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Original article

The effect of yeast *Yarrowia lipolytica* on the antioxidant indices and macro- and microelements in blood plasma of turkey hens

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Abstract

The aim of the study was to assess the effect of different amounts of *Yarrowia lipolytica* yeast on the redox response and content of macro- and microelements in the blood plasma of turkey hens. The experiment was carried out on 240 turkey hens aged from 1 to 16 weeks. The birds were randomly assigned to 3 experimental groups of 80 birds each. Group I served as a control (K) and did not receive any experimental compounds. The turkey hens from the experimental groups (YL3 and YL6) were administered dried *Yarrowia lipolytica* yeast in their feed mixtures in the amount of 3% (YL3) or 6% (YL6).

Yarrowia lipolytica yeast in the feed mixtures for the turkey hens did not induce oxidation reactions in the organism of the birds. However, an increase in catalase activity and a reduction in the level of LOOH, MDA and vitamin C were observed in the blood plasma of the turkey hens whose diet was supplemented with YL yeast. In the case of other indices, such as superoxide dismutase activity and total antioxidant potential (FRAP), the additive caused no significant changes. Administering *Yarrowia lipolytica* yeast to turkey hens may stimulate the enzymatic response of the antioxidant system (e.g. increasing catalase activity), mainly by increasing the concentration of iron in the plasma.

Key words: turkey hens, yeast, antioxidant, mineral elements, blood plasma

Introduction

The use of yeast of the species *Yarrowia lipolytica* (YL) in poultry feeding is innovative and has not been fully researched. To this point the main species of yeast used as a feed supplement has been *Saccharomyces cerevisiae* (SC). The results of studies conduc-

ted on various animal species (mainly cattle and pigs) indicate that the use of this species of yeast has multifaceted benefits, due in part to its chemical composition and biochemical functions (Wójcik et al. 2008, Baurhoo et al. 2009, Cakiroglu et al. 2010, Przysiecki et al. 2010, Al-Mansour et al. 2011).

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According to Davis et al. (2004) and Wójcik et al. (2008), an immune activation effect can be attributed to *Saccharomyces cerevisiae*, while according to Mourfo et al. (2005) it has an inhibitory effect on the replication of pathogenic bacteria and their toxins. SC yeast also stimulate replication of the saprophytic microflora of the digestive tract, which has been demonstrated in studies by Mathew et al. (1998) and Baurhoo et al. (2009).

Because *Yarrowia lipolytica* has a similar chemical composition to that of *Saccharomyces cerevisiae*, it can be supposed to have similar functions in the organism. Our own preliminary research (Merska et al. 2013, Czech et al. 2014) has shown that this yeast, in the amount of 3% and, particularly, 6%, stimulates the immune defence mechanisms of the organism which is evidenced by an increase in the plasma lysozyme level, the percentage of phagocytic cells (%PC) and the phagocytic index (PI), and a reduction in the H/L ratio in the blood of turkey hens. Moreover, it has no negative effect on the health condition of the birds, as indicated by their normal weight gain and biochemical indicators (Merska et al., 2013).

This reaction of the organism suggests that YL yeast may not only effectively stimulate immune processes, but also beneficially inhibit oxidation processes in the organism. The available literature, however, supplies little information regarding research on the possibility of using SC or YL nutrient yeast as a modulator of redox processes in food, or more importantly, in the organism of animals.

Hence the aim of the study was to assess the effect of different amounts of *Yarrowia lipolytica* yeast on the redox response and content of macro- and microelements in the blood plasma of turkey hens.

Materials and Methods

The experiment was carried out on 240 turkey hens of the Big 6 line, aged from 1 to 16 weeks. The birds were randomly assigned to 3 experimental groups of 80 turkeys each, with 5 replications of 16 birds each. The birds were kept in cages 2.5 x 4 m in size, under conditions recommended for turkey fattening according to the Regulation of the Ministry of Agriculture and Rural Development of 15 February 2010, No. 56, item 344. The experimental procedure was approved by the 2nd Local Ethics Commission for Experiments with Animals in Lublin (approval no. 19/2012).

