



COMPARISON OF THE EFFECTS OF PROBIOTIC-BASED FORMULATIONS ON GROWTH, FEED UTILIZATION, BLOOD CONSTITUENTS, CECAL FERMENTATION, AND DUODENAL MORPHOLOGY OF RABBITS REARED UNDER HOT ENVIRONMENTAL CONDITIONS

Mahmoud Alagawany¹, Samar S. Bassiony¹, Mohamed S. El-Kholy¹, Karima El-Naggar², Abeer E. El-Metwally³, Adham A. Al-Sagheer^{4*}

¹Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

²Nutrition and Veterinary Clinical Nutrition Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria 21521, Egypt

³Pathology Department, Animal Reproduction Research Institute, Giza 3514805, Egypt

⁴Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

*Corresponding author: adham_alsahat@hotmail.com

Abstract

The present study aimed to assess the effects of three probiotic-supplemented diets on growth, cecal fermentation, blood biochemical, and intestinal morphological features in growing rabbits reared under summer conditions. Rabbits were allotted into four groups: G1 rabbits were fed the basal diet (control), G2 rabbits received *Enterococcus faecium* (EF) and *Clostridium butyricum* (CB) complexes (1×10^8 and 2.5×10^6 cfu/kg diet, respectively), G3 rabbits were given CB (2.5×10^6 cfu/kg diet) and yeast complexes (1 g/kg diet), and G4 rabbits received EF (2×10^8 cfu/kg diet) and yeast (1 g/kg diet). G2 rabbits exhibited the highest performances in terms of enhanced body weight and weight gain, protein efficiency ratio and feed conversion ratio ($P < 0.05$). Serum total protein, globulin, immunoglobulin M, and high-density lipoprotein concentrations were higher in probiotic-fed rabbits than those in controls. Additionally, lipid profile parameters were significantly reduced in the probiotic-fed rabbits, with the lowest concentrations measured in G4 rabbits ($P < 0.05$). Rabbits given EF and CB had the highest total volatile fatty acid (VFA) and propionic acid levels and the lowest ammonia concentrations. Increased villi length and muscular layer thickness and reduced crypt depth were observed in rabbits receiving EF and CB compared with the values obtained in controls ($P < 0.05$). In summary, supplementing fattening rabbit diets with EF and CB, as a novel formulation, might be a promising and easy method to enhance growth performance under hot climate conditions by improving the feed utilization, immune response, serum lipid profile, cecal VFA production, and duodenal morphology.

Key words: rabbit, probiotic, hot climate, growth, volatile fatty acids, blood

Efforts are currently made to produce, in a sustainable manner, food resources of high quality in quantities meeting the world's demand. Rabbits are one of the livestock species that could contribute to bridging the gap between food demand and supply (Oladimeji et al., 2022). They are characterized by a short generation period and are widely accepted as a high-quality animal protein source. Moreover, rabbit production is easy owing to the low management cost, high efficiency of utilization of fibrous diets, and limited competition with humans for grains compared to other species such as poultry (Haque et al., 2016). However, rabbit production is greatly influenced by climatic changes, especially high environmental temperatures, which is one of the biggest challenges for rabbit production in open or semi-closed housing systems in subtropical regions like Egypt (Daader et al., 2018).

Rabbits are homeothermic animals highly sensitive to hot climates because of their dense fur and few sweat glands, which lower the heat dissipation from their body and decrease their ability to control their internal temperature for ambient temperatures above 35°C (Al-Sagheer

et al., 2021; Ferraz et al., 2019). Thus, rabbits are susceptible to thermal stress. Several adverse effects of heat stress (HS) on rabbit productivity, including welfare, feed intake (FI) and utilization, growth, health status, and reproductive function, have been reviewed by Oladimeji et al. (2022). Currently, research is focused on management and dietary strategies to alleviate these adverse environmental conditions and maintain rabbit bodies' health and productivity. Ameliorative approaches, either managemental interventions, such as using well-ventilated housing facilities, lowering the stocking density, feeding at the cooler periods of the day (Abdel-Monem et al., 2009; Badr, 2015), or nutritional supplementation of feed or water using additives such as vitamins, natural antioxidants, and probiotics (Ayyat et al., 2018, 2021 a, b) have been effective practical measures against HS.

Because of widespread concerns about limiting the use of antibiotics and pharmaceuticals in the animal production industry, several investigations have been devoted to identifying alternative substances with a growth-promoting and immune-stimulating activity. Probiotic products are potential growth enhancers for use in the

rabbit production industry. They stimulate the immune system, promote feed digestion and absorption by producing enzymes and vitamins, improve gut functions by favoring gut colonization with beneficial bacteria, reducing pathogens, and lowering gut pH by producing lactic acid, and improve the intestinal barrier function (Chen et al., 2018; Abd El-Hack et al., 2020; Mancini and Paci, 2021). Commonly used probiotic strains include *Streptococcus* spp., *Lactobacillus* spp., *Saccharomyces* spp., enterococci, and *Bacillus* spp. They are used as feed bio-additives in different animal species. Previous studies have demonstrated that probiotics are beneficial for combating toxic gut flora, promoting immune system resistance against infectious agents, and improving feed digestion and absorption and digestive tract development (Chen et al., 2018). In the present work, three probiotic formulations were added to the diet of growing rabbits reared under summer conditions to assess their effects on growth performance, nutrient utilization, cecal fermentation, blood components, and intestinal morphology.

Material and methods

Rabbit care and experimental design

Rabbits were housed in galvanized wire cages measuring $35 \times 40 \times 60$ cm on a conventional indoor farm. These cages contained fresh, clean water delivered by automatic nip drinkers and manual feeders. Feed and water were presented *ad libitum*. The animals were housed in a semi-closed building with windows equipped with wire netting (0.9 m high from the floor) on the east side to enable natural ventilation. All rabbits were reared in the summertime, under natural temperature and light conditions (photoperiod cycle of 13.5/10.5 h light/dark). On average, the humidity and air temperature inside the farm were 58.05% and 32.56°C, respectively. The temperature–humidity index was 30.19 ± 0.38 throughout the experimental period, indicating that the rabbits had been exposed to acute HS according to Marai et al. (2001). Before starting the experiment, rabbits were acclimatized for one week, during which they were fed a basal diet (BD) (control diet without any probiotic supplements). The formulation of the BD followed the recommendations of De Blas and Mateos (2020) as shown in Table 1. The BD's chemical composition was determined based on the Association of Official Analytical Chemists (AOAC, 2006) standards. Rabbits in all groups were kept for 8 weeks under the same environmental, management, and sanitary conditions.

