Effect of lemon pulp powder on growth performance, serum components and intestinal morphology of broilers exposed to high ambient temperature

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Journal of Livestock Science (ISSN online 2277-6214) 8: 155-162
Received on 6/4/2017; Accepted on 28/6/2017

Abstract

The main aim of the current experiment was to assess the potential of dietary lemon pulp powder(LPP)as promoters of broiler resistance to high ambient temperature.The experiment was conducted as a completely randomized design with 4 levels of LP (0,0.2, 0.6 and 1 percent in diet). At d 25, a total of 192 Ross 308 broilers were randomly assigned to 4 dietary treatments with 4 replicates of 12 broiler chickens each. The temperature was increased to 34 ºC with 50% relative humidity for 5 h daily starting from d 28 until d 38. At the end of the trial (d 42), 2 broiler chickens per pen were sampled for determination of serum components and variables of intestinal morphology.Dietary LP did not affect weight gain, feed intake, and feed conversion ratio of broiler chickens. The inclusion of %0.6 LPP increased serum albumin, but reduced serum lactate dehydrogenase activity in broiler chickens. Lemon pulp powder supplementation decreased the activity of lactate. No differences in the other blood characteristics and intestinal traits were observed. These results indicate that LPP might modify some blood, but without beneficial effect on growth performance of broiler chickens under hot conditions.

Keywords: blood chemical; broiler; gut histology; heat stress; lemon pulp powder

Introduction

Iran has a hot, dry climate characterized by long, hot, dry summers and short, cool winters. Daily temperatures can be very hot; on some days, temperatures can reach easily 40°C or more. The deleterious effects of high ambient temperature during some months of the year on poultry production have been of great concern. It has been well documented that exposing broiler chickens to continuously high ambient temperatures, especially during the finisher phase, leads to chronic heat stress (Sahin et al., 2003; Ahmad et al., 2008) and could exert profound effects on performance, health and overall physiology of birds (Han et al., 2010; Melesse et al., 2011; Quinteiro-Filho et al.,2010). Elevated ambient temperature also causes a disruption in the structure and function of the intestinal epithelium, including reduced regeneration (Burkholder et al., 2008) and integrity (Meddings & Swain, 2000) of the intestinal epithelium. However, good intestinal health in broilers is of utmost importance to achieve target growth rates and feed efficiency (Montagne et al., 2003). Several management approaches have been used to minimize the deleterious impacts of elevated temperatures. Undoubtedly, diet manipulation can be a viable option to resolve this issue (Sahin et al., 2002, 2003; Dai et al., 2011). Rahimi and Khaksefidi (2006) reported that diet supplementation with virginiamycin has a significant effect on body weight gain and FCR during the heat stress. But the use of antibiotics in poultry feed as growth promoter and for health maintenance can cause drug resistance bacteria and antibiotic residue effects. Use of antibiotics in aviculture is considered as a risk factor to human health as their residues may be found in tissues and as they may cause cross-resistance for pathogenic bacteria in humans. It has previously been reported that adding plant extracts to broiler chickens diet under optimal environmental conditions has either a positive or no effect on their growth performance (Botsoglou et al., 2002; Lee et al., 2003a). Lee et al. (2003b) reported that dietary plant extracts could stimulate growth performance in broilers fed a suboptimal diet. Plant extracts may stimulate crypt cell proliferation and subsequent tissue turnover and, thus, result in a healthier gut (Brenes and Roura, 2010; Incharoen et al., 2010). Studies have shown that phenolic compounds (PC) may exert antimicrobial and antioxidant effects when fed to poultry (Akbarian et al., 2011; Viveros et al., 2011). These compounds are found in many plants such as fruits and vegetables. The fruit byproducts use as energy source in animal feed and these feedstuffs are economical and environmentally sound way for food processors to reduce waste discharges and cut waste management cost. Selling by-products can also produce additional revenue (Crickenberger, 1991). The poor state of economy in developing countries has made consumption of high protein foods out of reach of more than 65-70% of the people. One of the ways of solving this problem is to use unconventional sources of feed ingredients to supplement the diets of man and farm animals (Nworgu, 2004). Lemon (*Citrus limon*) pulp are common by-products of the food and juice extraction industry and the most widely consumed citrus in the world (Ghasemi et al., 2009). The pulp from citrus fruit represents approximately one-fourth of whole fruit mass and is obtained after the extraction of juice and removing the remaining pulp inside mechanically (Braddock, 1999). These by-products are available at low cost in most seasons in some countries like Iran. During citrus juice processing, a considerable quantity of wastes or by-products is generated. Though large quantities of citrus pulps are used for animal (ruminant) feeds, the majority of the processing residue is thrown out, and consequently pollutes the environment. Therefore, citrus-processing industries have been searching for applications of these by-products. Currently, almost few information is available on feeding lemon pulp to broiler chickens under hot conditions (eg. Akbarian et al., 2013). Therefore, the hypothesis was tested whether the dietary inclusion of lemon pulp powder (LPP) could relieve some of the metabolic and digestive disturbances induced by elevated environmental temperature.

