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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Effect of various doses of probiotic Vetom 1 comprised of a *Bacillus subtilis* strain on relative weights of some internal organs in Pharaon quails

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**ABSTRACT**
*Poultry meat production is still an area of extensive research aimed at finding efficacious non-chemical antibiotic replacers. Probiotics are considered as an integral component of industrial livestock and poultry farming nowadays. The trial described here was performed to investigate the effect of B. subtilis strain DSM 32424 (Vetom 1™) on the relative weights of quails' internal organs. The probiotic was added in drinking water for forty 40-day-old Pharaon quails at a range of doses once a day during 28 days. A dose-dependent effect of Vetom 1™ on the relative weight of the studied quails' viscera was shown. The greatest medians for the relative weight of liver and small intestine were observed in the birds that received the probiotic at the dose of 100 mg/kg of live weight on day 28 from the beginning of its supplementation. The findings obtained in the current research might have at least theoretical implication for the poultry industry.*
***Keywords:*** *gizzard, liver, poultry, proventriculus, small intestine.*

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## INTRODUCTION

Poultry meat production is still an area of extensive research aimed at finding efficacious non-chemical antibiotic replacers [1]. Currently, probiotics are considered as an integral component of industrial livestock and poultry farming. The implementation of probiotic drugs helps to optimize the gut microecology, after which they are quickly eliminated from the organism without the cumulative effect, causing no harm to the environment or the host, due to their natural origin. One of the most crucial advantages of organic antibiotic substitutes is the lack of the resistance phenomenon [2].

Among the microorganisms most commonly used as probiotics is an aerobic gram-positive endosporous bacterium *Bacillus subtilis*. Studies [3, 4] have established the capability of some *B. subtilis* strains to secrete a human epidermal growth factor, which is a polypeptide stimulating the proliferation of epidermal and epithelial tissue, contributing to wound healing in diabetic patients. This is consonant with the presumption that the entrance of probiotics into a bird's organism increases the relative weights of immune-competent organs [5].

The vast majority of studies in the field of effects of various probiotics, including *B. subtilis*, on poultry that have been published to date are focused predominantly on birds' live weight growth and the gastrointestinal microbiota, but there is a paucity of studies assessing dose response of quails' internal organs to *B. subtilis* dietary supplementation.

Numerous literature data point to a positive effect of *B. subtilis* on the gut microbial landscape, immune reactivity, and growth performance in poultry. The safety and efficacy of several *B. subtilis* strains for poultry have been repeatedly approved by the Panel on Additives and Products or Substances used in Animal Feed [6]. At the same time, it must be stated that there is extremely few evidence addressing the effects of *B. subtilis* on quails' viscera in the existing scientific literature. As an attempt to fill this gap, the present research was undertaken in order to evaluate whether *B. subtilis* addition in drinking water could affect the relative weights of Pharaon quails' internal organs.

## MATERIAL AND METHODS

The study was carried out on a poultry farm owned by the Novosibirsk State Agrarian University (Novosibirsk, Russia) in compliance with Directive 2010/63/EU, and it was approved by the local Ethics Committee. Forty 40-day-old unvaccinated female Pharaon quails were randomly distributed to four groups, ten birds each: a no-treatment control group (CON) and three trial groups that were given powder of *B. subtilis* strain DSM 32424 (Vetom 1™) containing at least 1 × $10^6$ colony forming units (CFU)/g, dissoluted in drinking water at doses of 50 (T1), 75 (T2) and 100 (T3) mg/kg live weight, once a day for 28 days. The quails received water and a commercial feed (its detailed composition is provided in [7]) ad libitum. On the 14th and the 28th days from the start of the probiotic supplementation, the birds were slaughtered by decapitation in accordance with GOST 52837-2007. After dissecting the birds, their liver, proventriculus, gizzard, and small intestine were weighed. Organ weights and were expressed as a percentage of the overall bodyweight. For statistical analysis of the data, ANOVA test was utilized via StatsDirect 3.2.7 using Dunnett's post-hoc test. Differences were considered statistically significant at $P<0.05$. Descriptive statistics of continuous variables included the calculation of the median with its standard error, interquartile range, and variance coefficient.

## RESULT AND DISCUSSION

According to the results of the experiment, changes in the relative weight of the internal organs of the quails were as follows (Table 1).

