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Effect of the use of *Yarrowia lipolytica* and *Saccharomyces cerevisiae* yeast with a probiotic in the diet of turkeys on their gut microbiota and immunity

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Abstract: An experiment was carried out to determine whether the yeast species *Yarrowia lipolytica* added to compound feeds for turkeys would have a more beneficial effect on their immunity and gut microflora composition than the commonly used species *Saccharomyces cerevisiae*. An additional aim of the study was to test whether the addition of a probiotic (*Bacillus licheniformis* and *Bacillus subtilis*) to the feed containing *Yarrowia lipolytica* or *Saccharomyces cerevisiae* yeast would enhance their effect. The experiment was carried out on growing turkey hens aged 7 to 112 days and randomly divided into six group/s (each n = 80). The birds in the control group (C) and group P were fed standard feeds, but group P additionally received a probiotic. Groups Y and YP received the feed containing the *Yarrowia lipolytica* fodder yeast, and the probiotic for the YP group. Similarly, in groups S and SP, the turkeys received the feed with the *Saccharomyces cerevisiae* fodder yeast, and the probiotic was additionally added to the feed for the SP group. *Yarrowia lipolytica* yeast can be an alternative to the commonly used yeast *Saccharomyces cerevisiae* in turkey feeds. *Yarrowia lipolytica* favourably influenced the intestinal microbiota (reduced the number of microorganisms – $P < 0.001$, fungi – $P < 0.001$, and coliforms – $P < 0.001$, including *E. coli*), and stimulated erythropoiesis (increased Hb content – $P = 0.049$ and RBC count – $P = 0.027$; increased Ht – $P < 0.001$) and immune mechanisms (increased the %pc – $P = 0.021$, NBT value – $P = 0.013$ and lysozyme content – $P = 0.013$; decreased IgM concentration – $P = 0.049$). The combined use of a probiotic with yeast, particularly *Yarrowia lipolytica*, has a more beneficial effect on the gut microbiota than the use of *Yarrowia lipolytica* alone. The combined use of a probiotic with a yeast, particularly *Yarrowia lipolytica*, has a more beneficial effect on the gut microbiota than the use of *Yarrowia lipolytica* alone.

Keywords: blood; *Bacillus*; immunological parameters

Currently, there is an increasing interest in the feed industry in yeast distinguished by rapid growth on lipid substrates, such as *Yarrowia lipolytica* (*YL*). This strain uses the glycerol fraction generated during the biofuel production to produce a biomass, which is important in terms of environmental protection (Michalik et al. 2013). In comparison with *Saccharomyces cerevisiae* (*SC*), *YL* yeast has three times the content of fat and valuable amino acids, including lysine, which makes it a more valuable

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source of protein for animals. Moreover, the fat accumulated in the cells contains over 90% unsaturated fatty acids, and 28–44% are essential fatty acids. *Yarrowia lipolytica* is currently used as a valuable protein source for livestock and for the production of carotenoids, organic acids and enzymes.

Benefits of the use of *YL* yeast have been demonstrated in turkey hens, in which it has had a positive effect on the erythrocyte indicators, stimulated immune mechanisms (Czech et al. 2014), and antioxidant mechanisms (Merska et al. 2015). The immunostimulatory effect of yeasts, which has been confirmed in many experiments, is mainly due to the presence of beta-glucans in their cell wall (Russo et al. 2012). Hence the use of the yeast, in combination with the probiotic bacteria, referred to as a symbiotic, is justified and can be more beneficial than the use of these additives individually. The use of symbiotics in poultry has been shown to improve the growth performance (Li et al. 2011), strengthen the immune mechanisms, and beneficially influence the gastrointestinal morphology (El-Sissi and Mohamed 2011). The combined administration of a probiotic with a prebiotic can also more effectively prevent constipation and diarrhoea. Due to the high selectivity of their activity, choosing the right combination of a probiotic and prebiotic to achieve the intended results in poultry is crucial and requires thorough research. The hypothesis, therefore, was that the yeast species *Yarrowia lipolytica* added to compound feeds for turkeys would have a more beneficial effect on their immunity and gut microflora composition than the commonly used species *Saccharomyces cerevisiae*.

Therefore, an experiment to this effect was carried out. An additional aim of the study was to test whether the addition of a probiotic to the feed containing *Yarrowia lipolytica* or *Saccharomyces cerevisiae* yeast would enhance their effect.

MATERIAL AND METHODS

Animals

For the experiment, one-week-old female 480 BIG6 turkey hens were used. They were randomly divided into six groups of 80 (five repetitions of 16 turkeys) and raised to the age of 112 days.

