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Full Length Article

Effect of *Lawsonia inermis* as a Growth Promoter in Broiler Chicks

Mousavi Kiavandani[1], Faramin Javandel[1], Jose Raduan Jaber[2], Alireza Seidavi[1] and Myriam R Ventura[3*]
[1]*Department of Animal Science, Rasht Branch, College of Agriculture, Islamic Azad University, P.O. Box: 41335-3516, Rasht, Iran*
[2]*Departamento de Morfologia, Campus de Arucas, Universidad de Las Palmas de Gran Canaria, 35413, Arucas, Spain*
[3]*Departamento de Patología Animal, Producción Animal, Bromatología y Tecnología de los Alimentos, Universidad de Las Palmas de Gran Canaria, 35413, Arucas, Spain*
[*]For correspondence: myriam.rodriguezventura@ulpgc.es
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Abstract

In the European Union, the utilization of antibiotics in livestock farming as growth promoters have been banned to avoid bacterial resistance. However, antibiotics suppression can originate certain animal diseases as well as increased feed conversion rates. Therefore, this study was conducted to evaluate *Lawsonia inermis* in broiler chicks and its effects on feed intake, growth gain, feed conversion ratio, carcasses quality, clinical biochemistry, immunity and intestinal flora during a 42-day trial. Five experimental groups with 3 replicates each were used: commercial diet (CD), CD + flavophospholipol (a conventional antimicrobial growth promoter), CD + *Lawsonia inermis* (*LI*) at 0.15%, CD + *LI* at 0.20% and CD + *LI* at 0.25%. *Lawsonia inermis* added to the diet of broilers did not cause an apparent adverse effect on palatability, carcasses' traits, biochemical profiles, or mortality. When it was added at 0.15 and 0.20%, the body weight was higher than broilers fed the control diet and that broilers fed the control diet + flavophospholipol. The immune response, total aerobic count and *Lactobacilli* count gave better results on broilers fed *L. inermis* than broilers fed the CD plus flavophospholipol (AGPs**)**.

Keywords: Additive; Broilers chicks; Blood biochemical parameters; Carcass quality; Clinical biochemistry; Growth performance; Henna; Immune system; Intestinal flora

Introduction

The current production of poultry meat worldwide has continuingly increased to feed a growing human population. The main reasons for this increase are genetic progress in poultry lines, a better understanding of nutrition fundamentals and progressive control of diseases (Ravindran 2013). The poultry industry demands the use of growth promoters to control pathogenic gastrointestinal microorganisms. In that sense, antibacterial medication has been administered in several animal species to get better growth rates and feed conversion productiveness, commonly known as antimicrobial growth performance promoters, or AGPs (Goetting *et al*. 2011; Huyghebaert *et al*. 2011). However, their use has been banned in the European Union (EC Regulation No. 1831/2003) and, in the United States of America, where alternative growth promoters are a progressive social demand to the poultry industry (Dibner and Richards 2005). In the European Union, the AGPs suppression has originated certain animal diseases and increased feed conversion rates (Huyghebaert *et al.* 2011). In this scenario and expecting that AGPs could be extensively forbidden worldwide, new growth promoters should be investigated to minimize digestive dysbacteriosis.

Lawsonia inermis, commonly known as "henna," belongs to the Lythraceae family and is the only species in the genus (Semwal *et al.* 2014). The Chemical names are Lawsone, 2-Hydroxy-1,4-naphthoquinone, and the molecular formula is C10H6O3 (NCBI 2018). Among several beneficial properties of *Lawsonia inermis*, anti-inflammatory and antibacterial activities have been documented. These effects are related to the plant powder and its extract. Other products such as ethyl acetate, aqueous, chloroform, petroleum ether and ethanol extracts from flowers, fruit and whole plant showed excellent activity against Gram-positive and Gram-negative bacteria (Semwal *et al.* 2014).

