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Research Article

Dietary Supplementation by some Phytogenic Substances in Albino Rats: Their Effects on Growth Performance and Serum Interlukin-6

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Abstract

Background and Objective: Medicinal plants are widely used as home remedies and raw materials for the pharmaceutical industries. The influence of dietary supplementation of black seed (*Nigella sativa*) and turmeric (*Curcuma longa* Linn.) on serum IL-6, growth performance parameters and feed utilization efficiency of Albino rats diets was investigated. **Materials and Methods:** In this study a total of 45 Albino rats were allotted into three equal treatments with five equal replicates for each throughout the experimental period (40 days). The rats in the first treatment fed on basal diet and served as a control while rats of the second and third treatments kept on basal diet contained 0.5% black seed and 0.5% turmeric, respectively. **Results:** Diet containing black seed and turmeric cleared a significant increase in final body weight, feed conversion ratio, reduced feed intake and immunological parameters when compared with the control group. Where experimental group contained 0.5% black seed was the best followed by that containing 0.5% turmeric. **Conclusion:** Dietary inclusion of black seed and turmeric relatively improved the growth performance, feed utilization efficiency and interleukin 6 (IL-6).

Key words: Black seed, turmeric, growth performance, IL-6, phytogenic substances

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbal remedies are used in the prevention, treatment and cure of disorders and diseases since ancient times[1]. The uses of natural products as black seed (*Nigella sativa*) and turmeric (*Curcuma longa* Linn.) have culminated in the development of a variety of drugs that are medically proven for their therapeutic effectiveness against a wide range of diseases[2]. Previous studies evoked that dietary supplementation by either black seed or turmeric were effective in improvement the body gain percent, feed utilization efficiency, serum biochemical and immunological parameters in rats[3], *Mugil cephalus* fish[4], Pekin ducklings[5] and Japanese quails[6]. In several studies the effect of *Nigella sativa* oil on the immune system has been investigated[7,8]. All these studies have shown that the oil of *Nigella sativa* inhibits many inflammatory mediators and may be useful in ameliorating inflammatory and autoimmune conditions. Chronic administration of *Nigella sativa* may change pro and anti-inflammatory cytokines profiles. Also, it may act as a balancing factor on Th1/Th2 lymphocytes in different exercise loads and act an inhibitory factor on Th2 phenotype in control animals[9].

Secretion of key pro-inflammatory mediators as IL-6, TNF-α and NO by primary macrophages is suppressed by aqueous extract of *N. sativa*, indicating that *N. sativa* exerts anti-inflammatory effects *in vitro*. Finally, experimental evidence indicates that the aqueous extract of *N. sativa* enhances Natural Killer (NK) cytotoxic activity against tumor cells YAC-1, suggest the anti-tumor effect of *N. sativa* may be attributed to its ability to serve as a stimulant to NK anti-tumor activity. It was anticipated that *N. sativa* employed as effective therapeutic agent in regulation of diverse immune reaction implicated in various diseases as cancer[10]. *Nigella sativa* stimulate bone marrow and immune cells and raises the interferon production, protects normal cells against cell destroying effects of viruses, destroys tumor cells and raises the number of anti-bodies producing B cells[11]. Turmeric is a rhizomatous perennial herb that belongs to the family Zingiberaceae, native to South Asia and is commonly known as turmeric. Turmeric supplementation decreases blood levels of IL-6, MCP-1, TNF-α, hyperglycemia and oxidative stress by using a cell-culture model and a diabetic rat model[12]. At the gene level, turmeric (curcumin) also dose-dependently blocked the mRNA expression of IL-6 in the LPS induced vascular smooth muscle cells *in vitro*. These results clearly demonstrated that curcumin significantly affected the expression of IL-6 at protein and gene levels. Therefore, we further determined whether curcumin interfered signaling mechanisms mediated IL-6 gene expression in LPS-induced vascular smooth muscle cells *in vitro*[13]. The current study aimed to investigate the effects of dietary supplementation of black seed and turmeric on IL-6, growth performance parameters and feed utilization efficiency of Albino rats diets.

MATERIALS AND METHODS

Ethical approval: We carried out the experiment after approval from the Institutional Animal Ethics Committee.

