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Effects of Different Levels of Dried Lemon (*Citrus aurantifulia*) Pulp on Performance, Carcass Traits, Blood Biochemical and Immunity Parameters of Broilers

Research Article

A. Nobakht[1*]

[1] Department of Animal Science, Maragheh Branch, Islamic Azad University, Maragheh, Iran

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*Correspondence E-mail: anobakht20@Yahoo.com

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ABSTRACT

This experiment was conducted to evaluate the effects of different levels of dried lemon pulp (DLP) on performance, carcass traits, blood biochemical and immunity parameters of broilers. In this experiment 240 of broilers (Ross-308) were used in a completely randomized design with 4 treatments and 3 replicates (with 20 birds in each replicate) from 1 to 42 days. Experimental diets included: 1) control group with no DLP, in 2 until 4 treatments the levels of DLP were 0, 1.5, 3 and 4.5 percent. Inclusion different levels of dried lemon pulp on broilers diets had significant effects on their performance, carcass traits and blood biochemical parameters (P>0.05). Adding DLP into broiler diets significantly increased the amount of feed intake in starter, grower and whole experiment periods. Also it significantly increased the amount of daily weight gain in starter and whole experiment periods; however they were not observed any significant difference between treatments in daily weight gain in grower period. As the rate of feed intake to daily weight gain was high, especially in grower period in treatments, which contained DLP, it had adverse effect on feed conversion and led to be increased, in contrast with control group. There were no significant differences between experimental groups regarding to feed conversion in starter period (P>0.05). Using 3% DLP significantly decreased the abdominal fat and blood low density lipoprotein (LDL), whereas the lowest percent of gizzard was obtained by inclusion 1.5% DLP. Adding different levels of dried lemon pulp could not significantly affect the hematological parameters of broilers. According to these experimental results, using DLP up to 4.5% of broiler diets in starter period, would be possible without any adverse effects on their performance, however these levels in grower period increased significantly the ratio of daily feed intake to daily weight gain and had adverse effect on feed conversion. Moreover 1.5% DLP had desirable effects on reduce of abdominal fat and blood low density lipoproteins.

KEY WORDS broiler, carcass quality, dried lemon pulp, immunity, performance.

INTRODUCTION

The agro-industrial processing was resulted some wastes as vegetable and fruit by-products. The fruit byproducts use as energy source in animal feed and these by-products 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).

The active antioxidant compounds in citrus pulp are flavonoids, isoflavones, flavones, anthocyanins, coumarins,

lignans, catechins and isocatechins. In addition, some compounds are found in natural foods such as vitamin C (Prior, 2003).

Flavonoids are good for keeping human and animals healthy. Pectin is a kind of carbohydrate gel, a component of plant cell wall. Therefore it has high water absorption property and can be used for treating diarrhea, as well as its viscosity has significant health benefits. It has reported that flavonoids play role in reducing cholesterol, because the structure of flavonoids contains numerous OH group, which can supply H atoms to quench free radical.

This property makes it a strong antioxidant, meanwhile the anti-tumor activity, may play a role in prevention of cancer, heart disease, circulation and Alzhemers' disease (Shahelian, 2005).

It was found that digestible energy content of sugar beet pulp, soybean hulls, wheat bran and citrus pulp was 12.52, 7.06, 11.21 and 7.23 MJ/kg DM, respectively (Papadomichelakis *et al.* 2004). Content of sweet orange rind (SOR) for vitamin C was 3.88 mg/100 g vs zero mg/100 g for maize, and it could be used for replacing of dietary maize up to 15% (Oluremi *et al.* 2006). It was shown that in broilers, dietary substitute of sweet orange fruit peel for maize up to 20% level without any adverse effects on their performance and carcass traits was possible (Agu *et al*. 2010). Similar results were reported for rabbits (Hon *et al.* 2009).

It was found that inclusion of juice wastes mixture (carrot, apple, mango, orange, melon and tree tomato) up to 20% could be possible for the broiler diet (Rizal *et al.* 2010). In the other experiment, inclusion citrus pulp up to 5% into broiler diets gave similar results with control groups, where there was a significant ($P<0.05$) reduction in the blood cholesterol level with citrus peel feeding. Reduction in triglycerides and glucose concentration was statistically non-significant (Chaudry *et al.* 2004). Moreover, it was resulted that sweet orange fruit pulp meal could be used as replacement feedstuff for maize in the ration of growing rabbits up to 20%.

