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EFFECT OF DIETARY SUPPLEMENTATION BY CINNAMON AND GARLIC POWDER ON GROWTH PERFORMANCE, INTERLEUKIN-6 AND SERUM BIOCHEMISTRY IN ALBINO RAT DIETS

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ABSTRACT

Aim:The current study aims to examine the effect of dietary supplementation with cinnamon and garlic powder as growth promoter agents on growth performance, immune response, and biochemical parameters in rats.

Materials and Methods: A total of 45 adult rats (118-127gm)were randomly assigned to three treatments for 30 days feeding period; group I (control group) received normal commercial basal diet, group II (Cinnamon group) fed with basal diet contained 5 % cinnamon powder, and group III (Garlic group) fed with basal diet contained 5 % garlic powder. Growth performance, serum triglycerides, total cholesterol, HDL (High density lipoprotein), LDL (Low density lipoprotein), malondialdehyde (MDA), total proteins,

albumin and total globulins were determined. In addition to IL-6 (Interleukin-6) titre was estimated in rat serum by using ELISA.

Results: Our results revealed that dietary supplementation with 5% garlic significantly increased the final body weight, weight gain and feed conversion ratio followed by group fed 5% cinnamon compared with control group fed basal diet only (P< 0.05). Serum biochemical analysis showed that triglycerides, total cholesterol, LDL and MDA in cinnamon and garlic groups recorded a significant variance in comparison with control. Moreover, cinnamon and garlic groups significantly increased serum total globulins. Also, data revealed a significant decrease in inflammatory cytokines IL-6 level in cinnamon and garlic groups, in comparison with control group.

Conclusion: Application of Cinnamon and garlic powder in the diet proved to have positive influence on growth performance, immune response, serum lipid profile and oxidative stress.

Keywords: Cinnamon, Garlic, Interleukin-6, Lipid profile, MDA, growth performance INTRODUCTION

Antimicrobials at subtherapeutic doses have been broadly used in animal diets as growth promoters to enhance animal performance growth and production. In the presence of low levels of antimicrobials, resistant cells survive and grow producing an antimicrobials resistant population in the final products. So, the utilization of antimicrobials as growth promoters in the animal feed has been banned in the European Union since January 2006. As a consequence of this boycott in European Union and increasing animal production in the world, alternative substances and strategies for animal growth promotion and disease prevention are being explored, among which phytogenic and herbal products have gained expanded consideration since they have obtained more acceptability among consumers as a natural additive [1]. Beneficial effects of bioactive plant substances in nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response antibacterial, antiviral and antioxidant actions [2, 3]. Cinnamon is one of the most common spices used worldwide flavouring agents, preservatives, and pharmacological agents [4]. Dietary inclusion of Cinnamon powder improves growth performance, feed efficiency and have positive influence on carcass abdominal fat and serum triglyceride concentration at slaughter age of broiler

chickens [3, 5, 6]. Cinnamon protects protein and lipids against oxidation [7]. Procyanidins extracted from cinnamon possess antioxidant activities [8,9]. On the other hand, Garlic is used as flavouring agent and traditional medicine for healing. Recently, many medicinal properties attributed to garlic and garlic supplement [**10**].Growth performance and efficiency enhancement effects of garlic powder have been reported in broiler chickens [11,12]. Hypolipidemic, hypocholesterolemic, antiatherosclerotic, antioxidant, antitumor immuneand modulatory effects of garlic were recorded [13,14]. Despite its inhibition of IL-2 transcript, cinnamon enhanced IL-2 Also, secretion. Cinnamon treatment caused a reduction in the sub-G1 phase, accompanied by an increased ratio of apoptotic cells to necrotic cells and was able to down-regulate IFN-y expression in activated T cells without altering IL-2 production [15].Low dose of cinnamon (10mg/kg) produced only an increase in serum immunoglobulins levels while the high dose of cinnamon bark (100mg/kg) decreased Pasteurellamultocida-induced mortality by 17%, increased the phagocytic index, increased neutrophil adhesion, increased serum immunoglobulin levels and antibody titre values [16]. Cinnamon extract Lower plasma concentrations of IL-

