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Abstract. This experiment was conducted to explore the effect of fenugreek seeds (FSP) and synbiotic (SP) powders in diet as antioxidant factors on antioxidative, physiological, immunological indicators of serum and anatomical properties during exposing to the oxidative stress (OS) induced by hydrogen peroxide (H_2O_2) in drinking water of broiler chickens from 2nd until 35th day of age. Totally, 300 chicks Ross 308 at one day old of age were randomly allocated into five treatments with three equal replicates in each treatment (20 chicks/replicate). The treatments were distributed into negative control without feed additives (T1) whereas T2 was adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water as stressed positive control. The dietary additive (0.5%) each of FSP and SP was added in stressed groups (T3 and T4), respectively and dietary combination of 0.25% both FSP and SP was added in stressed group (T5). The results referred that T3, T5 and T1 registered an increased level in catalase ($p \leq 0.05$) and glutathione peroxidase ($p \leq 0.01$) activity and there was increased amounts in superoxide dismutase and packed cells volume in T4 ,T5 and T1 ($p \leq 0.01$) in comparison to T2. The T3, T4, T5 and T1 achieved a decreased malondialdehyde ($p \leq 0.05$) with considerable decrease ($p \leq 0.01$) in heterophils/lymphocytes ratio, uric acid, creatinine, alanine and aspartate aminotransferases compared with T2. In comparison to T2, all stressed treatments and T1 had high antibody titers against Newcastle disease ($p \leq 0.05$) and infectious bursal disease ($p \leq 0.01$). Moreover, high proportional weights of lung and heart ($p \leq 0.01$) were in T5 whereas T1 achieved high ($p \leq 0.01$) kidneys weight and large intestine length. Thus, dietary FSP and SP supplement added synergistically or individually could promote antioxidant status, regulate liver and kidney function, and improve immune system of stressed chickens.

Keywords. Oxidative stress, Broiler chickens, Fenugreek, Synbiotic.

1. Introduction

Under normal condition, oxygen molecule can be reduced into intermediate compounds which called reactive oxygen species (ROS) and nitrogen reactive species (NRS) [1,2]. Most of these reactive compounds are free radicals which are toxic to cells when they overpass the required level through their excessive accumulation in certain sites of the cells. This would lead to cellular instability and loss of the cells' mechanism to remove them, which in turn leads to the occurrence of oxidative stress incidence [3,4]. Oxidative stress (OS) is one of the most deleterious phenomena to cellular metabolism,whose passive consequences is not restricted to deterioration of productive and physiological performance but also affects animal welfare and final evaluation of produced meat and

eggs [5]. In other word, OS is imbalance state between prooxidant and antioxidant netwrok system (redox) which causes lipid peroxidation, protein oxidation , DNA injury and abnormal regulation of cellular signaling pathways [6]. Thus, for alleviating stressful OS, it needs to find out alternative and safe materials offered in diet, drinking water or other practical approaches that might be capable of eliminating and scavenging the offensive free radicals.

Fenugreek (*Trigonella foenum-graecum*) is one of the effective medicinal herbs that is distinguished by antitoxic, anti-microbial, anti-stress and hypoglycemic activity because of its constituents of many flavonoids, overall polyphenols, saponins, alkaloids, proteins, vitamins, fatty acids and minerals such as, zinc, iron magnesium calcium, and phosphorous [7,8]. Fenugreek used as phytogenic feed additives instead of antibiotics in poultry nutrition because it proved its efficacy to increase productive performance by ameliorative digestibility, powerful intestinal absorption and removal of pathogens inhabitants in the digestive system [9-11]. Moreover, fenugreek extracts stimulate immune system [12] and antioxidant defense mechanism in egg yolk [13] as well as improve the meat quality and protein metabolism in broilers [14].

