Investigation of the effect of tribulus terrestris extract on the main biochemical and haematological indices of the blood in guinea fowls (*Numida meleagris*)

ПРОУЧВАНЕ ВЛИЯНИЕТО НА СУХ ЕКСТРАКТ ОТ TRIBULUS TERRESTRIS ВЪРХУ ОСНОВНИ БИОХИМИЧНИ И ХЕМАТОЛОГИЧНИ ПОКАЗАТЕЛИ НА КРЪВТА ПРИ ТОКАЧКИ (*NUMIDA MELEAGRIS*)

Christo CHRISTEV[1], Matina NICKOLOVA[1], Dimo PENKOV[1], Rumiana IVANOVA[1], Desislava ABADJIEVA[2], Svetlana GRIGOROVA[2]

[1]Agricultural University, Mendeleev Str. 12, 40000 Plovdiv, Bulgaria

[2]Institute of Animal Science – Kostinbrod

ABSTRACT

The aim of the present research was to investigate the effect of Bulgarian additive Vemoherb-T (dry extract of the annual plant Tribulus terrestris – L), produced by Vemo 99 Ltd Company, Sofia on main biochemical characteristics and hematological parameters of the blood in guinea fowl (*Numida meleagris*). An experiment was carried out with 30 Pearl-gray Guinea fowl (32 weeks old), distributed in two groups – a control and an experimental, 12 female and 3 male each. All birds were fed the same mixture for breeder guinea fowl. Vemoherb-T was supplemented to the compound feed of the experimental group in a daily dose of 10 mg/kg body weight for a period of 12 weeks.

The tested product decreased significantly the levels of total triglycerides ($P < 0.05$), total cholesterol ($P < 0.01$) and glucose ($P < 0.01$; $P < 0.001$ in male and female birds respectively) in the blood serum. It was established significantly higher total protein- (($P < 0.001$) and calcium ($P<0.01$) values in the blood serum of the treated birds.

The addition of Vemoherb-T increased significantly hemoglobin level, the number of erythrocytes and leukocytes and decreased the number of eosinophils in guinea fowl from the both sexes.

KEYWORDS: guinea fowl, Tribulus terrestris, biochemical characteristics,

hematological parameters of blood

РЕЗЮМЕ

Целта на настоящото изследване беше да се установи влиянието на Българския продукт Vemoherb-T (сух екстракт от едногодишното растение Tribulus terrestris-L), произведен от фирма Вемо 99 ООД, София, върху основни биохимични характеристики и хематологични показатели на кръвен серум при токачки (*Numida meleagris*). Беше проведен научен експеримент с 30 Бисерносиви токачки (на 32 седмична възраст), разпределени в две групи – опитна и контролна, 12 женски и 3 мъжки всяка. Всички птици получаваха една и съща смеска за разплодни токачки. Vemoherb-T беше добавян към комбинирания фураж на опитната група ежедневно в доза 10 mg/kg жива маса в продължение на 12 седмици.

Изпитваният продукт понижава достоверно нивата на общите триглицериди ($P < 0.05$), общия холестерол ($P < 0.01$) и глюкозата ($P < 0.01$; $P < 0.001$ съответно за мъжките и женски птици) в кръвния серум. Бяха установени достоверно по-високи стойности на общия протеин (($P < 0.001$) и на калция ($P < 0.01$) в кръвния серум на третираните птици.

Добавката на Vemoherb-T повишава достоверно нивото на хемоглобина, броя на еритроцитите и левкоцитите, и понижава броя на еозинофилните левкоцити при токачките и от двата пола.

КЛЮЧОВИ ДУМИ: токачки, Tribulus terrestris, биохимични характеристики, хематологични показатели на кръвта

ПОДРОБНО РЕЗЮМЕ

През последните десетилетия важна задача на фуражната индустрия в световен мащаб е съвместно с научно-изследователските колективи да търси алтернатива на нутритивните антибиотици и синтетичните хормони във фуражите за селскостопанските животни и птици. Една от тези алтернативи са билковите екстракти, които са натурални продукти, не са токсични и влияят положително върху здравето, продуктивността и репродукцията на животните, особено в условията на стрес от различен характер. Фуражните добавки на билкова основа отговарят на съвременните изисквания за биологично земеделие и екологично чиста животинска продукция.

Една от тези алтернативи е едногодишното билково растение Tribulus terrestris L., представител на сем. Zygophyllaceae. Негови основни биологичноактивни субстанции са сапонините от фуростанолов тип протодиосцин и протограцилин. Приложението му при селскостопанските животни е свързано главно с

подобряване на репродуктивната функция.

Целта на настоящото изследване беше да се установи влиянието на Българския адитив Vemoherb-T (сух екстракт от едногодишното растение *Tribulus terrestris-L*), произведен от фирма Вемо 99 ООД, София, върху основни биохимични характеристики и хематологични показатели на кръвен серум при токачки (*Numida meleagris*).

