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Comparison of Using Ionophore and Non-Ionophore Coccidiostats on Performance, Carcass Characteristics, Blood Biochemical Parameters and Gut Microbial Flora in Broiler Chickens

Research Article

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ABSTRACT

The effect of ionophore and non-ionophore coccidiostats on performance parameters, carcass characteristics, serum biochemical parameters and gut microbial floras in broilers was investigated. A total of 300 one-day-old broilers were randomly allocated into five treatments including: (1) basal diet (control group); (2) basal diet with 60 ppm salinomycin; (3) basal diet with 3.75 ppm maduramicin; (4) basal diet with 1 ppm Diclazuril; and (5) basal diet with 125 ppm amprolium. Supplementing the diet with diclazuril improved weight gain compared to all diets containing ionophore coccidiostats (P<0.05). Additionally, feed intake significantly increased with broilers fed on diclazuril containing feed compared with broilers fed on maduramicin at 1 to 42 days. For female chicks, the highest carcass yield value was recorded in the control group, followed by the salinomycin, and maduramicin treatments respectively; however, thigh yield was higher in the diclazuril treatment group (P<0.05). Highest carcass and breast yields for male broilers were obtained in the diclazuril treatment group (P<0.05). *Lactobacilli* and *Coliform* bacterial populations were significantly higher in the diclazuril and control groups when compared to the salinomycin group at 28 and 42 days (P<0.05). Blood glucose and total protein were significantly increased by feeding diclazuril diets in both male and female broilers (P<0.05). Blood sodium and potassium concentrations were affected by treatment in male chickens (P<0.05). The use of diets containing non-ionophore coccidiostats, particularly diclazuril, had beneficial effects on the overall growth performance of broiler chickens.

KEY WORDS broiler, ionophore coccidiostats, non-ionophore coccidiostats, performance.

INTRODUCTION

Coccidiosis is the most important parasitic disease of the poultry industry, the protozoan parasite, *Eimeria*, is responsible for coccidiosis disease. *E. necatrix*, *E. tenella*, *E. acervulina*, *E. maxima*, *E. praecox*, *E. brunette*, and *E. mitis* are all pathogenic species which cause coccidiosis, but *E. necatrix*, *E. maxima*, and *E. praecox* are the most common causative agents in poultry (Vrba *et al.* 2011; Barbour *et al.*

2015). Various coccidiostats are utilized in broiler chicken farms in order to prevent coccidiosis (Shirley, 1986; Vrba *et al.* 2011; Barbour *et al.* 2015). Supplementation with coccidiostats as feed additives has been an important factor in the growth of the poultry industry over the past 50 years because coccidiostats are the principal means for controlling coccidiosis in the broiler industry. Currently, the poultry industry is largely dependent on coccidiostats for the control of coccidiosis disease (Allen *and* Fetterer, 2002;

Asadi Iraee *et al.* 2015).Globally, the use of coccidiostats success in controlling a avian disease, the poultry industry has been under constant pressure to decrease its dependence on antimicrobials including coccidiostats (Abdelrahman *et al*. 2014).

Coccidiostats are divided into two groups: Ionophore and non-ionophore (Varga *et al.* 2017). Ionophore coccidiostats, such as salinomycin, lasalocid, monensin, maduramicin, and narasin, are monocarboxylic polyether antibiotics (Ebrahimnezhad *et al.* 2010), which can bind any available mono-divalent and divalent cations to form dimeric groups that facilitate metal ions crossing hydrophobic membranes. Therefore, ionophore antibiotic dietary supplements may change the bioavailability of some nutrients, affecting intestinal absorption in the body (Elsasser, 1984; Ebrahimnezhad, 2010).

Ionophores are toxic to human cells and are therefore not used except for veterinary purposes (Food and Drug Administration, 2014). However, these compounds hinder many bacteria activity. Development of resistance against ionophores resistance in bacteria to more than one ionophore has also been noted (Ebrahimnezhad *et al*. 2010). Some studies document consistent improvements in the performance of chickens fed diets containing ionophore coccidiostats (Elwinger *et al.* 1998; Ebrahimnezhad *et al.* 2010). However, other researchers observed no significant differences in the performance of chickens given diets with ionophore antibiotics (Ebrahimnezhad and Pourreza, 2005; Ebrahimnezhad *et al.* 2010). In contrast, other studies have shown that the use of ionophore antibiotics, even at the recommended levels, can have undesirable effects on growth performance (Ebrahimnezhad and Pourreza, 2005; Ebrahimnezhad *et al.* 2010). Moreover, Keshavarz and McDougald (1982) observed that the use of ionophore coccidiostats at high levels decreased the performance of broiler chickens; this effect is likely due to diminished feed intake or a change in nutrient absorption. Dietary supplemented ionophore coccidiostats may form complexes with metallic elements and alter the transport, absorption, and toxicity of nutrient metals, which can be a safety concern for agricultural animals (Ammerman *et al.* 1977).

The ionophore coccidiostats salinomycin and maduramicin are the most widely utilized coccidiostats in the poultry industry due to the positive effects on broiler chicken performance (Elwinger *et al.* 1998; Ebrahimnezhad *et al.* 2010). In addition, the researchers found the use of salinomycin, even at recommend levels in the diet, had no impact on or even reduced growth performance of broiler chickens (Pearson *et al.* 1990) and could result in reduced water consumption (Radu *et al.* 1987).

