



Dietary Supplementation of *Silybum marianum* or *Curcuma spp* on Health Characteristics and Broiler Chicken Performance

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ABSTRACT

This study was carried out to investigate the effect of *silybum marianum* (SM), *Curcuma spp* (CP), or their mixture (PM) on intestinal microflora and broiler chicken performance. A total of 180 unsexed broiler chicken (Ross-308) were randomly assigned to 4 diets with 3 replications of set 15 chickens in each. Diets were included control or the inclusion of SM, CP, or PM (equal amount) at level of 0.5% in diets. Feed intake, body weight gain, and feed conversion ratio were significantly increased by diets contained CP and PM rather other diets at 42 days of age ($P<0.05$). In contrast, the inclusion of SM and PM in diets resulted in significantly decreases in total number of bacteria, gram-negative bacteria, and coliforms bacteria in ileum rather other diets at 42 days of age ($P<0.05$). The inclusion of tested medicinal plants in diets led to significantly decreases in pH value in ileum and significantly increases in intestinal weight and length rather control ($P<0.05$). The results of present study have shown that the inclusion of 0.5% *Curcuma spp* or *Silybum marianum* in diets boost up broiler chicken performance and reduced ileum pathogenic bacteria. The equal mixture of tested medicinal plants showed mutual effects which could help to improve intestinal health and well being of poultry.

Key words: broiler chicken, intestinal microflora, medicinal plant

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INTRODUCTION

Antibiotic resistance and unreliable antibiotic therapy in poultry (Joerger, 2002) have lead to ban the use of antibiotic in many countries (Patterson and Burkholder, 2003). Increasing investigations regarding alternatives to antibiotics were widely carried out to achieve gut health and best growth performance. It is well documented that effect of antibiotics alternatives are mediated by intestinal microflora (Joerger, 2002). Today, there is accelerating trend toward the

development of using alternative ingredients particularly those from plants which are perceived as natural and safe ingredients. Medicinal plants are being used as feed additive to improve growth performance, to manipulate gut functions and microbial habitat of domestic animals (Panda *et al.*, 2000). The positive effects of medicinal plants were shown on health condition and production performance (Yakhkeshi *et al.*, 2012; Elmakki *et al.*, 2013). A variety of medicinal plants have been widely used to maintain and improve health of birds (Yakhkeshi *et al.*, 2012; Jadalla *et al.*, 2014) as prohibitory the growth of inward baneful bacteria through the digestion system (Hernandez *et al.*, 2004). They can modulate microflora population and improve growth performance (Chen *et al.*, 2003; Garca *et al.*, 2007; Yakhkeshi *et al.*, 2012).

Curcuma longa is a perennial herb, and a member of *Zingiberaceae* family. *Curcuma longa* has been reported to have antimicrobial, anti-inflammatory, anti-viral, antioxidant, and anti-cancer effect (Gandhi *et al.*, 2011). The main active component of *Curcuma longa* is curcumin (diferuloylmethane) which commonly used in Iran and Indian cuisine as a spice and food-coloring.

Silybum marianum is a member of *Asteraceae* family. *Silybum marianum* have been used as a natural medication for the liver and biliary duct since ancient times. Pharmacologically effective substance of silymarin includes four main ingredients: silybin, silychristin, silydianin, and isosilybin (Ding *et al.*, 2001) which have hepatoprotective, anti-inflammatory, cytoprotective and anti-carcinogenic effects (Manna *et al.*, 1999).

Although, there are many inconsistent results regarding substitution of medicinal plants for antibiotic and clarifying roles of these additives in poultry production, but some of these additives have been reported to have a great potential to replacement of antibiotics. Therefore, the present study was carried out to determine whether *Silybum marianum* and *Curcuma spp* or their mixture would influence the growth performance and intestinal microflora of broiler chicken to get health condition.

MATERIAL AND METHODS

Experimental Design and Birds

A total of 180 unsexed 1-day-old broiler chickens (Ross 308) were randomly divided to 4 treatments with 3 replications of 15 birds in each. Treatments were included of control (without medicinal plant) and the inclusion of *Silybum marianum* (SM), *Curcuma spp* (CP), or equal amount of plant mixture (PM) at level of 0.5% in diets. Diets were designed as starter (1 to 21 days of age) and grower (22 to 42 days of age) based on NRC (1994) recommendations to meet their nutrient requirements (Table 1). Feed and water were offered *ad libitum* in all period of experiment. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality were measured. The lighting schedule was 23 h light / 1 h darkness at 32°C the first day. This was subsequently reduced 3°C each week until third week. Thereafter it was constant.