Настоящото проучване беше проведено в Учебно-експерименталната база на катедра Животновъдни науки при Аграрен университет – Пловдив с 30 Бисерносиви токачки (на 32 седмична възраст), разпределени в две групи – опитна и контролна, 12 женски и 3 мъжки всяка. Птиците бяха отглеждани върху дълбока несменяема постеля в боксове с дворчета волиерен тип. Опитът продължи 13 седмици - 1 седмица подготвителен и 12 седмици опитен период. Всички птици получаваха една и съща смеска за разплодни токачки. Vemoherb-T беше добавян към комбинирания фураж на опитната група, ежедневно в доза 10 mg/kg жива маса в продължение на 12 седмици.

В края на опитния период беше взета кръв от всички птици от vena cutanea ulnaris сутрин преди залагане на фуража. Биохимичните показатели в кръвния серум (триглицериди, общ холестерол, глюкоза, общ протеин, калций) бяха определени с помощта на биохимичен анализатор Pentra 400. Червените и бели кръвни клетки, еритроцитните индекси (MCH-средно съдържание на хемоглобин в 1 еритроцит и MCHC-средна концентрация на хемоглобин в еритроцитите) бяха определени по Ангелов и др., 1998; концентрацията на хемоглобина - по цианметхемоглобиновия метод, а еозинофилите - по метода на Dunker.

Изпитваната фуражна добавка понижава достоверно съдържанието на общите триглицериди ($P < 0.05$); общия холестерол ($P < 0.01$) и глюкозата ($P < 0.01$; $P < 0.001$ съответно за мъжките и женски птици) в кръвния серум. Бяха установени достоверно по-високи стойности на общия протеин ($P < 0.001$) и на калция ($P < 0.01$) в кръвния серум на токачките от опитната група.

Добавката на Vemoherb-T повишава достоверно нивото на хемоглобина, броя на еритроцитите и левкоцитите, и понижава броя на еозинофилните левкоцити при токачките и от двата пола.

INTRODUCTION

Extensive use of antibiotics results in residual medication of poultry products as well as bacterial antibiotic resistance and tolerance. This situation represents a considerable risk to human health [11]. The banning of the use of nutritive antibiotics has accelerated and led to investigations of alternative feed additives in poultry production [15]. Plant extracts can play a role in supporting both performance and health status of the poultry. Beneficial effect of herbal extract or active substances in poultry nutrition may include the stimulation of appetite and feed intake, the

improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial antiviral antioxidant and antihelminthic actions. They are also seen to have a great potential in practical application.

One of the most popular phytoproducts is the extract of the flowering annual herb Tribulus terrestris Linn. (*Zygophilaceae*). This extract is harmless for humans and animals and contains biologically active substances as saponins, flavonoids, [17,20], glycosides, phytosterols [22], alkaloids and other constituents [23]. Its main active components are saponins of the furostanol type, termed protodioscin [10]. Figure 1 presents the mechanism of protodioscin action. Flavonoids have anti-inflammatory effect and improve the overall physiological status of the animals. Tannins influence gastrointestinal microflora and exert astringent effect due to their antibacterial properties [19]. Our earlier investigations have shown that dry extract of Tribulus terrestris increase significantly calcium level in the blood serum in broilers parent [7]; decrease significantly glucose blood level in laying hens [8]; decline total serum cholesterol in laying hens and broiler parents [7,8]. This changes are related with increased growth rate and egg performance. On the other hand it is well known that growth performance is closely related with poultry health status. There are no data concerning the effect of Tribulus terrestris extract on blood biochemical parameters in other poultry species.

The objective of this research was to investigate how the addition of Bulgarian dry extract of Tribulus terrestris commercially known as Vemoherb-T produced by Vemo 99 Ltd Company, Sofia changes main biochemical characteristics and hematological

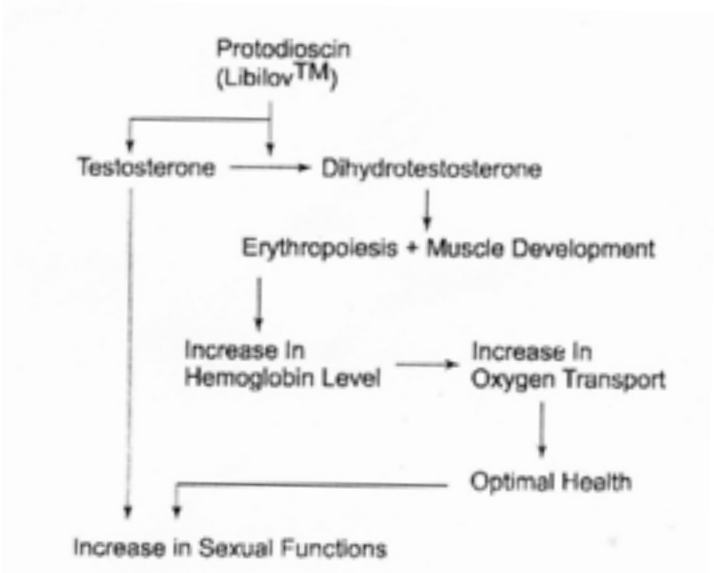

Figure 1. Mechanism of protodioscin action
Фигура 1. Механизъм на протодиосциновото действие

