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ORIGINAL RESEARCH

# Effect of dietary graded levels of dried lemon (*citrus aurantifulia*) pulp on performance, intestinal morphology, and humoral immunity in broiler chickens

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**Abstract**
*Purpose* Dried lemon pulp (DLP) is a by-product of fruit processing industry and is containing active antioxidants such as flavonoids, isoflavones, and flavones. Thus, current experiment was carried out to evaluate the effect of dietary graded levels of DLP on performance, intestinal morphology, and humoral immunity in broiler chickens.
*Methods* Accordingly, a total of 280-day-old broiler chickens (Ross 308) were assigned to 4 treatments and 5 replicates of 14 chicks each. Dietary treatments included control (CON) with no additive as well as DLP1: 2.5, 5, and 7.5%; DLP2: 5, 7.5, and 10%; and DLP3: 7.5, 10, and 12% in starter, growing, and finisher phases, respectively. Subsequently, performance, intestinal morphology, and humoral immunity were evaluated throughout the experiment.
*Results* Body weight of chickens decreased when using graded levels of DLP during different periods of the experiment as compared with CON ($P < 0.05$). Also, daily weight gain was lower in those supplemented by 7.5% DLP than CON across finishing period ($P < 0.05$). Therefore, feed conversion ratio of broilers was impaired when using graded levels of DLP across the entire production phase compared to the birds in CON group ($P < 0.05$). Antibody titer against influenza disease virus and sheep red blood cells decreased when using DLP3 and DLP2, respectively ($P < 0.05$). Jejunal crypt depth decreased in chickens fed on DLP3 compared with control.
*Conclusion* Dietary graded levels of DLP modified intestinal segments while deteriorated growth performance of chickens. Furthermore, DLP3 decreased jejunal crypt depth. Thereby, the use of DLP particularly at high levels is not recommended.

**Keywords** Broiler chickens · Dried lemon pulp · Immunity · Intestinal morphology · Performance

## Introduction

Generally, about 60–80% of expenses in poultry production is related to feeding (Chaudry et al. 2004). As such, replacing traditional feed ingredients with waste by-products may contribute to the development of the cost efficiency in poultry diets. Besides, agro-industrial waste is a matter of great concern and a big problem for fruit- and vegetable-based industries. The wastes left after processing, are, however, rich in some essential nutrients that have the potency to be supplemented in animal diets as by-products. Incorporation of fruits and vegetable wastes in animal feeds may improve palatability of diet and consequently increase the feed consumption in addition to decrease the cost of the feed (Chaudry et al. 2004). Iran is producing about 3.5% of the world total citrus production and subsequently has been encountered with enormous citrus wastes. The major by-products of citrus family include dry pulp, molasses, washed pulp solids, and essential oils. Citrus pulp contains active antioxidants encompass of flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins (Nobakht 2013). Flavonoids may decrease the blood

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cholesterol and quench free radicals (Hougee et al. 2005). Dietary replacement of the feed corn by sweet orange up to 15% has been previously reported (Oluremi et al. 2006). Agu et al. (2010) substituted dietary maize by sweet orange up to 20% with no deleterious impact on the performance and carcass characteristics in broiler chickens. The nutrient digestibility improved, when the yellow corn was replaced up to 20 and 40% with the lemon pulp and with 20 and 60% orange pulp in rabbit diets (Ibrahim et al. 2011). Furthermore, Nobakht (2013) indicated that dried lemon pulp (DLP) improved growth performance of chickens across the entire production period. Moreover, citrus pulp was indicated to expand the gastrointestinal tract in broiler chickens (Mourão et al. 2008). Nevertheless, effects of dried lemon pulp (DLP; *citrus aurantifulia*) on the morphology of small intestine and humoral immunity in broilers are less investigated. Thus, the objective of current trial was to evaluate the effect of dietary graded levels of DLP on growth performance, intestinal morphology, and humoral immunity in broiler chickens.

