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Testing of The individual And Synergistic Effect of Adding Two Medicinal Plants to The Diet On Blood Parameters of Heat Stressed Broilers

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Abstract: *The aim of this study was to evaluate the efficacy of adding Capparis spinose (Cs) fruitsor Withaniasomnifera (Ws) leaves or their combinationto broiler diet in alleviating heat stress through studying their effect on blood parameters.A total of 180, day old, Ross 308 broiler chicks were distributed into 4 experimentalgroups (3 replicate/group) and submitted to high environmental temperature (29-35◦C). The experimental groups were as follows: T0=control group without any addition, T1=adding 2g Cs/kg diet. T2=adding 2gWs/kg diet and T3=adding a combination of 1g Cs+1g Ws/kg diet. Main results obtained showed that all plantsaddition treatments led to significant reduction in uric acid ($p<0.05$),urea ($p<0.05$), total cholesterol ($p<0.01$) and LDL-cholesterol ($p<0.01$) in comparison with control. The individual addition of Wsled to significant reduction in AST ($p<0.01$) and ALT ($p<0.05$). Results also showed that plantcombinationtreatment led to:significant ($p<0.01$) reductionin AST in comparison to control, significant ($p<0.05$) increasing in albumin in comparison to Cs group, significant ($p<0.01$) increasing in blood glucose in comparison to individual addition of Cs or Ws groups, and, significant ($p<0.05$) reduction in creatinine in comparison to Ws group. The individual addition of Cs or Ws reduced significantly the level of ALP while their combination didn't affected it.We can concluded from the results of recent study that there were a synergistic effect between the bioactive compounds presented in Capparis spinose fruits and Withania somnifera leaves which led to reduce the levels of uric acid, urea, total cholesterol and LDL-cholesterol in serum of broilers submitted to high environmental temperature while the individual addition of Withania somnifera leaves reduced significantly AST and ALT.*

Keywords*: Capparis spinose, Withania somnifera, heat stress, broilers, haemabiochemical parameters.*

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I. Introduction

There are a lot of external stressors that affect the stability and equilibrium of the organism in case of non-adaptation, and this will be reflected in how long it remains, its reproduction and production. Poultry are one of those organisms that can be strained by many factors, including environmental temperature degree disorders that leads to heat stress [30], causes a change in body physiology that is reflected in its long-lasting production [9]. Heat stress is known to be a major reason for increasing the free radicals for reactive oxygen species (ROS) which causes injury to the cells through the occurrence of lipid peroxidation and the occurrence of oxidative damage to protein and DNA [25,22]. To find solutions to this problem, many researchers have conducted experiments on this subject with a view to finding appropriate solutions, among those solutions were the use of medicinal plants and herbs and their extracts as dietary addition [19] because of its active biochemical compounds content that help to withstand stress, tissuesprotection and enhancement of cell survival [36]. There are a number of plants within the class called adaptogens are defined in herbal medicine as a natural substance having the ability to helping the body to adapt and resist the negative influences of a wide range of stressors. *Withaniasomnifera*(*Ws*), family Solanaceae is one of these plants proved to be an excellent adaptogene and antistress agent [11] have multifaceted medicinal properties including antioxidant [37]. It is located in Iraq as a wild plant. The leaves contain flavonoids, withanaloides and alkaloids[20].[40]found thatrats administrated with equal concentration of the active constitutesof this plant, namely, SitoindosidesVII-Xa and Withaferin A led to increase activity of superoxide dismutase, catalase and glutathione peroxidase in the brain. Caper is one of the most widely dispersed plants in nature and is the common name of the Capparis-spgenus that belongs to the Capparaceae family.The type located in Iraq is *Capparis spinose* L (*Cs*) [5].It is of high nutritional value and is used in treating many diseases [32]. In view of the fact that it contains many effective compounds of biological efficacy [39] including Glucosinolates, phenols, Tocopherols and Carotenoides, which are known for their antioxidant and antimicrobial efficacy [18, 34]. Plant flower buds have been taken care of more than the rest of the parts, so the fruits of the plants are very important in studying their biological efficacy.

