



The Effect of Dietary Inclusion of Whole Yeast, Extract, and Cell Wall on Production Performance and Some Immunological Parameters of Broiler Chickens

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ABSTRACT

A total number of 192 male one-day-old broilers chickens were randomly divided into four treatment groups of 48 chickens. Chickens of group one fed a plain diet without any supplement (control), while the diets in groups two, three, and four were supplemented with Whole Yeast (WY, *Saccharomyces cerevisiae*, 0.1%), Yeast Cell Wall (YCW, 0.3 %), and Yeast Extract (YE, 0.07 %), respectively. At the end of the experimental period (35 days), the bodyweight of chickens and the feed intake of each cage were measured, and then the feed conversion ratio was calculated. Blood samples were also collected to measure the serum components and relative spleen, bursa of Fabricius, and thymus gland. The results obtained indicated that all productive performance parameters improved in response to the feeding supplementation. Blood parameters indicated that the treated groups had a significantly higher level of serum total protein and albumin as well as significantly lower serum total lipids and cholesterol. The enzyme activities of ALT, AST, and ALP were significantly reduced by WY, YCW, and YE supplementation. The relative organ weights of the spleen, bursa of Fabricius, and thymus increased significantly in broilers fed with WY, YCW, and YE, and the highest values were observed in the chickens fed with WY. It can be demonstrated that the supplementation of WY or its derivatives in the diet of broiler chickens improves the production performance as well as the physiological and immunological parameters, and consequently produce a healthier chicken.

Keywords: Broilers, Immunity, Yeast, Yeast cell wall, Yeast extract

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INTRODUCTION

The use of antibiotics as growth promoters was completely banned in 1999 by the European Union (EU). This was due to increases in microbial resistance to antibiotics and residues in chicken meat products which might be harmful to consumers (Koc et al., 2010). Numerous Natural Growth Promoters are used or have been proposed as agents to suppress pathogens or to improve growth and feed conversion, including predominantly organic acids, probiotics, prebiotics, synbiotics, phytochemicals, feed enzymes, and immune stimulants (Jaiswal et al., 2017). Probiotics is a live microbial feed supplement that benefits the host animal by enhancing its microbial intestinal balance (Gibson et al., 2017). Probiotics have a major effect on the efficiency of the broiler, such as intestinal microflora modification, pathogen inhibition, intestinal histological changes, immunomodulation, certain haemato-biochemical parameters, sensory characteristics of dressed broilers improving (Kabir, 2009).

The prebiotic is a carbohydrate derived from Yeast Cell Walls (YCW) that can block the proliferation of pathogenic bacteria and stimulate the non-specific immune system, thereby improving birds' health and growth efficiency (Gibson et al., 2017). Yeast Extract (YE) is a source of protein extracted from the live yeast's cell material. High levels of nucleotides, inositol, and glutamic acid (Zarei et al., 2016). Nucleotides, especially in infant diets, are traditionally used in human diets; they favor the development of the gastrointestinal tract and immune functions and preserve the gut flora (Alizadeh et al., 2016).

The objective of the present study was to investigate the effect of Whole Yeast (WY, *Saccharomyces cerevisiae*), YCW, and YE on the improvement of production performance and the immune response in commercial broiler chickens.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in Poultry Research Unit, Biological Application Department, Radioisotopes Applications Division, in the Inshas area, the Nuclear Research Center, Egyptian Atomic Energy Authority October to

December 2017. The experimental protocols were approved and carried out according to the ordinance and guidelines of the Ethics Committee of Cairo University for the Care and Use of Experimental Animals in Education and Scientific.

Animals and housing

One day-old male Cobb 500 broiler chicken (n=192) were used in the present study. The test period extended from one day of age up to slaughter (35 days). The chickens were allotted into four equal groups, each consisting of 48 chickens, and assigned into four equal replicates of 12 chickens. All groups ran from one day of the set to 35 days of age simultaneously. Chickens in group one fed on a plain diet without any supplementation (control), while the diets of groups 2, 3, and 4 were supplemented with WY (0.1%), (YCW) (0.3 %), and YE (0.07 %), respectively.