Eighty New Zealand White (NZW) rabbits, aged 35 days, were divided into four equal groups. Each group comprised a total of 10 replicates, each with two animals. The experimental groups were as follows: group 1 (G1) was fed the BD lacking probiotic supplements (control), G2 rabbits were fed the BD supplemented with *Enterococcus faecium* NCIMB 11181 (EF) and *Clostridium butyricum* (CB) complexes at 1×10^8 and 2.5×10^6 col-

ony-forming units (cfu)/kg diet, respectively, G3 rabbits received the BD supplemented with CB (2.5×10^6 cfu/kg diet) and yeast complexes (1 g/kg diet), and G4 rabbits were given the BD supplemented with EF (2×10^8 cfu/kg diet) and yeast complexes (1 g/kg diet). We purchased EF from Probiotics International Ltd. Company, UK, and CB (Clostri-mixVR) was obtained from Cheil Bio Company, Ltd. (Youngdungpo-Gu, Seoul, South Korea). Yeast was a commercial product (10×10^9 cells/g; Levitan, Dox-al Italia S.P.A., Italy). The doses utilized in the present study were based on earlier studies (Bassiony et al., 2021; Belhassen et al., 2016).

Table 1. Formulation and chemical analysis of the basal diet fed to the growing rabbits

Ingredient	g/kg diet
Alfalfa hay	340
Yellow corn	80
Barley grains	170
Wheat bran	220
Soybean meal	140
Cane molasses	30
Sodium chloride	5
Dicalcium phosphate	10
Mineral and vitamin mixture ¹	4
DL-methionine	1
Chemical analysis ²	
organic matter	913.7
crude protein ($n \times 6.25$)	170.9
ether extract	29.8
neutral detergent fiber	344.9
acid detergent fiber	176.5

¹Premix containing the following vitamins and minerals (per kilogram of diet): vitamin A, 8000 IU; vitamin D₃, 600 IU; vitamin E, 34 mg; vitamin K₃, 1.32 mg; vitamin B₁, 1.32 mg; vitamin B₂, 4.0 mg; vitamin B₆, 1.32 mg; vitamin B₁₂, 0.01 mg; pantothenic acid, 13.32 mg; biotin, 0.13 mg; folic acid, 3.32 mg; choline chloride, 800 mg; manganese 32 mg; zinc 60 mg; iron 120 mg; copper 16 mg; iodine 2 mg; selenium 0.4 mg; and cobalt 0.4 mg.

²According to AOAC (2006).

Performance parameters and carcass traits

All animals were separately weighed at 35 (initial weight), 63, and 91 days of age, and the body weight gain (WG) was calculated. Each cage's FI was monitored. Feed conversion ratio (FCR) was measured as the ratio of feed to WG (both in g). The protein efficiency ratio (PER) was the ratio of WG to protein intake (both in g). The relative growth rate (RGR) was obtained as follows:

$$RGR = \frac{(W_1 - W_0)}{1/2 (W_0 + W_1)} \times 100$$

where W_1 and W_0 are the final and initial weights, respectively.

Five rabbits per group were weighed and slaughtered at the end of the feeding experiment to evaluate the car-

cass qualities. The weights of the carcass and some internal organs, including the liver, spleen, heart, kidneys, and lungs, were documented. The dressing percentage was measured as follows:

$$\text{dressing percentage} = \frac{\text{carcass weight}}{\text{live weight}} \times 100$$

The relative organ weights were measured in g/kg of the rabbit's live weight.

Blood sampling

At the end of the feeding experiment, five rabbits per group were used for blood sampling from the lateral ear vein. Two separate blood samples per rabbit ($n = 2/\text{rabbit}$) were collected. The first sample was collected into ethylenediaminetetraacetic acid (EDTA) containing tubes to assess the blood picture. The second sample was used to isolate serum by centrifugation at 3,000 rpm for 20 min.

Hematological and biochemical parameters

Hematological parameters, including hemoglobin (Hb), total erythrograms (red blood cells [RBCs]), and total leukograms (white blood cells [WBCs]), were obtained according to Jain (1986). The mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined. In the serum samples, the total protein and protein fraction levels, immunological indices, and lipid profiles were spectrophotometrically estimated using commercial kits following the manufacturer's procedures.

Cecal volatile fatty acid (VFA) analysis

Cecal contents were directly obtained after rabbit slaughtering ($n = 5$ rabbits/group). Four layers of cheese-cloth were used to transport the contents of the cecum to a beaker. Then, microcentrifuge tubes containing 200 μL meta-phosphoric acid 25% (w/v) were filled with cecal content (1 mL) and centrifuged at $30,000 \times g$ (15,000 rpm, JA-17 rotor) for 20 min. Gas chromatography was utilized to analyze the supernatant's VFA content (Palmquist and Conrad, 1971).

Intestinal morphology

At 91 days of age, animals were sacrificed, and the small intestine was carefully removed from the abdomen. Each rabbit's duodenum was dissected into 2 cm-long segments, washed in saline, and fixed in 10% formalin solution for 24 h. Samples were prepared and stained with hematoxylin and eosin (Bancroft et al., 2012). Five sections per rabbit were examined under a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

Data were analyzed using one-way analysis of variance followed by Tukey's *post hoc* tests with SPSS21® software (IBM Cooperation, Armonk, NY, USA). If the

probability (P) was less than 0.05, the difference was considered significant. The assumptions of homogeneity and normality of variance were tested before analyzing data using Levene's test and Shapiro–Wilk test, respectively.

Results

Table 2 shows the rabbit growth and efficiency of feed utilization in response to probiotic supplementation. Probiotic addition resulted in significant differences ($P < 0.05$) in the body weight (BW) and WG of rabbits from all the groups over the eight experimental weeks compared with those of the control rabbits fed the probiotic-free diet (G1). G2 rabbits (receiving EF and CB mixture) had the highest BW and WG, which were lower for G4 and G3 rabbits and the lowest in the control group. The FI was not significantly different among all groups at all ages studied; however, there was a trend toward a reduced, albeit not significant, FI in the probiotic-fed groups. On the other hand, FCR was significantly improved in the probiotic-fed rabbits ($P < 0.05$) as it was decreased at 1–4 and 1–8 weeks of age, with the lowest FCR recorded in G2 rabbits. The PER was increased in rabbits who received probiotic supplements in their diet ($P < 0.05$), and the highest values were measured in G2 rabbits. A non-significant increase was observed in the RGR in rabbits given supplemental probiotics ($P > 0.05$). Neither the dressing percentage nor the internal organ relative weights were significantly affected by the supplementation with probiotics ($P > 0.05$) (Table 3).

Table 4 shows the cecal fermentation parameters. Probiotic addition to the rabbit diet modified the total VFA and ammonia concentrations in the cecum compared with those found in control rabbits fed the probiotic-free diet ($P < 0.05$). The total VFA concentration was the highest in G2 (EF + CB complex) and G3 (yeast + CB complex) rabbits, whereas it was the lowest in control rabbits. Similar results were obtained for the cecal propionate concentration as it increased significantly in response to the probiotic supplementation ($P < 0.05$). Acetic, butyric, and valerate acid concentrations were not significantly different among the different groups compared with those in control rabbits ($P > 0.05$), although they tended to increase in probiotic-fed rabbits. Additionally, the cecal ammonia concentration was significantly reduced in animals given probiotics, with the lowest value obtained in G2 rabbits.