Group I served as a control (K) and did not receive the yeast *Yarrowia lipolytica*. The turkey hens in the experimental groups (YL3 and YL6) were administered dried *Yarrowia lipolytica* yeast in two different

amounts, 3% (YL3) and 6% (YL6) of the feed mixtures. Birds from all groups received feed mixtures composed of wheat, triticale, soybean meal, rapeseed cake, potato protein (Starter – 1-3 weeks) and soybean oil. All the mixtures were isonitrogenous and isoenergetically balanced. The nutrient content in 1 kg of feed mixture given to the turkey hens was as follows: TP – 28.76%; ME – 2,700 kcal kg⁻¹; Lys – 1.9%; Met+Cys – 0.6%; Ca – 1.4%; P – 0.7% (1-3 weeks); TP – 24.5%; ME – 2,800 kcal kg⁻¹; Lys – 1.6%; Met+Cys – 0.6%; Ca – 1.3%; P – 0.65% (4-7 weeks); TP – 22.50%; ME – 2,800 kcal kg⁻¹; Lys – 1.4%; Met+Cys – 0.5%; Ca – 1.15%; P – 0.6% (8-12 weeks); and TP – 18.50%; ME – 2,900 kcal kg⁻¹; Lys – 1.1%; Met+Cys – 0.4%; Ca – 1.0%; P – 0.5% (13-16 weeks) (Czech et al., 2014). All birds were kept under the same management, environmental and hygienic conditions except for the different dietary levels of yeast. The mineral content of *Yarrowia lipolytica* yeast is presented in Table 1. Feed and fresh water were provided *ad libitum* throughout the experimental period.

Table 1. Content of minerals in *Yarrowia lipolytica* yeast.

Parameter	Unit	<i>Yarrowia lipolytica</i>
Phosphorus	g kg ⁻¹	4.78 ± 0.55
Magnesium	g kg ⁻¹	1.82 ± 0.24
Calcium	g kg ⁻¹	4.26 ± 0.49
Copper	mg kg ⁻¹	6.53 ± 0.60
Zinc	mg kg ⁻¹	71.63 ± 3.07
Iron	mg kg ⁻¹	109.94 ± 5.80

Blood for analysis was sampled from the brachial vein of 10 birds from each group at the end of the experimental period (16th week of the birds; life). Blood was collected into 10 ml heparinized test tubes in volume under the supervision of a veterinarian.

Biochemical analysis

Activity of superoxide dismutase (SOD, EC 1.5.1.1) in the blood plasma of the birds was measured spectrophotometrically using the adrenaline assay method following Misra in Greenwald (Clairborne, 1985), with a modification of the wavelength to 320 nm (Bartosz, 2013). Catalase (CAT, EC 1.11.1.6) activity was determined according to Bartosz (2013). Total antioxidation potential of the plasma (FRAP) was measured according to Benzie and Strain (1996). Plasma concentrations of lipid peroxidation products were determined as follows: hydroperoxide (LOOH) according to Gay and Gębicki (2002) and malondialdehyde (MDA), as the final product of lipid oxidation,

Table 2. Level of redox parameters potential in the blood plasma of the turkey hens.

Item	Experimental groups			SEM
	K	YL3	YL6	
LOOH $\mu\text{mol l}^{-1}$	1.59 ^b	1.17 ^a	1.23 ^{ab}	0.055
MDA; $\mu\text{mol l}^{-1}$	0.732 ^b	0.481 ^a	0.473 ^a	0.078
CAT; U ml^{-1}	3.34 ^a	4.44 ^b	4.59 ^b	0.132
SOD; U ml^{-1}	40.45	45.91	40.38	0.799
FRAP; $\mu\text{mol l}^{-1}$	34.58	35.55	37.48	1.099
Vitamin C; mg l^{-1}	0.056 ^b	0.044 ^a	0.050 ^{ab}	0.008

^{a, b} – values in the same rows with different letters differ at $p \leq 0.05$

Table 3. Content of macro- and microelements in the blood plasma of the turkey hens.

Item	Experimental groups			SEM
	K	YL3	YL6	
Phosphorus; mmol l^{-1}	1.92	1.95	1.94	0.018
Magnesium; mmol l^{-1}	0.958 ^b	0.841 ^a	0.860 ^a	0.012
Calcium; mmol l^{-1}	2.06 ^a	2.73 ^b	2.55 ^b	0.024
Copper; $\mu\text{mol l}^{-1}$	4.14 ^a	4.90 ^b	5.00 ^b	0.047
Zinc; $\mu\text{mol l}^{-1}$	18.38	18.04	18.25	0.229
Iron; $\mu\text{mol l}^{-1}$	25.68 ^a	28.25 ^b	28.59 ^b	0.059

^{a, b} – values in the same rows with different letters differ at $p \leq 0.05$

according to Ledwozyw et al. (1986). The content of vitamin C was determined colorimetrically in a reaction with 2,6-dichlorophenolindophenol following the method of Omaye et al. (1979).