Herbal plants: Black seed and turmeric roots powder were bought on a local market in Hail City, King Saudi Arabia. The whole seeds and roots were crushed in a blender and mixed well with the basal diet before their administration.

Experimental animals: In the present study, adult male Albino rats weighing 122-126 g were obtained from the Animal House in King Saud University, Riyadh, Saudi Arabia. The rats were acclimatized in ventilated, clean, sterile, plastic cages with wood shavings under conventional conditions and had free access to feed and water. The animal room was well ventilated and subjected to a photoperiod of 12 h light/12 h darkness during the period of experimental study (40 days).

Experimental design and diets: A total 45 Albino rats were randomly distributed into three equal treatments with three replicates. Rats of the first treatment fed on basal formulated diet and served as a control whereas rats of the second and third treatments fed on basal formulated diet contained 0.5% black seed and 0.5% *Curcuma* powder, respectively. The formulated experimental diets met the requirements of rats according to Nutrient Requirements of Laboratory Animals, Fourth Revised Edition in 1995 (Table 1).

Growth performance parameters: At the beginning of experiment and at the end of the experimental period weights of rats from each replicate per treatment group were recorded to estimate the growth performance parameters and feed intake per each using the following formulae:

$$\text{Weight gain (g rat}^{-1}) = W_f - W_0$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{FI}{W_f - W_0}$$

where, W_f and W_0 are the final and initial weights of the rats per group, respectively and FI is feed intake.

Serum samples collection: Blood were obtained from orbital venous plexus. Blood was centrifuged at 3000×g for 10 min

Table 1: Formula and calculated analysis of the experimental diets

	Treatments		
Experimental diets	T1	T2	T3
Ingredients (g kg^{-1} diet)			
Alfalfa meal (17%)	143.0	143.0	143.0
Yellow corn, grain	465.0	460.0	465.0
Soybean meals (48%)	92.0	92.0	92.0
Wheat grain screenings	200.0	200.0	200.0
Dried skim milk (33%)	55.0	55.0	55.0
Barley, grain	32.0	32.0	27.0
Vegetable oil	5.0	5.0	5.0
Black seed	0.0	5.0	0.0
Turmeric	0.0	0.0	5.0
Salt	5.0	5.0	5.0
Vit. and Min. Premix*	3.0	3.0	3.0
Total	1000.0	1000.0	1000.0
Calculated analysis**			
Dry matter (%)	88.99	88.56	88.54
Crude protein (%)	15.02	14.98	14.96
Ether extract (%)	3.51	3.49	3.50
Crude fiber (%)	6.27	6.26	6.25
Metabolizable energy (kcal kg^{-1})	2908.52	2891.77	2894.17

*Vit and Min. Premix: Mineral, mg kg^{-1} diet: Cobalt carbonate 0.44, copper sulphate 4.40 mg, iron sulphate 132.3, manganous oxide 66.20, zinc oxide 17.60, calcium iodate 1.54, vitamin, kg^{-1} diet: Stabilized Vit. A palmitate 6,060.00 IU, Vit D3, 5,070.00 IU, Vit K 3.9 mg, α-tocopheryl acetate 22.10 mg, choline chloride 617.00 mg, folic acid 2.43 mg, niacin 33.10 mg, Ca-d-pantothenate 19.80 mg, pyridoxine-HCL 1.87 mg, riboflavin supplement 3.75 mg, thiamine mononitrate 11.00 mg, d-biotin 0.15 mg, vitamin B12 0.004 mg, **Calculated according to feed stuffs ingredient analysis table 2014 edition

using centrifuge. Sera were separated and were collected using dry Pasteur pipette. Labeled and stored in the refrigerator at -20°C for analyses.

Measurement of serum IL-6 level: Interleukin-6 levels were analyzed by using validated enzyme linked immunosorbent assay (ELISA) kits (ENZO® life sciences) according to Chard[14].

Statistical analysis: All values are given as Means±Standard Error (SE) and statistical analyses were performed by using SPSS VERSION 22 using one way ANOVA test for multiple group's comparison. Differences among means were analyzed using Duncan's new multiple range test[15] with $p<0.05$ considered as significant.

