https://doi.org/10.17221/24/2023-CJAS

Ameliorative effect of yeast cell walls on broiler chickens' performance and gut health under coccidiosis challenge

*Shawkat Abdulrazaq M'Sadeq**

Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, Duhok, Iraq

**Corresponding author: smsadeq@uod.ac*

Citation: M'Sadeq S.A. (2023): Ameliorative effect of yeast cell wall on broiler chickens' performance and gut health under coccidiosis challenge. Czech J. Anim. Sci., 68: 346–355.

Abstract: This study was conducted to evaluate the efficacy of yeast cell wall parts (YCW) in reducing the severity of coccidiosis in broiler chickens. One-day-old Ross 308 chicks (n = 320) were randomly allocated to 32 floor pens in two rooms with a 2 × 4 factorial arrangement of treatments. Factors were (1) challenge: negative or positive by room, (2) feed additive: control (none), anticoccidial (salinomycin at 60 mg/kg), YCW 0.1% or 0.2%. On day 8, none-challenged room was inoculated with saline, and challenged room was inoculated with 5 000 *Eimeria tenella* oocysts. Performance results showed that in challenged birds, feed conversion ratio (FCR) and weight gain (WG) were poorer than in unchallenged birds on day 24 and 35. Birds given anticoccidial and YCW had improved FCR and WG (P = 0.01) over the control group. Challenge and additive interactions were observed on day 24 and day 35 for FCR and WG (P = 0.01) and for feed intake on day 24 (P = 0.01). Challenged birds fed YCW on days 24 and 35 had higher WG and better FCR compared with both challenged and non-challenged controls and anticoccidial treatment. Coccidial challenge increased crypt depth (CD), villous tip and base width, and villous surface area and decreased villous height to crypt depth ratio (VH : CD) and villous height (VH). Birds fed YCW exhibited significantly decreased CD, villous tip width, villous base width, villous surface area and increased VH, VH : CD in comparison to the control group. Challenged birds fed YCW had significantly lower bursa of Fabricius follicle length compared to birds fed the control diet. Challenged birds had significantly increased serum alanine transaminase (ALT) and aspartate aminotransferase (AST) concentrations than non-challenged birds. In conclusion, these results demonstrated that the yeast cell wall has the ability to control coccidiosis.

Keywords: yeast part; *E. tenella*; gut histomorphology; serum biochemicals; bursa of Fabricius histomorphology

Gut microflora is involved in numerous functions such as digestion, absorption, preventing pathogen infection, shaping the immune system of the gastrointestinal mucosa and providing nutrients (Yegani and Korver 2008). The gut microflora balance, as well as the micro and macro structure of gut integrity, are interlinked with the overall gastrointestinal health status of poultry. Therefore, the maintenance of gastrointestinal health is of utmost importance, as minor alterations can often lead to disturbances in gastrointestinal function, ultimately impacting the overall performance of poultry.

Enteric diseases are the most significant ones in the commercial poultry industry from a welfare

https://doi.org/10.17221/24/2023-CJAS

and economic point of view. Enteric diseases have been associated with a variety of pathogens, including parasites, viruses and bacteria and other non-infectious and infectious agents (M'Sadeq et al. 2015). Coccidiosis is a protozoal disease caused by *Eimeria* spp. that mainly effecting the gastrointestinal tracts of animals (Qaid et al. 2022). The disease results in significant economic losses of approximately USD 0.21 per chicken in the context of commercial poultry production (Blake et al. 2020). Coccidiosis causes intestinal leakage of plasma protein, malabsorption, villous atrophy, interruption of nutrient digestion, decreased weight gain (WG), reduced feed and water intake, increased feed conversion ratio (FCR), increased mortality rates, increased intestinal passage, increased susceptibility to other diseases and increased medication expenses (Williams 2005; Martins et al. 2022). Various chemical anticoccidials have been used for controlling coccidiosis and has proven to be successful over the past few decades (Felici et al. 2021). However, due to raised concerns regarding the safety of anticoccidials and their potential impact on animal health, human health, and the environment in recent years led researchers to propose and develop alternative agents.