Due to the scarcity of studies that deal with the effect of treating broiler chickens with fruits of caper as feed additive and for its importance as food and as an antioxidant, in addition to, the importance of

*Withaniasomnifera*as adaptogens herbs,thus, this study is conducted to testing of the efficacy of the individualand the combinationaddition of both the fruits of the *Capparis spinose*,and theleaves of*Withaniasomnifera*to broilers diet to alleviate heat stress by studying their effect on some of the blood parameters.

II. Materials And Methods

2.1Birds' management

The trial was conducted under high environmental temperature at poultry farm in a wood shavings floor pins house. The average house temperature along the treating period (21-42 day of age)was 29-35◦C. A total of one hundred and, eighty, dayold, un sexed Ross 308 broiler chicks were reared together from 1-21 day of age, then, they were randomly distributed into 4 treated groups with 3 replicates per each (15 chicks/replicate).Chicks were fed *ad libitum* from 1-21 day of age on basal diet (starter diet) containing 23% crude protein and 3027 kcal Metabolizabl energy (ME)/ kg diet, then on grower basal diet (22-42 day of age) containing 20% crude protein and 3195.3 kcal ME/kg diet (Table 1). The diet was formulated according to [26]. An artificial lighting regime was used to provide 23L: 1D/day along the experimental period. The birds were vaccinated against Newcastle disease (ND) on the first and 11th day of age (B1 strain),ND (Lasota strain) at 29 and 32 days of age and Gumboro disease at 20 days of age.

2.3The experimental plants and treatments

Capparis spinosefruits and Withaniasomnifera leaves was collected from the westerner part of Baghdad (Abu Ghraib). The fruits and leaves were washed using tab water,then, air dried in shades, after that, crashed by an electric grinder on need. The experimental treatments were as follows: T0=control group without any adition,T1= adding 2g dry Capparis spinosefruits/kg diet, T2=adding 2g dry Withaniasomniferaleaves/kg diet and T3=adding a combination of 1g dry Capparis spinosefruits+ 1g dry Withaniasomnifera leaves/kg diet.

Table 1. Ingredients and chemical calculations of the basal diet.

Ingredients	Starter %	Grower%
Yellow corn	30	40
Wheat	28.25	24
Soybean meal (48%)	31.75	24.8
Protein concentrate	5	5
Vegetable oil	2.9	4.4
Limestone	0.9	0.6
Dicalcium phosphate	0.7	0.9
Salt	0.3	0.1
Premix	0.2	0.2
Total	100	100
Chemical calculated analysis*		
Crude protein	23	20
ME (kcal/kg diet)	3027	3195.3
Lysine	1.2	1.1
Methionine	0.49	0.46
Cysteine	0.36	0.32
Methionine+cysteine	0.85	0.78
Calcium	0.85	0.79
Available phosphorus	0.45	0.49
C/P ratio	131.61	159.77

III. *According To NRC [26]

3.1 Blood collection and tests

At the end of experiment (42 days of age) blood was obtained from the brachial vein (three birds/replicate) and immediately collected into two sterile tubes, the first one was with anticoagulant in order to estimate packed cell volume (PCV), hemoglobin (Hb) concentration and the total count of white blood cells (WBC), while the other tube was without anticoagulant to obtain serum by centrifugation it at 4000 rpm for 10 minutes, then serum kept in – 20◦C until be used to estimate the biochemical tests.

Serum biochemical tests were estimated by following the steps illustrated in the commercial kits: total protein, albumin, glucose,bilirubin, uric acid, urea, creatinine, total cholesterol,triglycerides,high density lipoprotein (HDL-cholesterol), aspartate aminotransferase (AST), alanine aminotransferase (ALT)) and alkaline phosphatase (ALP).