Diets

Chickens fed *ad libitum* a commercial starter diet (23% crude protein and 3000 kcal ME/kg diet) during the first week of age, a commercial grower diet (22% crude protein and 3150 kcal ME/kg diet) from two to four weeks of age, and then commercial finisher diet (19% crude protein and 3200 kcal ME/kg diet) until the end of the experiment. The chickens had free access to water. The diet compositions are indicated in Table 1, while the vaccination program is shown in Table 2.

Probiotics and prebiotics

Viable yeast contains 10 billion live yeast cells (*Saccharomyces cerevisiae*) per gram that are used as a probiotic. As shown in Table 3, yeast cell walls, extracted from *Saccharomyces cerevisiae* and used as a prebiotic, are rich in manno-oligosaccharides and beta-glucans (Kwiatkowski et al., 2009). Yeast extract which is considered as a protein source derived from the cell content of live yeast and has high nucleotide, inositol, and glutamic acid levels used as another source for the prebiotics.

Measurements

Production performance

The body weight (BW) of the chickens and the feed intake of each cage were measured at the end of the experimental duration (35 days), and then the Feed Conversion Ratio (FCR) was estimated. After slaughtering the weight of the spleen, bursa, and thymus were individually recorded and presented as a percentage of living body weight.

Blood biochemical assay

Fasted blood samples were collected were obtained by cervical cutting from four birds per pen (n=16). Blood samples were centrifuged rapidly at 4000 rounds per minute (rpm) for 15 minutes. The serum was separated and stored in a freezer at -20°C until the biochemical analysis. Total Protein (TP) and Albumin were determined calorimetrically, however, Globulin was calculated by subtracting serum albumin from total serum protein. Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) activities were colorimetrically determined (Bergmeyer et al., 1978), where alkaline phosphatase (ALP) activities were assayed using commercial kits (Bio-Merieux Co. Marcy. L. Eoile chorbouneries, France). Total cholesterol and Total Lipids (TL) were determined according to Pesce and Bodourian (1976).

Immunological measurements

Haemagglutination test against sheep red blood cells

At 21 days of age, four chickens from each group were randomly selected and housed in separated cages. Sheep Red Blood Cells (SRBC) were collected from three egyptian sheep using a hparenized syringe and washed three times using phosphate buffer saline (PBS). The saline was discarded and SRBC sample were diluted with normal saline solution to form a concentrate of 5% SRBC that was used immediately for injection. Each chicken was intravenously primary injected with 0.5 ml of 5 % SRBC suspension in the right or left brachial vein. Blood samples were collected from the brachial vein of each bird at four, seven, and 10 days after the primary injection to detect the primary antibodies titer according to the method described by Wegman and Smithies (1966).

Haemagglutination inhibition test against newcastle disease virus

At 28 days of age, five chickens from each experimental group were randomly chosen and blood samples were collected from the brachial vein of each chicken at three, seven, and ten days post-vaccination. The Haemagglutination Inhibition (HI) antibody titer against Newcastle Disease Virus (NDV) was determined by the HI test according to the method proposed by Majiyagbe and Hitchner (1977).

Statistical analysis

The data obtained were statistically analyzed on a one-way design basis using the SAS® software statistical analysis program (SAS, 2001). The significant differences between the four treatment groups (Control, WY, YCW, and

YE) for all parameters were analyzed by Duncan's multiple range test. The significance level was set at $p \leq 0.05$. The results are expressed as Least Square Means (LSM) \pm SEM.

Table 1. Composition of the three-phase broilers diet and its calculated chemical composition (on as fed basis)

Ingredients	Starter (kg)	Grower (kg)	Finisher (kg)
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix*	3	3	3
Calculated analysis			
Crude protein (%)	23.0	22.0	19.0
Metabolizable energy (kcal/kg)	3000	3150	3200

*Each gram of mineral mixture contained: vitamin A (transretinyl acetate) 9,000 IU; vitamin D3 (cholecalciferol) 2,600 IU; vitamin E (dl- α -tocopheryl acetate) 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride) 300 mg; nicotinic acid 30 mg; pantothenic acid (d-calcium pantothenate) 10 mg; folic acid 0.6 mg; biotin 0.07 mg; manganese (MnO) 70 mg; zinc (ZnO) 60 mg; iron (FeSO₄ H₂O) 40 mg; copper (CuSO₄ 5H₂O) 7 mg; iodine (Ca(IO₃)₂) 0.7 mg; selenium (Na₂SeO₃) 0.3 mg.

