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# Effects of direct-fed microbial on productive performance, qualitative traits of eggs, blood biochemicals, and ileal microflora of laying hens fed a wheat-soybean-based diet

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A B S T R A C T

This study investigated the effects of direct-fed microbes on qualitative traits of eggs and productive performance, ileal and cecal microflora, and pH of hens fed wheat-based diets from 60 to 68 wk of age. The total number of 240 Hy-Line W-36 laying hens were randomly distributed between 40 cages, and the five experimental diets including (A) corn-soybean meal-based control diet, (B) wheat-based diet with no microbial additive, (C) wheat-based diet supplemented with *Bacillus velezensis*, (D) wheat-based diet supplemented with *S. cerevisiae* and (E) wheat-based diet supplemented with *B. velezensis* + *S. cerevisiae* were assigned to hens with eight replicate cages per diet and six hens per each replicate. Increased egg weight and production (EP), feed intake (FI), improved feed conversion ratio (FCR), and decreased pH of ileum and caecum were observed in layers fed the diet included *B. velezensis* + *S. cerevisiae* ($P<0.05$). Improved intestinal morphology characteristics were observed in hens fed the microbe-added diets ($P<0.05$). Increased plasma protein, albumen, and HDL in hens fed microbe-added diets ($P<0.05$). Adding *B. velezensis* + *S. cerevisiae* to the diet modulated the ileal and caecal microflora composition by decreasing the numbers of Salmonella and increasing the numbers of Lactobacilli. Based on the results of the current study, it can be concluded that adding *B. velezensis* + *S. cerevisiae* to the wheat-based diet improves performance and intestinal morphology characteristics of laying hens, decreases plasma levels of cholesterol, triglyceride, and LDL, and the intestinal pH and number of Salmonella.

***Keywords:*** *Bacillus velezensis, Intestinal health, Organ pH, Saccharomyces cerevisiae, wheat*

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## 1 Introduction

In recent years, due to the energy crisis, grain crops have been used as the solution to the feed shortage (1). Laying hen efficiency is influenced by nutrition, according to Figueiredo et al. (2). The hen's distribution of nutrition to the egg can be gauged by evaluating egg size and composition (3). The use of probiotics/direct-fed microbial (commonly used microbes include Lactobacilli, Streptococci, Bifidobacterium, and Yeast (saccharomyces)) as supplements in hens feeds has been increasing as a result of the aspiration for a more "natural" product (4). The highest cost in the poultry industry was related to the nutrition sector. Therefore, using feed additives that can improve digestion and absorption of food improves poultry performance (5). Wheat has become an important energy source in poultry diets due to supply shortages and rising corn prices, even in markets that do not traditionally rely on wheat (6). One way to improve the nutritional value of cereals (such as wheat) is to add exogenous enzymes that can improve poultry performance and reduce feed costs and environmental pollution (7). Numerous studies have shown that *Saccharomyces cerevisiae* fermentation products can improve feed efficiency, phosphorus utilization, and egg quality (8-10). Feed supplementation with *S. cerevisiae* has been reported to improve humoral immune response, growth rate, and feed efficiency (11). Previous studies demonstrated that the presence of non-starch polysaccharides (NSP) in wheat (5.2% arabinoxylan in corn, 8.1% arabinoxylan in wheat) negatively affects nutrient utilization and performance of poultry (12, 13). To our knowledge, few studies have investigated the impact of bacterial or fungal enzymes on laying hens. Therefore, this experiment aims to evaluate the impact of bacterial or fungal enzyme sources on productive efficiency, egg quality, biochemical blood indices, and intestinal microflora in layers.

## 2 Materials and Methods

The experiment was conducted at the Faculty of Agriculture at Razi University, Kermanshah, Iran. The Animal Welfare Committee of Razi University, approved all experimental procedures.. All hens were weighed and arranged into the replicate cages with similar body weights. A total of 240 laying hens (Hy-Line W36) 60 weeks of age were randomly divided into 40 cages and assigned to receive one of the five experimental diets with eight replicates and six hens per replicate. All hens were supplied with feed and water *ad libitum* in the 8-week trial period. The five experimental diets including (A) corn-soybean meal-based control diet, (B) wheat-based diet with no microbial additive, (C) wheat-based diet with 1 × 109 cfu/kg of *B. velezensis*, (D) wheat-based diet with 1 × 105 cfu/kg of *S. cerevisiae* and (E) wheat-based diet with *B. velezensis* + *S. cerevisiae* were assigned to hens with eight replicate cages per diet and six hens per each replicate.