In addition, Ebrahimnezhad *et al.* (2010) found that diets supplemented with salinomycin improved feed intake conversion when compared to diets supplemented with maduramicin. In addition, Abdelrahman *et al.* (2014) showed that broilers that received the salinomycin in their diet performed very similar to broilers that received probiotics in terms of overall performance.

Sharma *et al.* (2005) reported that supplementing the diet with 5 and 10 mg/kg maduramicin for 21 days reduced the performance of broilers and led to the development of diarrhea, depression, and macrocytic anemia (increased red blood cell volume).

Synthetic drugs, such as the non-ionophore coccidiostats diclazuril, amprolium, halofuginone, nicarbazin, robenidine, and decoquinate have specific response mechanisms against parasitic metabolism (Mortier *et al.* 2005).

For example, amprolium blocks the absorption of thiamine by *Eimeria* spp. (Rogers, 1962; Reid, 1972). When compared to supplementation with ionophore coccidiostats, dietary supplementation with synthetic antibiotics (non-ionophore coccidiostats) was more effective against *Eimeria* and improved broiler performance (Peeters *et al.* 1994).

Another non-ionophore coccidiostat (chemical synthetic antibiotic) is diclazuril, which is used to prevent the onset of coccidiosis. Diclazuril has been used to prevent coccidiosis in broilers since 1989 and is known to be effective against all *Eimeria* spp. in poultry (Vanparijs *et al.* 1989; El-Banna *et al.* 2005; Mortier *et al.* 2005). Moreover, Asadi Iraee *et al.* (2015) reported that non-ionophore coccidiostats like diclazuril improved performance of chickens. In addition, Conway *et al.* (2001) indicated that diclazuril is more efficacious in preventing coccidiosis than the other widely utilized chemical and ionophore anticoccidials. Researcher McDougald *et al.* (1990) and Conway *et al.* (2001) found dietary supplementation of diclazuril improved the performance of broiler chickens.

Globally, various coccidiostats (ionophore or non-ionophore) are commonly used in poultry diets for prevention of coccidiosis diseases across the world. There are contradictory reports about using these coccidiostats (non-ionophore or ionophore), even at recommended levels, for broiler chickens.

Some researchers have stated, care should be taken when using the ionophore coccidiostats drug because it affects the growth, feed consumption, mineral bioavailability, sulfur amino acid requirements, anion-cation balance and metabolism of birds under certain conditions (Keshavarz and McDougald, 1982; Elsasser, 1984; Versteegh *et al.* 1990; Ebrahimnezhad *et al.* 2010).

Therefore, the purpose of this study was to further examine and compare the effects of ionophore and non-ionophore coccidiostats on performance, carcass characteristics, blood biochemical parameters and gut microbial flora in broiler chickens.

MATERIALS AND METHODS

Animals, breeding and nutrition

The research protocol utilized in this study was recommended by the Animal Care and Use Committee of Islamic Azad University Ethics Committee. A total of 300 one-day-old broiler chicks of the Ross 308 strain (Aviagen, 2018) were purchased from a commercial hatchery. All broiler chicks were housed in 1.5 × 1.5 m floor pens each equipped with a pan feeder and manual drinker. Stocking densities are based on the EU broiler welfare Directive (2007), 33 kg/m2 (Aviagen, 2018). Male and female chicks were separated by the feather sexing method and weighed on day one. Chicks were distributed to cages so that the average body weight was approximately equal. Birds received 22L:2D throughout the experiment period. There were 25-floor pens in this completely randomized design study utilizing five treatment groups and five replicates with 12 chicks (six males and six females) in each replicate. The basal diet formulated to the nutritional requirements of NRC (1994), Table 1. The five experimental groups were as follows:

Group 1) basal diet (control group), group 2) basal diet with 60 ppm salinomycin (ionophore coccidiostats), group 3) basal diet with 3.75 ppm maduramicin (ionophore coccidiostats), group 4) basal diet with 1 ppm diclazuril (non-ionophore coccidiostats) and group 5) basal diet with 125 ppm amprolium (non-ionophore coccidiostats).

The salinomycin in this study was Salinocox® (recommend level 500 grams per ton), which is contained 120 grams salinomycin sodium per kg. Maduramicin in this work was Maduracarb® (recommend level 500 grams per ton), which is contained 7.5 grams maduramicin per kg, diclazuril in this study was Qilucoxid Rooyan® (recommend level 200 grams per ton), which is contained 5 grams Diclazuril per kg, Amprolium in this experiment was Ethoamprox® (recommend level 500 grams per ton), which is contained 250 grams amprolium per kg.

This study lasted 42 days. Feed and water were available *ad libitum* throughout the study and broilers were kept under management conditions recommended for the Ross 308 strain. Feed form in this study was mash.