During the experiment, the birds in all the groups received *ad libitum* complete feeds balanced according to NRC (National Research Council 1994) for each rearing period (Starter: 1–4 weeks; Grower 1: 5–8 weeks; Grower 2: 9–12 weeks; Finisher: 13–16 weeks).

The birds in all the experimental groups received feed based on wheat (45.3–47.5% for all the rearing periods) and soybean meal (19.5–40.5% for all the rearing periods) and additionally triticale (10% – Grower 2 and Finisher), rapeseed cake (4% – Grower 1; 6% – Grower 2; 9% – Finisher) and potato protein (5% – Starter). Soybean oil was added to all the feeds. The results of the chemical analyses of the feeding mixtures are presented in Czech et al. (2020).

The birds in the control group (C) and group P were fed standard feeds without the addition of yeast, but group P additionally received a probiotic which was a mixture of *Bacillus licheniformis* – 1.6×10^9 CFU/g and *Bacillus subtilis* – 1.6×10^9 CFU/g in the amount of 0.05%. Groups Y and YP received the feed containing 3% *Yarrowia lipolytica* (Scotan S.A, Poland) fodder yeast, and the probiotic was added to the feed in the amount of 0.05% (0.5 kg per ton of feed) for the YP group.

Similarly, in groups S and SP, the turkeys received the feed with 3% *Saccharomyces cerevisiae* fodder yeast, and the probiotic was added to the feed in the amount of 0.5 kg per tonne of feed (Table 1) for the SP group.

Table 1. Experimental design

Feeding group		Yeast		
		without (control)	3% *Yarrowia lipolytica*	3% *Saccharomyces cerevisiae*
Probiotic	without (control)	C	Y	S
	Bacillus licheniformis (1.6×10^9 CFU/g) and *Bacillus subtilis* (1.6×10^9 CFU/g) in the amount of 0.05%	P	YP	SP

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Experimental and laboratory procedures

Blood profile. At the end of the experiment, at 112 d of age, blood was drawn from the wing vein of 10 turkeys from each group. The blood was collected after eight hours with no access to the feed.

In the samples of the whole blood, the haematocrit (Ht) was determined by the microhaematocrit method, and the haemoglobin content (Hb), leukocyte count (WBC) and erythrocyte count (RBC) were determined by standard methods. The percentage composition of the white blood cells (leukogram) was determined as well, i.e., the number of heterophils (HET), lymphocytes (LYM), monocytes (MON), eosinophils (EOS) and basophils (BAS), by Pappenheim staining.

In the blood of the turkeys, the phagocytic activity of the leukocytes was determined against the *Staphylococcus aureus* strain 209P and expressed as the percentage of the phagocytic cells (%pc) and the phagocytic index (PI) according to Siwicki et al. (1994). The ability of the heterophils to absorb and reduce nitrotetrazolium blue (NBT test) was determined according to Park et al. (1968). The serum lysozyme was measured by the turbidimetric method (Siwicki and Anderson 1993). The total serum IgY and IgM concentrations were determined using a commercial Turkey ELISA (Enzyme-linked immunosorbent assay) kit (KA2515, Abnova, Taiwan).

Microbiological analysis. The contents of the small intestine were collected from ten individuals from each group after slaughter. The following indicators were analysed in the homogenised material: the total number of mesophilic aerobic bacteria – on a nutrient agar; the total number of fungi and moulds – on a DG18 agar; the total number of coliform bacteria – on a VRBL agar; the total number of *Escherichia coli* bacteria – on an mFC agar. After a specified incubation time, the colonies were counted with an automatic counter. To identify the yeast-like fungi, the colonies were evaluated macroscopically and then transferred to a Sabouraud agar. The colonies were assessed macro- and microscopically and Gram staining was performed. The final identification was made using an API 20 C AUX test (bioMérieux Polska; Warsaw, Poland). All the tests were performed in accordance with the standards and EN-ISO 7218:2007/A1:2013.

Statistical analysis. The statistical calculations of the microbiological analysis of the intestinal contents and the analysis of the blood parameters were performed by the one-way analysis of variance (ANOVA), taking the influence of the feeding group described in Table 1 into account. The calculations were performed using general linear models (SAS v9.4). The research hypotheses (comparisons of the effects of the experimental factors) were verified using predefined orthogonal contrasts with the Bonferroni correction.

