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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*

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Abstract

The aim of the study was to determine whether the alternative yeast species *Yarrowia lipolytica* in turkey feed would have a more beneficial effect on growth performance and intestinal histology than the commonly used species *Saccharomyces cerevisiae*. An additional objective of the study was to test whether the addition of a probiotic to feed containing *Yarrowia lipolytica* or *Saccharomyces cerevisiae* yeast would enhance its effect on growth performance and intestinal histology in turkeys. The experiment was carried out on 480 turkey hens randomly divided into six groups. Birds from the control group (C) and group P were fed standard feeds but group P additionally received a probiotic (0.05%). Groups Y and YP received feed containing *Yarrowia lipolytica* fodder yeast (3%), and the YP group received also the probiotic (0.05%). Similarly, in groups S and SP, the turkeys received feed with *Saccharomyces cerevisiae* fodder yeast (3%), and for the SP group the probiotic was added to the feed (0.05%). *Yarrowia lipolytica* yeast added in the amount of 3% to the turkey feed may be an alternative to the commonly used *Saccharomyces cerevisiae* yeast, because it improved growth performance, and above all, had a more beneficial effect on intestinal histology. The use of *Yarrowia lipolytica* alone can be beneficial for growth performance, while the combined use of 3% *Yarrowia lipolytica* in the feed and a 0.05% addition of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* has a more beneficial effect on gastrointestinal histology.

Key words: *Yarrowia lipolytica,* probiotic, turkey hens, performance, intestinal histology

The last decade has seen increased interest in alternatives to *Saccharomyces cerevisiae* yeast cultures, such as *Yarrowia lipolytica*. These strains use biofuel by-

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products (e.g. glycerol) to produce yeast biomass, and therefore can become a link between biodiesel by-products and animal feed, which is extremely important in the context of environmental protection (Czech et al., 2016). In addition, they have unique physical properties (colour, aroma and sweet flavour) and chemical properties (essential amino acids, B vitamins, and a rich mineral composition) (Merska et al., 2015). Therefore, their use in poultry feeding seems to be justified, and previous research suggests that a 3% share in the feed is optimal in turkey diets (Merska et al., 2013).

To enhance the activity of fodder yeast, combinations of yeast (as a prebiotic) with probiotics are used, which in the nutritional nomenclature are called synbiotics (Popović et al., 2015). Their combined use can provide a number of benefits, such as improved productivity (Li et al., 2012), an immunostimulatory effect (An et al., 2008; Czech et al., 2014), and a beneficial effect on digestive tract function in birds (Spring et al., 2000). These qualities suggest that the presence of yeasts and probiotics in feeds for turkeys may have a positive effect on their health and digestive tract function, which leads to improved metabolism and thus improved fattening performance (Czech et al., 2018).

Therefore, the aim of the study was to determine whether the alternative yeast species *Yarrowia lipolytica* in turkey feed would have a more beneficial effect on growth performance and intestinal histology than the commonly used species *Saccharomyces cerevisiae*. An additional objective of the study was to test whether the addition of a probiotic to feed containing *Yarrowia lipolytica* or *Saccharomyces cerevisiae* yeast would enhance its effect on growth performance and intestinal histology in turkeys.

Material and methods

Chemical analysis of yeast and feed

The chemical composition of *Yarrowia lipolytica* and *Saccharomyces cerevisiae* yeast, i.e. the content of total protein, dry matter, and crude ash, was analysed using AOAC (2012). These nutrients, as well as the content of crude fat, were also analysed in the compound feeds according to AOAC (2012). We also determined the quantitative composition of amino acids (Lys, Met, Thr, Trp, Cys, Leu, Ile, His, Arg and Phe) in the yeast and the content of lysine and methionine + cysteine in the feeds by ion-exchange chromatography with spectrophotometric detection (IEC-Vis) (AOAC, 2012). The content of macro- and micronutrients, i.e. Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} and Cu^{2+}, in the yeast and the content of calcium and sodium in the feeds were determined by flame atomic absorption spectrometry (AOAC, 2012). Total phosphorus content in the yeast was determined by spectrometry according to the Fiske and Subbarow (1925) method, and available phosphorus in the feeds according to Oberleas (2006). The analyses were carried out in three batches of *Yarrowia lipolytica* and *Saccharomyces cerevisiae* yeast in duplicate, while feeds were analysed twice in duplicate.