The hematological parameters are presented in Table 5. The erythrogram and leukogram pictures of blood showed no significant changes among the different groups ($P > 0.05$). The WBC count was lower in G2 (EF + CB) and G3 (yeast + CB) rabbits, whereas it was increased in G4 (yeast + EF) compared with that in the control group ($P > 0.05$). Serum total protein levels were significantly increased in rabbits fed the probiotic-containing diets ($P < 0.05$) compared with those in the control rabbits, and the highest concentration was obtained in G4 (yeast +

EF) animals (Table 6). On the other hand, albumin and globulin levels and the albumin/globulin ratio (A/G ratio) were not significantly affected by the experimental treatments ($P>0.05$), although albumin concentration and A/G ratio were increased in G2 animals, whereas they were reduced in rabbits fed on the yeast-supplemented diets (G3 and G4) ($P>0.05$). In contrast, the serum globulin concentration was lower in G2 rabbits and the highest in G4 and G3 animals ($P>0.05$). Moreover, lipid profile parameters (total triglycerides [TG], total cholesterol [TC], low-density lipoprotein [LDL], and very-low-density lipoprotein [VLDL]) varied significantly with the probiotic addition ($P<0.05$). All these parameters were reduced in the probiotic-fed rabbits compared with the levels found in control rabbits, and the lowest concentrations were measured in G4 (yeast + EF) animals (Table 6). On the other hand, the concentration of serum high-density lipoprotein (HDL) was higher in G4 (yeast + EF) rabbits compared with that in the other groups ($P>0.05$). Regarding the immunological indices measured, serum immunoglobulin M (IgM) concentration was significantly increased by the supplementation with probiotics

compared with that in control rabbits ($P<0.05$), and the higher values were found in animals given EF (G4 and G2 rabbits). Additionally, a trend toward a higher IgG concentration was observed in probiotic-fed rabbits compared with that of control rabbits ($P>0.05$).

Table 7 shows the histomorphometry measurements in the intestine of growing rabbits in response to the different treatments. Rabbits that received EF and CB (G2) showed the greatest intestinal villi length, which was intermediary in G3 and G4 (yeast-containing diets) animals compared with those measured in the control group ($P<0.05$). Unlike the villi length, the villi width was reduced in probiotic-fed rabbits, with the smallest width found in G4 (yeast + EF) rabbits ($P<0.05$). Moreover, probiotic supplementation reduced the other morphological parameters, including the crypt depth, submucosal layer thickness, and tunica serosa thickness, with the lowest values recorded in G2 (EF + CB) animals compared with those measured in the other treatment groups. Additionally, the muscular layer thickness was greater in G2 rabbits than that in control rabbits (G1) or rabbits fed other probiotics (G3 and G4) ($P<0.05$).

Table 2. Growth performance of rabbits receiving or not probiotics and reared under summer conditions (n = 20/group)

Parameter	Experimental groups				SEM	P-value
	control	<i>E. faecium</i> + <i>C. butyricum</i>	yeast + <i>C. butyricum</i>	yeast + <i>E. faecium</i>		
Body weight (g)						
initial weight	679	685	682	681	9.97	0.988
4 weeks	1217	1392	1353	1351	28.94	0.185
8 weeks	1682 b	1950 a	1847 a	1897 a	31.61	0.014
Daily weight gain (g)						
1–4 weeks	19.21	25.08	23.96	23.93	1.02	0.210
5–8 weeks	16.65	19.94	17.64	19.48	0.71	0.360
1–8 weeks	17.93 b	22.51 a	20.80 ab	21.71 a	0.60	0.038
Feed intake (g/day)						
1–4 weeks	74.40	67.22	67.49	65.82	1.56	0.236
5–8 weeks	119.85	113.75	122.24	126.06	2.92	0.544
1–8 weeks	97.12	90.48	94.86	95.94	1.83	0.660
Relative growth rate (%)						
1–4 weeks	56.14	67.27	65.90	65.58	2.12	0.268
5–8 weeks	32.39	33.52	31.13	33.73	1.33	0.902
1–8 weeks	84.72	95.36	92.26	94.10	1.82	0.192
Feed conversion ratio						
1–4 weeks	4.09 a	2.82 b	2.85 b	2.80 b	0.18	0.021
5–8 weeks	7.20	5.81	7.26	6.54	0.25	0.162
1–8 weeks	5.48 a	4.10 b	4.57 b	4.44 b	0.15	0.007
Protein efficiency ratio						
1–4 weeks	1.51 b	2.21 a	2.08 a	2.15 a	0.11	0.077
5–8 weeks	0.81 b	1.03 a	0.84 ab	0.91 ab	0.04	0.151
1–8 weeks	1.08 b	1.48 a	1.29 ab	1.33 a	0.05	0.026

a–b – means in the same row with different letters are significantly different ($P<0.05$).

SEM, standard error of the mean.

Feed conversion ratio = feed intake (g)/weight gain (g); Protein efficiency ratio = weight gain (g)/protein intake (g).

Table 3. Carcass traits and relative weight of internal organs of growing rabbits receiving or not probiotics and reared under summer conditions (n = 5/group)

Parameter (g/100 g BW)	Experimental groups				SEM	P-value
	control	<i>E. faecium</i> + <i>C. butyricum</i>	yeast + <i>C. butyricum</i>	yeast + <i>E. faecium</i>		
Dressing	58.88	59.60	58.85	59.58	0.20	0.370
Liver	4.46	4.35	4.14	4.54	0.08	0.339
Kidneys	1.29	1.21	0.98	1.08	0.06	0.247
Heart	0.47	0.58	0.40	0.48	0.04	0.639
Lungs	1.05	1.07	0.97	1.07	0.05	0.890
Spleen	0.07	0.07	0.06	0.07	0.004	0.277

BW, body weight; SEM, standard error of the mean.

Table 4. Cecal fermentation parameters measured in growing rabbits receiving or not probiotics and reared under summer conditions

Parameter	Experimental groups				SEM	P-value
	control	<i>E. faecium</i> + <i>C. butyricum</i>	yeast + <i>C. butyricum</i>	yeast + <i>E. faecium</i>		
Cecal VFA concentration (mmol/100 ml)						
total	102.88 b	124.45 a	113.36 a	110.15 ab	2.79	0.028
acetate	80.01	94.96	89.59	82.83	2.43	0.111
propionate	6.64 c	10.42 a	8.67 a	7.49 ab	0.44	0.002
butyrate	15.21	17.99	13.93	18.61	0.75	0.065
valerate	1.02	1.08	1.17	1.22	0.05	0.473
Cecal VFA proportions (%)						
acetate	77.67	76.18	78.88	75.25	0.60	0.132
propionate	6.43	8.42	7.63	6.84	0.29	0.058
butyrate	14.93	14.54	12.46	16.81	0.73	0.212
valerate	0.978	0.86	1.03	1.11	0.04	0.084
Ammonia (mg/100 g)	16.26 a	10.10 b	12.57 b	11.86 b	0.77	0.006

a–c – means in the same row with different letters are significantly different (P<0.05).

