# INFLUENCE OF TRIBULUS TERRESTRIS EXTRACT SUPPLEMENTATION ON LAYING PRODUCTIVITY AND EGGS QUALITY IN JAPANESE QUAILS

# ВЛИЯНИЕ НА ДОБАВКАТА НА ЕКСТРАКТ ОТ TRIBULUS TERRESTRIS ВЪРХУ НОСЛИВОСТТА И КАЧЕСТВОТО НА ЯЙЦАТА ПРИ ЯПОНСКИ ПЪДПЪДЪЦИ

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## ABSTRACT

The aim of the current work was to examine the influence of Bulgarian phytoproduct VemoHerb T (dry extract of Tribulus terrestris –TT) on laying productivity of Japanese quails (Coturnix coturnix japonica) and their egg morphological and sensor properties. A trial was organized with 52 female and 16 male Japanese quails from the breed Faraon at the age of 44 days randomly divided in four groups – control and three experimental groups, 13 female and 4 male each. All birds were fed ad libitum the same compound feed for Japanese quails. The trial lasted 10 weeks. The experimental groups received with the drink water the tested product in following daily doses: 4mg/kg body weight (10weeks); 10mg/kg body weight (the first 5 weeks of the trial); 10mg/kg body weight (10 weeks) for I$^{st}$, II$^{nd}$ , III$^{nd}$ experimental groups respectively.

The addition of TT-extract improved significantly the laying productivity. It was found significant higher values of egg weight, albumen - and yolk weight in quails from II$^{nd}$ and III$^{rd}$ experimental groups. There was a tendency to increase the egg shell weight and egg shell thickness in all treated groups in comparison to the control group. The use of VemoHerb T did not aggravate the sensor properties of the quails' eggs.

KEY WORDS: Tribulus terrestris, Japanese quails, egg capacity, egg quality

## РЕЗЮМЕ

Целта на настоящата работа беше да се проучи влиянието на Българския фитопродукт VemoHerb T (сух екстракт от Tribulus terrestris –TT) върху носливостта на японски пъдпъдъци, както и върху морфологичните и органолептични качества на яйцата им. Беше проведен опит с 52 женски и 16 мъжки Японски пъдпъдъци (Coturnix coturnix japonica) от породата Фараон на 44 дневна възраст, разпределени в четири групи-контролна и три опитни, по 13 женски и 4 мъжки всяка. Опитът продължи 10 седмици. Всички птици получаваха ad libitum един и същ комбиниран фураж за пъдпъдъци. Към питейната вода на опитните птици беше добавян ежедневно изпитвания продукт в следните дневни дози: 4mg/kg жива маса (10седмици); 10mg/kg жива маса (първите 5 седмици от опита); 10mg/kg жива маса (10 седмици) съответно за I$^{ва}$, II$^{ра}$ и III$^{та}$ опитни групи. Добавката на екстракт от TT подобрява достоверно носливостта на птиците. Установено беше статистически доказано нарастване на яйчната маса, масата на белтъка и жълтъка. Наблюдавана беше тенденция на увеличаване масата и дебелината на на черупката при птиците от всички опитни групи в сравнение с тези от контролната. Приложението на VemoHerb T не влошава вкусовите качества на яйцата.

КЛЮЧОВИ ДУМИ: Tribulus terrestris, японски пъдпъдъци, носливост, качество на яйцата

