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Growth performance and gut health parameters of finishing broilers supplemented with plant extracts and exposed to daily increased temperature

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Abstract

The effects of three plant extracts, *i.e.* lemon peel extract (LPE), orange peel extract (OPE) and *Curcuma xanthorrhiza* essential oil (CXEO), on the performance and gut health parameters of broilers exposed to high temperature was investigated. A total of 336 unsexed Ross 308 broilers were distributed to seven dietary treatments, a control diet and six diets containing 200 or 400 mg kg^{-1} feed of one of the three products between d 25-38 (12 chicks per pen, four replicates). To induce chronic heat stress, the temperature was increased to 34°C with 50% relative humidity for 5 h daily starting from d 28 until d 38. At d 38, four animals per pen were sampled for morphological characteristics (duodenum, jejunum and ileum) and microbial counts (ileo-cecal contents). Plant extracts did not affect the bird performance. The bursa weight of the control birds was lower ($p < 0.05$) comparing to those fed 400 mg kg^{-1} OPE and 200 and 400 mg kg^{-1} CXEO diets. Feeding 400 mg kg^{-1} of LPE decreased the duodenal villus:crypt ratio compared to control and 200 mg kg^{-1} OPE fed birds. Plant extracts did not have effect on ileal histo-morphology. Feeding with 400 mg kg^{-1} of LPE and CXEO caused a decrease in coliform counts in ileum and feeding of 400 mg kg^{-1} CXEO diet decreased coliform counts in caecum compared to control birds ($p < 0.05$). These results elucidate that CXEO, OPE and LPE might modify some microbial and intestinal traits, but without beneficial effect on performance of broilers under heat stress.

Additional key words: chickens; *Curcuma xanthorrhiza* essential oil; gut bacteria; heat stress; lemon peel extract; orange peel extract.

Introduction

The effect of high ambient temperature during some months of the year on poultry production has been of great concern in many countries. In south of Iran, maximum air temperatures of 35 to 45°C during the months of April to September is normal, and performance of birds is reduced drastically. During the finishing phase, the suitable ambient temperature for poultry is between 16 and 25°C (Sahin *et al.,* 2001). It has been well documented that exposing broiler chickens to continuously high temperature especially during the finisher phase leads to chronic heat stress (Sahin *et al.,* 2003; Ahmad *et al.,* 2008). Heat stress

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Abbreviations used: BWG (body weight gain); CXEO (*Curcuma xanthorrhiza* essential oil); FCR (feed conversion ratio); FI (feed intake); LPE (lemon peel extract); MRS Agar (de man rogosa sharpe agar); OPE (orange peel extract); PBS (phosphate buffered saline); PC (phenolic compounds); PCA (plate count agar); RSDBS (reduced sterile dilution blank solution); VRB Agar (violet red bile agar).

induces profound effect on overall physiology and animal health which can lead to changes in body composition. The gastro intestinal tract is particularly responsive to stressors like heat stress, which modify the normal and protective microbiota (Bailey *et al.,* 2004) and decreased integrity of the intestinal epithelium (Lambert, 2009) which in turn can affect its barrier function and the absorption of nutrients, impairing productive performance of animals (Liu *et al.,* 2009).

Numerous techniques have been proposed as possible therapies to offset the consequences of heat stress. Dietary manipulation could be a feasible option. In this regard, the possibility of using new plant derived additives in animal diets is being researched. Among the natural products, phenolic compounds (PC) seem to be potential candidates. The phenolic compounds show the ability to protect the microvilli, which are responsible for the absorption of nutrients (Pearlman & Lee, 1974). These functions of the intestinal mucosal layer are connected with their intrinsic antioxidative activity at both cell and tissue levels (Rhodes, 1996). In addition, phenolic compounds exhibit considerable antimicrobial activity. Their antimicrobial ability may modulate the gut ecosystem to affect feed efficacy (Si *et al.,* 2006). These ingredients are found in many plants such as fruits and vegetables. Studies showed that fruits of the Citrus family (particularly orange and lemon), and herbs of the *Zingiberaceae* family (particularly turmeric), are rich in phenolic compounds. Orange (*Citrus aurantium*) and lemon (*Citrus limon*) peel are common by-products of the food and juice extraction industry and the most widely consumed citrus in the world (Ghasemi *et al.,* 2009). They are also available at low cost in most seasons in some countries like Iran, and currently there is no information available about feeding orange and lemon peel extracts to broiler chickens under heat stress conditions. *Curcuma xanthorrhiza* (commonly known as temu lawak or Javanese turmeric in Indonesia), grows in southeast Asia and is found both wild and cultivated in Indonesia. It is traditionally used for medicinal purposes.

