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RESEARCH ARTICLE

Effects of Dietary Yeast Cell Wall Supplementation on Performance, Carcass Characteristics, Antibody Production and Histopathological Changes in Broilers

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Summary

This study was carried out to determine the effects of dietary yeast cell wall supplementation on growth performance, carcass traits, antibody production to sheep red blood cells (SRBC) and histopathological changes in broilers. A total of 272 Ross 308 male broiler chicks aged one day were allocated into one control group and three treatment groups each containing 68 chicks. A basal diet was supplemented with 0, 1, 2 and 3 g/kg yeast cell wall (InteMos) to obtain dietary treatments. The experimental period lasted 6 weeks. Dietary yeast cell wall increased body weight gain during the first three weeks ($P<0.001$). Feed conversion during the first three weeks ($P<0.001$) and during the overall experimental period ($P<0.01$) were improved with the dietary supplementation of yeast cell wall. No differences were observed in feed intake, carcass yield and the relative weights of gizzard, liver and heart. Yeast cell wall supplementation decreased the relative weight of abdominal fat ($P<0.05$) and increased antibody titres to SRBC ($P<0.01$) in broilers. Control and treatment groups had similar histological grade; hepatic lobular inflammation, steatosis and cell ballooning scores and, cardiac steatosis. It is concluded that yeast cell wall was an effective feed additive in broiler feeding due to the increased growth performance, increased humoral immune response and the reduction in abdominal fat.

Keywords: *Broiler, Carcass traits, Histopathological changes, Performance, Yeast cell wall*

Broyler Karma Yemlerine Maya Hücre Duvarı İlavesinin Performans, Karkas Özellikleri, Antikor Üretimi ve Histopatolojik Değişiklikler Üzerine Etkileri

Özet

Bu araştırma broyler karma yemlerine maya hücre duvarı ilavesinin büyüme performansı, karkas özellikleri, koyun eritrositine karşı antikor üretimi (SRBC) ve histopatolojik değişiklikler üzerine etkilerini belirlemek amacıyla yapılmıştır. Toplam 272 adet günlük Ross 308 erkek broyler civciv her biri 68 adet içeren bir kontrol ve üç deneme grubuna ayrılmıştır. Bazal karma yeme 0, 1, 2 ve 3 g/kg düzeyinde maya hücre duvarı (InteMos) ilave edilerek deneme karma yemleri oluşturulmuştur. Deneme 6 hafta sürdürülmüştür. Karma yeme maya hücre duvarı ilavesi ilk üç haftada canlı ağırlık kazancını artırmıştır ($P<0.001$). Denemenin ilk üç haftası ($P<0.001$) ve deneme süresince ($P<0.01$) yem dönüşüm oranı maya hücre duvarı ilavesi ile olumlu yönde etkilenmiştir. Yem tüketimi, karkas randımanı ile relatif taşlık, karaciğer ve kalp ağırlıkları bakımından gruplar arasında farklılık gözlenmemiştir. Maya hücre duvarı ilavesi broylerlerde relatif abdominal yağ ağırlığını azaltmış ($P<0.05$) ve SRBC'ye karşı antikor titresini ($P<0.01$) artırmıştır. Kontrol ve deneme gruplarında hepatik lobüler inflamasyon, yağlanma ve hücre balonlaşma skorları ile kalp yağlanma oranlarını içeren histolojik skorlamalar benzer bulunmuştur. Sonuç olarak, büyüme performansındaki artış, humoral immun cevaptaki artış ve abdominal yağdaki azalmadan dolayı maya hücre duvarı etkili bir yem katkı maddesidir.

Anahtar sözcükler: *Broyler, Histopatolojik değişiklikler, Karkas özellikleri, Maya hücre duvarı, Performans*

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INTRODUCTION

Yeast cell walls have been used increasingly in poultry diets as a feed additive after the ban on the use of antibiotic growth promoters in the EU [1]. Yeast cell walls contain prebiotic oligosaccharides such as fructooligosaccharides, mannanoligosaccharides and β-glucans that beneficially affect gut health [2,3] and modulate immunity [4,5]. Dietary β-1,3/1,6-glucan, derived from yeast *(Saccharomyces cerevisiae)* cell walls, increases performance by improving average daily gain, reducing the feed/weight gain ratio and enhancing immunological response [6].