## ПОДРОБНО РЕЗЮМЕ

Съвременното животновъдство включва фитобиотиците като заместители на антибиотиците и други алопатични медицински препарати, което се дължи до голяма степен на опасенията свързани с бактериалната резистентност. Фитобиотиците са продукти от растителен произход и предизвикват все по-голям интерес сред изследователите и животновъдите. Такъв продукт е българският сух екстракт от едногодишното тревисто растение Tribulus terrestris с търговско наименование VemoHerb T, производство на фирма Vemo-99, ООД, София, България. Той е богат на биологично активни субстанции:фуростанолови сапонини (предимно протодиосцин и протограцилин), танини, флавоноиди, гликозиди и др. През периода октомври-декември 2008 година беше проведен научен експеримент с 52 женски и 16 мъжки полово зрели японски пъдпъдъци (Coturnix coturnix japonica) от породата Фараон с цел установяване на влиянието на VemoHerb T върху яйчната продуктивност, морфологичните и органолептични качества на яйцата. Птиците бяха отглеждани в мрежести едноетажни клетки и разпределени в четири групи - контролна и три опитни, по 13 женски и 4 мъжки всяка.Опитът продължи 10 седмици. Всички групи получаваха един и същ комбиниран фураж за разплодни пъдпъдъци. Към питейната вода на опитните групи беше добавян ежедневно изпитвания продукт в следните дневни дози: I$^{ва}$ опитна – 4mg/kg жива маса в продължение на 10 седмици; II$^{ра}$ опитна – 10 mg/kg жива маса през първите пет седмици на опита и III$^{та}$ опитна - 10mg/kg жива маса в продължение на 10 седмици. По време на опита беше контролирана ежедневно носливостта.В началото (II$^{ра}$ седмица), средата (V$^{та}$ седмица) и края (X$^{та}$ седмица)на опита на 50 яйца от група, събирани в продължение на няколко последователни дни бяха измерени: маса на яйцето, маса на жълтъка и белтъка, маса на черупката, индекс на формата¸ дебелина на черупката, Хаф единици и цвят на жълтъка ( по Roche). В края на опита беше проведена дегустация на 80 варени в продължение на 4min яйца (по 20 от група) от десетима души. Добавката на екстракт от ТТ подобрява достоверно носливостта на птиците. Не бяха констатирани статистически достоверни изменения на изследваните параметри при добавяне на 4mg/kg жива маса/ден от изпитвания продукт (I$^{ва}$ опитна група). Пъдпъдъците от II$^{ра}$ и III$^{та}$ опитна групи имаха статистически доказани по-висока носливост, яйчна маса, маса на белтъка и жълтъка в сравнение с тези от контролната и I$^{ва}$ опитна група. Наблюдавана беше тенденция на увеличаване масата и дебелината на черупката при птиците от всички опитни групи в сравнение с тези от контролната. Не бяха установени различия по отношение на показателите индекс на формата, единици на Хаф и цвят на жълтъка между яйцата на пъдпъдъците от опитните и контролната групи. Приложението на VemoHerb T не влошава вкусовите качества на яйцата.


## INTRODUCTION

The main research interest in the recent years accentuates on the ecological animal production [6,13,15] and organic technologies of animal raising [8,9,10]. The modern animal husbandry incorporated natural feed additives as substitute for nutritive antibiotics and other allopathic medicine largely due to concerns over bacterial resistance. Such additives are phytobiotics. They are products of plant origin added to the feed or to the drink water in order to improve performance, gut health and immunity [11]. To this group of products belongs Bulgarian dry extract of the annual herb Tribulus terrestris (Zygophyllaceae) commercially known as VemoHerb-T, produced by the firm "Vemo 99" Ltd, Sofia. It is made from the whole aerial plant's part harvested during the flowering period by extraction in water-ethanol mixtures. The TT extract is easy soluble in water and has strong bitter taste. The biologically active substances of VemoHerb T are: steroidal saponins of furostanol type determined as protodioscin (55 to 65%); dubbing substances, calculated as tanin (not more than 10%); flavonoids, determined as rutin (not more than 10%).The qualitative indices of this product are controlled in accordance with company documentation [17]. TT extract improves the reproductive functions [4,7], productivity [17,18] and health [5,14] in animals.Our earlier studies demonstrated positive effect of VemoherbT addition on the laying capacity and egg quality in hens [2,3] and Guinea fowls [12]. To our knowledge there are no information regarding the effect of TT supplementation on this parameters in quails.