The objective of the current study was to determine the effect of dietary lemon peel extract (LPE), orange peel extract (OPE) and *C. xanthorrhiza* essential oil (CXEO) on performance, body composition traits, intestinal microflora and morphology of broiler chickens exposed to high ambient temperature. It was expected that selected plant extracts would relieve the deleterious effects of heat stress.

Material and methods

Animals, diets and experimental design

The experimental protocol was approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. A total number of 336 unsexed Ross 308 broiler chicks was obtained from a commercial hatchery (Quchan, Mashhad, Iran) and commercially raised for first 25 days before the commencement of the study. Chicks were vaccinated for infectious bronchitis on the first day, Newcastle disease and avian influenza on d 7 and infectious bursal disease on d 14. At d 25 of age, the birds were weighed, and randomly allotted to 28 floor pens with 12 birds each. Each pen (1 m^2) was equipped with a manual feeder and two nipple drinkers, and the floor was covered with clean wood shavings. The ventilation rate was 0.12 m s^{-1} during the whole period. Light with approximately 20 lux was made available around the clock. The initial house temperature was 32°C and then gradually decreased to reach 22°C at 21 d of age. The birds were given a finishing diet from d 25 to 38. The basal diet was formulated to meet or exceed the nutrient requirements of the broiler chickens as recommended by Ross 308 broiler management guide (Aviagen, 2011). Ingredient and chemical composition of the basal diet are shown in Table 1.

A completely randomized design was used with seven dietary treatments replicated in four pens each. The dietary treatments were: a basal diet (control treat-

Table 1. Composition of basal diet fed to birds from 25 to 38 d of age (g kg^{-1} unless otherwise stated)

Ingredients		Calculated nutrients[2]	
Maize	607.8	ME[3] (MJ kg^{-1})	12.76
Soybean meal	319.7	Crude protein	193.0
Vegetable oil	36.8	Calcium	8.1
Limestone	10.4	Available phosphorus	4.0
Dicalcium phosphate	14.2	Lysine	10.5
Common salt	4.3	Methionine	4.0
L-Lysine HCl	0.8	Methionine + Cystine	7.2
DL-Methionine	1.0	DCAB[4] (mEq kg^{-1})	211.0
Vitamin and mineral premix[1]	5.0		

[1] Vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 9,790 IU; vitamin E, 121 IU; vitamin B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg. [2] Based on NRC (1994) feed composition tables [3] Metabolizable energy. [4] DCAB = Na + K – Cl.

ment) and the same diet supplemented with either OPE, LPE or CXEO at two different levels (200 and 400 mg kg^{-1}). The extracts and essential oil were first mixed very well with the associated corn oil and then gradually added to the basal diet which was provided as mash form. Feed and water were offered *ad libitum.* The feeding experiment period lasted 13 days (25-38 d of age). In order to accustom to the experimental diets; a 3-d adaptation period was included before imposing chronic heat stress. To induce chronic heat stress, birds were exposed to an ambient temperature of 34°C with 50% relative humidity for 5 h daily (from 10:00 AM until 15:00 PM) between 28 and 38 d of age as reported by Aksit *et al.* (2006).

