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The effects of dietary biotechnological products of *Saccharomices cerevisiae* on growth performance, health status, and meat composition in broiler chickens

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Abstract: The present study was conducted to investigate the effects of three biotechnological products of *Saccharomyces cerevisiae* (organic selenium, concentrated mannan of *S. cerevisiae*, and living cells of the same yeast) used in broiler diet on growth performance, health status, and meat quality and composition. One hundred day-old broiler chickens were divided into four groups (control and three experimental groups using the above-mentioned diet supplements) and fed basic feed mixture (developed by the Nutrition Department at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania) for 42 days. After this period, from each group, five birds were sacrificed and measurements for slaughter yield, percent of different body parts, and chemical composition of meat were determined. At the end of the research period (42 days), average values of corporal mass show very significant differences between the experimental groups and the control group ($P < 0.001$). Registered data show that the administration of 0.03% organic selenium in the broilers' diet resulted in a significant difference ($P < 0.001$) in the meat content of selenium towards control. No significant differences in the chemical composition of breast meat were registered between the experimental groups. The results of the current study indicate that different organic prebiotic and probiotics supplementation in broiler chickens' diet improves growth performance and affects immunological parameters (albumins and globulins). Organic selenium supplementation improves breast meat quality by increasing the selenium level.

Key words: Organic additives, broiler chickens, health status, meat composition

1. Introduction

Global demographic increases have resulted in an accelerated and forced development of the food industry around the world, and the increasing of the world's population at this accelerated pace causes serious problems in providing food for all people on the planet. Under these circumstances, the need to ensure enough animal protein in food has materialized in the use of various growth promoters, the most widespread being those based on antibiotics. However, if the use of these growth promoters has had a significant impact on animal productivity, we cannot say the same thing about the quality of food products, which has reached such a point that their consumption over a long period may endanger the health of the consumer.

In recent years, there has been a growing tendency to replace feed supplements based on antibiotics due to the possible risks associated with their use in animal feed, namely residual waste and the occurrence of antibiotic resistance in consumers.

For this reason, researchers around the world have tried and managed to find alternative feeding strategies based on organic additives, which are used in animal feed, resulting in improved productivity, product quality, animal health and, implicitly, the health of consumers (1,2).

Keeping birds in good health with high welfare standards results in high quality carcasses and meat products (3,4).

In recent times, consumers have become more aware of the relationship between the quality of meat and animal welfare (5), but different problems have surfaced in the last decades in poultry growing, and this has given rise to some negative effects on consumers' perspectives (6) about including meat in their daily diet.

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Overall, poultry meat fits the current consumer demand because it includes lower contents of fat cholesterol and sodium and has higher levels of unsaturated fatty acids (7). Broiler chickens are raised for their rapid development in a short time, readiness for scarification, and high yield of meat versus bone.

Diet is a very important factor in this process (8) and using feed additives made from natural sources that improve health and shorten the development period is a challenge for any breeder. Poultry meat quality is affected by a series of factors such as genotype, diet, and age at the time of slaughter (9,10).

Selenium is an important microelement with antioxidant properties, both in animals and humans. The importance of selenium in animal feed is a known effect of maintaining the cellular integrity of muscle tissue (11). This is an important characteristic for meat because water loss and tissue damage during meat cooking denote a low quality of meat.

A biotechnological product containing an organic form of yeast and selenium mixture, made for a better absorption, storage, and use of selenium in animal organisms compared to inorganic selenium, is used in animal feeding (11).

Prebiotic product from concentrated mannan oligosaccharide, derived from the mannan fraction of a specific strain of *Saccharomyces cerevisae* developed through nutrigenomics, is used in animal feeding, helping animals to reach their maximum genomic potential without any antibiotic supplementation in their diet. Its use in the animal diet (especially in chickens) also results in growth and intestinal improvements such as villus height, villus height to crypt depth ratio, and goblet cell counts (12).

If probiotic products containing living cells of specifically selected *Saccharomyces cerevisiae* are used in animal feed, they improve digestion by increasing cellulolytic bacteria and improving the performance responses of animal to diet containing this type of product (13). This probiotic has been used mainly in beef, dairy, calf, and equine feeds (14) but, lately, new experiments have been conducted by using it in broiler chicken breeding (13,15).