The synbiotic preparations are synergistic combination of probiotics and prebiotics that are utilized in various levels as a feed supplements to support the overall growth performance and public health of poultry. The probiotics are consisting of advantageous microbes that are obtained from the intestinal microflora of adult avian gut. These microflora are able to prevent pathogenic bacteria from achieving adhesion sites in epithelial cells present in intestine via influential impact of beneficial microflora to envelop the receptors of epithelial cells and expel the harmful bacterial outside the host body [15]. Besides, the powerful microorganisms participate to the microbial modulating of intestinal flora during challenging birds by stressful environment such as, heat and toxic stresses or other diseases [16,17]. Prebiotics are nonviable and non-digestible feed component such as oligosaccharides which used as effective agents to reinforce, enhance and nourish populations of useful bacteria in the digestive system of different poultry species [18]. These additions are basically extracted from the cell wall of microorganisms such as, some bacteria, yeasts and molds and different herbs that have nutritive and preventive effects [19]. These complex sugars of prebiotics are intaked by intestinal bacteria and have pivotal roles in blocking the receptors found on the deleterious bacteria wall surface. Therefore, prebiotics act for inhibiting harmful microbial adhesion to the epithelial cells of the digestive canal, decreasing the chance of disease infection and promoting the immune system [20]. Interestingly, synbiotic products demonstrated its valuable influence in histomorphological alterations after digestion by augmentation the villi length and epithelial layer thickness of intestine via its role in ameliorating the microbial equilibrium of whole gut [21,22].

The objective of the study was aimed to possible using of fenugreek seeds and synbiotic powders as dietary antioxidants for stressed broiler Ross 308 challenged by adding hydrogen peroxide (H_2O_2) in drinking water and its impacts on some antioxidative, biochemical and immunological indices in serum as well as anatomical characteristics at 35 days old of age.

2. Materials and Methods

2.1. Experiment Scheme

This study was implemented for 35 days in poultry farm of Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Babylon /Iraq and laboratories in Mosul Ethical approval No. um.VET.2021.5. In total, 300 chicks broilers Ross 308 at one day old of age with an average initial weight (44.5 ±0.5 g) were used. The chicks were randomly distributed into five treatments with three equal replicates in each treatment (20 chicks/replicate). All birds were raised on floor system under controlled standard conditions and fed balanced diet based on NRC [23]. The chicks except for negative control group were challenged to OS from 2 days old until finishing of the study. The experimental treatments were divided into *ad libitum* feeding without any feed additives (negative control, T1) whereas T2 was adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water as positive control. In T3, T4 and T5, the additives of 0.5% fenugreek seed powder (FSP), 0.5% synbiotic powder (SP) and combination of 0.25% for each FSP and SP were added to diet respectively plus adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water.

2.2. Feed Supplements

The FSP was obtained from the local market and its biologically active compounds were detected and analyzed by routine laboratory tests (table 1) based on protocol of Anhawange *et al.* [24], Trease and Evans [25], Mradu *et al.* [26] using the high performance liquid chromatography (HPLC) technique. Regarding to synbiotic powder (SP), was in powdery form with fragrant odor consisting of both mixture of probiotic and prebiotic products under the name (Iraqi synbiotic). The SP is permissible for using in animal nutrition and other scientific trends according to manufacturer's instructions and was made in and obtain from food technology labs of the College of Agricultural Engineering Sciences/ University of Baghdad, Iraq. Each 1 gm of SP contains 10^8 *Bifidobacterium*, 10^8 *Lactobacillus acidophilus*, 10^9 *Bacillus subtilis* and 10^9 *Saccharomyces cervisia*. Besides, prebiotic part in synbiotic mixture represents complex sugars isolated from cell wall of dry yeast which called *Saccharomyces cerevisiae*. Hydrogen peroxide solution (H_2O_2, Panreac Quimica S.L.U., Barcelona, Spain, 50%) with specific irritating odour was applicated as pro-oxidant substance to provoke experimentally OS *in vivo*. To obtain the prescribed dose (0.5%), H_2O_2 was diluted with sterile distilled water. H_2O_2 preparation was done daily to preserve its chemical purity and oxidation power also it was stored in black plastic closely tight containers and placed far away from deleterious influences of sunlight. H_2O_2 was offered to birds daily by taking 1 ml of 0.5% and supplemented in 1 L of drinking water [4,6].

Table 1. Detection of phytoconstituents in fenugreek seed powder.