## Methods

### Birds and management

Two-hundred-eighty-day-old mixed sex broiler chicks (Ross 308) were purchased from a local hatchery, weighted individually on arrival, and randomly allocated to 4 treatments and 5 replicates of 14 chicks each in completely randomized design. The effect of DLP was evaluated through the 2.5, 5, and 7.5% addition of DLP (DLP1), 5, 7.5, and 10% (DLP2), and 7.5, 10, and 12% (DLP3) to the basal diet (CON) in the starter (1–14), growing (14–28), and finisher (28–42) periods, respectively. Chicks housed in 1.2 × 1.2 wire floor pens covered with paper roll and had free access to mash feed and water throughout the trial. Experimental diets were formulated to provide more or exceed nutritional requirements for broiler chickens based on Ross Broiler Manual (Aviagen 2009). The ambient temperature was gradually decreased from 33 to 25 °C on day 21 and was then kept constant. Ingredients and nutrient specifications of experimental diets are shown in Table 1. All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch.

### Preparation of dried lemon pulp and analysis

Wet lemon pulp were collected from juice counters and dried under sunlight, which were afterwards mixed well together, and their chemical composition was determined (Table 2). Crude protein of diet samples were determined using method 990.03 (AOAC 2000). Crude fiber was measured by sequential extraction with diluted acid and alkali (method 978.10) and Ca and P by spectrophotometry (methods 968.08 and 965.17) as indicated by AOAC (2000). Ether extract (EE) of the fiber sources was analyzed by Soxhelt fat analysis after acid hydrolysis (method 954.02) as described by AOAC (2000). The neutral detergent fiber (NDF) was determined as described by Van Soest et al. (1991) and expressed on ash-free basis. The moisture and ash contents were determined based on methods reported by Debon and Tester (2001). Content of non-fiber carbohydrates (NFC) was determined using the formula: $NFC = 100 - (CP + ash + EE + NDF)$. Nitrogen-free extract (NFE) was determined by $NFE = 100 - (CP + ash + CF + EE)$.

### Performance parameters

Broilers were weighed on days 1, 14, 28, and 42 of age. Daily feed intake (DFI) and daily weight gain (DWG) of chicks in each pen were recorded in different phases of experiment. The feed conversion ratio (FCR; feed intake/weight gain) was calculated.

On days 24 and 42 of experiment, two male birds close to the mean body weight (BW) of pen were selected, individually weighed, and slaughtered. Liver and heart were collected, weighed, and expressed as a percentage of live BW on day 24. Also, noted organs as well as carcass yield and abdominal fat were reported as a percentage of live BW on day 42. Proportions of digestive organs including pancreas, gizzard, small intestine, and cecum were calculated for both days 24 and 42 of age. The length of intestinal segments consisted of duodenum, jejunum, ileum, and cecum were also measured and recorded.

### Morphology of small intestine

On day 24 of age, two male birds of each pen were slaughtered and intestinal samples were taken immediately from the jejunum; midway between the point of entry of the bile ducts and Meckel's diverticulum, ileum; 10 cm proximal to the ileo-cecal junction were taken to evaluate the villus height, crypt depth, and villus height:crypt depth ratio. Segments which were 1.5 cm in length were flushed with saline and fixed in 100 g/l buffered formalin (pH = 7.0). The fixed intestinal samples embedded in paraffin were then sectioned (5 μm) and stained with hematoxylin-eosin and examined by light microscope (Olympus CX31, Tokyo, Japan). Villus height (μm) was measured from the tip of the villus to the villus crypt junction and crypt depth was measured from the base upward to the region of transition between the crypt and villus. Villus height-to-crypt depth ratio (V/C) was then calculated.

