

EFFECT OF DIETARY *TENEBRIO MOLITOR* MEAL ON
THE PRODUCTION PERFORMANCE AND EGG QUALITY
OF LAYING HENS

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Abstract

The study aimed to investigate the effect of the incorporation of *Tenebrio molitor* meal (TM) as partial replacement of soybean meal in the diet of layer hens on the production performance and quality characteristics of eggs. The trial included 90 layers at the age of 33 weeks allocated to 5 dietary groups. Each group consisted of 6 replicate cages with 3 layers per cage. The layers were fed a basal diet (C) and diets including TM in amounts of 1% (T1), 2% (T2), 3% (T3), and 4% (T4) for a period of 6 weeks. The results demonstrated different effect of the incorporation of TM in the layers' diet depending on the dose. After the first week of the trial, it was associated with significant reduction of the egg production and impaired feed conversion ratio in the birds fed 2% TM, which was also observed in T4 from the 4th till the 6th week of the experiment. The weight of the eggs and the albumen showed linear response to the inclusion of the TM in the diet, as both decreased with the increasing levels of the insect meal. The latter also affected the colour of the yolk, which was lighter in all the groups consuming TM in comparison to the control group.

Key words: *Tenebrio molitor* meal, layers, productive performance, egg quality

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Introduction. Globally, the poultry sector is one of the most rapidly growing livestock sectors. This is driven by the increasing demands for chicken meat and eggs being an affordable source of high-quality protein, vitamins and minerals, necessary for a healthy and balanced diet. To meet the growing demands, world poultry meat production escalated from 9 to 133 million tonnes between 1961 and 2020, and egg production has increased by 150 percent for the last three decades [1]. This growth in poultry production is having a profound impact on the demand for feed and raw materials, and the availability of low-priced, high-quality feeds is critical for poultry production to remain competitive and continue to grow and meet the demand for animal protein [2]. Providing balanced energy and high-quality protein is essential in poultry diets. Soybean meal has been the main preferred plant-based protein [3], however, soybean production is associated with significant negative impacts such as extensive deforestation and clearance of natural vegetation, as well as considerable water pollution due to intensive agricultural practices [4]. Insects are becoming popular as a soybean meal substitute, especially in poultry nutrition, due to their high protein content [5], as well as rapid and sustainable production [6]. *Tenebrio molitor* or yellow mealworm, is among the insect species that are widely produced in the world [7]. It has been incorporated in the poultry diets as a meal, and more recently also as live larvae [8]. The studies on its application are mainly focused on broilers, and although increasing in number, the experiments with laying hens still remain limited. So far, trials on the incorporation of *Tenebrio molitor* meal in the diet of laying hens did not demonstrate adverse effects on the egg quality or laying performance [9, 10]. Improvements in feed conversion and nutrient absorption have been reported [11], suggesting a possible enhancement of digestive efficiency [12]. The aim of the study was to evaluate the effect of practical application of TM in the diets of laying hens on the production performance and egg quality thus providing information about the possibilities for development of sustainable and economically viable poultry production.

Material and methods. Experimental birds and diets. The study was carried out in the experimental poultry facility of the Institute of Animal Science - Kostinbrod, Bulgaria. The trial involved a total of 90 ISA-Brown layers at the age of 33 weeks. The birds were randomly allocated to 5 dietary groups. Each group consisted of 6 replicate cages with 3 layers per cage. The birds were fed a basal diet (C) and diets including TM in amounts of 1% (T1), 2% (T2), 3% (T3), and 4% (T4). The composition of the diets is presented in Table 1.

The TM meal was supplied by VBF Pro Ltd., Petrich, Bulgaria. It was derived from larvae and its chemical composition was analyzed in the Laboratory for Feed Analysis at the Institute of Animal Science - Kostinbrod, according to the methods of AOAC [13]. The TM contained: crude protein 56.07%, moisture 5.77%, crude fat 24.16%, crude fibre 10.35%; crude ash 6.01%; nitrogen free extracts 4.51%; Ca 0.055%; P 0.663%.