Therefore, this work aimed to assess the use of *Lawsonia inermis* in feeding intake, growth gain, and feed conversion ratio of broilers during 42 days from hatching to 6 weeks of age. Different parameters such as carcass characteristics, blood plasma biochemistry, immunity, and intestinal flora of broilers were analyzed during the trial.

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Materials and Methods

Location, housing and animals

The experimental trial was done in a commercial chicken farm located in Abkenar (37° 27' 35" North, 49° 19' 53" East, 18 m below sea level). Before the investigation, the farm was cleansed, including the water dispensers and the bird feeders. Continuous controlled temperature (22–25°C), air humidity (55 to 65%) and ventilation were implemented following the recommendations done for Ross 308 broilers (Aviagen, Newbridge, Scotland, UK 35805). Chicks were one-day-old male, Ross 308 strain (Aviagen, Newbridge, Scotland, UK 35805), with similar body weights in all groups. The animal experiments conducted in this study complied with the Ethical Committee of the Azad University and the parameters mentioned as ARRIVE (Animals in Research: Reporting *In vivo* Experiments). Special considerations were taken to minimize the stressful conditions of the animals.

Experimental design, feedstuffs and treatments

The research was an entire randomized lock, with five treatments, three replicates per treatment with 15 animals per replicates (a final amount of 225 male chicks). The experiments lasted 42 days. All chicks were fed a commercial diet following the manufacturer's recommendation (Table 1). Food and water were provided *ad libitum*. The commercial diet composition in the starter (1^{st}–21^{st} days of age) and finisher (22^{nd}–42^{nd} days of age) periods are shown in Table 1. The treatments were: (1) Control group CD (commercial diet); (2) Commercial diet plus flavophospholipol 0.6% (Flavomycin, at the recommended dose); (3) CD plus *Lawsonia inermis* powder (0.15%); (4) CD plus *Lawsonia inermis* powder (0.20%); (5) CD plus *Lawsonia inermis* powder (0.25%). Flavophospholipol 0.6% (Flavomycin® 0.6%) is an antibiotic used as conventional AGPs for animal production, including broilers (Barros *et al.* 2012) and it was prepared from Damyaran Pharmacy Co. and *Lawsonia inermis* leaf powder.

Evaluated parameters

Performance: Diets were offered *ad libitum* to the chicks throughout the 42 days of the experimental period. Bodyweight and feed intake of all broilers were recorded weekly for six weeks and calculated on days 21 and 42. Feed conversion ratio (FCR) was calculated as the ratio (g/g) of average feed intake and average body weight gain.

Carcass traits: Once finished the experiment and 4 h later of fasting for complete evacuation of the intestinal content, one broiler from each replicate was euthanized and treated following the standard protocols. These broilers were collected to measure the carcass yield, the meat and gastrointestinal tract characteristics. Broilers were pecked using standardized dry pecking methods. Feet at tibiotarsal joint, neck, wingtips, intestine, and hepatic tissue were taken out from the carcass. Later on, the corpse was weighted and gut segments were measured and recorded. Different sections of the corpse were analyzed and separately weighted. Cook carcass yield were ascertained 5 h after euthanized following the protocol suggested by Cason *et al*. (1997). Chicken breast samples were weighed and packaged, then steam-cooked in a water-bath at 85°C for half an hour. Later on, these tissues were cooled at ambient temperature and re-weighed.