LDL and VLDL were calculated as follows [15]:
VLDL= Triglycerides/5
LDL= Total cholesterol- (HDL+VLDL)
Globulin= Total protein- albumin

3.2 Statistical analysis

Statistical Analysis System (SAS) [31] was used to analyze data with a Completely Randomized Design (CRD). To test the significant differenceamong treatments, Duncan's multiple rang test [13] was used. Statistical significance was set at $p<0.05$ and $p<0.01$.

IV. Results And Discussion

Results showed that treating heat stressed broilers with *Cs* or *Ws* or their combination didn't affected significantly blood hematological parameters: PCV, hemoglobin (Hb) and the total count of white blood cells WBC (`Table 2).

Table 2. Effect of adding individual and combination ofmedicinal plantsto broiler diet on blood parameters

Parameters	Treatment groups				P-value
	T0	T1	T2	T3	
Hb(g/dL)	9.00±**0.58**	10.33±**0.88**	9.33±**0.88**	10.33±**0.33**	NS
PCV %	28.33±**1.20**	31.67±2.33	30.00±2.64	33.0**0**±1.0	NS
WBC count (10^3/Mm^3)	15.60±2.65	12.10±3.23	13.73±**2.70**	9.53±**0.63**	NS

NS: Non significant, **T0: control group, T1, T2 and T3:2gCs, 2gWs and1gCs+1gWs/kg diet respectively.**

Statistical analysis of data presented in Table 3 showed that there were insignificant differences between treatments in the levels of total protein, globulin and bilirubin. Serum albumin was increased significantly ($p<0.05$) in thecombination treatment (T3) in comparison with dietary *Cs* fruits treatment ((T1), from other hand, all supplemented groups (T1, T2 and T3) didn't differ significantly with control group. Serum albumin was increased significantly ($p<0.05$) in the combination treatment (T3) in comparison with dietary individual Cs fruits treatment ((T1), from other hand, all supplemented groups (T1, T2 and T3) didn't differ significantly with control.With respect to serum glucose, results showed that the combination treatment (T3) was not differ significantly with control, but it led to significant ($p<0.01$) increasing in its concentration as compared to the individual addition (T1 and T2). As well as, the combination treatment has led to significant ($p<0.05$) reduction in the value of the creatinine compared to other experimental treatments and this reduction was significant in comparison with *Ws* leaves group (T2) while all herbs supplemented groups didn't differ with control.

Table 3.Effect of adding individual and combination of medicinal plants to broiler diet on serum biochemical tests.

Parameters	Treatment groups				P-value
	T0	T1	T2	T3	
Total protein(g/dL)	2.233±0.41	1.43±0.10	1.7±0.079	2.2±0.233	NS
Albumin(g/dL)	1.07±0.16ab	0.75±0.05b	0.92±0.04ab	1.11±0.06a	0.05
globulin(g/dL)	1.16±0.25	0.68±0.06	0.87±0.02	1.11±0.16	NS
glucose(mg/dL)	210.00±3.21a	162.67±6.96c	183.33±4.37b	201.67±3.17a	0.01
Uric acid(mg/dL)	4.19±0.09a	3.22±0.33b	3.42±0.24ab	4.05±0.26ab	0.05
Urea(mg/dL)	2.94±0.14a	1.88±0.14b	2.33±0.38ab	2.27±0.19ab	0.05
Creatinine(mg/dL)	0.30±0.05ab	0.26±0.08ab	0.46±0.88a	0.20±0.05b	0.05
Bilirubun(mg/dL)	10.26±0.98	9.12±3.17	7.98±2.48	6.84±0.98	NS

[A,b,c]Mean(±standard error) in the same row with different letters are significantly different at $p<0.05$ and $p<0.01$,NS:Non-significant. T0: control group, T1, T2 and T3:2gCs, 2gWs and1gCs+1gWs/kg diet respectively.