Evaluation of growth performance

Broiler chicks were weighed and feed intake (FI) was re-corded at the end of each period. Body weight gain (BWG), FI, and feed conversion ratio (FCR) were calculated based on performance values. European broiler index (EBI) from day 1 to day 42 was calculated using the following equation (Ghasemi-Sadabadi *et al.* 2019):

European broiler index (EBI)= [average daily gain (g/bird) × survival rate (%)] / [feed conversion ratio × 10]

Slaughtering method and carcass characteristics

Six birds (three males and three females) from each pen were randomly sampled for carcass evaluation at 42 days; each was slaughtered, dissected manually and weighed. The chickens were electrical stunned individually on the head using 70 V prongs. Thereafter, heads were decapitated from the neck using a sharp knife. Feathers were plucked and carcasses gutted by hand. The live body weight, eviscerated carcass, breast, thigh, wings, abdominal fat, liver, and intestines were excised and individually weighed. Carcass yields were calculated as a percentage of the pre-slaughter live body weights. Internal organ weights were expressed as a percentage of live body weight. Small intestinal length was expressed in centimeters.

Gut microbial flora

Two birds (one male and one female) on 24 days of age and six birds (three males and three females) on 42 days of age, from each pen were randomly selected for the measurement of intestinal microbial flora. For this purpose, one gram of the composite gut sample from each chicken was diluted with 9 mL of 0.9% saline solution and mixed on a vortex. Viable counts of bacteria in the gut samples were then conducted by plating serial tenfold dilutions (in 1% peptone solution) into *Lactobacilli* de Man, Rogosa and Sharpe agar plates and MacConkey agar plates (to isolate the *Lactobacillus* and *Coliforms*), respectively. The *Lactobacilli* de Man, Rogosa and Sharpe agar plates were then incubated for 48 h at 37 °C under anaerobic conditions. The *Lactobacillus* and *Coliforms* colonies were conducted immediately after removal from the incubator as described by (Proietti *et al.* 2008; Ghasemi-Sadabadi *et al.* 2019). Also, reiforced Clostridia agar was used for the enumeration of the anaerobic bacteria. The plates were incubated in anaerobic jars (Oxoid) with Anaerogen (Oxoid) at 37 °C for 48h. Bacterial colony forming units (CFU) in the petri dishes were counted using a colony counter. The counts were expressed as log10 colony forming units per gram of digesta (log10 CFU/g) (Proietti *et al.* 2008; Ghasemi-Sadabadi *et al.* 2016; Ghasemi-Sadabadi *et al.* 2019).

Table 1 Composition of the basal diet (ingredients and nutrients) given to broiler chickens for 6 weeks

Ingredient, g/kg	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	566.4	570.5	611.6
Soybean meal, 44% CP	367.4	351.2	312.0
Soybean oil	09.5	32.0	32.2
Dicalcium phosphate (DCP)	1.89	16.5	15.3
Oyster shell	13.5	11.2	10.8
Sodium bicarbonate	2.6	2.6	2.6
Common salt	2.3	2.3	2.3
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
DL-Methionine	4.2	2.6	2.5
L-lysine monohydrochloride	3.8	1.1	1.2
L-threonine	6.4	5.0	4.5
Calculated nutrient			
Metabolizable energy, kcal/kg	2850	3000	3050
Crude protein %	22.14	20.95	19.54
Calcium %	1.05	0.90	0.85
Available phosphorus %	0.50	0.45	0.42
Sodium %	0.18	0.18	0.18
Potassium %	0.90	0.87	0.81
Chlorine %	0.17	0.17	0.17
Met + Cys %	1.07	0.90	0.86
Lysine %	1.43	1.18	1.09
Threonine %	0.94	0.80	0.74
Tryptophan %	0.94	0.29	0.26

[1] Vitamins mixture provide per 2.5 kilogram of diet: vitamin A: 12000000 IU; vitamin B_1: 4000 mg; vitamin B_2: 6000 mg; vitamin B_3: 18000 mg; vitamin B_6: 3000 mg; vitamin B_{12}: 15 mg; vitamin D_3: 5000000 IU; vitamin E: 50000 IU; vitamin K_3: 3000 mg; vitamin B_9: 1500 mg; vitamin B_5: 70000 mg; vitamin H_2: 100 mg and Choline chloride: 400000 mg.
[2] Mineral mixture provide per 2.5 kilogram of diet: Mn: 120000 mg; Zn: 100000 mg; Fe: 40000 mg; Cu: 20000 mg; I: 1000 mg and Se: 300 mg.

Blood biochemical parameters

At the end of experiment, six birds (three males and three females) from each pen starved 12 h before blood sampling from the brachial vein in nonheparinized tubes. The blood was centrifuged at 2000 × g for 15 min to obtain serum. All the samples were properly labeled and stored for further analysis at -20 ˚C.

Serum biochemical parameters including glucose, total protein (TP), albumin, uric acid, calcium and phosphorus were calculated using a Technicon RA-1000 Auto-analyzer (Technicon Instruments Corporation, Tarrytown, New York, USA) and the kit package (Pars Azmoon Co Tehran, Iran).

Plasma globulin concentration was determined by the difference between total protein and albumin (Coles, 1986). Blood sodium and potassium concentration was analyzed by AOAC (2005) methods with a Flame photometer (Ebrahimnezhad *et al.* 2010).

Statistical analysis

Data were analyzed in a completely randomized design using the General Linear Models procedures of SAS (SAS, 2003) and significant differences between treatments were separated using Tukey (Tukey, 1949) range test at (P<0.05).