Clinical biochemistry: A total of 5 mL of blood was obtained from the ulnar blood vessel and preserved in anticoagulant ethylene diamine tetraacetic acid (EDTA). Blood samples were collected from two broilers belonging to each replicate. Before blood samples were obtained, the feed was withdrawn for a period of four hours in order to stabilize serum parameters in all broilers. Blood samples were collected in the early hours to reduce the circadian variations in specific serum parameters. At the laboratory, pieces were centrifuged at 2000 rpm for 20 min to obtain plasma and reserved at -20°C for further analysis. Total plasma cholesterol and triglyceride levels were obtained by enzymatic tests (Teif Azmoon Pars, Co., Tehran, Iran) following the protocol done by Schmid and Forstner (1986). Cholesterol fractions (HDL and LDL) were evaluated directly with HDL-C and LDL-C diagnostic kits (Teif Azmoon Pars Co., Tehran, Iran). The colorimetric index of cholesterol was also assessed with the cholesterol oxidase test (Schmid and Forstner 1986). Plasma glucose was determined with a glucose oxidase kit based on oxidase-peroxidase procedure (Teif Azmoon Pars, Co., Tehran, Iran) according to Trinder (1969) and Barham and Trinder (1972). Plasma uric acid was measured using a uric acid-uricase enzyme kit, the uricase-TOOS method (Teif Azmoon Pars, Co., Tehran, Iran) (Kato *et al.* 2000).

Immunity assessment: The humoral immune response to the Newcastle and Avian Influence vaccinations at 28 and 42 days old was determined based on hemagglutination inhibition (HI) method in two broilers from each replicate. A second humoral test was done with the inoculation of sheep red blood cells (SRBC) according to the literature (Pourhossein *et al.* 2015) and the sampling times were at 28 and 42 days of age.

Digestive bacterial count: Digestive bacteria were counted according to Dibaji *et al*. (2014). MRS agar (Man Rogosa Sharpe agar, 1.10660 for *Lactobacilli*, Eosin Metilan Blou (EMB, 1.01347.0500) for *E. coli*, MacConkey agar (105465.0500) for coliforms, and nutrient agar (1.05450.0500) was used to culture total aerobic bacteria. A suspension of microorganisms obtained from gut material from two broilers of each replicate was prepared. *Lactobacilli* bacteria were cultivated at 37ºC in the anaerobic ambiance for 72 h. Total aerobic bacterial count incubated at 37ºC in the aerobic ambiance for 48 h.

Table 1: Ingredients and estimated nutritive value of the commercial diet (CD)

Ingredients	Starter period[A]	Finisher period[B]
Corn	56.9	58.7
Soybean meal (43% CP)	33.1	30
Fish meal	3.4	3.5
Soybean oil	2	3.5
Di Calcium Phosphate	1.55	1.55
Oyster shell	1.03	1.18
DL-methionine	0.01	0.01
Vitamin premix[C]	0.5	0.5
Mineral premix[D]	0.5	0.5
Salt	0.26	0.26
Sand (as empty space)	0.75	0.75
Calculated nutritional content		
ME (Kcal/kg)	2910	3030
Crude protein (N×6.25) (%)	20.1	19.0
Crude Fat (%)	4.6	6.14
Calcium (%)	0.95	0.90
Total phosphorus (%)	1.23	1.06
Available phosphorus	0.45	0.36
Metionine	0.5	0.38
Lysine	1.01	1.00
Met + Cys	0.83	0.71

[A]1 -21 days of age; [B]22 - 42 days of age; [C] (vitamin A, 3600000 IU; D3, 800000 IU; vitamin E, 7200 IU; vitamin B1, 710 mg; vitamin B2, 2640 mg; vitamin B6, 1176 mg; vitamin B9, 400 mg; vitamin B12, 6 mg; vitamin k3, 800 mg; pantothenic acid, 3920 mg; vitamin Biotin, 40 mg; vitamin Niacin, 12000 mg and choline chloride, 200000 mg); [D] (Mn, 40000 mg; Fe, 20000 mg; Zn, 33900 mg; Cu, 4000 mg; I, 400 mg and Se, 80 mg); ME (Kcal/kg), metabolizable energy; Met + Cys, Metionine + Cystene

Microorganisms were counted with a colony counter (UFC). Microorganism's counts were related as logarithm number of bacteria/g sample.