1 β , IL-6 and TNF- α and decrease the liver concentrations of IL-1β, IL-6, and TNF-α [17]. On the other hand, Garlic enhance the functioning of the immune system by stimulating certain cell types, such as macrophages, lymphocytes, natural killer (NK) cells, dendritic cells and eosinophils, by mechanisms including modulation of cytokine secretion, immunoglobulin production, phagocytosis, and macrophage activation [18]. The present study aims to study the effect of cinnamon and garlic powder on growth performance, immune response and serum lipid profile in rat.

MATERIALS AND METHODS

Ethical approval

All institutional and national guidelines for the care and use of laboratory animals were followed

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Hail.

Animals and Experimental Design

Adult male albino rats were purchased from the animal house in King Saud University; Riyadh, Saudi Arabia weighing about(118-127gm) and the rats were acclimatized in ventilated, clean,

sterile, plastic cage with wood shavings under conventional conditions. The animal room was well ventilated with a 12 hrLight/12 hrdark cycle throughout the experimental period (30 days). Then the rats were randomly divided into three experimental groups, with three replicates in each group and each replicate contained 5 rats (i.e. per group: $5\times3=15$ rats) and the rats had free access to feed and water and amount of feed consumed was determined. Group I (control group) rats received normal commercial basal diet (table 1). Group II (Cinnamon group) rats fed with basal diet contained 5 % Cinnamon powder (5gm / 100gm basal diet). Group III (Garlic group) rats fed with basal diet contained 5 % Garlic powder (5gm / 100gm basal diet).

Growth performance parameters

Weights of rats from each replicate per group were recorded at the beginning of experiment and at the end of the experimental period and also the feed consumed to estimate the growth performance using the following formulae: weight gain (WG; g/rat) = W_f - W_0 ; feed conversion ratio(FCR) = FI/ (W_f - W_0), where W_f and W_0 were the final and of the rats per group, respectively; and FI is feed intake.

Serum chemistry

Serum triglycerides and total cholesterol were determined according to method of [19, 20] respectively; high density lipoprotein (HDL) was measured [21] then low density lipoprotein (LDL) was calculated. Serum malonaldehyde (MDA) was determined according to method of [22]. Totalprotein and albumin were measured by method of [23,24] respectively then globulins were calculated.

Determination of IL-6 in rat serum samples

IL-6 levels were measured in collected serum by using ENZO[®] life sciences ELISA kits; the generated colour was read at 450nm. The measured optical density is directly proportional to the concentration of IL-6 in either standards or samples [25].

Statistical Analysis

All values are given as means \pm S.E. Statistical analyses were performed by using SPSS VERSION 22 using one-way ANOVA test for multiple groups' comparison. Differences among means were analysed using Duncan's test, with p < 0.05 considered as significant.

RESULTS AND DISCUSSION

Dietary supplementation effects of Cinnamon and garlic powder on growth performance and feed conversion ratio

The data in Table (2) shown that there were significant different between treatments (cinnamon and garlic 5%) when compared with control (untreated group) on final body weight, body gain, feed intake and feed conversion ratio, where the group fed diet supplemented with garlic 5% has highest final body weight and body gain followed by cinnamon 5% group than control group (215.30, 197.0, 188.37 and 93.07, 74.87, 68.73 respectively). However, untreated group (control) has highest results of feed intake and feed conversion ratio followed by garlic 5% group than cinnamon 5% (110.9, 96.90, 90.10 and 1.62, 1.05, 1.21 respectively).