VFAs, volatile fatty acids.

Table 5. Hematological parameters in growing rabbits receiving or not probiotics and reared under summer conditions

Parameter	Experimental groups				SEM	P-value
	control	<i>E. faecium</i> + <i>C. butyricum</i>	yeast + <i>C. butyricum</i>	yeast + <i>E. faecium</i>		
Erythrogram						
RBCs (10 ⁶ × μL)	4.85	4.66	4.97	4.48	0.12	0.537
Hb (g/dL)	11.17	11.63	12.13	10.67	0.28	0.310
Hct (%)	37.20	36.23	38.47	34.01	0.93	0.441
MCV (fL)	77.00	77.82	77.48	76.35	1.46	0.991
MCH (pg/dL)	23.07	24.97	24.44	23.87	0.34	0.237
MCHC (g/dL)	30.23	32.08	31.55	31.28	0.44	0.570
platelets (10 ³ /μL)	311.67	324.67	337.33	454.67	42.33	0.112
Leukogram						
WBCs (10 ³ /μL)	5.34	4.63	4.10	7.06	0.45	0.079
lymphocytes (%)	62.75	61.99	56.52	67.68	1.84	0.205
monocytes (%)	2.00	2.33	2.67	2.33	0.19	0.728
neutrophils (%)	28.25	33.35	38.81	26.65	2.20	0.198
eosinophils (%)	1.67	2.00	2.00	3.00	0.32	0.552

Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBCs, red blood cells; SEM, standard error of the mean; WBCs, white blood cells.

Table 6. Protein and lipid profiles and immunological indices in growing rabbits receiving or not probiotics and reared under summer conditions

Parameter	Experimental groups				SEM	P-value
	control	<i>E. faecium</i> + <i>C. butyricum</i>	yeast + <i>C. butyricum</i>	yeast + <i>E. faecium</i>		
Protein profile						
TP (g/dL)	5.27 b	6.62 a	6.59 a	7.24 a	0.25	0.010
ALB (g/dL)	2.91	3.77	2.92	3.05	0.33	0.552
GLOB (g/dL)	2.36	2.86	3.67	4.19	0.38	0.186
A/G (%)	1.23	1.32	0.80	0.73	0.34	0.537
Lipid profile						
TC (mg/dL)	228.02 a	189.67 b	201.01 ab	128.61 c	11.73	0.001
TG (mg/dL)	273.34 a	181.33 b	174.65 b	156.02 b	16.23	0.015
HDL (mg/dL)	38.69	48.31	46.34	52.04	2.51	0.317
LDL (mg/dL)	150.80 a	106.47 b	111.73 b	47.40 c	12.07	0.001
VLDL (mg/dL)	54.27 a	34.60 b	34.93 b	31.20 b	3.29	0.019
Immunological indices						
IgG (ng/mL)	16.47	20.13	24.33	24.27	1.40	0.126
IgM (mg/mL)	1.57 b	3.27 a	2.23 ab	3.37 a	0.28	0.039

a–c – means in the same row with different letters are significantly different ($P < 0.05$).

A/G, albumin/globulin ratio; ALB, albumin; GLOB, globulin; HDL, high-density lipoprotein; IgG, immunoglobulin G; IgM, immunoglobulin M; LDL, low-density lipoprotein; SEM, standard error of the mean; TC, total cholesterol; TG, total triglycerides; TP, total proteins; VLDL, very low-density lipoprotein.

Table 7. Histomorphometry measurements in the intestine of growing rabbits receiving or not probiotics and reared under summer conditions

Parameter	Experimental groups				SEM	P-value
	control	<i>E. faecium</i> + <i>C. butyricum</i>	yeast + <i>C. butyricum</i>	yeast + <i>E. faecium</i>		
Villus length (μm)	224.2 c	549.8 a	384.0 b	376.8 b	28.15	<0.001
Villus width (μm)	177.0 a	105.8 b	105.3 b	91.5 b	8.75	<0.001
Crypt depth (μm)	86.0 a	37.0 b	50.2 b	46.4 b	4.73	<0.001
Submucosa (μm)	131.0 a	70.4 b	84.0 b	83.4 b	5.75	<0.001
Muscle (μm)	69.4 b	103.6 a	71.8 b	70.6 b	3.68	<0.001
Serosa (μm)	60.6 a	36.0 b	50.0 ab	39.6 b	3.21	0.015

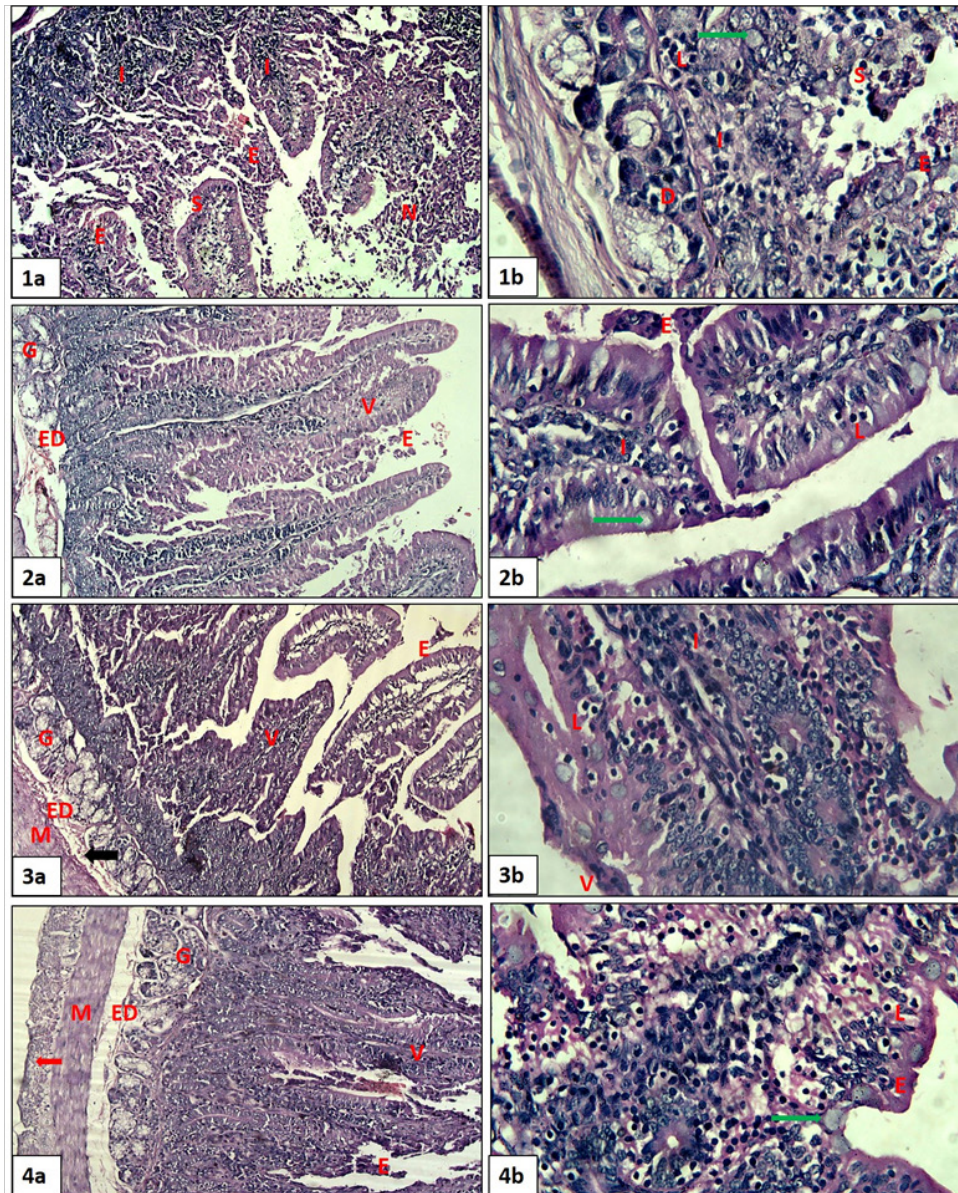
a–c – means in the same row with different letters are significantly different ($P < 0.05$).