Materials and methods

Lemon pulp (*C. limon*) were obtained from a commercial source (Zamani company of Agro-industry, Mashhad, Iran). The products were dried in a forced air oven at 35 ºC for 12 h. The dried samples were ground into 3 mm to 5 mm particles using a laboratory mill (Model 2001DL; Braun GmbH, Kronberg, Germany), then packed in polyethylene bags and stored until use in 5 ºC. The chemical composition of lemon pulp powder is shown in Table 1.

Animals, diets and experimental design

The experimental protocol was approved by the Animal Care Committee of Islamic Azad University. The study was conducted at Poultry Research Station, Islamic Azad University, Mashhad branch (Mashhad, Iran).A total of 192 unsexed Ross 308 broiler chickens was obtained from a commercial hatchery (Seamorgh Co., Quchan, Mashhad, Iran) and raised for 25 d before the commencement of the study, i.e., the feeding of the experimental diets. At d 25 of age, broiler chickens were randomly allotted to 16 floor pens with 12 birds each. Each pen (1^{m2}) was equipped with a manual feeder and 2 drinkers, and the floor was covered with clean wood shavings. The ventilation rate was

0.12 m/s during the whole period. The light was made available around the clock with an intensity of approximately 20 lux (23 h light:1 h dark). The initial house temperature was 32 ºC and was gradually decreased to reach 22 ºC at 21 d of age. The birds were given a finisher diet from d 25 to 42. The basal diet was formulated to meet recommended by Ross 308 broiler management guide (Aviagen 2009). Ingredient and chemical composition of the basal finisher diet are shown in Table 2. The experiment was conducted as a completely randomized design with 4 levels of LPP (0, 0.2, 0.6 and 1 percent in feed). Feed and water were offered *ad libitum*. To accustom to the experimental diets, a 3-d adaptation period was included before increasing house temperature. From d 28, a different temperature regimen was followed as reported by Aksit et al., (2006). The basal temperature was 22 ºC. Between 08:30 and 10:00, the temperature was gradually increased to 34 ºC and this high temperature was then maintained for 5 h (until 15:00). After that, the temperature was gradually decreased to the basal level by 16:30. Temperature control was executed by heating elements, air conditioner, and dynamic ventilation. Average relative humidity was kept at 50% during the experimental period and the rest of the day. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined during the experiment.

Table 1. Chemical composition of lemon peel powder

Constituents	**Percentage**
Dry matter	95.55
Gross energy (Kcal/Kg)	3374
Crude protein	2.09
Ether extract	0.78
Fiber	21.74
Ash	7.54
Calcium	0.49
Phosphorous	0.12
Sodium	0.34
Total phenols	1.1

Table 2. Ingredient and calculated nutrient composition of the basal finisher diet fed from 25 to 42 d of age.

Item	**Content (%)**
Ingredients (g/kg)	
Corn	64.7
Soybean meal	29.65
Vegetable oil	1.7
Limestone	1.25
Dicalcium phosphate	1.43
Common salt	0.3
DL-Methionine	0.16
L-LysineHCL	0.23
Vitamin and mineral premix[a]	0.5
Calculated composition	
ME (kcal/kg)	2980
CP (g/kg)	18.5
Ca (g/kg)	0.9
Available P (g/kg)	0.42
Lys (g/kg)	0.9
Met (g/kg)	0.4
Met+Cys (g/kg)	0.7
DCAB (mEq/kg)[b]	210

[a] Vitamin andmineralpremixsuppliedperkilogramofdiet:vitamin A: 10,000IU; vitaminD3: 9790IU;vitaminE:121IU;vitaminB12: 20 µg; riboflavin:4.4mg; calciumpantothenate:40mg;niacin:22mg;choline: 840 mg;biotin:30 µg; thiamin:4mg;zincsulfate:60mg;manganese oxide:60mg;sodiumselenite: 0.3mg;potassiumiodide:1mg;copper (II) sulfate:10mg;andironsulfate:50mg.
[b] DCAB=dietary cation- anion balance(Na+K- Cl).