RESULTS

The results of the quantitative microbiological analysis of the small intestinal microflora are presented in Table 2. The addition of the probiotic (C+Y+S *vs* P+YP+SP) or *YL* yeast (C+P *vs* Y+YP) reduced the number of microorganisms ($P < 0.001$), fungi ($P < 0.001$), and coliforms ($P < 0.001$ and $P = 0.042$, respectively), including *E. coli* ($P = 0.040$ and $P = 0.032$, respectively), relative to the groups not receiving these additives (Table 3). In contrast, while the addition of *SC* reduced the number of microorganisms, it caused an increase in the number of fungi relative to the groups whose feed did not include these yeasts. The biological material obtained from the turkeys receiving the *SC* yeast in their feed contained more microorganisms ($P < 0.001$), fungi $P < 0.001$), and *E. coli* bacteria ($P = 0.048$ and $P = 0.042$, respectively) than the material from the birds receiving the *YL* yeast (Y+YP *vs* S+SP; YP *vs* SP). The combined addition of the probiotic and yeast (Y+S *vs* YP+SP; Y *vs* YP) reduced the number of coliforms, including *E. coli*, in the material. No pathogenic bacteria of the genera *Salmonella*, *Proteus* or *Pseudomonas* or β-haemolytic *Escherichia coli* were found in the material (Table 3).

The values of the immunological indicators are presented in Table 2. The addition of *YL* increased the %pc ($P = 0.021$), the NBT value ($P = 0.013$) and the lysozyme content ($P = 0.013$), but it decreased the IgM ($P = 0.049$) concentration (C+P *vs* Y+YP). The addition of *SC*, as in the case of *YL*, increased the %pc, the PI and decreased the serum IgM concentration (Table 3).

The comparison of the effect of *YL* with that of *SC* (Y+YP *vs* S+SP) shows that the addition of *SC* reduced the %pc ($P = 0.028$), and increased the PI ($P = 0.005$), as well as the NBT value ($P = 0.008$) (Table 3).

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Table 2. Microbiological analysis of the intestinal contents and haematological and immunological indicators in the turkey's blood

Item	Feeding groups[1]					
	C	P	Y	YP	S	SP
Microorganisms (CFU/g)						
Microbes	9.70×10^4	7.62×10^4	1.26×10^4	1.28×10^4	2.86×10^4	2.60×10^4
Fungi	3.2×10^2	1.7×10^2	2.4×10^2	1.5×10^1	2.9×10^2	4.8×10^2
Coliforms	2.7×10^2	1.1×10^2	1.8×10^2	1.5×10^2	2.0×10^2	1.7×10^2
E. coli	1.1×10^2	8.7×10^1	9.1×10^1	7.9×10^1	1.0×10^2	9.2×10^1
Immunological indicators						
%pc	46.33	49.15	52.33	46.50	48.42	54.67
PI	4.58	5.42	4.58	5.67	6.67	4.83
NBT	22.93	26.89	28.08	25.08	24.50	24.92
Lysozyme (μg/ml)	0.336	0.346	0.404	0.382	0.371	0.350
IgY (ng/ml)	10.21	11.42	10.43	12.65	10.32	11.02
IgM (ng/ml)	41.33	45.09	40.54	43.76	40.22	42.98
Red blood cell indicators						
Ht (l/l)	0.25	0.25	0.27	0.27	0.26	0.27
Hb (mmol/l)	7.53	7.49	7.76	8.02	7.21	7.38
RBC (10^{12}/l)	2.91	3.08	3.46	3.91	3.31	3.45
White blood cell indicators						
WBC (10^9/l)	31.92	30.87	27.05	33.59	27.51	24.19
HET (%)	45.50	42.67	37.67	42.17	49.33	48.50
LYM (%)	44.67	46.67	52.00	48.00	44.50	40.67
H : L	1.04	0.94	0.73	0.89	1.12	1.20
MONO (%)	4.67	6.33	5.83	6.50	3.50	7.17
EOS (%)	3.83	4.00	4.50	2.80	3.67	3.00
BAS (%)	2.67	2.50	1.80	3.00	1.25	1.75

[1]C = control; P = the addition of the probiotic to the feed; S = 3% share of *Saccharomyces cerevisiae* in the feed; SP = the addition of the probiotic and the 3% share of *Saccharomyces cerevisiae* in the feed; Y = 3% share of *Yarrowia lipolytica* in the feed; YP = the addition of the probiotic and the 3% share of *Yarrowia lipolytica* in feed

The addition of the probiotic (C+Y+S *vs* P+YP+SP) caused a significant increase in the serum IgY and IgM concentration ($P = 0.037$ and $P = 0.004$, respectively).

The addition of the probiotic together with the *SC* yeast contributed to a significant increase in the %pc and IgM concentration ($P < 0.001$ and $P = 0.012$, respectively), but decreased the PI value ($P = 0.046$) (S *vs* SP). In contrast, the combined use of *YL* with a probiotic (Y *vs* YP) caused a significant reduction in the NBT value ($P = 0.003$), the %pc ($P = 0.001$) and an increase in the PI ($P = 0.025$). It also contributed to a significant increase in the serum IgY and IgM concentration ($P = 0.017$ and $P = 0.011$, respectively).