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured during the trial. Feed intake was determined from the difference between supplied and residual feed in each pen. The FCR was calculated from the ratio between feed intake and weight gain of chick, in each pen and was adjusted for mortality. Dead birds were weighed and recorded daily. At 38 d of age, four chicks per pen (16 chicks per treatment) were randomly selected and killed by cervical dislocation, to determine the body composition traits. The heart, empty proventriculus, empty gizzard, liver, spleen, pancreas, visceral fat, bursa of Fabricius and both carcass sides of the breast and thigh muscles (without skin) of each chick were weighed.

Curcuma xanthorrhiza essential oil and citrus peel extracts

C. xanthorrhiza essential oil was obtained from PT. Phytochemindo Reksa (Bogor, Indonesia). According to the compositional data provided by the supplier the main bioactive compounds were: ar-curcumene (approximately 11.4%), β-curcumene (approximately 8.5%) and xanthorrhizol (hydroxy-ar-curcumene) (approximately 28%). Xanthorrizhol is a sesquiterpenoid compound and the antioxidant properties of *C. xanthorriza* essential oil are ascribed to the phenolic structure of this compound. This essential oil of *C. xanthorriza* was used as such in the feeding experiment.

Fruit peels of orange (*C. aurantium*) and lemon (*C. limon*) were obtained from the fields of Mashhad in Khorasan Razavi province of Iran on October 2010. The products were dried in an air draught oven at 40°C for 12 h. The dried samples were ground into 3-5 mm particles using a laboratory mill (Braun, Model 2001DL, Germany), then packed in polyethylene bags and stored at –20°C until use. Fifty grams of each ground sample (orange peel and lemon peel) were extracted three times with 500 mL of ethanol using a Teflon-coated magnetic stir bar and stir plate for 6 h at room temperature. Extracts were filtrated through Whatman No. 1 filter paper. The combined filtrates from the three extractions were concentrated in a rotary evaporator at 40°C to a final volume of 100 mL crude extract and stored at –20°C until use. These extracts were used in the feeding experiment.

Total phenolic compounds were determined with Folin-Ciocalteu reagent using tannic acid as standard according to the method described by Taga *et al.* (1984). Results are expressed as mg tannic acid equivalents g^{-1} dried extract. Separation of phenolic compounds was carried out as described by Ricardo-da-Silva *et al.* (1993) using HPLC.

To test for the presence of flavonoid compounds in the samples, a modified colorimetric aluminum chloride method was used as reported by Woisky & Salatino (1998). Separation of flavonoids was performed using HPLC according to the method described by Baldi *et al.* (1995).

Histomorphometry of the small intestine

Intestinal tissues were obtained immediately after slaughter. Segments were removed from the duodenum, jejunum, and ileum as follows: 1) intestine from the gizzard to pancreatic and bile ducts was referred to as the duodenum, 2) the jejunum was defined as the portion of intestine extending from the bile duct entrance to Meckel's diverticulum, 3) the ileum was defined as the region from Meckel's diverticulum to a point 40 mm proximal to the ileo-cecal junction. Tissue samples (3 cm) were taken at the midpoint of each section and immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3 μm), stained by hematoxyline-eosin, and analyzed under a light microscope to determine morphometric indices using image-analysis software. The morphometric variables included villus height, crypt depth, villus width, tunica mucosal, tunica submucosal and tunica muscularis. The ten longest and straightest villi and associated crypts were measured from each segment. Measurements for the villi height were taken from the

tip of the villus to the villus-crypt junction. The crypt depth was defined as the depth of the invagination between adjacent villi and the villus width was measured at the top and bottom of villi. Tunica mucosal was measured from top of the villus (epithelium) until the lower part of muscularis mucosa. Tunica submucosal was measured from under part of muscularis mucosa until internal muscular layer and tunica muscularis measurement was performed from under part of submucosa until outer part of external muscularis layer. The mean from 10 measurements per sample was used as the average value for further analysis.

