EFFECTS OF INACTIVATED BREWER'S YEAST (*SACCHAROMYCES CEREVICIAE*) ON EGG PRODUCTION, SERUM ANTIBODY TITRES AND CHOLESTEROL LEVELS IN LAYING HENS*

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Abstract. The aim of this study was to investigate the effects of dietary supplementation with inactivated brewer's yeast (*Saccharomyces cereviciae*) produced from waste of beer industry, on egg production, feed efficiency, serum antibody titers and cholesterol levels in laying hens. A total of 320, twenty two wks old laying hens (Lohmann Brown) were randomly divided into 4 equal groups (each of them 80 hens) with 4 replicates according to the diet regimen; birds were supplemented for 16 wks with 1, 3, 5% *Saccharomyces cereviciae* whereas in the control group, layers were not supplemented. All diets were formulated to meet or exceed the National Research Council (NRC, 1994) recommended layer requirements for all nutrients and study was carried out for 16 wks. Egg production, feed intakes and feed efficiency were determined biweekly whereas serum antibody titers and cholesterol levels were explored on wks 2 and 16 in 15 birds from each group. At the end of the study, there were no significant effects of yeast supplementation on egg production, feed intake and feed conversion ratio in laying hens whereas there were significant differences ($P<0.05$) between control and other treatment groups about feed conversion ratio parameters at 28–29th wks of study. Similar to serum antibody titers, cholesterol levels of laying hens also were not altered by yeast supplementation. As a conclusion, *Saccharomyces cereviciae* had no beneficial effect on production parameters of hens fed with optimal diets and reared under proper management conditions.

Keywords: antibody, cholesterol, egg yield, layers, yeast.

INAKTYVUOTŲ ALAUS MIELIŲ (*SACCHAROMYCES CEREVICIAE*) ĮTAKA VIŠTŲ DEDEKLIŲ PRODUKTYVUMUI, SERUMO ANTIKŪNŲ TITRAMS IR CHOLESTEROLIO KIEKIUI

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Santrauka. Šio darbo tikslas – išsiaiškinti inaktyvuotų alaus mielių (*Saccharomyces cereviciae*) papildo, gaminamo iš alaus pramonės atliekų, įtaką vištų dedeklių produktyvumui, serumo antikūnų titrams ir cholesterolio kiekiui. 320 dvidešimt dviejų savaičių vištų dedeklių (Lohmann Brown) buvo suskirstyta į ketrias grupes (po 80 vištų kiekvienoje) pagal atitinkamą dietą. 16 savaičių trijų tiriamųjų grupių paukščiai su lesalais gaudavo 1, 3 ir 5 proc. *Saccharomyces cereviciae.* Kontrolinės grupės paukščiai šio papildo negaudavo. Visos dietos buvo sudarytos taip, kad atitiktų Nacionalinės mokslinių tyrimų tarybos (NRC, 1994) reikalavimus maisto medžiagoms. Tyrimas tęsėsi 16 savaičių. Dedeklių produktyvumas, sunaudotų lesalų kiekis ir lesinimo poveikis buvo nustatomi kas dvi savaites, o serumo antikūnų titrai ir cholesterolio kiekis – antrą ir šešioliktą savaitę penkiolikai paukščių iš kiekvienos grupės. Tyrimo pabaigoje didesnė mielių papildo įtaka dedeklių produktyvumui, sunaudotų lesalų kiekiui ar maisto pasisavinimo greičiui pastebėta nebuvo. Taip pat nepastebėta įtaka serumo antikūnų titrams ir cholesterolio kiekiui. Galima daryti

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išvadą, kad *Saccharomyces cereviciae* tinkamai laikomų ir optimaliai lesinamų dedeklių produktyvumo parametrams teigiamos įtakos neturi.

Raktažodžiai: antikūnas, cholesterolis, dedeklės, produktyvumas, mielės.

Introduction. Administration of low doses of Growth Promoter Antibiotics (GPA) for long periods result stronger microorganisms which are more resistant to antibiotics (Jones and Ricke, 2003). For this reason in 2006, the GPA in animal diets have been banned in the European Community. This situation brings new obligations to look for natural alternatives to GPA, such as prebiotics and probiotics (Patterson and Burkholder, 2003).