Blood sampling and determination of serum components

At the end of the experiment (42 d of age and after the 5 h of high ambient temperature), 2 birds from each pen (8 per treatment) were randomly selected and weighed individually after a 3-h fasting period. Blood samples were collected from the wing vein. Serum was obtained by centrifugation of the coagulated blood (3000 g for 10 min at 4 ºC) within 30 min after sampling to measure serum chemical components. Following variables were analyzed: total protein (TP), fasting blood sugar (FBS), and albumin (Tietz, 1995), HDL, LDL, the activity of lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) (Young, 1995). These were analyzed by an automatic analyzer (Furuno, CA-180, Japan).

Histomorphometry of the small intestine

Following blood sampling the selected broiler chickens were killed by cervical dislocation. Intestinal tissues were obtained immediately after slaughter. Intestinal segments were defined as follows: (1) duodenum as the intestine from the gizzard (pylorus) to pancreatic and bile ducts, (2) jejunum as the portion of intestine extending from the bile duct entrance to Meckel′s diverticulum, and (3) ileum as the region from Meckel′s diverticulum to apoint 40 mm proximal to the ileo-cecal junction. After removing the intestinal contents, approximately 3 cm lengths of duodenum, jejunum and illeum were removed for gut morphological measurements. Intestinal samples from each section were immersed in formaldehyde, before fixation in Bouin's solution and paraffin embedding. Histological examinations were carried out according to the method of Iji et al. (2001). Paraffin sections at 6 µm

thickness were made from each sample, stained with hematoxylin and eosin, and examined by light microscopy.The morphometric variables (all expressed in micrometer) measured included villus height, crypt depth, and villus width at the top and the base, thickness of the mucosal and submucosal layer, and muscularis thickness as described by Akbarian et al. (2013). The 10 longest and straightest villi and associated crypts were measured from all segments. 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. Muscularis thickness was determined from the submucosa to the external layer of the intestine. The mean from 10 measurements per sample was used as the average value for further analysis.

Statistical analyses

All data were analyzed using the GLM model procedure of the SAS (Version 9.1; SAS Inst. Inc., Cary, NC). All statements of significance were based on the probability of $P<0.05$.

Results and Discussion

Lemon pulps powder were analyzed for dry matter, crude protein, ether extract, crude fiber, ash, phosphorus, calcium, sodium and gross energy (Table 1). The total content of phenolic compounds was 1.1±0.02 percent in dry matter. As discussed by Akbarian et al. (2013), the content of bioactive compounds in plants depends on several factors. The effects of dietary treatment on BWG, FI and FCR are given in Table 3. No mortality was observed throughout the trial.

Table 3. Effect of dietary lemon peel powder (LPP) on performance of broilers exposed to high ambient temperature during 28 to 42 d of age.

Treatment		BW (28 d)	BW (42 d)	FI (g/d)	BWG (g/d)	FCR
1		1151	1967	102.5	56.22	1.835
2		1201	1977	96.96	56.30	1.736
3		1144	2021	97.36	53.93	1.805
4		1102	1963	94.65	51.91	1.829
SEM		46.66	45.67	4.04	3.03	0.072
	Anova	0.53	0.79	0.49	0.71	0.9
P value	Linear	0.31	0.54	0.57	0.67	0.97
	Quadratic	0.34	0.47	0.23	0.32	0.91

1- Control, 2- Control+ %0.2 LPP, 3- Control+ %0.6 LPP, Control+ %1 LPP

Table 4. Effect of dietary lemon peel powder (LPP) on concentration of some serum components in broilers exposed to high ambient temperature during 28 to 42 d of age.