RESULTS AND DISCUSSION

Growth performance parameters: Results presented in Table 2 illustrated that the initial body weight (g) of experimental animals did not differ, indicating that all treatment groups were homogenous. However, the treatment group fed diet contained 0.5% black seed had a significant ($p<0.05$) increase in final body weight (g) and body gain percent (60.81%) followed by that fed diet contained 0.5% *Curcuma* powder (53.81%) when compared with the control group without additives (47.36%). This was in line with Saad *et al.*[16] who indicated that the diets contained 0.5% of black seed and/or turmeric improved the growth performance parameters in sea bass.

Phytogenic compounds are an alternative to antibiotic growth promoters due to the recent bans and restriction on the use animal antibiotic growth promoters feed additives in diets[17]. Therefore, researchers tended to use natural additives which meet the requirements of good growth promoting agents. *Nigella sativa* supplementation improved significantly feed intake and growth performance[18]. This improvement in growth performance can be attributed to the nutritional effects of the main components of *N. sativa* contains high percentages of fatty acids and all essential amino acids that may promote growth rate of birds[19]. *Nigella sativa* has also a positive effect on digestive enzymes to enhance the utilization of dietary nutrients[17].

Data included in the Table 2 exposed also that the feed utilization efficiency in the terms of feed intake and Feed Conversion Ratio (FCR) had a significant differences between the all treatment groups where the treatment group fed diet contained 0.5% black seed showed positive improvement in feed intake and FCR followed by that fed diet contained 0.5% *Curcuma* powder in comparison with the control group. It was reported that the supplementation of phytogenic compounds in poultry feed decrease palatability of diet due to acrid odour, which depress the feed intake without changes in body weight gain percent, leading to an improved feed conversion ratio and these is agreed with our findings regarding feed intake and FCR[20]. Phytogenic feed additives such as black seed and turmeric increase the output of digestive enzymes from the pancreas, gut mucosa and increased bile flow. Furthermore, phytogenic feed additives improve the activity of digestive enzymes such as trypsin, amylase and increase the digestibility and absorption of nutrients favours the growth performance parameters and improvement of gut flora and health status in animals and this explicates these results where there were a significant increase in the final body weight and body gain percent of rats fed diets including 0.5% black seed and *Curcuma* powder[21]. Similarly, in Asian sea bass fed diets supplemented with black seed and turmeric mixture 5 g and 10 g kg^{-1}, increased in the growth performance parameters[22]. In conclusion, dietary supplementation with 0.5% black seed and *Curcuma* powder can improve the growth performance and feed utilization efficiency in rats.

Effect of turmeric on serum IL-6 level: In these results, turmeric had opposite effects on production of interleukin-6,

Table 2: Dietary supplementation effects of black seed and *Curcuma* powder on growth performance and feed conversion ratio of rats

Growth performance	Control	Black seeds	*Curcuma*	F-ratio and p-value
Initial body weight (g rat^{-1})	124.66±0.64	122.46±1.15	125.62±1.18	F = 2.517, p = 0.122
Final body weight (g rat^{-1})	183.78±1.11^{c}	196.90±1.11^{a}	193.16±0.90^{b}	F = 42.054, p = 0.001
Body gain (g rat^{-1})	59.02±1.25^{c}	74.44±0.50^{a}	67.54±1.26^{b}	F = 52.665, p = 0.001
Body gain (% rat^{-1})	47.36±1.14^{c}	60.81±0.79^{a}	53.81±1.43^{b}	F = 34.147, p = 0.001
Feed intake (g rat^{-1})	96.66±1.22^{a}	81.96±0.58^{b}	81.38±0.69^{b}	F = 97.357, p = 0.001
Feed conversion ratio	1.64±0.02^{a}	1.12±0.03^{c}	1.20±0.03^{b}	F = 123.749, p = 0.001

Values in the same row with different superscript letters are significantly different (p<0.05). Values are presented as Mean±SE (n = 15 rats in each group)

Table 3: Dietary supplementation effect of black cumin seed and *Curcuma* powder on amount of serum IL6

Parameter	Control	Black seeds	*Curcuma*	F-ratio, p-value
IL6 (pg mL^{-1})	1115.78±1.44^{b}	1512.22±1.70^{a}	1043.82±1.20^{c}	F = 29711.21, p = 0.0001