RESULTS AND DISCUSSION

Performance

The effects of using ionophore and non-ionophore coccidiostats on broiler chicken BWG, FI, FCR and EBI are shown in Table 2. Experimental treatments showed significant effects on broiler BWG at 1-10, 24-25, and 1-42 days (P<0.05). At 1-10 days, broilers supplemented with diclazuril displayed higher BWG than any other group. Broilers raised on diets supplemented with diclazuril had significantly higher BWG when compared to broilers reared on diets supplemented with ionophore coccidiostats (salinomycin and maduramicin) at 25-42 and 1-42 days. No significant differences were observed in FI among treatments at 1-10, 11-24 and 25- 42 days. Broiler FI for days 1-42 was significantly higher (P<0.05) for chickens supplemented with diclazuril when compared to chickens supplemented with salinomycin. There were no significant differences in FCR and EBI between treatments during any period.

Carcass characteristics

The effects of using ionophore and non-ionophore coccidiostats on carcass characteristics at 42 days on male and female broiler chickens are shown in Table 3.

Table 2 Comparison of using ionophore coccidiostats (maduramicin and salinomycin) and non-ionophore coccidiostats (diclazuril and amprolium) on performance of broiler chickens (1-42 d)

Traits	Control	Salinomycin	Maduramicin	Diclazuril	Amprolium	SEM	P-value
Body weight gain, g							
Period							
1-10 d	155.50^b	153.06^b	152.85^b	166.52^a	157.77^{ab}	6.68	0.0427
11-24 d	574.30	540.79	546.67	551.76	537.98	11.80	0.2535
25-42 d	1573.73^{ab}	1468.19^{bc}	1439.41^c	1657.59^a	1545.27^{abc}	40.71	0.0131
1-42 d	2303.53^{ab}	2162.04^c	2138.93^c	2375.87^a	2241.02^{bc}	41.14	0.0053
Feed intake, g							
Period							
1-10 d	212.31	204.54	209.70	217.50	212.64	4.52	0.3926
11-24 d	810.69	782.95	782.78	789.50	764.93	15.24	0.3648
25-42 d	3156.40	3115.31	3020.62	3385.00	3180.63	93.07	0.1354
1-42 d	4179.4^{ab}	4102.8^{ab}	4013.10^b	4392.00^a	4158.20^{ab}	98.29	0.0408
Feed conversion ratio							
Period							
1-10 d	1.36	1.33	1.37	1.30	1.35	0.02	0.2623
11-24 d	1.41	1.44	1.43	1.43	1.42	0.01	0.3795
25-42 d	2.00	2.12	2.10	2.04	2.05	0.03	0.2683
1-42 d	1.81	1.89	1.87	1.84	1.85	0.02	0.2869
European broiler index							
Period							
1-42 d	263.93	246.47	250.11	266.93	257.09	11.58	0.6965

European broiler index (EBI)= [average daily gain (g/bird) × survival rate (%)] / [feed conversion ratio × 10].
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Comparison of using ionophore coccidiostats (maduramicin and salinomycin) and non-ionophore coccidiostats (diclazuril and amprolium) on carcass characteristics of female and male broiler chickens at 42 days

Traits	Control	Salinomycin	Maduramicin	Diclazuril	Amprolium	SEM	P-value
Male carcass characteristics							
Carcass yield (%)	73.57^{ab}	72.53^{ab}	69.16^b	75.55^a	72.97^{ab}	2.17	0.0257
Abdominal fat (%)	1.74	1.39	1.55	1.59	1.53	0.12	0.4445
Liver (%)	2.35	2.75	2.72	2.59	2.72	0.22	0.6390
Small intestine (cm)	173.38	169.68	168.02	174.83	170.53	5.57	0.8292
Intestine yield (%)	4.11	3.38	3.22	4.28	3.88	0.24	0.0618
Breast yield (%)	32.23^b	32.85^b	32.93^b	35.72^a	33.59^{ab}	0.82	0.0204
Thigh yield (%)	28.59	27.87	26.49	29.14	28.96	0.65	0.1102
Wings yield (%)	6.66	6.44	6.13	6.99	6.30	0.72	0.2387
Female carcass characteristics							
Carcass yield (%)	74.40^a	69.55^b	69.68^b	72.67^{ab}	72.31^{ab}	2.14	0.0258
Abdominal fat (%)	1.58	1.60	1.58	1.53	1.68	0.14	0.9684
Liver (%)	3.01	2.96	3.58	3.00	3.27	0.24	0.1654
Small intestine (cm)	177.34	174.37	173.77	177.71	177.83	4.40	0.3716
Intestine yield (%)	4.15	3.91	3.31	4.16	3.68	0.34	0.4018
Breast yield (%)	33.36	31.34	31.37	31.85	33.47	1.70	0.8389
Thigh yield (%)	29.76^{ab}	28.07^{ab}	27.33^b	30.64^a	28.09^{ab}	0.70	0.0256
Wings yield (%)	6.98	6.07	6.74	6.25	6.00	0.37	0.0770

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

The results of this study showed that carcass and breast yield for male broilers and carcass and thigh yield for females were significantly affected by certain treatments at day 42 (P<0.05). Male diclazuril supplemented broilers presented significantly higher carcass yield than other groups (P<0.05).

