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EFFECT OF TRIBULUS TERRESTRIS EXTRACT ON SEMEN QUALITY AND SERUM TOTAL CHOLESTEROL CONTENT IN WHITE PLYMOUTH ROCK-MINI COCKS**

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**Original Scientific paper

Abstract: Tribulus terrestris extract was added to the water of 10 cocks from the population White Plymouth Rock – mini cocks once daily in dose 10mg/kg body weight for a period of 11weeks. The trial lasted 20 weeks-1week preparatory and 19 weeks experimental period. Eight weeks of the experimental period were intended to measure the aftereffect of the tested product.

It was found, that Tribulus terrestris extract improves cocks semen quality : volume of ejaculate, spermatozoids concentration and motility, and shortened the time of methylen blue decolouration.

Serum total cholesterol content in the experimental cocks was 9,24% lower ($p>0,05$) in comparison with the control group.

The aftereffect of Tribulus terrestris on the studied parameters was maintained for 8 weeks.

Key words: Tribulus terrestris extract, cocks, semen quality, serum cholesterol content

Introduction

Tr.terrestris L.(Zygophylaceae), also called " Puncture vine", is a prostrate annual herb native of Mediterranean region, but widely distributed in warm regions of Europe, Asia,Amerika, Africa and Australia *(Frohne, 1999*). This plant has been used in the folk medicine in many countries including Bulgaria for different purposes, but is popularly claimed to improve the sexual function in humans. It contains biologically active substances as steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, etc. (*Adaikan et al.,* 2000). The main active components of this plant are saponins of

the furostanol type, termed protodioscin (R=glucose; rhamnose-2:1) (*Tomova,1987, Kostova and Dinchev, 2005).*

The clinical investigations carried out in Bulgaria and in other countries show, that Tr. Terrestris extract improves the reproductive functions in humans, rats and mice (*Tomova, 1987; Viktorov et al.,1994; Gauthaman et al.2002,2003; Teuscher and Lindequist,1994). Kistanova et al.(2005*) observed a positive effect of the extract from Tribulus terrestris (produser Vemo Ltd, Bulgaria) on the spermatogenesis of rams. *Adaikan et al*.(2000) studied the proerectile pharmacological effect of Tribulus terrestris extract on the rabbits corpus cavernosum and established stimulatory effect on the erection.

Tribulus terrestris extract is commonly used in the folk medicine also for control of blood pressure and cholesterol. There are reports showing that this extract decreases blood cholesterol level in humans, rats and mice (*Li et al., 2001, Chu et al., 2003*).

There exist no data concerning the effect of Tribulus terrestris extract in poultry. The aim of this study was to investigate the effect of Tribulus terrestris extract, added to the water of White Plymouth Rock – mini cocks on semen quality and serum total cholesterol content.

Material and Methods

An experiment was carried out in the Poultry Experimental base of the Institute of Animal Sciences-Kostinbrod with 20 cocks at the age of 23 weeks from the population White Plymouth Rock-mini. They were divided in two groups - control and experimental, 10 birds in each. The experiment lasted 20 weeks – 1 week preparatory and 19 weeks experimental period.

The cocks from the two groups were fed the same diet containing 17,60 crude protein and 2720 kcal/kg metabolizable energy (Table1). The extract from Tribulus terrestris (producer Vemo Ltd, Bulgaria) contained 55% furostanol saponins of which protodioscin is the dominant saponin. This extract was added to the drinking water of the experimental cocks in a dose of 10 mg/kg body weight daily for a period of 11 weeks at the beginning of the experimental period. The next 8 weeks of the experimental period were envisaged to measure the aftereffect of Tribulus terrestris.

Semen was collected once weekly during the first four weeks and once per 4 weeks during the rest period. The sperm was received by the massage method of *Burrows and Quin* (1938).The following parameters were evaluated; reaction to massage (positive and negative); ejaculating cocks (%), volume (ml), spermatozoids motility (balls) concentration of spermatozoids (mlrd/ml), time of methylen blue decoloration (min).