The basal diet (mash form) was formulated according to (Hy-Line W-36 International 2015 recommendations) management guide. Ingredients and nutritional composition of the experimental diets are shown in Table 1.

**Table 1.** Ingredients and nutritional composition of the experimental diets.

| Ingredients (%) | Corn-based diet | Wheat-based diet |
|---|---|---|
| Corn | 53.10 | - |
| Soybean meal | 23.19 | 11.58 |
| Wheat | - | 70.66 |
| Wheat bran | 2.97 | - |
| Soybean oil | 3.50 | 3.50 |
| DCP | 1.78 | 1.72 |
| Limestone | 5.00 | 5.00 |
| Oyster shell | 5.33 | 5.40 |
| NaCl | 0.38 | 0.31 |
| Mineral premix[1] | 0.30 | 0.30 |
| Vitamin premix[2] | 0.30 | 0.30 |
| Methionine | 0.02 | 0.15 |
| L-Lysine-HCl, 78% | 0.12 | 0.57 |
| Threonine | 0.01 | 0.30 |
| ***Calculated analysis*** | | |
| AMEn (Mcal/kg) | 2.925 | 2.725 |
| CP | 15.30 | 15.30 |
| Ca | 4.40 | 4.40 |
| AP | 0.62 | 0.45 |

| | | |
|---|---|---|
| Na | 0.17 | 0.17 |
| SID Lysine | 0.69 | 0.96 |
| SID Methionine | 0.41 | 0.36 |
| SID Methionine +Cystine | | |
| SID Threonine | 0.50 | 0.48 |
| SID Valine | 0.64 | 0.62 |
| ***Determined analysis*** | | |
| DM | 94.0 | 93.5 |
| CP (N × 6.25) | 15.3 | 15.3 |
| CF | 3.12 | 2.93 |
| Ca | 4.40 | 4.40 |
| Ether extract | 5.40 | 5.00 |

Hens were allowed *ad libitum* access to feed and water throughout the experimental period. The light was provided for 16 h daily, and the temperature of the barn was maintained at 23 ± 4°C throughout the experiment. At the end of the experiment, egg production and weights were obtained daily by replicate, and feed consumption was obtained weekly by replicate to calculate egg production, egg mass, and feed conversion ratio (FCR). The eggs were individually weighed, and their external and internal quality was determined. The shell was separated from the yolk and albumen and weighed after drying overnight at 60°C. Shell thickness was measured using a digital micrometer (Echometer 1061, Robotmation Company, Tokyo, Japan), and albumen height was determined with an electronic height gauge (Futura Company, Lohne, Germany).

### *2.1 Blood biochemical analysis*

At the end of the experimental period, ten samples from each treatment were withdrawn for blood biochemical analysis and measured with appropriate laboratory kits (Zist Shimi, Tehran, Iran).

### *2.2 Ileal and cecal microflora composition and pH value*

For the microbial analysis, two layers from each replication were selected randomly, and one gram of the Ileal and cecal contents were homogenized with phosphate buffer saline using a homogenizer and subjected to serial dilution until ten at the end of the experimental period. Ileal and cecal contents were immediately obtained and then placed on ice for transportation to the laboratory. The counts of microflora were analyzed by the method of Wang and Kim (14). The digestive tract of each hen was removed, and the pH in segments (such as the crop, gizzard, small intestine, and caecum) was immediately measured using a pH meter (Corning Glass Works, Medfield, MA). The mean of the two measurements was used to evaluate the data statistically.

### *2.3 Intestinal morphology characteristics*

Sample sections (3 cm in length) were taken from the descending duodenum, the middle region of the jejunum, and the ileum region. Intestinal tissue samples were fixed in formalin and dehydrated, cleared, and impregnated with paraffin by Yu et al. (15). The processed tissue was then embedded in paraffin wax. Section was cut with a thickness of 5 µm (3cross sections from each sample) from the waxed tissue on a microtome, cleared of wrinkles by floating on warm water (55 to 60 ˚C) prior to mounting on 10.0% poly-L-lysine coated slides. Histological indices were determined by using a computer-aided light microscopic image analyzer (Motic Images, 2000 1.2, Scion Image, Tokyo, Japan). The villous height and crypt depth were measured, and a calculation was made for the villous height/crypt depth rate.

