



The Effect of Oral Consumption of guggul (*Commiphora Mukul*) Resin on Performance and Humoral Immunity Response of Broilers

Fariba Iranparast¹, Siamak Parsaei^{*2}, Mohammad Houshmand², Asghar Naghiha²

¹ Ms.c student of Animal Nutrition, Department of Animal Science, College of Agriculture, Yasuj University, Yasuj, Iran.

² Assistant professors of Department of Animal Science, College of Agriculture, Yasuj University, Yasuj, Iran.

ABSTRACT

This experiment was conducted to determine the effects of the oral consumption of guggul plant resin on the performance and humoral immunity response of broiler chicks. For doing this experiment, 320 one-day old chicks of the Cobb 500 strains, in a Completely randomized design with 4 treatments and 4 replicates were used. Experimental diets include: control diet (without the guggul), control diet + 200 ppm guggul, control diet + 400 ppm guggul, control diets + 600 ppm guggul. Live body weight, feed consumption and feed conversion ratio were recorded at 21 and 42 d of experiment. To determine antibody titers against sheep red blood cell (SRBC) 1 ml of 3% suspension of sheep erythrocyte in phosphate buffer saline was injected into the wing veins at 29 and 35-day chicks. Treatments had significant effects on daily gain, feed conversion and feed intake during growth period (22-42 d). Treatments had significant effects on total antibody titers and IgY ($p < 0.05$), but the effect of the treatments on the production of IgM was not significant. 6. The effect of the treatments on the bursa of Fabricius relative weight mean, was significant, but there was no significant effect on the spleen.

Key words: antibody, broilers, guggul, humoral immunity, sheep red blood cell.

1. INTRODUCTION

Birds have the natural resistance and the limited immunity against infection by pathogens. Industrial keeping of poultries in high extents and compressively, increases the possibility of the occurrence of the disease. In order to reduce the rate of occurrence of these diseases and also help to increase growth and improve the performance characteristics, various chemicals such as antibiotics are used in wide levels of broiler breeding units (Lee et al., 2003). Due to the possibility of establishing bacterial species resistant to antibiotics and also the notion of antibiotics staying in animal tissues, especially meat, use of growth stimulant antibiotics from the beginning of January 2006 in Europe was banned overall (Garcia et al., 2007). Therefore, antibiotics substituted materials including medicinal plants are used (Ipu et al., 2006). There are many researches regarding the effect of medicinal plants and their extracts on the performance and safety of the Immune system (Azeke and Evetc Ekpo, 2009; Al-ankari et al., 2004). Volatile oils extracted from medicinal plants, are a mixture of aromatic and volatile compounds that many of those properties are anti-microbial. The main and active components of these compounds are phenols and terpenes that the mechanism of these compounds action is to damage the glycolipid walls of bacterial

cells, which leads to leakage and reduction of cytoplasmic compositions. In the performed researches it is known that medicinal plants and their volatile oil have the characteristic to lower the cholesterol and improve the performance of broilers by increasing the intestinal enzymes (Dorman and Deans, 2000).

Jang et al. (2007) reported that the activities of trypsin and α -amylase in the pancreas and maltaz activity of the proximal part of the small intestine of broiler chickens fed on commercial mixture of medicinal plants ether extract, increased in comparison with the diet containing antibiotics and control diet. They also said that the activity of these enzymes and the growth performance of broilers fed with these compounds in comparison with the control group increased significantly. Thyme (*thymus vulgaris* L.), Purple Coneflower (*Echinacea purpurea* L.) and garlic (*allium sativum* L.) are plants with anti-microbial properties and improve the immune system (Schulte et al., 1967). Active pharmaceutical composition of Purple Coneflower are polysaccharides that are able to improve the adjustment of the immune system (Hobbes, 1989). Gershon (1998) reported, guggul increased white blood cells, phagocytosis and consequently, stimulating and strengthening the immune system. Guggul is as a purifier and an strengthening factor which helps the cells and tissues become rejuvenated (Ruitang, 2007). Satyavati (1988) has introduced the guggul as an anti-cancer, anti-inflammation and a disinfecting combination and has also referred to it as a drug for the treatment of inflammation. Recently, they have also found that this plant causes breast cancer cell toxicity and bone marrow in malignant tumor (Ichikawa, 2006). In accordance with existing experiments and reports, essential oils and extracted chloroform resin extract of guggul and other special compounds extracted from it has antibacterial effects and actively inhibits the growth of Gram (+) and Gram (-) bacterias (Asif Saeed and Sabir, 2004). This research was conducted to determine the effect of oral consumption of Commiphora Mukul plant resin on humoral immunity response and performance of broilers.