Female diclazuril supplemented broilers displayed higher breast yield when compared with salinomycin and maduramicin supplemented females. Both carcass and thigh yield for ionophore coccidiostat supplemented groups were significantly lower than those in the control group for female broiler chickens (P<0.05).

Gut microbial flora

The effects of using ionophore and non-ionophore coccidiostats on gut microbial flora at 24 and 42 days on male and female broiler chickens are shown in Table 4. No significant effects on gut microbial floras in either male or female broiler chickens were apparent at 25 days, nor in females at 42 days. Significant differences in gut microbial floras between ionophore and non-ionophore coccidiostat treatment groups were present at 42 days for male broilers only (P<0.05). Males supplemented with diclazuril had significantly higher *Lactobacilli* populations and lower *Coliform* bacteria populations than males supplemented with salinomycin (P<0.05).

Blood biochemical parameter

The effects of using ionophore and non-ionophore coccidiostats on blood biochemical parameters at 42 days on male and female broiler chickens are shown in Table 5. Blood glucose and albumin concentration in male and female broiler chickens were significantly affected by treatments; treatments also had a significant effect on blood sodium, potassium, and total protein concentration in male broiler chickens (P<0.05). Male broilers supplemented with diclazuril coccidiostats in diets had higher blood glucose concentrations than males supplemented with salinomycin. Female broilers supplemented with amprolium and diclazuril in diets showed higher blood glucose concentrations compared to females supplemented with salinomycin (P<0.05). Diclazuril coccidiostats significantly increased total protein concentrations in male broilers when compared to males supplemented with salinomycin and maduramicin (P<0.05). The results of this study showed that the use of salinomycin and maduramicin in diets significantly decreased blood sodium concentrations when compared to birds supplemented with diclazuril and those in the control group in male chickens (P<0.05). In addition, regarding the data, it can be concluded that supplementation of salinomycin and maduramicin resulted in significantly higher blood potassium concentrations in male chickens (P<0.05).

The results showed that the use of diclazuril had a better effect on BWG at 1-10 and 1-42 days compared to the ionophore coccidiostats and control group. In addition, the use of diclazuril had a better effect on carcass, breast, and thigh yield when compared to ionophore coccidiostats, although there was no significant difference between the diclazuril and control groups. Diclazuril is a well known and approved coccidiostats for the prevention and treatment of coccidiosis in broiler chickens. In similar case, Habibi *et al.* (2014) stated that the use of 1 ppm diclazoril in diets improved FCR in broiler chickens at the end of experiment period. McDougald *et al.* (1990) showed that the use of diclazuril in the diet improved final live weight and feed

conversion at 49-day broiler chickens. The positive effects of diclazuril on performance compared to ionophore coccidiostats is likely due to improved intestinal function due to reduction of *Eimeria* oocytes (Pirali-Kheirabadi *et al.* 2008). Researchers showed that the chicks were inoculated by *E. tenella* revealed higher BWG and a significant reduction in the oocyst counts in medicated group with diclazuril in comparison with a non-medicated group (Habibi *et al.* 2014). In same study, Pirali-Kheirabadi *et al.* (2008) demonstrated that the use of diclazuril significantly contributed to the destruction of oocytes when compared to treatment with salinomycin. The results of the present study were consistent with the results of Conway *et al.* (2001) who observed that supplementation with diclazuril at one ppm improved broiler chicken performance when compared to supplementation with ionophore coccidiostats. Other researchers, Asadi Iraee *et al.* (2015) showed that diclazuril had positive effect on body weight, WG and FCR of infected groups only in the 4th week of the experiment. Maes *et al.* (1988) found that the use of diclazuril at one ppm has negative, even lethal, effects on both sexual and non-sexual stages of *E. tenella*. It is well known that diclazuril approved for the prevention and treatment of coccidiosis in broiler chickens by Pirali-Kheirabadi *et al.* (2008). Jiang-Zhong (1999) showed that supplementation of diclazuril in drinking water to control *E. tenella* increased body weight in broiler chickens. In addition, researchers observed that the supplementation of diclazuril improved the pathological changes and anti-oxidant ability of the chickens by inhibiting the growth and reproduction of *E. tenella*. Therefore, it seems that the use of diclazuril can improve the performance of broiler chickens (Zhou *et al.* 2015). According to the results obtained from the Table 2, it can be concluded that the use of ionophore coccidiostats at recommended levels reduced the performance of broiler chickens compared to diclazuril. Such findings agree with Pirali-Kheirabadi *et al.* (2008) and Lee *et al.* (2012) who demonstrated that salinomycin significantly lowers broiler body weight when compared with diclazuril.

Jones *et al.* (1990) noted that salinomycin at a level of 60 mg kg-1 in feed caused a significant drop in hatchability, but not in egg production. Relatively low doses of ionophore can also be toxic to turkeys and their toxicity may be potentiated by other substances and notably by the antimicrobial drug tiamulin (Konstantinos *et al.* 2013). Addition of ionophore coccidiostats, especially salinomycin, into the diet caused inhibition of the activity of the enzymes amylase and lipase, which may explain the lack of growth performance in broiler chickens in this study (Radu *et al.* 1987; Harms *et al.* 1989). Ionophore coccidiostats have free radicals, which cause oxidative degradation in the body (Novilla, 1992).