Animals

The experiment was carried out on 480 BIG6 turkey hens randomly divided into six groups of 80 (five repetitions of 16 turkeys raised to the age of 112 days). During the experiment birds from all groups received *ad libitum* complete balanced feeds according to the recommendations of NRC (1994) for each rearing period (Table 1). All feeds were balanced in terms of nitrogen and metabolisable energy.

Birds from 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 feed containing 3% *Yarrowia lipolytica* fodder yeast, and for the YP group the probiotic was added to the feed in the amount of 0.05% (0.5 kg per tonne of feed). Similarly, in groups S and SP, the turkeys received feed with 3% *Saccharomyces cerevisiae* fodder yeast, and for the SP group the probiotic was added to the feed in the amount of 0.5 kg per tonne of feed.

The experimental procedure was approved by the Local Ethics Commission for Experiments with Animals in Lublin (approval no. 19/2012).

Experimental procedures and sample collection

During the experiment, the weight of the turkeys was monitored on the 7, 28, 56, 84 days of age (all birds were weighed at the beginning of the experiment). Feed intake was monitored as well. In the 16th week of age, 10 birds from each group were slaughtered, after which slaughter analysis of the carcasses was performed and the duodenum, caecum and jejunum were sampled for histopathological examination.

Histological analysis

Five-µm paraffin sections stained with hematoxylin and eosin were prepared from the caecal and duodenal tissues. The slides were evaluated by light microscopy. In addition, morphometric measurements were made of intestinal villus length (V) and intestinal crypt depth (IC) using Multi-Scan Base v.8 computer image analysis software operating in the Windows environment and coupled with a Zeiss Axiophot microscope. Ten villi cut lengthwise were measured in each intestinal sample. The thickness of the muscular layer (M) was determined as well.

Statistical analysis

Statistical calculations of the studied traits were performed by two-factor model with interaction, taking into account the influence of the feeding group described in Table 2. The calculations were made using general linear models (procedure GLM) in SAS 9.4 software (SAS Institute, Cary, NC, USA). The significance of differences between means was determined using Tukey's test.

Table 1. Ingredients (%) and chemical composition of feed for turkey hens

Group[1]	Starter			Grower 1			Grower 2			Finisher		
	1–4			5–8			9–12			13–16		
	C	Y	S	C	Y	S	C	Y	S	C	Y	S
Wheat	45.5	46.0	45.3	47.0	47.5	46.8	45.5	46.0	45.2	46.5	47.0	46.6
Triticale	–	–	–	–	–	–	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal (460 g/kg CP)	40.51	37.53	37.72	40.52	37.77	37.97	26.60	23.61	23.90	22.66	19.68	19.57
Rapeseed cake	–	–	–	4.0	4.0	4.0	6.0	6.0	6.0	9.0	9.0	9.0
Potato protein	5.0	5.0	5.0	–	–	–	–	–	–	–	–	–
Soybean oil	3.0	2.5	3.0	3.5	3.0	3.5	7.0	6.5	7.0	7.5	7.0	7.5
Yarrowia lipolytica	–	3.0	–	–	3.00	–	–	3.0	–	–	3.0	–
Sacharomyces cerevisiae	–	–	3.0	–	–	3.00	–	–	3.0	–	–	3.0
Calcium carbonate	1.8	1.8	1.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.05	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01
Probiotic	–	–	–	–	–	–	–	–	–	–	–	–
PREMIX[2]	3.5	3.5	3.5	3.0	3.0	3.0	3.0	3.0	3.0	2.5	2.5	2.5
Methionine	0.5	0.5	0.5	0.55	0.55	0.55	0.43	0.43	0.43	0.45	0.45	0.45
Lysine	0.10	0.10	0.10	0.35	0.10	0.10	0.44	0.44	0.44	0.35	0.35	0.35
NaCl	0.04	0.03	0.04	0.03	0.03	0.03	0.025	0.015	0.020	0.026	0.014	0.02
Nutrients												
Crude protein (%)	28.02	28.04	28.02	26.0	25.98	26.10	21.51	21.56	21.51	18.80	18.79	18.76
Crude fat (%)	4.22	4.18	4.16	5.17	5.15	5.22	8.75	8.70	8.76	10.49	10.46	10.49
Crude ash (%)	5.23	5.20	5.23	4.60	4.58	4.61	3.87	3.90	3.85	3.74	3.73	3.72
Crude protein (%)	2.83	2.84	2.80	3.31	3.29	3.32	2.95	2.96	2.95	3.12	3.11	3.10
Lysine (%)	1.58	1.59	1.58	1.44	1.45	1.43	1.20	1.22	1.18	0.95	0.95	0.94
Methionine + cysteine (%)	1.03	0.98	1.00	0.90	0.92	0.90	0.71	0.70	0.72	0.65	0.66	0.64