In the present study according to the results in Table 2 and Figure 1, the dietary supplementation with 5% garlic and 5% cinnamon for 30 days in rat diets significantly increased the final body weight, body gain and feed conversion ratio values in comparison to Control group fed on basal diet. Recently, dietary inclusion of 1% garlic [26] significantly increased the growth performance and feed efficiency in broiler chicken. The use of cinnamon powder had a great role in enhancing the growth performance, hematological and blood biochemical variables and reproductive performance [27] and these changes due to addition of cinnamon could be attributed to the presence of cinnamaldehydes which give cinnamon its aroma and bio-antioxidant properties and our results regarding the growth performance agreed with those.

Effect of cinnamon and garlic on serum triglycerides, total cholesterol, HDL, LDL and MDA

The feeding administration of garlic and cinnamon 5% to rat showed significantly changes in serum triglyceride, total cholesterol, HDL, LDL and MDA. Regarding the results in Table (3), it was observed that the triglyceride and total cholesterol contents were highest untreated group (control) followed by cinnamon and garlic 5% (57.73, 34.03, 39.68 and 86.74, 69.35, 66.08,mg/dl respectively).

The cinnamon and garlic 5% marked a significant increase in HDL content when compared with untreated group (35.63, 35.45 and 34.28 mg/dl respectively). However, obtained data showed decrease in LDL content after feeding with garlic and cinnamon 5% when compared with control (23.42, 28.59 and 40.80 mg/dl respectively).

On the other hand, MDA are the end products of lipid oxidation and the obtained data revealed that there were significant decrease in MDA serum content when treated with garlic and cinnamon 5%

when compared with control (3.5, 4.60 and 6.28 respectively).

Owing to our data in Table 3, Figure 2 which revealed a significant decrease of triglycerides, cholesterol and LDL by adding cinnamon (5%) and garlic (5%) to the diet of rats come in accordance with [28-31] who mentioned that cinnamon and garlic lower blood triglycerides and cholesterol. Cinnamon contain mevalonic acid derivatives [32,33], Inhibition of cholesterol biosynthesis by some mevalonic acid derivatives was reported [34]. On the other hand, garlic inhibit cholesterol synthesis [35,36] and the suppress LDL oxidation [37]. Our result data in Table 3 showed that cinnamon (5%) and garlic (5%) supplementation to rat significant decrease and highly significant decrease MDA respectively which concomitant with [29,38,39]. Cinnamon oil potentially exhibits superoxide-dismutase- (SOD) like activity. Also, isolated flavonoids of cinnamon have antioxidant properties with free-radical-scavenging activities Garlic extract exhibits antioxidant activities by scavenging the free radicals generated in rat kidney [39].

Effect of cinnamon and garlic on serum total proteins, albumin, and total globulins

The serum proteins and useful parameters to indicate impairment in the

functional capacity of the liver and kidney. In this study no alteration in the levels of total proteins, albumin was found (Table 4, Figure 3). However, Total globulin content was significantly different from the control group after the cinnamon and garlic 5% treatment (1.77, 2.33 and 2.35 g/dl respectively).

Concerning the result of serum total proteins, albumin, total globulins in table 4, there was a significant increase of total globulins with cinnamon and garlic groups, meanwhile no significant change of total proteins and albumin in comparison with control group.

Concerning to the data in Table 4 which revealed that cinnamon (5%) and garlic (5%) significantly increased serum total globulins. These results come in accordance with [41] who mentioned that cinnamon (0.5%) increased $\alpha 1$ and $\alpha 2$ and γ-globulins. Also [42] stated that garlic powder (3%) increased serum γ-globulins in broiler chicks, immuno-stimulant effect of garlic supplementation may be due to its component [43]. Moreover, this increasing in globulins may be attributed to their antioxidant activity which is cleared by our result of decreased MDA.

Effect of cinnamon and garlic on level of IL-6 in rat serum

The obtained data revealed that, there was a significant decrease in

inflammatory cytokines IL-6 level with cinnamon and garlic (5%) groups, in comparison with control group, as shown in Figure 4.