Intestinal microbial populations

Samples of the contents from the ileum and both caeca were immediately collected per chick (four chicks per pen, 16 chicks per treatment) into glass containers, sealed, and put on ice until they were transported to the laboratory for enumeration of microbial populations. One gram of mixed contents was blended into 9 mL of reduced sterile dilution blank solution (RSDBS). Further serial dilutions were made in RSDBS for aerobic bacterial enumeration. The initial dilution in RSDBS was also used as a source for serial dilutions in PBS for enumeration of aerobic bacterial populations. The samples from the ileum and caeca were diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. From each dilution, 0.1 mL was inoculated on agar plates for aerobics. Bacteria were enumerated on plate count agar (PCA) (total aerobes), MRS agar (facultative anaerobes *Lactobacillus* spp.), and VRB agar (coliform) (Merck, Germany). For bacterial growing, all the plates were incubated at 37°C. MRS agar plates were incubated anaerobically for 48 h (gas-pack container, AnaeroPack™, Tokyo, Japan), and other plates were incubated aerobically for 24 h. Total numbers of bacterial colonies were counted at each incubation period and expressed as $\log_{10}$ cfu g^{-1} digesta. The spread plate method for plate count determination was performed in accordance with the procedure recommended by the APHA (1993).

Statistical analysis

Data were analyzed by a linear model with the treatment as fixed effect using the General Linear Model procedure of the SAS Inst Inc (vers. 9.1, Raleigh, NC, USA). Tukey means separation test was used to determine significant differences between treatment mean values ($p < 0.05$).

Results

Composition of citrus peel extracts

The total content of phenolic compounds was 34.9 and 33.7 mg tannic acid equivalents g^{-1} dried extract for OPE and LPE respectively, with protocatchic accounting for approximately 80% in both extracts, catechol for 6.4 and 10% respectively and the other compounds each for less than 5% relative to the total content of phenolic compounds (Table 2). The total flavonoid contents were 3.68 and 4.52 mg quercetin g^{-1} dried extract in OPE and LPE, respectively.

Performance

The effects of dietary extracts on BWG, FI and FCR are given in Table 3. Throughout the experiment, there was only one case of mortality and it was in the control treatment. Because of this limited number of death

Table 2. Content of bioactive compounds of orange peel extract (OPE) and lemon peel extract (LPE)

	OPE	LPE
Total phenolic compounds (mg tannic acid equivalents g^{-1} dried extract)	34.92	33.71
Composition of phenolic compounds (%)		
Protocatchic	80.7	78.9
Catechol	6.4	10.0
Cinnamic	4.5	2.4
Caffein	3.3	1.7
Vanillic	2.9	2.1
Syringic	1.2	0.8
Chrisin	0.5	0.4
Coumarin	0.1	0.6
Total flavonoid compounds (mg quercetin g^{-1} dried extract)	3.68	4.52
Composition of flavonoid compounds (%)		
Quercetin	48.1	37.5
Luteolin	—	21.8
Rutin	18.5	15.6
Hesperetin	3.3	4.1
Unknown compounds	30.1	21.0

Table 3. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on performance parameters and mortality rate of broilers during 28-38 d of age

	Feed intake (g d^{-1})	Body weight gain (g d^{-1})	Feed conversion ratio (g g^{-1})
Control	177	91	1.96
OPE (mg kg^{-1})			
200	160	91	1.78
400	161	96	1.68
LPE (mg kg^{-1})			
200	168	93	1.80
400	163	94	1.74
CXEO (mg kg^{-1})			
200	163	93	1.74
400	166	94	1.77
p value	0.517	0.927	0.544
SEM[1]	5.84	3.27	0.09

[1] Standard error of mean. Values were taken from four replicates per treatment.

cases no statistical analysis was performed. Dietary OPE, LPE and CXEO did not affect BWG, FI and FCR of broiler chickens during 28-38 d of age.

Body composition traits

Effect of dietary supplemental plant extracts on different organ weights are presented in Table 4. Relative spleen, pancreas, gizzard, liver, proventriculus, thigh, breast, heart and visceral fat weight were not influenced by the use of plant extracts. The relative bursa weight (g kg^{-1} BW) of the control birds was lower ($p < 0.05$) compared to those fed 400 mg kg^{-1} OPE and 200 and 400 mg kg^{-1} CXEO diets. Also, the birds fed with 400 mg kg^{-1} CXEO had significantly heavier bursa than those fed 200 mg kg^{-1} OPE.

