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RESEARCH ARTICLE

Effects of Adding Different Dosages of *Saccharomyces cerevisiae* in Diet on Growth Performance, Carcass Characteristics, Intestinal Morphology, and Gut Microflora of Broilers

Željko MAKSIMOVIĆ [1,a] Marija STARČEVIĆ [2,b (*)] Dragan ŠEFER [3,c] Jelena JANJIĆ [2,d]
Anita RADOVANOVIĆ [4,e] Stamen RADULOVIĆ [3,f] Dejan PERIĆ [3,g] Radmila MARKOVIĆ [3,h]

[1] Feix Nutrition D.O.O., Bulevar Kralja Petra I 55/20, 21000 Novi Sad, SERBIA
[2] Department for Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobodjenja 18, Belgrade 11000, SERBIA
[3] Department of Nutrition and Botany, Faculty of Veterinary medicine, University of Belgrade, Bulevar Oslobodjenja 18, 11000 Belgrade, SERBIA
[4] Department of Histology and Embryology, Faculty of Veterinary medicine, University of Belgrade, Bulevar Oslobodjenja 18, 11000 Belgrade, SERBIA
ORCIDs: [a] 0000-0002-4269-857x; [b] 0000-0002-0857-5218; [c] 0000-0002-4394-6336; [d] 0000-0003-3351-7199; [e] 0000-0001-9195-7147
[f] 0000-0002-7250-537X; [g] 0000-0002-4752-7489; [h] 0000-0002-4479-5007

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Abstract: The aim of this study was to evaluate the effect of adding different dosages of live yeast *(Saccharomyces cerevisiae)* in diet on growth performance, carcass characteristics, intestinal morphology, and gut microflora of broilers. The study was conducted on 270 one-day old chickens (Ross 308) during a 42-day period. Broilers were randomly allocated to one of three dietary treatments that differed in the content of added yeast: no additional yeast; with 0.25 g/kg; and with 0.65 g/kg of added yeast. Each experimental group contained 90 animals. The results from our study showed that diet supplemented with 0.25 g/kg of yeast resulted in better growth performance and carcass quality (P<0.05), improved intestinal morphology (P<0.001), higher development of beneficial microflora *(Lactobacillus* spp.) (P<0.01), and reduction of pathogenic bacteria *(Escherichia coli)* in guts of broilers than in the group fed only with the basic diet (P<0.05). On the other hand, the group with the highest inclusion of yeast in diet (0.65 g/kg) achieved similar results for the examined parameters as did the group with no added yeast. Therefore, the adequate dose of *S. cerevisiae* in diet was 0.25 g/kg of yeast, while higher dose of live yeast in broiler diet (0.65 g/kg) did not achieve better results.

Keywords: *Broiler, Live yeast, Performance, Carcass characteristics, Intestinal morphology, Intestinal microflora*

Rasyona Farklı Dozlarda *Saccharomyces cerevisiae* İlavesinin Etlik Piliçlerin Büyüme Performansı, Karkas Özellikleri, Bağırsak Morfolojisi ve Bağırsak Mikroflorası Üzerine Etkileri

Öz: Bu çalışmanın amacı, etlik piliçlerde rasyona farklı konsantrasyonlarda canlı maya *(Saccharomyces cerevisiae)* ilavesinin büyüme performansı, karkas özellikleri, bağırsak morfolojisi ve bağırsak mikroflorası üzerine etkisini değerlendirmektir. Çalışma, 42 gün süre boyunca 270 adet bir günlük civcivler (Ross 308) üzerinde gerçekleştirildi. Etlik piliçler sırasıyla; maya içermeyen, 0.25 g/kg maya içeren ve 0.65 g/kg maya içeren üç farklı rasyon uygulama grupları içerisine rastgele dağıtıldı. Her deney grubunda 90 adet hayvan yer aldı. Çalışmamızdan elde edilen sonuçlar, sadece temel rasyonla beslenen gruba göre 0.25 g/kg maya ilaveli rasyon ile beslenen etlik piliçlerin, daha iyi büyüme performansı ve karkas kalitesi sergilediğini (P<0.05), gelişmiş bağırsak morfolojisine (P<0.001) ve daha yüksek oranda faydalı mikrofloraya *(Lactobacillus* spp.) sahip olduklarını (P<0.01) ve bağırsaklarında daha az oranda patojenik bakterilerin *(Escherichia coli)* yer aldığını gösterdi (P<0.05). Diğer taraftan, rasyonunda en fazla (0.65 g/kg) maya bulunduran grup, incelenen parametreler yönünden rasyonunda maya bulundurmayan grupla benzer sonuçlar verdi. Bu nedenle, broiler rasyonlarında *S. cerevisiae*'nin yeterli konsantrasyonu 0.25 g/kg iken, daha yüksek konsantrede (0.65 g/kg) canlı maya kullanımı daha iyi sonuçlar vermemiştir.