Yeasts and *Saccharomyces cerevisiae* cell walls are found among probiotics and prebiotics, respectively, which have been approved as safe microorganisms for animals (Nitta and Kobayashi, 1999). Yeasts have been fed to animals for more than ten decades, either in the form of yeast by-products from breweries or commercial yeast products for animal feeding (Stone, 1998). *Saccharomyces cerevisiae (SC)* yeast has biologically valuable proteins, vitamin B-complex, important trace minerals and several unique "plus" factors such as ability to enhancement of phosphorus availability (Brake, 1991; Moore et al., 1994) and reduction of infectious diseases (Line et al., 1997). It has also many beneficial effects on hens; improves feed consumption, egg weight, egg mass and size (Nahashon *et al*., 1992; Jin *et al*., 1997) and depresses serum and egg yolk cholesterol concentrations (Mohan *et al*., 1995; Kurtoglu *et al*., 2004). The addition of probiotics such as *Saccharomyces cerevisiae* to diets showed their benefit effects on host animals by stimulating appetite (Nahashon *et al*., 1992) and immune system (Toms and Powrie, 2001), improving intestinal microbial balance and decreasing pH in gut (Fuller, 1989). It was concluded that addition of yeast culture products to diets had resulted in significant improvement in performance and antibody responses in broiler and laying hens (MacDonald, 1995; Savage *et al*., 1996; Spring *et al*., 2000; Cotter *et al*., 2002). Yeast products contain 1.3/1.6 β-glucans which are recognized as immune modulator substances for animals. Thus, supplementation of yeast products to poultry diets results in an improvement of immune system, body weight gain and feed conversion (Abel and Czop, 1992; Parks *et al*., 2001). Several studies have reported that addition of yeast culture products to hen diets improved feed efficiency (Liu and Yoon, 2002; Tangendjaja and Yoon, 2002), increased egg production (Thayer *et al*., 1975) or egg weight and decreased egg yolk cholesterol without affecting performance and egg traits (Yalcın *et al*., 2008). Inclusion of probiotic (*Saccharomyces cerevisiae*) supplementation has been shown to reduce the cholesterol concentration in egg yolk (Abdulrahim et al., 1996; Haddadin et al., 1996) and serum in chickens (Mohan et al., 1996; Jin et al., 1998). In addition, previous reports suggest that manan oligosaccharide (extracted from yeast wall) supplementation resulted in significant improvement of antibody responses in broiler and layers (Raju and Devegowda, 2002; Cotter *et al*., 2000). Furthermore, researchers have noticed that supplementation of manan oligosaccharide to diets had a positive effect on antibody titers and all the production traits in broiler breeders (Shashidhara and Devegowda, 2003). On the other hand, other researchers (Day *et al.,* (1987) and Nursoy *et al.,* (2004)) reported no effect of dietary yeast culture on feed consumption, egg production, egg weight, and feed efficiency in laying hens. Similarly, Dizaji and Pirmohammadi (2009) reported that addition of yeast products to layer diets resulted with decrease of egg production.

Results of different experiments on the effects of supplementation of this yeast culture product (*Saccharomyces cerevisiae*) to poultry diets have been inconsistent. Several studies reported that addition of yeast or yeast culture products to diets resulted with better feed efficiency (Liu *et. al.* 2002; Tangendjaja and Yoon, 2002), increased egg weight (Yalcın *et al.*, 2008) and improved internal egg quality (Miles and Bootwalla, 1991) in hens. In contrast, other studies (Day *et al.,* 1987; Nursoy *et al.,* 2004) reported there was no effect of dietary yeast culture on feed consumption, egg production, egg weight, and feed efficiency in laying hens.

Summarizing it can be said that there are still conflict reports on the beneficial effect of yeast products supplementation in poultry diets. Therefore, the objective of our study was to investigate the effects of dietary supplementation with inactivated brewer's yeast (*Saccharomyces cereviciae*), produced from waste of beer industry, on egg production, feed efficiency, serum antibody titers and cholesterol levels in laying hens.

Materials and Methods

Birds and Experimental Diets

A total of 320 laying hens (Lohmann Brown), 16 wk of age, were used in this study. Hens were housed in 80 laying cages (50 x 59 x 60 cm) with 4 hens per cage in a windowed poultry house with a light regimen of 16L:8D. Four groups of 80 hens (4 replicates with 20 hens per group) were randomly assigned to 4 dietary treatments. Feed and water were provided ad libitum during the entire 16 wks experimental period. Corn and soy bean meal based diets (Table 1) were prepared and basal diet was supplemented with 1, 3 and 5% inactivated brewer's yeast (*Saccharomyces cereviciae*), respectively. The diets were formulated to be isocaloric and isonitrogenous and to meet or exceed the nutrient requirements of laying hens of the National Research Council (NRC, 1994).