Statistical analysis

Information was analyzed using a randomized experimental design involving 225 chickens with five treatments, three replicates per treatment, with 15 animals per replicates. The SPSS Statistical package v. 20 was used to analyze the randomized block design followed up with Duncan's multiple range tests when the overall treatment was significant ($P < 0.05$).

Results

Feed intake, body weight and feed conversion ratio

The broilers showed good health and expected behavior during the experiment. There were no differences in feed intake among the diet groups in the starter (1–21 days), the finisher (22–42 days) and in the total period (1-42 days) (Table 2). Regarding bodyweight (Table 2) in the starter period, the groups fed flavophospholipol and 0.15% *Lawsonia inermis*, gained significantly ($P=0.02$) more body weight than the control group fed the CD alone. In the finisher and total periods, the highest bodyweight gain was achieved by broilers feeding at total of 0.15 and 0.20% *Lawsonia inermis* and was significant ($P=0.02$). In this way, the weight at 42 days of chickens provided 0.15 and 0.20% *Lawsonia inermis* increased 253 and 312 g per day, respectively; compared with the chicken's weight gain fed the CD alone. The weight at 42 days of the chickens fed 0.15 and 0.20% *Lawsonia inermis* increased 51 and 110 g respectively and significant ($P < 0.05$) in comparison with the weight gain of the broilers fed the CD plus flavophospholipol (AGPs). The feed conversion ratio was not significant ($P > 0.005$) in any period among the groups (Table 2).

Carcass traits

At 42 days old, there were no significant differences ($P > 0.005$) in carcass yield, thighs, and breast, neck, or wing gains. The weight of the gizzards and small intestines (Table 3) of broilers fed flavophospholipol, 0.15 and 0.20% *Lawsonia inermis* groups differed significantly ($P = 0.035$ and $P = 0.009$, respectively). Weights were significantly lower in the gizzard and were markedly higher in small intestine compared to CD. At 0.25%, the *Lawsonia inermis* group had lower weight in heart ($P = 0.007$), proventriculus ($P = 0.014$), and the small intestine ($P = 0.009$), than the rest of the groups (Table 4).

Clinical biochemistry, immunity assessment and digestive bacterial count

No significant differences ($P > 0.05$) were found for biochemical profiles (Table 5). Related to immunity assessment (Table 6), the immune response was higher in broilers fed *L. inermis* at 0.20 and 0.25% compared to broilers fed the CD plus flavophospholipol (AGPs**)**. Table 7 shows digestive bacterial count, no differences ($P > 0.05$) were found among the groups in *E. coli* and coliforms count in gut observed at 42 days old chicks, but there was a lower ($P = 0.02$) total aerobic bacteria count in broilers fed *L. inermis* than broilers fed the control diet and CD plus flavophospholipol (AGPs**)**. Regarding lactobacillus count, significant differences ($P = 0.01$) were found among the groups, lactobacillus count was higher in broilers fed *L. inermis* and in the CD plus flavophospholipol (AGPs**)** than broilers fed the control diet without *L. inermi.*

Discussion

Some alternative growth promoters evaluated in the poultry industry include exogenous enzymes, organic acids, probiotics, prebiotics, etheric oils and some plants (Huyghebaert *et al.* 2011). *L. imermis* possesses a wide range of beneficial properties in humans in whom both the oral and topical routes have treated diseases. Moreover, aromatic herbs are frequently affirmed (Zeng *et al.* 2015) to improve the flavor and palatability of the feed, hence increasing feed intake followed by improved weight gain.