SEM, standard error of the mean.

As illustrated in Figures 1 a and b, duodenal tissues of the control group presented obvious histological alterations. They were characterized by complete or partial enterocytes sloughing from the villi together with villus thickening, shortening, and flattening and fusion of neighboring villi. In addition, masses of necrosis were found inside the intestinal lumen, and there was localized necrosis, villi erosion, and lamina propria exposure. Evidence of epithelial vacuolar degradation was also found. There was mild hyperplasia and polyp development in the epithelial cells lining some of the villi. Lymphocytosis could be seen inside the epithelium. Some areas of the crypt revealed signs of atrophy, hyperplasia, and inflammation. In the control group, goblet cell loss was predominantly observed between enterocytes. Broadening of the stroma around the villi was apparent, and it was accompanied by infiltra-

tions of leukocytes, such as mononuclear cells and dispersed neutrophils, sometimes extending all the way to the submucosa. Congested and edematous submucosae with atrophy in the intestinal glands were evident. Partially thick serosa and a thinning of the muscle layer were found in some of the tissue samples (Figures 1 a and b).

These morphological alterations were attenuated in rabbits reared under HS conditions of the summer season and fed diets supplemented with yeast and EF or yeast and CB. Numerous fingerlike villi and crypts with some desquamation of the epithelial cell lining were observed in the mucosa. Goblet cells became more prominent. Mild edema and inflammatory cells appeared in the stroma and submucosa, and the thickness of the muscular layer and serosa had returned to apparently normal ranges (Figures 2 a and b; Figures 3 a and b).



D, vacuolar degeneration; E, epithelium; ED, edema; G, intestinal glands; I, inflammatory cell infiltrations; L, intraepithelial lymphocytosis; M, muscular layer; N, necrotic masses; S, erosions; V, villi. Green arrow, goblet cell; red arrow, serosa; black arrow, congestion.

Figure 1 a. Representative photograph of the duodenum of heat-stressed animals showing desquamation of the villi epithelium (E), erosion (S), short-fused villi, necrotic masses of intestinal lumen (N), stroma broadening, crypt loss, and inflammatory cell infiltrates (I) in mucosa and sub-mucosa (hematoxylin and eosin [H&E] staining, magnification: x100)

Figure 1 b. Representative photograph of the duodenum of heat-stressed animals showing damaged epithelium with desquamation (E), vacuolar degeneration of the epithelium (D), epithelial necrosis and erosions (S), marked villous stroma broadening accompanied with inflammatory cell infiltrations (I), intraepithelial lymphocytosis (L) and few goblet cells (green arrow) (H&E staining, magnification: x400)

Figure 2 a. Representative photograph of the duodenum of heat-stressed animals treated with yeast and *C. butyricum* showing mild epithelial dysregulation and desquamation (E), long villi (V), moderate sub-mucosal edema (ED), and moderate increase of intestinal glands (G) (hematoxylin and eosin [H&E] staining, magnification: x100)

Figure 2 b. Representative photograph of the duodenum of heat-stressed animals treated with yeast and *C. butyricum* showing mild epithelial desquamation (E), mild inflammatory cell infiltrations in the stroma (I), intraepithelial lymphocytosis (L), and moderate goblet cell loss (green arrow) (H&E staining, magnification: x400)

Figure 3 a. Representative photograph of the duodenum of heat-stressed animals treated with yeast and *E. faecium* showing villus dysregulation (V), epithelial desquamation of some villi (E), mild submucosal edema (ED), moderate hyperplasia of intestinal glands (G), thick muscular layer (M), and congested blood vessels (black arrow) (hematoxylin and eosin [H&E] staining, magnifications: x100)

Figure 3 b. Representative photograph of the duodenum of heat-stressed animals treated with yeast and *E. faecium* showing mild inflammatory cell infiltrations in the stroma (I) and intraepithelial lymphocytosis (L) (H&E staining, magnification: x400)

Figure 4 a. Representative photograph of the duodenum of heat-stressed animals treated with *E. faecium* and *C. butyricum* showing villi and crypts with mild sloughing of the epithelium (E), elongated villi (V), increased intestinal glands (G), mild submucosal edema (ED), thick muscular layer (M), and mild subserosal inflammatory cells (red arrow) (hematoxylin and eosin [H&E] staining, magnification: x100)

Figure 4 b. Representative photograph of the duodenum of heat-stressed animals treated with *E. faecium* and *C. butyricum* showing villi with nearly normal epithelial lining (E), normal goblet cell distribution (green arrow), and intraepithelial lymphocytosis (L) (H&E staining, magnification: x400)

Interestingly, the animals subjected to HS and given EF and CB had apparently normal intestinal crypts and villi. Thus, the use of EF and CB ameliorated the destructive effects of HS. The mucosal layer was characterized by numerous fingerlike villi that protruded into the lumen of the duodenum and lined with normal enterocytes and goblet cells. There was also a low stromal cellularity. Furthermore, microvilli lined the enterocytes bearing brush border membranes. In some villi, there was a mild sloughing of the epithelium. Intestinal glands, the muscular layer, and serosa appeared normal (Figures 4 a and b).

Discussion

Under hot summer temperatures, superior rabbit production is significantly restricted compared with that of other animals. Therefore, supplementing the diet or water is necessary to promote growth and stimulate the immune system of rabbits, thus improving their tolerance to these stressful conditions. Dietary supplementation with EF and CB mixed cultures alleviated the adverse effects of HS conditions by enhancing the rabbits' growth and blood biochemistry, stimulating their immunological response, and improving intestinal morphology.