Table 2. Vaccination program of broiler chickens in Poultry Research Unit, Egyptian Atomic Energy Authority

Age (days)	Vaccines	Method used
8	IB + MA5 vaccine	Eye drop
10	Newcastle disease + H5N2 (oil killed vaccines) 0.5 cm	Subcutaneously into the lower back part of the neck
14	Infection bursal disease (Gumboro)	Eye drop
19	Newcastle disease vaccine (clone 30)	Drinking water

Table 3. The components of yeast cell wall

Component	Cell wall mass (% dry weight)
(1 \rightarrow 3)- β -D-glucan	50-55
(1 \rightarrow 6)- β -D-glucan	5-10
(1 \rightarrow 4)- α -(1 \rightarrow 3)- β -D-glucan	3-7
* Mannoprotein complex	35-40
Chitin	2

RESULTS AND DISCUSSION

Production performance

The improvement in broilers' productive performance affected by dietary WY, YCW, and YE supplementation is shown in Table 4. The WY group harvested the highest BW, body weight gain, and feed intake as well as the best FCR, followed by those fed 0.07 % YE, and 0.03 % YCW. The control group, on the other hand, had inferior values. Koc et al. (2010), who indicated that the addition of *Saccharomyces cerevisiae* at 0.2 percent increased the broiler chickens' weight gain and feed intake, agreed with the results obtained in broiler chickens, the beneficial effects of YC on efficiency have also been shown (Zhang et al., 2005). However, other studies reported that yeast products did not affect the production performance in turkeys (Bradley and Savage, 1995). Differences in animal response can be correlated with differences in the formulations of product. Yeast products are categorized as active dried yeast, live YC, or fermented YC interchangeably, rendering comparisons between studies difficult.

Blood biochemical assays

The current results explained that the WY group had the significantly highest total protein concentration while the control group had the lowest. However, the total globulin of treated groups did not differ from one another but was significantly higher, compared to the control group (Table 5). This finding is in agreement with Abou El-Naga (2012), who found a significantly higher total serum protein and serum albumin in the WY group than the control group.

The enzyme activities of ALT, AST, and ALP were significantly reduced by WY, YCW, and YE supplementation ($p < 0.05$, Table 5). The lowest values for ALT and ALP were observed in chickens fed a diet with WY, followed by those fed YE and YCW. However, the lowest values of AST were recorded in chickens fed a diet with yeast, followed by those fed YCW and then YE. These results agreed with Nikpiran et al. (2013), who showed that the activity of ALP in males depressed by probiotic (yeast) consumption ($p < 0.05$). The present results indicated that the WY group had a

significantly ($p < 0.05$) lower total serum cholesterol, whereas the control group had the significantly highest value (Table 5). The YCW and YE groups, on the other hand, had almost identical values and were in the center, slightly different from the other two groups.

The results also revealed that the total serum lipids concentration of the WY group was the lowest while the control group had the highest value and was significantly different from the WY and YE groups. Similar observations were recorded by [Abou El- Naga \(2012\)](#). [Fukushima and Nakano, \(1995\)](#) indicating that probiotic microorganisms were known to inhibit hydroxymethylglutaryl coenzyme A, an enzyme that is involved in the cholesterol synthesis pathway, thereby decreasing cholesterol synthesis.

Immunological parameters

The data presented in Table 6 indicate that the spleen, bursa, and thymus weights in broiler chickens that were fed either WY, YCW, or YE diets were significantly higher, compared to the control group ($p \leq 0.05$). [Abou-Zeid et al. \(2019\)](#) found that the relative weights of bursa of fabricius and thymus were significantly ($P \leq 0.05$) improved in groups fed 2g yeast containing diet and they found that in conclusion, yeast (*Saccharomyces cerevisiae*) could be safely used in broiler feeding as natural feed additives at 2g/kg feed with superior effects on their productive and immune response.

