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NUTRITIONAL VALUE OF *YARROWIA LIPOLYTICA* YEAST AND ITS EFFECT ON GROWTH PERFORMANCE INDICATORS IN PIGLETS*

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Abstract

***Yarrowia lipolytica* yeast can be a valuable component of feed compounds due to its high content of protein and essential amino acids, as well as minerals. The objective of the study was to verify the research hypothesis that *Yarrowia lipolytica* (YL) yeast, having a more valuable chemical composition than *Saccharomyces cerevisiae* (SC), can improve growth performance in piglets. An attempt was made to determine what proportion of YL yeast in a compound feed for piglets would produce measurable effects in terms of production and health. The experiment was carried out on 46-day-old piglets receiving experimental mixtures for 39 days. The piglets in the experimental groups were fed the same mixture as the control, but with 3% or 6% fodder yeast of the strain *Yarrowia lipolytica* A-101 in place of soybean meal. The YL fodder yeast had significantly higher content of alanine (by about 50%), lysine, isoleucine and tryptophan (over 30%), leucine, valine, glycine and glutamic acid (over 20%), and tyrosine (by about 17%) in comparison with SC yeast. Content of crude fat was three times higher than in SC yeast. YL yeast had significantly higher content of Na^{+}, K^{+}, Ca^{+2}, Mn^{+2}, S, Mg^{+2} and Zn^{+2} than SC, as well as significantly higher content of vitamins B_2 and E. *Yarrowia lipolytica* fodder yeast introduced to compound feed in the amount of just 3% improved growth performance (an increase in daily weight gain and a decrease in feed conversion per kg weight gain). These results indicate that YL in the amount of 3% in compound feed can be successfully used in the diet of piglets in place of the commonly used SC.**

Key words: *Yarrowia lipolytica*, yeast, piglets, performance

Yarrowia lipolytica (YL) has been used for many years in numerous branches of the food industry. It is involved in the ageing of products such as cheeses and sausages (Iucci et al., 2007) and is a valuable source of many enzymatic proteins, such as protease, esterase or lipase (Fickers et al., 2006; Kim et al., 2007). It can also produce large quantities of organic acids, such as citric, isocitric, pyruvic and alpha-ketoglutaric acid, as a source of carbon (Coelho et al., 2010; Zhou et al., 2010). Cur-

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rently these microbes have also found wide application in production of protein-rich or lipid-rich biomass (Rymowicz et al., 1997). As a result of research on protein-rich biomass, on 20 September 2010 the FEFAC (European Feed Manufacturers' Federation) authorized *Yarrowia lipolytica* yeast, as a fodder material produced from the glycerine fraction derived from biofuel production, for marketing in the European Union. Its use in animal diets was intended not only to enrich feed compounds as a valuable source of protein, vitamins and minerals, but also to supply biomolecules with prebiotic or probiotic properties (Erasmus et al., 2005). In this regard research is being conducted on the use of *Yarrowia lipolytica* yeast in raising animal species such as turkeys (Czech et al., 2014; Merska et al., 2015) or fish (Hatlen et al., 2012; Berge et al., 2013). However, we have found no studies conducted on piglets.

Currently the most popular yeast on the market for use in animal feed is produced on a base of *Saccharomyces cerevisiae* and molasses. These preparations contain considerable quantities of crude protein (approx. 35.9–40.5%), and the amino acid composition of yeast includes large amounts of glutamic acid, aspartic acid and lysine (Fadel et al., 2013). The nutritional value of yeast depends mainly on its chemical composition, which is influenced by such factors as production technology, the type of strains used and the quality of the medium serving as a source of substrates. For this reason the chemical composition of yeast of different strains may vary.

The objective of the study was to verify the research hypothesis that *Yarrowia lipolytica* (YL) yeast, having a more valuable chemical composition than *Saccharomyces cerevisiae* (SC), can improve growth performance in piglets. An attempt was made to determine what proportion of YL in a compound feed for piglets would produce measurable effects in terms of production and health.

Material and methods

The experiment was carried out on 264 piglets (165 barrows and 99 gilts), from litters of line 990 sows and boars, which were assigned to three experimental groups according to their body weight and age. The experimental group consisted of 88 piglets distributed in 11 pens (8 piglets per pen). The piglets in group K (control) received a base compound feed (Table 1) with no fodder yeast. The piglets in groups YL3 and YL6 received 3% and 6% fodder yeast of the strain *Yarrowia lipolytica* A-101 in their feed in place of soybean meal (group YL3 – 30 kg of yeast per tonne of feed; group YL6 – 60 kg of yeast per tonne of feed).