Intestinal pH and Characteristics

Two birds from each replicate were randomly selected and sacrificed by cervical dislocation at 42 days of age. Weights and length of small intestinal as well as pH value of intestinal were measured. The digesta diluted nine-fold (w/v) with distilled water were stirred for 5 min and the pH of the suspensions were measured using a calibrated pH meter.

Microbial Sampling and Incubation

On day 42 of the experiment, 2 birds from each replicate were slaughtered by cervical dislocation and ileum contents were collected. Contents were gently removed into sterile sampling tubes and immediately transferred on ice to the laboratory. Serial dilutions of 1 g sample (10^{-4} to 10^{-7}) were made. Selective media of Nutrient Agar, MacConkey Agar, Eosin-methylene Blue Agar, and Xylose Lysine Deoxycholate Agar were inoculated to detect the total number of bacteria, coliforms, gram-negative bacteria, and Salmonella, respectively. Total number of bacteria and coliforms were counted after aerobic incubation for 24 h at 37°C. Gram-negative bacteria were counted after incubation for 48 h at 37°C and Salmonella were counted after incubation for 24 h at 37°C.

Table 1. Diet composition at different periods of the experiment¹

Ingredient (% or as stated)	Starter (1-21 d)	Grower (22-42 d)
Corn grain	53.80	60.74
Soybean meal (45% CP/kg)	38.70	32.22
Soybean oil	3.0	3.0
Calcium carbonates	1.63	2.05
Dicalcium phosphate	1.72	1.15
Premix ²	0.50	0.50
Common salt	0.44	0.23
DL-Met	0.14	0.06
L-Lys	0.07	0.05
Calculated Analysis		
Metabolizable energy (kcal/kg)	3,000	3,055
Crude protein	21.54	19.09
Calcium	0.93	0.85
Available phosphorus	0.45	0.33
Calcium: Phosphorus	2.07	2.57
Energy: Protein	139.27	160.03

¹*Silybum marianum*, *Curcuma spp.*, or plants mixtures (equal amount) were added to the basal diet at 0.5% to make the respective diets for each experiment, respectively. ²Supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (DL-alpha-tocopheryl acetate), 25 IU; menadione, 1.5 mg; vitamin B₁₂ (cyanocobalamin), 0.02 mg; biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 15 mg; pyridoxine (pyridoxine_HCl), 4 mg; riboflavin, 10 mg; and thiamin, 3 mg (thiamin mononitrate); 10 mg of copper (CuSO₄); 1.0 mg of iodine Ca (IO₃)₂; 80 mg of iron (FeSO₄·H₂O); 100 mg of manganese (MnSO₄·H₂O); 0.15 mg of selenium (NaSeO₃); 80 mg of zinc (ZnSO₄·H₂O); and 0.5 mg of cobalt (CoSO₄).

Statistical Analyses

All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure of GLM procedure of SAS software (SAS Inst. Inc., Cary, NC). A completely randomized design was employed. Pen was used as the experimental unit and data were analyzed by GLM procedure. Logarithmic (log₁₀) transformation was applied for microbial colony forming unit (CFU). Duncan's multiple range test were used for comparison of means (P<0.05).

RESULTS

The effect of dietary treatments on broiler chicken performance is shown at Table 2. The results indicated that the diets contained CP or PM led to significantly increases in FI and BWG as well as significantly decreases in FCR rather other diets (P<0.05). Diets contained medicinal plants resulted in significantly decreases in mortality rather control (P<0.05). Table 3 showed the effect of diets on ileum microflora population at 42 days of age. Diet contained SM or PM caused

to significantly decreases in total number of bacteria, gram-negative bacteria, and coliforms bacteria rather other diets ($P<0.05$). Moreover, none of samples had shown Salmonella. The effect of diets on the intestinal characteristics, pH, intestinal weight, and length, are presented at Table 4. The inclusion of tested medicinal plants in diets led to significantly decreases in pH value in ileum rather control ($P<0.05$). Same diets induced significantly increases in intestinal weight and length rather control ($P<0.05$).

Table 2. Effect of diets on broiler chicken performance at 42 days of age

Treatment	FI ¹ (g/d per bird)	BWG ² (g/d per bird)	FCR ³	Mortality (%)
Control	112.9 ^b	50.4 ^b	2.2 ^b	3.3 ^a
SM ⁴	103.2 ^c	45.3 ^c	2.3 ^c	2.0 ^b
CP ⁵	116.2 ^a	53.3 ^a	2.2 ^a	1.7 ^b
PM ⁶	116.0 ^a	53 ^a	2.2 ^a	1.7 ^b
SEM	2.82	1.28	0.06	0.35
P value	0.023	0.051	0.081	0.011

Means with common letters in the same column are not significantly different ($P<0.05$). SEM: Standard error of the means. ¹Feed intake, ²Body weight gain, ³Feed conversion ratio, ⁴*Silybum marianum*, ⁵*Curcuma spp.*, and ⁶Plants mixture.