Anahtar sözcükler: *Broiler, Canlı maya, Performans, Karkas özellikleri, Bağırsak morfolojisi, Bağırsak mikroflorası*

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(*) Corresponding Author

Tel: +381 11 3615 436; Fax: +381 11 2685 936
E-mail: marijadok@gmail.com (M. Starčević)

Introduction

In the past twenty years, annual poultry meat production has increased from 40 mt to 132 mt (annual increase 3.5% - 4.7%) and the average annual consumption of poultry meat in the world is 15 kg per capita [1]. Reasons for increasing world poultry production could be found in the high nutritional value and favorable content of protein and fat in meat, the meat's acceptability by many cultures and regions, and low cost. Moreover, poultry meat is easily affected by modifications in feed ingredients that influence the health and growth performance of poultry, gaining meat with beneficial effects on human health [2,3]. Recently, probiotics and prebiotics have been introduced to poultry feed in order to achieve many beneficial effects in poultry. Several studies indicated the importance of probiotics and prebiotics as potential substitutes for antibiotics in order to improve bacteria-related immune dysfunction, intestinal morphology disruption, and growth performance in broilers [4-8]. Probiotics comprised of live yeast contain many biologically valuable proteins, functional nucleic acids, vitamin B-complex, mannanoligosaccharide, immune enhancers such as β-glucan, and growth promoting factors [8,9]. Therefore, live yeast *(Saccharomyces cerevisiae)* has been reported to have several beneficial effects on poultry, such as achieved gut microbial balance, improved humoral immune response and intestinal morphology, and favorable growth performance results [10,11].

However, various researchers have reported different effective doses of yeast *(S. cerevisiae)* in broiler feed on poultry health, growth performance results, and carcass characteristics [8]. Therefore, the aim of this study was to evaluate the effect of adding different dosages of live yeast *S. cerevisiae* in diet (no additional yeast; with 0.25 g/kg; and with 0.65 g/kg of added yeast) on growth performance, carcass characteristics, intestinal morphology, and gut microbiota of broilers.

Material and Methods

Ethical Statement

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (Resolution number: 01-511-2/2020/07/23).

Animals, Housing, and Feeding

The study was conducted on 270 one-day-old chickens of both sexes and the same origin (Ross 308) during a 42-day period. At the beginning of the study, broilers were randomly allocated to one of three dietary treatments. Each experimental group contained 90 animals housed in groups of 15 birds per pen in six repetitions (stocking density = 0.15 m^2/head). Conditions in the facility (ventilation, heating, lighting, and relative humidity) were according to the technological standards and recommendations for this hybrid [12]. Pens were bedded with straw and provided with fresh water and feed *ad libitum*. At the beginning of the trial, the temperature of the room was 32°C and then was gradually lowered to 22°C, which was maintained until the end of the study. During the trial, relative humidity was 60-70%. A continuous period of six hours of dark was provided during the night, and for the rest of the day, artificial light was uniformly distributed throughout the house. Broilers were not identified by sex at any time during the study, so we assumed an approximately equal ratio of males and females was distributed in the experimental groups.

From the start of the trial, each group of broilers was fed with one of three experimental diets which comprised the same basal diet, but differed only in the content of added *S. cerevisiae*. Basal diet was formulated according to the recommendations for Ross 308 strain [13] *(Table 1)*. Diets were fed from days 1 to 42 including starter (days 1-10), grower (days 11-24), and finisher (days 25-42). All components of the diets were analyzed for dry matter [14] (ISO 6496, 1999), crude protein [15] (ISO 5983-1, 2005), crude fat [16] (ISO 6492, 1999), crude fiber [17] (ISO 6865, 2000), ash [18] (ISO 5984-1, 2002), calcium [19] (ISO 6490-1, 1985), and phosphorus [20] (ISO 6491, 1998) *(Table 1)*. Yeast was added to the diets in the microspherule form of live yeast concentrate *(S. cerevisiae*, new strain, 1 x 10^{10} CFU/g, produced by Lesaffre, France) at different levels: the first group with no additional yeast (background only); the second with 0.25 g/kg; and the third with 0.65 g/kg of added yeast. Levels of active yeast in the three experimental diets were: 0 CFU/kg (group with no additional yeast), 2.5 x 10^9 CFU/kg (group with 0.25 g/kg of added yeast), and 6.5 x 10^9 CFU/kg (group with 0.65 g/kg of added yeast).