Reisinger et al.[7] observed that the yeast derivative (contains 0.017% mannan and 0.025% glucan) when fed at 0.1% of the diet positively influenced the final body weight, the daily body weight gain, feed conversion ratio and jejunum goblet cell density and reduced the number of apoptotic enterocytes. They [7] concluded that increased goblet cell density might have protected the broilers against primary infections and this could have been a reason for the improved performance. Chae et al.[8] reported that dietary levels of β-glucan (derived from *Saccharomyces cerevisiae*) at 0.02% and 0.04% improved weight gain, nutrient retention and immunity in broilers. However the feed conversion ratio was not improved with β-glucan supplementation [8]. Reports on the effects of yeast cell wall as prebiotics on the gut pH, antibody titer to SRBC and histopathological changes in broilers are lacking. Therefore the present study was designed to determine the effects of dietary yeast *(Saccharomyces cerevisiae)* cell wall on performance, carcass characteristics, humoral immune response and histopathological changes in broilers.

MATERIAL and METHODS

Animals and Diets

A total of 272 Ross 308 male broiler chicks aged one day were randomly assigned to one control group and three treatment groups each containing four replicate groups of 17 chicks. Chicks of each replicate groups were placed in separate floor pen measured as 170 x 94 x 90 cm, width x length x height, respectively. Each pen had wood shavings litter, two nipples and one hanging suspended feeder. Feed in mash form and water were provided *ad libitum* during 42 days. Continuous lighting was applied during the whole experiment. Average room temperature was 32±2°C on the first week and then gradually lowered to average 24-26°C and this temperature was maintained up to slaughter age. All animal use protocols were in accordance with the Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010 on the protection of animals used for scientific purposes [9].

Basal diets were supplemented with the yeast cell wall derived from bakers yeast, *Saccharomyces cerevisiae* (InteMos, NCYC R 625, Integro Food and Feed Manufacturing Company, İstanbul, Turkey) at the level of 1, 2 and 3 g/kg for the diets of the first, second and third treatment groups, respectively. Yeast cell wall had 227.2 g/kg crude protein, 37.4 g/kg ether extract, 4.3 g/kg crude fibre, 64 g/kg crude ash, 7.39 g/kg calcium and 5.91 g/kg phosphorus. The basal diet was formulated according to the commercial management guide (Ross 308 Broiler). The ingredients and chemical composition of the basal diet are presented in *Table 1*.

Measurements, Sample Collection and Laboratory Analysis

Moisture, crude ash, crude fibre, ether extract and crude protein contents of basal diet was determined according to the AOAC [10]. The samples were ashed in a muffle furnace prior to the analysis of calcium and total phosphorus [11,12]. Metabolizable energy levels of samples were estimated using the Carpenter and Clegg's equation [13].

Chicks were weighed individually at the beginning of the experimental period and weekly for calculating body weight gains. The birds were observed daily for evaluating mortality. Feed consumption was recorded weekly and

Table 1. *Ingredients and chemical composition of the basal diets*
Tablo 1. *Bazal karma yemlerin yapısı ve kimyasal bileşimi*

Ingredients (g/kg)	Starter Diet 1-14 days	Grower Diet 15-28 days	Finisher Diet 29-42 days
Corn	490.5	544.5	544.5
Soybean meal	240.0	190.0	160.0
Full fat soya	165.0	165.0	195.0
Meat and bone meal	40.0	40.0	40.0
Sunflower oil	30.0	26.0	26.0
Limestone	15.0	15.0	15.0
Dicalcium phosphate	12.0	12.0	12.0
Salt	2.5	2.5	2.5
DL-Methionine	2.0	2.0	2.0
Lysine	0.5	0.5	0.5
Vitamin mineral premix[1]	2.5	2.5	2.5
Chemical composition (Analyzed)			
Metabolizable energy[2] (MJ/kg)	13.15	13.28	13.46
Crude protein (g/kg)	223.0	205.0	202.8
Calcium (g/kg)	14.2	14.1	14.1
Total phosphorus (g/kg)	8.2	8.0	8.0