Table 2: Effects of the experimental diets on feed intake (g), body weight (g) and feed conversion ratio (g feed/g body weight) of the broilers up to the age of 42 days

	CD [A]	Flav [B]	0.15% *LI* [C]	0.20% *LI* [D]	0.25% *LI* [E]	SEM	*P*-Value
Feed intake (g)							
Starter[F]	1194.4	1232.5	1292.1	1180.3	1197.7	16.3	ns
Finisher[G]	3384.2	3588.4	3656.8	3581.1	3174.6	123.1	ns
Total [H]	4578.7	4821.0	4858.9	4761.5	4362.4	123.8	ns
Body weight (g)							
Starter[F]	739.9 b	822.4 a	812.2 a	766.7 b	740.0 b	16.6	0.02
Finisher [G]	1321.1 b	1440.4 b	1501.9 ab	1606.4 a	1351.1 b	42.5	0.02
Total [H]	2061.1b	2262.9a	2314.13a	2373.1a	2091.2b	97.5	0.04
Feed conversion ratio (g feed/g body weight)							
Starter[F]	1.61	1.49	1.59	1.53	1.60	0.03	ns
Finisher[G]	2.56	2.49	2.43	2.22	2.34	0.10	ns
Total [H]	2.22	2.13	2.09	2.00	2.08	0.05	ns

[A] CD Control diet (commercial diet); [B] CD + Flavophospholipol; [C] CD + 0.15% *Laswsonia inermis;* [D] CD + 0.20% *Laswsonia inermis;* [E] CD + 0.25% *Laswsonia inermis;* [F] Period of 1-21 days of age; [G] from 22 - 42 days of age; [H] Total period of 42 days
Means with different letters within treatments differ significantly ($P < 0.05$); SEM, Standard error of the mean (n = 44 chicks for each diet); ns, non-significant ($P > 0.05$)

Table 3: Effects of the experimental diets on carcass characteristics (g) of the broilers at 42 days' old

	CD [A]	Flav [B]	0.15% *LI* [C]	0.20% *LI* [D]	0.25% *LI* [E]	SEM	*P*-Value
Carcass yield	1563.6	1771.0	1753.6	1666.3	1547.6	66.3	ns
Cooked Carcass yield	1298.3	1486.3	1407.6	1383.0	1233.0	60.3	ns
Breast	451.6	511.0	492.6	489.6	400.6	28.8	ns
Thighs	425.6	474,0	442.3	458.6	420.3	19.5	ns
Wing	117.3	135.3	126.6	128.0	119.0	5.4	ns
Neck	55.6	62.3	55.3	60.6	49.3	3.3	ns

[A] CD Control diet (commercial diet); [B] CD + Flavophospholipol; [C] CD + 0.15% *Laswsonia inermis;* [D] CD + 0.20% *Laswsonia inermis;* [E] CD + 0.25% *Laswsonia inermis*
SEM, Standard error of the mean; ns, non-significant ($P > 0.05$)

Table 4: Effects of the experimental diets on the organ weights (g) of the broilers at 42 days old

	CD [A]	Flav [B]	0.15% *LI* [C]	0.20% *LI* [D]	0.25% *LI* [E]	SEM	*P*-Value
Liver and biles	46.6	53.6	56.3	50.6	47.6	4.41	ns
Pancreas	5.0	6.3	7.0	6.6	5.6	1.0	ns
Heart	12.0a	12.6a	14.3a	12.3a	9.3 b	0.69	0.007
Spleen	5.0	2.3	2.3	2.0	1.6	1.80	ns
Thymus	4.3	5.0	3.3	4.6	2.6	0.74	ns
Bursa	2.0	1.6	2.0	2.0	1,0	0.39	ns
Proventriculus	9.6a	11.0a	10.6a	10.6a	7.3 b	0.64	0.014
Gizzard	68.0 a	53.6 b	64.0 ab	57.6 ab	53.3 b	3.24	0.035
Small intestine	72.0 c	82.3bc	104.3 a	92.6 ab	69.0c	5.93	0.009
Large intestine	22.0	33.3	26.0	27.6	22.3	5.90	ns

[A] CD Control diet (commercial diet); [B] CD + Flavophospholipol; [C] CD + 0.15% *Laswsonia inermis;* [D] CD + 0.20% *Laswsonia inermis;* [E] CD + 0.25% *Laswsonia inermis*
SEM, Standard error of the mean; ns, non-significant ($P > 0.05$); Within rows, means with different letters within treatments differ significantly ($P < 0.05$)