Table 3. Ileum microflora in response to diets at 42 days of age (Log10 cfu/g of digesta)

Treatment	Total number of bacteria	Gram-negative	Coliforms	Salmonella
Control	10.00 ^a	9.33 ^a	9.00 ^a	Negative
SM ¹	8.33 ^b	7.33 ^b	6.00 ^c	Negative
CP ²	9.50 ^a	9.33 ^a	7.17 ^b	Negative
PM ³	8.83 ^b	7.83 ^b	6.50 ^c	Negative
SEM	0.23	0.21	0.18	NA ⁴
P value	0.0001	0.0001	0.0001	NA

Means with common letters in the same column are not significantly different ($P<0.05$). SEM: Standard error of the means. ¹*Silybum marianum*, ²*Curcuma spp.*, ³Plants mixture, and ⁴Not applicable.

Table 4. Effect of diets on the intestinal characteristics of birds at 42 days of age

Treatment	pH	Weight (g)	Length (cm)
Control	6.69 ^a	71.70 ^b	198.50 ^b
SM ¹	5.58 ^b	82.80 ^a	225.60 ^a
CP ²	5.87 ^b	80.50 ^a	219.60 ^a
PM ³	5.82 ^b	85.30 ^a	232.70 ^a
SEM	0.15	2.19	5.41
P value	0.004	0.003	0.004

Means with common letters in the same column are not significantly different ($P<0.05$). SEM: Standard error of the means. ¹*Silybum marianum*, ²*Curcuma spp.*, and ³Plants mixture.

DISCUSSION

Various parameters such as plant parts, physical properties, genetic variation, age, used dosage, extraction method, harvest time, and compatibility with the other ingredients can influence performance of broiler chicken fed with medicinal plant (Yang *et al.*, 2009). Based on obtained results, it posses that SM and CP had different effect on measured traits. The inclusion of CP in diets (as single or mixed with SM) could promote performance traits (FI, BWG, and FCR), while the inclusion of SM in diets (as single or mixed with CP) could decrease detrimental bacteria colonization in ileum. Dietary supplementation of CP exhibited a significantly positive

effect on FI, BWG, and FCR which is in accordance with several reports (Durrani *et al.*, 2006; Kumari *et al.*, 2007; Rajput *et al.*, 2013), who validated positive effect of *curcuma longa* / curcumin on broiler performance. These positive effects might be due to the well reported antiinflammatory, antioxidant, antibacterial activities (Chattopadhyay *et al.*, 2004), prebiotic like effects (Niamsa and Sittiwet, 2009), enhanced secretions of amylase, trypsin, chymotrypsin, and lipase enzymes by curcumin (Platel and Srinivasan, 2000). Moreover, Suchy *et al.* (2008) reported that the *Silybum marianum* treated groups of broiler showed significantly better performance as compared to the untreated group which is in agreement with current study. On the other hand, it is reported that mixture of medicinal plants resulted in better performance in broiler chicken (Mushtaq *et al.*, 2013). It is appear that inclusion of PM in diets had an intermediate effect as midway of tested medicinal plants.

Many medicinal plant oil and extracts have been reported to have antimicrobial properties (Lawless, 1995). It is proposed that plant antibacterial properties are related to their lipophilic characters (Farg *et al.*, 1989). The major mechanism of medicinal plants is adhesion and thrust of bacterial membrane which inhibits bacterial enzymes activation (Shapiro and Guggenheim, 1995; Stiles *et al.*, 1995). These reactions can reduce pathogenic populations in the intestine which was also seen in the present study by reducing gram-negative bacteria, coliforms, and total number of bacteria in ileum by the inclusion of SM or PM in diets. Results are in agreement with other studies (Guo *et al.*, 2004; Sarica *et al.*, 2005). One another possible mechanism of antimicrobial effect of medicinal plants is reducing of intestinal pH which confirm by obtained results of present study. Any factor that increases the activity of an organ above threshold levels can lead to increases in organs weight and length by hypertrophy and hyperplasia of the related organs (Yakhkeshi *et al.*, 2012). It seems that tested medicinal plants induce intestinal activation and increases intestinal weight and length.

CONCLUSION

The results of present study have shown that the inclusion of 0.5% *Curcuma spp* in diets improve broiler chicken performance while the inclusion of 0.5% *Silybum marianum* in diets reduce ileum pathogenic bacteria. The equal mixture of tested medicinal plants showed mutual effect.

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