Growth Performance and Carcass Characteristics

To study the effect of added yeast in the diet on growth performance of broilers, all animals were weighed on days 1, 10, 24, and 42 to obtain average body weight and daily weight gain. Feed consumption per pen was recorded during periods 1-10 days, 11-24 days, 25-42 days, and for the overall study duration (1-42 days). Feed conversion ratio (FCR) was calculated as the ratio between feed intake and weight gain.

At the end of the study, broilers were transported to the slaughterhouse, electrically stunned and immediately slaughtered by severance of the jugular veins. Subsequently, animals were processed following standard industrial techniques. Carcasses were stored in a ventilated cold room at 2°C. After 24 h of chilling, 30 carcasses from each

Table 1. Ingredients and chemical composition of diets

Ingredient (g/kg)	Starter (0-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Maize	458.70	494.10	548.00
Rye	50.00	50.00	50.00
Soybean meal (CP 44%)	226.50	147.80	67.40
Full fat soya	218.90	266.70	293.10
Monocalcium phosphate	13.20	13.50	11.80
Limestone	14.00	11.50	12.70
Salt	3.50	3.10	3.10
Lysine L-79	3.00	1.40	1.70
Methionine DL-99	2.20	1.90	2.20
Vitamin-mineral premix[a]	10.00	10.00	10.00
ME (MJ)	12.50	12.97	13.39
Chemical Composition (g/kg)			
Dry matter	898.20	901.40	896.30
Crude protein	224.60	215.00	178.40
Ether extract	59.30	64.90	74.90
Crude fiber	33.10	34.90	29.30
Ash	64.10	59.30	48.60
Calcium	9.80	9.70	9.50
Total phosphorus	7.90	8.40	6.90

[a] Vitamin-mineral premix provided per kg of diet: Vit. A: 10.000 IU; Vit. D_3: 4.000 IU; Vit. E: 55 mg; Vit K_3: 3 mg; Vit B_1: 3 mg; Vit. B_2: 8 mg; Vit. B_3: 65 mg; Vit. B_5: 20 mg; Vit. B_6: 5 mg; Vit. B_7: 0.3 mg; Vit B_9: 2 mg; Vit. B_{12}: 0.02 mg; Iron ($FeSO_4$): 80 mg; Copper ($CuSO_4$): 8 mg; Manganese ($MnSO_4$): 60 mg; Zinc ($ZnSO_4$): 40 mg; Iodine (KI): 0.33 mg

group were measured to calculate dressing percentage on cold carcass weight. Furthermore, carcasses were separated into breasts and drumsticks with thighs that were weighed, and their percentage of total cold carcass weight was calculated.

Morphological and Histological Analyses

For morphological and histological analyses, tissue samples of the ileum and cecum were collected from 12 birds in each experimental group. Samples were fixed in 10% buffered formalin saline, dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), and then infiltrated with xylene and embedded in paraffin. Paraffin sections of 2 µm thickness were cut with a rotary type microtome and placed on glass slides. Sections were stained with Mayer's hematoxylin and eosin (HE) and with a combination of periodic acid Schiff stain and Alcian blue (PAS-AB) [21,22]. Histological sections were examined using a light microscope (Olympus BX53) with the objective magnifications x 4 and x 10. Morphometric examinations were carried out using Olympus cellSens software *(http://www.olympusamerica.com)*. For parameters of gut integrity (villus height, crypt depth, villus height/crypt depth ratio), 15 measurements for each parameter per animal were made (five villi per slide; three slides per animal). Villus height was measured over the vertical distance from the villus tip to villus-crypt junction level. Crypt depth was measured over the vertical distance from the villus-crypt junction to the lower limit of the crypt. Goblet cells were enumerated on 10 different villi along 500 µm of each villus surface, and goblet cell density was calculated.