Table 5: Effects of the experimental diets on clinical biochemistry of the broilers at 42 days old

	CD[A]	Flav [B]	0.15%*LI* [C]	0.20% *LI* [D]	0.25% *LI* [E]	SEM	*P*-value
Total protein (g/dL)	4.4	4.5	4.5	4.0	4.6	0.35	ns
Albumin (g/dL)	1.6	2.1	1.3	2.3	2.0	0.33	ns
Glucose (mg/dL)	207.4	209.1	207.1	205.4	208.7	11.1	ns
Cholesterol (mg/dL)	138.1	145.4	151.5	153.9	157.1	7.1	ns
Triglyceride(mg/dL)	27.1	23.9	36.9	39.7	24.7	13.7	ns
HDL (mg/dL)	79.6	82.3	73.0	82.6	77.0	7.1	ns
LDL (mg/dL)	49.0	58.3	67.1	63.3	75.2	13.8	ns
VLDL (mg/dL)	11.3	9.4	7.9	4.9	4.7	2.7	ns
UricAcid (mg/dL)	5.4	4.9	4.3	6.1	3.72	0.79	ns
ALP (U/L)	1463	1410	831.7	1683.3	1436.7	265.7	ns

[A] CD Control diet (comercial diet); [B] CD + Flavophospholipol; [C] CD + 0.15% *Laswsonia inermis;* [D] CD + 0.20% *Laswsonia inermis;* [E] CD + 0.25% *Laswsonia inermis*
SEM, Standard error of the mean; ns, non-significant ($P > 0.05$); HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, Very low density lipoprotein; ALP, Alkaline phosphatase

However, the results of the present investigation show that there was no significant difference ($P > 0.05$) in feed intake among the groups, the addition of *Lawsonia inermis* leaf powder at concentrations of 0.15, 0.20 and 0.25% did not affect palatability, intake, neither acceptability of the feed by chicks. But, Adedeji *et al.* (2019) found significant differences on daily feed intake of broiler birds fed different levels of *Lawsonia inermis.*

Table 6: Immune response: antibody titers against SRBC (sheep red blood cells), IgG and IgM (log10) values of broilers; antibody titers against Anti-Newcastle disease hemagglutination-inhibition (log10) and anti-avian influenza hemagglutination-inhibition (log_{10}) titers of broilers at different diets, at 28 and 42 days of age

	CD [A]	Flav [B]	0.15% *LI* [C]	0.20%*LI* [D]	0.25%*LI* [E]	SEM	*P*-Value
Immune response for total SRBC, IgG and IgM (log10) values of broilers at different diets at 28 and 42 days of age.							
SRBC(28d)	2.3b	4.0ab	4.0ab	5.3ab	6.3a	1.09	0.02
IgG (28 d)	1.0b	2.3ab	2.0ab	3.3ab	4.0a	0.84	0.02
IgM (28 d)	1.3	1.6	2.0	2.0	2.3	0.44	ns
SRBC (42 d)	4.3b	5.0ab	4.33b	6.0ab	6.6a	0.63	0.01
IgG (42 d)	2.6	3.3	2.6	4.3	4.3	0.49	ns
IgM (42 d)	1.6	1.6	1.6	2.3	2.3	0.42	ns
Anti-Newcastle disease hemagglutination-inhibition (log10) and anti-avian influenza (*Avian* I.) hemagglutination-inhibition (log10) titers of broilers at different diets at 28 and 42 days of age							
Newcastle disease (28d)	4.0ab	4.3ab	2.6b	4.0ab	4.6a	0.51	0.014
Newcastle disease (42d)	2.3ab	1.6ab	1.0b	2.6ab	3.0a	0.57	0.018
Avian influenza (28 d)	1.6	1.3	1.3	2.3	2.6	0.49	ns
Avian influenza(42 d)	0.3	1.0	0.3	1.0	1.3	0.44	ns