Treatment		TP (g/dL)	Albumin (g/dL)	FBS (mg/dL)	LDL (mg/dl)	HDL (mg/dl)	LDH (IU/L)	CPK (IU/L)
1		3.60	1.872^{ab}	75.00	28.253	31.273	783.97^{ab}	194.39
2		3.50	1.400^{b}	95.00	26.278	39.370	714.49^{b}	174.16
3		3.60	1.850^{a}	73.50	26.748	36.493	784.50^{ab}	158.23
4		3.58	1.615^{ab}	92.50	24.193	38.088	780.33^{a}	187.52
SEM		0.13	0.129	22.15	28.253	3.0278	30.722	13.883
	Anova	0.86	0.12	0.77	0.93	0.29	0.2	0.89
P value	Linear	0.51	0.72	0.73	0.86	0.11	0.52	0.73
	Quadratic	0.45	0.28	0.82	0.69	0.52	0.59	0.96

Note: Within column means with different superscript letter are significantly different ($P < 0.05$). 1- Control, 2- Control+ %0.2 LPP, 3- Control+ %0.6 LPP, Control+ %1 LPP

Dietary LPP did not affect BWG, FI, and FCR of the broiler chickens. Although many studies have been conducted with plant extracts rich in phenolic compounds on broiler performance, the data obtained from these studies are controversial. It has been shown that using high doses of propolis rich in phenolics and vitamin C could partially overcome the depression in growth and carcass quality caused by high temperature in broiler chickens (Seven et al., 2008). In agreement with our results, Reisinger et al. (2011) found that supplementation of broiler chicken diets with a mixture of phytogenic feed additive containing oregano, anise, and citrus peel oils did not affect FI or FCR. The same result was found in a study of Akbarian et al. (2013) who reported no differences in FI, BWG and FCR of broiler chickens fed lemon citrus extract. Lee et al. (2003b) also reported no differences in FI, BWG and FCR of broiler chickens fed thymol, cinnamaldehyde and a commercial mixture of essential oil components. Elsewhere, incorporation of a blend of plant oils derived from oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds, and citrus peel into laying hens diet during the summer season, did not exert a pronounced effect on FI but an improved FCR for supplemented groups compared to their control was observed (Çabuk et al., 2006). Several reasons might explain these inconsistencies. The efficacy of plant extracts to impart on animal performances depends on several factors, e.g., dose of the plant extract used and concentration and profile of active components present in the extracts, physiological state of the animal, background diet, housing conditions, etc. The plant composition is in turn determined by storage method, soil and growth conditions of the plants, etc. (Basmacioğlu et al., 2010; Brenes & Roura 2010; Lee et al., 2003a, b).

The effects of dietary LPP on serum components of broiler chickens are given in Table 4. The inclusion of LPPhas significant difference on albumin and the activity of LDH ($P<0.05$). Fasting blood glucose, total protein,LDL, HDL and CPK were not affected by the treatments.

A number of enzymes are used in the clinical biochemistry as tools for differential diagnosis, such as CPK and LDH. Since the bulk of each is located in different tissues, their abnormal appearance in the blood can give a

hint to specific muscle or organ damage (Pech-Waffenschmidt, 1992). In broiler chickens, CPK is released into the circulation following changes in the permeability of the sarcolemma in response to various pathologies and exposure to environmental stressors (Mitchell & Carlisle, 1992; Mitchell & Sandercock, 1995). In addition, overt muscle damage in birds is associated with an increase in the plasma activity of the intracellular muscle isoenzyme CPK (Eraslan et al., 2007). Publications about the effect of high environmental temperature on the CPK activity are not consistent. Even in broiler chickens of the same age, size, and breed, large variations were observed in their responses to elevated temperature, as evaluated by blood composition and behavior. In this connection, Sandercock et al. (2001) and Bogin et al. (1996) observed a significant increase in CPK activity in the plasma of broiler chickens exposed to high ambient temperature reflecting heat stress-induced myopathy. Similarly, Yalçin et al. (2009) reported an increase in plasma CPK activity on broiler chickens exposed to a daily cyclic heat treatment. According to the results obtained by Bogin et al. (1996) heat exposure increased the activities of CPK in different organs like brain, breast muscle, and heart. They also found a significant increase in the LDH activity in the heart muscle. This was also supported by previous findings of Melesse et al. (2011), who reported that the activity of LDH was increased by long term high temperature in the laying hen. On the other hand, according to the results obtained by Pech-Waffenschmidt et al. (1995), heat exposure did not change the enzyme activities in the broiler broiler chickens serum. This was also supported by findings of Ward and Peterson (1973), who reported that the activity of CPK was not influenced even by acute heat exposure. Hence, the decreasedLDH and CPK activity found in our study, supports that LPPmay act to decrease the harmful effects of high temperature in broilers which might be related to the antioxidant capacity of the PC present in the lemon peel powder (e.g., Farrell et al., 1965).The highest reduction of LDH activity was obtained in treatment 2. The metabolic changes induced in broiler chickens by high temperature include impairment of endocrine functions (Sinurat et al., 1987) and reduced serum protein concentrations (Khan et al., 2002). Regarding total protein, consistent with our results, Eraslan et al. (2007) observed an increase in total protein in the serum of rats fed propolis, which is rich in flavonoid and phenolic compounds. One mechanism through which LPP may exert its hyperproteinemia action is via transamination through its phenolic compounds. The phenol and its derivatives can alter protein metabolism by altering the transamination rate of amino acids by enhancing the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Abdel-Hamid, 2007). We found that fasting blood glucose, LDL and HDL were not influenced by the treatments. Related to the results for glucose levels, this is not surprising since Bogin et al. (1981) and Arad and Marder (1983) showed that glucose levels were not influenced by elevation in house temperature in broiler chickens and laying hens. Also, Seven et al. (2008) reported that using propolis rich in flavonoid and phenolic acids, had no influence on biochemical parameters of blood including glucose and albumin of broiler chickens exposed to an increased temperature. Several reports have shown that using PC had no influence on serum glucose of broiler and fish (Biavatti et al., 2003; Roche & Boge, 2000).