Antibody titer

The findings in Table 6 also showed that on post-immunization days, either WY, YCW, or YE were almost the highest antibody titers against SRBCs and NDV. The present findings were in line with those studies by [Yalçın et al. \(2010\)](#), who found that in laying hens fed diets containing 2, 3, or 4 g/kg of yeast autolysate had a higher antibody titer. Furthermore, [Mohiti-Asli et al. \(2007\)](#) stated that laying hens with multi-strain probiotic supplementation and yeast supplementation had a greater immune response than the control group.

These indicators relating to the weight of the lymphoid organs and the humoral immune response reflect the immunologic system status of broilers. The bursa of Fabricius is the primary site of B-cell development in chickens ([Ratcliffe, 2006](#)). In accordance with the findings of the present research, [Gheisari and Kholeghipour \(2006\)](#) observed a significant increase in relative bursa weight in broilers fed a diet containing yeast powder, compared to other treatments. Moreover, [Wang et al. \(2017\)](#) reported that KM (yeast: *Kluyveromyces marxianus*) had a positive effect on the broiler's immune organs.

Macrophages have receptors (CR3) for $\beta 1, 3/1, 6$ branched glucans. By recognizing specific sugars found in glycoproteins of the epithelial surface, prebiotics would bind to macrophage reception sites, causing a cascading reaction that would activate macrophages and release cytokines, thus triggering the immune response acquired and causing higher antibody responses to antigens ([Bohn and BeMiller, 1995](#)). Following that, innate and acquired responses are increased by T immune lymphocyte proliferation ([Swiatkiewicz et al., 2014](#)). These results might be used as an indicator of the good health status of chickens fed dietary yeast or its derivatives supplementations.

Table 5. Effect of whole yeast, cell wall, or extract supplementations on some blood serum parameters of broiler chickens (mean \pm standard error).

Treatments	Control	WY	YCW	YE	p Value
Total protein (g / dL)	3.75 \pm 0.043 ^d	4.27 \pm 0.037 ^a	3.98 \pm 0.038 ^c	4.10 \pm 0.122 ^b	0.0001
Total albumin (g / dL)	1.78 \pm 0.025 ^c	2.01 \pm 0.173 ^a	1.82 \pm 0.041 ^{bc}	1.88 \pm 0.038 ^b	0.001
Total globulin (g / dL)	1.97 \pm 0.065 ^b	2.26 \pm 0.024 ^a	2.16 \pm 0.018 ^a	2.22 \pm 0.043 ^a	0.002
ALT (I μ / L)	131.75 \pm 0.66 ^a	124.33 \pm 1.55 ^b	127.45 \pm 1.23 ^b	126.31 \pm 1.07 ^b	0.005
AST (I μ / L)	121.15 \pm 0.88 ^a	110.33 \pm 0.35 ^b	119.37 \pm 0.79 ^a	120.49 \pm 1.24 ^a	0.0001
ALP (I μ / L)	308.22 \pm 2.72 ^a	283.89 \pm 3.53 ^c	297.42 \pm 3.03 ^b	286.00 \pm 3.41 ^c	0.001
Total lipids, (mg /dl)	634.87 \pm 15.25 ^a	452.03 \pm 6.81 ^c	478.88 \pm 20.32 ^{ab}	510.28 \pm 3.22 ^b	0.0001
Total cholesterol, (mg /dl)	156.43 \pm 2.86 ^a	135.37 \pm 1.72 ^c	144.28 \pm 1.65 ^b	148.58 \pm 1.15 ^b	0.0001

^{a, b, c}: The mean values, which are followed by different superscripts, within trait within age are significantly different ($P \leq 0.05$).

Table 4. Effect of whole yeast, cell wall, or extract supplementations on broiler performance at 35 days of age (mean \pm standard error).