pH and Microbiological Analyses

After evisceration, contents from the ileum and cecum were collected in sterile bags by squeezing the intestine gently. The pH of these contents was measured using a hand-held pH-meter Testo 205 (TestoAG, Lenzkirch, Germany). Collected ileum and cecum contents were held under the cold conditions (2°C) until microbiological analyses of total aerobic bacterial count, *Lactobacillus* spp. count, *Enterococcus* spp. count, and *Escherichia coli* count were conducted. A sterile stick was used to put 1 g of intestinal content into a sterile test tube together with buffered peptone-water, and this was resuspended by vortexing. Each pooled sample (0.1 mL) was serially diluted via 10-fold dilutions (from 10^{-1} to 10^{-9}). Duplicate plates of selective media were inoculated with 0.1 mL of each dilution to determine the bacterial species defined by standard laboratory methods. The total aerobic count was determined using standard plate count agar medium (OXOID). The plates were incubated at 30°C, aerobically for 24-48 h. Total *Enterococcus* spp. and *E. coli* were counted on UTI agar (urogenital tract infections agar) (HiMedia) after incubation for 24 h at 37°C, while total *Lactobacillus* counts were determined on selective MRS agar (de Man, Rogosa, and Sharpe) agar supplemented with 20 mg/mL vancomycin (Sigma Aldrich) and 2 mg/mL cefotaxime (Sigma Aldrich), after incubation for 72 h at 30°C. Micro-aerophilic atmospheres, used for lactobacilli growth on agar plates, were produced using the AnaerocultR C (Merck KgaA, Darmstadt, Germany). Bacterial colonies were counted immediately after removing plates from the incubator, and the bacterial numbers were expressed as $\log_{10}$ CFU per gram of digesta.

Statistical Analyses

Statistical analysis of the results was elaborated using software GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and pooled standard error of means (SEM). One-way ANOVA with Tukey's post hoc test was performed to assess the significance of differences among experimental groups. Statistical significance was considered at a level of $P<0.05$.

Results

Growth Performance of Broilers

Body weight of broilers did not differ on days 1 and 10 across the treatment groups *(Table 2)*. On days 24 and 42,

body weight of the group with 0.25 g/kg of added yeast was higher (P<0.05) than the group with no added yeast (0 g/kg). For the overall period of the trial, higher daily weight gain (P<0.05) was found in the group with 0.25 g/kg of added yeast than in the group fed with basic diet. During the third period (days 25-42) and for the overall period of the study, feed intake was higher (P<0.05) in the group with 0.25 g/kg of added yeast than in the two other experimental groups. Moreover, broilers fed only with basal diet had higher (P<0.05) feed conversion ratios during the second period (days 11-24) and for the overall period of the study compared to those supplemented with 0.25 g/kg and 0.65 g/kg of added yeast.

Carcass Quality of Broilers

A higher dressing percentage (P<0.05) was determined in the group with 0.25 g of added yeast/kg than in the group with no added yeast in diet *(Table 2)*. Supplementation of yeast significantly improved yields and the proportion of carcass cuts (breast and drumsticks with thighs), where the group with 0.25 g of added yeast/kg had higher (P<0.05) weight of breast and drumsticks with thighs compared to the control group. In addition, the third group (0.65 g/kg of yeast) did not differ in carcass quality parameters compared to the two experimental groups (0 g/kg and 0.25 g/kg of added yeast).

Intestinal Morphology of Broilers

In ileum, villus height was significantly higher in broilers supplemented with yeast (0.25 g/kg and 0.65 g/kg of added yeast) than in broilers fed with basic diet *(Table 3)*. Lower crypt depth (P<0.05) was found in the group supplemented with 0.25 g/kg of yeast compared to the control group of birds (0 g/kg of added yeast). In ileum, the villus height to crypt depth ratio was the highest in the experimental group with 0.25 g/kg of added yeast. Goblet cell density in ileum was higher in the group supplemented with 0.25 g/kg of yeast than in the control group. Supplementation of yeast with 0.25 g/kg resulted in higher villus height in cecum than in birds fed with basic diet. Moreover, crypt depth was lower and the villus height to crypt depth ratio was higher (P<0.05) in cecum of broilers with supplemented yeast (0.25 g/kg and 0.65 g/kg of added yeast) than in broilers with no yeast supplement. Goblet cell density in cecum did not differ among experimental groups.