The primary finding was that there were significant falls in IL-6 level, in the cinnamon group (P<0.05) and these findings were consistent with the data from the randomized controlled trials, which showed a reduction in an acute-phase inflammatory response, and pain, after daily intake of cinnamon [44]. Garlic extract induced a variety of Immunomodulatory activities on leukocyte cytokine production. Leukocyte and inflammatory cytokine

production was reduced significantly in the presence of garlic extract, revealing a potential therapeutic use in inflammatory conditions. Garlic derivatives have both a stimulatory [45] and inhibitory [46] effect lymphocyte proliferation on and lipopolysaccharides induced TNF-α generation. Our results suggest that garlic has an overall inhibitory effect on IL-6 production (P<0.05), these result agreed with [47] who mentioned that garlicderived compounds inhibit the transcription of several cytokine genes involved in proinflammatory responses, such as IL-6.

Table 1: Commercial basal diet used and its composition

Ingredients	Concentrations (g kg ⁻¹)
Alfalfa pellets	350
Maize Broken	150
Barley	150
Wheat	100
White Sorghum	100
Sunflower white	50
Sunflower black	50
Safflower	50
Total	1000
Calculated analysis*	
Crude protein (%)	17%
Metabolizable energy (Kcal/kg)	2415

^{*}calculated according to feed stuffs ingredient analysis Table 2012 edition

Table 2: Dietary supplementation effects of cinnamon and garlic on growth performance and feed conversion ratio of

		rats		
	Control	Cinnamon	Garlic	F-Ratio;
				P-Value
Initial Body weight (g/rat)	119.63±1.10	122.13±2.38	122.23±2.32	F=0.529;P=0.614
Final body weight (g/rat)	188.37 ± 1.5^{c}	197.0 ± 1.88^{b}	215.30±3.45 ^a	F=32.073; P=0.001
Body Gain (g/rat)	68.73 ± 2.19^{b}	74.87 ± 3.02^{b}	93.07 ± 5.74^{a}	F=10.24; P=0.012
Feed intake (g/rat)	110.93 ± 1.00^{a}	90.10 ± 0.87^{c}	96.90 ± 3.10^{b}	F=29.67; P=0.001
Feed conversion ratio	1.62 ± 0.04^{a}	1.21 ± 0.04^{b}	1.05 ± 0.03^{c}	F=66.49; P=0.0001

Values in the same row with different superscript letters are significantly different (P < 0.05). Values are presented as mean \pm SE (n = 15rats in each group).

Table 3: Effect of cinnamon and garlic on serum triglycerides, total cholesterol, HDL, LDL and MDA

	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	MDA (nmol/ml)
Control	57.73± 2.98 ^a	86.74± 2.35 ^a	34.28±2.28	40.80±1.14 ^a	6.28±0.28 ^a
Cinnamon	34.03±1.43 ^b	69.35±2.50 ^b	35.63 ± 1.89	28.59 ± 1.60^{b}	4.60±0.23 ^b
Garlic	39.68±2.48 ^b	66.08±2.41 ^b	35.45 ± 2.08	23.42±1.20°	3.56 ± 0.23^{c}
F-Ratio;	F=26.95	F=21.019	F=0.123	F=45.100	F=30.71
P-Value	P=0.001	P=0.002	P=0.887	P=0.0001	P=0.001

The groups which have different superscript letters in the same column are significantly different where the high significant group which has superscript a followed by b then c. (P<0.05)

Table 4: Effect of cinnamon and garlic on serum total proteins, albumin, and total globulins

	Total proteins(g/dl)	Albumin (g/dl)	Total globulins (g/dl)
Control	5.49± 0.04	3.64±0.12	1.77±0.06 ^b
Cinnamon	5.71±0.45	3.84±0.16	2.23 ± 0.03^{a}
Garlic	5.69±0.17	3.52±0.10	2.35 ± 0.04^{a}
F-Ratio;	F=3.48	F=1.66	F=179.2
P-Value	P=0.099	P=0.267	P=0.0001

The groups which have different superscript letters in the same column are significantly different where the high significant group which has superscript a followed by b then c. (P<0.05).