The objective of the current study was to examined the influence of VemoHerb T supplementation on laying productivity in Japanese quails and on their eggs' morphological and sensor properties

## MATERIALS AND METHODS

The study described in the present paper was conducted in October-December 2008 in the Experimental base of the Agricultural University, Plovdiv, Bulgaria with 52 female and 16 male Japanese quails (44 days old) from the breed Faraon, kept in cages. The poultry are randomly divided into four groups: control and three experimental groups, 13 female and 4 male in each. The duration of

Table1 Composition and nutritive value of the compound feed for Japanese quail
Табл.1 Компонентен и химичен състав на комбинирания фураж за пъдпъдъци

| Ingredients | % |
|---|---|
| Maize | 62.0 |
| Soybean meal (44CP) | 20.0 |
| Fishmeal (60%CP) | 4.5 |
| Sunflower expeller (27%CP) | 5.0 |
| Limestone | 7.0 |
| D-C-P | 1.0 |
| Salt | 0.2 |
| L lysine | 0.2 |
| DL methionine | 0.1 |
| Total | 100% |
| Die diet contains: | % |
| Crude protein | 18.6 |
| Lysine | 0.115 |
| Methionine | 0.74 |
| Table Calcium | 3.12 |
| Phosphorus, total | 0.65 |
| Metabolizable energy, MJ/kg | 11.5 |

the trial was 10 weeks. All birds were fed ad libitum the same compound feed for Japanese quails (Table 1). The fodder nutritive value was determined by the Weende analysis. The experimental groups received VemoHerb T with the drink water in following daily doses: 4mg/kg body weight for a period of 10 weeks ($I^{st}$ group); 10mg/kg body weight during the first fife weeks of the trial ($II^{nd}$ group); 10mg/kg body weight for a period of 10 weeks ($III^{rd}$ groups).

Daily laying intensity and capacity were controlled. Daily laying intensity were calculated by the formula:

I = Nex100/Nd, where I is laying intensity, Ne – number of eggs/day, Nd – quails' number;

Fifty eggs from each group, laid within few consecutive days were taken at the start (at the beginning of $II^{nd}$ week), in the middle ($V^{th}$ week) and at the end (10 week) of the experiment and following measurement were made:

- The weight of the egg, yolk and egg shell with the shell membrane were measured with electronic scales OHAUS

2000 within 0.01g;

- The albumen weight was determined for greater precision in the following way: the sum of the shell weight and the yolk weight was deducted from the value of the egg weight;

- Form index was calculated by the formula: $I_{sh}$=d/Dx100, where d is the small egg diameter and D – the big egg diameter;

- Haugh unit was calculated by the formula: HU=100lg ($h + 7.17 – 1.7W^{0.37}$) using his method (1937), where h is the height of the thick albumen (in mm); W – the egg weight;

-The shell thickness (in mm) without the shell membrane was measured by a micrometer Amer 25EE;

Visually was measured the egg color (according to the 15 Roche Color Fan having 15 degrees scale) and the albumen color as well as the presence of any blood stains and other not typical inclusions in them.

At the end of the trial 80 eggs boiled for 4 min (20 from each group) were tasted by the ten people who were given sampling cards prepared in accordance with the requirements of BDS 4336-73 [1]. The taste and smell of the boiled eggs was determined while the eggs were warm.

Statistical analysis of the obtained results was performed using Software product Excel 7f. All values are presented as mean ± SEM.