On the other hand, in comparison with the control treatment, increases in feed intake occurred in broilers consuming diets with 5 or 10% citrus pulp, which led to significantly higher feed conversion rates with the 10% level (Mourao *et al*. 2008).

In another experiment it was resulted that 30% replacement of maize by the fermented sweet orange fruit peel meal significantly depressed body weight gain, feed intake and live weight in broiler starter diets (Oluremi *et al*. 2010).

Recently, it was reported that replacing lemon pulp at levels of 20 and 40% and orange pulp at levels of 20 and 60% for yellow corn in rabbit diets achieved best nutrients digestibility and nutritive value (Ibrahim *et al.* 2011).

The present experiment was conducted to determine the effects of different levels of dried lemon pulp (DLP) on performance, carcass traits, blood biochemical, and immunity parameters of broilers.

MATERIALS AND METHODS

Animals and dietary treatments

This experiment was conducted to evaluate the effects of different levels of DLP on performance, carcass traits, blood biochemical, and immunity parameters of broilers. In this experiment 240 of broilers (Ross-308) were used in a completely randomized design with 4 treatments and 3 replicates (with 20 birds in each replicate) from 1 to 42 days. Experimental diets included: 1) control group with no dried lemon pulp (0%), 2) three other treatments included different levels of dried lemon pulp; 1.5, 3 and 4.5 percent. Wet lemon pulp collected from juice counters and dried under sunlight, afterwards well mixed together, one sample prepared for determine chemical composition. The compositions of DLP (Table 1) were determined according to AOAC (1990) and after fine milling, mixed with other ingredients.

The diets were formulated (Tables 2 and 3) to meet the requirements of broilers as established by the NRC (1994) in two breeding periods (starter 1-21 days, and grower 22-42 days). The diets and water was provided *ad libitum*. The lighting program during the experimental period consisted of a period of 23 hours light and 1 hour of darkness. Environmental temperature was gradually decreased from 33 ˚C to 25 ˚C on day 21 and was then kept constant.

Performance parameters

Body weight, feed intake and feed conversion were determined weekly on bird bases. Mortality was recorded.

Blood biochemical and immunity parameters

At 42 day of age two birds from each replicate (male and female) were randomly chosen for blood collection and approximate 5 mL blood samples were collected from the brachial vein. One ml of collected blood was transferred to tubes with EDTA for determination of immunity parameters include: red blood cells, hemoglobin, packed cell volume, white blood cells and lymphocytes (Gross and Siegel, 1983).

The remaining 4 mL blood was centrifuged to obtain serum for determination the blood biochemical parameters including: glucose, cholesterol, triglyceride, albumin, total protein and uric acid. Kit package (Pars Azmoon Company; Tehran, Iran) were used for determination the blood biochemical parameters using Anision-300 auto-analyzer system.

Table 1 The analysis results of dried lemon pulp (as 100% dry base) in 100 gram

Moisture (%)	Crude Protein (%)	Crude fat (%)	Crude fiber (%)	Calcium (%)	Phosphorous (%)
86.89	8.22	7.47	28.3	0.61	0.34

Table 2 The ingredients and nutrients composition of starter diets of broilers (1-21 days)

Diets	Control group	1.5% DLP	3% DLP	4.5% DLP
Ingredients (%)				
Yellow corn	55.15	53.11	51.07	49.04
Soybean meal	37.74	37.87	38	38.13
Canola oil	3.39	3.81	4.25	4.66
DLP	0	1.5	3	4. 5
Oyster shell	1.37	1.37	1.36	1.36
Dicalcium phosphate	1.40	1.39	1.37	1.36
Salt	0.28	0.28	0.28	0.28
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL-methionine	0.17	0.17	0.17	0.17
Calculated composition				
Metabolisable energy (kcal/kg)	3000	3000	3000	3000
Crude protein (%)	21.56	21.56	21.56	21.56
Calcium (%)	0.94	0.94	0.94	0.94
Available phosphorous (%)	0.42	0.42	0.42	0.42
Sodium (%)	0.14	0.14	0.14	0.14
Crude fiber (%)	3.97	4.26	4.56	4.85
Laysin (%)	1.23	1.23	1.23	1.23
Methionine + Cysteine (%)	0.87	0.87	0.87	0.87
Thryptophan (%)	0.28	0.28	0.28	0.28

