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Blood biochemical changes and sperm quality in bulls fed diet supplemented with dry extract from *Tribulus terrestris*

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Introduction

Plant extracts have aroused much scientific interest over the past few years. Most of the studies have been performed on the extracts of herbs and used in the feed of different type and categories monogastrics and ruminants because of that many herb show prophylactic and therapeutic effects by regulating the functioning of the internal organs of animals. Bulgarian scientists have recently discovered the potential of a dry extract from annual herb *Tribulus terrestris L. (Zygophilaceae)* in animal nutrition as ecological additive to improve the production, reproductive functions and quality of products from animal origin, so as to get better their composition and the health in general. The folk medicine uses this herb in human as aphrodisiacs, diuretics and as a medicine decreasing blood pressure, cholesterol and glucose (Nikolov, 2007). *Tribulus terrestris* (TT) extract contains biologically active substances as steroids, saponins, flavonoids, alkaloids, tannins, vitamins, unsaturated fatty acids etc. (Asenov et al., 1998). The main active components are saponins of the furostanol type: protodioscin and protogracilin (Kostova and Dinchev, 2005).

Plant *Tribulus terrestris*

Bulgarian product has trade name *Vemoherb-T,* produced by firm Vemo 99 Ltd., Sofia, Bulgaria. Up to now, Bulgarian experimental works showed that Vemoherb-T has:

- Significant enhancement of production in rabbits (Valchev et al., 2008) and in poultry (Nikolova et al., 2009);

- Sure effect on reproductive capacity and sperm quality in rams (Kistanova et al., 2005), in White Plymouth Rock mini cocks (Grigorova et al., 2007, Kashamov, 2007);

- Significant enhancement of calcium blood level in broilers'parent (Grigorova et al., 2008) and Guinea fowls (Christev et al., 2009);

- Significant decrease of glucose blood level in poultry (Grigorova et al., 2008a, b; Christev et al., 2009);

- Sure effect on blood triglycerides and total cholesterol content in poultry (Grigorova et al., 2007, Grigorova et al., 2008 a, Christev et al., 2009).

Objectives

There exist no data concerning the effect of *Vemoherb-T* in bulls of service. The present study was conducted to asses the influence of this Bulgarian product on the biochemical changes and sperm both quality and quantity in bulls of service.

Material and methods

The used in this investigation 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% flavonoids determined as rutin; not less than 10% tannins. *Vemoherb-T* is harmless for humans and animals. His heavy metals content is ≤ 0.001%.

The experiment was carried out in the period December 2008 – February 2009 in the Station of Artificial Insemination, town Sliven (SAIS), Bulgaria with nine bulls of service from three different breeds: 5xBlack and White Breed (BWB), 2xBrown Breed (BB), 2 Bulgarian Rhodopean Breed (BRB) and different ages (4x3 years, 2x4years and 3x5year). The experiment lasted 129 days – 40 days preparatory, 40 days treatment with *Vemoherb-T* and 49 days after effect control. During the whole experiment all bulls were fed the same total mixed ration: 65% meadow hay and 35% compound feed (12% barley, 15% corn, 35% oat, 25.4% wheat bran, 8% sunflower meal, 1.6% chalk and 3% premix). The chemical composition of feedstuffs was determined in accordance with international standards AOAC (1990) and is presented in Table 1. *Vemoherb-T* was added to the bulls' compound feed once daily in dose 3mg/kg body weight for a period of 40 days.

Table 1
Chemical composition of experimental feeds for sires

Items	**Compound feed**	**Meadow hay**
Dry matter, kg	0,934	0,937
In % of DM		
Crude protein	16,68	5,95
Crude fats	2,71	0,45
Crude fiber	9,21	35,62
Crude ash	7,08	6,12

The blood hemoglobin content and hematocrit, as well as the serum levels of total protein, glucose, calcium, GOT, GPT, testosterone were determined in clinical laboratory Cibalab estimated one day before the treatment and at the end of treatment after 40 days. The blood was taken from *V. jugularis*, morning before feeding.
180 ejaculates were examined in the experiment: 77 during the preparatory period, 45 during the treatment and 58 during the after effect control. Semen was collected with an artificial vagina. Sperm parameters (volume, motility and concentration) were determined in SAIS laboratory.
Statistical analysis of obtained data was performed by Excel 2000, single factor, ANOVA program. Values are expressed as means ± SEM.

Results and Discussion

The total protein, glucose, calcium, GOT, GPT, testosterone in the blood, as well as hemoglobin and haematocrit values are pointed in Table 2. The authentic decrease (P <0.001) of blood glucose level established by us in bulls at the end of the *Vemoherb-T* treatment is analogous to the results reported by Amin (2006) in human and by Grigorova et al.(2008 a, b) in poultry. The hypoglycemic effect of the TT extract can be explained by glyconeogenesis depression (Kohli et al., 2004).