The comparison of the use of yeast with and without a probiotic (Y+S *vs* YP+SP) revealed that the combination of the probiotic with the yeast led to a significant increase in the IgY and IgM concentration in the serum ($P = 0.039$ and $P = 0.018$, respectively) (Table 3).

The values of the haematological variables are presented in Table 2. It should be noted, that the haematological profile of the turkeys in all the experimental groups was within the ranges presented by other authors (Ozsoy and Yalcin 2011).

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Table 3. Estimators of the differences and their *P*-values in the mean experimental factors of the microorganisms in the intestinal contents and the immune indicators in the serum

Contrast[1]	C+P *vs* Y+YP		C+P *vs* S+SP		Y+YP *vs* S+SP		C+Y+S *vs* P+YP+SP		Y *vs* YP		S *vs* SP		YP *vs* SP		Y+S *vs* YP+SP	
Trait	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*
Microbes	73 900	< 0.001	59 300	< 0.001	−14 600	< 0.001	7 733	< 0.001	−200	0.885	2 600	0.069	−13 200	< 0.001	1 200	0.226
Fungi	119.31	< 0.001	−140.3	< 0.001	−259.6	< 0.001	61.53	< 0.001	223.2	< 0.001	−188.0	< 0.001	−465.2	< 0.001	17.60	0.095
Coliforms	19.50	0.042	2.50	0.124	−17.00	0.139	71.33	< 0.001	27.00	0.051	29.00	0.047	−16.00	0.319	28.00	0.019
E. coli	8.80	0.032	−2.10	0.123	−10.90	0.048	11.33	0.040	11.40	0.042	8.40	0.086	−12.40	0.038	9.90	0.045
%pc	−1.50	0.021	−3.63	0.024	−2.12	0.028	−1.19	0.081	5.83	0.001	−6.25	< 0.001	−8.16	< 0.001	−0.21	0.620
PI	−0.125	0.229	−0.925	0.017	−0.750	0.005	−0.028	0.895	−1.09	0.025	1.84	0.046	0.840	0.173	0.526	0.076
NBT	−1.75	0.013	0.125	0.656	1.88	0.008	−0.472	0.402	3.00	0.003	−0.417	0.669	0.168	0.864	1.29	0.063
Lysozyme	−0.051	0.013	−0.019	0.350	0.032	0.116	0.011	0.505	0.022	0.446	0.021	0.459	0.032	0.269	0.022	0.289
IgY	−0.725	0.108	0.145	0.399	0.870	0.201	−1.38	0.037	−2.22	0.017	−0.700	0.087	1.63	0.033	−1.46	0.039
IgM	1.06	0.049	1.61	0.033	0.550	0.185	−3.25	0.004	−3.22	0.011	−2.76	0.012	0.780	0.136	−2.99	0.018

[1]Orthogonal contrast: C+Y+S *vs* P+YP+SP: ⅓(C+Y+S) − ⅓(P+YP+SP); C+P *vs* Y+YP: ½(C+P) − ½(Y+YP); C+P *vs* S+SP: ½(C+P) − ½(S+SP); Y+YP *vs* S+SP: ½(Y+YP) − ½(S+SP); S+Y *vs* SP+YP: ½(S+Y) − ½(SP+YP)

ED = the estimators of the differences in the mean experimental factors; *P* = *P*-value (the probability of rejecting the null hypothesis)

The addition of the *YL* yeast (C+P *vs* Y+YP) caused a significant ($P = 0.049$) increase in the Hb content and the RBC count ($P = 0.027$). In addition, the turkeys receiving the *YL* yeast additive showed an increased Ht ($P < 0.001$). Similar dependencies were noted in the turkeys receiving *SC* (C+P *vs* S+SP), but with no changes in the Hb content (Table 4). The turkeys from these groups (S+SP) had a lower Hb content than the birds receiving the feed with *YL* (Y+YP) ($P = 0.009$). Comparison of the use of the yeast with and without a probiotic (Y+S *vs* YP+SP) revealed that the combination of the probiotic with the yeast caused an increase in the RBC count ($P = 0.017$). The addition of the probiotic (C+Y+S *vs* P+YP+SP) caused an increase in the RBC count ($P = 0.024$) (Table 4).