[1] 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 (B_1): 1.8 mg; Riboflavin (B_2): 6.6 mg; Panthothenic acid (B_5): 10 mg; Pyridoxine (B_6): 3 mg; Cyanocobalamin (B_{12}): 0.015 mg; Niacin (B_3): 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant 100 mg.
[2] Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe): 50 mg; Mn (MnSO4.H2O, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu (CuSO4.5H2O): 10 mg; I (KI, 58% I): 1 mg and Se (NaSeO3, 45.56% Se): 0.2 mg.

Carcass components

At the 42th day of age, two birds per replicate were randomly chosen, slaughtered and percents of carcass, abdominal fat, gizzard, breast, thigh and liver percents to total weight were calculated.

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2005). Means were compared using the Duncan multiple range test. Statement of statistical significance was based on $P<0.05$.

RESULTS AND DISCUSSION

Performance and carcass traits

The effects of different levels of DLP in feeds on performance of broilers are summarized in Table 4.

Feed intake

In starter period adding 4.5% DLP and in grower and whole periods inclusion 1.5 to 4.5% DLP significantly increased the amounts of daily feed intake ($P<0.05$). Increase in daily feed intake by adding DLP was in agreement with findings of Mourao *et al.* (2008) and Rizal *et al.* (2010) and was not in agreement with report of Chaudry *et al.* (2004).

Rizal *et al.* (2010) were found that adding up to 20% of juice wastes mixture (carrot, apple, mango, orange, melon and tree tomato) in broiler diets, increased the amount of feed consumption. This was probably due to the increase in the palatability of diets caused by the acid content in the diet. According to the previous report (Cave, 1984), the addition of propionate to the diet increased feed consumption of broiler chickens, whereas, on the base of Chaudry *et al.* (2004), addition 5% citrus pulp into broiler diets did not have significant effects on feed consumption. Increasing the amount of feed intake as a result of adding (DLP) might be related to high dietary content of crude fiber. The negative effects of dietary fiber on broiler performances were intimately associated with a decrease in the digestion of nutrients and with a decrease of the diet AME (Tabook *et al.* 2006), which resulted from the birds' limited capacity to degrade non starch polysaccharides (NSP) in the small intestine (Hesselman and Aman, 1986; Pettersson and Aman, 1989). Insoluble fiber acted primarily by diluting the nutrients concentration, increasing digesta passage rate and reducing nutrient digestibility (Hetland *et al.* 2004).

Daily weight gain

In contrast with control group, inclusion DLP into broiler

Table 3 The ingredients and nutrients composition of grower diets of broilers (22-42 days)

Diets	Control group	1.5% DLP	3% DLP	4.5% DLP
Ingredients (%)				
Yellow corn	66.77	64.69	62.62	60.54
Soybean meal	29.19	29.30	29.41	29.53
Canola oil	0.63	1.1	1.56	2.02
DLP	0	1.5	3	4.5
Oyster shell	1.25	1.25	1.25	1.25
Dicalcium phosphate	1.31	1.31	1.31	1.31
Salt	0.3	0.3	0.3	0.3
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL-methionine	0.05	0.05	0.05	0.05
Calculated composition				
Metabolisable energy (kcal/kg)	3000	3000	3000	3000
Crude protein (%)	18.75	18.75	18.75	18.75
Calcium (%)	0.85	0.85	0.85	0.85
Available phosphorous (%)	0.38	0.38	0.38	0.38
Sodium (%)	0.14	0.14	0.14	0.14
Crude fiber (%)	3.61	3.92	4.21	4.51
Laysin (%)	1.02	1.05	1.07	1.11
Methionine + Cysteine (%)	0.67	0.67	0.67	0.67
Thryptophan (%)	0.24	0.79	0.79	0.79

[1] 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 (B_1): 1.8 mg; Riboflavin (B_2): 6.6 mg; Panthothenic acid (B_5): 10 mg; Pyridoxine (B_6): 3 mg; Cyanocobalamin (B_{12}): 0.015 mg; Niacin (B_3): 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant 100 mg.
[2] Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe): 50 mg; Mn (MnSO4.H2O, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu (CuSO4.5H2O): 10 mg; I (KI, 58% I): 1 mg and Se (NaSeO3, 45.56% Se): 0.2 mg.