Total Ca (%)	1.20	1.21	1.20	1.01	1.02	0.99	0.85	0.85	0.84	0.80	0.82	0.81
Av. P %	0.55	0.56	0.50	0.56	0.53	0.55	0.41	0.39	0.40	0.40	0.41	0.39
Total Na (%)	0.18	0.18	0.19	0.15	0.14	0.15	0.12	0.10	0.13	0.12	0.13	0.11
Calculated												
ME (kcal/kg)		2790			2890			2980			3090	

[1]C – control; Y – 3% share of *Yarrowia lipolytica* in feed; S – 3% share of *Saccharomyces cerevisiae* in feed

[2]Mineral-vitamin premix containing the following in 1 kg in respective feeding periods (weeks 0–4; weeks 5–8; weeks 9–12; weeks 13–16): vitamin A (IU) – 1 450 000, 1 650 000, 1 650 000, 2 000 000; vitamin D_3 (IU) – 320 000, 370 000, 370 000, 445 000; vitamin E (mg) – 3300, 3700, 3100, 3700, vitamin K_3 – 500, 500, 350, 300; vitamin B_1 (mg) – 900, 800, 700, 800; riboflavin (mg) – 2000, 1100, 950, 1100; vitamin B_6 (mg) – 1500, 1500, 1200, 1400; vitamin B_{12} (mg) – 0.001, 0.002, 0.001, 0.002; folic acid (mg) – 300, 300, 300, 3500; biotin (mg) – 68, 63, 35, 45; niacin (mg) – 17 000, 17 000, 17 000, 17 000; choline (mg) – 460 000, 460 000, 370 000, 460 000; calcium pantothenate (mg) – 4 200, 4 200, 4 200, 4 200; Mn (mg) – 16 000, 19 000, 19 000, 23 000; Zn (mg) – 19 000, 20 500, 16 000, 15 000; Fe (mg) – 18 000, 16 000, 16 500, 20 000; Cu (mg) – 2000, 2500, 1800, 2500; I (mg) – 200, 200, 200, 200; Se (mg) – 60, 60, 60, 60; Co (mg) – 20, 20, 20, 20; Ca (g) – 15, 15, 5, 15.

Table 2. Experimental design

Feeding group		Yeast		
		– (Control)	*Yarrowia lipolytica*	*Saccharomyces cerevisiae*
Probiotic	– (Control)	C	Y	S
	Bacillus licheniformis and *Bacillus subtilis*	P	YP	SP

Results

Chemical composition of *Yarrowia lipolytica* and *Saccharomyces cerevisiae* yeast

The content of basic nutrients in yeast of the species *Yarrowia lipolytica* and *Saccharomyces cerevisiae* was similar (Table 3). *Yarrowia lipolytica* fodder yeast had significantly higher content of lysine, methionine, leucine and isoleucine than *Saccharomyces cerevisiae* yeast, while the latter had significantly higher arginine content. *Yarrowia lipolytica* had nearly twice the amount of calcium as *Saccharomyces cerevisiae*. The content of iron and zinc in *Yarrowia lipolytica* was also higher than in brewer's yeast. Only the content of phosphorus was higher in *Saccharomyces cerevisiae* yeast than in *Yarrowia lipolytica*.

