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Inclusion of Phytogenic Feed Additives in Diet of Growing Rabbits: Effects on Antioxidant Enzymes and Immunoglobulins

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ABSTRACT

This study was performed to investigate the effects of dietary turmeric powder, ginger powder, fenugreek seeds powder, dried lemon powder on antioxidant enzymes and total immunoglobulins and their fractions of growing rabbits. A total of 30, New Zealand White rabbits (NZW) at 5 weeks of age were randomly assigned to five treatments with three replicates. The dietary treatments consisted of 5 groups as follows; the basal diet as control, phytogenic additives groups were supplemented with 0.5% turmeric powder, 0.5% ginger, 1.0% fenugreek seeds and 1.0% dried lemon added to the basal diet. The data revealed that, there were there were significant differences ($p \leq 0.01$) among all dietary treatments on the values of catalase (CAT), glutathione peroxidase (GSH-Px) and Malondialdehyde (MDA). However, there were no significant differences ($p > 0.05$) among all dietary treatments on glutathione (GSH) and Hepatic Superoxide dismutase (SOD). Rabbits fed dietary lemon recorded the highest ($p \leq 0.01$) value of Tig, IgG and IgM followed by fed on fenugreek seeds. Rabbits fed control diet recorded the lowest values of Tig and IgG compared with supplemented diets.

Abbreviations: NZW: New Zealand White Rabbits; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSH: Glutathione, CAT: Catalase; Ca: Calcium; P: Phosphorus; Mg: Magnesium; K: Potassium; S: Sulfur; Na: Sodium; Fe: Iron; Mn: Manganese; Ni: Nickel; B: Boron; Si: Silicon; Cu: Copper; Zn: Zinc; Mo: Molybdenum; Se: Selenium; Co: Cobalt ; Cr: Chromium ; Ge: Germanium; As: Arsenic; Tig: Immunoglobulin; IgG: Immunoglobulin G; IgM: Immunoglobulin; IgA: Immunoglobulin A

Introduction

A part of rabbit farms profitability depends on the efficacy of weanling rabbits to grow efficiently and to rescue from morbidity and mortality during the fattening period. For this reason, antibiotic growth promoters are commonly included in the diets of growing rabbits [1]. In last decades, there are increasing concerns about using natural feed additives as antibiotic alternatives for decreasing development of antimicrobial resistance bacteria and for producing safer animal products with minimal antibiotic residues. Of natural feed additives, those from phytogenic source including different parts of the plants or their extracts are being increasingly included in animal nutrition due to their impressive range of phytochemicals. This enables them to be potentially antimicrobial candidates with multiple mechanisms of action [2]. Additionally, phytogenic compounds are not only used to control pathogenesis, but also they have been reported to improve appetite, intestinal microflora, immune functions, oxidative status, growth and carcass traits when included in animal diets [3]. Considering the affordability of these plant materials, they could be widely used as growth promoters in the livestock sector worldwide [4]. Most of the active phytochemicals identified in plant materials are of alkaloids, terpenes, flavonoides and glucosinolates [4,5]. However, each plant has a unique combination of these phytochemicals, and therefore their biological effects are expected to be different.

Among potential phytogenic feed additives, propolis is a product of plant resinous substances collected by honeybees. It has substantial levels of phenolic compounds including flavonoids,

vitamins, minerals, and enzymes [6]. It has been found to have strong antioxidant, anit-inflammatory and immunomodulation activities [7]. Also, Moringa oleifera Lam is a tropical/subtropical plant with a highly nutritive value [8]. Each part of this plant has been reported to contain considerable biologically active phytochemicals particularly glucosinolates [9], and thus promising therapeutic properties [10,11]. As recently reviewed by [3] the potential biological effects of different combinations of phytochemicals on growth performance, antioxidant and antibacterial activities and blood metabolites in rabbits are not fully scrutinized. The aim of this study was to evaluate the consequence of adding some natural feed additives i.e. turmeric, ginger, fenugreek and dried lemon to rabbit diets at the levels of 0.5, 0.5, 1.0, and 1.0% respectively, on antioxidant enzymes and total immunoglobulins and their fractions of growing rabbits.

Materials and Methods

Experimental Animals

A total number of 30 males,5 weeks old growing New Zealand white rabbits were used to study the effect of some natural feed additives on antioxidant enzymes and total immunoglobulins and their fractions of growing rabbits.. Rabbits distributed into (5 treatments x 3 replicates x 2 rabbit = 30 rabbits). All rabbits were housed in open house. The rabbits were allocated in a cage with slatted floor of iron. The dimensions of the cage were (45 × 45 × 38cm) for length, width and high, respectively. Feed and water given to the rabbits ad-libitum during the experimental periods.