The addition of the probiotic, as well as *YL* yeast caused an increase in the WBC count relative to the groups not receiving these supplements (C+Y+S *vs* P+YP+SP; $P = 0.006$; Y+S *vs* YP+SP – $P = 0.040$). The effect of the combined use of the probiotic and *YL* (YP) or *SC* (SP) to the yeast alone (group Y or S, respectively) depended on the species of the yeast, as the use of *YL* with the probiotic resulted in an increase in the WBC count ($P < 0.001$), while the use of *SC* with the probiotic reduced it ($P < 0.001$). The comparison of these two groups (YP *vs* SP) shows that the WBC count in the blood of the turkeys from the YP group was higher than in the SP group ($P = 0.007$) (Table 4).

The turkeys from the groups receiving *YL* had a higher %LYM ($P = 0.012$) and a lower WBC count ($P = 0.033$) than the groups that did not receive these yeasts (C+P *vs* Y+YP), and also in comparison to the turkeys from the groups receiving *SC* (Y+YP *vs* S+SP). The %LYM in the turkeys from this group (YP) was higher than in the birds from the SP group ($P = 0.003$). Reverse relationships were observed for the %HET, as the addition of *YL* reduced the number of these cells in relation to the groups not receiving these yeasts (C+P *vs* Y+YP; $P = 0.031$) and the group receiving *SC* (Y+YP *vs* S+SP; $P < 0.001$)) (Table 4).

This was reflected in the H : L ratio, which was higher in the turkeys from the C+P groups and in the turkeys receiving *SC* (C+P *vs* S+SP; $P = 0.024$) than in the groups of the birds receiving *YL* (C+P *vs* Y+YP; $P = 0.024$). The use of the probiotic together with the *YL* yeast (Y *vs* YP) caused an increase ($P = 0.045$) in the H : L ratio relative to the yeast alone.

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Table 4. Estimators of the differences in the mean experimental factors of the haematological indicators in the blood

Contrast[1]	C+P vs Y+YP		C+P vs S+SP		Y+YP vs S+SP		C+Y+S vs P+YP+SP		Y vs YP		S vs SP		YP vs SP		Y+S vs YP+SP	
Trait	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*	ED	*P*
Ht	–2.56	< 0.001	–2.08	< 0.001	0.482	0.297	–0.404	0.284	–0.288	0.659	–0.846	0.196	0.203	0.756	–0.567	0.220
Hb	–0.374	0.049	0.213	0.138	0.587	0.009	–0.136	0.153	–0.274	0.184	–0.174	0.479	0.637	0.054	–0.224	0.057
RBC	–0.690	0.027	–0.384	0.027	0.306	0.107	–0.251	0.024	–0.452	0.052	–0.131	0.546	0.467	0.260	–0.291	0.017
WBC	1.08	0.033	0.55	0.069	4.53	0.050	–1.61	0.006	–6.54	< 0.001	3.32	< 0.001	9.40	0.007	–3.39	0.040
HET	4.167	0.031	–4.833	0.013	–9.00	< 0.001	–0.278	0.858	–4.50	0.098	0.833	0.757	–6.33	0.021	–1.83	0.338
LYM	–4.33	0.012	3.08	0.071	7.42	< 0.001	1.94	0.162	4.00	0.097	3.83	0.112	7.33	0.003	3.92	0.023
H : L	0.175	0.024	–0.176	0.024	–0.351	< 0.001	–0.045	0.471	–0.159	0.045	–0.080	0.462	–0.311	0.005	–0.119	0.122
MONO	–0.667	0.398	0.167	0.832	0.833	0.292	–2.00	0.002	–0.667	0.550	–3.67	0.001	–0.667	0.550	–2.17	0.007
EOS	0.267	0.699	0.583	0.421	0.317	0.667	0.733	0.212	1.70	0.090	0.667	0.539	–0.200	0.831	1.18	0.110
BAS	0.183	0.755	1.083	0.052	0.900	0.117	–0.511	0.271	–1.20	0.170	–0.500	0.497	1.25	0.168	–0.850	0.138

[1]Orthogonal contrast: C+Y+S *vs* P+YP+SP: ⅓(C+Y+S) – ⅓(P+YP+SP); C+P *vs* Y+YP: ½(C+P) – ½(Y+YP); C+P *vs* S+SP: ½(C+P) – ½(S+SP); Y+YP *vs* S+SP: ½(Y+YP) – ½(S+SP); S+Y *vs* SP+YP: ½(S+Y) – ½(SP+YP)

ED = the estimators of the differences in the mean experimental factors; *P* = *P*-value (the probability of rejecting the null hypothesis)

