Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057 OPEN ACCESS 2020 15 (2): 13-20

Portulaca Oleracea Leaves in Broiler Diets: Effect on Chicken Performance and Health

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Received: 20 April 2020 ▪ Revised: 07 June 2020 ▪ Accepted: 17 August 2020

Abstract: **Background:** Portulaca oleracea L. which is commonly called purslane is a herbaceous weed that is widely distributed throughout the world. Purslane is listed in the World Health Organization as one oaf the most used medicinal plants. The purslane is the richest vegetable sources of omega-3 fatty acids, flavonoids (kaempferol, quercetin and apigenin), vitamins A, C, E and minerals. To study the impact of using Portulaca oleracea in boiler diet on performance and health of chicken. **Method:** Chemical composition of Portulaca oleracea leaves was performed, then five iso-nitrogenous and iso-caloric experimental broiler diets were formulated as MOL0%, POL5%, POL10%, POL15% and POL20%, respectively and supplemented to broilers (10 chicks in each concentration) for 42 day. After 42nd day. **Results:** The chemical composition of Portulaca oleracea leaves showed average proportions of crude fiber, crude lipids, crude protein, total sugars, reducing sugar and non-reducing sugars. The highest effect of supplementation of Portulaca oleracea poultry diets on body weight of broiler, was 2024, 2112 and 2341gm, of treatments (10, 15 and 20% of POL), respectively. Also, the more effective treatment was 20% of POL of blood biochemical, lipid profile, Liver function and haematological parameters, comparing with normal diets.

Keywords: Portulaca Oleracea, Broiler Diets, Chicken, Performance, Health.

INTRODUCTION

Portulaca oleracea L. (Purslane) is a common type of weed in field crops and turf grass areas [1,2]. Purslane is annual, succulent, with fleshy green purplish stem and alternate fleshy leaves, either erect or decumbent herbaceous, it grows up to 30 cm in high [3,4].It belongs to the family Portulacaceae, it is a small family involving 21 genera and 580 species [5].There are varieties of purslane grows in wide range of regions and climate under several names [3]. Purslane is distributed around the world, Portulaca oleracea is native of man regions in Europe, and it can be found also in the West and East Indies, Japan, China, and Ascension Island [6]. Portulaca oleraceahas been cultivated in the Middle East in the middle ages [7].Purslane is used for the treatment of headache, burns, disease related to the intestine, stomach, liver, shortness of breath, cough, and arthritis[3].Several activities of succulent was reported in literatures; including antioxidant, anticancer, neuroprotective, antiulcer, anti-inflammatory, nephroprotective, hepatoprotective, antidiabetic, hypocholestremic, antifungal, and antibacterial activities [4]. Purslane was demonstrated to have better nutritional quality compared to the major vegetables which contain higher ascorbic acid, alpha-linolenic acid, and beta-carotene[8]. Alpha-linolenic acid is an omega-3 fatty acid which plays an important role in the development and growth of the human, as well as preventing diseases [9]. Purslane contains more amount of omega-3 fatty acids compared to other leafy vegetable plants [7]. Purslane also contains carbohydrates, vitamins, dietary minerals, coumarins, flavenoids, alkaloids, and saponins [7].Purslane has been reported to be used in a feed supplement in poultry [10]. Aydin&Dogan [11] suggested that adding dried purslane with diets of laying hens resulted in increased the production of egg and egg weight. Another study by Zhao et al [12]reported that supplementation of 0.2% purslane extract to the broiler resulted in improvement of

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gaining weight, and feed conversion ratio. This study aims to investigate the effect of using Portulaca oleracea in boiler diet on performance and health of chicken.

MATERIALS AND METHODS

Plant Sample

The air dried leaves of the plant was ground into a fine powder. Leaves were obtained from Agricultural Research Center, Giza, Egypt.

Chemical Composition of Investigated Leaves

Determination of Moisture Content

It was determined according to the method illustrated by AOAC (2000)[13].2gm of air dried Leaves was further dried to a constant weight at 105°C using an air drying oven. Moisture content percentage was then calculated.

Determination of Ash Content

It was estimated according to AOAC (2000)[13], as follow: in a silica crucible, a 2g of air dried leaves were placed and ignited at 600ºC in a muffle furnace till a constant weight, calculation of ash percentage was done.