Table 2. *Growth performance and carcass characteristics of broilers receiving diets containing different levels of yeast (Saccharomyces cerevisiae)*

Parameter	Day	Level of Yeast in Diet			SEM	P Value
		0 g/kg	0.25 g/kg	0.65 g/kg		
Body Weight (g/bird) (n=90	1	42.12	42.88	42.12	0.55	0.1561
	10	277.70	274.50	280.70	5.38	0.6708
	24	1071.00^{A}	1150.00^{B}	1113.00^{AB}	24.56	0.0005
	42	2499.00^{A}	2611.00^{B}	2548.00^{AB}	55.84	0.0511
Daily Weight Gain (g/bird) (n=90)	1-10	23.56	23.16	23.86	0.85	0.6039
	11-24	56.65^{A}	62.57^{B}	59.45^{C}	1.18	<0.0001
	25-42	84.00	85.94	84.41	2.01	0.4770
	1-42	58.50^{A}	61.14^{B}	59.64^{AB}	1.33	0.0532
Daily Feed Intake (g/bird) (n=6	1-10	34.50	33.78	34.31	1.18	0.7428
	11-24	80.14^{A}	82.43^{B}	81.14^{AB}	0.93	0.0288
	25-42	166.94^{A}	170.65^{B}	166.18^{A}	1.15	0.0005
	1-42	103.33^{A}	104.52^{B}	102.52^{A}	0.56	0.0018
Feed Conversion Ratio (n=6)	1-10	1.46^{A}	1.42^{B}	1.44^{AB}	0.02	0.0148
	11-24	1.42^{A}	1.32^{B}	1.36^{C}	0.01	<0.0001
	25-42	1.99	1.98	1.97	0.01	0.1694
	1-42	1.76^{A}	1.71^{B}	1.73^{C}	0.01	<0.0001
Carcass Weight (g) (n=30)	42	1899.00	2005.00	1959.00	19.19	0.1076
Dressing Percentage (%) (n=30)	42	73.99^{A}	74.90^{B}	74.49^{AB}	0.12	0.0175
Breast (g) (n=30)	42	737.60^{A}	805.30^{B}	758.60^{AB}	10.27	0.0175
Breast (%) (n=30)	42	38.33^{A}	39.27^{B}	38.59^{AB}	0.14	0.0323
Drumsticks with Thighs (g) (n=30	42	525.60^{A}	577.00^{B}	553.00^{AB}	7.25	0.0265
Drumsticks with Thighs (%) (n=30)	42	28.03^{A}	28.74^{B}	28.19^{AB}	0.11	0.0314

^{A,B,C} *Means within a row with the different superscript letter significantly differ at P<0.05; Tukey's tests were applied to compare means, SEM=Standard error of the mean*

Table 3. *Intestinal morphology of broilers receiving diets containing different levels of yeast (Saccharomyces cerevisiae)*

Segment of Intestine	Intestinal Morphology	Level of Yeast in Diet			SEM	P Value
		0 g/kg	0.25 g/kg	0.65 g/kg		
Ileum (n=12)	Villus height (μm)	1192.00^{A}	1448.00^{B}	1349.00^{B}	60.45	<0.0001
	Crypt depth (μm)	138.30^{A}	114.80^{B}	126.00^{AB}	6.18	0.0002
	Villus height/Crypt depth ratio	8.72^{A}	12.70^{B}	10.80^{C}	0.79	<0.0001
	Goblet cell density (cells/500 μm)	89.90^{A}	100.60^{B}	96.40^{AB}	4.47	0.0205
Cecum (n=12)	Villus height (μm)	198.00^{A}	225.60^{B}	212.80^{AB}	7.47	0.0004
	Crypt depth (μm)	42.20^{A}	32.20^{B}	31.40^{B}	1.66	<0.0001
	Villus height/Crypt depth ratio	4.74^{A}	7.06^{B}	6.81^{B}	0.34	<0.0001
	Goblet cell density (cells/500 μm)	49.50	54.20	53.60	2.99	0.1252

^{A,B,C} *Means within a row with the different superscript letter significantly differ at P<0.05; Tukey's tests were applied to compare means, SEM=Standard error of the mean*

Table 4. *Bacterial counts (log_{10} cfu/g) and pH value in ileal and caecal digesta of broilers receiving diets containing different levels of yeast (Saccharomyces cerevisiae)*