TABLE 1: THE RELATIVE WEIGHT OF INTERNAL ORGANS OF VETOM-FED AND NON-TREATED QUAILS DURING THE EXPERIMENTAL PERIOD

| **The first slaughter, day 14** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Internal organs** | **CON** | | | **T1** | | | **T2** | | | **T3** | | |
| | Me ± SE | IQR | VC | Me ± SE | IQR | VC | Me ± SE | IQR | VC | Me ± SE | IQR | VC |
| Liver | 3.08 ± 1.85 | 0.20 | 17.63 | 3.74 ± 2.75 | 0.40 | 24.87 | 3.41 ± 1.85 | 0.79 | 17.60 | 2.94 ± 3.26 | 1.40 | 29.0 |
| Glandular stomach | 0.46 ± 0.34 | 0.11 | 21.62 | 0.57 ± 0.39 | 0.06 | 22.21 | 0.42 ± 0.26 | 0.09 | 18.54 | 0.46 ± 0.23 | 0.12 | 15.30 |
| Gizzard | 2.86 ± 0.80 | 0.42 | 9.31 | 2.95 ± 1.68 | 0.53 | 18.75 | 2.95 ± 0.69 | 0.19 | 7.36 | 2.42 ± 2.19 | 0.56 | 25.11 |
| Small intestine | 3.53 ± 1.02 | 0.45 | 8.65 | 3.88 ± 1.50 | 0.27 | 11.52 | 3.71 ± 0.48 | 0.21 | 4.14 | 4.36 ± 2.29 | 0.60 | 17.94 |
| **The second slaughter, day 28** | | | | | | | | | | | | |
| **Internal organs** | **CON** | | | **T1** | | | **T2** | | | **T3** | | |
| | Me ± SE | IQR | VC | Me ± SE | IQR | VC | Me ± SE | IQR | VC | Me ± SE | IQR | VC |
| Liver | 3.03 ± 2.11 | 0.89 | 20.91 | 3.30 ± 1.49 | 0.40 | 13.75 | 3.69 ± 1.71 | 0.82 | 19.74 | 4.20 ± 1.40* | 0.67 | 10.73 |
| Glandular stomach | 0.44 ± 0.36 | 0.10 | 24.98 | 0.37 ± 0.56 | 0.25 | 41.65 | 0.45 ± 0.26 | 0.17 | 23.78 | 0.41 ± 0.19 | 0.06 | 14.48 |
| Gizzard | 2.79 ± 1.61 | 0.17 | 19.35 | 2.63 ± 1.41 | 0.18 | 15.93 | 2.60 ± 1.08 | 0.57 | 16.13 | 2.62 ± 0.17 | 0.03 | 2.11 |
| Small intestine | 3.13 ± 1.10 | 0.28 | 10.93 | 3.10 ± 3.76 | 1.28 | 30.61 | 3.44 ± 1.61 | 1.0 | 17.96 | 4.54 ± 1.28* | 0.67 | 9.21 |

* ANOVA test ($P<0.05$)

Note: Me=median. SE=standard error of the median. IQR=interquartile range. VC=variance coefficient

At the time of the first slaughter, as may be seen from Table 1, the medians of liver relative weights in T1 and T2 exceeded one in the intact group, but that of T3 was lower when compared to the controls. On trial day 28, T1 and T2 had greater medians in relation to CON, while in T3 livers now outweighed those from the untreated birds significantly ($P<0,05$). These findings can be regarded as being in line with those of [8], who detected greater ($P<0.05$) relative liver weights for broiler chickens that were receiving a probiotic throughout a 5-week period as opposed to synbiotic-fed birds.

With respect to glandular and muscular stomachs, no significant differences between Vetom-fed quail and the controls were recorded on day 14 as well as day 28. These findings are consistent with results obtained in an investigation [9], which identified that chicken fed a multibacterial probiotic had a heavier proventriculus when compared to those untreated over a 12-week growth period. Also, some studies [10, 11] revealed that supplementing the diets of broiler chicks with *B. subtilis* had not affected ($P>0.05$) relative weight of their gizzard.

As of the first slaughter, the medians of the relative weight of the small intestine of quails in the test groups insignificantly ($P>0.05$) exceeded this value observed in the non-treated birds. It follows from the results of the second slaughter that the relative weights of quails' small intestine in T1 was less as opposed to the quails in the control group, whereas the birds in T2 had a greater median than those not

fed the probiotic. For T3, this indicator was significantly greater in comparison with CON (P<0.05). It was impossible for us to retrieve any trial evaluating the impact of *B. subtilis* on quails' relative weight of small intestine, except an experiment [12] reporting that the feeding of 80 day-old quails with *B. subtilis* in a variety of concentrations resulted in no significant difference (P>0.05) in their intestine development. Interestingly, a 126-day research performed on turkeys [13] demonstrated that on day 28, birds fed the diet with $2^8$ CFU *B. subtilis* per kg of feed had higher (P<0.05) relative weight of small intestine in relation to birds that were given greater or lower levels of the probiotic, which is in contrast to our study.

## CONCLUSION

Thus, a dose-dependent effect of Vetom 1™ on the relative weight of the studied quails' viscera was shown. The greatest medians for the relative weight of liver and small intestine were observed in the birds that received the probiotic at the dose of 100 mg/kg of live weight on day 28 from the beginning of its supplementation. The results presented in this investigation might have at least theoretical implication for the poultry production, providing new knowledge regarding the impact of *B. subtilis* addition in daily drinking water on internal organs of Pharaon quails.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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