Determination of Crude Fiber Content

Crude fiber was evaluated based on AOAC (2000)[13] method. Mixing of 2gm air dried leaves with 0.5g asbestos was done, then addition of 200ml of sulphuric acid (1.25%v/v H2SO4), was done. The mixture was boiled under reflex for 30 minutes, the mixture was filtered using Gooch crucible. The residue was boiled again with 200ml, 1.25%w/v of aqueous sodium hydroxide solution for 30 minutes, then filtration was repeated as the previous filtration illustrated. Washing of the residue was performed using hot water then diethyl ether and dried at 110ºC to constant weight. The content of Gooch crucible was then ignited in the muffle furnace at 600ºC to a constant weight. Fiber content was obtained by the subtraction of ash content from the weight of digested sample, then the percentage was calculated.

Determination of Crude Protein Content

Determination of crude protein content was done according to the official Kjeldahl method [13], as follow: 0.5 gm of air dried leaves was digested using 8ml. of concentrated sulphuric acid in the presence of (2.14g) digestion mixture which contain 1kg potassium sulphate and 60g of mercuric oxide red in a Kjeldahl flask. The solution was treated with 10ml of 40% sodium hydroxide solution after digestion process. 10 ml of 1% boric acid was used to receive the liberated NH3 in the presence of 2 drops of Tachero indicator (1.25g methyl red+0.32g methylene blue in one litre of 90% ethanol). Titration of the received ammonia was performed using 0.01N sulphuric acid. The total nitrogen and its percentage were estimated and the crude protein content was evaluated by using 5.25 as a factor of protein. Erian et al., (1994)[14].

Determination of Crude Lipid

AOAC (2000)[13] was used for the determination of crude lipid.2 gm of leaves‘powder was extracted using n-hexane as a solvent in soxhlet apparatus, extraction process lasted for 24 hours. The percentage of the crude lipid was estimated after the removal of the solvent.

Determination of Soluble Carbohydrate Content

A slightly modified phenol–sulphuric acid method according to Masuko et al., (2005)[15] was used for estimation of carbohydrate content. The method involved mixing of 50µml of crude polysaccharide solution with 150µml of concentrated H2So4 to initiate the color reaction, then immediate addition of 30µml of 5% phenol was done. The reaction mixture was incubated for 5 min at 90C. The reaction mixture then cooled to room temperature, then absorbance was measured at 490 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. D-glucose was used as standard for the total carbohydrate content calculation.

Determination of Reducing Sugar

The reducing sugar was determined by the modified method of Miller., (1959)[16]. 0.5 ml of 1% 3,5-dinitrosalicylic acid (DNS) was added to an aliquot of sample (20–500µml), and the volume was completed to 5 ml with distilled water. The mixture was shaken then a water bath of boiling water was used to heat the mixture for 5 min, the mixture then was cooled to room temperature; after cooling,

addition of 2.5 ml of distilled water to the mixture was done. The absorbance of the mixture was measured at 540 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. D-glucose was used as the standard for estimation of the total reducing sugar.

Calculation of Non-Reducing Sugars

Insoluble sugars were calculated according to the following equation: Non-reducing % = Total sugars % - reducing sugars %.

Experimental Animals

Fifty healthy broiler chicks with a weigh range of 40-45gm were obtained from Science Academy of Experimental Researches, Mansoura, Egypt. A battery system with good environmental conditions was used for the housing of chicks and each of the replicate of two birds was put in a separate cages.

Experimental Protocol

Five iso-nitrogenous and iso-caloric experimental broiler diets were formulated and designated as POL 0%, POL 5%, POL 10%, POL 15% and POL 20%, respectively Zanu et al., (2011)[17].The study was performed on five groups; each group included 10 chicks and their feeding was as following;

Group1: broiler chicks fed normal diets+(0% POL).

Group2: broiler chicks fed normal diets + (5% POL).

Group3: broiler chicks fed normal diets + (10% POL).

Group 4: broiler chicks fed normal diets+ (15% POL).

Group 5: broiler chicks fed normal diets+ (20% POL).

The five experimental diets were randomly assigned after one week of acclimatization and then fed to the chicks ad libitum for a total of six weeks (42 days).

Body Weight

The ten broiler in each group were weighted individually at the start of the experiment and after two weeks post supplementation (corresponding 42thday). The diet consumed by the chicks were recorded for estimation of weight gain and feed conversion rate Allam et al., (2016)[18].