In view of the fact that no studies have been conducted on piglets using *Yarrowia lipolytica* yeast, the experiment was performed on somewhat older and stronger individuals. The average age of the piglets on the first day of the experiment was 46 days (the piglets were weaned from the sows in the third week after birth). The experiment lasted for 39 days.

The piglets in the experimental groups received complete mixed rations: Starter I (days 1 to 21 of the experiment) and then Starter II (days 22 to 39 of the experiment). The nutrient content in each period was in accordance with NRC (1998). *Yarrowia*

lipolytica A-101 is a commercial product. It was produced on glycerol from rapeseed biofuel production.

The piglets were fed *ad libitum*, with permanent access to drinking water. Nutrient content in the feed mixtures was calculated (Table 1). Yeast in powder form was added to the feed mixtures during production of the mixtures.

Table 1. Composition of compound feed and nutrient content during each period of the experiment

Item	Experimental group					
	days 1–21 of experiment			days 22–39 of experiment		
	K	YL3	YL6	K	YL3	YL6
Barley meal	42	42	42	35	35	35
Wheat meal	35	35	35	35	35	35
Soybean protein concentrate	6	6	6	0	0	0
Soybean meal	9	6	3	20	17	14
YL yeast	0	3	6	0	3	6
Wheat bran	3.95	3.95	3.95	5.61	5.61	5.61
Soya oil (20% linolenic acid)	0.5	0.5	0.5	1	1	1
Premix [(1)]	0.5	0.5	0.5	0.5	0.5	0.5
Calcium formate	1.4	1.4	1.4	1.3	1.3	1.3
MCP – $CaHPO_4$	0.57	0.57	0.57	0.5	0.5	0.5
Salt	0.4	0.4	0.4	0.4	0.4	0.4
DL-methionine	0.03	0.03	0.03	0.04	0.04	0.04
Lysine HCl	0.42	0.42	0.42	0.4	0.4	0.4
L-threonine	0.12	0.12	0.12	0.14	0.14	0.14
Xylanase	0.1	0.1	0.1	0.1	0.1	0.1
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Nutrient content of the diets (%)						
Metabolizable energy (MJ)	12.9	13.0	13.0	12.9	13.0	13.0
Crude protein (g)	181	180	180	181	180	180
Lysine HCl 98% (g)	12.0	11.9	11.8	12.0	11.9	11.8
Methionine+Cystine (g)	6.40	6.40	6.40	6.40	6.40	6.40
Threonine (g)	7.55	7.55	7.55	7.55	7.55	7.55
Calcium (g)	7.04	7.04	7.04	7.04	7.04	7.04
Phosphorus (g)	5.19	5.12	5.28	5.19	5.12	5.28
Sodium (g)	1.76	1.75	1.73	1.76	1.75	1.73

[(1)] Vitamins and minerals (content in 1 kg): vitamin A: 15,000 IU, vitamin D_3: 2,000 IU, vitamin E: 75 mg, vitamin B_1: 2.5 mg, vitamin B_2: 4 mg, vitamin B_6: 4 mg, vitamin B_{12}: 0.04 mg, vitamin PP: 30 mg, vitamin K_3: 2.5 mg, choline: 350 mg, Fe: 120 mg, Cu: 160 mg, Zn: 149 mg, Mn: 50 mg, I: 0.5 mg, Se: 0.30 mg, Co: 50 mg, folic acid: 1 mg, pantothenic acid: 14.00 mg.

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

Samples of *Yarrowia lipolytica* and *Saccharomyces cerevisiae* yeast were collected for analysis in accordance with Commission Regulation (EC 152/2009) and Commission Regulation (EC 691/2013) amending Regulation (EC 152/2009) as regards methods of sampling and analysis. They were analysed for content of basic nutrients, i.e. crude protein, dry matter, crude ash, and crude fat, according to the AOAC

procedure (2000). We also determined the quantitative composition of amino acids (by ion-exchange chromatography with spectrophotometric detection – IC-UV); content of vitamins, i.e. E (high-performance liquid chromatography with photodiode array detection – HPLC-DAD), B_1, B_2, B_6 and B_{12} (high-performance liquid chromatography with fluorescence detection – HPLC-FLD); content of macro- and microelements, i.e. Na^+, K^+, Mg^{+2}, Ca^{+2}, Fe^{+2}, Mn^{+2}, Zn^{+2}, S (flame atomic absorption spectrometry – AAS) and phosphorus (by spectrophotometry); and content of heavy metals (Cd^{+2} and Pb^{+2} by electrothermal atomic absorption spectrometry – ETAAS, Hg^{+2} by flame atomic absorption spectrometry – AAS, and As^{+3} by hydride generation atomic absorption spectrometry – HGAAS).