In the current study, the effects of three organic additives, biotechnological products of *Saccharomyces cerevisiae* (one probiotic, one prebiotic, and organic selenium), used in broiler chickens feed on production performance and quality parameters of meat were studied.

2. Materials and methods

This study was conducted on the broiler chicken ROSS-308 hybrid from a farm in Bihor County, Romania, which produces day-old chickens for marketing. According to the Ross Broiler Management Manual (Scotland, UK), the nutritional demands for this hybrid are the following: metabolizable energy (Kcal/kg): starter 3000, grower 3100–3150, finisher 3200; crude protein (%): starter 22, grower 20–19, finisher 18–17.5.

One hundred day-old broiler chickens (Ross 308) were divided into four groups (control and three experimental groups), housed in pens with a concrete floor and wood shavings. The dimensions of every pen were as follows: 1.25-m high, 1.55-m long with a total surface of 1.93 m^2. During the experiment, continuous lighting was provided using incandescent light. The temperature was set at 30 °C at the beginning of the experiment and gradually reduced in the first 20 days until ambient temperature of the pen was reached.

Chicken diet was used according to the following phases: days 1–14 (starter), days 15–35 (grower), and days 36–42 (finisher). Feed mixture was purchased at the beginning of each of the three phases; thereby, for Phase I, every experimental group of chickens was provided with 14 kg of feed; for Phase II an amount of 60 kg/experimental group; and for Phase III 29 kg feed/experimental group. The feed for the control and experimental groups was stored separately.

Group 1 (control) received a standard broiler diet (Table 1) without any supplementation; experimental group 1 (L1) received a diet supplemented with 0.1% organic selenium; experimental group 2 (L2) received a diet supplemented with 0.08, 0.04, and 0.02% prebiotic; and experimental group 3 (L3) received a diet supplemented with 0.1% probiotic additive.

In order to assess the effect of additives used in the experiments on broiler chickens, a series of determinations and lab analyses were made. Parameters on health, morbidity, and mortality, as well as the overall behavior of all groups, were assessed by daily observations of every group.

Different methods of statistical and physiological processing and interpretation of the obtained data were applied, according to the procedures specified in the literature.

At 42-days old, 5 birds from each group were slaughtered to determine their body components. Before the slaughtering period, birds from every group were weighed for living body mass and, after slaughter, carcass weight, as well as edible internal organs, were measured. Each individual bird was marked after determination of the living body weight to determine the slaughter and commercial yields after scarification. Commercial yield represents the carcass weight together with edible organs (liver, heart, and gizzard). To highlight the possible effect of used feed additives on production performance in broiler chickens, the following determinations were carried out:

Table 1. Basal diet for broiler chicken according to growing phase (adjusted from ROSS 308 Broiler Nutrition Specifications, 2014).

Material	Phase I	Phase II	Phase III
Feed structure (g/kg feed mixture)			
Corn	410.0	433.7	478.4
Wheat	120.0	120.0	50.0
Barley	-	-	50.0
Soybean meal (46% BP)	290.0	200.0	180.0
Sunflower meal (36% BP)	-	90.0	80.0
Corn gluten	80.0	60.0	60.0
Vegetal fat	50.0	50.0	60.0
L-lisin HCl	3.5	3.0	1.3
Dl-metionin	1.1	0.3	0.3
Dicalcium carbonate	20.0	18.0	16.0
Monocalcium phosphate	12.4	12.0	11.0
Salt	3.0	3.0	3.0
Zoofort	10.0	10.0	10.0
Nutritional characteristics of feed (g/kg calculated based on composition)			
Crude protein	227.6	213.1	199.4
Lisin	12.8	11.5	9.3
Metionin	4.9	4.4	3.9
Met + Cistein	7.5	7.9	7.1
Triptofan	2.4	2.3	2.1
Ca	10.6	9.8	8.8
P	6.9	7.3	6.7
ME (kcal/kg)	**3029.1**	**3134.0**	**3144.0**

body weight monitoring, feed mixture consumption, and feed conversion ratio, as well as the average daily gain.