Blood Samples

Blood samples were collected from the eyes of chicks in heparinized tubes after 42 days from the beginning of the experiment. The obtained blood sample was divided into two tubes; one was treated with 10% of ethylene diamine-tetracetic acid (EDTA) with gentle shaking to estimate complete blood count (CBC), whereas the other sample was left to allow blood clot and then centrifuged to obtain the blood serum in order to estimate the liver function (ALT, and AST), and the lipid profile (Triglycerides, total cholesterol, HDL-c, LDL-c and VLDL-c). Serum samples were preserved in a freezing condition until used.

Chemical Analysis of Blood

Lipid Profile: Was determined by enzymatic colorimetric method of Richmond (1973)[19] described in a commercial kits by Human (Germany).

Liver function: Was determined according to Reitman and Frankel (1957)[20] using commercial kits by Randox (United Kingdom).

Haematological analysis (Hb, RBC, PCV, MCV, MCHC, Plt, MPV, PCT, PDW, WBC, LYM, MON, GRA) were determined according to Nakul et al., (2003)[21] bya fully automated Haematological analyzer named ABX Micros 60 from Sysmex Corporation International Company.

Histological Observations

Tissue samples from Spleen, thymus and Bursa, were collected from all the groups at the end of the experiment to study the histological changes associated with the different diets. 10% formalin-saline solution was used for sample fixation.

All sections were dehydrated in ascending grades of ethanol, cleared in xylene and then embedded in paraffin wax. Transverse sections (4-5 microns, thickness) were taken, mounted on glass slides and stained with haemotoxyline and eosin (H and E) stains. All sections were microscopically examined Bancroft et al., 2012[22].

Statistical Analysis

The statistical software package (CoStat, 2005)[23] was used for statistical analysis. One way analysis of variance (ANOVA) was used for comparison and significant differences between treatment means were determined using Duncan's multiple range test at $P<0.05$ as the level of the significance (Duncan, 1955)[24].

RESULTS AND DISCUSSION

The chemical composition of the investigated leaves is shown in table (1), figure1. There were 9.35% moisture, 5.89% ash, 17.41% fiber, 25.37% protein, 2.44% lipid, 39.02% total sugars, which included 13.62% reducing sugars, and 21.4% no-reducing sugars. The chemical composition revealed that total sugars represented the highest component of Portulaca oleracea leaves, followed by proteins, and fibers, whereas lipid represented the least common component. Aberoumand [25] found that the stem and leaves of Portulaca oleracea contained 22.66% ashes, 40.67% fibers, 23.47% crude protein, and 5.26% lipid. The variations in the proportions reported in the previous study compared to our study can return to the difference in the method of investigation and the amount used of the plant. Another study by Ezekwe et al [26]reported that the proximate composition of Portulaca oleracea leaves was27% ash, 2.17% crude fiber, 22.9% crude protein, and 6.9% lipid.

Table 1: Chemical Composition of Investigated Leaves

Plant leaves	Moisture %	Ash %	Fiber %	Protein %	Lipid %	Total sugars %	Reducing sugars %	Non reducing sugars %
Portulaca oleracea	9.35	5.89	17.41	25.37	2.44	39.02	13.62	21.40

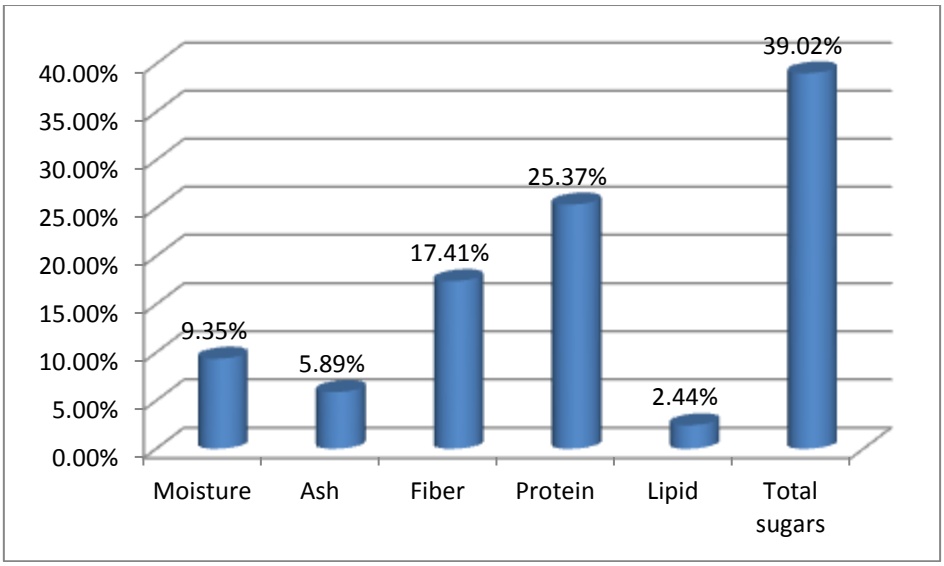

Fig. 1: Chemical Composition of Investigated Leaves

Effect of Supplementation of Portulaca Oleracea Poultry Diets on Body Weight of Broiler