From recent results, it can be clearly observed that adding a combination of *Cs* fruits and *Ws*leavesseems to has apositive effect on some blood biochemical parameters because it led to a significant increase in both albumin and glucose accompanied by significant reduction in the creatinine ascompared to the individual addition and this is could be considered as an evidence to the synergistic effect between the active constitutes presented in both herbs. Under heat stress condition, increasing the level of albumin in avian blood is important, because of its role in keeps blood volume and help birds to resist the factors that cause the change in body temperaturewhich is considered to be backed up in the abnormal cases that birds being exposed to it, in addition to, its important role in the transport of metal elements, vitamins and fatty acids, as well as the hormonal transport of thyroxin and corticosterone[2]. The increase in the albumin in recent study has been accompanied by a natural increase in the blood sugar (glucose), which means an improvement in the bird's body's response to stress.Body's needs of sugar as an energy especially the brain and the nervous system and this spare the birds from exposure to heatfatigue [33].The increase in blood glucose is due to the increase in glycogen decomposition as a result of the increase in hormone secretion that analyzedcollagen (epinephrine, norepinephrine and glucagon), in addition to the increase in the rate of sugar formation from non-carbonic sources in response to the increase in the secretion rate of corticosterone hormone from the adrenal cortex [16, 21]. Lowering the level of creatinine in current study is a reflection of the low protein catabolism, as exposing birds to different environmental stressors leads to rise creatininein birds'blood because of the increased rate of protein catabolism for the purpose of forming glucose from protein [23].

The individual and combinationaddition of experimental plants has led to significant ($p<0.05$) reduction in serum uric acid and urea in comparison to control, while these treatments didn't differ significantly among each other.

Uric acid is the main product of the protein catabolism, non-protein nitrogen and purine in birds [6]. Bird in the case of normal nitrogenic balance and acid-base balance will be secreted by 80% of total nitrogen as uric acid, 15% as ammonia and 1-10% as urea.The level of uric acid is increased in the blood of birds(hyperuricemia) when there is a severe tissue demolition [3] , one of the causes of this case is the exposure of birds to heat stress, thus, the significant decrease in uric acid and urea in our current experimentinbroilerssupplemented withCs fruits and *Ws* leaves either in individual or combination formis evidence of the good impact of the bioactive compounds in them to help the bird's body face heat stress, which appeared through reduced tissue catabolism, and, the greater role was referred to *Withania somnifera* leaves which led to a significant reduction in AST and ALT levels as well as, to the role of the plant combination in the AST reduction.Results concerning serum lipids profile are presented in Table 4.

Table 4.Effect of adding individual and combination of medicinal plants to broiler diet on serum lipids profile

Serum lipids	Treatment groups				p-value
	T0	T1	T2	T3	
Triglycerides	64.19±8.41	56.11±10.64	67.93±7.81	70.88±8.86	NS
Cholesterol	93.93±7.16a	70.97±5.79b	59.23±3.50b	69.48±2.22b	0.01
HDL-cholesterol	45.03±7.16	46.03±3.72	36.03±3.40	46.32±4.45	NS
LDL-cholesterol	36.05±3.94a	13.52±3.82b	9.68±0.34b	8.99±3.34b	0.01
VLDL	12.84±1.68	11.22±2.12	13.59±1.56	14.17±1.77	NS

[A,b,]Mean(**±standard error) in the same row with different letters are significantly different at p<0.05 and p<0.01,NS:Non-significant. T0: control group, T1, T2 and T3:2gCs, 2gWs and1gCs+1gWs/kg diet respectively.**

Individual and combination addition of the studied plants (*Cs* and *Ws*) had led to a significant reduction in the concentration of total cholesterol ($p<0.01$) and LDL-cholesterol ($p<0.05$) in broilers blood serum in compared to control, while, triglycerides, HDL-cholesterol and VLDL didn't affected significantly due to experimental treatmentsThe exposure of birds to high environmental temperatures leads to increase serum cholesterol due to the decline in thyroid gland activity. Thyroid gland is considered the most important gland with regard to control of cholesterol metabolism.Thyroid hormones in natural conditions acts on cholesterol formation and increase the liver's capability to excrete it in bile's[28,21].Another cause of high cholesterol at high environment temperature is; the increase in corticosterone excretion[14] and the high levels of epinephrine, norepinephrine and glucagon in the blood leading to hyperlipidemia due to glycolysis of fatty tissue [21].