[A] CD Control diet (commercial diet); [B]CD + Flavophospholipol; [C] CD + 0.15% *Laswsonia inermes*; [D] CD + 0.20% *Laswsonia inermis;* [E] CD + 0.25% *Laswsonia inermis*
SRBC, sheep red blood cells; IgG, immunoglobulin G; IgM, immunoglobulin M; SEM, Standard error of the mean; ns, non-significant (*P > 0.05*); Within rows, means with different letters within treatments differ significantly (*P < 0.05*)

Table 7: Effects of the experimental diets on count of *E. coli*, coliform bacteria, Lactobacilli, and total aerobic bacteria (log CFU g-1) in intestinal tract at 42 days old

	CD [A]	Flav [B]	0.15% *LI* [C]	0.20% *LI* [D]	0.25% *LI* [E]	SEM	*P*-Value
E. Coli	5.6	6.9	6.7	6.7	6.5	0.45	ns
Coliformus	6.5	8.0	8.0	6.7	7.9	0.44	ns
Lactobacilli	6.7b	7.7a	7.4ab	7.7a	7.8a	0.27	0.01
Total aerobic bacteria	8.1ab	8.6a	7.7b	7.8ab	7.9ab	0.24	0.02

[A] CD Control diet (commercial diet); [B] CD + Flavophospholipol; [C] CD + 0.15% *Laswsonia inermes;* [D] CD + 0.20% *Laswsonia inermis;* [E] CD + 0.25% *Laswsonia inermis*
CFU, colony forming units; SEM, Standard error of the mean; ns, non-significant (*P > 0.05*); Within rows, means with different letters within treatments differ significantly (*P < 0.05*)

Nevertheless, a variation was observed regarding weight gain; broilers fed *L. inermis* at 0.15 and 0.20% of the total diet gained significantly (*P < 0.05*) more bodyweight than those fed the CD and fed the CD plus flavophospholipol (AGPs), even though no significant differences (*P > 0.05*) were found on feed conversion rate. These results are agreeing with (Adedeji *et al.* 2019) which found significant differences (*P < 0.05*) on growth performance parameters (daily weight gain, total weight gain, final weight) and also on feed conversion rate of broiler birds by inclusion of different levels of *Lawsonia inermi*s in their feed. As (Denil *et al.* 2003) reported, the increase in the body weight may be due to the positive effect of supplementation of additives in broiler diets with improved nutrient utilization. But for us the increase in the body weight could be due to the increase of the *lactobacillus* count caused by the addition de *L. inermis* on the diets as we will see later on.

Regarding the effects of the experimental diets on carcass characteristics and organ weights, even though no differences (*P > 0.05*) were found among the groups, some significant differences (*P < 0.05*) were found on some organ weights (heart, proventriculus, gizzard and small intestines). Although we did not find an explanation for this, the biological properties described for *Lawsonia inermis* could explain these findings in broiler chicks. Despite the lower weight found in small intestines in broilers fed a CD without growth promoters might be due to an intestinal syndrome commonly referred to as 'dysbacteriosis,' which has emerged in some areas where the AGPs have been banned. Although this syndrome has not been fully described, it resembles 'wet litter', 'bacterial overgrowth,' or 'malabsorption'. Therefore, a thinning of the small intestine occurs, accompanied by higher water content of feces and decreased digestibility of feed with undigested food identified in the feces (Huyghebaert *et al.* 2011).

The effects of the experimental diets on clinical biochemistry of the broilers at 42 days' old did not show significant differences (*P > 0.05*) for biochemical profiles. A low total protein and albumin level can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. However, total protein was standard and similar to the total protein reported by (Adedeji *et al.* 2019). Results on albumin and total cholesterol were higher than values found by (Adedeji *et al.* 2019) in all the broilers fed *L. inermis*, this may be due to the race of the birds used on the study. Glucose plasma levels, HDL and uric acid values were within the normal ranges in poultry (Campbell 2004) and hence it may not be of any practical relevance.