Table 3. Chemical composition of *Yarrowia lipolytica* and *Saccharomyces cerevisiae*

Nutrient	*Yarrowia lipolytica*	*Saccharomyces cerevisiae*
Crude protein (%)	45.5	40.34
Crude fat (%)	1.47	0.51
Dry matter (%)	97.30	97.44
Ash (%)	7.71	8.03
Amino acids (g kg^{-1}DM)		
Lysine	30.5	26.88
Methionine	6.94	6.01
Threonine	15.85	13.21
Tryptophan	4.01	3.98
Cysteine	4.23	4.66
Leucine	28.0	24.55
Isoleucine	18.9	14.77
Histidine	9.78	8.98
Arginine	17.51	20.98
Phenylalanine	18.53	19.31
Minerals		
Calcium (g kg^{-1})	4.11	2.98
Phosphorus (g kg^{-1})	4.87	9.44
Magnesium (g kg^{-1})	1.77	1.69
Iron (mg kg^{-1})	110.8	99.05
Zinc (mg kg^{-1})	70.76	65.87
Copper (mg kg^{-1})	10.41	11.62

Table 4. Results of growth performance parameters

Trait	Day of age	Probiotic			Yeast				Probiotic × Yeast						
		–	+	SEM[1]	–	S	Y	SEM	C	P	Y	YP	S	SP	SEM
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Body weight (kg)	7	0.154	0.155	0.002	0.155	0.155	0.154	0.002	0.154	0.156	0.153	0.155	0.154	0.155	0.003
	28	1.41	1.42	0.015	1.41	1.41	1.41	0.018	1.41	1.42	1.41	1.41	1.41	1.41	0.025
	56	4.09	4.12	0.031	4.19 a	4.01 b	4.12 ab	0.038	4.19 a	4.19 a	4.15 ab	4.08 ab	3.93 b	4.08 ab	0.054
	84	6.83	6.97	0.049	7.00 a	6.76 b	6.95 ab	0.060	7.01 a	6.99 a	6.92 ab	6.98 a	6.58 b	6.94 a	0.085
	112	9.88	9.91	0.062	9.91	9.82	9.95	0.076	9.96	9.86	9.92	9.98	9.75	9.88	0.108
Body weight gains (kg)	7–28	1.26	1.26	0.013	1.26	1.26	1.26	0.016	1.26	1.26	1.26	1.26	1.26	1.26	0.001
	29–56	2.68	2.7	0.021	2.78 a	2.59 b	2.71 a	0.025	2.78 a	2.77 a	2.74 ab	2.67 a	2.52 b	2.66 a	0.003
	57–84	2.74 a	2.85 b	0.031	2.81	2.75	2.84	0.038	2.81 ab	2.8 ab	2.77 ab	2.9 a	2.64 b	2.86 ab	0.005
	85–112	3.04 a	2.93 b	0.036	2.91 b	3.06 a	3 ab	0.044	2.95 ab	2.86 b	3.00 ab	3.00 ab	3.17 a	2.94 ab	0.009
	7–112	9.72	9.75	0.061	9.75	9.66	9.8	0.075	9.8	9.7	9.77	9.83	9.6	9.72	0.004
Feed intake (kg/day)	7–28	0.079	0.079	0.001	0.081	0.079	0.078	0.001	0.079	0.082	0.078	0.078	0.079	0.078	0.023
	29–56	0.183 a	0.177 b	0.002	0.188 a	0.174 b	0.178 b	0.002	0.197 a	0.179 b	0.178 b	0.177 b	0.173 b	0.175 b	0.036
	57–84	0.234 a	0.244 b	0.003	0.245 a	0.241 ab	0.232 b	0.003	0.239 ab	0.251 a	0.227 b	0.236 ab	0.236 ab	0.245 ab	0.054
	85–112	0.394	0.383	0.005	0.389	0.391	0.385	0.006	0.397	0.380	0.386	0.385	0.397	0.385	0.062
	7–112	0.241	0.24	0.003	0.245	0.239	0.236	0.003	0.247	0.242	0.235	0.237	0.24	0.239	0.106

Table 4 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
FCR (kg/kg)	7–28	1.32	1.32	0.010	1.34	1.31	1.31	0.012	1.33	1.36	1.3	1.31	1.33	1.3	0.018
	29–56	1.91 a	1.83 b	0.015	1.90	1.88	1.84	0.018	1.98 a	1.81 c	1.82 bc	1.86 bc	1.92 ab	1.84 bc	0.026
	57–84	2.39	2.43	0.027	2.45 ab	2.45 a	2.34 b	0.033	2.38 ab	2.51 a	2.29 b	2.38 ab	2.50 a	2.39 ab	0.047
	85–112	3.62	3.66	0.030	3.74 a	3.58 b	3.59 b	0.037	3.77 a	3.72 a	3.6 ab	3.59 ab	3.50 b	3.67 ab	0.052
	7–112	2.60	2.58	0.020	2.63 a	2.60 ab	2.53 b	0.024	2.65	2.62	2.53	2.54	2.62	2.58	0.035

a, b, c – means in rows with different letters differ significantly at $P \leq 0.05$.
[1]Standard error of the mean.