The body weight of each group was estimated during 1-14 days, 14-28 days and 28-42 days. During 1-14 days, the mean ±SD of body weight of (group1, 2,3, 4 and 5), was 448±0.0, 510±1.21, 580±21.99, 620±5.44, and 623±8.71, respectively. During 14-28 days, the mean ± SD of body weight of group1,2,3,4,5 was 1500±23.76, 1590±12.01, 1635±29.75, 1720±0.38, and 1735±27.19, respectively. During 28-42 days, the mean± SD of body weight of (group1,2,3, 4 and 5), was 2110±23.16,2125 ±83.11,2250±63.6,2300±0.42, and 2320±2.28, respectively. The mean body weight of each group during the three periods is shown in table2.In the first period (1-14 days), the mean of body weight of each group is increasing through groups from group 1 to group 5 by increasing the concentration of POL, the same can be noted in the other two periods; days 14-28, and days 28-42, where the highest mean of body weight was found regarding group 5 which administrated higher concentration of POL. It was reported that supplementing of 0.2% Portulaca oleracea extract to broiler feed resulted in improvement in the weight gain [12].Also, supplementing diets with dried Portulaca oleracea increased the production of eggs and their weight [11]. Another study conducted on broiler chickens with induced ascites reported that

addition of Portulaca oleracea powder to the diet could improve the oxidative status and reduced the incidence of ascites without impairing the growth performance [27].

Table 2: Effect of Portulaca Oleracea on Body Weight of Broilers

Groups	Number of Birds	Average body weight		
		Days 1-14	Days 14-28	Days 28-42
Group 1	10	448^{c}±0.00	1500^{d}±23.76	2110^{b}±23.16
Group 2	10	510^{b}±01.21	1590^{c}±12.01	2125^{b}±83.11
Group 3	10	580^{b}±21.99	1635^{b}±29.75	2250^{a}±63.06
Group 4	10	620^{a}±05.44	1720^{a}±00.38	2300^{a}±00.42
Group 5	10	623^{a}±08.71	1735^{a}±27.19	2320^{a}±02.28
LSD=0.05	4.02			

Group 1: Level of dietary of poultry (0% POL), Group 2: Level of dietary of poultry (5% POL), Group 3: Level of dietary of poultry (10% POL), Groups 4: Level of dietary of poultry (15% POL), Group 5: Level of dietary of poultry (20% POL), respectively.

Effect of Supplementation of Portulaca Oleracea Poultry Diets on Lipid Profile

The mean ±SD of total cholesterol for group one was 200.08±0.15, and it was decreased through groups reaching to group5, where the mean ± SD of total cholesterol level was178.55±0.04. Also, there was a reduction in the mean± SD level of LDL from group1; 100.14 ±0.09, through groups reaching to group 5; 80±0.02. The mean ±SD of vLDL level was decreased through groups from group1; 88±0, reaching to group5; 82.15±0.04. Triglycerides increased from group one with a level of 340±0.12 through groups reaching to group five 382±0.14. Whereas HDL mean ±SD levels were increased from group one 100.14±0.09 through groups reaching to the highest level in group five 92±0.04. The details of the mean values of the lipid profile in the five groups are shown in table (3). HDL is referred as good cholesterol, whereas LDL is known as bad cholesterol, the increase in the total cholesterol, LDL and vLDL lead to atherosclerosis which affect the health, whereas increased the levels of HDL result in good health. The findings in this study revealed a protective effect of Portulaca oleracea leaves against hyperlipidemia on the health of chicken by administrating the leaves in their diet. In a previous study [28] the ethanolic extraction of Portulaca oleracea leaves showed anti-hyperlipidemic activity; however it should be noted that the previous study was conducted on rats. Ahmad et al [6] reported that the hydoalcoholic extract of Portulaca oleracea leaves resulted in reduction in the total cholesterol and LDL in all animals treated with Portulaca oleracea extract, these findings are in agreement with ours.