Microbiological analysis of the feed samples was performed as well (*Salmonella*; number of *Clostridium perfringens* per g; presence of coagulase-positive staphylococci in 25 g – horizontal methods; and the number of *Enterobacteriaceae* – plate method). Physical properties of the yeast were determined (organoleptic evaluation by the visual method and pH by the potentiometric method).

The yeast samples were also tested for the presence of mycotoxins. The contents of ochratoxin A, deoxynivalenol, and aflatoxin B1, B2, G1 and G2 were determined by high-performance liquid chromatography methods.

Growth performance

Growth performance in the piglets was evaluated on the basis of measurements of their body weight, feed consumption and conversion, as well as frequency of diarrhoea.

Statistical analysis

Statistical computations were carried out using Statistica v. 6.1.G software. The significance of differences between means was determined by one-way analysis of variance (ANOVA) at significance levels of 0.05 and 0.01.

Results

The content of crude protein in the *Yarrowia lipolytica* yeast was slightly higher than in *Saccharomyces cerevisiae* yeast. It is particularly noteworthy that the content of crude fat in YL yeast was three times the content in SC ($P \leq 0.01$). Content of crude ash in the two species was similar, at about 8% (Table 2).

The YL fodder yeast had significantly higher content of lysine (by about 30%) than SC yeast ($P \leq 0.01$). YL also had significantly higher content of 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%) than SC yeast. SC yeast had significantly higher content of arginine (by about 17%) and proline (by about 41%) than YL yeast. The content of sulphuric amino acids – methionine and cystine – was similar in the two species of yeast.

Table 2. Content of nutrients (%) and amino acids (g kg^{-1} D.W.) in fodder yeast

Parameter	*Yarrowia lipolytica*			*Saccharomyces cerevisiae*		
	min.	max.	mean	min.	max.	mean
Crude protein	39	48	43.50	31.14	48.98	40.06
Dry matter	95	98	96.50	89.77	96.23	93.00
Crude ash	7.79	8.33	8.01	7.39	8.61	8.00
Crude fat	0.5	2.5	1.5 A	0.45	0.55	0.50 B
Amino acids						
Aspartic acid	25.39	46.21	35.80	30.02	38.88	34.45
Threonine	10.22	29.98	20.10	15.85	18.77	17.31
Serine	16.68	19.68	18.18	18.44	21.26	19.85
Glutamic acid	49.81	71.56	60.68 a	45.87	53.35	49.61 b
Proline	14.70	19.62	17.16 b	20.09	28.45	24.27 a
Glycine	15.52	23.74	19.63 a	14.64	18.00	16.32 b
Alanine	32.63	40.05	36.34 A	22.65	25.63	24.14 B
Valine	22.33	25.47	23.90 a	16.87	20.45	18.66 b
Isoleucine	18.67	21.07	19.87 a	12.63	17.05	14.84 b
Leucine	29.91	32.15	31.03 a	21.79	27.15	24.47 b
Tyrosine	13.52	16.42	14.97 a	11.09	14.53	12.81 b
Phenylalanine	15.44	18.98	15.44	12.49	19.05	15.77
Histidine	8.93	10.23	9.58	8.01	10.29	9.15
Lysine	27.27	38.41	32.84 A	23.62	26.84	25.23 B
Arginine	12.31	23.89	18.10 b	19.05	23.29	21.17 a
Cystine	2.55	6.21	4.38	4.38	5.08	4.73
Methionine	4.73	9.69	7.21	5.99	7.71	6.85
Tryptophan	5.99	7.04	6.51 a	4.03	5.67	4.85 b

a, b – average values with different letters differ significantly at $P \leq 0.05$.
A, B – average values with different letters differ significantly at $P \leq 0.01$.

YL has nearly twice the content of calcium and sodium and as much as 4 times the manganese content as SC yeast ($P \leq 0.01$). Content of sulphur, potassium, magnesium and zinc was also significantly higher in the YL yeast ($P \leq 0.05$) than in brewer's yeast. Only phosphorus content was significantly ($P \leq 0.01$) higher in SC yeast than in YL (Table 3). YL yeast had significantly ($P \leq 0.01$) higher content of vitamin B_2 (more than two-fold) and vitamin E (over 60%), whereas SC yeast had significantly ($P \leq 0.01$) higher content of vitamin B_1 (by about 21%). Content of B_6 in the two species was similar. In regard to the sensory properties analysed, the yeast had favourable properties of colour, odour, form, pH and density (Table 4). Microbiological analysis found no *Salmonella*, *Clostridium perfringens* or *E. coli* (Table 4).