## RESULTS AND DISCUSSION

Figure 1 presents the weekly average values of quails laying intensity from control and experimental groups. Laying intensity in $II^{nd}$ and $III^{rd}$ experimental group was higher during the $3^{rd}$ ($P<0.001$), $4^{th}$ ($P<0.001$), $7^{th}$ ($P<0.05$) and $8^{th}$ ($P<0.05$) week of the trial relatively to control and $I^{st}$ group. First and $III^{rd}$ group had significantly higher laying intensity at $6^{th}$ ($P<0.05$) and $9^{th}$ ($P<0.05$) week as compared to control group. During the last week of the experiment only $II^{nd}$ group surpassed significantly laying intensity of the control group. Peak laying intensity was lowest in control (85.71%) and $I^{st}$ group (86.90%) at $5^{th}$

Table2. Laying capacity and laying intensity of quails from the control and experimental groups
Таблица 2. Носливост и интензивност на снасяне на пъдпъдъците от контролната и опитна групи

| Parameters/Показатели | Control group Контролна група n=13 | Group Iexp. | Group II | Group III |
|---|---|---|---|---|
| Laying capacity, egg number/hen | 44.80±3.65 a1a2b1 | 49.76±1.90 b1b2 | 55.28±2.11 a1c1 | 57.92±2.83 a2b2c1 |
| Laying intensity (%) | 70.64±3.63 a1a2 | 71.62±3.08 a3b1 | 81.46±2.27 a1b1 | 82.47±2.64 a2a3 |

Significant in each row: a - p<0.001; b - p<0.01; c - p<0.05

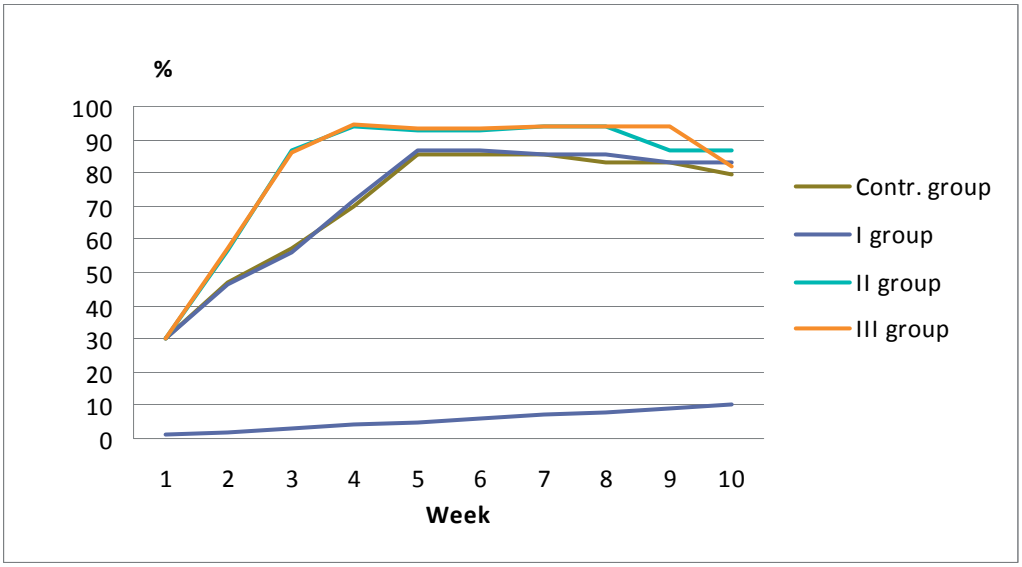


Figure 1. Laying intensity dynamics of quails from the control and experimental groups
Фигура 1. Динамика на носливостта на пъдпъдъците от контролната и опитна групи

week, and highest – in III$^{rd}$ group (94.51%) at 4$^{th}$ week. The most abrupt fall of laying intensity (11.91%) was observed in III$^{rd}$ group, whereas the laying intensity in the other groups declined gradually . The obtained in the III$^{rd}$ group sharp decrease of laying intensity at the end of the trial could be due to physiological fatigue of the ovaries by the supplemental TT extract. Analogical date reported other authors in hens (2) and Guinea fowl (12) given 10 mg/kg body weight/day VemoHerb T for a period of 12 weeks. Tribulus terrestris addition should not continue more than 6-7 weeks in laying poultry.