Compound	Value	Unit
Trigonelline	135.69	ppm
Total flavonoids	20.45	mg rutin/gm
Total phenols	30.69	mg gallic acid/gm
Total tannins	5.36	%
Total saponins	0.58	%
Total terpinoids	11.36	%
Total alkaloids	13.25	%

2.3. Studied Parameters

2.3.1. Blood Sampling

Blood was collected twice for cellular and biochemical tests from the brachial vein at 35 days of 2 males and 2 females per replicate before had been slaughtered (12 birds per treatment). One collection of blood (2 ml) was preserved in K_3EDTA tubes for determination cellular components (packed cells volume (PCV) and heterophils/ lymphocytes ratio (H/L)). Another blood collection was put in serum separator tube, centrifuged at 1000 RPM for 10 minutes to obtain serum and then stored at – 25°C in freezing until performing the antioxidative, biochemical and immunological analyses in serum involving glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase, malondialdehyhe (MDA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, uric acid and antibody titers against infectious bursal disease (IBD) and Newcastle disease (ND).

2.3.2. Test of Blood Cellular Values

The PCV was determined using the microhematocrit centrifuge method, heparinized capillary tubes and microhematocrit reader [27] whereas H/L was enumerated using method reported by Gross and Siegel [28].

2.3.3. Test of Serum Biochemical Indices

The ALT and AST activities were measured using available kit (Randox, England) based on colorimetric procedure coined by Reitman and Frankel [29]. A commercial kit (Biolabo, French) was used for determination both creatinine and uric acid levels in serum by following protocols of Tietz [30]. A spectrophotometer (Shenzhen, China) was used for determination the wavelengths for the biochemical tests.

2.3.4. Test of Serum Antioxidant Indices
The GPX activity was determined according to the method of Hafemann et al. [31] by calculating the lowering in glutathione level after exposing the specimen to H_2O_2 and NaN_3. The SOD was determined according to the competitive reaction between the autoxidation of pyrogallol by $O2\bullet^{-}$ and the dismutation and inhibition of this free radical by SOD [32]. Catalase activity was measured depending upon spectrophotometric assay of H_2O_2 and formation of its non reactive complex with ammonium molybdate [33]. With respect to MDA, it was estimated by thiobarbituric acid (TBA) assay method according to Buege and Aust [34] which depends on reaction of one molecule of MDA with two molecules of TBA to result a red MDA-TBA complex which can be measured at 535 nm wavelength. All these analyses were performed by using specific kits (Sigma-Aldrich, CO, USA) and spectrophotometer (Shenzhen, China).

2.3.5. Test of Immunological Indices
The ready kits (ProFLOK® IBD-T ELISA, USA) and (ProFLOK® NDV-T ELISA, USA) were used to determine antibody titers against IBD and ND, respectively by using an automated micro-plate reader (BioTek Epoch 2, USA) with following the manufacturer guidelines.

2.3.6. Evaluation of Anatomical Traits
At the end of the experiment (35 days), 2 females and 2 males were randomly selected from each replicate group (12 in each group) and exposed to withdrawal of feed for 6 hours to stabilize the body weights and record their final body weight . Thereafter, these birds were decapitated by euthanasia procedure, defeathered and eviscerated to obtain their internal functional organs involved lungs, heart, kidnyes, pancreas, liver, gizzard, small intestine, large intestine, whole gut, and weight of immune organs (bursa of Fabricius, thymus gland and spleen). All these organs were cleaned, separately weighed using electronic sensitive scale (W&J Instrument Co., Ltd/China) to register their weights in relation to live body weight. Absolute lengths of whole gut, small intestine and large intestine were measured individually by using tape measure and electronic vernier. Moreover, proportional length of small intestine was calculated in relation to whole gut length. Additionally, bursa of Fabricius index was calculated by dividing proportional weight of bursa of Fabricius in each chicken of experimental group over the proportional weight mean of bursa of Fabricius in negative control group [35].

