 25^{a}	295± 24^{a}
LPT (µm)	123±7^{ab}	113 ±9^{ab}	142 ±12^{a}	143 ± 20^{a}	141 ± 24^{a}	74±6^{b}	104 ± 6^{ab}
MMT (µm)	30±3^{a}	26 ±2^{abc}	23 ±1^{bc}	26±1^{abc}	25±1^{abc}	30 ±1^{ab}	21 ±1^{c}
MET (µm)	159±24^{a}	154.6±12^{ab}	102 ± 7^{bc}	107 ±5^{abc}	114 ± 14^{abc}	119 ±13^{abc}	89 ± 4^{c}

$^{a–c}$within the same row, means with different superscripts are significantly different ($P < 0.05$). Values are shown as mean±SEM.

Table 9. Effect of *C. roseus* aqueous extracts (leaf and root) on histomorphometry of ileum in 35-day old broilers.

Parameters	TREATMENT GROUPS						
	Control	0.05%ALE	0.1%ALE	0.2%ALE	0.05%ARE	0.1%ARE	0.2%ARE
VH (µm)	451±25^{b}	456±56^{b}	431±19^{b}	638±18^{a}	611±48^{a}	455±23^{b}	544±37^{ab}
VW (µm)	100± 9^{cd}	81 ±8^{d}	168 ±11^{ab}	175 ±13^{ab}	216 ±19^{a}	144 ±7^{bc}	154 ±8^{b}
CD (µm)	124±9^{b}	121±12^{b}	146±11^{ab}	208±22^{a}	197±25^{a}	150±6^{ab}	143±12^{ab}
VH:CD	3.94±0.51	3.77±0.32	3.04±0.17	3.54±0.56	3.44±0.37	3.08±0.24	3.89±0.18
VSA (mm^2)	146± 22^{cd}	117 ±17^{d}	224 ±12^{cd}	355 ± 31^{ab}	435 ± 63^{a}	203 ±10^{cd}	259 ±17^{bc}
LPT (µm)	91±10^{b}	44 ±7^{c}	127 ±10^{ab}	135 ±12^{ab}	169 ±17^{a}	110 ±7^{b}	117±8^{b}
MMT (µm)	32 ±3	26 ±2	24 ±1	27.5 ±4	32 ±4	28 ±2	26 ±1
MET (µm)	193 ±23	177±28	168 ±10	170 ±37	185 ±30	184 ±13	179 ±17

$^{a–d}$within the same row, means with different superscripts are significantly different (P<0.05). Values are shown as mean±SEM. CR represents *Catharanthus roseus*, ALE (Aqueous leaf extract), ARE (Aqueous root extract), VH (Villus height), VW (Villus Width), CD (Crypt Depth), VH:CD (Villus/ Crypt ration), VSA (Villus Surface Area), LPT (Lamina Propria thickness), MMT (Muscularis mucosae), MET (Muscularisexterna thickness)

The feed consumption (FC) of all the groups, including the dietary aqueous leaf and root extracts of *C. roseus* remained unchanged throughout the trial. These results are in agreement with Oleforuh-Okoleh*et al.* (2015) who reported that supplementation of bitter leaf (*Vernoniaamygdalina*) has no effect on feed intake in broilers. Windisch *et al.* (2009) investigated the effects of phytobiotics supplementation and concluded that on average phytobiotics supplementation, like plant extracts, reduces feed intake by 2.1% (-8% to +3%), improves feed conversion ratio by 3.4% (-17% to +2%) without affecting body weights (-8% to +14%) in broilers. Similarly, Chauhan *et al.* (2012) reported that *C. roseus* has no effect on feed intake in rodents. Supplementation

with *C. roseus* causes dose-dependent effects in different species. During stressful conditions, the effect of phytobiotic supplementation is enhanced compared to the corresponding effect in a controlled environment.

The Feed conversion ratio (FCR) remained the same in all the supplemented groups compared to the control birds. Habibi & Firouzi (2017) reported that the supplementation of phytobiotics (*E. purpurea*) did not improve FCR. FCR was unaffected in broilers supplemented with *Moringa oleifera* as a phytobiotic feed additive (Paguia *et al.,* 2014). However, the absence of a positive effect of *C. roseus* extracts may be due to using a low dose which was insufficient to induce its effect on poultry.