**Table 1** Ingredients and nutrient specifications of experimental diets applied in starter, grower, and finisher periods

| | Starter (1–14 days) | | | | Grower (14–28 days) | | | | Finisher (28–42 days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 | CON | DLP1 | DLP2 | DLP3 | CON | DLP1 | DLP2 | DLP3 |
| Ingredients | | | | | | | | | | | | |
| Corn (8% CP) | 50.10 | 48.96 | 48.06 | 43.78 | 51.48 | 51.70 | 50.69 | 47.40 | 55.70 | 55 | 53.95 | 50.61 |
| Soybean meal (44% CP) | 39 | 39.40 | 39.70 | 39.70 | 33.60 | 34.60 | 34.90 | 34.90 | 27.40 | 28.60 | 29 | 29.50 |
| Dried lemon pulp | 0 | 2.50 | 5 | 7.50 | 0 | 5 | 7.50 | 10 | 0 | 7.50 | 10 | 12 |
| Wheat bran | 4 | 2 | 0 | 0 | 8 | 2 | 0 | 0 | 10 | 2 | 0 | 0 |
| Soybean oil | 2.70 | 3 | 3.20 | 4.10 | 3.43 | 3.33 | 3.60 | 4.45 | 3.60 | 3.80 | 4 | 4.90 |
| Dicalcium phosphate | 1.70 | 1.70 | 1.70 | 1.70 | 1.45 | 1.45 | 1.45 | 1.45 | 1.22 | 1.23 | 1.23 | 1.22 |
| Calcium carbonate | 1.05 | 1 | 0.90 | 0.85 | 0.96 | 0.83 | 0.76 | 0.70 | 0.87 | 0.68 | 0.62 | 0.55 |
| DL-Methionine | 0.33 | 0.34 | 0.34 | 0.35 | 0.16 | 0.17 | 0.18 | 0.18 | 0.23 | 0.25 | 0.25 | 0.26 |
| L-Lysine | 0.23 | 0.21 | 0.21 | 0.22 | 0.08 | 0.08 | 0.08 | 0.08 | 0.15 | 0.15 | 0.15 | 0.16 |
| L-Threonine | 0.09 | 0.09 | 0.09 | 1 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.05 |
| Vitamin premix[a] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral premix[b] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Sodium chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.25 | 0.25 | 0.25 | 0.25 |
| Calculated composition | | | | | | | | | | | | |
| ME (kcal/kg) | 2850 | 2850 | 2850 | 2850 | 2900 | 2900 | 2900 | 2900 | 2940 | 2940 | 2940 | 2940 |
| Crude protein (%) | 21.8 | 21.8 | 21.8 | 21.8 | 20.1 | 20.1 | 20.1 | 20.1 | 17.9 | 17.9 | 17.9 | 17.9 |
| Lysine (%) | 1.37 | 1.37 | 1.37 | 1.37 | 1.09 | 1.09 | 1.09 | 1.09 | 1.06 | 1.06 | 1.06 | 1.06 |
| Threonine (%) | 0.92 | 0.92 | 0.92 | 0.92 | 0.77 | 0.77 | 0.77 | 0.77 | 0.72 | 0.72 | 0.72 | 0.72 |
| Met + Cys (%) | 1.03 | 1.03 | 1.03 | 1.03 | 0.81 | 0.81 | 0.81 | 0.81 | 0.83 | 0.83 | 0.83 | 0.83 |
| Calcium (%) | 0.91 | 0.91 | 0.91 | 0.91 | 0.81 | 0.81 | 0.81 | 0.81 | 0.72 | 0.72 | 0.72 | 0.72 |
| Available phosphorous (%) | 0.46 | 0.46 | 0.46 | 0.46 | 0.41 | 0.41 | 0.41 | 0.41 | 0.36 | 0.36 | 0.36 | 0.36 |
| Fiber (%) | 4.27 | 4.47 | 4.69 | 5.04 | 4.36 | 4.64 | 4.86 | 5.23 | 4.71 | 4.74 | 4.96 | 5.27 |

CON: control; DLP1, 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively

[a] Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; 100 mg ethoxyquin as antioxidant

[b] Mineral premix per kg of diet: Fe ($FeSO4{\cdot}7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO4{\cdot}H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO4{\cdot}5H_2O$), 10 mg; I (KI, 58% I), 1 mg; Se (NaSeO3, 45.56% Se), 0.2 mg