Extensive research efforts in feed additives have been conducted in order to evaluate the effects of different products, including fungal extracts, organic acids, essential oils, medical plants, yeasts, probiotics and prebiotics, on productivity, immune response, animal health and controlling coccidiosis (Peek and Landman 2011). Among the different products, the cell walls of *Saccharomyces cerevisiae* have been widely used. The yeast cell walls (YCW) are rich in D-mannose, β-glucans, α-methyl-D-mannoside, polysaccharides mannanoligosacarides (MOS), and several other compounds. The YCW components, including MOS and β-glucans have immunomodulatory effects in many animal species, including poultry (Shanmugasundaram et al. 2013). The administration of YCW stimulates gut microflora by competitive exclusion of pathogens by competing for nutrients, antimicrobial metabolite production, or adhering to receptor sites that pathogens otherwise would occupy (Vandeplas et al. 2010). Thus, this study aimed to evaluate the efficacy of YCW products in reducing the severity of coccidiosis in broiler chickens.

MATERIAL AND METHODS

The experiment was approved by the Animal Ethics Committee of the College of Agricultural Engineering Sciences, Animal Production Department (approval No.: AEC120120201).

Animal husbandry

A total of 320 one-day-old mixed-sex Ross 308 chicks were randomly assigned to eight treatments, each with four replicate pens and ten birds per pen in the University of Duhok, College of Agricultural Engineering Sciences, Animal Production Department. Treatments were arranged in a 2 × 4 factorial challenge: with (+) or without (–); and feed additive in the diets: control, anticoccidial, yeast cell wall (YCW) 0.1% and YCW 0.2%. Floor pens were assigned to two rooms according to the challenge (16 pens per room) in the identical environmentally controlled facility. Environmental management, including lighting and temperature, was adjusted according to the Ross 308 guidelines (Aviagen 2018). Feed and water were provided *ad libitum* for each pen. During the trial period, starter diets were fed during days 0–10, grower diets between days 10–24, and finisher diets between days 24–35. On days 10, 24, and 35, chickens and leftover feed were weighed as pen-based and feed intake, WG, and FCR were assessed as the main performance parameters.

Dietary treatments

Experimental diets were formulated according to Ross 308 nutrient specifications (Table 1). The nutrients and broiler premix composition used in this study were identical to those used by Hussein et al. (2021). Treatments were arranged in a 2 × 4 factorial, challenge: with (+) or without (–); and feed additives: control diet (no additive), control diet supplemented with salinomycin at 60 mg/kg, control diet supplemented with 0.1% YCW and control diet supplemented with 0.2% YCW. Diets were formulated using the Concept 5 feed formulation program (Creative Formulation Concepts, LLC, Annapolis, MD, USA). The Alimaya CatchMyco premix (YCW, sepiolite and kaolinitic clays) was purchased from the Alimaya company, Neuillyen-Donjon, France. Salinomycin (Sacox 120) was purchased from

https://doi.org/10.17221/24/2023-CJAS

Table 1. Ingredient and nutrient composition of the basal starter, grower, and finisher diets as percentage

Ingredients	Starter	Grower	Finisher
Corn	53.1	56.9	61.7
Soybean meal	31.5	32.9	28.2
Fish meal	4.00	–	–
Vegetable oil	3.00	4.48	4.57
Limestone	2.00	1.39	1.35
Di-calcium phosphate	2.72	0.950	0.823
Salt	0.108	0.190	0.770
DL-Methionine	0.376	0.320	0.243
L-Lysine HCl	0.505	0.260	0.207
L-Threonine	0.252	0.130	0.086
Broiler premix[1]	2.50	2.50	2.50
Nutrient composition			
ME (MJ/kg)	12.55	13.18	13.40
Crude protein	23.0	21.0	19.16
Crude fiber	2.25	2.38	2.33
D. arginine	1.29	1.14	0.990
D. lysine	1.29	1.14	0.990
D. methionine cystein	0.870	0.840	0.730
D. tryptophan	0.226	0.236	0.210
D. isoleucine	0.868	0.805	0.727
D. threonine	0.820	0.730	0.630
D. valine	0.989	0.916	0.850
Calcium	1.60	0.900	0.850
Phosphorus available	0.844	0.450	0.420
Sodium	0.160	0.160	0.160
Chloride	0.350	0.312	0.230
Linoleic acid	2.18	2.64	2.73