The content of heavy metals in the yeast was low and did not exceed acceptable levels (Regulation of the Minister of Agriculture and Rural Development, 2012) (Table 5). The results of the mycotoxin tests were within acceptable norms (EC 576/2006) (Table 5).

Table 7 presents the frequency of occurrence of diarrhoea during the experiment in each of the experimental groups. In the animals from group YL6, receiving com-

pound feed with 6% YL yeast, diarrhoea was observed more frequently and lasted longer (number of days with diarrhoea = 1.45%; in the first days of the experiment 10 piglets had diarrhoea for 5 days). However, the diarrhoea had no significant effect on the growth of the animals. In comparison with the control (group K) and group YL6, daily weight gain was significantly higher in the piglets receiving feed containing 3% YL fodder yeast, with lower feed conversion from days 22 to 39 of the experiment (P≥0.05) (Table 6).

Table 3. Content of minerals and vitamins in the dry weight of fodder yeast

Parameter	*Yarrowia lipolytica*			*Saccharomyces cerevisiae*		
	min.	max.	mean	min.	max.	mean
Sodium (g kg^{-1})	12.81	19.38	16.11 A	7.87	8.93	8.40 B
Potassium (g kg^{-1})	19.10	25.14	22.12 a	16.41	18.27	17.34 b
Sulphur (g kg^{-1})	3.53	6.21	4.87 a	3.33	4.07	3.70 b
Calcium (g kg^{-1})	3.04	5.48	4.26 A	1.99	2.47	2.24 B
Phosphorus (g kg^{-1})	3.40	5.28	4.34 B	8.00	12.04	10.02 A
Magnesium (g kg^{-1})	1.82	2.04	1.93 a	1.30	1.66	1.48 b
Manganese (mg kg^{-1})	12.03	18.21	15.12 A	3.35	4.33	3.84 B
Zinc (mg kg^{-1})	57.97	82.57	70.47 a	55	66.86	60.93 b
Iron (mg kg^{-1})	76.88	143.00	109.94	98.9	103.24	101.07
Vitamins (mg kg^{-1} D.W.)						
Vitamin E	6.04	7.52	6.78 B	37.9	52.1	45.00 A
Vitamin B_1	80.76	115.24	98.00 B	100.8	136.8	118.8 A
Vitamin B_2	11.01	21.47	16.24 A	5.83	6.37	6.10 B
Vitamin B_6	25.43	31.41	28.42	19.3	33.7	26.50
Vitamin B_{12} (μg kg^{-1} d.m.)	52.80	60.00	56.40 A	3.52	5.44	4.48 B

a, b – average values with different letters differ significantly at P≤0.05.
A, B – average values with different letters differ significantly at P≤0.01.

Table 4. Organoleptic and microbiological evaluation of fodder yeast

Parameter	*Yarrowia lipolytica*			*Saccharomyces cerevisiae*		
	min.	max.	mean	min.	max.	mean
Density (kg m^{-3})	400	460	430	390	430	410
Specific weight (N m^{-3})	3924	4415	4169.5	4001	4501	4251
pH	3.8	6.0	4.9	4.0	6.1	5.01
Odour	yeast-like			yeast-like		
Colour	beige			pale yellow		
Form	powder			powder		
Salmonella (presence in 25 g)	not detected			not detected		
Clostridium perfringens (presence in 0.1 g)	not detected			not detected		
E. coli (presence in 0.1 g)	not detected			not detected		

Table 5. Content of heavy metals (mg kg^{-1}) and mycotoxins (μg kg^{-1}) in fodder yeast

Parameter	*Yarrowia lipolytica*			*Saccharomyces cerevisiae*		
	min.	max.	mean	min.	max.	mean
Arsenic	0.025	0.36	0.29	0.022	0.034	0.028
Cadmium	0.028	0.050	0.039	0.034	0.039	0.037
Mercury	0.006	0.009	0.007	0.003	0.007	0.005
Lead	0.050	0.057	0.053	0.047	0.058	0.052
Deoxynivalenol	50.72	53.52	52.12	48.37	50.55	49.50
Ochratoxin A		none			none	
Aflatoxin B1		none			none	
Aflatoxin B2		none			none	
Aflatoxin G1		none			none	
Aflatoxin G2		none			none	

