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Immune-modulatory effects of dietary *Saccharomyces cerevisiae* cell wall in broiler chickens inoculated with *Escherichia coli* lipopolysaccharide

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Abstract 1. An experiment was conducted to study the effect of yeast (*Saccharomyces cerevisiae*) cell wall (YCW) supplemented in diets of broiler chickens challenged with *Escherichia coli* lipopolysaccharide (LPS).
2. One-day-old broiler chicks were randomly distributed into 24 cages (6 replicate cages; 8 birds/cage) and were inoculated with 0 or 1 mg/kg body weight *E. coli*-LPS (d 4 and 9) and 0 or 500 mg YCW/kg feed, resulting in a 2 × 2 factorial arrangement of treatments. Experimental diets did not include coccidiostats, in-feed antibiotics or enzymes.
3. On d 21, the inoculation of *E. coli*-LPS reduced weight gain and feed intake and increased feed conversion ratio (FCR) of birds, an effect maintained until 28 d. In contrast, chickens given diets with YCW improved the FCR at both 21 and 28 d of age.
4. *E. coli*-LPS challenge reduced the relative weight of bursa of Fabricius, except when chickens were given YCW, which resulted in an interaction. Supplementation of broiler diets with YCW exacerbated the cellular immune response as measured by the delayed cutaneous hypersensitivity response test.
5. The results of this study suggested a benefit on feed efficiency when YCW was added to diets fed to broiler chickens challenged with *E. coli*-LPS. Part of the mode of action of YCW might be related to better maintenance of immune status in response to microbial challenge.

INTRODUCTION

The presence of microbial challenges in commercial poultry farms causes important economical loses for the poultry industry. The control of stress and challenges appears to be critical to maintain profitability and reduce losses. Moreover, maintaining the appropriate functioning of the immune system is essential to protect birds from environmental challenges (Klasing, 1998). When broilers were experimentally infected with a pathogenic *Escherichia coli* strain, yeast β-1, 3/1, 6-glucan supplementation attenuated the reduction in performance associated with the challenge (Huff *et al.*, 2006). During the last 20 years, the immuno-modulatory capacity of yeast β-1, 3/1, 6-glucans has been well documented in birds and other animal species (Dritz *et al.*, 1995; Chang *et al.*, 2000; Guo *et al.*, 2003; Morales-Lopez *et al.*, 2009; Cox *et al.*, 2010*a*, 2010*b*). Beta-1, 3/1, 6-glucans and other polysaccharides such as mannanoligosaccharides are important constituents of *Saccharomyces cerevisiae* cell wall (Aguilar-Uscanga and François, 2003); thus, yeast cell wall (YCW) can be used as a source of immune-modulatory polysaccharides (Morales-Lopez *et al.*, 2009). Studies have shown a positive effect of dietary YCW on productivity and health of birds kept under challenge conditions (Hooge *et al.*, 2003; Stanley *et al.*, 2004; Sun *et al.*, 2005; Morales-Lopez *et al.*, 2010; Huff *et al.*, 2011). In addition, bacterial endotoxins, such as lipopolysaccharide (LPS), an integral part of the external membrane of gram negative bacteria (e.g. *E. coli* and *Salmonella* spp), are powerful immuno-stimulants and are commonly present in organic dust in commercial poultry farms (Petkov and Tsutsumanski, 1975; Zucker and Muller, 2000; Huneau-Salaün *et al.*, 2011). LPS has been employed in experimental animal models focused in the evaluation of chicken susceptibility to

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environmental microbial challenges (immune stress). LPS can reduce productivity through stimulation of acute phase inflammatory interleukins (IL-1 and IL-6) (Klasing *et al.*, 1987; Wang *et al.*, 2003; Mireles *et al.*, 2005).

The aim of the experiment was to study the effect of YCW (*S. cerevisiae*)-supplemented diets fed to broiler chickens inoculated with 0 or 1 mg/kg of LPS of *E. coli* in order to elucidate a possible mechanism for the previously reported benefits of feeding YCW.