2.4. Statistical Analysis
Data were analyzed by using the statistical analysis software [36] to find out the influence of various treatments on the variables by application a completely randomized design, and the mean differences among the treatments were compared according to Duncan's multiple range test [37]. To test the hypothesis, the levels of $P<0.05$ and $P<0.01$ were considered statistically significant.

3. Results and Discussion

3.1. Results
Based on results presented in table 2, it showed that T3, T5 and T1 obtained high levels in serum catalase ($p\leq0.05$) and GPX ($p\leq0.01$) activity compared to T2 which in turn did not differ significantly from T4. High SOD activity ($p\leq0.01$) was in T4, T5 and T1 compared to T1 and T3. All experimental groups achieved lowering in MDA value ($p\leq0.05$) compared to T2.
Table 3 showed that T4, T5 and T1 registered high significant differences ($p\leq0.01$) in PCV compared to T2. Also, there was obvious lowering ($p\leq0.01$) in H/L and serum uric acid, creatinine, ALT and AST levels in favor of T3, T4, T5 and T1 compared to T2.

Table 2. Effect of fenugreek and synbiotic and their combination added to diet on serum oxidative markers of stressed broiler chickens (mean± standard error).

Parameters	Treatments					Significance
	T1	T2	T3	T4	T5	
catalase (kU/L)	48.448± 8.29 a	31.58± 2.34c	39.92± 6.45 ab	32.34± 3.49 bc	45.29± 7.29 a	*
GPX (U/L)	8.469± 1.82 a	4.816± 1.18 c	7.69± 1.21 ab	5.53± 1.20 bc	6.78± 1.99 b	**
SOD (U/mL)	53.56± 4.11a	42.79± 11.30 c	47.98± 4.67 bc	49.31± 3.88 ab	53.37± 2.30 a	**
MDA (mmol/L)	10.83± 2.72 c	26.79± 3.98 a	21.04± 3.42 b	21.81± 6.61 b	17.97± 2.87 bc	*

T1: negative control, T2: positive control (adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water); T3, T4 and T5: adding 0.5% fenugreek seed powder, 0.5% synbiotic powder and combination 0.25% each of fenugreek seed and synbiotic powders in diet respectively plus adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water.
Different letters in the same row indicate to significant difference among treatments, * at ($p \leq 0.05$), ** at the level ($p \leq 0.01$).

Increase in antibody titers against ND ($p \leq 0.05$) and IBD ($p \leq 0.01$) was recorded for T3, T4 ,T5 and T1 in comparison to T2. No significant differences were observed among groups in relative weight of bursa of Fabricius, thymus and spleen and bursa of Fabricius index (table 4).

Table (5) demonstrated that high body weight ($p \leq 0.05$) was in all experimental treatments in comparison to T2. High ($p \leq 0.01$) lung weight was in T3 and T5 whereas high ($p \leq 0.01$) heart weight was in T5 compared to T2. The T1 obtained higher ($p \leq 0.01$) kidneys weight and large intestine length than T2 whereas other groups did not differ from T2 in these traits. Lack of significant differences were clear among treatments for other parameters measured.

4. Discussion

The best significant improvement in antioxidant mechanism defense was achieved by T5 followed by T3 and T4 through enhanced serum catalase, GPX and SOD levels synchronized with decreased serum MDA compared with T2 (table 2). This is due partly to the presence of powerful rich phytochemicals in FSP represented by polyphenols, flavonoids and others (table 1) and influential content of synbiotic preparation from beneficial bacterial and sugars. The FSP is characterized by scavenging the oxygen–derived free radicals and lowering of lipid peroxidation levels in heaptic mitochondrial membranes [38,39] and stimulation the secretion of SOD, glutathione, catalase and glutathione S-transferase in renal tissue [40] which have been implicated in multiple stressful disorders. Moreover, dietary FSP proves its influence on decreasing inducible nitric oxide synthase in ileum mucosa which could avoid intestinal injury, and inhibit inflammatory reaction related to OS in chickens [11].