2. MATERIALS AND METHODS

2.1. Birds and diets

A Total of 320 one-day old male and female chickens (Cobb 500 strains) were randomly distributed among the four treatments. Each treatment had 4 replicates and 20 chickens were placed in each pen (115*150*150 cm). All chicks had ad libitum access to diets and water, continuous lighting, and controlled ventilation. Temperature was maintained at 32 to 34°C for the first 7 days and then gradually reduced according to normal management practices until a temperature of 21 to 23°C. At first guggul resin milled and dissolved in a adequate amount of the liquid oil and was added to the diet from the 7 days of age to the end of experimental period. Experimental diets were: control diet (without the guggul), control diet + 200 ppm guggul, control diet + 400 ppm guggul, control diets + 600 ppm guggul. The diets used in this experiment were based on corn and soybean meal and formulated based on NRC 1994 (Table 1). Starter diet fed until 21 d of age and grower diet fed from 22 to end of the experiment.

2.2. Growth performance

Feed intake and body weight were recorded at 21 and 42 d of experimental period. In days 21 and 42, one bird from each replicate, slaughtered and abdominal fat pad weighted. Spleen and bursa of Fabricius in 42 d were weighted. (Relative organ weights were calculated as organ weight (g)/ 100 g live weight).

Table 1: Formulation and Composition of the basal diets (as feed basis).

Item	Starter(g/kg)	Grower(g/kg)
Corn	550	625
Soyben meal	361.4	302
Soybean oil	45	30
Dicalcium phosphate	18	15
CaCo ₃	14	13
Salt	3.6	3
Vit. and Min. Premix ^a	5	10
DL-Met	3	2
Calculated analysis		
ME (kj/kg)	13455.74	13174.38
Crude protein (g/kg)	223.8	187.6
Calcium (g/kg)	10.6	9.3
Available phosphorus (g/kg)	5.5	4.7
Methionine+cysteine (g/kg)	5.9	5.4
Lysine (g/kg)	11.5	9.9
Threonine (g/kg)	8.3	7.6
Tryptophan (g/kg)	2.6	2.4

^a One kg of premix contained the following according to the supplier: retinol acetate 3.7 mg, cholecalciferol 0.06 mg, DL- α -tocopherol acetate 9 mg, vitamin K3 2.5 mg, thiamine 20 mg, riboflavin 10 mg, pyridoxine 20 mg, cyanocobalamin 12 mg, niacin 70 mg, pantothenic acid 25 mg, folic acid 10 mg, Fe 160 mg, Zn 90 mg, Cu 16 mg, Mn 150 mg, Se 0.1 mg, I 0.8 mg.

2.3. Antibody Response

Sheep red blood cells (SRBC) were used as T-dependent antigens to quantify the antibody response. In 28 and 35 d of experiment, one bird per replication were injected intravenously with SRBC (3% suspension in PBS, 1 mL/ chick). Blood samples were collected at 42 day old. The serum from each sample was collected, inactivated at 56°C for 30 min and then analyzed for total, mercaptoethanol-sensitive (MES) IgM and mercaptoethanol-resistant IgY anti-SRBC antibodies, as previously described (Delhanty and Solomon, 1966; Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 μ L of serum was added in an equal amount of PBS in the first column of a 96-well v-shaped bottom plate, and the solution was incubated for 30 min at 37°C. A serial dilution was then made (1/2 to 1/4096), and 50 μ L of 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 min of incubation at 37°C. Logarithm of the highest dilution that showed complete hemagglutination was set as antibody titer. For MES (IgM) response, 50 μ L of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the forementioned procedure. The difference between the total and the IgY response was considered to be equal to the IgM antibody level and expressed as the log₂ of the reciprocal of the highest serum dilution giving complete agglutination (Cheema et al., 2003).

2.4. Statistical Analysis

Data of this experiment were analyzed by analysis of variance using ANOVA procedures of SAS. The means were compared by Duncan's Multiple Range Test. The level of significance was reported at P<0.05. The statistical model is as follows:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

In this model, Y_{ij} is the observed data, μ is the population mean, T_i is the treatment effect, and ϵ_{ij} is the error of the experiment.

Results

The effect of different levels of guggul on feed intake, weight gain and feed conversion ratio of the chicks during the starter, grower and total period (1 to 42 days) are shown in table 2.