Table 5. Results of statistical analysis of carcass analysis of turkey hens

Feeding groups	Probiotic			Yeast				Probiotic × Yeast						
Trait	–	+	SEM	–	S	Y	SEM	C	P	Y	YP	S	SP	SEM
Breast	27.46	27.22	0.20	27.67	27.27	27.08	0.25	27.74	27.61	27.12	27.04	27.51	27.03	0.35
Thigh	10.99	11.54	0.23	11.28	11.34	11.17	0.28	10.59	11.97	10.88	11.47	11.49	11.18	0.40
Drumstick	8.94 a	9.27 b	0.11	9.48 a	9.08 ab	8.77 b	0.13	9.22 ab	9.73 a	8.56 b	8.97 ab	9.04 ab	9.12 ab	0.19
Liver	1.45	1.42	0.02	1.40	1.46	1.44	0.02	1.41 ab	1.40 ab	1.52 a	1.36 b	1.43 ab	1.48 ab	0.04
Stomach	1.23	1.26	0.02	1.18 a	1.25 ab	1.32 b	0.02	1.15 c	1.21 bc	1.26 abc	1.37 a	1.29 ab	1.21 bc	0.03
Heart	0.45	0.45	0.01	0.46	0.44	0.46	0.01	0.46 ab	0.46 ab	0.48a	0.45 ab	0.42 b	0.46 ab	0.01
Abdominal fat	0.48 a	0.6 b	0.03	0.65 a	0.39 b	0.58 a	0.04	0.63 abc	0.66 ab	0.41 bc	0.74 a	0.38 c	0.40 bc	0.06

a, b, c – means in rows with different letters differ significantly at P≤0.05.

Table 6. Results of statistical analysis of intestinal histology

Feeding groups		Probiotic			Yeast				Probiotic × Yeast						
Trait		–	+	SEM	–	S	Y	SEM	C	P	Y	YP	S	SP	SEM
Duodenum	V	2355 a	2553 b	41	2212 b	2526 a	2624 a	50	2105 c	2319 bc	2460 b	2788 a	2501 ab	2551 ab	71
	IC	437 a	352 b	7	450 a	474 a	259 b	9	546 a	354 c	279 d	240 d	486 b	462 b	13
	M	515 a	588 b	6	502 b	501 b	652 a	8	438 d	565 b	605 b	699 a	50 c	498 c	11
	V/IC	6.0 a	8.0 b	0	5.2 b	5.4 b	10.3 a	0	3.9 d	6.6 c	8.8 b	11.8 a	5.2 cd	5.5 c	0.4
Caecum	V	528 a	568 b	9.1	551 b	489 c	605 a	11.2	512 cd	591 ab	555 bc	654 a	518 cd	460 d	16
	IC	240 a	189 b	6	227 a	243 a	175 b	8	259 a	194 bc	197 bc	152 c	264 a	222 ab	11
	M	508 a	552 b	7	522 ab	517 b	550 a	8	508 b	537 ab	523 b	577 a	492 b	543 ab	12
	V/IC	2.3 a	3.2 b	0.1	2.5 a	2.0 b	3.6 c	0.1	2.0 c	3.1 b	2.9 b	4.3 a	2.0 c	2.1 c	0.2
Jejunum	V	1855 a	1925 b	19	1815 b	1904 a	1950 a	23	1760 b	1870 ab	1904 a	1996 a	1899 ab	1909 ab	33
	IC	396 a	342 b	7	388 a	388 a	332 b	8	439 a	336 cd	376 bc	287 d	373 bc	402 ab	12
	M	507 a	541 b	7	506 b	478 b	587 a	9	467 b	546 ab	586 a	589 a	468 b	489 b	13
	V/IC	4.7 a	5.8 b	0.1	4.8 b	4.9 b	6.0 a	0.1	4.0 c	5.6 b	5.1 b	7.0 a	5.1 b	4.8 bc	0.2

a, b, c, d – means in rows with different letters differ significantly at P≤0.05.

Growth performance of turkeys

Results of growth performance parameters are presented in Table 4.