Analyses of the chemical composition (dry matter, crude protein, total lipids, and ash following standard analysis methods) of the chicken meat were carried out in the Faculty of Animal Science and Biotechnology of the University of Agricultural Sciences and Veterinary Medicine following standard methods.

The chemical composition of the breast and legs (thigh) was determined. This was evaluated on the basis of 5 samples for each individual anatomical region from each group (control, L1, L2, and L3) and a total of 40 samples. The following investigations on the chemical composition of meat were made: dry weight (gravimetric) (16), total proteins (Kjeldahl method) (17), total lipids (Soxhlet method) (18), ash content (calcination) (19), and selenium content (atomic absorption spectrometry) (11).

Hematological investigations were performed on blood samples from 5 birds from each experimental group (control, L1, L2, and L3), harvested in sterile vaccutainers containing Li-Heparin by puncture of the vein. Blood proteinogram (total proteins and albumins and γ-globulins), following specific protocols (20), were determined in a specialized laboratory.

All analyses were performed in triplicate. The results are presented as the mean ± SD. In addition, standard error of mean (SEM) was provided. Significant differences ($P < 0.05$), distinct significant differences ($P < 0.01$), and very significant differences ($P < 0.001$) between samples were analyzed with one-way ANOVA post hoc tests, and pairwise multiple comparisons were conducted using Duncan's multiple range tests and the GraphPad InStat 3.05 program.

3. Results

The mean values of the body weight of broiler chickens during the experimental periods are presented in Table 2. After 7 days, the control group presented an average weight of 174.2 g, L1 group 177.9 g, L2 174.9 g, and L3 182.7 g. After 14 days, significant differences could be

observed. The highest weight was registered in L3 followed by L1, L2, and control. After 21 days from the beginning of the experiment, the evolution of body weight gain presented the same trend and all experimental groups had higher values compared to control but not statistically significant between the groups. High averages of body weight were registered after 28 days: L3 was situated at the top (1426.7 g), followed by L2 (1398.2 g), L1 (1366.9 g), and control (1313.6 g). At the end of the study (42 days), differences between groups could be observed: L3 and L2 presented an average body weight higher than 2500 g and L1 and control had lower means (2342.1 g and 2159.2 g, respectively).

Table 3 presents feed intake and the feed conversion rate. The lowest feed conversion ratio was registered in control and the highest in L2. Higher mean daily gain was registered in L2 and L3 (59.00 and 58.69g/day), followed by L1 and control. The same classification in the feed conversion ratio was also observed. The best feed conversion ratio was in L2 (1.87) and the weakest conversion ratio in was in the control group (2.71). At the end of the experimental period, individuals from L2 and L3 had the highest measured body weight (over 2500 g) and also highest carcass weight (over 2000 g).

From a commercial point of view, carcass yield (carcass weight/living weight × 100) and commercial yield (carcass weight + edible organs/living weight × 100) are very important. These parameters were higher in the treatments of L1 and L3 compared to control (Table 3).

The basic chemical composition of breasts and thighs from the 4 groups did not differ significantly (Table 4). The chemical composition of thighs from L3 presented

Table 2. Average body weight in broiler chickens at the end of every week of experiment and experimental variant (g/bird).

Age (weeks)	Control	L1	L2	L3	Significance
0	43.6 ± 0.62^{a}	43.9 ± 0.48^{a}	43.5 ± 0.43^{a}	45.1 ± 0.43^{a}	NS
1	174.2 ± 2.43^{b}	177.9 ± 2.55^{ab}	174.9 ± 2.51^{b}	182.7 ± 2.05^{a}	NS
2	420.6 ± 6.35^{b}	441.8 ± 5.88^{a}	439.7 ± 6.15^{a}	451.7 ± 7.07^{a}	0.015
3	804.2 ± 10.52^{c}	811.4 ± 14.08^{bc}	840.9 ± 15.55^{ab}	848.9 ± 15.18^{a}	0.027
4	1313.6 ± 19.53^{c}	1366.9 ± 17.78^{b}	1398.2 ± 20.38^{ab}	1426.7 ± 24.82^{a}	0.006
5	1932.7 ± 43.73^{b}	1987.6 ± 35.65^{ab}	2038.4 ± 39.85^{a}	2035.2 ± 41.53^{a}	0.044
6	2159.2 ± 33.23^{c}	2342.1 ± 19.48^{b}	2518.2 ± 39.55^{a}	2510.4 ± 43.06^{a}	<0.001