Thus polyphenolic compounds in FSP may exert as powerful radical scavengers which might promptly react, quench or terminate the peroxidation pathways and finally ameliorate the blood antioxidant profile. In Arbor Acres broilers, similar conclusion were reported by Tao et al. [14], that feeding 6% FSP has elevated serum total antioxidant capacity at 42 days and high serum SOD activity was reordered in 9% FSP at 21 day although no influence on the serum MDA contents was stated. The broad and superior possibilities of antioxidant compounds to neutralize or prevent free radicals chain or coordinate redox.

Homeostasis in the host cell could be activated by dietary synergistic effect of probiotic and prebiotic as synbiotic [18,19]. It is also mentioned that synbiotic can affect *in vivo* antioxidant stimulation by generating antioxidant enzyme system and antioxidtive metabolites with modulation intestinal microbiota and regulation the antioxidant related-signaling pathways of transcription nuclear factors [15]. Thus, there was improvement in antioxidant profile by enhanced set of antioxidative enzymes (SOD, CAT, GPx, glutathione reductase) and decreased indicator of lipid oxidation (thiobarbituric acid reactive substances; TBARS) in serum and liver of broilers offered diet contains 0.2% mannan-oligosaccharides along with *Lactobacillus acidophilus* at 106 or 107 CFU/g [41]. Our data was in

harmony with conclusions of Song et al. [42] who revealed that was increased total antioxidant status in serum of broiler chickens represented by lowered MDA and high SOD during feeding on combination of synbiotic composed of 3 g/kg fructooligosaccharide and 0.1 g/kg microencapsulated *Lactobacillus plantarum* as a substitute for dietary antibiotic. Different data to ours showed that using 1 g synbiotic composed of *Lactobacillus reuteri*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, and fructooligosaccharides per kg of diet mitigated the heat stress reactions and regulation of stress consequences of broiler males exposed from day 15 to 42 to 32°C for 9 hours daily by improved GPX activity in plasma and spleen, enhanced transcription factor (Nrf-2) [43]. Moreover, the results of Li et al. [44] were not in line with current data, who declared that serum MDA and SOD in male Partridge shank chicks were no affected by feeding for 50 days at 1.5 g per kg of diet on synbiotic composition (*Clostridium butyricum*, *Bacillus subtilis* and *Bacillus licheniformis*) as probiotic and (yeast cell wall and xylooligosaccharide) as prebiotics, however, there was only registered decrease in hepatic MDA level.

Table 3. Effect of fenugreek and synbiotic and their combination added to diet on blood cellular and biochemical values of stressed broiler chickens (mean± standard error).

Parameters	Treatments					Significance
	T1	T2	T3	T4	T5	
PCV (%)	30.00± 4.50 a	25.55± 0.2b	27.33± 1.45ab	28.66± 1.20a	31.66± 3.84a	**
H/L	0.21± 0.00 b	0.31± 0.02a	0.22± 0.01b	0.22± 0.01 b	0.22± 0.01 b	**
uric acid (mg/100 ml)	7.33± 0.77 c	12.79± 1.60a	10.47± 0.67 b	9.88± 2.32 b	9.87± 0.46 b	**
creatinine (mg/100 ml)	0.42± 0.02 c	0.85± 0.07 a	0.61± 0.12 b	0.52± 0.04 bc	0.57± 0.08 b	**
ALT (U/L)	4.85± 1.35 d	10.86± 7.13a	2.92 ± 5.82 c	7.95± 3.88 b	7.37± 4.47 b	**
AST (U/L)	21.14± 2.69 c	48.69± 7.56a	23.66± 5.20 c	38.02± 9.05b	37.44± 4.25 b	**

T1: negative control, T2: positive control (adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water); T3, T4 and T5: adding 0.5% fenugreek seed powder, 0.5% synbiotic powder and combination 0.25% each of fenugreek seed and synbiotic powders in diet respectively plus adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water.
Different letters in the same row indicate to significant difference among treatments, **at the level ($p \leq 0.01$).

Table 4. Effect of fenugreek and synbiotic and their combination added to diet on serum antibody titers against ND and IBV diseases and lymphoid organs weights of stressed broiler chickens (mean± standard error).