[1] Supplied the following per kilogram of diet: 12.000 IU vitamin A, 2.400 IU vitamin D_3, 30 mg vitamin E, 2.5 mg vitamin K_3, 2.5 mg vitamin B_1, 6 mg vitamin B_2, 4 mg vitamin B_6, 20 µg vitamin B_{12}, 25 mg niacin, 8 mg calcium-D-panthotenate, 1 mg folic acid, 50 mg vitamin C, 50 µg D-biotin, 80 mg Mn, 60 mg Zn, 60 mg Fe, 5 mg Cu, 1 mg I, 0.5 mg Co, 0.15 mg Se;
[2] Metabolizable energy content of diets was estimated according to the equation of Carpenter and Clegg [11]

expressed as g per bird per week and the feed conversion ratio was calculated as kg feed per kg body weight gain.

To collect excreta, broilers in each replicate were put on cleaned plastic sheet in a separate pen during 5-10 min at day 40. Then excreta samples of each replicate pen were collected and mixed. The samples were dried in an air-forced oven at 60°C until reaching constant weight, and then the moisture content of samples was determined according to the AOAC [10].

At day 36, 20 broilers from each diet group (5 from each replicate) were randomly selected from each pen and injected with 0.1 ml of 0.25% suspension of sheep erythrocytes (SRBC, provided from a healthy male sheep) in phosphate buffer saline. Circulating anti-SRBC antibody titers were determined by the microhemagglutination technique from samples taken at 5 days after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution [14].

Blood samples were collected from vena brachialis under the wing from 20 fed broilers randomly chosen from each group (five from each replicate) at day 41 and centrifuged at 3.220 x g for 8 min. Serum was collected and stored at -20°C for determination of total protein, albumin, uric acid, triglyceride, cholesterol, and levels of aspartate amino transferase (AST) and alanine amino transferase (ALT) by an autoanalyser (Product code 680-2153, Vitros 350; Johnson-Johnson Company, New York, USA) using their accompanying commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics; Johnson-Johnson Company).

At the end of the experiment (on day 42) 16 broilers from each group (4 from each replicate) were weighed and slaughtered by severing the jugular vein. Their gastrointestinal tracts were excised. Hot carcasses were weighed to determine the carcass yield. Absolute and relative weights of abdominal fat, liver, heart, gizzard, spleen and bursa of Fabricius were determined. Duodenal, jejunal, ileal and caecal digesta contents were pooled and homogenized. pH values of digesta contents were measured immediately by pH meter (Selecta pH meter, pH 2004, J.P. Selecta, Barcelona).

For histopathological analysis, half of the liver and heart samples were frozen in -80°C in liquid nitrogen and cut at 5 µm and stained with haematoxylin and eosin and oil-red (-O) stain. The other halves were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax and cut 5 µm and stained with haematoxylin and eosin and trichrome stains. Histopathological examination was carried out by independent investigators blinded to treatment groups using light microscopy in 30 high-power fields per sample with a magnification of 200x. Histopathological features of hepatic steatosis were evaluated using a semiquantitative, histopathology scoring adapted from the recently accepted AASLD criteria for steatosis staging, and then scored for steatosis, lobular inflammation, and hepatocyte ballooning using the NAFLD activity score [15].

Statistical Analysis

Statistical analysis were done using SPSS program (SPSS Inc., Chicago, IL, USA). The experimental unit was the cage (n = 4). The normality of data distribution was checked using the Kolmogorov-Smirnov test. Values were reported as means ± SEM. The significance of mean differences among groups was tested by Duncan [16]. Level of significance of $P<0.05$ was used.

RESULTS

The effects of dietary yeast cell wall on growth performance and excreta moisture are shown in *Table 2*. Supplementing diets with yeast cell wall increased the weight gains during the starter period ($P<0.001$) in broilers. Dietary treatments did not significantly affect feed intake. Feed conversion during the starter period ($P<0.001$) and during the whole period ($P<0.05$) was improved by yeast cell wall supplementation. During the experimental period, 2 (2.9%), 1 (1.5%), 2 (2.9%) and 1 (1.5%) broilers died in the control group and groups fed with diets containing yeast cell wall at the level of 1, 2 and 3 g/kg, respectively. Dietary yeast cell wall supplementation had no effect on excreta moisture.