Table 6. Growth performance results and cases of diarrhoea in piglets

	Group		
	K	YL3	YL6
Age at start of experiment (days)	46.75	45.71	45.62
Body weight (kg):			
start of experiment	10.78	11.15	11.04
21 days of experiment	19.10	19.98	19.61
39 days of experiment	30.19 b	32.98 a	30.07 b
Daily weight gain during period (kg), AGD			
1–21 days of experiment	0.397	0.421	0.408
22–39 days of experiment	0.613 b	0.722 a	0.581 b
1–39 days of experiment	0.498 b	0.560 a	0.489 b
Mean daily feed intake (kg), ADFI			
1–21 days of experiment	0.631	0.620	0.581
22–39 days of experiment	0.993	0.984	1.023
1–39 days of experiment	0.798	0.788	0.785
Feed conversion in kg per kg weight gain, FCR			
1–21 days of experiment	1.59	1.47	1.42
22–39 days of experiment	1.62 b	1.36 c	1.76 a
1–39 days of experiment	1.60 a	1.41 b	1.61 a
Number of animals on the first day of the experiment	88	88	88
Number of animals that died during the experiment	2	3	2
Number of animals excluded from the experiment	1	0	1
Number of days with diarrhoea	1.00	0.82	1.45

a, b – values in the same rows with different letters differ significantly at $P \leq 0.05$.

Discussion

The fodder market offers many kinds of fodder yeast, with substantially different chemical compositions. The results of our study show that in terms of chemical

composition and nutritional value *Yarrowia lipolytica* yeast is not inferior to *Saccharomyces cerevisiae*, which is currently on the market. Differences in nutrient content may be due to factors such as production technology or the type of cultures or substrates used. It is particularly noteworthy that YL yeast has significantly higher content of lysine than SC yeast, which makes it a more valuable source of complete fodder protein. Because YL yeast is grown on glycerol, a by-product of production of biofuels from rapeseed (Rymowicz et al., 2010), it has substantially higher energy content than SC yeast. It should be noted that glycerol is a completely safe source of carbon and energy for YL yeast because it is registered as a feed additive (E 422) in the category 'technological additives' and there are no quantitative limits on its application in the animal species for which it is used (EC 1831/2003, 2008). According to a study by Rymowicz et al. (1997), *Yarrowia lipolytica* has the ability to accumulate large quantities of fat in its cells, in which over 90% of fatty acids are unsaturated, with a high percentage of essential fatty acids (28–44%).

Fodder yeast and yeast-like preparations can act by a variety of mechanisms, such as modulation of the immune system (Davis et al., 2004), binding of pathogenic bacteria and their toxins (Mourão et al., 2005), or interactions with the digestive tract (Mathew et al., 1998). By enhancing immune processes and thereby the health of animals, they indirectly contribute to improvement in growth performance. Positive effects of the use of fodder yeast in the diet of piglets have been presented by Shen et al. (2009). Using SC yeast the authors of the study obtained higher daily weight gain, which was reflected in greater feed intake. Van der Peet-Schwering et al. (2007) observed higher daily weight gain and better feed consumption per kg of weight gain in a group of piglets receiving SC yeast in their feed than in a control group. A study by van Heugten et al. (2003) also showed better growth performance following the use of live SC yeast cells. These results are consistent with the results of our study, as during the second period of the experiment (days 22–39) the piglets receiving feed containing 3% YL yeast had a faster growth rate and lower feed conversion rate per kg of weight gain than the control and group YL6. Moreover, during the entire experiment the fewest cases of diarrhoea were noted in the group of piglets fed a mixture with 3% YL yeast. In the piglets fed a mixture containing 6% yeast, diarrhoea was observed more often and lasted longer. It is difficult to interpret the reduction in growth performance and the increase in the frequency of diarrhoea in the piglets receiving feed with 6% *Yarrowia lipolytica* yeast. This may have been due to the body's adaptation to a larger dose of a supplement which not only contains basic nutrients but is also a rich source of numerous other bioactive substances. An excessive dosage of yeast could also cause uncontrolled fermentation processes in young piglets, which may have induced this type of response. The use of a smaller amount of fodder yeast is also more economical.

Yarrowia lipolytica yeast is a rich source of basic nutrients and has a high content of valuable amino acids (lysozyme, tryptophan, tyrosine, leucine, isoleucine, valine, alanine, glycine and glutamic acid), as well as minerals (Ca^{+2}, Zn^{+2}, Na^{+}, Mn^{+2}, S, K^{+2} and Mg^{+2}), and can be successfully used in the amount of 3% of compound feed for piglets instead of the commonly used yeast *Saccharomyces cerevisiae.*

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