Parameters	Treatments					Significance
	T1	T2	T3	T4	T5	
antibody titers against ND	6812± 2.01 a	4603± 1.22 c	6222± 2.01b	8800± 3.15a	6793± 1.65ab	*
antibody titers against IBV	3292± 0.37 ab	2019± 0.90 c	2876± 0.84b	4435± 1.99a	3286± 1.48ab	**
bursa of Fabricius (%)	0.16± 0.00	0.12± 0.02	0.17± 0.02	0.17± 0.01	0.15± 0.00	NS
bursa of Fabricius index	0.99± 0.00	0.75± 0.02	1.073± 0.02	1.082± 0.03	0.948± 0.03	NS
thymus (%)	0.10± 0.01	0.08± 0.00	0.10± 0.02	0.08± 0.00	0.09± 0.00	NS
spleen (%)	0.13 ± 0.01	0.09± 0.00	0.11± 0.01	0.10 ± 0.01	0.10± 0.01	NS

T1: negative control, T2: positive control (adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water); T3, T4 and T5: adding 0.5% fenugreek seed powder, 0.5% synbiotic powder and combination 0.25% each of fenugreek seed and synbiotic powders in diet respectively plus adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water.
Different letters in the same row indicate to significant difference among treatments, * at ($p \leq 0.05$), ** at the level ($p \leq 0.01$), N.S: non-significant.

No alterations in the PCV level was in T3 compared to T2 might be resulted from lack of hematopoietic regulatory action occurring in the bone marrow with the single inclusion of fenugreek in the diet. Contrary, the combination of fenugreek with synbiotic (T5) had potential influence on haemopoietic process and this reflected on ameliorative erythropoiesis by inhibited formation of free radicals that cause destruction of erythrocytes and haemoglobin and thus could reduce PCV value [7]. As compared with T1, similar result was recently reported by Paneru et al. [45] who found that was no changing in PCV value, H/L counts in broiler chickens at 6 weeks fed diet containing 5 g/kg FSP.

Decreasing in H/L was synchronized with lowered levels in serum biochemical analytes for indices of kidney test (uric acid, creatinine) and liver test (ALT, AST) in all stressed birds fed FPS (T3), synbiotic (T4) and their mixture (T5) as well as T1 compared to T2. This might be presence number of detected bioactive compounds in FSP (table 1) which paly multiple physiological effects which ameliorated the OS conditions [10,12]. FSE affects modulation of inflammatory factors and stressful environmental conditions via its impacts on intestinal microflora population, intestinal morphology and overall nervous system functionality in addition to increased feed appetite and palatability of birds [11]. This led to obvious decrease in H/L ratio which could express as indicator for long-term changes in response to excessive stress in broilers' environment [28].

Thus, effect of FSP was proved in improved public health through lowered activity of hepatic enzymes and renal function indicators. High levels of ALT, AST, uric acid and creatinine present in blood stream refer to damaged or disrupted hepatocytes and epithelial renal cells. This result was confirmed by Alsieni et al. [40], who concluded that administration of aqueous extract of FSP at 200 mg/kg of body weight through gastric gavage could reduce ALT, alkaline phosphatase, lactate dehydrogenase, creatinine, uric acid and urea in serum of diabetic male rats fed high fat diet and challenged by intraperitoneal injection at 65 mg/kg body weight of streptozotocin for 8 weeks. In contrast, Amein et al. [46] proved that adding 0.2 and 0.5% FSP in diet had no influence on serum uric acid of broiler chicken at 42 days. Likewise, improved hematological and biochemical references for T4 or T5 indicate to improving the public health and ameliorative resistance against OS. This is due to compositional activity of synbiotic used as antioxidant factor in diet. High PCV in T3 and T5 could be related to positive infulence of synbiotic composition for stimulation of morphological changes in intestine and increase the absorption capacity and feed efficiency which may in turn induce the increase of red blood cells and PCV for accelerate metabolism under stress [18].