Treatments	Control	WY	YCW	YE	p Value
Initial body weight (1 day) (g)	52.87 \pm 0.48	53.19 \pm 0.52	53.08 \pm 0.45	53.15 \pm 0.42	0.9618
Final Body weight (35) (g)	1769.10 \pm 6.26 ^d	1991.08 \pm 23.42 ^a	1838.791 \pm 18.56 ^c	1928.10 \pm 17.26 ^b	0.0001
Body weight gain (1-35 day) (g)	1716.24 \pm 6.40 ^d	1937.89 \pm 23.58 ^a	1785.71 \pm 18.58 ^c	1874.95 \pm 17.42 ^b	0.0001
Feed intake (1- 35) (g)	2883.66 \pm 4.50 ^c	2972.41 \pm 17.52 ^a	2913.31 \pm 9.33 ^{bc}	2935.29 \pm 2.62 ^b	0.0001
Feed conversion ratio (1- 35) (g/g)	1.68 \pm 0.007 ^a	1.53 \pm 0.028 ^c	1.63 \pm 0.032 ^{ab}	1.57 \pm 0.012 ^{bc}	0.003

^{a, b, c, d}: The mean values, which are followed by different superscripts, within the age characteristic between treatments, are significantly different ($p \leq 0.05$). Where WY is whole yeast, YCW is yeast cell wall and YE is yeast extract.

Table 6. Effect of whole yeast, cell wall, or extract supplementations on immunological parameters of broiler chickens

Treatments	Control	WY	YCW	YE	P Value
Spleen	0.1203 ± 0.0029 ^d	0.1988 ± 0.0052 ^a	0.1390 ± 0.0012 ^c	0.1602 ± 0.0077 ^b	0.0001
Bursa	0.0601 ± 0.0325 ^d	0.1146 ± 0.0525 ^a	0.0730 ± 0.0128 ^c	0.0932 ± 0.0463 ^b	0.0001
Thymus	0.2730 ± 0.0187 ^c	0.6106 ± 0.0354 ^a	0.3885 ± 0.0141 ^b	0.4198 ± 0.0386 ^b	0.0001
HA titer against SRBCs (days post-immunization)					
3	4.03 ± 0.125 ^c	6.20 ± 0.163 ^a	5.60 ± 0.248 ^{ab}	5.48 ± 0.309 ^b	0.0001
7	6.88 ± 0.14 ^c	9.23 ± 0.20 ^a	8.73 ± 0.30 ^a	7.73 ± 0.36 ^b	0.0001
10	4.28 ± 0.18 ^b	5.45 ± 0.20 ^a	4.38 ± 0.13 ^b	4.73 ± 0.30 ^b	0.008
HI titer against NDV (days post-immunization)					
3	3.90 ± 0.14 ^c	6.95 ± 0.38 ^a	5.90 ± 0.19 ^b	5.63 ± 0.18 ^b	0.0001
7	5.88 ± 0.18 ^c	8.50 ± 0.27 ^a	7.58 ± 0.25 ^b	7.35 ± 0.12 ^b	0.0001
10	5.20 ± 0.11 ^c	8.25 ± 0.34 ^a	7.08 ± 0.27 ^b	6.60 ± 0.21 ^b	0.0001

*Means followed by different superscripts, between treatments, within trait within age are significantly different ($p \leq 0.05$). Where WY is whole yeast, YCW is yeast cell wall, YE is yeast extract, HA is haemagglutination, SRBCs is sheep red blood cells, HI is haemagglutination inhibition and NDV is Newcastle disease virus.

CONCLUSION

It can be demonstrated that the supplementation of whole yeast at 0.1 percent or yeast cell wall at 0.03 percent and yeast extract at 0.07 percent increased the broiler chickens' body weight and feed conversion. Also, the inclusion of 0.1 whole yeast or its derivatives improved the immunological parameters, such as the antibody titer and immunological organ weights.

DECLARATIONS

Authors' contribution

Engy Youssry Abd El-Salam collected the data, performed the data analysis, and wrote the draft of manuscript. A. M. Atta designed the study, and revised the manuscript. M. A. EL-Mannawy was responsible for the scientific material collection that was used in the experiment and revised the manuscript. A. M. Abo-Taleb was responsible for the laboratory analysis. All authors have read and approved the final data and manuscript. Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

Competing interests

The authors declared that they have no competing interests.

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Ethical considerations

All authors took responsibility for the integrity of the data and the accuracy of the data analysis as well as ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy). All authors confirmed the final edition of the article and declared that they did not use any related data of this article on any other publications.

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