Table 4 Comparison of using ionophore coccidiostats (maduramicin and salinomycin) and non-ionophore coccidiostats (diclazuril and amprolium) on intestinal microbial flora of male and female broiler chickens. (Log_{10} cfu/g)

Traits	Treatments					SEM	P-value
	Control	Salinomycin	Maduramicin	Diclazuril	Amprolium		
Male							
Intestinal microbial flora at 24 day							
Total bacteria	6.14	7.00	6.61	6.44	6.80	0.80	0.9469
Coliforms	4.07	4.56	4.94	4.42	4.24	0.91	0.9686
Lactobacillus	4.90	4.08	4.76	4.61	4.72	0.86	0.9680
Male							
Intestinal microbial flora at 42 day							
Total bacteria	5.36	6.89	6.45	5.85	6.86	0.75	0.5609
Coliforms	5.03[b]	7.05[a]	6.34[ab]	5.21[b]	5.82[ab]	0.51	0.0491
Lactobacillus	7.72[a]	4.06[c]	4.74[bc]	6.89[ab]	6.74[abc]	0.64	0.0048
Female							
Intestinal microbial flora at 24 day							
Total bacteria	5.27	5.99	5.88	5.40	5.33	0.75	0.9332
Coliforms	5.03	5.92	5.65	5.83	4.77	0.96	0.8848
Lactobacillus	5.56	5.16	5.36	5.87	5.14	0.80	0.9556
Intestinal microbial flora at 42 day							
Female							
Total bacteria	5.96	6.08	6.83	5.35	5.97	0.92	0.8552
Coliforms	5.62	6.43	6.52	5.85	6.14	0.52	0.7010
Lactobacillus	5.83	5.40	5.22	5.95	6.03	0.76	0.9118

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

In addition, Assen (2006) showed that the use of 20-30 ppm salinomycin in feed caused difficult and incoordinated movements, paralysis of leg and neck muscles, dysponea and diarrhea and even 34.5% mortality in turkey (Rizvi *et al.* 2008). The researchers showed that continued use of salinomycin, even at the recommended level, for protection of coccidiosis can decreased growth performance. The growth reduction at 60 ppm in layer chicks is in congruence with findings of various researchers (Prohaszka and Rozsnyai, 1990; Thompson *et al.* 2005). Similarly, Novilla (1992) found that supplementation with high-levels of ionophores caused poisoning in poultry and Konstantinos *et al.* (2013) reported that the use of high levels of salinomycin decreased the performance of turkeys and broiler breeders. Other studies have confirmed that the use of salinomycin decreased the growth performance of broiler chickens (Migaki and Babcock, 1979; Patel *et al.* 1980). Harms *et al.* (1989) found that the use of high levels of salinomycin in diets decreased the amount of feed intake and body weight in Cobb 500 broiler chickens. Researchers have shown that long-term consumption of salinomycin even at recommended levels can reduce the growth performance of poultry (Ebrahimnezhad *et al.* 2010). Although, Pearson *et al.* (1990) reported that the use of 40-80 ppm salinomycin in diets had no significant effects on body weight.

Rizvi *et al.* (2008) and Harms *et al.* (1989) observed lower feed intake and body weight with different levels of salinomycin supplementation in broiler chickens. Radu *et al.* (1987) and Engberg *et al.* (2000) reported that birds fed salinomycin was not heavier than control birds up to 42 d of age in a floor pen trial. It seems that the decline in the growth associated with the use of this antibiotic at high levels is due to its inherent properties, which ultimately reduced feed intake (Keshavarz and McDougald, 1982). However, the results of this experiment did not match the results of these other researchers (Keshavarz and McDougald, 1982; Leeson and Summers, 1983). In the present study, the carcass yield significantly improved in chickens supplemented with diclazuril, whereas the relative weights of the breast on male broiler and thigh in female broiler were significantly higher in diclazuril group.

It seems that this may be related to the high BWG of broiler chickens in diclazuril groups. Regarding the data, it can be concluded that the use of ionophore and non-ionosphere coccidiostats did not significantly affect abdominal fat, liver, small intestine, and intestine yield. Our results were in agreement with the findings of Ebrahimnezhad *et al.* (2010) and Khan (2005) who indicated that the supplement of ionophore in diets did not affect heart and liver weight.

Table 5 Comparison of using ionophore coccidiostats (maduramicin and salinomycin) and non-ionophore coccidiostats (diclazuril and amprolium) on blood biochemical parameters of male and female broiler chickens