T a b l e 1
Composition of the diets

Component, %	C	T1	T2	T3	T4
Wheat	48.79	49.64	50.50	51.09	51.83
Soybean meal	10.20	9.00	8.10	6.75	5.70
Maize	15.00	15.00	15.00	15.00	15.00
Sunflower meal	11.00	10.70	10.00	10.00	9.50
Limestone (coarse-grained)	5.30	5.30	5.30	5.30	5.30
Limestone	3.50	3.50	3.50	3.50	3.50
DDGS ¹	3.00	3.00	3.00	3.00	3.00
Sunflower oil	1.20	0.86	0.60	0.30	0.10
Dicalcium phosphate	0.30	0.30	0.30	0.30	0.30
Aminoacids	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.24	0.23	0.23	0.23	0.24
Salt	0.19	0.19	0.18	0.17	0.16
DL-Methionine	0.13	0.13	0.13	0.13	0.13
Sodium bicarbonate	0.12	0.12	0.13	0.15	0.16
Acidifier	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.07	0.07	0.07	0.07	0.07
Essential oils+tributyrin	0.05	0.05	0.05	0.05	0.05
Lysolecithin	0.05	0.05	0.05	0.05	0.05
Antioxidant	0.01	0.01	0.01	0.01	0.01
Vitamin mineral premix	0.50	0.50	0.50	0.50	0.50
TM	0.00	1.00	2.00	3.00	4.00
Chemical composition					
Crude protein, %	17.01	17.01	17.01	17.02	17.01
Crude fat, %	2.89	2.78	2.76	2.69	2.72
Crude fibre, %	4.02	4.05	4.02	4.09	4.09
Crude ash, %	12.06	12.04	12.01	12.04	12.01
Metabolizable energy, kcal/kg	2727.71	2753.16	2786.19	2809.27	2844.25

¹ Dried distillers grains with solubles
The diets contain 3.74% Ca and 0.44% P

The trial period lasted 6 weeks as the feed and water were provided ad libitum to the hens.

Production performance. During the trial, the mortality of the birds as well as the feed consumed and the eggs for each replicate were daily controlled. These parameters were further used to calculate the egg production, eggs per hen and feed conversion as follows:

Egg production, % = (Total number of eggs produced for the period / Number of hens × days of the period) × 100;

Eggs per hen = Total number of eggs for the period / Number of hens;

Feed conversion ratio, g/g = Feed intake, g/g eggs.

Egg quality traits. At the last day of the experiment the eggs from all the replicates were collected and subjected to analysis of the quality traits. Each egg was individually numbered and weighed using digital balance with accuracy of 0.01 g. Further the width (mm) and length (mm) of the egg were measured through digital caliper and used to calculate the egg shape index as: (width/length) $\times$ 100.

The albumen was characterized by measuring its weight and Haugh units. The Haugh units were calculated as described by EISEN et al. [14]: $HU = 100 \times \log (H + 7.6 - 1.7 W^{0.37})$, where H is the height of the thick albumen (mm), measured with a tripod micrometer, and W is the weight of the egg (g). The albumen weight was registered after careful separation from the yolk and shell on digital scales.

The yolk was evaluated by the yolk index, weight and colour. The yolk index was calculated as the ratio of the yolk height (mm), measured by the tripod micrometer to its diameter (mm), measured using a digital caliper. The yolk was then separated and weighed. The colour of the yolk was determined using Roche fan.

The shell weight was measured after washing and air-drying the shells for 48 hours at ambient temperature. Shell thickness was measured to the nearest 0.01 mm using a micrometer.

Statistical analysis. The statistical processing was performed using the JMP pro 17 software package [15]. The normality of distribution for the data was checked by Shapiro–Wilk test. The cage was the experimental unit for the growth performance and egg quality. Data were analysed through one-way ANOVA followed by post-hoc comparisons (Tukey HSD, $P < 0.05$) to assess the significance of the differences. Polynomial contrasts were used to assess the linear (L) and quadratic (Q) effects of the levels of TM in the diet of the layers on the examined performance and egg quality traits. Data was presented as Mean and Standard error of the mean (SEM).

Results and discussion. The incorporation of TM in the diet of the layers affected significantly the production performance (Table 2), as the effect was mostly observed after the first week of the trial period.

At the beginning of the experiment, the productive performance traits remained unaffected by the dietary inclusion of TM with exception of the Feed Conversion Ratio (FCR).

The egg production and the number of the eggs laid per hen showed quadratic response to the inclusion of the insect meal on Week 2 ($P = 0.0089$) and Week 3 ($P = 0.0139$) of the experiment. Significant differences were observed between the dietary groups, with T2 having the lowest egg production during these weeks (62.70% and 66.67%, respectively, for Week 2 and 3). The egg production and

the number of the eggs laid showed significant linear decrease in Week 4 ($P = 0.0003$), Week 5 ($P < 0.0001$) and Week 6 ($P = 0.0005$). During these weeks, the parameters remained consistently lower in T4 in comparison to the rest of the groups, while in Weeks 5 and 6 they showed marked decrease in T2 as well.