Table 1.Composition and nutritive value of the feed mixture of parents
Tabela 1. Sastav i hranljiva vrednost smeša za roditelje

Components / Sastojci	**Percent/procenat (%)**
Maize / Kukuruz	30,00
Wheat / Pšenica	29,80
Sunflower meal /Suncokretova sačma	14,00
Soybean meal / Sojina sačma	14,50
Sunflower oil / Suncokretovo ulje	2,00
Limestone /Krečnjak, kreda	7,55
D-C-P	1,60
Salt / so	0,20
Premix 3k / premiks 3k	0,20
L-lysine HCl /L-lizin HCl	0,05
DL- methionine/ DL-metionin	0,05
Enzyme/Enzim	0,05
Total/Ukupno:	**100.00**
Content/Sadržaj:	
Crude protein/Sirovi protein	17,60
Lysine/Lizin	0,70
Methionine + Cystine/Metionin+cistin	0,70
Calcium/Kalcijum	3,13
Phosphorus, available/Fosfor, dostupni	0,70
Metabolizable energy,kcal/kg/Metabolička energija, kcal/kg	2,72

Serum total cholesterol content was determined at the beginning, in the middle and in the end of the trial in both groups of cocks. The cholesterol content was determined by the method of *Watson* (1960)

Statistical estimation was conducted by Student t-test.

Results and Discussion

Table 2. Ejaculating cocks (%)
Tabela 2. Udeo petlića koji su imali ejakulaciju (%)

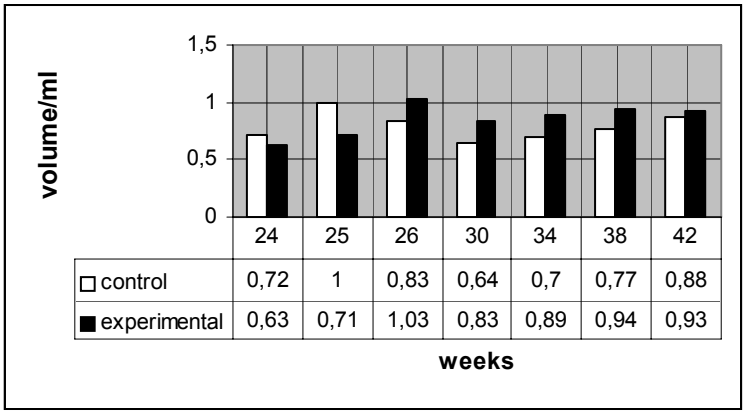
Groups/Grupe	**Weeks/Nedelje**						
	24	**25**	**26**	**30**	**34**	**38**	**42**
Control/kontrola	80.00	46.67	60.00	73.33	73.33	73.33	60.00
Experimental/ogled	86.66	53.33	46.67	66.66	80.00	86.60	73.33

The positive massage response in experimental and control cocks during the first test, carried out at the age of 23 weeks, was 53,3% and 73,3 % respectively. The observed differences to the massage – induced ejaculation

throughout the experiment could be due to the individual stress susceptibility of cocks to the handling (see table 2).

The age dynamics of the quantitative and qualitative characteristics of ejaculate in both groups was studied.