This was confirmed recently by Astuti et al. [47], that 0.25 and 0.50% of synbiotic led to enhance synthesis of erythrocytes which reflect on increased hemoglobin and PCV of broilers. Low H/L could be explained by effect of synbiotic to relieve stress and improve resistance against NC and IBD (table 4) and this was similar to what obtained by Abdel Razek and Tony [48]. Later , Mohammed et al. [49] indicted also that H/L was decreased in stressed broilers offered diet containing the synbiotic composed of *Bifidobacterium animalis*, *lactobacillus reuteri*, *Enterococcus faecium*, *Pediococcus acidilactici* and fructooligosaccharides at 1.0 g/kg during exposing to 32˚C for 9 hours from 15 until 42 days daily. Incompatible data were achieved by Sozcu and Ipek [50] that was changing in ALT activity and increased AST activity in serum of Cobb 500 broiler fed 500 and1000 g/ton of synbiotic (*Saccharomyces cerevisiae*, glucan-oligosaccharide and mannan- oligosaccharide) for 42 days of age. More recently, Dev et al. [41] found also no effect of different doses of synbiotic (*Lactobacillus acidophilus* and mannan-oligosaccharides) on serum AST and ALT activity in broiler chickens.

Table 5. Effect of fenugreek and symbiotic and their combination added to diet on visceral weight and length of stressed broiler chickens (mean± standard error).

Parameters	Treatments					Significance
	T1	T2	T3	T4	T5	
body weight (g)	2381.87± 42.57ab	1819.03± 18.04 c	2366.78± 13.28 b	2354.36± 19.89 b	2447.13± 10.82 a	*
lung weight (%)	0.57± 0.01 ab	0.48± 0.02 b	0.58± 0.03a	0.56± 0.00 ab	0.61± 0.03 a	**
heart weight (%)	0.56± 0.04ab	0.52± 0.03b	0.63± 0.04ab	0.63± 0.01ab	0.65± 0.03a	**
kidney weight (%)	0.43± 0.02 a	0.30± 0.00 b	0.30± 0.04 b	0.39± 0.03 ab	0.34± 0.02 ab	**
pancreas weight (%)	0.21± 0.02	0.16± 0.01	0.24± 0.04	0.19± 0.00	0.22± 0.04	NS
liver weight (%)	2.19± 0.06	2.15± 0.13	2.53± 0.13	2.23± 0.041	2.52± 0.15	NS
gizzard weight	1.58± 0.12	1.26± 0.03	1.35± 0.10	1.43± 0.18	1.56± 0.14	NS
small intestine weight (%)	4.69± 0.24	4.74± 0.57	5.65± 0.53	5.17± 0.38	5.64± 0.47	NS
large intestine weight (%)	0.85± 0.08	0.69± 0.07	0.85± 0.13	0.86± 0.06	0.72± 0.05	NS
gut weight (%)	8.49± 0.47	8.22± 0.87	9.27± 0.98	9.08± 0.53	9.42± 0.47	NS
gut length (cm)	276.16± 4.47	234.71± 3.19	264.68± 9.70	273.91± 12.17	267.18± 20.71	NS
small intestine length (cm)	202.17± 3.11	179.66± 3.58	200.68± 9.79	209.66 ± 8.42	203.66± 16.42	NS
large intestine length (cm)	51.66± 1.42 a	35.75± 2.38 b	44.33± 1.45 ab	43.41± 3.83 ab	43.50± 6.89 ab	**
Small intestine length (%)	73.21± 0.55	76.54± 1.06	75.76± 1.12	76.57± 0.75	76.21± 1.92	NS

T1: negative control, T2: positive control (adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water); T3, T4 and T5: adding 0.5% fenugreek seed powder, 0.5% synbiotic powder and combination 0.25% each of fenugreek seed and synbiotic powders in diet respectively plus adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water.
Different letters in the same row indicate to significant difference among treatments, * at ($p \leq 0.05$), ** at the level ($p \leq 0.01$), N.S: non-significant.