Table 5. Effect of dietary lemon peel powder (LPP) on morphological characteristics of the jejunum (µm) in broilers exposed to high ambient temperature during 28 to 42 days of age.

Treatment		Villus Height	Villus Width	Crypt Depth	Mucosal Layer	Submucosal Layer	Muscularis Thickness	VH/CD	Villus Surface
1		1622.8	192.00	232.25	209.00	41.25	175.75	7.733	11590^{a}
2		1359.8	181.75	209.75	215.50	49.50	167.25	7.604	8538^{b}
3		1377.3	199.00	201.50	214.00	53.75	187.25	7.000	9481^{b}
4		1479.8	232.75	198.75	198.00	41.50	200.25	8.401	12530^{a}
SEM		150.78	23.319	26.893	19.239	6.146	18.476	0.86	611.53
	Anova	0.6	0.47	0.81	0.62	0.42	0.91	0.99	0.002
P value	Linear	0.16	0.09	0.6	0.68	0.20	0.64	0.65	0.008
	Quadratic	0.16	0.02	0.45	0.75	0.72	0.25	0.97	0.149

Note: Within column means with different superscript letter are significantly different ($P < 0.05$).
1- Control, 2- Control+ %0.2 LPP, 3- Control+ %0.6 LPP, Control+ %1 LPP

The treatments did not have any effect on the jejenum traits with an exception for villus surface which had significant differeance and was reduced when LPP was added to the diet (linear, $P<0.008$) (Table 5). Several observations support the hypothesis that herbal feed additives may favorably affect gut functions (e.g., enzyme activity, microbial eubiosis) in vitro (Liu et al., 2011). Phytogenic compounds enhanced mucus production and thickness in the stomach and jejunum suggesting a potential protective against colonization by gut pathogens (Jamroz et al., 2006). It is likely that changes in cell proliferation would be observed first in the stem cells of the crypt rather than the villus because of the high proliferative activity of the crypt (Yamauchi et al., 1995). Garcia et al. (2007) indicated that addition of 200 mg/kg plant extract comprising a blend of oregano, cinnamon, and pepper

essential oil increased villus height. It has been reported that birds subjected to 30 ºC for 24 h had reduced crypt depth compared with birds at 23 ºC, but villus height and the villus height:crypt depth were unchanged in birds exposed to 30 ºC (Burkholder et al., 2008). It has been shown that mucus content of small intestine during a short-term feed withdrawal of broiler chickens as a stressor was reduced (Thompson & Applegate 2006). It is suggested that the balance between tissue irritation and beneficial effects of intestinal hygiene may determine the overall impact of plant products on gut morphology. In the present study, feeding with LPP at the 0.6 % apparently improved some histomorphological criteria that are negatively affected by increased temperature. There were no differences between treatments in the deodnum and ileum parts.

There are several possible reasons why middle intestine characteristics were unchanged in our study in response to elevated temperature, including the short duration of the heat shock and the resistance of these parts to structural.

Conclusion

Adding lemonpulp powder to the finisher diet might modify some blood components, but without beneficial effects on performance of broiler chickens under hot conditions. Further studies are required to fully explore dose-response effects on broiler chickens performance and the broiler chickens performance and physiology.

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