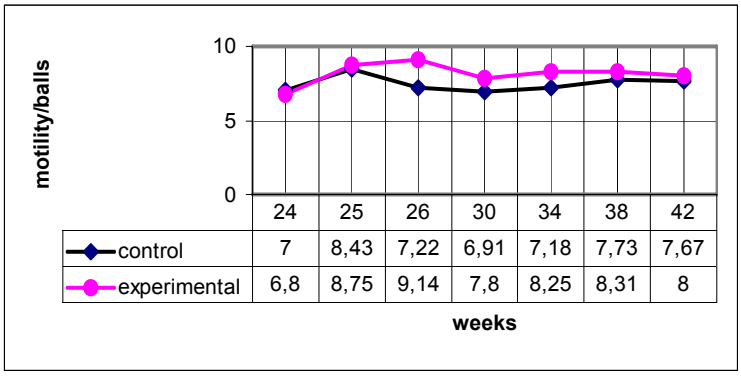
The most considerable effect of Tribulus terrestris extract was observed on the volume of ejaculate (Fig1). During the treatment of the experimental cocks (24-33 week of age) this parameter increased up to 29% in relation to the control group. Eight weeks after cessation of the treatment ejaculate volume was maintained higher in experimental cocks as compared to that in control cocks.



	24	25	26	30	34	38	42
control	0,72	1	0,83	0,64	0,7	0,77	0,88
experimental	0,63	0,71	1,03	0,83	0,89	0,94	0,93

Figure 1. Volume of ejaculate, ml
Slika 1. Zapremina ejakulata, ml

Sperm motility and concentration in the experimental group were higher ($p>0,05$) throughout the experiment (Fig.2 and Fig.3).



	24	25	26	30	34	38	42
control	7	8,43	7,22	6,91	7,18	7,73	7,67
experimental	6,8	8,75	9,14	7,8	8,25	8,31	8

Fig 2. Motility of sperms, balls
Slika 2. Pokretljivost sperme, kuglice

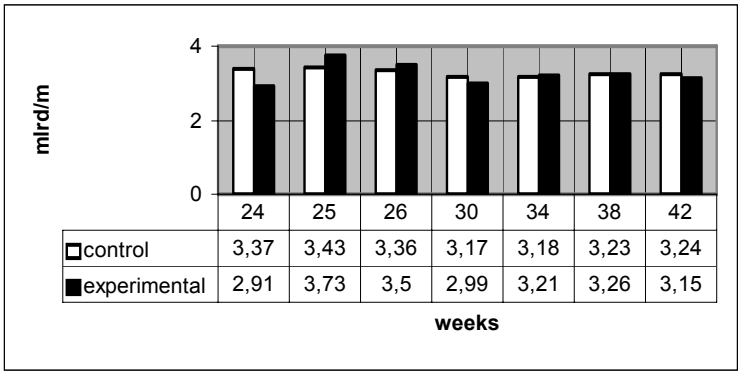


Figure 3. Concentration of sperms, mlrd/ml
Slika 3. Koncentracija sperme, mlrd/ml

The time of methylen blue decolouration is presented in Fig.4.This parameter shows the spermatozoids respiratory activity and depends on their motility and concentration. The experimental cocks, which had a higher spermatozoids concentration and motility were with a shorter time of methylen blue decolouration.

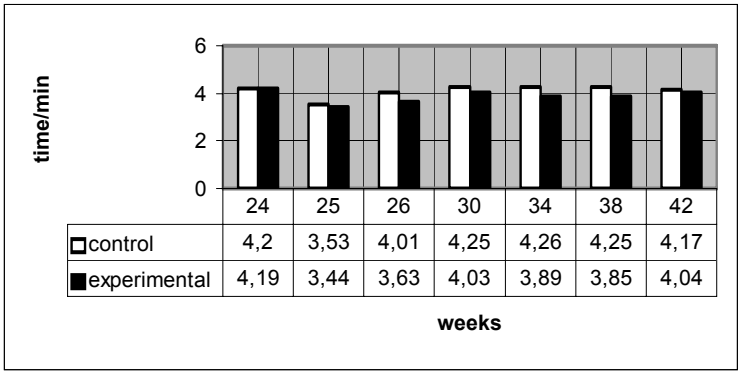


Fig.4. Time of methylen blue decoloration, min
Slika 4. Vreme metilen plave dekoloracije , min

The obtained results are in agreement with the data of *Kistanova et.al*.(2005), who observed an improvement of the semen quality in rams, given once daily 1,5g Tribulus terrestris extract per head for a period of 40 days.