The effects of dietary yeast cell wall on anti-SRBC titer and blood serum parameters in broilers are shown in *Table 3*. Dietary yeast cell wall supplementation increased antibody titers to SRBC ($P<0.01$), increased serum protein concentration ($P<0.05$) and decreased total cholesterol and triglyceride concentrations ($P<0.001$). No differences were observed in serum albumin, uric acid, ALT and AST among groups.

Carcass yield and the relative weights of gizzard, liver, heart, spleen and bursa of Fabricius were not affected by the yeast cell wall supplementation *(Table 4)*. However the relative weight of abdominal fat was decreased with dietary yeast cell wall. Intestinal pH *(Table 4)* tended to be decreased in broilers supplemented with yeast cell wall and the jejunal pH and ileal pH was significantly lower than that of the control group ($P<0.05$).

No significant differences were seen in histological grade; hepatic lobular inflammation, steatosis and cell ballooning scores and cardiac steatosis among groups *(Table 5)*.

DISCUSSION

Dietary yeast cell wall supplementation increased the weight gains during the starter period ($P<0.001$) in broilers. Total live weight gain and weight gain during the grower

Table 2. *The effects of dietary supplementation of yeast cell wall on growth performance and excreta moisture in broilers*
Tablo 2. *Karma yemlere maya hücre duvarı ilavesinin broylerlerde büyüme performansı ve dışkı nemi üzerine etkileri*

Items	Yeast Cell Wall (g/kg)				SEM	P-value
	0	1	2	3		
Live weight gain (g)						
1-21 days	838b	879a	880a	893a	6	<0.001
22-42 days	1831	1895	1896	1900	16	0.375
1-42 days	2669	2775	2776	2793	19	0.059
Feed intake (g)						
1-21 days	1080	1074	1071	1079	2	0.253
22-42 days	3436	3428	3400	3414	8	0.428
1-42 days	4516	4501	4471	4492	8	0.268
Feed conversion ratio (g/g)						
1-21 days	1.29a	1.22b	1.22b	1.21b	0.01	<0.001
22-42 days	1.88	1.81	1.79	1.80	0.02	0.219
1-42 days	1.69a	1.62b	1.61b	1.61b	0.01	0.030
Excreta moisture (g/kg)	797.0	803.6	804.2	803.8	1.3	0.149

[a-b] Means with different superscript in the same row are different at P<0.05 in instances with significant interaction; n = 4

Table 3. *The effects of dietary supplementation of yeast cell wall on anti-SRBC titers and blood serum parameters in broiler*
Tablo 3. *Karma yemlere maya hücre duvarı ilavesinin broylerlerde SRBC'ye karşı antikor düzeyi ve kan serum parametreleri üzerine etkileri*

Items	Yeast Cell Wall (g/kg)				SEM	P-value
	0	1	2	3		
Anti SRBC titer (log_2)	5.20b	5.95a	6.10a	6.10a	0.11	0.004
Total protein (g/L)	30.3b	32.6ab	34.0a	33.3a	0.5	0.026
Albumin (g/L)	13.5	13.7	13.8	14.0	0.1	0.585
Uric acid (mg/L)	56.7	56.9	57.3	56.6	0.8	0.995
Total cholesterol (g/L)	1.26a	1.16b	1.08c	1.10c	0.01	<0.001
Triglyceride (g/L)	1.29a	1.14b	0.96c	0.94c	0.02	<0.001
ALT (U/L)	14.6	14.1	14.8	13.9	0.3	0.637
AST (U/L)	226	221	225	235	3	0.344

[a-b] Means with different superscript in the same row are different at P<0.05 in instances with significant interaction; n = 20