The current study revealed that relative weights of gizzard and pancreas were lower ($P < 0.05$) in the 0.1% ALE supplemented birds. Qorbanpour *et al.* (2018) reported that supplementation of phytobiotics (*Zingiber officinale*) decreased gizzard weight. The relative length of the small intestine was lower in the 0.05% ALE and 0.05% ARE supplemented groups compared with the control birds. However, relative weights of liver, proventriculus, heart, spleen, bursa, small intestine, and ceca and relative length of caecum remained unchanged. Many studies have reported that essential oils, herbal extracts, and powders when supplemented, do not affect the characteristics of viscera (Hernandez *et al.,* 2004; Demir *et al.,* 2008). Early development of the gut and other organs such as the heart, liver, and skeleton are very important to supply nutrients to the body and promote growth performance (Huber, 2018). In poultry, the liver is the primary site of fatty acid synthesis, thus efficiency in the protein turnover and energy utilization across treatments may be attributed to its health status.

The small intestine is involved in the absorption of nutrients, so its development is important for broiler performance (Attia *et al.,* 2017). Larger villi result in rapid growth rates in broilers. To support the nutritional effects on gastrointestinal physiology, measures of villus condition are commonly undertaken. However, it is documented many times that villus height or crypt depth shows significant positive correlations in live performance improvements (Lilburn and Loeffler, 2015). In the current study, the VH in the duodenum and jejunum was higher in the 0.05% ALE group whereas, in the ileum, higher VH was observed in the 0.2% ALE supplemented birds as compared with the control birds. VW in the ileum was higher in the 0.05% ALE supplemented group. Increased VH is an indicator that the surface area available for nutrient absorption has also been increased (Attia *et al.,* 2017). Longer villi are related to active cell mitosis by which the absorptive potential of villi is increased for nutrients (Samanya *et al.,* 2002). Current results are in accordance with the study of others (Hashemi *et al.,* 2014; Nkukwana *et al.,* 2014) who reported that herbal plant supplementation causes an increase in VH and VSA. Ganguly (2013) has reported that the products derived from plants increased the height of villi in the small intestine and reduced the harmful bacteria. It was reported that phytobiotic feed additives stimulate secretion of intestinal mucus in broilers thus impairing the adhesion of pathogens to the intestinal epithelium (Vidanarachchi *et al.,* 2005). The improvement in villus height may be attributed to the antimicrobial and antioxidant effects of *C. roseus* which have biologically active components such as various flavonoids, phenolic acids, and their derivatives (Renjini *et al*., 2017; Zheng and Wong, 2001).

The crypt depth was deeper ($P < 0.05$) in the 0.2% ALE supplemented birds in the ileum while in the duodenum CD was deeper in the 0.05% ALE supplemented group when compared with the control group. The villi crypts are understood as being the villus factory and rapid tissue turnover is indicated by large crypts with high demand for new tissue (Choct, 2009). Deeper crypts indicate that if there is a need for renewal of the intestinal villi the tissue metabolism increases (Hamedi *et al.,* 2011).

The current study demonstrates that VH:CD was higher in the 0.1% ALE supplemented group in the duodenum and jejunum when compared to the control group. The digestive capacity of the small intestine is indicated by the VH:CD ratio. With the increase in the VH:CD, the digestion and absorption rate are also increased (Montagne *et al.,* 2003). Therefore, enhanced VH:CD is generally associated with improved performance by means of greater nutrient absorption (Attia *et al.,* 2017).

Lamina propria consists of dendritic cells and its thickness is a sign of gut health. Dendritic cells prevent infection by adjusting mucin production, increasing gut motility, and adaptive immune response activation (Macpherson and Harris, 2004). In the present study, LPT was higher in the duodenum with the 0.05% ALE and 0.05% ARE supplemented groups in the ileum when compared with the control. Miles *et al.* (2006) used bacitracin methylene disalicylate an AGP in feed, which increases the thickness of lamina propria in the duodenum. Lamina propria plays an important role in the regulation and transportation of nutrients to the blood and eviction of secretions into the lumen (Mescher, 2009). Thus increase in the thickness of lamina propria by supplementation of *C. roseus* may have improved the nutrient absorption.

The absorption rate of nutrients is increased when the thickness of muscularis mucosa is decreased (Miles *et al.,* 2006). The current study shows that MMT and MET in the duodenum and jejunum were lower in the group supplemented with 0.2% ARE when compared with the control group. The present results are in agreement with the study of Anwar (2013) in which the

supplementation of *C. roseus* decreased the thickness of muscularis mucosae and that of muscularis externa.

Conclusion: The results demonstrated that the use of plant extracts from *C. roseus* as a feed supplementation resulted in improvement of histomorphometric parameters including VH, VW, CD, VH:CD, and LPT. However, the supplementation of feed with this plant extract has no effects on body growth, weight gain, feed consumption, FCR, or viscera development. Therefore, we concluded that *C. roseus* can be used as a feed additive for organic poultry production. It may replace antibiotics and be used as a phytobiotic for the production of antibiotic-free meat for human consumption.

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