MATERIALS AND METHODS

Animal housing and experimental diets

Commercial 1-d-old Ross 308 male broiler chicks were obtained for a commercial hatchery and placed into cages (Petersime batteries) in a house with forced ventilation, heating and artificial illumination. The temperature inside the house was initially 33–35°C and was decreased by 3°C each week to 22°C. The lighting programme was 23 h of light for the first 4 d, 20 h until 10 d and 18 h thereafter. Feed and water were provided *ad libitum* throughout the duration of the trial. The efficacy of *S. cerevisiae* cell wall (YCW) (Lesaffre Feed Additives, Marcq-en-Baroeul, France) was tested in a single diet in mash form, formulated to meet or exceed National Research Council (1994) requirements (Table 1). The experimental basal diets did not include antibiotics, coccidiostats or exogenous enzymes. The YCW supplements (Table 2) were included in the basal diet with the vitamin and mineral premix to replace maize starch. All experimental conditions and animal protocols were approved by the Ethical Commission of IRTA (Catalonia, Spain).

A 2 × 2 factorial arrangement of treatments was used and included the inoculation of *E. coli*-LPS at 0 or 1mg/kg of body weight (BW) on d 4 and 9 and 0 or 500 mg YCW/kg feed. Experimental treatments were replicated 6 times with 8 chickens in each replicate cage.

E. coli-LPS inoculation

E. coli-LPS (LPS 055:B5, Sigma Chemical Co, Sr. Louis, MO, USA.) was dissolved with 1 mg/ml sterile physiological saline solution (PSS). Chickens were inoculated with *E. coli*-LPS inoculum (1mg/kg BW) by intra-peritoneal and subcutaneous injections at 4 and 9 d of age, respectively, according to methods of Webel *et al.* (1998) and Mireles *et al.* (2005). Chickens from the control group were inoculated with PSS instead of *E. coli*-LPS inoculum.

Table 1. *Composition of the experimental diet as feed basis and calculated nutrient analysis*

Ingredient	Amount (g/kg) (1–28 d)
Maize	250.0
Soyabean meal (48% CP)	271.3
Barley	241.0
Wheat	100.0
Soyabean full fat	40.0
Soyabean oil	47.5
Calcium carbonate	17.0
Dicalcium phosphate	17.7
DL-Methionine (99.0%)	3.2
L-Lysine HCL (78.8%)	3.0
L-Threonine (98.5%)	0.8
Sodium chloride	3.4
Choline (50.0%)	0.6
Minerals and vitamins[1]	4.0
YCW fraction or carrier[2]	0.5
Total	1000.0
Calculated composition (g/kg)	
ME (MJ/kg)	12.54
CP	215
Total Lys	127
Total Met	62
Total Met + Cys	96
Total Ca	120
Available P	45
Analysed nutrients (g/kg)	
CP	201
Crude fat	76
DM	905

[1]Provided per kilogram of feed: vitamin A, 12 000 IU; vitamin D3, 2400 IU; vitamin E, 30 mg; vitamin K3, 3 mg; thiamine, 2.2 mg; riboflavin, 8 mg; pyridoxine, 5 mg; cyanocobalamin, 11 μg; folic acid, 1.5 mg; biotin, 150 μg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn (manganese sulphate), 60 mg; Zn (zinc oxide), 40 mg; I (potassium iodate), 0.33 mg; Fe (ferrous sulphate), 80 mg; Cu (copper sulphate), 8 mg; Se (sodium selenite), 0.15 mg; ethoxyquin, 150 mg.
[2]YWC, yeast cell wall (*S. cerevisiae*).

Table 2. *Analytical composition of experimental yeast cell wall* (S. cerevisiae)

Composition (g/kg)[1]	Yeast cell wall
Dry matter	976
Beta-glucans	260
Manans	216
Crude fat	130
Crude protein	249

[1]Conducted at Bio-Springer Maisons-Alfort-Cedex, France, by the Eurasyp (2007) methodology, $n = 3$ analyses per sample.

Animal performance

Chickens were weighed by pen at 1, 21 and 28 d of age. As growth performance parameters, average BW, daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR, feed to gain ratio) were calculated for 1–21 d, 21–28 d and 1–28 d of age. Mortality (%) was calculated for the total length of the experiment.

Cell-mediated immune response

The effect of different treatments on cell-mediated immune response was determined by the cutaneous basophilic hypersensitivity test at 14 d (Corrier and DeLoach, 1990). Briefly, an intradermic inoculation of phytohaemagglutinin-P (PHA-P) (150 µg/0.1 ml of PHA-P, Sigma Chemical, St. Louis, Mo) was carried out in the inter-digital membrane of the 3rd and 4th phalanges of the right inferior extremity and PSS (0.1 ml) of the left foot (6 chickens per treatment). Twenty-four hours post-inoculation, the thickness of the inter-digital membrane was measured in millimetres using a micrometre.