D. = digestible; ME = metabolisable energy

[1]The broiler premix contained per kg: arginine, 2.2 g; valine, 1.4 g; threonine, 58.9 g; tryptophan, 0.4 g; methionine and cystein, 113.9 g; methionine, 113.5 g; lysine, 113.2 g; choline, 10 411.2 mg; choline chloride, 12 000 mg; D-pantothenate, 600 mg; calcium-D-pantothenate, 600 mg; vitamin K_3, 100 mg; folic acid, 40 mg; niacin, 1 600 mg; vitamin B_{12}, 1 400 mcg; biotin, 4 mg; vitamin B_6, 160 mg; vitamin B_2, 280 mg; vitamin B_1, 120 mg; vitamin E, 1 200 mg; vitamin D_3, 160 000 IU; vitamin A, 400 000 IU; Se, 10 mg; I, 40 mg; Fe, 2.0 g; Zn, 2.4 g; Mn, 3.2 g; Cu, 0.4 g; chloride, 64.0 g; sodium, 50.0 g; available phosphorus, 121.3 g; calcium, 62.0 g; mycotoxin binder, 40 g; citric acid (E330), 0.2 g; propyl gallate (E310), 0.112 g; BHT (E321), 1.34 g; endo-1,4-beta-xylanase, 10 800 IU activity; endo-1,3(4)-beta-glucanase, 2 800 IU activity (Hussein et al. 2021)

Huvepharma Canada Corporation Inc. (Ottawa, Ontario, Canada).

Coccidial challenge

Coccidia oocysts were isolated from fresh, blood-stained caecal droppings of broiler chickens in the animal production department farm, College of Agricultural Engineering Science, University of Duhok. The coccidial inoculant was prepared at the University of Duhok, College of Veterinary Medicine (Hussein et al. 2021). The sample was placed in a 2 ml microfuge tube with saline and centrifuged at 6 000× *g* for 5 min before the supernatant was discarded. Oocysts were allowed to sporulate in a 2.5% (w/v) potassium dichromate solution for three days at 27 °C. On day 8, challenged birds inoculated with 5 000 *E. tenella* sporulated oocysts in 1 ml 1% (w/v) of sterile saline by crop needle. Non-challenged birds received 1 ml of 1% (w/v) saline.

Coccidial oocysts in excreta

Droppings from positive and negative controls were gathered over a 24-h period on days 7, 8, 9, 10, and 11 after inoculation. A light microscope was employed to test oocysts in the excreta samples.

Sample collection

On day 24, two birds from each pen were randomly selected, weighed, and euthanised by cervical dislocation. For serum biochemical assays, 5 ml of clotted blood was centrifuged at 3 000 rpm for 5 min, and the serum was stored at –20 °C. For the morphometric study, approximately 1 cm of the jejunum tissue and the whole bursa of Fabricius for each chicken were collected. Jejunum tissues were gently flushed and cleaned with phosphate buffered saline (pH 7). The jejunum and bursa of Fabricius samples were fixed in 10% neutral buffered formaldehyde for histomorphological analysis.

Measurements and analyses

Jejunum and bursa of Fabricius histomorphology. Fixed samples were dehydrated, cleaned, and embed-

https://doi.org/10.17221/24/2023-CJAS

ded in paraffin wax. Using a microtome (Thermo Scientific, Rockville, MD, USA), consecutive longitudinal sections (7 µm) were cut, individually mounted on Superfrost® slides, and stained with Haematoxylin and Eosin (M'Sadeq et al. 2015). Light microscopy (Olympus CX41 microscope; 10× objective, Tokio, Japan) and colour video camera (DinoCapture v2.0, ANMO Electronics Corporation, Taiwan) were used to analyse the sections. The height of 10 villi (VH) and the depth of 10 crypts (CD) were determined in each replicate. From VH and CD means, villous height to crypt depth ratio (VH : CD) was measured. The villous surface area, villous tip width and villous base width were measured according to Iji et al. (2001). The width of 10 bursal follicles per replicate was measured with DinoCapture software by tracing around the selected follicle.

Serum biochemical analyses. At day 24 of age, prepared serum was used for determination of cholesterol, globulin, protein, alanine transaminase (ALT) and aspartate aminotransferase (AST) by automatic Cobas Integra 400 Plus analyser and Cedex Bio HT analyser (Roche Diagnostics Deutschland GmbH Manheim, Germany).

Statistical analysis. Statistical significance was assessed using the SAS statistical software v9.3 (Proc GLM; SAS Inst., Cary, NC, USA). Duncan's multiple range test was used when significant effects were observed ($P < 0.05$). The difference between the means was declared significant at $P < 0.05$ or highly significant at $P < 0.01$ or $P < 0.001$.