parameters of the blood in guinea fowl (*Numida meleagris*).

MATERIAL AND METHODS

The used in the current study herbal product is standardized and contains (in percent of dry matter): not less than 60% saponins of furostanol type defined as protodioscin; not less than 10% tannins; not less than 10% flavonoids determined as rutin. The heavy metals content of Vemoherb-T is ≤ 0.001%.

The present investigation was conducted in the period April – June, 2008 in the Experimental base of Agricultural University, Plovdiv, Bulgaria with 30 Pearl gray guinea fowl (32 weeks old), raised under extensive conventional method of production on a deep litter pen [13]. The birds were randomly distributed in two groups – a control and an experimental, 12 female and 3 male each. The trial duration was 13 weeks – one week adaptation and 12 weeks experimental period. All birds were fed the same mixture for breeder guinea fowl (Table 1). The nutritive value

Table 1 Ingredients and chemical composition of compound feed for breeder Guinea fowl
Таблица 1. Компонентен и химичен състав на комбинирания фураж за разплодни токачки

Ingredients %	%
Maize	64.10
Soybean meal, solvent 44% CP	22.00
Sunflower meal	5.00
D-C-P (Dicalcium Phosphate)	1.60
Limestone	6.20
Salt	0.20
Vitamin Premix	0.50
Mineral premix	0.20
DL-Methionine	0.20
TOTAL	100.0
Chemical composition	%
Metabolizable energy, MJ/kg	11.80
Crude protein	17.77
Crude fat	4.21
Crude fiber	5.41
Lysine	0.90
Methionine + Cystine	0.82
Calcium	2.75
Phosphorus, available	0.43

of the diet was determined by traditional Weende analysis. The metabolizable energy was calculated according to WPSA, 1989 [21]. Vemoherb-T was supplemented to the compound feed of the experimental group in a daily dose of 10mg/kg body weight for a period of 12 weeks.

The blood for this study was taken from vena cutanea ulnaris at the end of the trial, morning before feeding. The biochemical parameters in the blood serum (triglycerides, total cholesterol, glucose, total protein and calcium) were measured with Pentra 400 biochemical analyzer. The red and white blood cells, the erythrocyte indices (mean corpuscular haemoglobin or MCH and mean corpuscular hemoglobin concentration or MCHC) were measured by Angelov et al., 1998 [2]. The hemoglobin concentration was determined by the cyanmethemoglobin method, the eosinophils – by the method of Dunker, described by Christev, 2007 [5].

Statistical analysis of obtained results was performed by Excel 2000, single factor, Anova program. Values are expressed as means ± SEM.

RESULTS AND DISCUSSION

The triglycerides, total cholesterol, glucose, total protein and calcium values measured in the blood serum of guinea fowl are shown in Table 2. The levels of total triglycerides and total cholesterol in the blood serum of the treated birds were significant lower ($P < 0.05$; $P < 0.01$ in male and female birds respectively) in comparison with the control group. Similar date reported other authors in mice [6], in bulls [14] and in poultry [7,8]. The Tribulus terrestris mode of action might be explained with the enhancement of testosterone level, witch has a proved cholesterol decline effect [12]. The cholesterol, witch synthesis is taken principally in the liver is a precursor of all steroid hormones including the sexual hormones.

The serum glucose value in treated group was significant lower ($P < 0.01$; $P < 0.001$ in male and female birds respectively) compared to the control birds. Analogical results have been obtained by Grigorova et al. [8] in hens given10 mg/kg body weight/day Vemoherb T for a period of 12 weeks. The extract of Tribulus terrestris reduces the serum glucose level perhaps by inhibiting gluconeogenesis [1,9].

It was found a significant enhancement of the serum total protein content in male and female guinea fowl from experimental group ($P < 0.001$) in relation to the control group. The established increase of serum total protein level in treated guinea fowl was in agreement with the data of Petkova et al. [14], who observed higher serum protein value in bulls fed Vemoherb-T supplemented diet in dose 3 mg/kg body weight/day for 40 days. Probably the increased level of this parameter is due to the main effect of protodioscin contained in Tribulus terrestris extract, witch is expressed in improvement of vitality and metabolism [18].