Growing rabbits fed with the EF and CB complex under hot summer conditions showed the highest performance in final BW, WG, and RGR. The supplementation also improved the feed efficiency utilization determined using the FCR and PER. The growth-promoting effect of EF and CB may result from the synergistic effects of these probiotics. Additionally, the enhanced growth and feed efficiency might be attributed to the improved intestinal morphology as rabbits given EF and CB presented increased villi height and decreased CD and to a better intestinal microbial balance, resulting in better feed digestion and absorption. Indeed, Bassiony et al. (2021) found enhanced growth performance in rabbits fed EF or EF and CB and linked this response to the improvement in the intestinal mucosa's structure, which consequently promotes nutrient absorption (Samli et al., 2007). Pogány Simonová et al. (2020 b) also found that the administration of *E. faecium* EF9a in drinking water (1.0×10^9 cfu/mL in 500 µL/d/animal) of rabbits resulted in higher final body weight at 77 days of age and greater average daily WG compared with those of control rabbits. In addition, EF probiotic use ameliorates pig growth performance by improving the intestinal barrier function, resistance to infection, and the jejunal mucosa's absorptive and secretory ability (Kreuzer-Redmer et al., 2016). As one probiotic component, CB participated in the effect of the supplementation possibly by augmenting VFA production in the caecum, especially that of butyric acid, which is highly efficacious in improving feed utilization by harmonizing gut microbiota (Nakanishi et al., 2003). Indeed, Liu et al. (2019) found that CB dietary addition enhanced the WG of weaned Rex rabbits. This effect has been linked to enhanced activity of digestive enzymes,

including α -amylase, chymotrypsin, trypsin, and cellulase, and improved small intestinal mucosa morphology as shown by the increased villi length and reduced CD. Moreover, the growth performances of rabbits receiving yeast-based probiotics (yeast + CB or yeast + EF) were lower than those of animals given the bacterial-based probiotic (EF + CB), suggesting that there was an antagonistic effect between the yeast and bacteria supplemented in the same mixture. Similarly, El-Badawi et al. (2017) found that supplementation with 0.1% live yeast (*S. cerevisiae*) resulted in better performance of NZW rabbits than those given a mixed culture containing *S. cerevisiae* and *B. subtilis* and demonstrated that there was a competition between yeast and bacteria on sites of digestion and absorption through the gastrointestinal tract.

The fermentation process occurs in the rabbit cecum, and the final products, known as VFAs, are crucial to the animal's performance and the efficiency of feed utilization. In the present study, probiotic supplementation affected the total VFA, propionic acid, and ammonia concentrations. The cecum of rabbits fed EF and CB-supplemented diet under hot climates condition contained the highest total VFA and propionic acid levels and the lowest ammonia concentration. These findings were indicative of increased fermentation activity and cecal microbial production, better intestinal health, and high nitrogen retention. These results are in agreement with previous studies investigating the effects of adding EF, CB, or both to the diet of pigs (Wang et al., 2019), broiler chickens (Han et al., 2018), and broiler rabbits (Bassiony et al., 2021). Additionally, Cao et al. (2019) found that CB-based probiotic supplementation of weaned piglets' diet stimulates propionic and butyric acid production in the colon. Moreover, probiotic supplementation has been associated with lower ammonia concentration in the cecum, which might result from an increased ammonia utilization in the liver for protein production.

In the present study, hematological parameters, including the erythrogram and leukogram, were not different among groups suggesting that the supplemental doses of probiotics used here sustained the normal hematopoietic function of rabbits. Additionally, serum total protein and globulin concentrations were increased in probiotic-fed rabbits, especially in those given a EF and yeast-containing diet. Bassiony et al. (2021) reported the same response in rabbits that received EF and/or CB-supplemented diets. The present data suggested that supplemental probiotic strains influence protein metabolism (El-Shafei et al., 2019). Additionally, dietary supplementation with probiotics given to growing rabbits raised in summertime had a hypolipidemic effect (lower levels of TC, TG, LDL, and VLDL). This effect was the strongest in rabbits fed a diet supplemented with EF and yeast mixed culture. Our results are in line with those obtained by Bassiony et al. (2021). Additionally, Abd El-Aziz et al. (2021) found that adding 0.12 g *Saccharomyces cerevisiae* per kg of diet from growing rabbits resulted in reduced blood TG and TC levels and increased TP and

albumin concentrations and A/G ratio. Various hypotheses, such as an assimilation of cholesterol by probiotics (Pereira and Gibson, 2002), attachment of cholesterol to the probiotic cell walls (Liong and Shah, 2005), coprecipitation of cholesterol and deconjugated bile (Liong and Shah, 2006), enzymatic deconjugation of bile acids by probiotics bile-salt hydrolase (Lambert et al., 2008), and conversion of cholesterol into coprostanol (Lye et al., 2010), have been proposed to explain the cholesterol-lowering effect of probiotics.

Probiotics improve the immune response by stimulating the mucosal immune system, maintaining the intestinal barrier by modulating the gut microbiota, and producing microbial inhibitory compounds (Fortun-Lamothe and Boullier, 2007). In the present study, growing rabbits that received a probiotic-supplemented diet showed markedly enhanced immunoglobulin (IgG and IgM) levels compared with those in non-supplemented rabbits. In particular, the highest serum IgM concentration was obtained in rabbits given EF (with CB or yeast), suggesting that EF helps enhance specific immune functions in rabbits. Likewise, Bassiony et al. (2021) found an improvement in the non-specific immune response in probiotic-supplemented animals. This effect was shown to be mediated by higher lysozyme activity and serum complement component C3, the levels of which are increased in rabbits fed EF or EF and CB compared with those in control rabbits. Wu et al. (2019) proposed that modifying the expression of anti-inflammatory and pro-inflammatory cytokines and other immune mediators is a key mechanism driving EF immunomodulatory effects in poultry. Similarly, Pogány Simonová et al. (2020 b) reported that the administration of *E. faecium* EF9a in drinking water from growing rabbits is associated with an enhanced non-specific immune response shown by increased phagocytic activity and index. The immunomodulatory effect of these probiotics might also be linked to the connection between VFAs and immunological functions (Kelly et al., 2015). In this context, Wang et al. (2019) confirmed that CB and EF increase the synthesis of VFAs by modifying the gut flora's structure and enhancing immunity in weaned pigs. Moreover, Liu et al. (2019) found that supplementing Rex rabbit diet with CB stimulated the immune response by regulating cytokine synthesis through the Toll-like receptor 2/4 (TLR2/4) and improving the intestinal barrier function. Further research is required to determine the mechanisms underlying the immunomodulatory effects of EF and/or CB on rabbit health especially those related to VFAs.