The effect of treatment on feed intake during the period of 1-21 and 22-42 days and in the entire period showed a significant difference ($P<0.05$). The highest rate of feed intake during starter period corresponds to 600 ppm treatment and the lowest corresponds to the control and 200 ppm treatments that the difference was significant ($P<0.05$). In the grower period, feed intake in 400 ppm treatments was significantly greater than the control group. Also, in the entire period there was a significant differences between 400 and 600 ppm and control group.

Table2: Feed intake, Body weight gain and Feed conversion ratio of broiler chicks fed on control and guggul diets

Treat.	1-21 days			22-42days			1-42 days		
	Feed intake (gr)	Body weight gain(gr)	FCR	Feed intake (gr)	Body weight gain (gr)	FCR	Feed intake (gr)	Body weight gain (gr)	FCR
control	752 ^b	626	1.2	2379 ^b	1210 ^b	1.94 ^a	3131 ^b	1911	1.64
200ppm	757 ^b	629	1.2	2492 ^{ab}	1357 ^a	1.83 ^b	3249 ^{ab}	1942	1.67
400ppm	770 ^{ab}	655	1.17	2556 ^a	1332 ^a	1.91 ^{ab}	3327 ^a	2037	1.63
600ppm	835 ^a	660	1.2	2515 ^{ab}	1364 ^a	1.84 ^b	3351 ^a	2067	1.62
±SEM	23.7	21.42	0.016	44.3	34.8	0.031	50.1	47.6	0.037
Sig.	*	n.s	n.s	*	*	*	*	n.s	n.s

Means within the same column with different superscripts differ significantly ($P<0.05$).

Treatments had no significant effect on body weight gain during starter and total of experimental period, but in the grower period (22 to 42 d), there was significant differences between control and other groups (Table 2, $P<0.05$). The highest mean body weight gain corresponds to the highest level of guggul (600 ppm) and the lowest Body weight was related to control group. According to table 2, the feed conversion ratio in Starter courses and total period didn't go under the effect of the treatments. In the grower period there was a significant difference between 600 ppm and control treatments, but there wasn't any difference between 200 and 400 ppm ($P<0.05$). The results of the effect of oral intake of guggul resin on concentration of blood immunoglobulins in 42 days chicks is reported in table 3. Total antibody titers to SRBC significantly affected by applied dietary treatments ($P<0.05$). Broilers fed by 400 ppm guggul, had the highest level of total antibody titers to SRBC. According to the table 3, between treatments that recieved Guggul, there wasn't any significant difference in terms of total antibody titers, but there was significant difference between control and other treatments. The rate of IgY was affected by treatments and there was significant differences between control and other treatments ($P<0.05$). There was not any difference between the treatments consuming guggul.

Table 3-Effect of the treatments on the antibody titers against SRBC in chicks of 42 days (Log₂)

Treatment	Ig total	IgM	IgY
Ctrl	3.00 ^b	0.75	2.25 ^b
200 ppm	6.75 ^a	2.5	4.25 ^a
400 ppm	7.00 ^a	2.2	4.75 ^a
600 ppm	6.5 ^a	1.5	4.75 ^a
±SEM	0.4	0.55	0.25
Sig.	**	n.s	**

Means within the same column with different superscripts differ significantly ($P<0.05$).

According to table 3, the effects of treatments on the rate of the IgM, was not significant. Table 4, was showed the effects of guggul on relative weight of bursa of Fabricius and spleen of broilers. Treatments had no significant effect on the weight of the spleen, but the effect of treatment on average relative weight of the bursa of Fabricius was significant ($P<0.05$). The highest bursa of Fabricius weight corresponds to 600 ppm treatment and the lowest was related to control.

Table 4-Effect guggul on mean relative weight of Fabricius burs and spleen (% of body weight)

Treatment	spleen	Bursa of Fabricius
Ctrl	0.12	0.03 ^c
200 ppm	0.09	0.04 ^{bc}
400 ppm	0.12	0.05 ^{ab}
600 ppm	0.09	0.06 ^a
SEM	0.011	0.003
Sig.	n.s	*

Means within the same column with different superscripts differ significantly ($P<0.05$).

According to table 5, the effects of treatments on abdominal fat pad in 21 and 42 d was not significant.

Table 5-Effect guggul on abdominal fat pad (% of body weight)

Treatment	21 d	42 d
Ctrl	1.70	2.14
200 ppm	1.51	2.17
400 ppm	1.53	2.12
600 ppm	1.61	2.05
SEM	0.15	0.14
Sig.	n.s	n.s

Means within the same column with different superscripts differ significantly ($P<0.05$).