Table 2
Biochemical changes in the blood of sires (x*±SE)

Items	***- Vemoherb T***	***+ Vemoherb T***
Total protein, g/l	63,75 1,75	64,75 1,83
Relatively effect	**100,0**	**101,57**
Glucose, mmol/l	3,03 0,06	2,92 0,03***
Relatively effect	**100,0**	**96,37**
Ca, mmol/l	2.21	2,36
Relatively effect	**100,0**	**106,79**
GOT, U/l	39,35 0,18	40,29 0,42
Relatively effect	**100.0**	**102,39**
GPT, U/l	9,88 0,26	10,06 0,28
Relatively effect	**100,0**	**101,82**
Hemoglobin, g/L	111,08 3,93	132,75 5,02
Relatively effect	**100,0**	**119,50**
Hematocrit, L/L	0,139 0,02	0,164 0,01
Relatively effect	**100,0**	**117,98**
Testosterone, nmol/l	52,84 3,02	76,58 5,30**
Relatively effect	**100,0**	**145,34**

*every x is average from 9 estimations; ** P< 0.01; *** P <0.001

The blood testosterone level in bulls was significant higher (P< 0.01) at the end of *Vemoherb-T* treatment in relation to the first investigation (a day for the treatment).This

result is in accordance with the experiment published by Kashamov (2007), who established 29% increase of serum testosterone in White Plymouth Rock – mini cocks, given *Vemoherb-T* in dose 10mg/kg body weight. This positive effect of the tested product might be explained by the fact, that the main active substance of TT - protodioscin - increase blood testosterone level and undergo changes in organism to dihydrotestosterone and its sulfate ester. The dihydrotestosterone stimulate erythropoiese, which increase hemoglobin level (Barcelo et al, 2007). Similarly can be explained the observed significant higher ($P<0.05$) hemoglobin level in bulls at the end of the TT treatment in the present study.

Table 3 shows the change of blood lipid profile during the experiment. It was found a statistical decline of triglycerides ($P< 0.05$) and LDL cholesterol ($P < 0.05$), and total lipids ($P > 0.05$), but trend of increase of HDL cholesterol. Similar results have been reported by Nikolova and Stanislavov (2000) in men, Chu et al. (2003) in mice, Nikolov (2007) in rabbits. The TT mode of action on the lipid metabolism might be explained with the increase of testosterone level, which has a proved cholesterol decline effect (Malkin et al., 2004). The cholesterol, which synthesis is taken principally in the liver, is a precursor of all steroid hormones including the sexual hormones. *Tribulus terrestris* extract stimulate the liver functions and hormones synthesis from cholesterol (mostly LDL fraction). In this way decrease the total cholesterol level and LDL cholesterol level.

Table 3
Blood lipid profile in sires (mmol/l) (n=9) (x*± SE)

Items	- *Vemoherb T*		+ *Vemoherb T*	
HDL cholesterol	1,80	0,26	1,944	0,09
LDL cholesterol	1,20	0,11	0,872	0,08*
Triglycerides	0,22	0,02	0,17	0,006*
Total lipids	3,22	0,02	2,98	0,20

*P< 0.05

It was found a trend ($P > 0.05$) of increase of the blood total protein, calcium, GOT and GPT levels at the end of the treatment in relation to the preparatory period. Probably the increased values of the above parameters are due to the main effect of protodioscin contained in the TT, which is expressed in improvement of vitality and metabolism (Tomova, 1987). The observed values of the investigated by us indices are in physiological norms (Ibrishimov et al., 1995).

Tables 4, 5 and 6 reflect the data about bulls` individual values of ejaculate volume, sperm motility and concentration. By sperm investigation of the bulls the individual reaction was observed. About the volume of ejaculate (Table 4) this parameter was the highest in two bulls; in 5 bulls it was the highest during the post control period.

Table 4
Volume of ejaculate (ml), individual results

Bull's name and breed	n	Before treatment		n	Treatment		n	Post effect	
Monarch - BWB	9	4.67	0.17	5	4.60	0.24	7	5.14	0.26
Simba - BWB	9	5.00	0.24	5	4.60	0.67	7	4.70	0.29
Pompey - BWB	9	4.67	0.29b	5	4.60	0.40a	7	5.57	0.20ab
Socrat - BWB	9	4.56	0.18	5	4.40	0.24	6	4.50	0.34
Batemberg - BWB	9	4.22	0.22	5	4.80	1.32	7	4.29	0.42
Aron - BB	9	3.44	0.18	5	3.80	0.20	5	4.20	0.49
Zinedin - BB	9	4.89	0.36	5	4.20	0.49	7	5.86	0.73
King - BRB	9	4.60	0.24	5	4.40	0.40	7	4.86	0.14
Wiking - BRB	5	3.40	0.24	5	3.60	0.24	5	3.20	0.20

Significant: a, b – $P < 0.05$

Similar were the results concerning sperm motility (Table 5) and concentration of spermatozoids (Table 6). The obtained individual values of sperm both quality and quantity as well as summarized results (Fig. 1) in conditions of our experiment give a reason to make a conclusion, that the different breeds and ages used in the trial had no any influence on the effect of TT on semen quantity and quality. Kistanova et al. (2005) and Grigorova et al. (2007) observed an improvement of quantitative sperm parameters in rams and White Plymouth Rock – mini cocks respectively given *Vemoherb-T.* Unfortunately our results do not demonstrate convincingly and were not unidirectional in all bulls used in the trial regarding the influence of TT on research sperm indicators. Probably the different doses and types of animals could be the reason of these results.