Data Collection

Nutrient composition of diets was determined according to the AOAC (2000). Metabolizable energy content of the diets was estimated using the Carpenter and Clegg equation (1956): ME, kcal/kg = 53 + 38 [(crude protein, %) + (2.25 x ether extract, %) + (1.1 x starch, %) + (sugar, %)].

Table 1. **Composition and analyzed results of experimental diets for laying hens**

Feed ingredients	Experiment Groups			
	C (Control)	Yeast (1%)	Yeast (3%)	Yeast (5%)
Corn	51.55	51.55	51.55	51.55
Soy bean meal	27.00	26.00	24.00	22.00
Soybean, full fat	7.00	7.00	7.00	7.00
Meat and bone meal	3.00	3.00	3.00	3.00
Yeast	-	1	3	5
Vegetable oil	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00
Limestone	7.50	7.50	7.50	7.50
DL-Methionine	0.25	0.25	0.25	0.25
DL-Treonine	0.15	0.15	0.15	0.15
Common salt	0.35	0.35	0.35	0.35
Vitamin premix[1]	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10
	Analyzed Results			
Dry matter, %	92.18	91.60	92.10	90.34
Ash, %	11.12	11.71	11.82	11.23
Crude protein, %	17.58	17.60	17.53	17.83
Ether extract, %	5.54	5.97	5.60	5.43
Crude fiber, %	2.56	2.67	2.58	2.37
Phosphor, %	0.45	0.45	0.46	0.47
Calcium, %	3.58	3.58	3.61	3.60
Metabolically energy, kcal/kg	2817	2819	2808	2814

[1]Vitamin premix provided per kilogram of diet; vitamin A, 15000 IU; vitamin D_3, 5000 IU; vitamin E, 50 mg; vitamin K_3, 10 mg; vitamin B_1, 4 mg; vitamin B_2, 8 mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg; niacin, 50 mg; pantothenic acid, 20 mg; folic acid, 20 mg; biotin, 0.25 mg; choline, 175 mg

[2]Mineral provided per kilogram of diet; manganese, 100 mg; zinc, 150 mg; iron, 100 mg,; cupper, 20 mg; iodine, 1.5 mg; cobalt, 0.5 mg; selenium, 0.2 mg; molybdenum, 1mg; magnesium, 50 mg

Mortality was recorded as it occurred. Eggs were collected daily and egg production was expressed on a hen-day basis. Feed intake was recorded biweekly and calculated as g/hen per d. Feed conversion ratio was calculated as kg feed/kg egg.

All groups were vaccinated with viable *Newcastle* Disease Virus vaccine (Lasota strain) using a commercially available vaccine sprayer at second and last wks of trial. Then blood samples (10 ml) were obtained from *Vena brachialis,* under the wing from 15 hens randomly chosen from each treatment. These samples were allowed to clot at room temperature for 6 hours and then they were centrifuged at 1500 g for 10 minutes at room temperature. Sera were carefully harvested and stored at -20°C until analysis. The serum cholesterol concentrations were analyzed by using commercial kit (Teco Diagnostic, 1286 Anaheim, CA 92807). The serum antibody titers were detected by the hemagglutination-inhibition (HI) test. HI test was performed in U-bottomed micro titer plates according to the suggestions of Allan and Gough (1974). Eight hemagglutination units (HAU) were preferred in the HI tests. La Sota vaccine strain (Intervet, Boxmeer, the Netherlands) of NDV (clarified from the allantoic fluid of 9–11 days old specific pathogen free eggs) were preferred for the HI tests. Back titration of antigen was included in all tests to verify the number of HAU used. The HI titer was set as the highest dilution of serum causing complete inhibition of 8 HAU of antigen. Titers were expressed as log2 values of the highest reciprocal of the dilution. The validity of the results was assessed against a negative and positive control serum.

Statistical Analyses

Data were analysed using the one way ANOVA procedure of SPSS to determine the treatment effects on performance parameters, antibody titres and serum cholesterol levels. Significant differences among means were separated by the Duncan test (1955).

Results

The feed intake, feed conversion ratio (FCR) and egg yield results for laying hens according to the dietary treatments are summarized in the Table 2. All these parameters showed no significant differences between treatment groups. However, the lowest feed intake for the first 6 weeks period was observed in birds supplemented with 1% yeast but in the following periods, this parameter was quite similar between groups. Evaluation of all experiment period for daily feed intake, 5% addition of yeast to diet caused numerically less feed intake compare with control group (1.7%).