The weight of the eggs in this experiment was within the range 56.00–58.58 g (Table 2) for the group fed basal diet. It can be noted that the weight is lower than the average reported for ISA Brown at the respective age. This is due to the selected type of diet for maximum intake (AFI = 125 g/d/ bird). It has the lowest methionine content and is used for prevention of peritonitis and oviductal atony that is induced by stress. In addition, for the purpose of this experiment we have selected actively laying hens transferred to the cages that had maximal laying performance with smaller eggs to prevent the above-mentioned conditions. The egg weight remained unaffected during the first two weeks of the trial, however, linear decrease in this trait was observed in Week 3 ($P = 0.0338$), Week 4 ($P = 0.0002$), Week 5 ($P < 0.0001$), and Week 6 ($P = 0.0007$). The differences in the weight were negligible among the dietary groups during Week 3, however, it showed marked decrease in T4 during Week 4, 5 and 6, and T2 during the last week of the trial.

The feed intake of the birds was affected by the dietary incorporation of TM during Week 3 (linear, $P = 0.0029$) Week 4 (linear, $P = 0.0010$) and Week 5 of the experiment (linear, $P = 0.0005$). The feed intake differed significantly between the dietary groups during these weeks showing considerably lower values in T4 and also in T2 in Week 5.

The feed conversion ratio in the first week of the trial differed between the groups with higher values in group C. However, we observed significant impairment of this trait in T2 in Week 2 ($P = 0.0477$) and Week 3 (quadratic, $P = 0.0330$). During the last three weeks of the trial, we observed significant response of the FCR towards the dietary incorporation of TM. The values showed marked increase in Week 4 (linear, $P = 0.0041$) and Week 5 (linear, $P = 0.0051$) with highest values in T4. In the last week of the experiment, both T2 and T4 showed the highest values of FCR, indicating considerable impairment of this parameter.

The results of this experiment showed different effect of the dietary inclusion of TM on the production performance during the different weeks of the trial with certain disadvantages when included in amounts 2% and 4%. In an experiment with layers receiving TM in amounts 1%, 2%, and 4% over a period of 4 weeks, KADDOUR et al. [16] did not find significant impairment of the laying rate of the hens, however, it tended to drop in the group fed 4% TM. This partially agrees with our observations suggesting a possible threshold beyond which the egg production might be negatively impacted. In contrast with our results, no significant effect on the performance traits was observed in layers when fed up to 3% TM [10], or receiving 5% TM with or without turmeric supplement [17]. Furthermore, SEDGH-GOOYA et al. [11] reported significant improvement of the

T a b l e 2

Effect of the dietary TM incorporation on the production performance in layers

Trait	C	T1	T2	T3	T4	SEM	ANOVA	L	Q
Week 1									
Egg production, %	84.13	83.33	85.72	85.71	84.92	9.41	0.9898	0.7333	0.8649
Eggs per hen	5.88	5.83	6.00	5.99	5.94	0.66	0.9897	0.7316	0.8680
Egg weight, g	56.36	56.61	55.72	54.74	55.41	2.21	0.6032	0.1801	0.8198
Feed intake, g/d	119.68	100.00	103.49	112.34	107.22	13.46	0.1291	0.5063	0.1207
FCR, g/g	2.54 ^a	2.13 ^b	2.19 ^{ab}	2.38 ^{ab}	2.28 ^{ab}	0.210	0.0194	0.2232	0.0425
Week 2									
Egg production, %	90.48 ^a	85.72 ^{ab}	62.70 ^b	82.54 ^{ab}	88.10 ^a	13.66	0.0123	0.7117	0.0089
Eggs per hen	6.33 ^a	6.00 ^{ab}	4.39 ^b	5.78 ^{ab}	6.17 ^a	0.95	0.0124	0.7113	0.0089
Egg weight, g	56.70	57.02	54.61	55.29	54.37	2.89	0.4028	0.0876	0.8884
Feed intake, g/d	121.67	122.26	117.78	112.15	126.03	18.95	0.7618	0.9542	0.3790
FCR, g/g	2.38 ^a	2.58 ^a	3.90 ^b	2.48 ^a	2.66 ^a	0.91	0.0477	0.7376	0.0773
Week 3									
Egg production, %	95.24 ^a	84.92 ^a	66.67 ^b	88.89 ^a	82.54 ^{ab}	9.97	0.0007	0.2210	0.0139
Eggs per hen	6.67 ^a	5.94 ^a	4.67 ^b	6.22 ^a	5.78 ^{ab}	0.70	0.0007	0.2208	0.0139
Egg weight, g	57.15	56.13	56.10	54.61	54.88	2.26	0.3138	0.0338	0.7452
Feed intake, g/d	128.85 ^a	120.71 ^{abc}	123.26 ^{ab}	111.35 ^{bc}	107.86 ^c	12.57	0.0456	0.0029	0.7877
FCR, g/g	2.37 ^b	2.53 ^{ab}	3.54 ^a	2.30 ^b	2.40 ^b	0.59	0.0074	0.6722	0.0330