period tended to be increased in broilers supplemented with yeast cell wall but differences with not supplemented group were not statistically significant. Dietary yeast cell wall supplementation improved feed conversion during the starter period (P<0.001) and during the whole period (P<0.05) however had no effect on feed intake. This improvement in yeast cell wall supplemented groups might be due to the improvement of the intestinal lumen health and thereby increasing the absorption and utilization of the dietary nutrients [17,18]. Zhang et al.[19] reported that the live weight gains by yeast cell wall fed broilers were greater than those of the control broilers from 4 to 5 weeks of age and from 0 to 5 weeks of age. Live weight gain [20,21], feed intake [19,20] and feed conversion [21] were not affected by using yeast cell wall in some studies. The differences in animal response may be related to the differences in the type and dose of yeast cell wall and diet composition. In the present study 2 (2.9%), 1 (1.5%), 2 (2.9%) and 1 (1.5%) broilers died in the control group and groups fed with diets containing yeast cell wall at the level of 1, 2 and 3 g/kg, respectively during 42 days. Similarly some researchers [7,20,22] reported that dietary supplementation of yeast cell wall had no effect on mortality. Dietary yeast cell wall supplementation did not significantly affect excreta moisture, as previously reported with yeast in broilers [23].

Antibody responses have been used as measures of the humoral immune status of poultry [24]. As shown in

Table 4. *The effects of dietary supplementation of yeast cell wall on carcass yield, relative organ weights and intestinal pH in broilers*
Tablo 4. *Karma yemlere maya hücre duvarı ilavesinin broylerlerde karkas randımanı, relatif organ ağırlıkları ve bağırsak pH'sı üzerine etkileri*

Items	Yeast Cell Wall (g/kg)				SEM	P-value
	0	1	2	3		
Carcass yield (%)	72.7	72.8	72.8	72.9	0.2	0.983
Gizzard (%)	1.33	1.33	1.34	1.32	0.02	0.960
Heart (%)	0.52	0.52	0.53	0.51	0.01	0.743
Liver (%)	1.91	1.89	1.92	1.88	0.02	0.792
Spleen (%)	0.11	0.10	0.11	0.11	0.01	0.261
Bursa Fabricius (%)	0.17	0.18	0.18	0.19	0.01	0.796
Abdominal Fat (%)	1.49a	1.33b	1.33b	1.27b	0.03	0.024
Duodenum pH	5.64	5.58	5.52	5.55	0.04	0.723
Jejunum pH	5.63a	5.54ab	5.46b	5.43b	0.03	0.048
İleum pH	6.52a	6.30b	6.32b	6.29b	0.03	0.041
Caecum pH	6.50	6.37	6.40	6.44	0.04	0.672

[a-b] Means with different superscript in the same row are different at P<0.05 in instances with significant interaction; n = 16

Table 5. *The effects of dietary supplementation of yeast cell wall on cardiac and hepatic histopathology in broilers*
Tablo 5. *Karma yemlere maya hücre duvarı ilavesinin broylerlerde kalp ve karaciğer histopatolojisi üzerine etkileri*

Items	Grade	Yeast Cell Wall (g/kg)				P-value
		0	1	2	3	
Cardiac steatosis(%)	0	75.0	87.5	100.0	93.8	0.218
	1	18.8	12.5	0	0	
	2	6.3	0	0	6.3	
Hepatic histopathology (%)						
Histological grade (%)	0	81.3	81.3	68.8	62.5	0.599
	1	18.8	18.8	31.3	31.3	
	2	0	0	0	6.3	
Hepatic steatosis (%)	0	81.3	93.8	100.0	87.5	0.500
	1	12.5	6.3	0	6.3	
	2	0	0	0	6.3	
	3	6.3	0	0	0	
Cell ballooning (%)	0	81.3	93.8	87.5	87.5	0.767
	1	18.8	6.3	12.5	12.5	
Lobular inflammation (%)	0	0	18.8	25.0	25.0	0.444
	1	81.3	62.5	50.0	62.5	
	2	18.8	18.8	25.0	12.5	

Table 3, dietary yeast cell wall supplementation increased antibody titers to SRBC (P<0.01). This might be due to the glucans and the mannans present in the yeast cell wall on the immune system [4,5,25]. It can be assumed that prebiotics would bind to macrophage reception sites by recognizing spesific sugars found in glucoproteins of the epithelial surface, triggering a cascading reaction that would eventually activate macrophages and release cytokines, thereby activating the acquired immune response [26,27]. Higher antibody responses in broiler breeders fed MOS were observed in the study of Shashidhara and Devegowda [5]. Chae et al.[8] showed that CD8 and TCR I cells were higher in the 0.04% β-glucan supplemented diet as compared with non-added diets. Similarly, Yalçın et al.[23] also reported greater antibody titre in broilers fed diets containing 1, 2, 3 or 4 g/kg of yeast autolysate.