Blood plasma samples were also used to determine the concentration of macroelements– phosphorus, magnesium and calcium, and microelements – zinc, copper and iron, using test kits developed by Cormay.

The content of minerals was determined in the yeast. Phosphorus was determined by spectrophotometry, and calcium, magnesium, copper, zinc and iron by flame atomic absorption spectrometry (ASA).

Statistical computations were carried out using Statistica v. 6.1.G software. For all analysed dependent variables, one-way analysis of variance ANOVA was conducted according to the following formula:

$$y_{ij} = \mu + \alpha_i + e_{ij}$$

where:

μ – total mean,

α_i – stable effect of i -th additive for $i = 0, 1, \dots, 3$ (for control $i = 0$),

e_{ij} – random error has normal distribution $N(0,)$.

Results

Data referring to the redox potential in the blood plasma of turkey hens on a diet supplemented with dried yeast *Yarrowia lipolytica* are presented in Table 2. The content of lipid hydroperoxida (LOOH) and malondialdehyde in the YL3 and YL6 groups was significantly lower ($p \leq 0.05$); the difference was about 34% in the case of LOOH and 24% for MDA with respect to the control (K) (Table 2). The addition of *Yarrowia lipolytica* yeast to the feed mixtures led to a significant ($p \leq 0.05$) increase in catalase (CAT) activity in both experimental groups with respect to the control, by 32% and 37%, respectively. Analysis of vitamin C content in the plasma of the turkey hens showed a decrease in the YL3 group with respect to the control ($p \leq 0.05$). The addition of *Yarrowia lipolytica* to the feed mixtures did not significantly ($p > 0.05$) affect SOD activity or the total antioxidant potential of the plasma (FRAP).

The content of macro- and microelements in the blood plasma of the turkey hens are presented in Table 3. A significantly ($p \leq 0.05$) lower concentration of magnesium was noted in the blood plasma of the turkey hens receiving mixtures containing 3% or 6% *Yarrowia lipolytica* yeast. The addition of YL yeast to the feed mixtures in the amount of 3% and 6% caused a significant ($p \leq 0.05$) increase in the concentration of calcium, copper and iron in the blood plasma of the turkey hens in comparison with the control. The aver-

age differences obtained were about 28% (Ca^{+2}), 19% (Cu^{+2}) and 10% (Fe^{+2}) (Table 3). However, no statistically significant ($p>0.05$) differences were noted in the content of phosphorus and zinc.

Discussion

Nutrient yeast, due to its beneficial amino acid composition, high content of B vitamins, choline, biotin, niacin and other bioactive components (such as enzymes, L-carnitine and the natural antibiotic malvidin) (Woźnica and Czech, 2010) stimulate immune reactions in the organism, directly improving the health of animals and indirectly improving production results (Czech et al. 2014). Moreover, yeast has the capacity to stimulate antioxidant reactions or to inhibit oxidation reactions (Aluwong et al. 2013, Pyka and Kopczyńska 2013). The properties of yeast, including those contributing to maintenance of a reductive environment in the organism, can lead to neutralization of free radicals by transforming them into less active derivatives. This can delay or even inhibit oxidation processes and thus restore and maintain homeostasis in the organism (Mandal et al. 2009).

Antioxidant functions in the organism are performed by numerous low-molecular-weight antioxidants, including bilirubin, creatinine, urea, uric acid, the reduced form of h-lipoic acid, glutathione, active forms of vitamins E and C, dissociated cations of transition metal groups (Cu^{+2} , Zn^{+2} , Mn^{+2} , Fe^{+3} and Se^{+2}) and others (Bartosz 2013). Our own research has shown that *Yarrowia lipolytica* affects the plasma level of UA, UREA, BIL and CREAT, which indicates an antioxidant response of the turkey hen organism to this additive (Czech et al. 2014). In addition to low-molecular-weight antioxidants, enzymes play a fundamental role in antioxidant defence of the organism. The most important enzymes with antioxidant activity are superoxide dismutase, glutathione peroxidase and catalase (Ognik and Krauze 2012). The action of these enzymes can be enhanced by feed additives such as herbs, probiotics, prebiotics or nutrient yeast, to which immunostimulatory functions are attributed (Świątkiewicz and Koreleski 2007, Cakiroglu et al. 2010, Przysiecki et al. 2010, Aluwong et al. 2013).