The values are the mean of 25 samples (N = 25), measured individually ± standard deviation. Different letters within a line denote significant differences (P < 0.05), distinct significant differences (P < 0.01), and very significant differences (P < 0.001); NS: not significant; L1: experimental group fed with organic selenium and *S. cerevisiae*; L2: experimental group fed with concentrated mannan oligosaccharide of *S. cerevisiae*; L3: experimental group fed with living cells of *S. cerevisiae*.

Table 3. Feed conversion ratio per kg of body weight during entire experimental period, average daily gain, and slaughtering indices of all experimental variants.

Parameter	Control	L1	L2	L3	Significance
Average feed conversion ratio (kg/kg)	2.71 ± 0.05^{a}	2.20 ± 0.03^{b}	1.87 ± 0.09^{c}	1.92 ± 0.09^{c}	<0.001
Average daily gain (g)	50.79 ± 0.97^{c}	54.71 ± 1.58^{b}	59.00 ± 1.10^{a}	58.69 ± 1.66^{a}	<0.001
Final body weight (g)	2187.40 ± 45.81^{c}	2355.80 ± 49.45^{b}	2591.40 ± 48.67^{a}	2520.20 ± 50.08^{a}	<0.001
Carcass weight (g)	1656.20 ± 35.80^{c}	1879.20 ± 38.36^{b}	2033.60 ± 41.69^{a}	2007.60 ± 40.38^{a}	<0.001
Carcass yield (%)	75.32 ± 1.92^{b}	79.68 ± 1.58^{a}	78.46 ± 1.63^{a}	79.62 ± 1.56^{a}	0.029
Commercial yield (%)	80.29 ± 1.94^{b}	84.06 ± 1.50^{a}	82.60 ± 1.65^{ab}	83.69 ± 2.05^{a}	NS

The values are the mean of 25 samples (average feed conversion ratio, daily gain, and final body weight) and 5 samples (carcass weight, carcass yield, and commercial yield) analyzed individually ± standard deviation. Different letters within a line denote significant differences (P < 0.05), distinct significant differences (P < 0.01), and very significant differences (P < 0.001); NS: not significant; L1: experimental group fed with organic selenium and *S. cerevisiae*; L2: experimental group fed with concentrated mannan oligosaccharide of *S. cerevisiae*; L3: experimental group fed with living cells of *S. cerevisiae*.

Table 4. Chemical composition of meat (thigh and breast) in control and experimental groups.

Parameter	Control	L1	L2	L3	Significance
Chemical composition of thigh (%)					
Dry matter	33.58 ± 0.93^{b}	34.96 ± 1.18^{a}	33.38 ± 0.39^{b}	31.33 ± 0.98^{c}	0.01
Crude protein	58.33 ± 2.10^{a}	55.47 ± 2.28^{b}	54.43 ± 0.63^{b}	59.96 ± 2.15^{a}	0.01
Crude fat	34.80 ± 1.89^{a}	29.90 ± 0.28^{c}	31.81 ± 0.82^{b}	35.25 ± 1.67^{a}	<0.001
Ash	2.82 ± 0.12^{b}	2.55 ± 0.17^{d}	2.69 ± 0.15^{c}	3.00 ± 0.12^{a}	<0.001
Chemical composition of breast (%)					
Dry matter	28.64 ± 0.79^{a}	28.53 ± 0.51^{a}	28.71 ± 0.34^{a}	28.74 ± 0.42^{a}	NS
Crude protein	80.34 ± 3.02^{b}	83.81 ± 1.03^{a}	83.11 ± 2.00^{ab}	83.58 ± 1.04^{a}	NS
Crude fat	13.40 ± 0.68^{b}	12.74 ± 0.55^{c}	14.43 ± 1.17^{a}	13.21 ± 1.02^{bc}	<0.001
Ash	4.52 ± 0.06^{b}	4.64 ± 0.02^{ab}	4.79 ± 0.13^{a}	4.58 ± 0.25^{b}	0.041