Obviously, the FSP, SP and their mixture proved their efficacy to increase serum antibody titers against NC and IBV at 42 days with no passive changing in proportional weight in lymphoid organs under stress effect (table 4). This is probably that different concentrations of FSE consumed in diet could stimulate immune system and immunoglobulins secretion such as (IgA, IgM, IgG) [40], increase the serum anti-inflammatory cytokines such as interleukin-1,10 and interferon-g with augment signaling pathways of mRNA expression of nuclear factor-kB (NF-kB) in the spleen tissue and thus potentiate resistance against inflammation and stress in broiler chickens [12]. In addition, FSP might depress pathogenic microfloa in the digestive system and increase the proliferation of beneficial bacteria composition responsible for immunomodulatory action. Our result agreed with previous study of Weerasingha et al. [51] who indicated that gradual feeding of FSP at 1,2,3,4 and 5% in diet from 3th week till 38 days did not show any significant differences in relative weight of bursa of Fabricius and spleen of broilers. Also, adding 3%, 6% and 9% FSP in diet did not show any significant change in indices of bursa of Fabricius and thymus at 21 days with no changing in indices of thymus, spleen, and bursa of Fabricius at 42 days [14]. However, it was found that no influence both of 1 and 2 % FSE and fenugreek extract supplemented in diet on primary and secondary immune responses which

represented by measuring antibody titers against sheep red blood cells at 5th and 7th weeks of trial in Leghorn laying hens [52]. One of the most important function of synbiotic is to promote the immune response against invading injurious microbial pathogens such as *Escherichia coli* and coliforms and increase commensal bacteria such as Bifidobacterium spp. and Lactobacillus spp in broiler cecum because of synbiotic' content of probiotic microorganism and prebiotic sugars [43]. That is may be attributed to influence of synbiotic powder in increased production of serum immunoglobulin A (IgA) , immunoglobulin G (IgG) , and interleukin -2 (IL-10) and high spleen index with reduced amounts of tumor necrosis factor-alpha (TNF-a) in chickens [42]. In this regards, and in line with our results, it was found that synbiotic (Biomin Imbo) containing *Enterococcus facium*, fructo-oligosaccharides and extracts of sea algae stimulated acquired humoral immune after vaccination against ND, infectious bronchitis (IB) and IBD in broiler chickens [53]. Likewise, using 250 and 500 g/ton of combined synbiotic and specific enzymes (Avi-bac®) as dietary adjuvant stimulated phagocytic activity against Newcastle virus by elevated serum antibody titers for ND as well as IBD [48]. Also, Dizaji et al. [54] stated that synbiotic (Amax4x) supplemented diet at 1kg per ton for six weeks did not change the absolute weights of spleen, bursa, proventriculus, gizzard and liver of broiler.

There was stability in the most relative weights of visceral organs and absolute length of gut and intestines among experimental groups although high body weights were registered in favor of all stressed birds supplemented with feed additives (T3, T4 and T5) and T1 compared with T2. However, increased weights of lungs in T3 and T5 with large weight of heart in T5 might be attributed to activity of these dietary antioxidative additives to detoxify the harmful H_2O_2 accumulative in the functional tissues in attempt to alleviate the stress incidence [4,6]. The same outcome was stated by Elamin et al. [55], that were no significant differences in relative and absolute weights of liver, gizzard and intestine of broiler chickens Ross 308 at 6 weeks fed diet containing 0.50 and 0.75% FSP. Also, this observation corroborated the data of Astuti et al. [47], who found that 0.25, 0.5 and 1% dietary synbiotic (FERMIX) comprising mixture of red rice and aromatic ginger as prebiotic fermented anaerobically using *Lactobacillus casei* (probiotic) had no effect on relative weights of heart, pancreas, gizzard, small intestine segments and ceaca although high liver relative weight was achieved by 0.5% FERMIX at 35 days of age in broilers.

Conclusion

This research provides apparent *evidence* about positively modulatory action of adding 0.5 % for both fenugreek seed or synbiotic powders in diet individually or synergistically (0.5% each of them) under stressed environment stimulated by adding 1 ml (0.5%) of H_2O_2 per 1 L of drinking water of broiler chickens . The antioxidative effects of fenugreek seed or synbiotic powders or their equal mixture in broilers serum was clearly by improved antioxidant defense systems and ameliorated function of liver and kidney and immune system against viral diseases. Taken together these results show that dietary supplement for these additives mitigated the oxidative stress in the broiler chickens.

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