Table 5
Motility of sperms (%), individual results

Bull's name and breed	n	Before treatment		n	Treatment		n	Post effect	
Monarch - BWB	9	78.89	1.11	5	80.00	0.00	7	77.10	1.84
Simba - BWB	9	76.67	1.67.	5	74.00	2.45	7	75.71	2.02
Pompey - BWB	9	73.33	1.67	5	72.00	2.00	7	71.43	1.43
Socrat - BWB	9	74.44	1.76	5	70.00	0.00	6	71.67	1.67
Batemberg - BWB	9	75.56	1.76	5	72.00	2.00	7	72.86	1.84
Aron - BB	9	70.00	0.00	5	70.00	0.00	5	72.00	2.00
Zinedin - BB	9	70.00	0.00	5	72.00	2.00	7	70.00	0.00
King - BRB	9	76.67	1.67a	5	70.00	0.00a,c	7	78.57	1.42c
Wiking - BRB	5	75.56	1.76	5	74.00	2.45.	5	77.14	1.84

Significant: a – $P < 0.05$; c – $P < 0.001$

Table 6
Concentration of spermatozoids (bill/ml), individual results

Bull's name and breed	n	Before treatment		n	Treatment		n	Post effect	
Monarch - BWB	9	1.478	0.014	5	1.500	0.032	7	1.486	0.014
Simba - BWB	9	1.411	0.020	5	1.280	0.010	7	1.414	0.026
Pompey - BWB	9	1.433	0.017	5	1.420	0.020	7	1.400	0.022
Socrat - BWB	9	1.416	0.012	5	1.400	0.000	6	1.400	0.000
Batemberg - BWB	9	1.444	0.018	5	1.300	0.010	7	1.321	0.067
Aron - BB	9	1.167	0.085	5	1.320	0.049	5	1.200	0.013
Zinedin - BB	9	1.433	0.017	5	1.480	0.032	7	1.457	0.030
King - BRB	9	1.356	0.060	5	1.340	0.112	7	1.443	0.043
Wiking - BRB	5	1.300	0.100	5	1.200	0.120	5	1.220	0.049

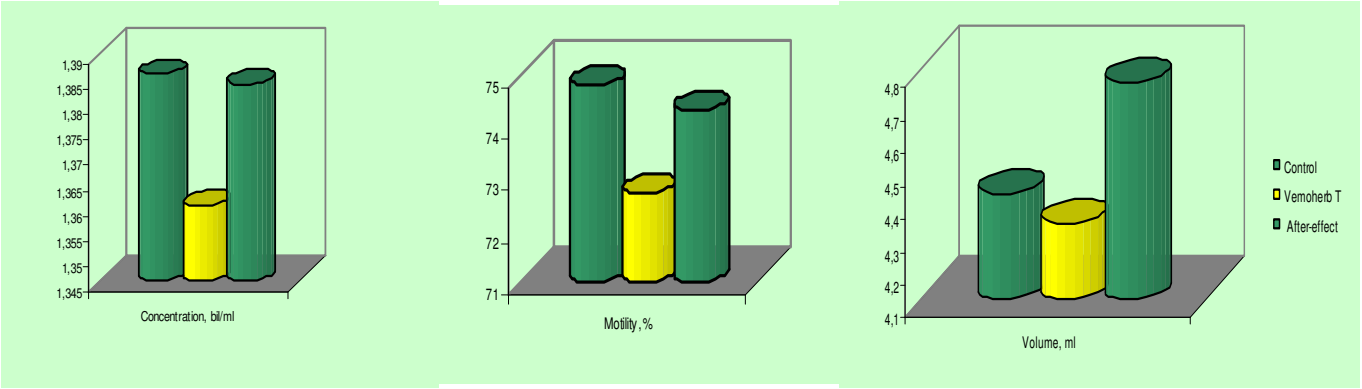

Fig. 1 Spermograms of the ejaculates from sires (summary results)

Conclusion

The obtained results are original on the biological effects of *Vemoherb-T* (dry extract of *Tribulus terrestris*) in amount 3mg/kg body weight with bulls of service.
The main effect is on the blood level of testosterone, which was increased by 45% ($P < 0.01$) under the influence of the tested product.
The blood lipid profile was positive reversed:

- LDL cholesterol was decreased by 27% ($P < 0.05$)
- Triglycerides were decreased by 23% ($P < 0.05$)
- HDL cholesterol was increased by 8% ($P > 0.05$)
- Total lipids were decreased by 6.9%

It was found the significant decrease ($P < 0.001$) of blood glucose.

The present investigation demonstrated that *Vemoherb T* is adjusted to use in bulls of service with sure benefit as a whole and gives opportunity to use greater amount from this additive with experimental aim.

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