DISCUSSION

The high stocking density of birds in poultry farming poses a number of threats associated with pathogens and gastrointestinal diseases. One solution to this problem is to add probiotics and/or prebiotics to the feed to increase the populations of beneficial microflora. The results of our microbiological tests indicate that the number of microorganisms and fungi in the intestinal contents of turkeys whose diets included a probiotic or *YL* was remarkably lower than in the control group or the group receiving feed with *SC*. However, it should be noted that in all the turkey groups these numbers were in line with the data presented by other researchers (Lutful Kabir 2009). The total number of *Escherichia coli* bacteria in the material was also favourable. The use of the probiotic or *YL* in the feeds was shown to inhibit the growth of the potentially pathogenic *E. coli* bacteria. Similar results for the number of *E. coli* bacteria and the total bacterial count have been reported by Konca et al. (2009) and Koc et al. (2010). In our study, no pathogenic bacteria of the genera *Salmonella*, *Proteus* or *Pseudomonas* or β-haemolytic *Escherichia coli* were found in the test material. These results could be linked to the presence of the β-glucans in the yeast cell wall, which protect the digestive tract against colonisation by very dangerous pathogens, such as *Salmonella enterica*, *Escherichia coli* and *Eimeria*, improve the intestinal status, and enhance the resistance to pathogens (Mantanovani et al. 2008; Shao et al. 2013).

Somewhat different patterns were observed in the case of *SC,* while the microbial count in the material from these birds was markedly lower than in the control group, the number of fungi was significantly higher. It should be noted, however, that no significant differences in the number of coliforms, including *Escherichia coli*, were found in the samples. This is in line with research by Koc et al. (2010).

The improvement in the microbiological composition of the digestive tract contents in the birds receiving a probiotic supplement could be due to the production of organic acids, hydrogen peroxide or bacteriocins that inhibit the growth of pathogenic microbes and reduce the capacity of other microorganisms, mainly pathogenic ones, to adhere to the intestinal epithelium.

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The results of our microbiological analyses correspond with the results of the research carried out using probiotics in broilers (Koc et al. 2010) or ducks (Li et al. 2011).

It should also be noted that the yeast used together with a probiotic significantly reduced the number of coliforms; this effect was stronger than that of the separate use of these two additives. In the group of birds receiving *YL* together with the probiotic, the number of *E. coli* bacteria was significantly reduced as well, which further underscores the effectiveness of their synergistic action. This may be due to the fact that the presence of *YL* affects the metabolic activity of the probiotic bacteria, with which they are in a symbiotic relationship. The beneficial effect of the yeast on the gut microbiota may be due, as mentioned above, to the presence of the β-glucans in their cell wall (Czech et al. 2010).

The addition of yeast and/or a probiotic, through their beneficial effect on the multiplication or stabilisation of the normal gut microflora in birds (Konca et al. 2009), may also enhance the body's immune defence mechanisms. In our experiment, the addition of *YL*, *SC* or a probiotic in the initial stage of the experiment caused the leukocyte count to significantly increase and then decrease, which may suggest the stimulation of immune responses in the younger birds, which are more susceptible to stressors or parasites than the adult birds, and whose immune system is not yet fully able to respond to pathogens (van Oers et al. 2010). In addition, stimulation of the immune system by the β-glucans present in the yeast cell wall may have increased the production of the leukocytes (Broadway et al. 2015).

A more sensitive indicator of the immune function and health status than the total leukocyte count is the ratio of heterophils to lymphocytes. A reduction in the H : L ratio has been observed by Beski and Al-Sardary (2015) in poultry receiving feed with a probiotic and a synbiotic. A significant reduction in this ratio, and thus an increase in the lymphocytes, was observed in the turkeys receiving *YL* in their feed relative to the control group and the group receiving *SC*. It can, therefore, be assumed that *YL*, and specifically the β-glucans present in its cell wall, stimulate the production of the lymphocytes, whose tasks include the participation in the acquired immune responses. As a result of the stimulation of the mechanisms of the humoral immune response, we can treat it as an increase in the level of the serum immunoglobulins, which was noted in turkeys receiving *YL* together with the probiotic, which indicates their synergistic effect. An increase in the humoral immune response in broilers receiving feed with yeast has been noted in many experiments (Chuka 2014).

The stimulation of non-specific immune mechanisms in the turkeys from the group receiving feed with *YL* or *SC* is also evidenced by the significantly higher NBT value, which is an indicator of the intracellular metabolic activity of the phagocytes, as well as the higher %pc. Stimulation of the non-specific immune mechanisms both in the turkeys receiving *YL* and to a lesser extent in those receiving *SC* may be associated with the best-known biological effect of the β-glucans, i.e., the intensification of phagocytic reactions, which are an element of non-specific immunity. The beta-glucans of the yeast cells do not exhibit any microbicidal activity, but only stimulate the immune system, especially the macrophages.