Small intestinal morphology

The effects of dietary OPE, LPE and CXEO on duodenal and jejunal criteria are presented in Table 5, respectively. Regarding the duodenal morphology, the inclusion of 200 mg kg^{-1} CXEO in the diet increased crypt depth compared to birds fed 200 and 400 mg kg^{-1} OPE or LPE diets ($p < 0.05$). Feeding 400 mg kg^{-1} of LPE diet decreased villus:crypt ratio compared to control and 200 mg kg^{-1} OPE fed birds ($p < 0.05$). There were significant differences between treatments for villus height of jejunum. In this regard, birds fed 400 mg kg^{-1} of LPE and CXEO diets had longer villi than those fed 200 and 400 mg kg^{-1} OPE diets ($p < 0.05$).

Table 4. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on relative weight of body composition traits (g kg^{-1} BW) of broilers at 38 d of age

Item	Heart	Breast (left and right)	Thigh (left and right)	Proventriculus	Gizzard	Liver	Spleen	Pancreas	Visceral	Bursa of Fabricius
Control	8.73	298	278	7.22	31	36	1.99	4.86	15	3.42^{c}
OPE (mg kg^{-1})										
200	8.84	298	282	6.80	30	35	1.94	4.94	16	3.79^{bc}
400	8.11	303	282	6.56	33	33	2.01	4.56	14	4.06^{ab}
LPE (mg kg^{-1})										
200	9.14	291	281	6.84	30	35	1.96	4.69	13	3.81^{abc}
400	8.38	290	275	7.31	31	33	2.05	5.01	15	4.02^{abc}
CXEO (mg kg^{-1})										
200	8.47	294	274	7.32	33	33	1.84	4.94	14	4.34^{ab}
400	8.13	291	293	6.93	32	34	2.00	4.79	13	4.43^{a}
p value	0.797	0.928	0.866	0.337	0.176	0.385	0.463	0.731	0.479	0.021
SEM[1]	0.53	8.90	10.09	0.26	0.92	1.16	0.07	0.20	1.13	0.19

[a-c] Means within a column with different superscripts are significantly different ($p < 0.05$). [1] Standard error of mean. Values were taken from four replicates per treatment.

Table 5. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on morphological criteria of the duodenum and jejunum of broilers at 38 d of age

Intestinal criteria (mμ)	Villus height		Villus width		Crypt depth		Tunica mucosal		Tunica submucosal		Tunica muscularis		Villus: Crypt ratio	
	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum
Control	1.119	604^{abc}	139	89	333^{ab}	294	1.506	727	47	48	275	162	4.06^{a}	2.16^{ab}
OPE (mg kg^{-1})														
200	1.143	570^{c}	139	80	288^{a}	277	1.326	688	45	56	276	190^{ab}	4.03^{a}	2.15^{ab}
400	1.047	582^{c}	115	106	297^{a}	257	1.472	856	50	54	260	207^{ab}	3.66^{ab}	2.28^{ab}
LPE (mg kg^{-1})														
200	1.103	606^{abc}	123	96	297^{b}	266	1.395	739	50	49	227	215^{a}	3.88^{ab}	1.81^{b}
400	1.107	695^{a}	115	99	276^{b}	263	1.517	778	51	43	297	190^{ab}	2.98^{b}	2.73^{a}
CXEO (mg kg^{-1})														
200	1.052	595^{bc}	139	82	372^{a}	335	1.386	832	52	53	256	201^{ab}	3.17^{ab}	1.81^{b}
400	1.163	687^{a}	121	100	333^{ab}	326	1.439	870	48	53	279	138^{b}	3.49^{ab}	2.47^{ab}
p value	0.663	0.036	0.146	0.371	0.028	0.138	0.760	0.525	0.852	0.582	0.401	0.044	0.046	0.048
SEM[1]	52.08	29.97	8.34	9.28	19.48	23.13	93.58	74.38	3.85	4.79	21.13	21.70	0.32	0.21

[a-c] Means within a column with different superscripts are significantly different ($p < 0.05$). [1] Standard error of mean. Values were taken from four replicates per treatment.