T a b l e 2
Continued

Trait	C	T1	T2	T3	T4	SEM	ANOVA	L	Q
Week 4									
Egg production, %	94.45 ^a	85.72 ^a	74.60 ^{ab}	84.13 ^a	57.14 ^b	13.38	0.0008	0.0003	0.4820
Eggs per hen	6.61 ^a	6.00 ^a	5.22 ^{ab}	5.89 ^a	4.00 ^b	0.94	0.0008	0.0003	0.4856
Egg weight, g	57.77 ^a	56.54 ^{ab}	56.59 ^a	54.07 ^{ab}	52.56 ^b	2.38	0.0051	0.0002	0.3842
Feed intake, g/d	125.48 ^a	107.50 ^{abc}	107.02 ^{bc}	115.52 ^{ab}	91.67 ^c	10.69	0.0002	0.0010	0.8882
FCR, g/g	2.29 ^a	2.34 ^{ab}	2.63 ^{ab}	2.55 ^{ab}	3.16 ^b	0.48	0.0359	0.0041	0.3117
Week 5									
Egg production, %	99.21 ^a	84.13 ^a	55.56 ^b	86.51 ^a	30.95 ^c	10.43	<0.0001	<0.0001	0.4730
Eggs per hen	6.94 ^a	5.89 ^a	3.89 ^b	6.06 ^a	2.17 ^c	0.73	<0.0001	<0.0001	0.4701
Egg weight, g	58.33 ^a	57.27 ^a	54.84 ^a	55.05 ^a	46.74 ^b	3.48	<0.0001	<0.0001	0.0410
Feed intake, g/d	123.81 ^a	116.31 ^a	89.01 ^b	107.26 ^{ab}	88.05 ^b	13.90	0.0003	0.0005	0.3728
FCR, g/g	2.14 ^b	2.42 ^b	3.18 ^b	2.23 ^b	7.86 ^a	2.56	0.0028	0.0051	0.0378
Week 6									
Egg production, %	99.21 ^a	80.95 ^a	34.92 ^b	81.75 ^a	35.71 ^b	15.31	<0.0001	0.0005	0.3313
Eggs per hen	6.94 ^a	5.67 ^a	2.44 ^b	5.72 ^a	2.50 ^b	1.07	<0.0001	0.0005	0.3317
Egg weight, g	58.58 ^a	56.49 ^{ab}	53.01 ^b	55.19 ^{ab}	53.00 ^b	2.35	0.0013	0.0007	0.1617
Feed intake, g/d	130.56	126.07	122.90	114.13	119.72	13.46	0.3019	0.0563	0.4760
FCR, g/g	2.25 ^b	2.96 ^b	8.60 ^a	2.53 ^b	9.19 ^a	4.59	0.0228	0.0478	0.9818

Means with different superscripts differ significantly ($P < 0.05$)

production performance in terms of egg production and feed conversion ratio in layers receiving 2.5% and 5% TM in the diet for 8 weeks. The authors, however, noted that the lower inclusion level was more effective. After evaluating the safety of TM in the diet of laying hens, STASTNIK et al. [12] found that the incorporation of the meal in proportion of 2% and 5% might be recommended. This was based on the lack of negative effect on the length of the villi and the microbiome of the caecum, as well as the highest digesta viscosity of the ileum in the group fed 5% TM, indicating a slower passage of the digestion through the digestive tract. The contrast of our results pointing to adverse effect of TM on the production traits of the layers with the previous experiments might be explained by the shorter duration of the trial period in our experiment, which is partially confirmed by the results of Kaddour et al. [16]. It can be suggested that if there is a negative impact of TM on some physiological processes affecting productive performance, it can be overcome after a certain period and in longer feeding trials.