Researches results have indicated that herbs are effective in reducing the risk of cardiovascular diseases through their effect on reducing the total cholesterol and triglycerides without affecting the concentration of HDL-cholesterol [17, 4]. This is the result that our current study reach it, which indicates that addingcaper fruits and *Withaniasomnifera* leaves has had effect in fat metabolism, this role may be through the role of some

effective biocompoundsin the inhibition of the activity of beta-hydroxy-beta-methylglutaryl Co enzyme (HMG-CoA) which is responsible for cholesterol biosynthesis in the liver [7,12] or the reason may be caused by increasing the rates of cholesterol degradation to yellow bile acids and neutral sterols.The results of recent study were supported by [1] who found that adding *Withaniasomnifera* leaves to broilers diet led to reduce the level of serum total cholesterol and malondialdehyde in liver while the levels of serum HDL, triglycerides and total protein didn't affected significantly and they concluded that adding herbs like *Withaniasomnifera* as natural antioxidant to broilers diet had positive effect in reducing lipid oxidation and minimize harmful effect of oxidation damage.It has been observed from the results illustrated in Table 5 that the addition of plants in their individual or combination form has reduced the level of AST, ALT enzymes in serum and the less significant reduction in AST was found in groups treated with *Ws* leaves (T2) followed by combination treatment (T3) in comparison to control which didn't differ significantly with group treated with caper fruits (T1) With regard to the level of ALT, results showed that adding*Ws*leaves (T2) had led to a significant reduction in it compared with control and combination groups, while it didn't differ significantly with the group treated with caper fruits.

The individual addition of each plants led to significant reduction in the value of serum ALP compared to control which didn't differ significantly with combination group (T3).

Researches has indicated that heat stress leads to oxidative stress [22], which means increasing the free radicals for reactive oxygen species (ROS), causes injury to cells through the occurrence of lipid peroxidation and the occurrence of oxidative damage to protein and DNA [24] and the mitochondria membranes are damaged in broilers skeletal muscle, resulting in a defect in the antioxidant defensive enzyme system in mitochondria and thus causing its reduced function [24]. AST, ALT is located in a high concentrations in the tissues of liver and kidney and released from it to blood serum after the tissue is destroyed [3]. Exposure to heat stress leads to increased AST and ALT in blood [27, 29],this increase is due to requirements for glucose generation from non-carbohydrate sources (amino acids), or these enzymes increase the range of amine group transport from alpha-type amino acids to ketone acids that are an important source of energyIncluded in the KBS cycle of power production in Mitochondria[38]. The secondary metabolites of plants (flavonoid and polyphenols) has the ability to free radical scavenging leading to modification of the enzyme system activity within the body through its interference with vital particles [10]),this effect will increase the antioxidant activity of increasing the levels of glutathione and catalase [35]. It will also protect the liver cells from damage and preserve the characteristics of its membrane, the most important of which are the selective permeability, leading to the non-infiltration and leakage of these enzymes (AST, ALT) from inside the cell to the outside and this mean reduces its blood level [8].

Table 5. Effect of adding individual and combination of medicinal plants to broiler diet on serum enzymes.

Serum enzymes	Treatment groups				p-value
	T0	T1	T2	T3	
AST(IU/L)	4.33±**0.66a**	3.00±**0.57ab**	1.00±**0.00c**	2.67±**0.33b**	0.01
ALT(IU/L)	6.33±**1.45a**	5.00±0.57ab	3.00±**0.57b**	6.00±**0.55a**	0.05
ALP(IU/L)	64.33±**6.33a**	37.67±1.75b	40.00±**5.29b**	53.00±**5.19ab**	0.01

[A,b,]Mean(**±standard error) in the same row with different letters are significantly different at p<0.05 and p<0.01,NS:Non-significant. T0: control group, T1, T2 and T3:2gCs, 2gWs and1gCs+1gWs/kg diet respectively.**

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