As shown in Table 2, at 7^{th} to 8^{th} weeks of trial the highest FCR result was observed in 5% yeast

supplemented group (P<0.05). The yeast addition to layer diets at level of 5% showed worst feed conversion ration at these weeks of treatment. But this significant difference had disappeared by the following week of trial and at the end of study only some numerical differences have been observed between control and yeast supplemented groups at a level of 1%. The FCR differed between control and 1% yeast added group, ranging from 1.94 to 1.91, respectively. These differences between groups did not get any significant meaning by statistical analyzed.

Table 2. **Some weekly performance parameters of laying hens**

	Weeks	Experiment Groups				
		C (Control)	Yeast (1%)	Yeast (3%)	Yeast (5%)	P
Feed intake (g day−1 per hen)	1-2	98.73	96.87	100.85	99.10	NS
	3-4	106.50	103.61	106.30	107.29	NS
	5-6	115.14	113.13	117.63	118.43	NS
	7-8	113.02	114.48	118.03	118.97	NS
	9-10	116.55	118.93	119.28	120.99	NS
	11-12	116.24	117.74	116.77	120.06	NS
	12-14	117.70	119.82	121.21	123.70	NS
	1-14	118.68	117.58	118.33	116.69	NS
Feed conversation ratio (kg feed kg−1 egg)	1-2	2.35	2.12	2.25	2.27	NS
	3-4	1.94	1.89	1.91	1.95	NS
	5-6	1.96	1.98	2.03	2.09	NS
	7-8	1.86^{b}	1.90^{b}	1.99^{ab}	2.03^{a}	*
	9-10	1.92	1.98	1.99	2.06	NS
	11-12	1.91	1.93	1.93	1.96	NS
	12-14	1.91	1.97	2.00	2.07	NS
	1-14	1.94	1.91	1.93	1.93	NS
Hen-day egg production (%)	1-2	76.79	82.37	82.79	79.00	NS
	3-4	93.83	93.04	93.67	92.70	NS
	5-6	96.42	93.20	95.50	94.42	NS
	7-8	97.62	96.45	95.58	94.42	NS
	9-10	96.04	94.83	95.67	95.29	NS
	11-12	95.50	95.87	95.46	94.87	NS
	12-14	96.75	95.42	95.62	93.58	NS
	1-14	96.37	97.00	95.92	94.12	NS

NS: not significant,
Different superscripts a,b in the same row indicate significant differences between groups.

The egg yield results were also reported in Table 2. However there were some numerical variances between control and yeast supplemented groups about egg production levels in different weeks of trial, these results did not present any significance among the groups by the end of experiment. As it is summarized in Table 3., *Saccharomyces cereviciae* addition to diets showed no important effects on antibody titres or serum cholesterol levels in layers. On the other hand, serum cholesterol levels were unexpectedly higher in groups which were supplemented with 1. 3 and 5 % yeast compared with control group at the end of the trial (9.3, 6.3 and 20.5 %, respectively).

Table 3. **Effects of *Saccharomyces cereviciae* addition to diets on serum antibody titres and serum cholesterol levels in laying hens**

Serum antibody titres (log_2)						
Weeks	C (Control)	Yeast (1%)	Yeast (3%)	Yeast (5%)	SEM	P
2^{nd} wk of trial	5.86	5.66	6.33	6.11	0.18	NS
16^{th} wk of trial	6.00	7.18	7.78	6.95	0.18	NS
Serum cholesterol levels (mg/dL)						
	108.04	119.15	115.31	135.90	5.153	NS

NS: not significant.

Discussion

In this research, the performance promoting effects of inactivated yeast (*Saccharomyces cerevisiae*) in different addition levels were examined in laying hens. At the end of 16 wks trial, no significant alterations were observed about egg yield, feed intake and feed efficiency in layers. There were numerous of studies about yeast addition to laying hen diets conducted and some of them found out, in parallel to our study, this application had no beneficial effects on feed intake and feed efficiency (Mutus et al., 2006; Yousefi and Karkoodi, 2007). Similarly, these results also are confirmed by Sehu et al. (1997) study which showed that inactivated brewer yeast addition to diets at levels of 5, 10 or 15% had no effect on feed intake and FCR in quails. On the contrary, numerous studies reported that manan oligosaccharides derived from cell walls of *Saccharomyces cerevisiae* have shown some beneficial effects on growth and feed efficiency in chickens (Onifade et al., 1999; Parlat et al., 2001; Yildiz et al., 2004) whereas Liu et al. (2002) noticed that yeast culture supplementation to the diet at a level of 0.2% resulted in feed intake decrease in laying hens.