The values are the mean of 5 samples (N = 5) analyzed individually in triplicates ± standard deviation. Different letters within a line denote significant differences (P < 0.05), distinct significant differences (P < 0.01), and very significant differences (P < 0.001); NS: not significant; L1: experimental group fed with organic selenium and *S. cerevisiae*; L2: experimental group fed with concentrated mannan oligosaccharide of *S. cerevisiae*; L3: experimental group fed with living cells of *S. cerevisiae*.

the highest amount of total proteins (59.96%), crude fat (35.25%), and ash content (3.00%) but presented the lowest dry matter content. Lower values were obtained for the control, L1, and L2 groups. With the exception of crude fat and ash, breast chemical composition analysis was very homogenous, with small differences showing between the control and experimental groups (Table 4).

The selenium content of control and L1 meat were measured in breast and thigh meat. The breast meat of L1 had the highest amount of selenium (222.46 µg/kg), compared to control (90.16 µg/kg). Smaller amounts were quantified in thigh meat, both in the L1 and in control groups (Figure).

Blood proteins (total circulating proteins, albumin, and **γ**-globulin) are presented in Table 5. Albumins were higher than normal in L2: 2.23g/dL and L3: 2.08g/dL, indicating an intense water activity in the organism. γ-globulins were lower in control group (0.80g/dL), highest values being registered in L2: 1.01g/dL, with significant differences towards control.

4. Discussion

Introduction of different biotechnological products of *Saccharomyces cerevisiae* in broiler feed as dietary supplements enhanced growth performances.

Results showing an improvement of body weight by Actigen administration were obtained on broilers and have been presented in different studies (21–24). Olejniczak and Nollet (21), using Actigen as a growth promoter in broilers, registered a positive growth in the experimental group regarding average daily gain, with significant differences. Results showing the positive effect of Actigen on average daily gain in birds were obtained by Culver et al. (22) in an experiment on broiler chickens. Munyaka et al. (23) studied the effect of feed supplementation with Actigen on production parameters and on the health immune status of broilers. In that study, the improvements of main production parameters were registered such as feed consumption, body weight, FCR, and reduced mortality (23). Concentrated mannan oligosaccharide administrated in broiler feed diet also resulted in the best feed conversion ratio (1.87) and higher daily gain (59.00 g) in our experiment. In the first two weeks, no significance between the groups was observed; however, at the end of the experimental period, significant differences were observed (Table 2).

Other studies (24), administrating the living yeast *Saccharomyces cerevisiae* to the diet of broiler chickens (2.5%) obtained good results in body weight (2459 g compared to 2378 g in the control group), average daily gain (57.5 g compared to 55.6 g in control) and feed conversion (1.95 compared to 2.03). The experimental group that received the probiotic containing living cells of *S. cerevisiae* 0.01% in our study (L3) also showed strong results in terms of final body weight (2510.5 g) and a second higher feed conversion ratio and average daily gain (1.92 and 58.69 g, respectively).

The effect of selenium in broiler nutrition is associated with maintaining the antioxidant system of the cells, with no significant effect on meat acceptability. Many research studies are available (25–27) confirming this through the positive effects on average daily gain and total body weight. Very good results, with significant differences, were obtained on feed conversion ratio, daily gain, final