Experimental Diets

Table 1: The composition and chemical analysis of the control diet for growing New Zealand White rabbits.

Ingredients	%
Ground yellow corn	54.0
Soyabean meal	20.3
Wheat bran	5.0
Limestone	0.3
Barseem hay	18.8
Dicalcium phosphate	1.0
Salt	0.3
Premix*	0.3
Total	100
Calculated Analyses%	
Crude protein	17.40
ME (Kcal/ kg)	2561.70
Crude fiber	7.80
Calcium	0.68
Available phosphorus	0.33
Methionine+ cysteine	0.57
Lysine	0.85

Growing rabbits were distributed to five dietary treatment groups. The first group fed control diet formulated to contain adequate levels of nutrients for growing New Zealand White rabbits as recommended by the National Research Council [12]. The formulation and chemical composition of control diet is shown in (Table 1). Chemical analysis of ingredients and diets was determined according to [13]. Four additional dietary treatment groups were formulated to contain control diet incorporated with feed supplementation according to the source of addition such as 0.5% curcuma, 0.5% ginger, 1.0% fenugreek and 1.0% dried lemon respectively.

Assay of Antioxidant Biomarkers in the Tissues and Serum

Samples were removed from −80 °C storage, diluted (v:v) with ice-cold isotonic physiological saline, to determine antioxidant indices such as glutathione (GSH), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) (Total-SOD, CuZn-SOD and SOD-Mn) in serum, using assay kits for antioxidant indices purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) [14].

Total Immunoglobulins and their Fractions

Three samples of serum from each group were used to determine serum total immunoglobulins and their fractions (TIg, IgG, IgM and IgA) were detected by using ELISA kits, according to the instructions of Anjing Jiancheng Bioengineering Institute, Nanjing, China [15].

Statistical Analysis:

Data were summarized using Microsoft® Excel 2010 (10.2614.2625) Microsoft Egypt. The general liner model (GLM) was applied to test the differences among the five experimental groups. P-values less than 0.05 were considered to be statistically significant [16]. The statistical analysis was calculated using the following equation:

$$Yij = \mu + Ti + Eij$$

Where:

Yij = Experiment observations.

μ = The overall mean.

Ti= The effect of dietary treatment.

i= T1, ----- T5.

Eij = The experimental error.

Duncan's test was used to examine the significance degrees among means [17].

Results and Discussion

Antioxidant Enzymes

Data of antioxidant enzymes of dietary treatments are presented in (Table 2). The results revealed that there were significant differences (p≤0.01) among all dietary treatments on the values of catalase (CAT), glutathione peroxidase (GSH-Px) and Malondialdehyde (MDA). However, there were no significant differences (p>0.05) among all dietary treatments on glutathione (GSH) and Hepatic Superoxide dismutase (SOD).The present study demonstrated that rabbits fed dietary fenugreek seeds increased (p≤0.01) CAT, followed by those fed on lemon. Hence, rabbits fed dietary lemon recorded the greatest improvement (p≤0.01) in GSH-Px followed by curcuma compared with other groups. The lowest value (p≤0.01) in MDA was recorded for rabbits fed lemon followed by fenugreek compared with other groups. Spices and herbs can have many benefits for the health of broilers and functions such as anti- oxidation ability [18], antimicrobial activity [19], enhance digestion by stimulating endogenous enzymes to the Brugalli at al. [20]. Ginger is widely used in many countries as a food spice and as an herbal remedy used [21]. Among the 81 chemical elements found in mammalian bodies, at least 19 are found in Citrus plants, including calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sulfur (S), sodium (Na), iron (Fe), manganese (Mn), nickel (Ni), boron (B), silicon (Si), copper (Cu), zinc (Zn), molybdenum (Mo), selenium (Se), cobalt (Co), chromium (Cr), germanium (Ge) and arsenic (As) [22]. Of these elements, Mn, Fe, Cu, Zn and Se have been reported to be related to the antioxidant activity of organisms [23]. For example, Se, an essential component of antioxidant enzyme GSH-Px, can destroy free radicals in the cytoplasm and protect the tissues against oxidative damage [24]. Over 60 flavonoids have been found in citrus. Flavonoids have a direct role in scavenging reactive oxygen species, which can counteract lipid oxidation *in vitro* and improve the body's antioxidant enzyme activity and decrease peroxide formation in vivo [25].