The addition of YL yeast to the turkey hen feed caused a significant increase in catalase activity which is usually caused by a reaction to the stress the birds are exposed to during the production process. In the experiment presented the increase in catalase activity did not, however, correspond to an increase in the activity of superoxide dismutase, which indicates that we are not dealing with a negative effect of yeast on

the organism of the birds. Since the level of LOOH and MDA in the groups receiving yeast additives was significantly lower than in the control, the increase in CAT activity can be presumed to be the effect of stimulation of the antioxidant system by iron-rich yeast. Grabowska-Bochenek et al. (2000) administered a selenium yeast preparation to rabbits and also noted a reduced level of malondialdehyde. This may have been due to the presence of β -glucans in the yeast, which are capable of stimulating immunomodulatory cytokines and regulating the level of interleukin 1β (IL- 1β), isoenzyme CYP7A1 of cytochrome P450, compounds that limit free radical generation in the organism (Hong et al. 2004, Chen and Raymond 2006).

Studies (Ilavarasan et al. 2003, Konyalioglu and Karamenderes 2005) indicate that an increase in catalase activity in the blood of turkey hens may result from the use of feed additives such as herbs, probiotics and others. A study by Aluwong et al. (2013) also shows that adding a probiotic to chicken feed together with *Saccharomyces cerevisiae* yeast can decrease catalase activity.

Ascorbic acid, which eliminates free radicals in the aqueous phase, has an active role in protecting cell membranes (McDowell et al. 2007). Turkeys are capable of synthesizing ascorbic acid, but during intensive rearing synthesis is often insufficient to meet metabolic needs (Świątkiewicz and Koreleski 2007). In the present study a significant decrease in vitamin C content was noted only in the group with 3% YL, so it was unlikely to be the effect of the yeast, as in the group receiving double this amount of yeast a similar correlation was not observed. The vitamin C concentrations observed correspond to the values noted in our previous research (Ognik and Krauze 2012) and by other authors (Gebert et al. 2006).

Among the numerous mineral elements that can take part in antioxidant reactions, ions of transition metal groups, such as copper, zinc and iron, merit particular attention, as they constitute catalytic centres of antioxidant enzymes (Pyka and Kopczyńska 2013). Their optimal concentration in the blood plasma ensures the proper functioning of enzymatic centres (Kleczkowski et al. 2004). A decrease in the concentration of these minerals may suggest that they are being utilized in the organism for antioxidant defence, while an increase may indicate that the organism is free of stress (Ognik and Krauze 2012).

Reduced ions of metals such as iron enter into reactions with hydrogen peroxide, leading to the production of highly reactive hydroxyl radicals or superoxide ions (Gebert et al. 2006). When such reactions are not initiated, iron and copper ions are bound by proteins present in the cell – ferritin, transferrin

and ceruloplasmin – and take part in erythropoietic processes (Kleczkowski et al. 2004). The present study shows that the YL yeast additive, both 3% and 6%, led to an increase in the concentration of iron and copper in the blood plasma of the turkey hens. This can be attributed to the better bioavailability of these elements in YL yeast, which were shown to be particularly rich in these microelements (Table 1).

Apart from microelements, yeast are also a valuable source of macroelements, such as calcium (0.5%), phosphorus (1.43 %) or magnesium (0.14 %) (Rodríguez et al., 2011). Their involvement in processes leading to inhibition of oxidation processes is small, but they are essential for maintaining homeostasis in the organism of birds. Magnesium ions influence the bioavailability of calcium ions, and vice versa. The interrelationship between the concentration and metabolism of Mg and Ca ions is very important, because the two elements behave antagonistically towards one another (Saris et al. 2000). This is confirmed by the results obtained in the experiment, as a significantly higher calcium concentration and lower magnesium concentration were observed in the turkey hens receiving the *Yarrowia lipolytica* yeast additive. The improvement in calcium utilization and associated magnesium content in the turkey hens is unfavourable in terms of health. It is difficult to definitively attribute the effect observed to the yeast because, as in the case of vitamin C, increasing the proportion of yeast in the feed did not cause a directly proportional increase in the calcium concentration in the plasma or a directly proportional decrease in magnesium content. Moreover, both the calcium and magnesium content noted in the experimental groups oscillated within the range of values obtained for these elements in other studies (Kleczkowski et al. 2004, Czech and Ognik 2014).

Conclusions

It can be concluded that the addition of *Yarrowia lipolytica* yeast to feed mixtures for turkey hens did not induce oxidation reactions in the organism of the birds. Administration of *Yarrowia lipolytica* yeast to turkey hens may stimulate the enzymatic response of the antioxidant system (e.g. increasing catalase activity), mainly by increasing the iron concentration in the plasma.

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