According to Chuammitri et al. (2011), the presence of the β-glucans yeast not only improves phagocytic activity, but above all – as observed in the turkeys receiving feed with *YL* – increases the lysozyme content.

The addition of a pro- and/or prebiotic stimulates the erythropoiesis (Chuka 2014), which was confirmed in our experiment. A significant increase in the erythrocyte count was observed in the turkeys receiving the probiotic additive, *YL* or *SC*. In addition, the haematocrit value was increased in the birds receiving *YL* or *SC*. The turkeys receiving feed with *YL* also had a significantly higher haemoglobin content in the blood compared to the control group and the group receiving feed with *SC*.

The stimulation of the haemoglobin synthesis may have been linked to the presence of the well-absorbed haematopoietic elements, i.e., iron, copper and zinc, of which *YL* is a valuable source (Czech et al. 2016). Moreover, yeasts are rich in other compounds that stimulate haemoglobin biosynthesis, such as folic acid or vitamin B_{12} (Beski and Al-Sardary 2015). Furthermore, the complete protein present in the *YL* yeast promotes absorption, mainly of iron, from the gastrointestinal tract (Czech et al. 2016).

In our experiment, no such relationship was observed in the case of the *SC* yeast, which was

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consistent with other studies (Saied et al. 2011). According to Mohamed et al. (2015), the addition of the *SC* yeast at 1% and 2% led to a slight increase in the haematocrit value, and a 3% addition even decreased it, while Saied et al. (2011) found no effect of the addition of the yeast on this parameter. An increase in the haemoglobin content and erythrocyte count was also induced by the addition of the probiotic alone and in combination with the yeast.

This effect can be attributed to the mode of action of the probiotics in the gut. By fermenting carbohydrates, these microorganisms contribute to the production of gases and organic compounds, including lactic acid, which reduces the pH of the intestinal contents. Lowering the pH promotes iron reduction from a trivalent form to a more digestible divalent form, as well as the production of substances with a bacteriostatic effect by probiotics (Hassanein and Soliman 2010).

In conclusion, the *Yarrowia lipolytica* yeast can be an alternative to the commonly used *Saccharomyces cerevisiae* yeast in turkey feeds. *Yarrowia lipolytica* favourably influences the intestinal microbiota, and also stimulates the erythropoiesis and immune mechanisms.

The combined use of a probiotic with a yeast, particularly *Yarrowia lipolytica*, has a more beneficial effect on the gut microbiota than the use of *Yarrowia lipolytica* alone (found to be more effective than *Saccharomyces cerevisiae*).

From a practical perspective, the use of *Yarrowia lipolytica* as a single additive or in combination with a probiotic should depend on the current needs of the poultry farmer. The use of *Yarrowia lipolytica* alone can be beneficial to the gut microbiota and the immune system of the birds. However, in the case of health problems in poultry, e.g., those resulting from an imbalance between the saprophytic and pathogenic microbes, it seems advisable to use *Yarrowia lipolytica* in combination with a probiotic, as their synergistic effect on the physiological functions of the digestive tract is greater than that of *Yarrowia lipolytica* alone.

In order to verify the results obtained, further research is needed using a greater number of birds.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Beski SS, Al-Sardary SY. Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. Int J Poult Sci. 2015 Jan 1; 14(1):31-6.

Broadway PR, Carroll JA, Sanchez NC. Live yeast and yeast cell wall supplements enhance immune function and performance in food-producing livestock: A review. Microorganisms. 2015 Aug;3(3):417-27.

Chuammitri P, Redmond SB, Kimura K, Andreasen CB, Lamont SJ, Palic D. Heterophil functional responses to dietary immunomodulators vary in genetically distinct chicken lines. Vet Immunol Immunopathol. 2011 Aug 15; 142(3-4):219-27.

Chuka E. Comparative study of the effects of probiotic and commercial enzyme on growth rate, haematology and serum biochemistry of broiler chicken. J Food Process Technol. 2014 Jan 1;5(9):1-5.

Czech A, Grela ER, Mokrzycka A, Pejsak Z. Efficacy of mannanoligosaccharides additive to sows diets on colostrum, blood immunoglobulin content and production parameters of piglets. Pol J Vet Sci. 2010 Jul 1;13(3):525-31.

Czech A, Merska M, Ognik K. Blood immunological and biochemical indicators in turkey hens fed diets with a different content of the yeast Yarrowia lipolytica. Ann Anim Sci. 2014 Oct 1;14(4):935-46.

Czech A, Smolczyk A, Ognik K, Kiesz M. Nutritional value of Yarrowia lipolytica yeast and its effect on growth performance indicators in piglets. Ann Anim Sci. 2016 Oct 1;16(4):1091-100.