Feeding both 200 mg kg^{-1} of CXEO and LPE diets decreased villus:crypt ratio in jejunum as compared to those fed 400 mg kg^{-1} LPE diet ($p < 0.05$). Jejunal muscular thickness was lower in birds fed 400 mg kg^{-1} CXEO diet and was higher in those fed 200 mg kg^{-1} LPE diet ($p < 0.05$). Plant extracts did not have a significant effect on ileum measured criteria (data not shown).

Intestinal microbiology

Effect of dietary supplemental plant extracts on bacterial counts in ileal and cecal digesta of broiler chickens are shown in Table 6. In this respect, feeding with both 400 mg kg^{-1} of LPE and CXEO caused a decrease in coliform counts in ileum compared to control group and feeding of 400 mg kg^{-1} CXEO diet decreased the number of coliforms in ceacum compared to those fed control diet ($p < 0.05$). Counts of *Lactobacillus* spp. and total aerobic counts of ileum and caecum were similar among the treatment groups.

Discussion

Composition of citrus peel extracts

The phenolic and flavonoid contents were lower than data reported by other authors (Wang *et al.,* 2008; Ghasemi *et al.,* 2009), *e.g.* using similar analytical methods. In this regard, Ghasemi *et al.* (2009) reported 131 mg gallic acid equivalent and 16.2 mg quercetin equivalent g^{-1} extract powder (following methanolic extraction) for total phenolic and flavonoids content respectively in lemon peel. Corresponding values for orange peel were 223 mg gallic acid equivalent and 7.7 mg quercetin equivalent g^{-1} extract powder. Wang *et al.* (2008) reported 32.7 mg rutin equivalents g^{-1} dried lemon peel (after methanolic extraction also) for total flavonoid contents, with hesperidin being the major flavonoid. These differences may be due to a number of reasons, *e.g.* variation in the agricultural soil profile and time of harvest. Fu *et al.* (2005) reported that similar samples produced in different countries could have different amount of bioactive compounds. However, the condition of the raw material and the extraction method has also a large impact on the yield of bioactive compounds, depending on the solvent type and concentration, time, temperature etc. (Lia *et al.,* 2006; Garau *et al.,* 2007).

Performance

Reported effects of dietary supplementation with plant extracts rich in phenolic compounds are inconsistent. Seven *et al.* (2008) found that using high doses

Table 6. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on ileal and cecal bacteria in broilers at 38 d of age

Bacterial group (log_{10} CFU g^{-1})	Ileum			Cecum		
	Lactobacilli	Coliforms	Total Aer. Count	Lactobacilli	Coliforms	Total Aer. Count
Control	3.99	3.93[a]	5.01	4.62	4.45[a]	5.62
OPE (mg k^{-1})						
200	4.15	3.69[ab]	5.46	5.16	4.22[ab]	5.89
400	4.12	3.73[ab]	5.38	5.14	4.42[ab]	5.96
LPE (mg kg^{-1})						
200	4.06	3.71[ab]	5.29	5.15	4.22[ab]	5.82
400	4.11	3.50[b]	5.10	4.92	4.12[ab]	6.01
CXEO (mg kg^{-1})						
200	4.40	3.71[ab]	5.47	5.19	4.14[ab]	5.93
400	4.55	3.42[b]	5.50	5.20	3.96[b]	6.41
p value	0.336	0.049	0.500	0.369	0.043	0.501
SEM[1]	0.18	0.12	0.20	0.20	0.14	0.25

[a-b] Means within a column with different superscripts are significantly different ($p < 0.05$). [1] Standard error of mean. Values were taken from four replicates per treatment.