All treated groups had higher overall laying intensity (P<0.05; P<0.001; P<0.001 for I$^{st}$,II$^{nd}$ and III$^{rd}$ experimental group respectively) and higher average value of laying capacity (P<0.01; P<0.001; P<0.001 for I$^{st}$,II$^{nd}$,$^{IIIrd}$ treated group respectively) across the trial than in control group (Table2). The obtained results were in agreement with the date of Surdjiiska et al. (16) who observed a tendency to increase the laying intensity in hens fed TT supplemented diet in dose 10 mg/kg body weight /day for 3 weeks.

The egg morphological characteristics of the control and experimental groups can be found in Table 3. There were no differences about the egg weight between control and I$^{st}$ experimental group throughout the whole trial. This parameter was significantly higher in II$^{nd}$ and III$^{rd}$ group in comparison to control and I$^{st}$ group regarding all measurements. The values of albumen weight of the birds from II$^{nd}$ experimental group increased significantly to the control (P<0.001, P<0.001, P<0.01 at start, in the middle and at the end of the trial resp.) and to the I$^{st}$ group (P<0.001; P<0.001; P<0.05 during the I$^{st}$, II$^{nd}$, and III$^{rd}$ measurement respectively).The same parameter had similar change in birds from III$^{rd}$ experimental group. It was found the following significant enhancement of yolk weight: between control and II$^{nd}$ group (P<0.05, P<0.001

Table.3. Morphological characteristics of quails' eggs from the control and experimental groups
Таблица 3. Морфологична характеристика на яйца от пъдпъдъци от контролната и опитна групи

| Parameters | Egg weight, g | Shape index, % | Albumen weight, g | Haugh Units | Yolk weight, g | Yolk color Roche | Egg shell weight, g | Egg shell thickness, mm |
|---|---|---|---|---|---|---|---|---|
| Controll group | | | | | | | | |
| Start of experiment n=50 | 11.06±0.24 a1a2 | 78.23±1.36 | 5.83± 0.12a1a3 | 97.75±0.64 a1 | 3.74±0.09 a1c1 | 10.92±0.27 | 1.49±0.05 | 0.18±0.005 |
| Midle of experiment n=50 | 12.93±0.18 b1b2 | 78.69±0.98 | 6.53± 0.14 a2a4 | 98.99±1.53 c1 | 4.46±0.11 | 10.17±0.24 | 1.89±0.08 | 0.19±0.004 |
| Final of exsperiment n=50 | 13.26±0. 17 a3 | 77.63±0.76 | 6.75± 0.14 b1a5 | 103.1±1.15 a1c1 | 4.55±0.09 a2b1 | 8.77± 0. 41 | 1.96±0.12 | 0.21±0.01 |
| Exp.group I | | | | | | | | |
| Start of exsperiment n=50 | 11.22±0.17 a4a7 | 78.29±1.24 | 6.20± 0.12 a6b2 | 96.67±0.74 a2 | 3.49±0.07 a3a4 | 9.57± 0.27 | 1.53±0.05 | 0.21±0.003 |
| Midle of experiment n=50 | 13.09±0.18 a5c1 | 78.39±0.42 | 6.34± 0.13 a7a8 | 99.43±1.02 c1 | 4.36±0.11 c2 | 8.55± 0.15 | 1.90±0.07 | 0.22±0.06 a |
| Final of experiment n=50 | 13.24±0.44 a6a8 | 78.61±0.94 | 6.96± 0.11 c1b3 | 102.7±0.93 a2c1 | 4.26±0.15a5b2 | 8.75± 0.29 | 1.99±0.13 | 0.22±0.07 |
| Exp.group II | | | | | | | | |
| Start of exsperiment n=50 | 12.78±0.28 a1a4 | 77.40± 0.33 | 6.98± 0.16 a1a6 | 96.78± 0.44 a3b1 | 4.15± 0.13 a3c1 | 9.92± 0.40 | 1.65±0.20 | 0.23±0.003 |
| Midle of experiment n=50 | 13.83±0.22 a5b1 | 79.15±0.67 | 7.18± 0.13 a2a7 | 101.10±1.03 | 4.93± 0.12 c2 | 9.78± 0.41 | 1.92±0.08 | 0.24±0.009 |
| Final of experiment n=50 | 13.78±0.2 a6 | 78.59±0.67 | 7.33± 0.13 b1c1 | 103.84±0.88 a3b1 | 4.91± 0.08 a2b2 | 9.15± 0.39 c2 | 1.94±0.25 | 0.25±0.09 |
| Exp.group III | | | | | | | | |
| Start of exsperiment n=50 | 13.06±0.11 a2a7 | 78.15± 0.88 | 6.87± 0.16 a3b2 | 97.40± 0.67 a4 | 4.33 ± 0.12 a1 a4 | 10.40±0.35 | 1.66±0.04 | 0.23±0.002 |
| Midle of experiment n=50 | 13.93±0.23 b2c1 | 78.55± 0.83 | 7.41± 0.12a4a8 | 104.80±1.23 a5 | 4.65± 0.17 | 10.23± 0.21 a3 | 1.89±0.11 | 0.25±0.009 |
| Final of experiment n=50 | 14.32±0.27 a3a8 | 78.99± 0.91 | 7.53± 0.15 a5b3 | 104.14±0.97 a4a5 | 5.07± 0.13 a5b1 | 9.67± 0.41 c3 | 2.14±0.15 | 0.25±0.07 |