Relative weight of lymphoid organs

At 21 d of age, 11 chickens from each treatment were selected at random and were killed by cervical dislocation. The spleen and bursa of Fabricius were excised and weighed. Afterwards, adherent fat from these organs was removed, and their relative weight (RW) expressed as a percentage of live BW was calculated.

Statistical analysis

The experiment was analysed as a randomised complete block design with 6 blocks and 4 experimental treatments in a 2 × 2 factorial arrangement (SAS Institute, 1996), with dietary YCW supplementation and *E. coli*-LPS inoculation as fixed factors. In case a significant interaction was found, differences between treatments were established by Duncan's test at $P < 0.05$ probability. When an interaction was not significant, the significance of main effects was obtained from the *F*-value in the ANOVA table. Before statistical analysis, percentage data were transformed to arcsine values.

RESULTS AND DISCUSSION

At 21 d, chickens challenged with *E. coli*-LPS had lower BW ($P < 0.01$), DWG ($P < 0.01$) and DFI ($P < 0.01$). From 21 to 28 d, chickens inoculated with *E. coli*-LPS tended to have increased BWG ($P = 0.08$) and improved FCR ($P = 0.09$) compared to unchallenged birds (Table 3). For the whole period (1 to 28 d), supplementation of broiler diets with YCW improved ($P < 0.05$) FCR, regardless of the challenge as represented by the non-significant interaction from d 0 to 21 and d 0 to 28. However, birds challenged with *E.coli*-LPS tended ($P = 0.07$) to be less efficient than non-challenged broilers from d 0 to 28 (Table 3). Conversely, chickens given YCW diets improved the FCR at both 1–21 d ($P < 0.05$) and 1–28 d ($P < 0.01$), but other growth performance parameters did not improve (Table 3). Other authors have reported a reduction in growth performance of broilers challenged with *E. coli*-LPS inoculation (immune stress) (Klasing *et al.*, 1987; Roura *et al.*, 1992; Webel *et al.*, 1998; Zhang *et al.*, 2011) that supports the present findings of decreased weight gain, feed intake and feed efficiency in broiler chickens under immune stress after LPS inoculation. The results also suggest compensatory growth after LPS challenge, as represented by the lack of differences in BW or DFI at d 28. Samuels and Baracos (1995) described a compensatory growth accompanied by greater feed

Table 3. *Effects of yeast cell wall (YCW) on growth performance parameters*[1] *of broiler chickens*[2] *inoculated with lipopolysaccharide (LPS) of E. coli*

		1 to 21 d				22 to 28 d				1 to 28 d			
Effect		BW 21 (g)	DWG (g)	DFI (g)	FCR (g/g)	BW 28 (g)	DWG (g)	DFI (g)	FCR (g/g)	DWG (g)	DFI (g)	FCR (g/g)	Mortality (%)
YCW													
0 mg/kg		717	32.0	43.8	1.38	1177	65.7	101	1.54	40.5	57.3	1.42	14.3
500 mg/kg		719	32.1	42.9	1.34	1179	65.8	100	1.52	40.6	56.5	1.39	14.8
LPS-*E. coli*													
Without challenge		747	33.5	44.6	1.33	1192	63.6	98	1.55	41.0	57.2	1.39	14.1
With challenge		689	30.7	42.1	1.37	1164	67.9	103	1.51	40.0	56.6	1.41	15.0
YCW	LPS-*E. coli*												
No	No	749	33.6	45.0	1.34[b]	1189	62.8	98	1.56	40.9	57.3	1.40[b]	16.2
Yes	No	744	33.4	44.3	1.33[b]	1195	64.3	98	1.53	41.1	57.2	1.39[b]	12.0
No	Yes	685	30.5	42.6	1.40[a]	1164	68.5	104	1.51	40.0	57.4	1.43[a]	12.5
Yes	Yes	693	30.9	41.6	1.34[b]	1164	67.3	101	1.50	40.0	55.8	1.39[b]	17.5
SE		15.7	0.74	0.89	0.01	22.1	2.3	3.8	0.21	0.78	1.27	0.01	4.0
Significance (*P*)													
YCW		0.92	0.93	0.36	0.05	0.91	0.96	0.78	0.39	0.90	0.51	0.02	0.52
LPS-*E. coli*		0.01	0.01	0.01	0.01	0.22	0.08	0.28	0.09	0.22	0.62	0.07	0.82
Interaction		0.67	0.66	0.85	0.14	0.90	0.56	0.69	0.67	0.90	0.60	0.17	0.25

[1]BW, body weight; DWG, daily weight gain; DFI, daily feed intake; FCR, feed conversion ratio (feed to gain ratio).
[2]$n = 6$ replicates of 6 birds from 0 to 21d and 5 birds from 21 to 28 d.
[a, b] Within a column, values not sharing a common superscript letter are significantly different ($P \leq 0.05$).