## Immune responses

On day 9 of age, Newcastle and influenza antigens were injected to chickens with 0.2 ml per chick with dual vaccine of Newcastle-influenza. Also, chicks were orally vaccinated against Newcastle Disease (Lasota) on day 19 of age. Two chickens per pen were selected randomly for intraperitoneal injection with a 1.0 ml of sheep red blood cells (SRBC) suspension diluted with phosphate buffered saline (pbs) on day 25. 5 days later, and the same wing-banded birds were bled to determine antibody titer against SRBC as well as influenza (IDV) and Newcastle disease viruses (NDV). Subsequently, antibody titer against SRBC was measured by hemagglutination assay method. Antibody titer against influenza and Newcastle separately was measured by hemagglutination inhibition method. Hemagglutination inhibition antibodies were then converted to $\log_2$. Antibody titers against SRBC were measured by the microtitre procedure described by Wegmann and Smithies (1966). On day 24 and at the end of the experiment, two birds were slaughtered after taking blood samples and then the spleen and bursa of Fabricius were weighed to evaluate the immune system development.

## Statistical analysis

Data were subjected to the analysis of variance appropriate for a completely randomized design using General Linear Model (GLM) procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). If a significant effect was detected, differences between treatments were separated using LSD test. Statements of statistical significance are based on a probability of $P < 0.05$.

**Table 2** Composition of the dried lemon pulp

| Dried lemon composition | (%) |
|---|---|
| Crude protein | 9.20 |
| Crude fiber | 17.50 |
| Ash | 6.10 |
| Ca | 1.06 |
| Total phosphorous | 0.16 |
| Moisture | 5.80 |
| Nitrogen-free extract | 57.70 |
| Neutral detergent fiber | 23.70 |
| Non-fiber carbohydrates | 51.50 |
| Ether extract | 3.73 |

## Results

### Performance parameters

According to Table 3, feeding graded levels of DLP decreased DWG of chickens during starter, finisher, and the entire production period compared with the CON group ($P < 0.05$). Therefore, chickens had lower BW than CON birds in different rearing periods ($P < 0.05$). Chickens fed on DLP1 had lower DFI than the other groups during the 28–42 and also 1–42 days of age ($P < 0.05$). These variations in DWG and DFI resulted in impaired FCR in chickens supplemented with DLP2 and DLP3 compared with CON across starter and growing periods ($P < 0.05$). Moreover, chickens received graded levels of DLP had inferior FCR than CON across 28–42 as well as the entire production period ($P < 0.05$).

### Carcass measurements and digestive organ relative weights

Data regarding the effects of dietary treatments on carcass measurements and digestive organ relative weights are summarized in Tables 4, 5, and 6. Feeding diets supplemented with DLP increased liver proportional weight compared with CON on day 24 of age ($P < 0.05$). Likewise, DLP3 elevated the liver relative weight on day 42 ($P < 0.05$). Dietary graded levels of DLP increased duodenum and jejunum weights compared with CON on day 24 of age ($P < 0.05$). In ileum, proportional weight increased in response to DLP2 and DLP3 compared with DLP1 and CON ($P < 0.05$). Furthermore, chickens had lengthened cecum in comparison to the birds in CON group ($P < 0.05$). Results observed on day 42 was similar to those displayed on day 24.

### Morphology of small intestine

Chickens receiving DLP3 had decreased jejunal crypt depth compared with CON and DLP1 ($P < 0.05$; Table 7). It resulted in greater V/C of them than the birds in CON and DLP1. Dietary treatments had no effect on the ileal morphometric features.

### Immune responses

Results of immune-related parameters are shown in Table 8. The lowest antibody titer against IDV was observed in chickens supplemented with DLP3 that was substantially lower than CON and DLP2 ($P < 0.05$). Moreover, dietary administration of DLP2 significantly decreased antibody titer against SRBC as compared with the chickens in CON group ($P < 0.05$). Relative weight of the spleen was significantly greater in birds applied with DLP2 than CON and DLP3, whereas both DLP1 and DLP2 increased the proportional weight of the bursa of Fabricius compared with CON ($P < 0.05$).