### *2.4 Statistical Analysis*

All data were submitted for analysis of variance using the General Linear Model (GLM) procedure of the SAS statistical package (16). Duncan's multiple range test was used to detect pairwise differences among treatment means at 0.05.

## 3 Results

The effects of direct-fed microbial supplementation on productive performance are shown in Table 2. Egg weight was increased in the direct-fed microbial treatments compared to those fed wheat-based diets ($P<0.05$). No significant differences were observed in feed intake between treatments ($P>0.05$). Increased production percent was detected in hens-fed microbe-added diets ($P<0.05$), but this increase was more significant in *S. cerevisiae* treatment. The highest egg mass was observed in the treatment *of B. subtilis* compared to other treatments ($P<0.05$). Improved feed conversion was observed in hens fed the microbe-added

diets (P<0.05). Significantly increased egg weight (Table 3) was shown by treatment containing *B. subtilis* in comparison to the based diet (P<0.05). No significant differences in the shape and yolk index, shell weight, or shell ratio were observed using direct-fed microbial. Increased Haugh unit, yolk color, and thick shell were detected in hens-fed microbe-added diets (P<0.05). The results have shown no significant effect of treatments on the pH values of crop, proventriculus, gizzard, and duodenum (P >0.05) (Table 4). PH values of jejunum were the same in all treatments but decreased in the control treatment compared to the others (P<0.05). Decreased pH values in the ileum and caecum were detected in hens-fed microbe-added diets (P<0.05). The effects of direct-fed microbial on intestinal morphology characteristics are shown in Table 5. The highest level of villus height and crypt depth were observed in *S. cerevisiae* treatment, but the highest villus/crypt ratio was observed in the control in duodenum (P<0.05). Dietary treatments did not affect villus height, crypt depth, and villus/crypt ratio in the duodenum (P > 0.05). They also, observed no significant effect of microbe-added diets on goblet cells across the small intestine (P>0.05). The results in the ileum showed that the lowest villus height was indicated in *S. cerevisiae* treatment in comparison to the others (P<0.05). Significantly increased crypt depth (in the ileum) was shown by treatments containing *B. subtilis* and *S. cerevisiae* in comparison to a wheat-based diet (P<0.05), but wheat consumption alone increased the villus/crypt ratio. The Effects of direct-fed microbial on blood biochemical parameters in laying hens fed a wheat-soybean meal-based diet can be seen in Table 6. Increased HDL, protein, and albumen plasma levels were detected in layers fed the diet, including *B. velezensis* + *Trichoderma* (P<0.05). The results have shown no significant effect of treatments on cholesterol and globulin (P >0.05). Decreased plasma triglyceride and highest phosphorous in hens fed *B. velezensis* added diets and increased plasma calcium were observed in layers fed microbials and wheat-based (P<0.05) in Table 7 shown status ileal and cecal microflora composition. The Lactobacillus counts were increased with direct-fed microbial supplementation (P< 0.05). On the other hand, no difference in *Escherichia coli* counts was found among treatments (P > 0.05). Increased Salmonella and total microbes were detected in layers fed the diet, including *B. velezensis* + *Trichoderma* (P<0.05).

**Table 2.** Effects of adding *B. velezensis* and/or *S. cerevisiae* to diet on performance of laying hens fed wheat-soybean meal-based diet (60 to 68 weeks of age)

| Treatments | Egg weight (g) | Feed intake (g/hen/day) | Production Percent (%) | Egg mass (g/day) | Feed Conversion Ratio (FCR) |
|---|---|---|---|---|---|
| Corn-based diet (CD) | 62.86$^{ab}$ | 109.90 | 83.39$^{d}$ | 52.29$^{d}$ | 2.10$^{b}$ |
| Wheat-based diet (WD) | 61.81$^{c}$ | 110.39 | 81.72$^{e}$ | 50.64$^{e}$ | 2.18$^{a}$ |
| WD + *B. velezensis* | 62.55 $^{b}$ | 110.40 | 84.26$^{c}$ | 52.72$^{c}$ | 2.09$^{b}$ |
| WD + *S. cerevisiae* | 63.01$^{a}$ | 110.36 | 84.53$^{b}$ | 53.24$^{b}$ | 2.07$^{c}$ |
| WD +*B. velezensis* + *S. cerevisiae* | 63.05$^{a}$ | 109.85 | 84.83$^{a}$ | 53.53$^{a}$ | 2.05$^{d}$ |
| SEM | 0.3193 | 0.7384 | 0.2142 | 0.2729 | 0.0192 |
| P - value | 0.0015 | 0.7825 | 0.0001 | 0.0001 | 0.0004 |

$^{a-c}$ The same letters in each column represent no significant difference between the averages. SEM: standard error of means.