The dietary incorporation of TM affected some of the quality characteristics of the eggs (Table 3). Linear response was observed in regard to the weight of the eggs ($P = 0.0336$) and albumen ($P = 0.0361$), that decreased with increasing the amount of TM. ANOVA revealed significant differences between the groups, with lower weights of the eggs and albumen in T2 and T4 in comparison to C. The index of the yolk also decreased linearly ($P < 0.0001$) with increasing the levels of dietary TM. Moreover, both T3 and T4 had significantly lower yolk index in comparison to C. The colour of the yolk was affected ($P < 0.0001$) and showed both linear ($P = 0.0014$) and quadratic ($P = 0.0019$) response to the TM treatment. The darkest colour of the yolk was registered in C, followed by T4, while T1, T2, and T3 had lighter yolks. The Haugh units remained unaffected by the TM in the diet.

Apart from the lower weight of the eggs and the corresponding lower weight of albumen in T2 and T4 there was no adverse effect of the dietary incorporation of TM on the egg quality. In line with our results, AIT-KAKI et al. [17] reported decreased egg weight in hens receiving 5% TM, however, the addition of turmeric powder (0.50%) to the meal increased the weight significantly. The quality of eggs was not adversely affected by the inclusion of TM either alone [11, 16] or in combination with probiotics [9]. The decreased index of the yolk found in the groups receiving higher levels of TM was not indicative of deterioration. Its values were within the range of 45.98–42.5%, and are well above the range indicating fresh and compact eggs [18].

The lighter colour of the yolk in the groups receiving TM observed in this study did not coincide with the results of KO et al. [10] reporting linear increase and darker yolk colour with increasing the percentage of dietary TM. In an experiment with free range hens, REMÓN et al. [19] did not find effect of TM meal on the yolk colour. The differences in the colour of the yolk might be due to the different kinds of pigments that accumulate and their proportion in the compo-

T a b l e 3
Effect of the dietary TM incorporation on the egg quality in layers

Trait	C	T1	T2	T3	T4	SEM	ANOVA	L	Q
Egg weight,g	59.07 ^a	57.23 ^{ab}	53.28 ^b	56.64 ^{ab}	53.66 ^b	4.31	0.0384	0.0336	0.1888
Egg shape index, %	76.53	77.90	77.25	77.92	77.36	3.78	0.8339	0.4199	0.5117
Albumen weight, g	37.76 ^a	36.31 ^{ab}	33.39 ^b	36.15 ^{ab}	33.49 ^b	3.10	0.0236	0.0361	0.2030
Yolk weight, g	15.46	14.96	13.86	14.95	14.17	1.34	0.1200	0.1834	0.0800
Yolk index, %	45.98 ^a	44.71 ^{ab}	43.77 ^{ab}	42.88 ^b	42.85 ^b	2.12	0.0012	<0.0001	0.0863
Shell weight, g	5.85	5.97	6.03	5.53	5.99	0.66	0.3266	0.1928	0.3054
Shell thickness, mm	0.42	0.43	0.43	0.41	0.46	0.03	0.0789	0.6338	0.6740
Haugh units	99.29	93.31	92.17	93.35	95.75	9.26	0.2822	0.1835	0.0515
Roche colour	11.47 ^a	8.92 ^b	9.33 ^b	8.94 ^b	9.75 ^{ab}	1.56	<0.0001	0.0014	0.0019

Means with different superscripts differ significantly ($P < 0.05$)

sition of the yolk [20], rather than impaired absorption of the pigments from the diet. ROMERO et al. [20] found significantly lighter colour of the yolk in hens fed BSF larvae meal, with significant content of carotenoids however predominantly containing zeaxanthin and lutein that were not associated with red hue.

Conclusion. The results of this study demonstrate different effect of the incorporation of *Tenebrio molitor* meal as a partial replacement of soybean meal in layers' diets depending on the dose. The obtained linear and quadratic responses indicate certain disadvantage of the insect meal in amounts 2% and 4% on the egg production for the period of 6 weeks. The increasing levels of TM were associated with decreased weight of eggs and albumen, as well as lighter colour of the yolk. Apart from this, the inclusion of the TM in the diet did not have negative effect on the quality of the eggs. Further studies with larger flocks and longer periods are needed to explore optimized inclusion levels and the TM meal long-term effects on the performance and sustainable production in layer hens.

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