Viktorov et al. (1994) established positive effect of Tribulus on the spermatogenesis in mice.

The positive effect of Tribulus terrestris on the reproductive system might be explained with the fact, that protodioscin in the Tribulus increases the level of luteinizing hormone, which in its turn stimulates testosterone secretion (*Tomova et al., 1981, Antonio et al., 2000).*

The data about serum total cholesterol content in the cocks of both groups are pointed in Table 3. The average value of cholesterol level within the experimental period was 9,24% lower (p>0,05) in experimental cocks than in control cocks. A great individual variability of this parameter among the cocks in the both groups was observed.

Table.3. Serum total cholesterol content in cocks (mg%)
Tabela3. Ukupni sadržaj holesterola u serumu petlića (mg%)

Stage/Faza	Control/Kontrola	Experimental/Ogled
At the start of the trial/ Početak ogleda	117.58 ± 9.44	108.94 ± 3.98
In the middle of the trial/ U sredini ogleda	88.00 ± 8.01	77.75 ± 5.12
In the end of the trial/ Na kraju ogleda	86.47 ± 7.30	78.93 ± 5.57

Chu et al. (2003) observed significant decline of serum total cholesterol level in mice, given Tribulus terrestris supplemented diet.

Similar results have been reported by *Li et al*. (2001) in mice given Tribulus terrestris decoction supplemented diet.

Conclusion

The addition of extract of Tribulus trrestris to the water of White Plymouth Rock–mini cocks had a positive effect on the quantitative and qualitative characteristics of their sperm. Tribulus terrestris increased ejaculate volume, spermatozoids motility, concentration and shortened the time of methylen blue decolouration.

Serum total cholesterol content in the experimental cocks was 9,24% lower (p>0,05) in comparison with the control group.

It is noteworthy that the aftereffect of Tribulus terrestris extract on the studied parameters was maintained for 8 weeks.

Uticaj ekstrakta *tribulus terrestrisa* na kvalitet semena i ukupni holesterol u serumu belih plimut rok-mini petlića

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Rezime

Izveden je ogled sa 20 petlića uzrasta od 23 nedelje iz populacije beli plimut rok – mini, podeljenih u dve grupe, kontrolnu I eksperimentalnu, sap o 10 ptica u svakoj grupi. Ogled je trajao 20 nedelja – 1 nedelja pripremna I 19 nedelja oglednog perioda. Tribulus terrestris ekstrakt je dodavan u vodu za ogledne pilića, jednom dnevno u dozi od 10mg/kg telesne mase u trajanju od 11 nedelja. U narednih 8 nedelja ogleda je ocenjivan uticaj testiranog proizvoda nakon završetka njegovog davanja.

Cilj ovog istraživanja je bio ispitivanje uticaja ekstrakta Tribulus terrestrisa u vodu na kvalitet semena i sadržaj ukupnog holesterola u serumu belih plimut rok – mini petlića.

Parametri semena i ukupnog holesterola u serumu petlića su ocenjivani. Sperma je sakupljana metodom masaže jednom nedeljno tokom prve četiri nedelje i jednom u 4 nedelje u preostalom periodu. Ispitivana je dinamika kvantitativnih i kvalitativnih karakteristika ejakulata u obe grupe petlića sa aspekta uzrasta.

Dodavanje ekstrakta Tribulus terrestris u vodu za napajanje petlića je imalo pozitivan uticaj na kvalitet sperme: povećana je zapremina ejakulata, koncentracija spermatozoida i njihova pokretljivost i skraćeno vreme metilen plave dekoloracije.

Sadržaj ukupnog holesterola u serumu kod oglednih petlića je bio za 9,24% niži ($p>0,05$) u poređenju sa kontrolnom grupom.

Uticaj Tribulus terrestris na ispitivane parametre se zadržao i 8 nedelja nakon prestanka njegove upotrebe.

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