## Discussion

In this study, feeding graded levels of DLP decreased DFI in broiler chickens. This lower feed intake might be due to the high pectin content of DLP. Similar results have been observed when chickens are given graded dietary levels of pectin (Langhout and Schutte 1996; Langhout et al. 1999). Pectin is a kind of soluble fiber that increases the intestinal viscosity and results in the enhanced retention time of the feed in gastrointestinal tract. Further, a negative correlation exists between the feed retention time and DFI in chickens of early ages (Almirall and Esteve-Garcia 1994). On the contrary, Chaudry et al. (2004) have shown that chickens receiving citrus peel had similar feed consumption to the birds in CON group. Otherwise, the increased DFI of broilers has been observed after dietary inclusion of citrus pulp or DLP (Mourão et al. 2008; Nobakht 2013). The lower DFI of chickens in our study might lead to reduced DWG and BW following supplementation with DLP. One more reason is possibly the decrease in nutrient digestibility, since soluble fiber alleviates diffusion rate of digestive enzymes into digesta and therefore decreases the nutrient utilization (Annison 1993). Different DFI and DWG of chickens consequently resulted in deteriorated FCR across various periods of production.

Although carcass characteristics such as carcass yield and abdominal fat were not influenced by dietary treatments, proportional weight of liver increased when using DLP in different levels on day 24 of age. These results are in contrast to those reported by Saki et al. (2011) who

**Table 3** Effects of dietary treatments on performance of broilers at different ages

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 |
| BW (g) | | | | |
| 14 days | 409.2$^{a}$ ± 7.41 | 384.7$^{b}$ ± 4.97 | 365.5$^{b}$ ± 5.84 | 369.8$^{b}$ ± 6.31 |
| 28 days | 1174.5$^{a}$ ± 1984 | 1091.4$^{b}$ ± 14.03 | 1031.3$^{c}$ ± 29.26 | 984.7$^{c}$ ± 12.98 |
| 42 days | 2404.7$^{a}$ ± 2.26 | 2086.1$^{b}$ ± 1.46 | 1957.4$^{c}$ ± 22.60 | 1899.1$^{d}$ ± 14.35 |
| DWG (g/day) | | | | |
| 1–14 days | 27.5$^{a}$ ± 0.65 | 24.9$^{b}$ ± 0.35 | 23.5$^{b}$ ± 0.42 | 23.8$^{b}$ ± 0.45 |
| 14–28 days | 53.4$^{a}$ ± 1.38 | 51.1$^{a}$ ± 0.30 | 47.7$^{b}$ ± 1.31 | 44.3$^{c}$ ± 0.48 |
| 28–42 days | 86.9$^{a}$ ± 0.87 | 71.8$^{b}$ ± 1.97 | 66.3$^{c}$ ± 2.02 | 65.7$^{c}$ ± 1.02 |
| 1–42 days | 55.4$^{a}$ ± 0.28 | 48.8$^{b}$ ± 0.67 | 41.7$^{c}$ ± 0.99 | 41.8$^{c}$ ± 0.05 |
| DFI (g/day) | | | | |
| 1–14 days | 36.4 ± 0.75 | 36.4 ± 0.93 | 36.7 ± 1.31 | 35.8 ± 1.08 |
| 14–28 days | 97.2 ± 0.24 | 98.5 ± 2.47 | 95.7 ± 1.82 | 96.6 ± 1.77 |
| 28–42 days | 182.8$^{a}$ ± 1.39 | 173.7$^{b}$ ± 0.69 | 183.3$^{a}$ ± 4.42 | 181.8$^{a}$ ± 1.40 |
| 1–42 days | 104.3$^{ab}$ ± 0.52 | 100.3$^{c}$ ± 0.05 | 105.8$^{a}$ ± 0.99 | 103.4$^{b}$ ± 0.75 |
| FCR | | | | |
| 1–14 days | 1.33$^{b}$ ± 0.03 | 1.47$^{ab}$ ± 0.05 | 1.56$^{a}$ ± 0.06 | 1.50$^{a}$ ± 0.05 |
| 14–28 days | 1.82$^{c}$ ± 0.05 | 1.93$^{bc}$ ± 0.05 | 2.01$^{ab}$ ± 0.08 | 2.18$^{a}$ ± 0.04 |
| 28–42 days | 2.10$^{c}$ ± 0.06 | 2.42$^{b}$ ± 0.06 | 2.76$^{a}$ ± 0.14 | 2.76$^{a}$ ± 0.06 |
| 1–42 days | 1.88$^{c}$ ± 0.01 | 2.05$^{b}$ ± 0.02 | 2.53$^{a}$ ± 0.09 | 2.47$^{a}$ ± 3.03 |