One of the most important measures of digestive health is intestinal histomorphology (Shamoto and Yamauchi, 2000), and the villous height and villous high to crypt depth ratio are indicators of intestinal architecture. Increased villi length is correlated with active cell proliferation (Parker et al., 2017). It may also enhance enzyme secretion and digestion and improve the digestive tract's system for transporting nutrients by expanding the surface available for absorption (Awad et al., 2009). On

the other hand, reduced villous height and deeper crypts result in lower absorption of nutrients, which in turn impairs animal growth (Xu et al., 2003). In our experiment, growing rabbits raised during the summer and fed a diet supplemented with EF and CB showed significantly increased duodenal villous heights with reduced crypt depths. These results are consistent with studies that investigated the effects of dietary supplementation with CB or EF on intestinal morphology in growing rabbits (Liu et al., 2019; Bassiony et al., 2021) and in weaning pigs (Galeano et al., 2016; Wang et al., 2019). The increased villi length indicates that the intestinal absorption area is augmented, which in turn improves nutrient absorption and utilization and, consequently, the rabbit growing performance. The decreased crypt depth also accelerates the enterocyte regeneration rate, thus contributing to the restoration of intestinal villi (Salim et al., 2013). Moreover, the muscular layer thickness was greater in rabbits given EF and CB, suggesting higher gut integrity, which contributes to protecting against various infections. Likewise, Salim et al. (2013) found a thicker ileal muscularis externa in broilers given direct fed microbials. The beneficial effects of EF and CB supplementation on intestinal parameters might explain the improved performance of rabbits given EF and CB. Similarly, Pogány Simonová et al. (2015) reported that administering the *E. faecium* CCM7420 strain to rabbits has a beneficial impact on their WG due to better feed utilization and a wider absorptive surface in the gut. The improved intestinal morphology in the probiotic-supplemented rabbits might be associated with the increased VFA production in these groups. Pogány Simonová et al. (2020 a) also hypothesized that there is a positive correlation between cecal fermentation and WG, improved jejunal morphology (gut functionality), and nutrient absorption.

Conclusions

The dietary addition of the probiotics tested here alleviated thermal stress-induced negative effects on growing rabbits reared during summer months as it had a growth-promoting activity and enhanced the rabbit immune response and intestinal health. The EF and CB mixture was the most effective. Supplementing the diet of growing rabbits with the combined EF and CB probiotic strains at 1×10^8 and 2.5×10^6 cfu/kg diet, respectively, was effective in enhancing rabbit performance under hot climate conditions by improving their growth and feed utilization, specific immune response, serum lipid profile, cecal VFA production, and duodenal morphology parameters.

Conflict of interest statement

No potential conflicts of interest declared.

Ethical approval

All the procedures for this study were conducted in accordance with a protocol approved by the institutional animal care and use committee of Zagazig University (approval number: ZU-IACUC/2/F/268/2022).

References

- Abd El-Aziz A.H., Mahrose K.M., El-Kasrawy N.I., Alsenosy A.E.A. (2021). Yeast as growth promoter in two breeds of growing rabbits with special reference to its economic implications. *An. Acad. Bras. Cienc.*, 93: e20190274.
- Abd El-Hack M.E., El-Saadony M.T., Shafi M.E., Qattan S.Y.A., Batiha G.E., Khafaga A.F., Abdel-Moneim A.-M.E., Alagawany M. (2020). Probiotics in poultry feed: A comprehensive review. *J. Anim. Physiol. Anim. Nutr.*, 104: 18351850.
- Abdel-Monem U., Mahrose K., Khalil B. (2009). Effects of cage density and climatic conditions on the performance of growing rabbits. *Zag. Vet. J.*, 37: 198–208.
- Al-Sagheer A.A., Abdel Monem U.M., Sayed-Ahmed E.E., Khalil B.A. (2021). Navel orange peel hydroethanolic extract as a phyto-genic feed supplement: impacts on growth, feed intake, nutrient digestibility, and serum metabolites of heat stressed growing rabbits. *Anim. Biotechnol.*, 112.
- AOAC (2006). Official Methods of Analysis of AOAC International. 18th edition. AOAC International, Arlington, VA, USA.
- Awad W.A., Ghareeb K., Abdel-Raheem S., Böhm J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Sci.*, 88: 4956.
- Ayyat M.S., Al-Sagheer A.A., El-Latif A., Khaled M., Khalil B.A. (2018). Organic selenium, probiotics, and prebiotics effects on growth, blood biochemistry, and carcass traits of growing rabbits during summer and winter seasons. *Biol. Trace Elem. Res.*, 186: 162–173.
- Ayyat M., Abd El-Latif K., Helal A., Al-Sagheer A. (2021 a). New Zealand White rabbits tolerance to chronic thermal stress at different dietary energy/protein levels. *Anim. Feed Sci. Technol.*, 278: 114992.
- Ayyat M.S., El-Latif A., Khaled M., Helal A.A., Al-Sagheer A.A. (2021 b). Interaction of supplementary L-carnitine and dietary energy levels on feed utilization and blood constituents in New Zealand White rabbits reared under summer conditions. *Trop. Anim. Health Prod.*, 53: 279.
- Badr A. (2015). Effect of feeding time and vitamin C levels on performance of rabbit does during the mild and hot seasons in Egypt. *Nat. Sci.*, 13: 25–29.
- Bancroft J.D., Layton C., Suvarna S.K. (2012). Bancroft's theory and practice of histological techniques. Elsevier, Churchill Livingstone, 7th ed., 6993 pp.
- Bassiony S.S., Al-Sagheer A.A., El-Kholy M.S., Elwakeel E.A., Helal A.A., Alagawany M. (2021). Evaluation of *Enterococcus faecium* NCIMB 11181 and *Clostridium butyricum* probiotic supplements in post-weaning rabbits reared under thermal stress conditions. *Ital. J. Anim. Sci.*, 20: 12321243.
- Belhassen T., Bonai A., Gerencsér Z.S., Matics Z.S., Tuboly T., Bergaoui R., Kovacs M. (2016). Effect of diet supplementation with live yeast *Saccharomyces cerevisiae* on growth performance, caecal ecosystem and health of growing rabbits. *World Rabbit Sci.*, 24: 191–200.
- Cao G., Tao F., Hu Y., Li Z., Zhang Y., Deng B., Zhan X. (2019). Positive effects of a *Clostridium butyricum*-based compound probiotic on growth performance, immune responses, intestinal morphology, hypothalamic neurotransmitters, and colonic microbiota in weaned piglets. *Food Funct.*, 10: 29262934.
- Chen L., Li S., Zheng J., Li W., Jiang X., Zhao X., Li J., Che L., Lin Y., Xu S., Feng B., Fang Z., De W. (2018). Effects of dietary *Clostridium butyricum* supplementation on growth performance, intestinal development, and immune response of weaned piglets challenged with lipopolysaccharide. *J. Anim. Sci. Biotechnol.*, 9: 62.
- Daader A., Al Sagheer A., Gabr H., Abd El Moniem E. (2018). Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants. *Span. J. Agric. Res.*, 16: e0610.
- De Blas C., Mateos G.G. (2020). Feed formulation. In: Nutrition of the rabbit, De Blas C., Wiseman J. (eds). CABI, Oxfordshire, London, UK, pp. 243–253.
- El-Badawi A.Y., Helal F.I.S., Yacout M.H.M., Hassan A.A., El-Naggar S., Elsabaawy E.H. (2017). Growth performance of male NZW rabbits fed diets supplemented with beneficial bacteria or live yeast. *Agric. Eng. Int.: CIGR J.*, pp. 220–226.
- El-Shafei A., Younis T., Al-Gamal M., Hesham A. (2019). Impact of probiotic (*Lactobacillus planterium*) supplementation on productive and physiological performance of growing rabbits under Egyptian conditions. *Egypt. J. Rabbit Sci.*, 29: 125–148.
- Ferraz P., Ferraz G., Barbari M., Majg S., Damasceno F., Cecchin D., Castro J. (2019). Behavioural and physiological responses of rabbits. *Agron. Res.*, 17: 704710.
- Fortun-Lamothe L., Boullier S. (2007). A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. *Livest. Sci.*, 107: 118.
- Galeano J., Herrera A., Suescun J. (2016). The probiotic *Enterococcus faecium* modifies the intestinal morphometric parameters in weaning piglets. *Rev. Fac. Nac. Agron. Medellín*, 69: 7803–7811.
- Han J., Wang Y., Song D., Lu Z., Dong Z., Miao H., Wang W., He J., Li A. (2018). Effects of *Clostridium butyricum* and *Lactobacillus plantarum* on growth performance, immune function and volatile fatty acid level of caecal digesta in broilers. *Food Agric. Immunol.*, 29: 797807.
- Haque A., Rahman M., Bora J. (2016). Effect of breed, weaning age and feeding regime on chemical composition of rabbit meat. *Int. J. Vet. Sci. Anim. Husband.*, 1: 12–13.
- Jain N.C. (1986). Schalm's veterinary hematology. Lea and Febiger, Philadelphia, USA.
- Kelly C.J., Zheng L., Campbell E.L., Saeedi B., Scholz C.C., Bayless A.J., Wilson K.E., Glover L.E., Kominsky D.J., Magnuson A., Weir T.L., Ehrentauf S.F., Pickel C., Kuhn K.A., Lanis J.M., Nguyen V., Taylor C.T., Colgan S.P. (2015). Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial hif augments tissue barrier function. *Cell Host Microbe*, 17: 662671.
- Kreuzer-Redmer S., Bekurtz J.C., Arends D., Bortfeldt R., Kutz-Lohroff B., Sharbati S., Einspanier R., Brockmann G.A. (2016). Feeding of *Enterococcus faecium* NCIMB 10415 leads to intestinal miRNA-423-5p-induced regulation of immune-relevant genes. *Appl. Environ. Microbiol.*, 82: 22632269.
- Lambert J.M., Bongers R.S., de Vos W.M., Kleerebezem M. (2008). Functional analysis of four bile salt hydrolase and penicillin acylase family members in *Lactobacillus plantarum* WCFS1. *Appl. Environ. Microbiol.*, 74: 47194726.
- Liong M.T., Shah N.P. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J. Dairy Sci.*, 88: 5566.
- Liong M.T., Shah N.P. (2006). Effects of a *Lactobacillus casei* synbiotic on serum lipoprotein, intestinal microflora, and organic acids in rats. *J. Dairy Sci.*, 89: 13901399.
- Liu L., Zeng D., Yang M., Wen B., Lai J., Zhou Y., Sun H., Xiong L., Wang J., Lin Y., Pan K., Jing B., Wang P., Ni X. (2019). Probiotic *Clostridium butyricum* improves the growth performance, immune function, and gut microbiota of weaning rex rabbits. *Probiotics Antimicrob. Proteins*, 11: 12781292.
- Lye H.S., Rusul G., Liong M.T. (2010). Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. *J. Dairy Sci.*, 93: 13831392.
- Mancini S., Paci G. (2021). Probiotics in rabbit farming: Growth performance, health status, and meat quality. *Animals*, 11: 3388.
- Marai I.F.M., Ayyat M.S., El-Monem A. (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Trop. Anim. Health Prod.*, 33: 451–462.
- Nakanishi S., Kataoka K., Kuwahara T., Ohnishi Y. (2003). Effects of high amylose maize starch and *Clostridium butyricum* on metabolism in colonic microbiota and formation of azoxymethane-induced aberrant crypt foci in the rat colon. *Microbiol. Immunol.*, 47: 951958.
- Oladimeji A.M., Johnson T.G., Metwally K., Farghly M., Mahrose K.M. (2022). Environmental heat stress in rabbits: implications and ameliorations. *Int. J. Biometeorol.*, 66: 111.
- Palmquist D., Conrad H. (1971). Origin of plasma fatty acids in lactating cows fed high grain or high fat diets. *J. Dairy Sci.*, 54: 10251033.