Discussion

As seem in table 2, feed intake significantly affected by treatments during starter, grower and the end of the experimental period. Several reports had stated that the aromatic medicinal plant and plants extracts accelerate digestion and shorten the length of the time material passes through the gastrointestinal tract (Alcicek et al., 2003). The main ingredients of the guggul resin include polysaccharids, Terpenoids, Furanosesquiterpenoids, Germacrene and guanine skeleton that causes the resin to be aromatic and fragrant.

The probably reason for the increase in feed intake with the applied guggul treatment, maybe from the existence of guanine composition and its fragrancy (Wang et al., 2004; Zhu et al., 2001). There was no further study in the field of communication between Guggul and feed intake. The other possible reason for the increased feed consumption in treatments using Guggul is due to the purgative characteristic of the Guggul (Malhotra et al., 1977) and consequently, reduction in time feed remaining in chicks' gastrointestinal tract. According to earlier studies, it was expected that consumption of Guggul reduces the weight of broiler chickens. Ruitang (2007) suggested that Guggul causes weight loss. Also in Ichikawa (2006) reported that the Guggul resin is useful for the treatment of obesity. But Khalili et al.

(1388) reported that consumption of guggul in diabetic mice were able to obviously lessen the weight loss of the mice, but in healthy mice it had no significant effect on the mice weight. The weight gain can be known as a result of increased feed intake, although it was not significant in the starter and the whole period of experiment.

The materials contained in medicinal plants and their antibacterial compounds are known as stimulating digestive. Medicinal plants create balance in the intestinal microbic echosystem and endogenous enzymes secretion, improves digestion of food and consequently the growth in poultries (Cross et al., 2007). The othere possibility reason for improvement in growth and feed intake of the chicks that had guggul in their diet, was the result of the anti-microbial compounds of this resin (Asif Saeed and Sabir, 2004), that reduced pathogenic bacteria in gastrointestinal. The datas in table 3 show the significant effect of guggul on the concentration of total antibody titer and immunoglobulin Y. This increase in the immune response in the treatments consuming Guggul resin can be related to its anti-bacterial (Asif Saeed and Sabir, 2004), and disinfectant relevancy effects of this resin (Satyavati, 1988). Volatile oils extracted from medicinal plants, are a mixture of various aromatic and volatile compounds that many of them have anti-microbial properties. The main and active components of these compounds are phenols and terpens that the mechanism of these compounds action is to damage the lipoprotein walls of bacterial cells which leads to leakage and reduccion of cytoplasmic compositions (Dorman and Deans, 2000). Gershon et al., (1998), also repoted the effects of Guggul on increasing white blood cells, phagocytosis and, consequently, stimulating and strengthening the immune system. Samman and Cook (1996) stated that the plants enriched by flavenoids and terpen compounds, strengthen the animals' immune system by increasing the activity of vitamin C and increasing their own anti-bacterial effects. Microorganisms digeste the polysaccharids and get the Probiotic virtue and causes the increase in *Lactic Acid* bacterias (Savage et al., 1996). Nie, and Zhang (1999) reported that the plant polysaccharids improve the secretion of antibodies. Also in other research has been stated that polysaccharids of Purple Coneflower (*Echinacea purpurea* L.), has the ability to adjust and improve the immune system in mice by increasing the secretion of macrophages, lymphocytes and antibody production especially IgY (Rehman et al., 1999; Bauer, 1996; Stimple et al., 1984). According to this evidence, and based on the compounds found in guggul, it is possible that the effect of Guggul on improving the chicks' immune system be the result of terpen and polysaccharid compounds of this resin. Also, according to Rehman (1999), antifungal and antibacterial effect and selective inhibitory activity and enhance beneficial bacteria of the guggul resin (Humphrey et al., 2002), indirectly enhances immunity. In performed researche, it is specified that the medicinal plants stimulatethe growth of immune organs (Hevener et al., 1999; Takahashi et al., 2000). The optimal growth of these organs, is a sign for higher efficiency in immunoglobulins production (Glick, 1977). In this experiment, the bursa of fabriciuse weight between various treatments was different and the difference between control and 600 ppm guggul resin was significant. This increase in weight can be an expressive improvement in immune response following consumption of guggul resin.

According to the provided conditions of growth and wide range of the climatic diversity of medicinal plants in the world and on the other hand the tendency of the world society to consume animal products free from the chemical compositions' residue, extensive research is needed in the fields of utilizing these plants and their products. This experiment indicate that the Guggul resin was able to improve the performance and significantly increases the proper response of humoral immunity in chickens against sheep erythrocytes. The best level of Guggul consumption can be considered 600 ppm. But for determining the best level, further experiments are needed.

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