The significant increase of calcium content in the blood serum of female guinea fowl from the treated group might be explained with significant higher egg productivity of

the treated group [13] as well as with the stimulating of calcium metabolism under the influence of the tested herbal extract. It is widely accepted that egg productivity correlates positively with blood estrogens level [4]. It is well known that estrogens modulate calcium metabolism [16]. About the level of calcium in the blood serum there is not difference between male guinea fowl from the both groups. Similar results are reported by Grigorova et al. [7] in broilers' parent given 10 mg/kg body weight/day Vemoherb-T to the diet.

Table 2. Levels of triglycerides, total cholesterol, glucose, total protein and calcium in blood serum of Guinea fowl
Таблица 2. Нива на триглицеридите, общия холестерол, глюкозата, общия протеин и калция при токачки

	CONTROL GROUPE		EXPERIMENTAL GROUPE	
INDICES	males	females	Males	females
Triglycerides, *mmol/L*	–	1.66± 0.03 *	–	1.54± 0.01 *
T. cholesterol, *mmol/L*	–	2.06±0.07 **	–	1.71±0.02 **
Glucose, *mmol/L*		9.69±0.20 ***	9.16±0.35 **	8.35±0.17 ***
Total protein, g/L		37.16±1.74 ***	70.2±2.80 ***	67.6±2.37 ***
Calcium, mmol/L	1.28±0.09	2.93±0.03 **	1.57±0.08	4.70±0.09 **

Significant in each row: * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$

The hematological parameters in the blood of guinea fowl from the control and experimental groups are given in Table 3. The hemoglobin level in the blood of experimental guinea fowl from both sexes was significantly higher ($P < 0.05$; $P < 0.01$ in male and female birds respectively) than those of the control group. Similar results were observed about number of erythrocytes ($P < 0.01$; $P < 0.001$ in male and female birds respectively), about MCH ($P < 0.001$) and MCHC ($P < 0.05$; $P < 0.01$ in male and female birds respectively).The positive effect of the tested product on erythropoiesis could be explained by the fact, that protodioscin in the Tribulus increases blood testosterone level and its transformation into dihydrotestosterone [3]. In turn dihydrotestosterone stimulates erythropoiesis, which leads to increase the

hemoglobin level (see Figure 1).

In the current research was established significant increase of the leukocytes number in the treated birds ($P < 0.06$; $P < 0.01$ in male and female birds respectively). The experimental group had significantly lower number of eosinophils ($P < 0.01$; $P < 0.001$) compared to the control group. The leukocytosis and eosinopenia are accepted as reliable indices of stress [5]. Therefore it could be suggested that the observed changes in leukocytes and eosinophils in female experimental birds due to their higher egg performance ($P < 0.05$) reported in our earlier investigation.

Bearing in mind that Tribulus terrestris extract has stimulating effect on the reproducibility, we assume that it improves metabolic interaction. Ultimately leads to the observed chances in our case.

Table 3. Main hematological indices in Guinea fowl from the both groups
Таблица 3. Основни кръвни показатели при токачките от двете групи

	CONTROL GROUP		EXPERIMENTAL GROUP	
INDICES	male	female	male	female
	132.00± .57	160.00±0.81		168.0±0.69
	*	**		**
	3.20±0.04	3.81±0.07	5.92±0.09	6.24±0.06
	**	***	**	***
	26.30±0.56	20.43±0.38		23.98±0.50
Leukocytes, ×10⁹/L	*	**		**
	77.61±1.93	69.35±1.08		56.78±0.71
	**	***		***
	41.25±0.94	42.0±0.51	23.2±0.78	26.9±0.56
MCH, pg	***	***	***	***
	257.8±11.15	300.0±13.18		
MCHC, g/L	*	**		

Significant in each row: * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$

CONCLUSIONS

The supplementation of 10mg/kg body weight/day Vemoherb T to the mixture of breeder guinea fowls for a period of 12 weeks decreased significantly the levels of total triglycerides ($P < 0.05$), total cholesterol ($P < 0.01$) and glucose ($P < 0.01$; $P < 0.001$ for male and female birds respectively) in the blood serum.

It was observed significant enhancement of the total protein- ($P < 0.001$) and calcium ($P < 0.01$) values in the blood serum of the treated birds.

The addition of the tested phytoproduct increased significantly hemoglobin level ($P < 0.05$; $P < 0.01$ for male and female birds respectively), the number of erythrocytes ($P < 0.01$; $P < 0.001$for male and female birds respectively) and leukocytes ($P < 0.05$; 0.01 for male and female birds respectively).

It was found, that the number of eosinophils decreased significantly in birds from the experimental group ($P < 0.05$; $P < 0.01$ for male and female guinea fowls respectively) in comparison to the control group.

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