CON: control; DLP1: 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively; BW: body weight; DWG: daily weight gain; DFI: daily feed intake; FCR: feed conversion ratio

[a–c] Means in the same row with different superscript differ significantly ($P < 0.05$)

**Table 4** Effects of dietary treatments on carcass measurements

| Carcass contents[A] | Dietary treatments | | | |
|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 |
| Carcass yield (%) | | | | |
| Day 42 | 70.9 ± 1.44 | 68.3 ± 1.11 | 68.0 ± 1.20 | 67.7 ± 0.54 |
| Abdominal fat (%) | | | | |
| Day 42 | 1.81 ± 0.09 | 1.98 ± 0.09 | 1.86 ± 0.10 | 2.01 ± 0.10 |
| Heart (%) | | | | |
| Day 24 | 0.58 ± 0.02 | 0.57 ± 0.02 | 0.56 ± 0.02 | 0.58 ± 0.02 |
| Day 42 | 0.52 ± 0.05 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.50 ± 0.04 |
| Liver (%) | | | | |
| Day 24 | 2.26$^{b}$ ± 0.06 | 2.78$^{a}$ ± 0.28 | 3.06$^{a}$ ± 0.15 | 3.29$^{a}$ ± 0.12 |
| Day 42 | 2.25$^{b}$ ± 0.17 | 2.27$^{ab}$ ± 0.20 | 2.06$^{ab}$ ± 0.08 | 2.51$^{a}$ ± 0.02 |

CON: control; DLP1: 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively

[A] Digestive organs are expressed as a percentage of live body weight

[a, b] Means in the same row with different superscript differ significantly ($P < 0.05$)

remarked depression in the liver weight of broilers along with addition of soluble fiber sources to the diet. Relative weight of the intestinal segments also increased with consumption of DLP. This could be in response to high pectin content of DLP that increases the digesta viscosity and induces the gastrointestinal tract to show a rapid

**Table 5** Effects of dietary treatments on digestive organs relative weights and length of the intestine on day 24

| Digestive organs | Dietary treatments | | | |
|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 |
| Gizzard (%) | 2.25 ± 0.15 | 2.38 ± 0.20 | 2.43 ± 0.13 | 2.63 ± 0.25 |
| Pancreas (%) | 0.39 ± 0.04 | 0.44 ± 0.04 | 0.43 ± 0.02 | 0.43 ± 0.01 |
| Proventriculus (%) | 0.61 ± 0.02 | 0.66 ± 0.04 | 0.65 ± 0.04 | 0.71 ± 0.03 |
| Duodenum (%) | $1.08^{b}$ ± 0.05 | $1.54^{a}$ ± 0.09 | $1.76^{a}$ ± 0.13 | $1.70^{a}$ ± 0.16 |
| Jejunum (%) | $2.06^{b}$ ± 0.11 | $2.79^{a}$ ± 0.13 | $2.77^{a}$ ± 0.22 | $3.12^{a}$ ± 0.13 |
| Ileum (%) | $1.48^{b}$ ± 0.09 | $1.89^{ab}$ ± 0.28 | $2.23^{a}$ ± 0.19 | $1.95^{a}$ ± 0.20 |
| Cecum (%) | 0.64 ± 0.06 | 0.68 ± 0.08 | 0.72 ± 0.07 | 0.60 ± 0.10 |
| Length of intestine (cm) | | | | |
| Duodenum | 57.4 ± 2.01 | 59.4 ± 2.56 | 63.2 ± 4.19 | 63.8 ± 1.88 |
| Jejunum | 51.8 ± 4.85 | 62 ± 4.18 | 60.2 ± 4.33 | 59.8 ± 3 |
| Ileum | 66.2 ± 0.73 | 67.2 ± 1.36 | 68.6 ± 1.91 | 67.2 ± 0.58 |
| Cecum | $24.8^{b}$ ± 0.37 | $26.2^{ab}$ ± 0.20 | $27.2^{ab}$ ± 1.80 | $29^{a}$ ± 0.31 |