Traits	Treatments					SEM	P-value
	Control	Salinomycin	Maduramicin	Diclazuril	Amprolium		
Male							
Blood biochemical parameters							
Glucose (mg/dL)	213.75[ba]	196.00[c]	198.25[bc]	216.25[a]	209.25[abc]	3.85	0.0035
Total protein (g/dL)	6.58[ab]	5.78[b]	5.49[b]	7.34[a]	6.65[ab]	0.42	0.0406
Albumin (g/dL)	4.20	3.31	3.55	4.37	3.77	0.26	0.0628
Globulin (g/dL)	2.38	2.47	1.94	2.97	2.88	0.48	0.5808
Uric acid (mg/dL)	4.07	5.00	4.90	4.10	4.80	0.59	0.6801
Calcium (mg/dL)	7.04	7.83	8.10	7.72	7.35	0.41	0.4328
Phosphorus (mg/dL)	5.42	5.92	5.92	4.92	5.30	0.35	0.2607
Sodium (mmol/L)	128.75[a]	115.00[b]	113.75[b]	127.75[a]	120.00[ab]	2.30	0.0006
Potassium (mmol/L)	3.60[b]	4.39[a]	4.36[a]	3.95[ab]	3.96[ab]	0.17	0.0277
Female							
Blood biochemical parameters							
Glucose (mg/dL)	206.50[ab]	194.75[b]	199.25[ab]	216.75[a]	215.75[a]	4.50	0.0034
Total protein (g/dL)	6.56[ab]	5.80[b]	5.70[b]	7.12[a]	6.13[ab]	0.34	0.0457
Albumin (g/dL)	3.61	3.03	3.19	4.16	3.70	0.29	0.1034
Globulin (g/dL)	2.95	2.77	2.51	2.96	2.43	0.31	0.6494
Uric acid (mg/dL)	4.02	4.50	4.47	4.05	4.15	0.86	0.9898
Calcium (mg/dL)	6.60	7.94	7.82	7.00	7.45	0.35	0.0909
Phosphorus (mg/dL)	4.82	5.65	4.87	5.35	4.72	0.29	0.1724
Sodium (mmol/L)	130.00	126.25	126.50	128.75	125.50	5.96	0.9805
Potassium (mmol/L)	3.88	4.11	4.18	4.00	3.86	0.15	0.5204

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

In this study, there were no significant differences in mean performance and carcass characteristics among the salinomycin and maduramicin and amprolium groups. These results are in agreement with Ebrahimnezhad *et al.* (2010) who did not observe differences between salinomycin and maduramicin at rearing period. The results of the this study showed that the use of diclazuril in the diet increased the population of *Lactobacilli* bacteria and reduced the population of *Coliform* bacteria, while the use of ionophore coccidiostats in the diet reduced the number of *Lactobacilli* bacteria population and increased coliform bacteria. Reducing the number of *Lactobacilli* by using salinomycin in male broilers may be due to the synergistic effects of this antibiotic with *Lactobacillus* bacteria (Engberg *et al.* 2000). Engberg *et al.* (2000) showed that *Lactobacillus salivarius* was present in high numbers among the lactic acid bacteria in broilers gut microflora, therefore *L. salivarius* was clearly inhibited in the whole intestinal tract due to the synergistic effect of ionophore coccidiostats. The previous study demonstrated that the supplementation of salinomycin in broiler diets resulted in lower concentrations of lactic acid, indicating the reduction of *L. salivarius* in the intestine (Engberg *et al.* 2000). Because of this, researchers have suggested that *L. salivarius* is the main bacteria in the chicken gut and it is highly susceptible to Salinomycin (Engberg *et al.* 2000; Adhikari and Kwon, 2017).

Dutta and Devriese (1984) indicated that ionophore coccidiostats strongly inhibited the growth of *Lactobacilli* in growth media (Engberg *et al.* 2000). Also, Czerwiński *et al.* (2012) reported that use of salinomycin reduced the number of *Lactobacillus* and *Enterococcus* in the ileum and cecum. However, there have been reports of ionophore coccidiostats, especially maduramicin, having antibacterial effects on many gram-positive bacteria (Sharma *et al.* 2005), such as *Lactobacilli* species (Klaenhammer and deVos, 2011). The present study found that the use of salinomycin in diets increased the coliform bacteria counts compared to the diclazuril and the control groups. Use of salinomycin can increase the number of *Coliform* bacteria population in the ileum and rectum of broiler chickens (Engberg *et al.* 2000). Therefore, the relatively high number of *Coliform* bacteria found in this study can be related to the reduction of the *Lactobacilli* bacteria population caused by the reduction of intestinal pH (Salminen and Salminen, 1997). Czerwiński *et al.* (2012) found that the use of salinomycin in diets reduced *Lactobacillus* population counts in the intestine. Furthermore, researchers have reported that ionophore coccidiostats have a direct effect on the activity of intestinal microorganisms (Czerwiński *et al.* 2012). In another study, Johansen *et al.* (2007) found that supplementation with ionophore coccidiostats raised the risk of necrotic enteritis in intestinal tissue.

While, a different study found that the use of monensin, an ionophore coccidiostat, did not show a significant effect on the level of *Salmonella* colonization in the intestine (George *et al*. 1981; Manning *et al*. 1994). In the present experiment, microbial flora of broilers was not significantly affected by diet supplementation with salinomycin and maduramicin and amprolium groups. There were no significant differences in mean microbial flora among the salinomycin and maduramicin and amprolium groups.