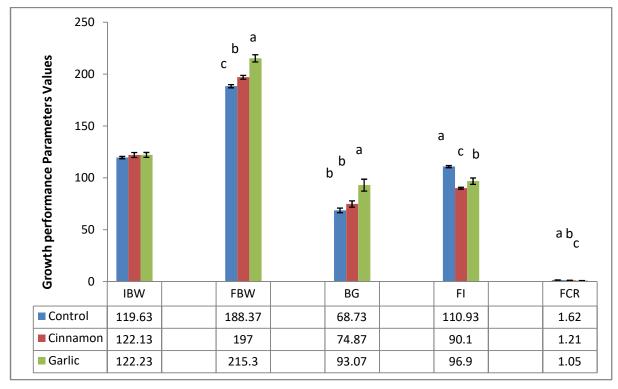


Figure 1: Dietary supplementation effects of cinnamon and garlic on growth performance and feed conversion ratio of rats.

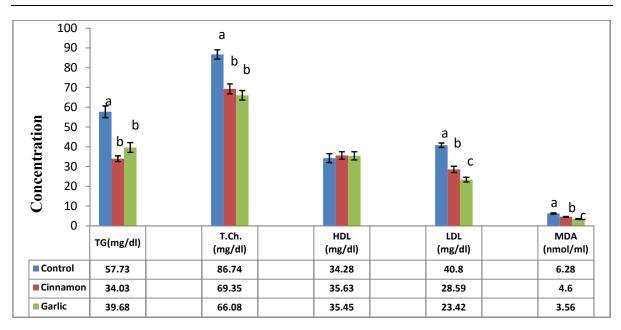


Figure 2: Effect of cinnamon and garlic on serum triglycerides, total cholesterol, HDL, LDL and MDA

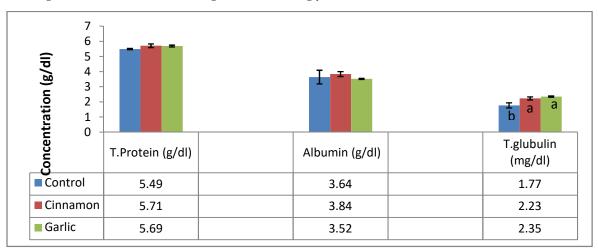


Figure3: Effect of cinnamon and garlic on serum total proteins, albumin, and total globulins

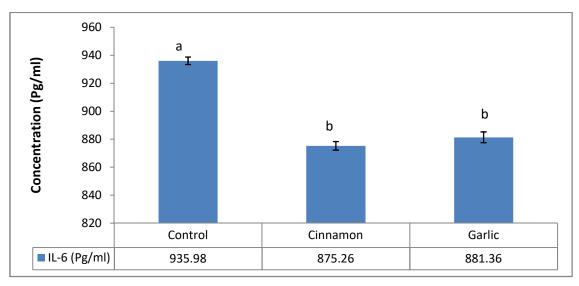


Figure 4: Dietary supplementation effect of cinnamon and garlic on amount of serum IL6.

CONCLUSION

According to the obtained results, it was found that supplementation of cinnamon and garlic powder by 5 percent to the rat diet led to improve the growth performance parameters, antioxidant status and increase serum total globulins could also reduce the serum levels of anti-inflammatory IL-6 and lipid profile. In general, the medicinal plants and their seeds could be used as a growth promoter to replace antibiotics in animal diets.

Authors' Contributions

This work was carried out in collaboration between all authors. SAAE, EAS and SYAA designed the study. Authors OFT and SYAA collected and processed the samples while SAAE, SYAA, EAS and **OFT** did the analysis of growth performance, serum immunological and biochemical parameters respectively. OFT and SYAA carried out the experimental work, laboratory analysis, While SAAE, EAS did data analysis. All authors wrote the first and final drafts of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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