**Table 3.** Effects of adding *B. velezensis* and/or *S. cerevisiae* to diet on qualitative traits of eggs of laying hens fed wheat-soybean meal-based diet (68 weeks)

| Treatments | Egg weight (g) | Shape Index (%) | Yolk index (%) | Yolk color | Haugh Unit (%) | Shell weight (g) | Ratio Shell (%) | Thick Shell (mm) |
|---|---|---|---|---|---|---|---|---|
| Corn-based diet (CD) | 63.31 $^{b}$ | 76.98 | 42.43 | 6.15 $^{a}$ | 89.08 $^{b}$ | 6.70 | 10.58 | 0.320 $^{b}$ |
| Wheat-based diet (WD) | 62.72 $^{c}$ | 77.30 | 41.69 | 5.77 $^{b}$ | 89.29 $^{a}$ | 6.71 | 10.70 | 0.318 $^{b}$ |
| WD + *B. velezensis* | 63.71$^{ab}$ | 76.87 | 42.35 | 6.13 $^{a}$ | b89.14$^{a}$ | 6.71 | 10.54 | 0.323 $^{b}$ |
| WD + *S. cerevisiae* | 63.52$^{ab}$ | 76.85 | 41.62 | 6.10 $^{a}$ | b89.21$^{a}$ | 6.77 | 10.67 | 0.331 $^{a}$ |
| WD +*B. velezensis* + *S. cerevisiae* | 63.91 $^{a}$ | 76.98 | 42.85 | 6.05 $^{a}$ | 89.21 $^{a}$ | 6.82 | 10.67 | 0.335 $^{a}$ |
| SEM | 0.1858 | 0.2407 | 0.4345 | 0.0961 | 0.0549 | 0.0394 | 0.0751 | 0.0019 |
| P - value | 0.0003 | 0.6898 | 0.2255 | 0.0403 | 0.0269 | 0.1957 | 0.4776 | 0.0001 |

$^{a-c}$ The same letters in each column represent no significant difference between the averages. SEM: standard error of means.

**Table 4.** Effects of adding *B. velezensis* and/or *S. cerevisiae* to diet on pH values of gastrointestinal tract segments in laying hens fed wheat-soybean meal-based diet (68 weeks)

| Treatments | Crop | Proventriculus | Gizzard | Duodenum | Jejunum | Ileum | Caecum |
|---|---|---|---|---|---|---|---|
| Corn-based diet (CD) | 5.80 | 5.63 | 4.73 | 5.64 | 5.80 $^{a}$ | 6.63 $^{a}$ | 6.32 $^{c}$ |
| Wheat-based diet (WD) | 5.78 | 5.59 | 4.67 | 5.59 | 5.79 $^{a}$ | 6.47 $^{b}$ | 6.30 $^{c}$ |
| WD + *B. velezensis* | 5.85 | 5.65 | 4.79 | 5.50 | 5.61 $^{b}$ | 5.87 $^{c}$ | 6.21 $^{d}$ |
| WD + *S. cerevisiae* | 5.75 | 5.49 | 4.77 | 5.73 | 5.89 $^{a}$ | 5.93 $^{c}$ | 6.53 $^{a}$ |
| WD +*B. velezensis* + *S. cerevisiae* | 5.79 | 5.52 | 4.81 | 5.60 | 5.83 $^{a}$ | 5.91 $^{c}$ | 6.43 $^{b}$ |
| SEM | 0.0458 | 0.0472 | 0.0405 | 0.0581 | 0.0484 | 0.0338 | 0.0159 |
| P - value | 0.6638 | 0.1335 | 0.1954 | 0.1488 | 0.0136 | 0.0001 | 0.0001 |

$^{a-c}$ The same letters in each column represent no significant difference between the averages. SEM: standard error of means.