The use of a probiotic resulted in a significant 138 g increase in the weight of the turkeys on day 84 of rearing relative to the groups not receiving this supplement (Table 4). The reverse was observed in the case of *Saccharomyces cerevisiae* yeast (187 g and 240 g on days 56 and 84, respectively). The turkeys receiving the *Yarrowia lipolytica* supplement had significantly higher body weights (especially on days 56 and 84–109 g and 193 g, respectively) compared to turkeys receiving *Saccharomyces cerevisiae*. The combined use of yeast and a probiotic contributed to higher body weight, which could be seen on day 84 with a difference of 213 g.

Weight gain between 29 and 56 days of rearing was significantly lower in turkeys receiving yeast (by 73 g for *Yarrowia lipolytica*; by 185 g for *Saccharomyces cerevisiae*). The addition of the probiotic also caused a significant 107 g reduction in weight gains compared to the groups not receiving the probiotic, but between days 85 and 112. Between days 85 and 112, weight gains in turkeys receiving *Saccharomyces cerevisiae* were significantly higher than in the groups not receiving this supplement. The combined addition of *Saccharomyces cerevisiae* with the probiotic contributed to higher body weight gains than in birds receiving feed with *Saccharomyces cerevisiae* alone, by 143 g in the initial fattening period between days 29 and 56 and by 216 g between days 57 and 84. A reverse and significant dependence (difference of 234 g) was recorded between days 85 and 112 of the experiment. Feed intake in turkey hens receiving *Yarrowia lipolytica* yeast in their feed, especially from days 7 to 84 of rearing, was significantly lower than in the groups not receiving *Yarrowia lipolytica*. This was noted in turkeys receiving the probiotic supplement together with *Saccharomyces cerevisiae*, but only between days 29 and 56. It is worth noting that the lower feed intake in turkeys receiving feed with *Yarrowia lipolytica* alone resulted in a significant reduction in feed conversion in the entire fattening period in relation to the groups without *Yarrowia lipolytica*. Feed conversion was significantly lower, by 159 g/g weight gain, in turkeys receiving *Saccharomyces cerevisiae* relative to the C and P groups between 85 and 112 days of rearing. The combined addition of yeast and the probiotic caused no changes in feed intake or conversion. It should be noted, however, that the combined use of *Saccharomyces cerevisiae* and the probiotic between 29 and 56 days of rearing reduced the feed conversion ratio compared to group S, but between 85 and 112 days of rearing the inverse relationship was observed, with a difference of 164 g/g weight gain. There were no deaths among the turkeys during the entire experiment.

Results of turkey carcass analysis

Results of carcass analysis of turkey hens are presented in Table 5. The statistical analysis shows that only the addition of *Yarrowia lipolytica* yeast caused a significant increase in the weight of the breast and drumstick muscle compared to the group receiving feed without these yeasts and the birds receiving *Saccharomyces cerevisiae*. It is noteworthy that the addition of *Yarrowia lipolytica* and also *Saccharomyces cerevisiae* resulted in a significant reduction in subcutaneous fat relative to the groups not receiving yeast. The combined use of yeast and a probiotic also reduced the amount of this tissue compared to birds fed with yeast alone, which corresponded to a higher dressing percentage. The addition of *Saccharomyces cerevisiae* to the turkey feed caused a reduction in stomach weight compared to birds from the group fed without yeast, as well as a reduction in heart weight compared to birds receiving *Yarrowia lipolytica*.

Results of histological analysis of turkey intestines

Results of intestinal histology are presented in Table 6. The turkeys receiving the probiotic (P+YP+SP) and those receiving *Yarrowia lipolytica* yeast (Y+YP) had significantly longer villi and a thicker muscular layer in all analysed gastrointestinal segments. Lower crypt depth was also noted in these birds, which translated into a significantly higher V/IC ratio. The addition of *Saccharomyces cerevisiae* yeast also caused an increase in the length of the villi relative to the groups whose feed did not include yeast, but only in the duodenum and jejunum. Comparison of the effect of *Yarrowia lipolytica* with *Saccharomyces cerevisiae* reveals that *Saccharomyces cerevisiae* significantly deepened the crypts and reduced the thickness of the muscular layer in all examined parts of the gastrointestinal tract. The ratio of villus length to crypt depth was also significantly lower in these birds. The combined addition of the probiotic and yeast (especially *Yarrowia lipolytica*) caused a reduction in crypt depth and an increase in the thickness of the muscular layer, especially in the duodenum and the caecum. This significantly increased the ratio of villus length to crypt depth, which was observed in all sections of the gastrointestinal tract.