Significant in each column: a - p<0.001; b - p<0.01; c - p<0.05

during the I$^{st}$ and III$^{rd}$ measurement respectively); between I$^{st}$ and II$^{rd}$ group (P<0.001, P<0.05, P<0.01during the I$^{st}$, II$^{nd}$ and III$^{rd}$ measurement respectively); between control and III$^{rd}$ group (P<0.001, P<0.01 during the I$^{st}$ and III$^{rd}$ measurement respectively), between I$^{st}$ and III$^{rd}$ group (P<0.001 during I$^{st}$ and II$^{nd}$ measurement).There was a tendency to increase the egg shell weight and egg shell thickness in all treated groups in comparison to the control quails. Probably the increased values of the aforesaid egg morphological parameters in treated groups were due to the improve ovary activity and overall stimulation of vitality and metabolism (17) caused by protodioscin ( the main active components of TT, which increase the estrogens value). The use of TT extract did not chance significantly the form index, Haugh unit and yolk color. Our results about egg morphological characteristics were in accordance with the date obtained from other authors (2, 3) in hens received TT extract in dose 10mg/kg body

weight for a period of 12 and 8 weeks respectively.

The visual evaluation of the yolk and albumen color did not establish any deviations from the normal color of the quails' eggs in all groups. The eggs from all groups did not have any blood stains and other not typical inclusions.

Boiled egg sampling did not demonstrate any difference in the egg taste and odor. It is noteworthy that the strong bitter taste of the tested product was not transferred into the eggs following 10 weeks long addition. This was in agreement with the study of Grigorova and Kashamov (3) concerning table eggs from hens receiving VemoHerb T in dose 10 mg/kg body weight/day for a period of 8 weeks.

## CONCLUSIONS

The addition of 4mg/kg body weight /day VemoHerb T for a period of 10 weeks did not change significantly the studied parameters except the average value of laying capacity ($P<0.01$).

The supplementation of the tested product in a dose of 10mg/kg body weight/day for a period of 5 weeks (II$^{rd}$ experimental group) and 10 weeks (III$^{rd}$ experimental group) improved significantly the average value of laying intensity and capacity, egg weight, albumen and yolk weight.

There was a tendency to increase the egg shell weight and egg shell thickness in all treated groups in comparison to the control quails.

Neither group exhibited any deviations from the normal yolk- and albumen color of quails' eggs. The eggs from the all groups did not have any blood stains and other not typical inclusions.

The use of the tested product did not aggravate the sensor properties of quails' eggs.

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