Table 4 The effects of different levels of lemon pulp on performance of broilers in different experimental periods

		Starter (1-22 days)			
Treatments	1	2	3	4	SEM
Parameters					
Feed intake (g)	38.48^{b}	40.88^{ab}	41.02^{ab}	43.53^{a}	1.40
Weight gain (g)	23.68^{b}	27.74^{a}	26.51^{a}	26.80^{a}	0.48
Feed conversion (g: g)	1.63	1.47	1.55	1.63	0.05
		Grower (22-42 days)			
Treatments	1	2	3	4	SEM
Parameters					
Feed intake (g)	134^{b}	162.27^{a}	161.29^{a}	160.41^{a}	4.35
Weight gain (g)	64.38	66.71	64.33	64.44	2.21
Feed conversion (g: g)	2.09^{b}	2.45^{a}	2.51^{a}	2.50^{a}	0.03
		Whole period (1-42 days)			
Treatments	1	2	3	4	SEM
Parameters					
Feed intake (g)	85.95^{b}	101.46^{a}	101.13^{a}	100.37^{a}	2.24
Weight gain (g)	43.96^{b}	48.22^{a}	45.42^{ab}	44.81^{ab}	1.09
Feed conversion (g: g)	1.96^{c}	2.11^{b}	2.23^{a}	2.24^{a}	0.03

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

diets significantly increased the amount of daily weight gain in starter and whole experimental periods (P<0.05), although it had no significant difference among groups regarding to daily weight gain in grower period. However in three experiment periods, the highest amount of daily weight gain was obtained in group 2 by inclusion 1.5% DLP.

With aging and increasing amount of feed consumption, the difference among control group and experimental groups in daily weight gain declined.

Hence, there was no significant difference between groups regarding to feed intake in grower period, however in whole experiment period, the control group showed significant difference with group 2. This might be associated with increasing of dietary crude fiber. Since the amount of feed consumption was increased with aging of broilers, high amount of dietary crude fiber could be intake, and dietary adverse effects of crude fiber had been discussed already. Increased of daily weight gain by inclusion of DLP in our experimental diets was in agreement with Rizal *et al.*

(2010) reported results, whereas not in agree with Mourao *et al.* (2008) findings.

Table 5 The effects of different levels of lemon pulp on carcass traits of broilers

Treatments	1	2	3	4	SEM
Carcass traits (%)					
Carcass	70.01	71.20	71.07	70.64	0.53
Abdominal fat	2.65^{a}	2.40^{ab}	2.06^{b}	2.72^{a}	0.15
Gizzard	2.25^{a}	1.91^{c}	2.22^{b}	2.48^{a}	0.07
Breast	21.54	23.04	23.84	22.68	0.78
Thigh	19.50	18.84	18.72	18.68	0.33
Liver	2.37	2.26	2.16	2.54	0.11

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.

Feed conversion

Since in experimental groups in contrast with control group, the rate of feed consumption was much higher than daily weight gain, feed conversion significantly increased in grower and whole experimental periods, by addition DLP (P<0.05), therefore the best feed conversion in these periods belonged to control group. In starter period unlike for the feed consumption and daily weight gain, there were not only no significant difference between groups in feed conversion, but also the best numerically feed conversion was resulted in group 2, by inclusion 1.5% DLP. Because the amount of daily feed intake in comparison with grower period was low in starter period, the lowest amounts of undesirable compounds like crude fiber and non starch polysaccharides were eaten, consequently, the performance especially feed conversion was not impaired. The observation of feed conversion in our study was in agreement with findings of Rizal *et al.* (2010), however it was not with Mour ao *et al.* (2008).