Concerning the immunity assessment, in 28 and 42d, antibody titer against Influenza vaccine was not markedly influenced by treatments in 28 and 42 d (*P < 0.05*), but antibody titer against Newcastle vaccine was higher in 0.25% LI treatment than other treatments (*P < 0.05*). Antibody production against SRBC affected by treatments in 28 and 42 d (*P > 0.05*) and was higher in 0.25% LI treatment than other treatments. The titer of IgM didn't

affect by treatments in 28 and 42 d ($P > 0.05$), but the titer of IgG was higher in 0.25% LI treatment than other treatments at 28 d ($P < 0.05$).

Immune stimulant activities have been demonstrated in mice treated with *L. inermis* as increased white blood cell counts or inhibition of drug-induced myelosuppression (Jinyvarghese *et al.* 2005) and macrophage stimulation phagocytic activity (Kulkarni and Karande 1998). However, we do not analyze this mode of action. The methods used to measure the immune response and the growth-promoting effect of *L. inermis* did not reveal its underlying mechanism. As (Zeng *et al.* 2015) state, there are different factors such as the species, harvest time, part of the plant used and method of isolation, even climatic conditions which could all affect the effectiveness of the plants used.

Different authors have stated that *Lawsonia inermi*s possesses antifungal, antibacterial, virucidal, antiparasitic or anti-inflammatory properties (Abdulmoneim 2007; Semwal *et al.* 2014; Al-Snafiea 2019). Moreover, some studies had demonstrated the inhibitory effects against *Escherichia coli* or aerobic bacteria like *Bacillus subtilis* or *Pseudomonas aeruginosa* (Jeyaseelan *et al.* 2012; Rahiman *et al.* 2013). Due to its antibacterial activity, this herb was selected as an alternative growth promoter for this study. In this work, no clear effects were observed on the intestinal microflora populations. But despite no differences ($P > 0.05$) were found among the groups in *E. coli* and coliforms count in gut observed at 42 days old chicks, there was lower total aerobic bacteria count in broilers fed *Lawsonia inermis*. This might be due to the fact that the effectiveness of a probiotic use depends on some different factors as dispensation level and concentration of plants, method of application, and farm hygiene (Wang *et al*. 2017).

As regards to *lactobacillus* count, significant differences ($P = 0.01$) were found among the groups, *lactobacillus* count was higher in broilers fed *L. inermis* than broilers fed the control diet. It is well known that *lactobacillus* used as probiotics enhance the growth and performance of animals, (Pham *et al*. 2003) found that feeding chickens with probiotics mix of *lactobacillus* increase the weight gain in broilers. This could be the main effect of the use of *Lawsonia inermis* L. in this work, *L. inermis* used in broiler feed, increase the *lactobacillus* count in ileal content of birds and hence increase the weight gains in broilers.

Conclusion

Although the beneficial role of AGPs is not fully understood, *Lawsonia inermis* may be used as a growth promoter replacing the antibiotic flavophospholipol because added to the diet at 0.15 and 0.20%, the body weight is higher than those animals fed the control diet and that animals fed the control diet + flavophospholipol. Furthermore, the immune response, total aerobic count and *lactobacillus* count gave better results in broilers fed *L. inermis* than broilers fed the CD plus flavophospholipol (AGPs) and did not cause apparent adverse effects on palatability, carcass traits, biochemical profiles, or mortality in chicks.

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Author Contributions

MRV and SA designed the investigation, interpreted the results, and wrote the manuscript. MK and FJ carried out the growth trial and the analysis. JRJ performed the statistical analysis, analysed the data and co-wrote the paper.

Conflict of Interest

The authors declare no conflict of interés

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials

Ethics Approval

The animal experiments conducted in this study complied with the Ethical Committee of the Azad University and the ARRIVE guidelines.

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