of propolis rich in phenolics and vitamin C could partially overcome the depression in growth and carcass quality caused by heat stress in broilers. Reisinger *et al.* (2011) conducted an experiment with broiler chickens fed a phytogenic feed additive containing a blend of essential oils from oregano, anise, and citrus peel. Parallel to our results, there was no difference for feed intake and feed conversion ratio among the treatments. Sinurat *et al.* (2009) used *Curcuma longa* and *C. xanthorrhiza* powder as a feed additive for broiler chickens. In agreement with our results, these authors reported that supplementation with these plant prouducts did not affect feed intake or feed conversion ratio. Lee *et al.* (2003) fed thymol, cinnamaldehyde and a commercial mixture of essential oil components to female broilers, and observed no differences in feed intake, weight gain and feed conversion ratio. Çabuk *et al.* (2006) used a blend of plant oils derived from oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds, and citrus peel for laying hens during the summer season. They did not find a significant effect of plant oils on the feed intake but they found an improved feed conversion ratio for supplemented groups compared to their control. The non-significant effects of plant extracts observed in the current study can be due to several reasons, consisting of either inappropriate doses used or short duration of heat stress exposure in both hours and days. Also, as reviewed by Brenes & Roura (2010), the extraction methods of plants, storing method and conservation duration of plant products could affect the results coming out from the plant extracts. Using higher levels of these plant extract could be taken into the account to see the positive effects on the broilers performance during hot weather of the year.

Body composition traits

In agreement with our results, Khaligh *et al.* (2011) indicated that supplementation of broiler diets with medicinal plant blends did not alter liver, gizzard and abdominal fat weight. On the other hand, Debersac *et al.* (2001) indicated that a herbal extract from rosemary, containing rosmarinic acid, flavones, and monoterpenes, enhanced hepatic metabolism and increased liver weight in rats.

The bursa of Fabricius, known as central or primary lymphoid organ, plays a crucial role in enzymatic maturation and acquisition of immunological competence of T- and B-lymphocytes (Rudrappa & Humphrey, 2007). Any disturbances in the development of bursa of Fabricius caused by stressors might result in significant deficiencies of the immune system functions in

chicken (Oznurlu *et al.,* 2010). Quinteiro-Filho *et al.* (2010) also observed that heat stress caused a decrease in the weight of the bursa of Fabricius. Zulkifli *et al.* (2002) reported that heat stress reduced antibody production in young chickens. A glucocorticoid-dependent mechanism during stress was reported to induce lymphoid organ involution (Shini *et al.,* 2008). The increased relative weight of bursa of Fabricius found in our study supports the assumption that dietary OPE and CXEO may act to decrease heat stress in broiler chickens.

Small intestinal morphology

Heat stress leads to generation of free radicals, which can induce lipid peroxidation and thereby damage cell structures (Altan *et al.,* 2003). Maintenance of normal morphology and structural integrity of the small intestine are imperative for preventing bacterial translocation from the intestinal tract. Heat stress could exert deleterious effects on the absorptive epithelium of the intestine, resulting in reduction in villus height and crypt depth (Yamauchi *et al.,* 2006). Burkholder *et al.* (2008) reported that birds subjected to 30°C for 24 h had reduced crypt depth compared with birds at 23°C. Smith *et al.* (1990) reported that villus height was reduced by 18.8% in heat stressed birds.

It has been suggested that some components of feeds can affect the mucosa thickness and villus height and intestinal brush border (Jamroz *et al.,* 2006). Concerning phytogenic feed additives literature does not draw a consistent picture. Based on literature, feeding broiler chickens and pigs with phytogenic products could cause to increased, unchanged as well as reduced villus length and crypt depth in gut (Namkung *et al.,* 2004; Nofrarías *et al.,* 2006; Oetting *et al.,* 2006; García *et al.,* 2007). In our study, there were no significant differences between control and treated groups, with exception for villus:crypt ratio in the duodenum part that was reduced when chickens were fed with 400 mg kg^{-1} LPE diet as compared to control birds and those fed with 200 mg kg^{-1} OPE. It is hypothesized that the overall impact of plant products on gut morphology depends on the balance between tissue irritation and beneficial effects on intestinal hygiene. On the other hand, heat stress is associated with intestinal irritation. Results found in this study could be explained in this way that selected plant extracts at the present doses were not able to improve histomorphological criteria that might be negatively affected by heat stress and exert positive effect on gut morphology.