**Table 5.** Effects of adding *B. velezensis* and/or *S. cerevisiae* to diet on intestinal histology of laying hens fed wheat-soybean meal-based diet (60 to 68 weeks)

| Treatments | Duodenum | | | | Jejunum | | | | Ileum | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Villus height | Crypt depth | Villus /crypt ratio | Goblet | Villus height | Crypt depth | Villus/crypt ratio | Goblet | Villus height | Crypt depth | Villus/ crypt ratio | Goblet |
| Corn-based diet (CD) | 889.40$^{bc}$ | 251.38$^{c}$ | 3.54$^{a}$ | 155.40 | 659.38 | 192.92 | 3.45 | 151.77 | 556.50$^{a}$ | 201.50$^{a}$ | 2.76$^{bc}$ | 138.25 |
| Wheat-based diet (WD) | 838.20$^{c}$ | 295.33$^{b}$ | 2.84$^{b}$ | 149.30 | 669.33 | 186.45 | 3.59 | 142.07 | 565.00$^{a}$ | 182.00$^{b}$ | 3.11$^{a}$ | 136.10 |
| WD + *B. velezensis* | 909.80$^{ab}$ | 329.90$^{ab}$ | 2.77$^{b}$ | 146.50 | 710.90 | 196.07 | 3.65 | 146.05 | 524.70$^{b}$ | 200.90$^{a}$ | 2.62$^{c}$ | 136.52 |
| WD + *S. cerevisiae* | 955.60$^{a}$ | 351.18$^{a}$ | 2.73$^{b}$ | 150.87 | 698.33 | 188.72 | 3.72 | 154.22 | 584.90$^{a}$ | 197.90$^{ab}$ | 2.95$^{ab}$ | 141.22 |
| WD +*B. velezensis* + *S. cerevisiae* | 909.30$^{ab}$ | 324.5$^{ab}$ | 2.83$^{b}$ | 154.32 | 747.33 | 208.32 | 3.58 | 148.25 | 584.00$^{a}$ | 205.40$^{a}$ | 2.84$^{abc}$ | 143.10 |
| SEM | 19.90 | 12.20 | 0.12 | 3.02 | 24.18 | 6.46 | 0.22 | 3.84 | 9.62 | 5.79 | 0.09 | 4.40 |
| P - value | 0.013 | 0.0004 | 0.001 | 0.265 | 0.13 | 0.19 | 0.93 | 0.24 | 0.002 | 0.09 | 0.01 | 0.75 |

$^{a-c}$ The same letters in each column represent no significant difference between the averages. SEM: standard error of means.

**Table 6.** Effects of adding *B. velezensis* and/or *S. cerevisiae* to diet on blood biochemical parameters in laying hens fed wheat-soybean meal-based diet (mg/dl)

| Treatments | Protein | Albumen | Cholesterol | Triglyceride | Calcium | Phosphorous | Globulin | LDL | HDL |
|---|---|---|---|---|---|---|---|---|---|
| Corn-based diet (CD) | 5.74$^{b}$ | 2.80$^{b}$ | 149.22 | 1806.20$^{a}$ | 26.05$^{b}$ | 6.18$^{ab}$ | 177.67 | 267.42$^{a}$ | 50.55$^{a}$ |
| Wheat-based diet (WD) | 5.68$^{b}$ | 2.57$^{c}$ | 144.35 | 1764.33$^{ab}$ | 27.30$^{a}$ | 5.94$^{b}$ | 174.57 | 266.60$^{a}$ | 48.68$^{b}$ |
| WD + *B. velezensis* | 6.40$^{a}$ | 2.97$^{a}$ | 151.37 | 1701.30$^{bc}$ | 27.05$^{ab}$ | 6.09$^{ab}$ | 173.62 | 227.55$^{c}$ | 50.48$^{a}$ |
| WD + *S. cerevisiae* | 6.23$^{a}$ | 3.01$^{a}$ | 153.52 | 1629.05$^{c}$ | 28.00$^{a}$ | 6.44$^{a}$ | 178.15 | 231.90$^{bc}$ | 51.10$^{a}$ |
| WD +*B. velezensis* + *S. cerevisiae* | 6.45$^{a}$ | 2.83$^{b}$ | 160.87 | 1739.63$^{ab}$ | 27.50$^{a}$ | 6.27$^{ab}$ | 173.07 | 236.05$^{b}$ | 50.02$^{a}$ |
| SEM | 0.0829 | 0.0396 | 4.6992 | 30.6660 | 0.3907 | 0.1179 | 1.6608 | 2.5536 | 0.3386 |
| P - value | .0001 | .0001 | 0.2087 | 0.0107 | 0.0357 | 0.0873 | 0.1476 | .0001 | 0.0018 |

$^{a-c}$ The same letters in each column represent no significant difference between the averages. SEM: standard error of means.