Czech A, Merska-Kazanowska M, Ognik K, Zieba G. Effect of the use of Yarrowia lipolytica or Saccharomyces cerevisiae yeast with a probiotic in the diet of turkey hens on growth performance and gut histology. Ann Anim Sci. Forthcoming 2020.

El-Sissi AF, Mohamed SH. Impact of symbiotic on the immune response of broiler chickens against NDV and IBV vaccines. Global J Biochem Biotechnol. 2011;6(4):186-91.

Hassanein SM, Soliman NK. Effect of probiotic (Saccharomyces cerevisiae) adding to diets on intestinal microflora and performance of Hy-Line layers hens. J Am Sci. 2010; 6(11):159-69.

Koc F, Samli H, Okur A, Ozduven M, Akyurek H, Senkoylu N. Effects of Saccharomyces cerevisiae and/or mannano-

https://doi.org/10.17221/145/2019-VETMED

ligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulg J Agric Sci. 2010 Oct 1;16(5):643-50.

Konca YU, Kirkpinar Fİ, Mert SE, Kayhan B. Performance, intestinal microflora, and blood constituents in finishing turkeys fed diets supplemented with dietary mannan oligosaccharide and live yeast. J Anim Feed Sci. 2009 Jun 24;18(3):508-17.

Li WF, Rajput IR, Xu X, Li YL, Lei J, Huang Q, Wang MQ. Effects of probiotic (Bacillus subtilis) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck. Int J Poult Sci. 2011; 10(8):583-9.

Lutful Kabir SM. The role of probiotics in the poultry industry. Int J Mol Sci. 2009 Aug 12;10(8):3531-46.

NRC – National Research Council. Nutrient requirements of poultry. 9th ed. Washington D.C.: National Academies Press; 1994. 155 p.

Mantovani MS, Bellini MF, Angeli JPF, Oliveira RJ, Silva AF, Ribeiro LR. beta-Glucans in promoting health: Prevention against mutation and cancer. Mutat Res. 2008 Mar-Apr; 658(3):154-61.

Merska M, Czech A, Ognik K. The effect of yeast Yarrowia lipolytica on the antioxidant indices and macro-and microelements in blood plasma of turkey hens. Pol J Vet Sci. 2015 Dec 1;18(4):709-14.

Michalik B, Jacyno E, Lubowicki R, Biel W. Biological evaluation of the protein nutritional value in the diets of rats based on cereals and the yeast Yarrowia lipolytica growing on industrial glycerol. Acta Agr Scand A-An. 2013 Sep 1;63(3):163-8.

Mohamed RE, Gadour MO, Adam I. The lowering effect of Gum Arabic on hyperlipidemia in Sudanese patients. Front Physiol. 2015 May 18;6:160.

Ozsoy B, Yalcin S. The effects of dietary supplementation of yeast culture on performance, blood parameters and immune system in broiler turkeys. Ankara Univ Vet Fak. 2011 Jun 1;58(2):117-22.

Park BH, Fikrig SM, Smithwick EM. Infection and nitroblue-tetrazolium reduction by neutrophils: A diagnostic aid. Lancet. 1968 Sep 7;292(7567):532-4.

Russo P, Lopez P, Capozzi V, De Palencia PF, Duenas MT, Spano G, Fiocco D. Beta-glucans improve growth, viability and colonization of probiotic microorganisms. Int J Mol Sci. 2012 May;13(5):6026-39.

Saied JM, Al-Jabary QH, Thalij KM. Effect of dietary supplement yeast culture on production performance and hematological parameters in broiler chicks. Int J Poult Sci. 2011;10(5):376-80.

Shao Z, Watanabe S, Christensen R, Jorgensen EM, Colon-Ramos DA. Synapse location during growth depends on glia location. Cell. 2013 Jul 18;154(2):337-50.

Siwicki AK, Anderson DP. Nonspecific defence mechanisms assay in fish II: Potential killing activity of neutrophils and monocytes, lysozyme activity in serum and organs and total immunoglobulin (Ig) level in serum. In: Siwicki AK, Anderson DP, Waluga J. Fish diseases diagnosis and prevention methods. Olsztyn, Poland: Inland Fisheries Institute Publisher; 1993. p. 105-111.

Siwicki AK, Anderson DP, Rumsey GL. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet Immunol Immunopathol. 1994 May 1;41(1-2):125-39.

van Oers K, Richardson DS, Saether SA, Komdeur J. Reduced blood parasite prevalence with age in the Seychelles Warbler: Selective mortality or suppression of infection? J Ornithol. 2010 Jan 1;151(1):69-77.

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