- Parker A., Maclaren O.J., Fletcher A.G., Muraro D., Kreuzaler P.A., Byrne H.M., Maini P.K., Watson A.J., Pin C. (2017). Cell proliferation within small intestinal crypts is the principal driving force for cell migration on villi. *FASEB J.*, 31: 636649.
- Pereira D.I., Gibson G.R. (2002). Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. *Appl. Environ. Microbiol.*, 68: 46894693.
- Pogány Simonová M., Lauková A., Žitňan R., Chrástínová L. (2015). Effect of rabbit origin enterocin-producing strain *Enterococcus faecium* CCM7420 application on growth performance and gut morphometry in rabbits. *Czech J. Anim. Sci.*, 60: 509–512.
- Pogány Simonová M., Chrástínová L., Lauková A. (2020 a). Autochthonous strain *Enterococcus faecium* EF2019(CCM7420), its bacteriocin and their beneficial effects in broiler rabbits – a review. *Animals*, 10: 1188.
- Pogány Simonová M., Lauková A., Chrástínová L., Plachá I., Szabóová R., Kandričáková A., Žitňan R., Chrenková M., Ondruška L., Bónai A., Maticszs ZS., Kovács M., Strompfová V. (2020 b). Beneficial effects of *Enterococcus faecium* EF9a administration in rabbit diet. *World Rabbit Sci.*, 28: 169.
- Salim H.M., Kang H.K., Akter N., Kim D.W., Kim J.H., Kim M.J., Na J.C., Jong H.B., Choi H.C., Suh O.S., Kim W.K. (2013). Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. *Poultry Sci.*, 92: 20842090.
- Samli H.E., Senkoylu N., Koc F., Kanter M., Agma A. (2007). Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. *Arch. Anim. Nutr.*, 61: 4249.
- Shamoto K., Yamauchi K. (2000). Recovery responses of chick intestinal villus morphology to different refeeding procedures. *Poultry Sci.*, 79: 718723.
- Wang K., Cao G., Zhang H., Li Q., Yang C. (2019). Effects of *Clostridium butyricum* and *Enterococcus faecalis* on growth performance, immune function, intestinal morphology, volatile fatty acids, and intestinal flora in a piglet model. *Food Funct.*, 10: 78447854.
- Wu Y., Zhen W., Geng Y., Wang Z., Guo Y. (2019). Effects of dietary *Enterococcus faecium* NCIMB 11181 supplementation on growth performance and cellular and humoral immune responses in broiler chickens. *Poultry Sci.*, 98: 150163.
- Xu Z.R., Hu C.H., Xia M.S., Zhan X.A., Wang M.Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Sci.*, 82: 10301036.

Received: 7 IX 2022

Accepted: 9 I 2023