**Table 7.** Effects of adding *B. velezensis* and/or *S. cerevisiae* to diet on the profiles of cecal microflora of laying hens fed wheat-soybean meal-based diet (60 to 68 weeks)

| Treatments | Lactobacillus | *Escherichia coli* | Salmonella | Total microbes |
|---|---|---|---|---|
| Corn-based diet (CD) | 7.44$^{ab}$ | 4.66 | 3.74$^{a}$ | 4.86$^{ab}$ |
| Wheat-based diet (WD) | 7.02$^{c}$ | 4.67 | 3.75$^{a}$ | 4.70$^{abc}$ |
| WD + *B. velezensis* | 7.26$^{b}$ | 4.68 | 3.17$^{ab}$ | 4.98$^{a}$ |
| WD + *S. cerevisiae* | 7.45$^{ab}$ | 4.73 | 3.06$^{ab}$ | 4.64$^{bc}$ |
| WD +*B. velezensis* + *S. cerevisiae* | 7.51$^{a}$ | 4.82 | 2.65$^{b}$ | 4.54 |
| SEM | 0.0604 | 0.1273 | 0.2669 | 0.0920 |
| P - value | 0.0002 | 0.8912 | 0.0474 | 0.0293 |

$^{a-c}$ The same letters in each column represent no significant difference between the averages. SEM: standard error of means.

## 4 Discussion

### *4.1 Productive Performance and Egg Quality*

In the study, egg weight, production percent, and egg mass were lower in laying hens fed by wheat. This can be explained by the adverse effects of wheat on nutrient absorption and viscosity of the intestine of laying hens, as reported previously (17, 18). When we compared the group's direct-fed microbial with the wheat-based diet, there were positive effects; that is, there was more excellent production of eggs and more significant weight and mass. Dietary treatments did not significantly affect feed intake but improved the feed conversion ratio with direct-fed microbials. Some researchers (18-21) also found that *S. cerevisiae* supplementation did not affect the body weight and feed intake of hens. However, other authors (17) have reported a considerable improvement in egg production in poultry-fed *S. cerevisiae*. This indicates that consuming *B. velezensis* and *S. cerevisiae* in the diet of laying hens improves the absorption of nutrients from the intestine (22). Consistent with the present study, Asli et al. (20) found that dietary supplementation with 1 g kg$^{-1}$ *S. cerevisiae* had no significant effect on egg production. This could be due to the *B. velezensis* and *S. cerevisiae* reducing the pathogenic bacterial load in the intestine, so the nutrients in the diet are efficiently diverted towards production in poultry direct-fed microbials, which might improve egg production in layers and breeders (23, 24).

Similarly, egg weight and production were reported to be increased by dietary supplementation with *S. cerevisiae* (25, 26). Consistent with our results, Ye et al. (15) showed a significant effect on egg production rate but could significantly reduce average daily feed intake and feed conversion ratio with an improved production performance. The Current study also has shown that *B. velezensis* and *S. cerevisiae* improved yolk color, Haugh unit, and thick shell. Improving egg quality by using probiotics in the bird's diet can be related to improving gastrointestinal health. Harmful microflora in the gastrointestinal tract have been shown to reduce the ability to digest and absorb nutrients (which are directly related to egg quality) in several ways (8, 20). The improvement observed in Haugh unit and thick shell in hens fed probiotic supplementing diets in the present experiment might be due to the supply of *S. cerevisiae* and probiotic treatment coupled with the supply of some essential micro-nutrients as reported by Khan et al. (7) that yeast and phytase are capable of increasing bioavailability of certain minerals such as Ca, Cu, Zn, Fe, Mn and even gross energy of the feed. The shape of poultry eggs become frequent in the field of interest of researchers. Shape may contribute to the solidity of the egg (27) and may affect gas transfer (28). The present study shows no effect of growth promoters on egg shape index. In this connection, the results of Ciftci et al. (29) showed that the diet type significantly affected the egg shape index, while the interaction between diet type and enzyme supplementation did not affect the egg shape index when triticale was used with either maize or wheat. Dipeolu et al. (30) reported that the egg shape index was uniform in the given layer diets containing either enzyme alone or enzyme/antibiotic groups. This shows that the treatments did not affect the formation of egg shape.