Values in the same row with different superscript letters are significantly different (p<0.05). Values are presented as Mean±SE (n = 15 rats in each group)

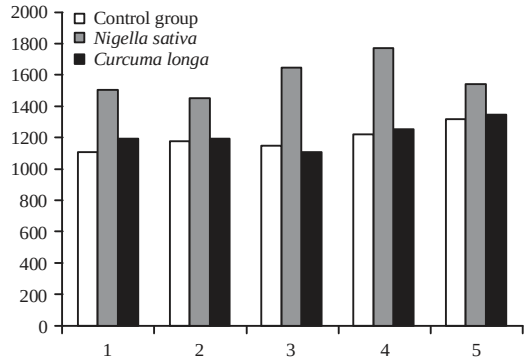

Fig. 1: Serum IL-6 in control, *Nigella sativa* and *Curcuma longa* groups

whereas there was significant decrease in serum interleukin-6 concentrations of the experimental group (supplemented with *Curcuma* powder) relative to the control group as shown in Fig. 1 and Table 3. Turmeric is extracted from the roots of the *Curcuma longa* plant (turmeric)[23]. Turmeric extract consists of three different curcuminoids: Curcumin, demethoxycurcumin and bis-demethoxycurcumin[24]. Curcumin (diferuloylmethane) is the most active component of turmeric. It is believed that curcumin is a potent antiinflammatory agent[25,26]. Curcumin supplementation resulted in potent anti-inflammatory effects mediated via inhibition of secretion of pro-inflammatory cytokines[27]. Numerous lines of evidence have indicated curcumin's ability to modulate multiple cell signaling molecules such as pro-inflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-1β, IL-6), apoptotic proteins, NF–κB, cyclooxygenase (COX)-2, STAT3, IKKβ, endothelin-1, malondialdehyde (MDA), C-reactive protein (CRP), phosphorylase kinase (PhK), transforming growth factor (TGF)-β, triglyceride and antioxidants[28].

This study demonstrates that rats had decreased serum levels of IL-6 in the group fed on diet contained 0.5% *Curcuma* powder. A similar dose of curcumin was shown to be effective in preventing nephropathy in animal studies[29]. This dose of curcumin is similar to that used in humans[30,31]. This study suggests that curcumin supplementation has the potential to reduce the blood levels of proinflammatory cytokines and thereby inhibit the pathogenesis of vascular inflammation. The evidence that curcumin can inhibit markers of vascular inflammation must be explored at the clinical level to see whether curcumin can reduce levels of proinflammatory cytokines in patient population. If so, then curcumin supplementation could be used as an adjuvant therapy for reduction of vascular inflammation.

Effect of *Nigella sativa* on serum IL-6 level: Obtained data revealed that, there was significant increase in serum interleukin-6 concentrations of the experimental group (supplemented with *Nigella sativa*) relative to the control group as shown in Fig. 1 and Table 3. This study, according to the best of our knowledge is the first to provide experimental evidence demonstrating that *Nigella sativa* has immunomodulatory properties against IL-6 level in rat. *Nigella sativa* plays an important role as anti-inflammatory activity[10,32]. Many studies shown that *N. sativa* has effect on immune system; it is able to inhibit many inflammatory mediators and could ameliorate inflammatory and autoimmune conditions[33,34]. *Nigella sativa* constituents have shown to down regulate B cell-mediated immunity[11,35]. *Nigella sativa* at the level of 2% would increase antibody response and duration of supplementation had a positive impact on immune response and reduced coliform count in the gut[24].

However, in this current study we clearly demonstrated the ability of *N. sativa* in a dose of 0.5% to induce significant increase in serum IL-6 level of rats. Chronic administration of *N. sativa* may change pro and anti-inflammatory cytokines profiles. Also, it may act as a balancing factor on Th1/Th2 lymphocytes and act as an inhibitory factor on Th2 phenotype[9].

CONCLUSION

The current study concluded that black seeds and turmeric are beneficial to be added in Albino rat diets where these phytogenic plants have positive effect in improving the overall growth performance parameters, feed utilization efficiency and some serum immunological parameters such as IL-6.

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