There was not any significant difference between the treatments in the ileum part. There are several possible reasons why ileal structure was unchanged in this experiment, including the resistance of the ileum to structural changes compared with other regions of the small intestine and possibly, the imposed high temperature stress was not severe enough so that plant extracts could exert a substantial effect. Yamauchi *et al*. (1996) indicated that morphological changes in response to stressors occur more rapidly in the proximal two-thirds of the small intestine than in the ileum.

Our findings on intestinal morphology indicate that feeding plant extracts which are high in phenolic compounds showed low activity and may not exert a proper effect on gut structure of broiler chickens under heat stress condition; therefore more studies with different doses and/or combination of selected extracts for inducing synergistic effects could be taken into account.

Intestinal microbiology

The indigenous gut microflora is a complex ecosystem that can benefit the host by serving as a barrier to pathogen colonization (Van der Waaij, 1989). Alteration of this protective barrier may leave the host more susceptible to colonization by enteric pathogens (Durant *et al.,* 1999). Neurohormones associated with stress can increase growth and virulence factor expression in pathogenic bacteria within the lumen (Lyte & Bailey, 1997). Hinton *et al.* (2000) showed an increase in intestinal *Enterobacteriaceae* and cecal aerobes with a concurrent decrease in lactic acid bacteria in broilers subjected to a 24-h feed withdrawal. Burkholder *et al.* (2008) noted that heat stress significantly decreased the intestinal bacterial populations of birds.

For many years, herbs and their extracts have been used as pharmaceuticals as a natural therapy, their antimicrobial ability may modulate the gut ecosystem to affect digestibility of feeds (Hernández *et al.,* 2004). In total, there is clear experimental evidence for an overall antimicrobial efficacy of phytogenic feed additives whether arising directly from an antimicrobial action or indirectly mediated by phytogenics to affect the microbiota: 1) The direct effect of plant oils is due to their lipophilic activities, adhesion and pass into the bacterial membrane which prevents acti-

vation of bacterial enzymes. Liu *et al.* (2008) concluded that phenolic compounds due to their hydrophobicity are able to disintegrate the outer membrane of gram-negative bacteria, and disturbing the bacterial structure. Likewise, Michiels *et al.* (2007) concluded that phenolic compounds can especially be used to reduce the bacterial population in the proximal and more acidic parts of the gastrointestinal tract. 2) Indirect effect of plant extracts have been reported due to reducing ileal pH value (which, unfortunately has not been executed in this experiment), while increasing the number of lactic acid bacteria and decreasing the co liform counts in the ileum and caecal contents of broiler chickens (Vidanarachchi *et al.,* 2006). In addition, it is mentioned that enhancement of activities of digestive enzymes by plant extracts could also increase nutrient digestibility and improve the regulation and stabilization of the gut microbiota. In this study, there was a significant reduction for coliform counts with no effect on other bacteria. In accordance with our results, Si *et al.* (2006) reported that it is possible to select plant bioactive compounds with a strong antimicrobial action against gut pathogens whilst not harming beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*.

Based on our results, reducing pathogenic bacteria such as coliforms by 400 mg kg^{-1} LPE and CXEO, could contribute to a balanced gut microflora, thus improving the ability to preserve intestinal integrity.

Under the conditions of this study, even though dietary CXEO, OPE and LPE did not have a significant effect on chickens performance and gut morphology, it can be stated that dietary CXEO and LPE at 400 mg kg^{-1} feed could be an option to use in broiler chickens diets during the finisher phase to prevent or diminish the stress-induced alteration of the intestinal microbiota and immunological functions.

Further studies are required to fully explore dose-response effects on the broiler performances and gut histomorphology.

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