According of Rizal *et al.* (2010), inclusion up to 20% dried juice wastes to broiler diets significantly improved the feed consumption, while according to Mourao *et al.* (2008), similar results in feed conversion were found in control group, as well as groups contained 5 and 10% citrus pulp.

Carcass traits

Among carcass traits, just the percents of abdominal fat and gizzard significantly affected by inclusion of DLP in broiler diets (P<0.05). The lowest percent of abdominal fat (2.06%) was observed in group 3, whereas the lowest percent of gizzard (1.91%) obtained in group 2. However, DLP had numerically beneficial effects on carcass and breast percents. The present findings in abdominal fat were in agreement with reports of Rizal *et al*. (2010). They reported the use of dried juice waste up to 20% in broiler diets significantly decreased the percentage of abdominal fat, although it had no significant effects on gizzard percent. The decrease in abdominal fat pad percentage was due to the increase in the fiber content of diets.

Cherry and Jones (1982) reported that the crude fiber in the diet could reduce the fat content of broilers. Our observation in gizzard percent was in contrast with reported results of Chaudry *et al.* (2004), who resulted that inclusion 5% and 10% citrus pulp in broiler diets, significantly increased the percentage of gizzard. They mentioned that high crude fiber of diets was the main factor for increase of gizzard percent.

Blood biochemical and immunity parameters

The only low density lipoproteins (LDL) was significantly affected by inclusion different levels of DLP in broiler diets (P<0.05).

Table 6 The effects of different levels of lemon pulp on blood parameters of broilers

Treatments	1	2	3	4	SEM
Blood parameters					
Cholesterol (mg/dL)	82.31	99.59	127.80	107.33	20.59
Triglyceride (mg/dL)	39.30	52.40	45.03	40.79	6.96
Uric Acid (mg/dL)	3.44	2.46	4.34	3.94	0.95
HDL (%)	85.44	73.96	87.36	73.60	8.64
LDL (%)	22.23^{ab}	13.74^{b}	31.70^{a}	25.60^{ab}	4.60

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean; HDL: high density lipoprotein and LDL: low density lipoprotein.

Table 7 The effects of different levels of lemon pulp on blood immunity cells of broilers

Treatments	1	2	3	4	SEM
Immunity parameters					
Red blood cell (×10^{9}/L)	2.63	2.63	2.73	2.95	0.27
Hemoglobin (g/dL)	8.6	10.84	10.73	9.44	0.8
Packed cell volume (%)	28	32.83	32.34	28.17	2.35
White blood cell (×10^{6}/L)	13167	17000	14833	14500	2060
Heterophil (%)	17.34	20	16.34	27.34	4.71
Lymphocyte (%)	80.67	78.67	81.34	72.67	4.92
Heterophil / lymphocyte	0.219	0.269	0.210	0.390	0.08

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean; HDL: high density lipoprotein and LDL: low density lipoprotein.

The lowest LDL (13.74 mg/dL) was obtained in group 2. There was not any significant difference between treatments in other biochemical and immunity parameters. Like other citrus pulps, DLP was a rich source of pectin (Baker, 1994). It was shown that pectin had desirable effect on blood biochemical parameters in human and animals. Dietary incorporation of pectin appeared to affect several metabolic and digestive processes; those of principal interest were the effects on glucose absorption and cholesterol levels. Numerous studies (Jenkins *et al*. 1976; Kanter *et al*. 1980; Levitt *et al*. 1980; Poynard *et al*. 1980; Schwartz *et al*. 1988) with human subjects and animal models showed that pectin added to a test meal, significantly reduced the rate of glucose uptake. In addition, dietary supplementation with pectin had been shown to reduce serum cholesterol levels.

CONCLUSION

According to this experiment's results, using DLP up to 4.5% of broiler diets in starter period could be possible without any adverse effects on their performance, although these levels in grower period increased significantly the ratio of daily feed intake to daily weight gain and had adverse effects on feed conversion. Moreover, 1.5% DLP showed desirable effects on reduction of abdominal fat and blood low density lipoproteins.

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