Digestive organs are expressed as a percentage of live body weight

CON: control, DLP1: 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively

[a, b] Means in the same row with different superscript differ significantly ($P < 0.05$)

**Table 6** Effects of dietary treatments on digestive organs relative weights and length of the intestine on day 42

| Digestive organs | Dietary treatments | | | |
|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 |
| Gizzard (%) | 1.40 ± 0.06 | 1.45 ± 0.05 | 1.44 ± 0.04 | 1.46 ± 0.06 |
| Pancreas (%) | 0.22 ± 0.01 | 0.21 ± 0.01 | 0.21 ± 0.01 | 0.24 ± 0.03 |
| Proventriculus (%) | 0.41 ± 0.02 | 0.42 ± 0.03 | 0.43 ± 0.02 | 0.47 ± 0.04 |
| Duodenum (%) | $1.08^{b}$ ± 0.05 | $1.54^{a}$ ± 0.09 | $1.76^{a}$ ± 0.13 | $1.70^{a}$ ± 0.16 |
| Jejunum (%) | $2.06^{b}$ ± 0.11 | $2.79^{a}$ ± 0.13 | $2.77^{a}$ ± 0.22 | $3.12^{a}$ ± 0.13 |
| Ileum (%) | $1.48^{b}$ ± 0.09 | $1.89^{ab}$ ± 0.28 | $2.23^{a}$ ± 0.19 | $1.95^{a}$ ± 0.20 |
| Cecum (%) | 0.64 ± 0.06 | 0.68 ± 0.08 | 0.72 ± 0.07 | 0.60 ± 0.10 |
| Length of intestine (cm) | | | | |
| Duodenum | 57.4 ± 2.01 | 59.4 ± 2.56 | 63.2 ± 4.19 | 63.8 ± 1.88 |
| Jejunum | 51.8 ± 4.85 | 62 ± 4.18 | 60.2 ± 4.33 | 59.8 ± 3 |
| Ileum | 68.2 ± 0.73 | 72.2 ± 1.36 | 68.6 ± 1.91 | 72.2 ± 0.58 |
| Cecum | $24.8^{b}$ ± 0.37 | $26.2^{ab}$ ± 0.20 | $27.2^{ab}$ ± 1.80 | $29^{a}$ ± 0.31 |

Digestive organs are expressed as a percentage of live body weight

CON: control; DLP1: 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively

[a, b] Means in the same row with different superscript differ significantly ($P < 0.05$)

adaptation in reaction to the lower diffusion rates (Iji et al. 2001). Our results are in line with Jiménez-Moreno et al. (2009) and González-Alvarado et al. (2010) in which greater weight of intestine following consumption of soluble fiber types was observed.

The crypt is considered as the villus cell producer. In this respect, a deeper crypt shows rapid tissue turnover and a high demand for new tissue (Choct 2009). Interestingly, DLP3 reduced crypt depth in this study; however, the larger crypt depth was expected. This might be attributed to the presence of active compounds such as tannins in the DLP. Feeding diets rich in soluble fiber usually lead to the presence of high viscosity digesta in the lumen, increase the rate of villus cell losses, and elevate the cell proliferation, subsequently increasing the crypt depth (Montagne et al. 2003).