Table 4. *Effects of yeast cell wall (YCW) on the relative lymphoid organ weight*[1] *and the delayed cutaneous hypersensitivity reaction of chicken*[2] *inoculated with LPS of E. coli*

Effect		21 d (g/100 g of body weight)		Delayed cutaneous hypersensitivity reaction 14 d (mm)
		Spleen	Bursa of Fabricius	
YCW				
0 mg/kg		0.125	0.290	0.301
500 mg/kg		0.112	0.304	0.441
LPS-*E. coli*				
Without challenge		0.117	0.331	0.326
With challenge		0.120	0.263	0.416
YCW	LPS-*E. coli*			
No	No	0.114	0.348[a]	0.238[c]
Yes	No	0.120	0.314[a]	0.414[a]
No	Yes	0.136	0.232[b]	0.365[b]
Yes	Yes	0.105	0.294[a]	0.467[a]
SE		0.016	0.024	0.050
Source of variation (*P*)				
YCW		0.42	0.53	0.01
LPS-*E. coli*		0.84	0.01	0.13
Interaction		0.24	0.031	0.44

[1] $n = 11$ chickens.
[2] $n = 8$ chickens.
[a, b] Within a column, values not sharing a common superscript letter are significantly different ($P \leq 0.05$).

consumption in young animals that had been subjected to a previous period of immune stress. The improvements in FCR by adding YCW have been reported previously in other broiler studies (Santin *et al.*, 2001; Zhang *et al.*, 2005; Morales-Lopez *et al.*, 2010).

At 21 d, LPS challenge significantly reduced bursa weight except in broilers given YCW, resulting in an YCW × LPS interaction ($P < 0.05$; Table 4). The increase in the relative spleen weight can be associated with greater activity of this organ, since the spleen is one of the main lymphoid organs involved in the production of antigens (Roura *et al.*, 1992). The effects that LPS can exert on the productivity and health of the birds are mediated by the production of acute phase inflammatory interleukins (IL-1, IL-6; Klasing, 1987). Models of immune stress in chickens show that LPS inoculation can increase IL-6 and corticosterone serum concentrations (Nakamura *et al.*, 1998; Zhang *et al.*, 2011). Indeed, Xie *et al.* (2000) suggested that the reduction in the RW of the bursa of Fabricius of chickens inoculated with LPS can be a consequence of the increased production of corticosteroids stimulated by LPS (immune-depressor effect), as an atrophic process of stress. On d 14, chickens given YCW displayed an increased ($P < 0.01$) cutaneous hypersensivity reaction, which is an indirect parameter of cell-mediated immune response (Table 4). The increase in the cutaneous hypersensivity reaction in the groups of chickens that consumed YCW suggests an improvement in the immune-competence of these birds. The cutaneous hypersensivity reaction indirectly evaluates the immune response (*in vivo*) mediated by cells (immune cellular response). It is believed that dietary YCW acts as a non-pathogenic modulator of immune activation. This is especially important in the digestive tract of chickens where a substantial number of immune cells are located (Baurhoo *et al.*, 2009) and may underlie the effect of a greater stimulation in the cellular immune response in chickens given YCW. It has been reported that the supplementation of purified fractions of β-1,3/1,6-glucans from YCW in the diet could increase the cutaneous hypersensivity reaction in Leghorn birds (Acevedo and Pedroso, 2001) and in broiler chickens (Guo *et al.*, 2003). For example, Guo *et al.* (2003) suggested receptors for beta-glucans in the phagocytes could be involved in their activation and production of cytokines or interleukins. In this case, the chemostatic activity of the cytokines would provoke the accumulation of heterophils, monocytes, macrophages and basophiles in the sites of injection of the PHA-P, increasing the local characteristic reaction (Chang *et al.*, 1994).

In conclusion, the results of this study suggest a positive effect of YCW on feed efficiency of chickens, particularly under conditions of an immunological challenge. YCW added to broiler chicken diets may prevent the relative reduction of the bursa of Fabricius caused by LPS, suggesting that YCW enhances the tolerance of the birds and thereby help them to overcome the microbial challenge. This effect of YCW could bring benefits to broiler chickens maintained under immune stress, in terms of restoring feed efficiency to levels seen in birds without immune stress.

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