Discussion

Studies by many authors indicate that fattening efficiency of poultry can be improved by including yeast in the diet (Houshmand et al., 2012; Priya and Babu, 2013). These effects are explained in part by the rich amino acid composition of yeast (mainly the content of digestible lysine) (Yirga, 2015), the capacity for immunomodulation and stimulation of immunity in the intestinal mucosa (Chichlowski et al., 2007), the ability to bind pathogenic bacteria and their toxins (Higgins et al., 2008), and modulation of the intestinal microbiota. An experiment on broilers conducted by Tabidi et al. (2013) demonstrated that even a small share of yeast (0.1, 0.2 or 0.3%) in poultry feed increases final body weight and reduces feed consumption. Some studies (Karaoglu and Durdag, 2005), however, indicate that yeast preparations (*Saccharomyces cerevisiae*) have a minor or even no significant effect on weight gain and feed intake in poultry. These results correspond to our own earlier study in which fattening turkeys received feed with a 3% or 6% share of *Yarrowia lipolytica*. In that study, birds whose feed contained a 3% share of *Yarrowia lipolytica* had similar growth performance parameters as the control group, while the turkeys receiving a 6% yeast supplement even weighed significantly less, perhaps because this dose was too high (Merska et al., 2013). The use of 6% *Yarrowia lipolytica* yeast in feed for pigs has also been shown to result in decreased growth performance and increased incidence of diarrhoea (Czech et al., 2016).

In the present study, a 3% share of *Yarrowia lipolytica* in the feed caused no significant differences in the body weight of turkeys relative to the control group, but feed intake in these birds up to 84 days of the experiment was lower and feed conversion was significantly lower. No such relationships were found in the case of *Saccharomyces cerevisiae*, which could be due in part to its palatability. Brewer's yeast has a slight bitter taste resulting from the production process, which is based on yeast biomass used to ferment hopped beer (Pretorius, 2003). Furthermore, these two yeast species are microbial cultures of differing chemical composition, which is influenced by differences in the production technology, drying method, and culture substrate, and thus they may influence production and health parameters in animals to different degrees (Tabidi et al., 2013).

The addition of a probiotic did not cause clear differences in growth performance relative to the control group. There was also no synergistic or antagonistic interaction with yeast. These findings differ somewhat from the results reported by other researchers. According to Saleh (2014) and Yirga (2015), the addition of a probiotic to the diet can favourably affect poultry fattening efficiency, increasing daily weight gains and significantly lowering feed intake, which translates into better feed conversion. According to Mountzouris et al. (2010), the addition of a probiotic in the amount of 10^8 CFU/kg increases body weight, reduces the feed conversion ratio, and reduces mortality in poultry as well. Research conducted on turkeys by Torres-Rodriguez et al. (2007) also found that a probiotic had a positive effect on production parameters and improved the economic value of the turkeys. According to Awad et al. (2009), dry yeast as a prebiotic interacting with a probiotic can be regarded as a synbiotic, and this combination is more potent than the probiotic or prebiotic alone. This has not been confirmed in the present study.

The slightly higher final weight of turkeys in the group receiving feed with *Yarrowia lipolytica* relative to the control group was reflected in a significantly higher percentage of breast muscle and drumstick muscle in these birds. No such relationship was found in birds whose feed contained *Saccharomyces cerevisiae*, which was consistent with research by Tabidi et al. (2013). On the other hand, turkeys whose feed contained *Saccharomyces cerevisiae* had significantly lower breast muscle and drumstick weight than birds whose feed included *Yarrowia lipolytica*. This may be due to the fact that *Yarrowia lipolytica* has significantly higher content of essential amino acids than *Saccharomyces cerevisiae*, i.e. of lysine (by about 30%), tryptophan (by about 34%), tyrosine (by about 17%), leucine (by about 26%), isoleucine (by about 34%), valine (by about 28%), alanine (by about 50%), glycine (by about 20%) and glutamic acid (by about 22%) (Czech et al., 2016).