On the contrary to the previous findings, one study revealed that the concentrations of cholesterol, LDL, and HDL didn't differ between the groups given purslane and the other groups given purslane with different concentration [29].

Table 3: Effect of Portulaca Oleracea Onlipid Profile in Poultry Diets

Groups	T-Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	vLDL (mg/dl)
Group 1	200.08±0.15	340.00±0.12	90.12±0.05	100.14±0.09	88.00±0.00
Group 2	195.00±0.21	350.02±0.12	88.04±0.02	93.77±0.03	85.12±0.01
Group 3	189.54±0.17	365.05±0.08	87.42±0.01	85.08±0.05	82.04±0.03
Group 4	180.08±0.09	375.00±0.03	90.03±0.03	82.01±0.01	80.00±0.05
Group 5	178.55±0.04	382.00±0.14	92.00±0.04	80.00±0.02	82.15±0.04
LSD=0.05	2.08	3.44	1.39	2.00	1.27

Effect of Supplementation of Portulaca Oleracea Poultry Diets on Liver Functions

The liver function can be investigated by estimation of the liver enzymes; ALT and AST, the values of liver enzymes of each group are shown in table (4). The mean± SD of ALT and AST level in group one was 50.17±0.15, and 88±0.01, respectively. These levels were decreased through the groups reaching to group five, where the mean ±SD level of ALT, decreased to 40±0.05, and 80.22±0.0, respectively.

The increase in the levels of liver enzymes reflects bad hepatic function, whereas decreased levels reflect good hepatic function, this indicates the role administrating Portulaca oleracea leaves in the diet of chicken. Portulaca oleracea leaves have good impact on the hepatic function of chicken and has a hepatoprotective activity. A study by Prabhakaran et al [30] performed on rats using Portulaca oleracea whole plant extract demonstrated that the plant extract could help the hepatic injury to be healed and the biochemical parameters were significantly restored. A study by Sudhakar et al [31] reported a hepatoprotective effect of the aqueous extraction of Portulaca oleracea aerial parts against cisplatin-

induced hepatotoxicity in chick embryonic liver, which were evidenced by the recovered levels of the measured biochemical parameters such as ALT, and AST.

Table 4: Effect of Portulaca Oleracea on Liver Functions in Poultry Diets

Groups	ALT(U/L)	AST(U/L)
Group 1	50.17±0.15	88.00±0.01
Group 2	49.00±0.22	87.66±0.04
Group 3	45.54±0.11	87.18±0.02
Group 4	40.08±0.07	82.54±0.09
Group 5	40.00±0.05	80.22±0.00
LSD=0.05	1.02	0.94

Effect of Supplementation of Portulaca Oleracea Poultry Diets on Haematological Parameters

The mean levels of hematological parameters are shown in table5. The mean ±SD level of Hb in group one was 9.40±0.2, and it increased through the groups reaching to the highest value in group five 9.5±0.44. The mean± SD number of RBCs was 3.55±0.22(10)6/µl in group one and it increased through the groups reaching to the highest mean number 3.72±0.18(10)6/µl in group five. The mean± SD of PCV was 32.04±0.19 in group one, but decreased through (groups2,3 and 4), whereas in slightly increased in group five to32.05±0.13. The MCV mean ±SD was 40±0.12 in group one and it increased through the groups reaching to 44.14±0.11 in group five. There was slight increase in MCHC from group one 32.02±0, compared to group five 33.16±0.12, the mean levels of MCHC were decreased in group 2,3 and 4 compared to group one and five. These results revealed that the highest concentration (20%POL) was the most effective concentration to be used in the diet.