### *4.2 pH of the GIT*

In our study, the pH of the crop, proventriculus, gizzard, and duodenum was not affected by *B. velezensis* + *S. cerevisiae* supplementation, but the pH of jejunum, ileum, and caecum decreased. It is well illustrated that probiotics can alter the gastrointestinal flora by reducing the pH, increasing the activity of intestinal enzymes, and nutrient

digestibility (31). The inclusion of probiotics in the diets exerts beneficial effects on nutrient availability in the digestive gut, improves the intestinal absorption of nutrients, and effectively reduces feed consumption because laying birds have adequate nutrients for maintenance and production (32). Adding probiotics (*S. cerevisiae*) to the diet of laying hens decreased the pH of the duodenum and jejunum (33).

### *4.3 Blood parameters*

This study showed a significant improvement in protein, albumen, calcium, and phosphorous by direct-fed microbial supplementation to layer diet. These results are similar to those reported by (7) that enzyme and probiotic to layers diet significantly increased plasma HDL concentration over the non-supplemented group. Such a decrease in plasma LDL concentrations and adding *B. velezensis* to the diet can prove that adding probiotics reduces cholesterol and triglyceride (34). The obtained results from our study regarding the beneficial effects of supplementary *S. cerevisiae* on triglyceride coincide with the findings of Ghasemi et al. (35), who stated that values were significantly higher for laying hens fed on the diets containing (S. *cerevisiae*). Similar results about serum triglyceride and protein Yalçın et al., (21). reported that baker's yeast *S. cerevisiae* seems to be the perfect organism for reducing triglyceride (36) reported that adding yeast culture (*S. cerevisiae*) did not affect serum cholesterol levels.

### *4.4 Histomorphology of the Gastrointestinal Tract*

The gut serves as a sizeable immune organ, gut-associated lymphoid tissue (GALT) is located in the digestive tract (37). An imbalance in intestinal microflora often accompanies lowering the body's defense mechanisms and can be an advantage to the host (38). In agreement with the present study, Zamanizadeh et al. (39) found dietary supplementation with yeast autolysate (*S. cerevisiae*) at 100 and 200 mg/kg in laying hens. In agreement with the present observations, Rahimi et al. (40) found that *S. cerevisiae* increased the villus height in broilers challenged with Salmonella and Campylobacter. Similar findings were found in a study by (41), where the villus height was significantly increased in laying Japanese quails receiving the *S. cerevisiae*-supplemented diets at 1.5%. These alterations may be related to dietary yeast affecting the local metabolism and increasing the synthesis of short-chain fatty acids (SCFAs), stimulating the proliferation of intestinal epithelial cells and resulting in broader and longer villi (41).

### *4.5 Ileal and cecal microflora composition*

The positive effects of yeast culture supplementation on the host animal's natural defense system, the biological regulation of its intestinal microflora, and the direct effect of the probiotic on health or the nutritional form of the probiotic have been previously reported (42, 43). Improved intestinal alterations due to dietary probiotics are related to their ability to develop a better environment for beneficial microbes (44). Similarly, Shah et al. (45) reported that lactobacillus bacteria may increase the villus height via the digestion of carbohydrates, production of VFAs, and consequent nourishment of the intestinal villi. Another study (42) reported that different levels (0.4, 0.8, 1.2, and 1.6%) of yeast supplementation to laying hen diets significantly reduced the number of total bacteria, especially in the 1.6% supplementation group. However, Wang et al. (46) found that yeast supplementation increased the total number of bacteria in the intestine of laying hens.

## 5 Conclusions

This study provides evidence that *S. cerevisiae* in the diet has some adverse effects on performance but improves intestinal microbial balance and ileal morphology of laying hens. Adding *S. cerevisiae* and B. *velezensis* to the diet reduces intestinal pH and modulates the intestinal bacterial population.

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**Conflict of Interest**

All authors declare that they have no conflicts of interest.

**Author Contributions**

All authors contributed to the original idea and study design.

**Data Availability Statement**

Data are available from the corresponding author upon reasonable request.

## Ethical Considerations

The Animal Welfare Committee of Razi University approved all experimental procedures.

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