**Table 7** Effects of dietary treatments on intestinal morphology

| Parameters | Dietary treatments | | | |
|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 |
| Jejunum | | | | |
| Villus height (μm) | 1666 ± 67.60 | 1710 ± 68.91 | 1712 ± 37.88 | 1580 ± 117.34 |
| Crypt depth (μm) | $266^{a}$ ± 21.08 | $251^{a}$ ± 21.05 | $216^{ab}$ ± 14.36 | $180^{b}$ ± 12.95 |
| V/C[b] | $6.55^{b}$ ± 0.40 | $7.30^{b}$ ± 0.48 | $7.33^{ab}$ ± 0.26 | $8.48^{a}$ ± 0.41 |
| Ileum | | | | |
| Villus height (μm) | 985 ± 32.39 | 925 ± 56.02 | 1091 ± 118.8 | 900 ± 80.6 |
| Crypt depth (μm) | 187 ± 12.22 | 153 ± 14.32 | 175 ± 17.58 | 161 ± 13.77 |
| V/C | 5.51 ± 0.34 | 6.14 ± 0.47 | 6.76 ± 0.89 | 5.71 ± 0.46 |

CON: control; DLP1: 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively; V/C: villus height/crypt depth

[a–c] Means in the same row with different superscript differ significantly ($P < 0.05$)

**Table 8** Effect of treatments on immune-related parameters and lymphoid organs

| Parameters | Dietary treatments | | | |
|---|---|---|---|---|
| | CON | DLP1 | DLP2 | DLP3 |
| IDV ($log_2$) | $5.50^{a}$ ± 0.17 | $5.10^{ab}$ ± 0.23 | $5.50^{a}$ ± 0.22 | $4.80^{b}$ ± 0.13 |
| NDV ($log_2$) | 5 ± 0.21 | 4.90 ± 0.38 | 5.20 ± 0.39 | 5 ± 0.30 |
| SRBC ($log_2$) | $7.50^{a}$ ± 0.27 | $6.90^{ab}$ ± 0.43 | $5.80^{b}$ ± 0.57 | $6.80^{ab}$ ± 0.39 |
| Spleen | | | | |
| Day 42 (%) | $0.13^{b}$ ± 0.01 | $0.16^{ab}$ ± 0.01 | $0.17^{a}$ ± 0.01 | $0.13^{b}$ ± 0.01 |
| Bursa of Fabricius | | | | |
| Day 42 (%) | $0.1^{b}$ ± 0.008 | $0.17^{a}$ ± 0.01 | $0.16^{a}$ ± 0.02 | $0.14^{ab}$ ± 0.02 |

CON: control, DLP1: 2.5, 5, and 7.5% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP2: 5, 7.5, and 10% dried lemon pulp in starter, growing, and finisher periods, respectively; DLP3: 7.5, 10, 12% dried lemon pulp in starter, growing, and finisher periods, respectively; IDV: influenza disease virus; NDV: newcastle disease virus; SRBC: sheep red blood cells

[a, b] Means in the same row with different superscript differ significantly ($P < 0.05$)

Chickens fed on DLP3 had lower antibody titer against IDV than CON birds. Moreover, antibody titer against SRBC decreased in broilers fed on DLP2. In contrast to our data, Abbasi et al. (2015) found that supplementing citrus pulp up to 2% of diet had no impact on humoral immunity of broiler chickens. Nobakht (2013) indicated that feeding 5% DLP tended numerically to increase the heterophil-to-lymphocyte ratio as a stress indicator that may impair immunity. These results imply that feeding high levels of DLP in broiler diets may apply negative impact on humoral immunity of broilers.

In conclusion, supplementing dietary graded levels of DLP to broiler diets deteriorated growth performance of chickens. Also, DLP developed the relative weight of intestinal segments and decreased jejunal crypt depth. Therefore, dietary consumption of DLP particularly at high levels is not recommended because it may compromise the growth performance of broiler chickens.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that there is no conflict of interest.



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