Table 5: Effect of Portulaca Oleracea Leaves on Haematological Parameters (Hb and RBCs) in Poultry Diets

Groups	Hb (g/dl)	RBCs (10^6/µl)	PCV (%)	MCV(µm^3)	MCHC (g/dl)
Group 1	9.40 ± 0.20	3.55 ± 0.22	32.04 ± 0.19	40.00 ± 0.12	32.02 ± 0.00
Group 2	9.28 ± 0.09	3.50 ± 0.14	30.01± 0.14	41.17 ± 0.04	30.04± 0.10
Group 3	9.15 ± 0.13	3.48 ± 0.03	31.03 ± 0.11	41.09 ± 0.08	31.00± 0.00
Group 4	9.20 ± 0.14	3.60 ± 0.33	31.07 ± 0.16	43.33 ± 0.15	31.12 ± 0.08
Group 5	9.50± 0.44	3.72 ± 0.18	32.05 ± 0.13	44.14 ± 0.11	33.16 ± 0.12
LSD=0.05	1.22	1.94	2.44	2.48	2.28

The effect of Portulaca oleraceaon Plt, MPV, PCT, and PDW is shown in table(6). The mean ± SD number of Plt increased from group one 1220±93.31(10)3/µl which administrated a concentration of (0%POL) through the groups and increased to 1288±90.88(10)3/µl in group five (20%POL). The mean ±SD of MPV was 9.1±0.22 in group one, while it showed reduction in group2 and 3, (8.9±0.33, 8.9±0.44, respectively), then showed the same level of group one 9.1±0.11, and showed slight increase in group five 9.2±14. The mean± SD of PCT was the same in group one and five 7.5±0, whereas it decreased in the group 2,3, and 4. The mean ±SD of PDW was 10.5±0.14 and it showed slight increase in group five 10.6±0.33, whereas it decreased in the group2,3, and4.

Table 6: Effect of Portulaca Oleracea Leaves on Haematological Parameters (Plt) in Poultry Diets

Groups	Plt (10^3/µl)	MPV (µm^3)	PCT (%)	PDW (%)
Group 1	1220 ± 93.31	9.1 ± 0.22	7.5 ± 0.00	10.5 ± 0.14
Group 2	1229± 17.55	8.9 ± 0.33	7.4 ± 0.02	10.2 ± 0.11
Group 3	1250± 16.29	8.9 ± 0.44	7.4 ± 0.04	10.2± 0.36
Group 4	1280 ± 77.09	9.1 ± 0.11	7.4 ± 0.18	10.4 ± 0.44
Group 5	1288 ± 90.88	9.2 ± 0.14	7.5 ± 0.00	10.6 ± 0.33
LSD=0.05	4.22	0.98	0.90	1.22

The effect of Portulaca oleracea leaves on WBCS, Lym, Mono, and GRA is shown in table (7). The mean± SD of the number of WBCs increased from group one 12 ±0.24(10)3/µl through the groups and the highest mean± SD number was found in group five 12.48±0.28(10)3/µl. The mean ± SD of Lym was 5.5±0.01(10)3/µl in group one and it increased to 5.66±0.14(10)3/µl in group five, whereas the other three groups showed a reduction. The mean ±SD of Monocytes was almost similar in group one and five; 0.48±0.11, and 0.48±0.45, respectively, whereas the value decreased in the other three groups.The mean ±SD of GRA showed slight increase from group one 38±0 through groups and the highest value was found among group five 38.5±0.04.In a previous study [10] it was reported that inclusion of Portulaca oleracea extract in the broiler chicken showed no effect on the immune response of chicken.

Table 7: Effect of Portulaca Oleracea Leaves on Haematological Parameters (WBCs) in Poultry Diets

Groups	WBCs (10^3/µl)	Lym (10^3/µl)	Mono (%)	GRA (%)
Group 1	12.00 ± 0.24	5.50 ± 0.01	0.48 ± 0.11	38.0 ± 0.00
Group 2	12.04 ± 0.20	5.48 ± 0.02	0.46 ± 0.12	38.1 ± 0.00
Group 3	12.22 ± 0.42	5.44 ± 0.24	0.46 ± 0.21	38.2 ± 0.00
Group 4	12.40±0.12	5.54 ± 0.30	0.47 ± 0.75	38.4 ± 0.00
Group 5	12.48 ± 0.28	5.66 ± 0.14	0.48 ± 0.45	38.5± 0.04
LSD=0.05	1.14	1.04	0.48	1.66

CONCLUSION AND RECOMMENDATIONS

Addition of Portulaca oleracea in a concentration of 20% in broiler diet results in improving the health of chicken that was observed through the reduction of bad cholesterol, improving hepatic functions, and enhancing the immunity of chickens. Also, the weight of chicken was improved by increasing the concentration of purslane; however, there was few studies conducted on the effect of purslane on the weight of chicken. So, this is the first study give details about the impact of Portulaca oleracea on the weight gain of chicken. Further studies should be performed including different concentrations of purslane and investigating other benefits of purslane.

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