Parameter	Segment of Intestine	Level of Yeast in Diet			SEM	P Value
		0 g/kg	0.25 g/kg	0.65 g/kg		
Total Aerobic Bacterial Count (n=12)	Ileum	6.84	6.69	6.70	0.19	0.5763
	Cecum	8.23^{A}	7.65^{B}	7.69^{AB}	0.27	0.0227
Lactobacillus spp. (n=12)	Ileum	6.99	7.16	7.06	0.26	0.7341
	Cecum	7.65^{A}	7.98^{B}	7.88^{AB}	0.12	0.0042
Enterococcus spp. (n=12)	Ileum	6.66	7.08	7.31	0.33	0.0664
	Cecum	7.65	7.60	7.52	0.18	0.4356
E. coli (n=12)	Ileum	6.50^{A}	5.95^{B}	6.09^{AB}	0.25	0.0281
	Cecum	7.84	7.34	7.36	0.26	0.0363
pH value (n=12)	Ileum	6.80^{A}	6.45^{B}	6.47^{B}	0.14	0.0066
	Cecum	7.20^{A}	6.78^{B}	6.73^{B}	0.09	<0.0001

^{A,B} *Means within a row with the different superscript letter significantly differ at P<0.05; Tukey's tests were applied to compare means, SEM=Standard error of the mean*

Bacterial Counts and pH Value in Ileal and Caecal Digesta of Broilers

Supplementation of yeast did not affect counts of total aerobic bacteria, *Lactobacillus* spp. or *Enterococcus* spp. in ileum *(Table 4)*. On the contrary, a higher (P<0.05) number of *E. coli* was found in ileum of the control group than in the group of broilers supplemented with 0.25 g/kg of yeast. Broilers supplemented with yeast (0.25 g/kg) had lower count (P<0.05) of total aerobic bacteria and higher count of *Lactobacillus* spp. (P<0.01) in cecum than control broilers (0 g/kg of added yeast). Moreover, experimental groups did not differ in the number of *Enterococcus* spp. or *E. coli* in cecum. In addition, the pH of digesta in ileum and cecum of birds fed with basic diet was higher (P<0.05) than in groups supplemented with yeast (0.25 g/kg and 0.65 g/kg of added yeast) *(Table 4)*.

Discussion

In our study, the addition of live yeast in diet improved growth performance results in broilers. The group supplemented with 0.25 g/kg of added yeast achieved higher body weight than the group with no added yeast. Similar results were found for daily weight gain, feed intake, and feed conversion ratio. Our results are consistent with those previously reported by other authors [7,23-26]. Live yeast could enhance growth performance in animals by reducing adverse effects of pathogen bacterial colonization in gut [27], but also by improving nutrient digestibility in broilers [11]. Live yeast is a good source of small peptides, free amino acids, and nucleotides that are necessary for animal growth and have a high rate of digestion and absorption [24,28]. Moreover, live yeast contains growth factors like pro-vitamins and/or micronutrients that stimulate broiler growth [24]. Although in the previously mentioned studies, rates of improved growth performance results were proportional to their viable yeast count, in our study the group with the highest level of live yeast (0.65 g/kg) achieved similar growth performance results to the group with no added yeast. Similarly to our results,

Wang et al.[5] and He et al.[11] used the same concentration of *S. cerevisiae* as we did (1 x 10^{10} CFU/g), but added in higher levels in feed (0.5 g/kg, approximately 5 x 10^{9} CFU/kg; 1 g/kg, approximately 1 x 10^{10} CFU/kg; and 5 g/kg, approximately 5 x 10^{10} CFU/kg), and did not find any effect of supplemented yeast on broiler growth. It seems that high levels of live yeast adversely affect growth performance [29]. According to Reisinger et al.[30], high inclusion of live yeast in diet decreases broiler performance because of a potential over-reaction of the immune system.

Regarding carcass quality parameters, higher dressing percentage, yield, and participation of the most valuable carcass parts (breast and drumsticks with thighs) were determined in the group with the medium level of added yeast in feed (0.25 g/kg) than in the group fed only with basic diet. Similarly to growth performance, in our study, the carcass quality parameters of the group with the highest dietary yeast level (0.65g/kg) did not differ from the control group. As previously mentioned, this could be a consequence of detrimental effects of higher dietary yeast levels on animal growth and consequently on carcass quality [29]. Moreover, different doses of probiotics result in variations of growth performance and carcass quality, suggesting that the optimal level of probiotics depends on the microorganisms added into broiler feed [31].