The use of *Yarrowia lipolytica* and *Saccharomyces cerevisiae* yeast as well as the combined use of yeast and a probiotic had a significant influence on abdominal fat content in the turkey hens. This fat, which accounts for about 15% of the total body lipids, is the most important fat deposit in the body. Its reduction in the carcass can affect the quality of poultry meat and at the same time arouse consumer interest (Hermier, 1997). A reduction in abdominal and intramuscular fat in the carcasses of broilers whose feed contained *Saccharomyces cerevisiae* has also been reported by Priya and Babu (2013). These results correspond to our own earlier research on fattening turkeys (Merska et al., 2013) receiving feed with a 3% or 6% share of *Yarrowia lipolytica*.

Achieving better production results is closely linked to intestinal integrity and intestinal villus height (Ghahri et al., 2013). In the turkeys receiving feed with the probiotic (P) alone or yeast *Yarrowia lipolytica* (Y) alone, there was a significant increase in villus length along the entire length of the small intestine (duodenum, jejunum and caecum) and in the V/IC ratio, which corresponded to a lower feed conversion rate relative to the control, while body weight was similar. In this case, the hypothesis was confirmed that yeast is a factor increasing villus length and the ratio of villus length to crypt depth. According to Ghahri et al. (2013), the ratio of crypt depth to villus height is a criterion for estimating the absorptive surface area of the small intestine and overall intestinal function. It is also an important indicator of intestinal health, regeneration and function, and therefore *Yarrowia lipolytica* and probiotics can be assumed to have a beneficial effect on intestinal function. This has also been directly correlated with increased regeneration of the epithelium. Therefore, it can be concluded that these additives beneficially affect the development of the intestinal epithelium (Fan et al., 1997).

A slightly different relationship was observed in birds whose feed contained *Saccharomyces cerevisiae*. Although the length of the villi in the duodenum and jejunum was significantly higher than in the control group, the V/IC ratio and the thickness of the muscular layer were not statistically significant. According to Xu et al. (2003), an increase in villus length with no effect on the V/IC ratio or the thickness of the muscular layer may result in poorer nutrient absorption, which leads to lower productivity. This was confirmed in our study, as the turkeys receiving *Saccharomyces cerevisiae* had the lowest body weight gains.

A similar effect was noted in the case of birds that received a probiotic in their feed in addition to *Saccharomyces cerevisiae*. This is puzzling, however, as many studies show that both a probiotic and *Saccharomyces cerevisiae* yeast should stimulate the length of the intestinal villi (Beski and Al-Sardary, 2015; Priya and Babu, 2013). Therefore, we can postulate that the results may have been influenced by an excessive amount of *Saccharomyces cerevisiae* (Ghahri et al., 2013) or an inappropriate choice of probiotic for use with these yeasts (Awad et al., 2009; Kota et al., 2012). Slightly different dependencies were observed in the case of *Yarrowia lipolytica* yeast; not only did the birds have significantly higher body weights than those receiving *Saccharomyces cerevisiae*, but the histology of the entire analysed length of the gastrointestinal tract was significantly better. This was in line with studies by Awad et al. (2009) and Kota et al. (2012), who suggested that the histology of the gastrointestinal tract may vary depending on the species of microorganisms or the part of the digestive tract. Taheri et al. (2010) showed that the use of *Pediococcus acidilactici* as a probiotic in the diet of broiler chickens stimulates growth of the villi in the duodenum and ileum, but does not affect their height in the jejunum. According to Gunal et al. (2006), the addition of a probiotic stimulates villus growth within the jejunum and ileum. Awad et al. (2009) report that in broiler chickens whose feed is enriched with *Lactobacillus* sp., crypt depth does not change in the duodenum but decreases in the ileum. These discrepancies are explained by insufficient knowledge of the extremely complex dynamics of the intestinal ecosystem of poultry (Rehman et al., 2007), but also by many other factors, including microbial composition (e.g. single strain or multi-strain), feed composition, the quantity of the additive, means and frequency of administration, the age of the individuals, conditions of the rearing environment, or other environmental factors.

Conclusions

Yarrowia lipolytica yeast added in the amount of 3% to the turkey feed may be an alternative to the commonly used *Saccharomyces cerevisiae* yeast, because it improved growth performance, and above all had a more beneficial effect on intestinal histology. The use of *Yarrowia lipolytica* alone can be beneficial for growth performance, while the combined use of 3% *Yarrowia lipolytica* in the feed and a 0.05% addition of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* has a more beneficial effect on gastrointestinal histology.

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