Blood serum biochemical parameters may provide useful information about the evaluation of the health status of broilers. Dietary yeast cell wall supplementation increased serum protein concentration (P<0.05) and decreased total cholesterol and triglyceride concentrations (P<0.001). Increased serum total protein in broilers fed diets supplemented with yeast cell wall may reflect a more intensive metabolism of the proteins and effective protein utilization in the broiler metabolism. This situation may also be explained by increasing weight gain. Similarly some researchers observed that serum total cholesterol and triglyceride concentrations were reduced by dietary MOS [28] or yeast autolysate [29]. Krasowska et al.[30] reported that *Saccharomyces* strains are able to remove cholesterol from the growth medium and that baker's yeast *Saccharomyces cerevisiae* seems to be the perfect organism for lowering cholesterol in the gastrointestinal tract. Nicolosi et al.[31] also indicated that yeast derived β-glucan lowered total cholesterol concentrations in hypercholesterolaemic men. However these results contradict the findings of Konca et al.[32] who reported that dietary MOS supplementation did not affect serum cholesterol and total protein concentration in turkeys. In the present study dietary supplementation of yeast cell wall had no effect on serum albumin, uric acid, ALT and AST. Yalçın et al.[23] also showed that serum uric acid, ALT and AST levels were not affected from dietary yeast autolysate.

No significant differences in the carcass yield and the relative weights of gizzard, liver, heart, spleen and bursa of Fabricius were observed among groups *(Table 4)*. However the relative weight of abdominal fat was significantly lower (P<0.05) in broilers fed with diets containing yeast cell wall than in birds fed with the control diet. This result shows a change in energy partitioning. It might be that the extra energy that was not being stored by the broilers fed yeast cell wall supplemented diets was being used to up regulate the immune system and increase the titer response to SRBC. In agreement with previous reports yeast cell wall or yeast cell wall ingredients had no significant effect on gizzard weight [21], relative weight of spleen and bursa of Fabricius [20]. The results of present study contradict the findings of previous work by Guo et al.[33] in which dietary supplementation of yeast β-glucan resulted in increased relative spleen and bursa weights. Corduk et al.[34] reported that MOS supplementation did not significantly affect carcass yield and the relative weights of abdominal fat and gizzard.

Intestinal pH tended to be decreased in broilers supplemented with yeast cell wall and the jejunal pH and ileal pH was significantly lower than that of the control group (P<0.05) as shown in *Table 4*. Low pH of the digesta of broilers fed yeast cell wall could improve utilization of the diets as reported in the study of Afsharmanesh et al.[35]. In contrast to the present study, some researchers observed that MOS supplementation did not affect [36] or increased [37] ileal pH. According to the findings of some studies, pH of duodenal contents [32,38] and pH of ileal and caecal contents [38-40] were unaffected by dietary MOS supplementation. The difference among the studies may be due to the diet composition, diet type, age of birds and method of pH measurement.

During the growth of broiler chickens by intensive feeding, some health problems occur, mainly limb defects, sudden death syndrome, or excessive fat deposition [41]. Maxwell et al.[42] reported that feed overconsumption in chicken results in fat deposition throughout the body, which leads to coronary and hepatic steatosis in chickens. In the present study control and treatment groups had similar histological grade; hepatic lobular inflammation, steatosis and cell ballooning scores and cardiac steatosis as shown in *Table 5*. However, these results do not exclude the possibility that yeast cell wall supplementation may be beneficial in other circumstances. In the previous study of Yalçın et al.[43] the dietary probiotic (Primalac 454; *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium thermophilus*) supplementation (at the dose of 0.05%) have significantly alleviated the development of a non-alcoholic fatty liver disease induced by dietary protein restriction.

The differences between the results of present study and those of previous reports may be the species, age, and sex of the birds, dietary nutrient composition, type, dose and composition of yeast cell wall or environmental conditions.

The results of this study indicate that dietary yeast cell wall at the level of 1, 2 and 3 g/kg improved body weight and feed efficiency, decreased abdominal fat and increased humoral immune response. Therefore it is concluded that yeast cell wall derived from bakers yeast (InteMos) was an effective and beneficial feed additive in broiler feeding.

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