In our study, adding live yeast to broiler diet notably improved intestinal morphology, both in ileum and cecum, in terms of higher villus height, lower crypt depth, and higher ratio of villus height to crypt depth. Our results are consistent with those found by other authors [10,11,29]. Probiotics induce the formation of short-chain organic acids that stimulate the proliferation of epithelial cells and lead to greater villus height [32]. The intestinal structure influences the absorptive capacity of gut, since greater villus height allows better contact with the digesta and absorption of nutrients. These changes are associated with better growth performance because of the larger absorption surface [33]. Moreover, chickens supplemented with yeast had higher expression of genes involved in differentiation of epithelial cells preferentially towards absorptive cells than secretory cells, resulting in an increase of villus height [29]. Moreover, in our study, goblet cell density in ileum was higher in the group supplemented with 0.25 g/kg of live yeast than in the control group. On the other hand, supplementation of live yeast did not affect goblet cell density in cecum of our broilers. Regarding yeast supplementation, other authors reported higher density of goblet cells in broilers fed diets supplemented with yeast or yeast products, suggesting that yeast induces proliferation of goblet cells as a defense mechanism [29,30,34]. In fact, Pascual et al.[29] found that higher density of the goblet cells was associated with their lower size, since they were constantly exposed to stimulus, producing and releasing mucin. A greater number of goblet cells and higher mucin production have protective effects under any challenging condition, protecting the epithelial cells from pathogenic microorganisms and mechanical damage [30,35]. Mucin produced by goblet cells is a key factor for normal intestinal function and the interaction between the immune system and intestinal microbiota [36]. Contrary to expectations, our group with the highest inclusion of yeast (0.65 g/kg) did not show any difference in goblet cell density compared to the control group, suggesting the high dose of *S. cerevisiae* (6.5 x 10^{9} CFU/kg) impaired goblet cell reproduction, which we speculate could reduce the broilers' resistance to pathogenic microorganisms.

In our study, supplementation of live yeast had a positive effect on the intestinal microbiota. We found lower total aerobic bacterial count and *E. coli* count, as well as higher *Lactobacillus* spp. count in both the ileum and cecum of the group with 0.25 g/kg of added yeast than in the control group. Beneficial changes of intestinal microflora after yeast supplementation in feed were also observed by other authors [5,34,37]. Other studies showed that supplementation of yeast improved the broiler performance due to inhibition of pathogen growth, seen for *E. coli* [10], *Salmonella* [38-40] and *Campylobacter* [38]. Beneficial effects of live yeast on intestinal microbiota could be ascribed to yeast's important components, mannanoligosaccharides and β-glucans. In fact, β-glucans could adsorb or bind toxins, viruses, and pathogenic bacteria, while mannanoligosaccharides act as prebiotics, providing nutrients for beneficial microbes in the gastrointestinal tract [28]. Recent studies have revealed direct binding of *S. cerevisiae* cell wall to pathogenic bacteria, *E. coli*, *Salmonella*, and *Listeria* [27]. Moreover, our broiler group with the highest level of added yeast achieved similar results, in terms of intestinal microbiota, as the control group, indicating this high dose of *S. cerevisiae* did not positively affect the intestinal microbiota. This could be a consequence of the very high concentration of yeast in the gut that compromised the growth of other beneficial microorganisms by competing for the same nutrients. However, the added yeast did increase the number of *Lactobacillus* in the gastrointestinal tract and reduced the pH in both ileum and cecum of our yeast-supplemented groups. One of the advantages of lower pH in guts is it creates a more unsuitable environment for the growth of pathogenic bacteria such as *E. coli* and *Salmonella* spp. [41].

In conclusion, the results from our study show that diet supplementation with yeast at a level of 0.25 g/kg results in better growth performance and carcass quality, improved intestinal morphology, and greater development of beneficial microbiota in guts of broilers than in the group fed only with the basic diet. We emphasize the importance of an appropriate level of *S. cerevisiae* in diet, since the

higher dose of live yeast used in our study (0.65 g/kg) produced broilers with similar growth performance, carcass quality, intestinal morphology, and gut microbiota as the group with no added yeast.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (M. Starčević).

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Ethical Statement

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (Resolution number: 01-511-2/2020/07/23).

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Competing Interests

The authors declare no conflict of interest.

Author Contributions

Experimental design was conceived by RM, DŠ and AR. Data were collected by ŽM, SR, and DP. Statistical analysis was conducted by JJ and MS. Original draft was written by MS and ŽM. All authors have contributed to the revision and final proof-reading of the manuscript.

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