Table 2: Effect of natural feed additives on antioxidant enzymes.

Treatment	CAT (U/mg protein)	GSH (µmol/g protein)	GSH-Px (IU)	MDA(nmol/mg protein)	T-SOD (U/mg protein)	SOD-Zn (U/ mg protein	SOD-Mn (U/ mg protein)
Control	59.93^{c}	12.12^{ab}	2525.68^{c}	11.81^{a}	555.48	444.88^{b}	110.60
Curcuma 0.5%	64.51^{c}	14.18^{a}	2900.13^{b}	8.34^{b}	611.84	462.83^{b}	149.01
Ginger, 0.5%	83.75^{b}	12.45^{ab}	2878.66^{b}	7.17^{c}	611.02	519.52^{a}	91.50
Fenugreek, 1.0%	102.99^{a}	12.69^{ab}	2651.63^{c}	5.99^{d}	622.29	543.13^{a}	79.16
Lemon, 1.0%	87.18^{b}	11.42^{b}	3473.14^{a}	4.83^{e}	535.48	448.68^{b}	86.81
±SE	3.24	0.69	69.32	0.2	26.09	12.50	21.48
Sig.	**	NS	**	**	NS	NS	NS

$^{a\text{-}e}$Values within the same column with different superscripts are significantly different (p≤0.05).SE:- standard error (±). NS: - Not significant. (**):- highly significant (p≤0.01).catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px), Malondialdehyde (MDA) and Hepatic Superoxide dismutase (SOD).

Decreasing lipid peroxidation by ginger treatment may be attributed to its antioxidant activity as it contains many phenolic compounds which have inhibitory effect on lipid peroxidation, these phenolic antioxidants may conserve the antioxidant enzymes but increase SH- containing compounds including glutathione. The depletion of antioxidant enzymes may be explained as ginger offered protection to cells against oxidative stress by scavenging free radicals [26]. This may be due to the presence of many antioxidative compounds like gingerols, shogaols, phenolic ketone derivatives, volatile oils and flavonoids in ginger, these antioxidant compounds may modulate spare the antioxidant enzymes [27]. These results are harmony with Zhang, et al. [28] showed that supplementation of ginger at the rate of 5g/kg significantly increased the activities of SOD and GSHPx and reduced MDA in broilers at the age of 21 and 42 days. The reduced level of MDA indicated that the addition of ginger alleviated the lipid peroxidative damage to the cell.

Humeral Immune Response (Immunoglobulin):

Data of immunoglobulin i.e. total immunoglobulin (Tig), immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA) of dietary treatments are presented in (Table 3). The results revealed that there was significant difference (p≤0.01) in all previous parameters among all dietary treatments. Rabbits fed dietary lemon recorded the highest (p≤0.01) value of Tig, IgG and IgM followed by fed on fenugreek seeds. However, the best (p≤0.01) IgA values were recorded for rabbits fed dietary lemon and control diet compared with other dietary treatments. Rabbits fed control diet recorded the lowest values of Tig and IgG compared with supplementaed diets. The improvement in immunoglobulin values as a result of adding feed supplementation to growing rabbits may be due to that herbal plants are rich in flavonoids such as garlic and turmeric extend the activity of vitamin C, act as antioxidants, and may therefore improve immune functions [29]. Moreover, Citrus pulp contain flavonoids and vitamin C which can present antioxidant properties [30,31], antibacterial [32] and immune stimulating activities [33-35].

Table 3: Effect of natural feed additives on immunoglobulin and their fraction of rabbits.

Treatment	Immunoglobulin			
	IgA	IgM	IgG	Tig
Control	12.25^{e}	9.95^{e}	0.3^{d}	0.53^{a}
Curcuma, 0.5%	14.73^{d}	11.83^{d}	0.32^{d}	0.59^{d}
Ginger, 0.5%	16.08^{c}	13.03^{c}	0.35^{c}	0.62^{c}
Fenugreek, 1.0%	17.43^{b}	14.23^{b}	0.38^{b}	0.65^{b}
Lemon, 1.0%	18.78^{a}	15.43^{a}	0.41^{a}	0.68^{a}
±SE	0.25	0.29	0.006	0.006
Sig.	**	**	**	**

$^{a-e}$Values within the same column have different superscripts are significantly different (p≤0.05).SE:- standard error (±). (**):- highly significant (p≤0.01).Total immunoglobulin (Tig), immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA).

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