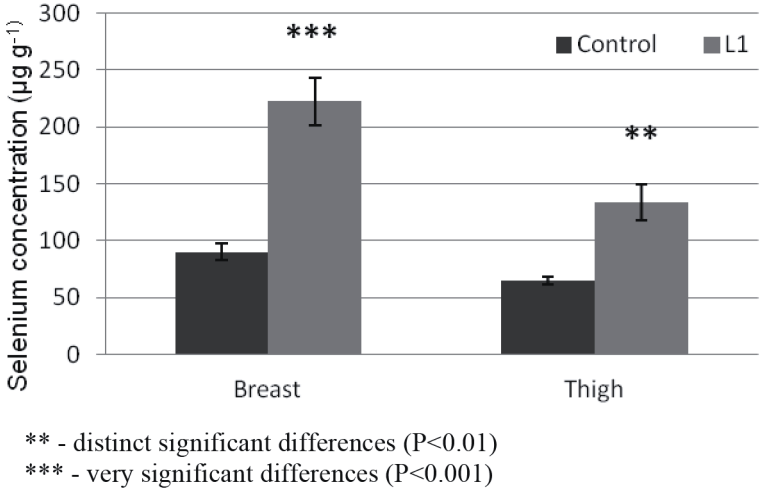

Figure. Selenium content in broiler chicken meat (breast and thigh) in the control group and L1 (experimental group with organic selenium supplementation).

Table 5. Proteinogram in broiler chickens from control and experimental groups.

Specification	Control	L1	L2	L3	Significance
Total proteins (g/dL)	3.15 ± 0.04^{d}	3.65 ± 0.50^{c}	4.17 ± 0.09^{b}	4.36 ± 0.10^{a}	<0.001
Albumins (g/dL)	1.37 ± 0.05^{d}	1.84 ± 0.03^{c}	2.23 ± 0.07^{a}	2.08 ± 0.60^{b}	<0.001
γ-globulins (g/dL)	0.80 ± 0.01^{d}	0.87 ± 0.02^{c}	1.01 ± 0.01^{a}	0.93 ± 0.06^{b}	<0.001

The values are the mean of three samples (N = 3) analyzed individually in triplicates ± standard deviation. Different letters within a line denote significant differences (P < 0.05), distinct significant differences (P < 0.01), and very significant differences (P < 0.001); L1: experimental group fed with organic selenium and *S. cerevisiae*; L2: experimental group fed with concentrated mannan oligosaccharide of *S. cerevisiae*; L3: experimental group fed with living cells of *S. cerevisiae*.

body weight, and carcass weight (Table 3). These main issues were followed and demonstrated in our experiment and are supported by other studies, meaning that using different biotechnological products of *Saccharomyces cerevisiae* in broiler diet improves the conversion of feed into healthy birds and good quality meat.

The chemical composition of the thigh meat presented significant differences and very significant differences in the experimental group compared to the control group (Table 4). The chemical composition of breast meat did not present significant differences between groups, except for crude fat and ash.

Total blood serum proteins vary by sex, physiological status, diet, temperature, and age (28). Albumins are hydrosoluble proteins that have the role of regulating the colloidal osmotic pressure of body fluids (29). A low level of albumin, correlated with a high aspartataminotransferase value, can provide clues about a potential liver injury. γ-globulins represent 12–18% of the total circulating proteins and play an important role in maintaining a healthy immune system (30). A proteinogram is helpful in establishing a clinical picture of health status.

Total proteins, albumins and γ-globulins presented very significant differences in the control group (Table 5) but the normal ranges also found in the literature (23).

Using organic selenium in broiler diet, Perić et al. (11) demonstrated the efficiency of this organic additive in the chicken diet and measured blood parameters and indicated the increasing protection against oxidative damage by improving the redox status of birds compared with those receiving inorganic forms of selenium or regular normal diets. The content of selenium in meat was increased compared to the control groups, and it was more abundant in the breast meat, similar to our experiment (Figure).

Registered data showed that the administration of 0.03% organic selenium in broiler diet resulted in very significant differences (P < 0.001) in the meat content of selenium.

In conclusion, the results of this study indicate that different biotechnological products of *Saccharomyces cerevisiae* supplementation in broiler diet improved growth performance and positively affected the animals' immune

system. As a result, the use of different types of prebiotic and probiotic additives in broiler diet is recommended; in terms of high body mass, daily gain, and feed conversion, we recommend a prebiotic product consisting of concentrated mannan oligosaccharide (L2) and living cells of *S. cerevisiae* supplementation (L3); for high carcass and commercial yields, we recommend organic selenium supplementation (L1).

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