Hassanein and Soliman (2010) have observed that feed conversion ratios (g feed/g egg) of layers fed yeast (*Saccharomyces cerevisiae*) levels of 0.4% and 0.8% had beneficial effects compared with controls. Some authors (Bageridizaj et al., 2006; Shugeng et al., 2010; Yalcin et al., 2010) also reported that yeast addition improve feed to egg ratio in laying hens. However, these effects were controversial since no effect of dietary yeast supplementation, especially on feed intake and FCR was reported in laying hens (Mahdavi et al., 2005; Asli et al., 2007; Chumpawadee et al., 2009). In addition, Yalcin et al. (2008) reported that 0 and 2 g/kg commercial yeast culture product (*Saccharomyces cerevisiae*) supplementation to diets also containing oilseed meal had no significant affect on feed intake and feed efficiency similar to egg production and serum cholesterol levels. Contradictions in different studies may be related to the variety of the product forms or different experiment conditions (etc: environmental stress).

Inclusion of yeast products in the layer diets had no significant effects on egg production data in the present study. In agreement with our findings, some researchers confirm that yeast culture supplementation had no beneficial effect on egg yield in laying hens (Day et al., 1987; Nursoy et al., 2004). In parallel, Bageridizaj et al. (2006) evaluated the effects of probiotic addition to diets at different levels (200, 300, 400 g t^{-1}) in layers and they also did not observe any positive effect of probiotic on egg production in hens. On the contrary, Abou et al. (1996) and Liu et al. (2002) have reported that egg production was improved by yeast culture supplementation in laying hens. Similarly, Hassanein and Soliman (2010) reported that 0.4 and 0.8% probiotic addition had beneficial effects on egg production in late period of laying hens. The inconsistence of results from different studies may explained by that biological additives have different effects on animals because of different gastro intestinal flora and varied environment conditions (Mahdavi et al., 2005).

The present results demonstrated that the inclusion of inactivated brewer's yeast in different addition levels in layer diets had no significant effect on cholesterol levels. Moreover, the groups which were fed with different levels of yeast (1, 3 and 5%) showed higher cholesterol levels compared with controls (119.15; 115.31; 135.90 compared with 108.04, respectively), these differences between treatment and control groups were not significant. There are some studies (Stanley et al., 2004; Bageridizaj et al., 2006; Asli et al., 2007) about yeast addition to layer diets which showed parallel results to our findings about serum cholesterol levels. Asli et al. (2007) tried to evaluate the effects of dietary probiotics, yeast, vitamin E and vitamin C supplementation on performance, serum and yolk cholesterol and immune response of heat stressed laying hens. They noticed that addition of 1 g yeast (*Saccharomyces cerevisiae)* to basal diet had no significant effects on performance, egg quality and serum cholesterol concentrations in 62 wks old hens. On the other hand, there are numerous studies concluding that addition of yeast to diets had significantly reduced serum cholesterol levels in layers (Mahdavi et al., 2005; Paryad and Mahmoudi, 2008; Yalcin et al., 2008 and 2010; Hassanein and Soliman, 2010). Mohan et al. (1995) examined the effect of probiotic supplementation on serum/yolk cholesterol and egg shell thickness in layers. Treatment groups were supplemented with probiotic at level of 100 and 150 mg/kg in their research. At the end of 10 wks trial, researchers noticed that the initial and final serum cholesterol concentrations significantly reduced with probiotic addition compared to control group. Environmental factors, genetic and interactions may be responsible for the conflicting results of different experiments.

Supplementation of hen diets with yeast had no significant variance for antibody titres in present trial. Even tough there were numerically higher results for 3% yeast addition compared with control group animals this result is not important. Asli et al. (2007) and Yalcin et al. (2010) found in their trials that addition of probiotic or yeast autolysate had significant increase of antibody titres in serum of laying hens. These authors concluded that this result could be explained by the supplementation of yeast products had beneficial effects on balancing of immune cells so that provided healthy environment for the immune system of layers. Disagreement about the results may be related with different yeast or probiotic source and different rearing periods of animals.

Conclusion

The results of the current study indicate that inactive brewer's yeast in different levels had effects neither on egg production nor on feed intake and FCR, serum antibody titres and cholesterol levels in laying hens. As a result, *Saccharomyces cereviciae* had no beneficial effect on production parameters of hens fed with optimal diets and reared under proper management conditions.

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