The results of this experiment show that the use of ionophore coccidiostats reduced the serum total protein concentration in both male and female broiler chickens. Previous studies have shown that serum total protein concentration was decreased by salinomycin treatment (Arun *et al*. 2003; Hussein and Abd-El-Rahman, 2005). This result agrees with Hussein and El-Rahman (2005) who indicated that supplementation with salinomycin had significant effects on serum total lipid, total protein, alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine, urea, calcium, and phosphorus groups. Similarity, it was demonstrated that the use of ionophore coccidiostats such as maduramycin increased ALT and alkaline phosphatase (ALP) activities, and triglyceride, uric acid and creatinine levels in broiler chickens. High serum ALP activity is observed in bone diseases, whilst increased creatinine levels are associated with damage to muscle tissue (Karabacak *et al*. 2015). Kamashi *et al*. (2004) state that the liver is the main organ for protein synthesis; therefore, it seems that the use of salinomycin at higher levels reduced protein synthesis, possibly due to oxidative damage to hepatocytes. Also, in this experiment, serum glucose concentration significantly decreased in salinomycin treatments compared to other groups. In addition, in our results, we also observed that glucose concentration in the Amprolium was significantly higher than salinomycin in the female broilers.

It seems that decreasing serum glucose concentration in this study can be related to the inhibition of amylase and lipase enzyme activity through salinomycin supplementation (Radu *et al*. 1987; Lee *et al*. 2012). Also, it is possible that the low blood glucose concentrations found in this study were due to alpha-amylase inhibition that reduces the digestion and absorption of starch (Tadera *et al*. 2006; Lo Piparo *et al*. 2008). Researchers investigated histopathological changes in skeletal muscle and observed degeneration and necrosis in various tissues as they analyzed poultry diet, concluding that salinomycin plays an important role in histopathologic changes in birds (Andreasen and Schleifer, 1995). It appears that histopathological changes caused by salinomycin can be related to variations in blood metabolites. Karabacak *et al*. (2015) stated that salinomycin could cause liver damage, which can explain the changes in the biochemical parameters. Also, researchers have noted

that the changes in the biochemical parameters can be related to degenerative disorders due to active oxygen production by ionophore coccidiostats (Karabacak *et al*. 2015). Based on these findings, it can be concluded that the use of ionophore coccidiostats decreased the serum sodium concentration in male chickens in comparison with control and diclazuril groups. Similarity, Ebrahimnezhad *et al*. (2010) demonstrated that the use of salinomycin and maduramicin in diets decreased blood sodium concentration in broiler chickens. It seems that the decrease in blood sodium levels found in this experiment is related to nutritional absorption impairment due to possible damage to the intestinal mucosa, which ultimately changed the absorptive mechanism of the digestive tract. Additionally, Boehmerle and Endres (2011) indicated that salinomycin causes the transfer of extracellular sodium to cell cytosol leading to increased cytosol sodium concentrations; consequently, blood sodium concentration drop due to the transfer of serum sodium to the intercellular region. Blood potassium concentrations in male chickens was significantly higher in the salinomycin and maduramicin treatments compared to the control group. The results of this research agree with the findings of other authors (Khan, 2005; Ebrahimnezhad *et al*. 2010) who reported that the use of ionophore coccidiostats significantly increased blood potassium. Khan (2005) and Ebrahimnezhad *et al*. (2010) confirmed that salinomycin and maduramicin have a strong affinity for binding potassium, and thus these coccidiostats induce the transfer of potassium from the intercellular to the extracellular space resulting in increased blood potassium concentrations. Previous studies showed that salinomycin tends to bind to potassium and sodium, causing a disruption of cation transport in mitochondrial membranes (Zinn, 1986). Ionophore coccidiostats, such as salinomycin, cause a decrease in the levels of sodium ions resulting in a change in cellular osmotic pressure (Zinn, 1986; Hosseini *et al*. 2013). Ionophore coccidiostats have a high affinity for combing with cations resulting in an increase in cation concentrations when compared to the control group because these antibiotics transferred cations from the inside to the outside of the cell (Ebrahimnezhad *et al*. 2010). The results of this experiment were in agreement with the results of (Khan, 2005). Previous studies have showed that ionophore coccidiostats, such as salinomycin, lasalocid, and monensin, combined with specific cations causing changes in the transfer of these cations in the cell membrane (Merchen and Berger, 1985). However, Dovorak *et al*. (1980) observed no difference in cation plasma concentration between monensin and control groups in bulls. Ionophore coccidiostats prevent the transmission of some electrolytes, thus these antibiotics disturb metabolism, causing toxicity and reduced function (Karabacak *et al*. 2015). In general, the low performance of

ionophore coccidiostats, such as the maduramicin and salinomycin used in this experiment can be related to the effects ionophore coccidiostats have on cation (K+, Na+, Ca+) transport across cell membrane. This ultimately leads to changing the entrance of cations into the cell, therefore causing disorders of the cell membrane and cell death (Sharma *et al.* 2005; Boehmerle and Endres, 2011).

CONCLUSION

Consequently, the results of this study indicated that the supplementation of diclazuril had improved BWG at 1-10 and 1-42 days and FI at whole experiment period. Regarding the data, it can be concluded that the use of diclazuril in diets had beneficial effects on intestinal bacteria population, and blood biochemical parameters compared to ionophore coccidiostats such as salinomycin and maduramicin in broilers. Generally, these experimental results showed that the use of non-ionophore coccidiostats especially diclazuril had better effects on male and female broiler chickens. Therefore, it seems that non-ionophore coccidiostats such as diclazuril could be considered more useful coccidiostats by farmers.

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