Table 2. Broiler chickens' performance fed different diets from day 0 to day 24

Treatment means	Feed intake (g/bird)	Weight gain (g/bird)	Feed/gain ratio
No challenge none	1 369.0^{c}	982.3^{b}	1.39^{b}
No challenge SM	1 432.5^{ab}	1 057.0^{ab}	1.36^{bc}
No challenge YCW 0.1%	1 420.0^{b}	1 114.8^{a}	1.28^{cd}
No challenge YCW 0.2%	1 404.0^{bc}	1 120.0^{a}	1.256^{d}
Challenge none	1 466.8^{a}	881.8^{c}	1.67^{a}
Challenge SM	1 417.5^{b}	1 053.8^{ab}	1.35^{bc}
Challenge YCW 0.1%	1 440.3^{ab}	1 053.8^{ab}	1.27^{b}
Challenge YCW 0.2%	1 372.0^{c}	10 163.8^{b}	1.35^{bc}
Pooled SEM	7.22	15.2	0.023
Main effects			
Challenge			
None	1 418.8	1097.3^{a}	1.30^{b}
Eimeria	1 409.9	1047.5^{b}	1.356^{a}
Additive			
None	1 417.8	932.0^{b}	1.53^{a}
SM	1 425.0	1 055.4^{a}	1.35^{b}
YCW 0.1%	1 430.1	1 084.1^{a}	1.32^{b}
YCW 0.2%	1 388.0	1 068.4^{a}	1.30^{b}
***P*-value**			
Challenge	0.495	0.010	0.019
Additive	0.162	0.001	0.001
Challenge × additive	0.001	0.001	0.001

None = no additive; SM = salinomycin; YCW = yeast cell wall (Alimaya CatchMyco; Alimaya, Neuillyen-Donjon, France)
$^{a–d}$Means sharing the same superscripts are not significantly different from each other at ($P < 0.05$)

RESULTS

Broiler performance

On day 10, no significant differences were observed among treatments. However, from days 0–24, the impact of the challenge was obvious (Table 2). The WG and FCR in challenged birds were significantly poorer than in unchallenged birds. The additives, anticoccidial, 0.1% YCW and 0.2% YCW, significantly improved WG and FCR when compared to birds fed a control diet.

Interaction of challenge × additive was noted for feed intake, WG and FCR. Challenged birds fed the control diet had higher feed intake relative to challenged birds fed anticoccidial and 0.2% YCW and non-challenged birds fed the control diet, and YCW ($P = 0.001$). The challenged birds fed the control diet had significantly lower WG and poorer FCR compared to challenged and non-challenged treatments with additives.

From days 0–35, the effects of challenge and feed additives on performance were markedly different (Table 3). Challenged birds significantly reduced their feed intake and WG and had an increased FCR compared to non-challenged birds. Feed additives resulted in an increased WG ($P = 0.01$) and an improved FCR ($P = 0.004$) over the control group.

Interaction of challenge × additive was observed for body weight gain and FCR. All challenged and non-challenged birds which received anticoccidial and YCW had significantly improved WG and FCR over challenged control. The WG and FCR of challenged birds fed YCW 0.1%, and 0.2% were not different than in challenged and non-challenged birds fed anticoccidial.

https://doi.org/10.17221/24/2023-CJAS

Table 3. Broiler chickens' performance fed different diets from day 0 to day 35

Treatment means	Feed intake (g/bird)	Weight gain (g/bird)	Feed/gain ratio
No challenge none	2 794.3	1 894.8^{b}	1.48^{b}
No challenge SM	2 829.3	1 957.0^{ab}	1.45^{b}
No challenge YCW 0.1%	2 888.2	1 966.3^{ab}	1.47^{b}
No challenge YCW 0.2%	2 940.8	2 002.0^{a}	1.47^{b}
Challenge none	2 859.0	1 698.0^{c}	1.69^{a}
Challenge SM	2 783.8	1 902.8^{b}	1.46^{b}
Challenge YCW 0.1%	2 806.5	1 893.5^{b}	1.48^{b}
Challenge YCW 0.2%	2 825.5	1 874.3^{b}	1.50^{b}
Pooled SEM	15.5	18.1	0.014
Main effects			
Challenge			
None	2 886.1^{a}	1 975.1^{a}	1.46^{b}
Eimeria	2 801.9^{b}	1 890.2^{b}	1.48^{a}
Additive			
None	2 826.6	1 796.4^{b}	1.58^{a}
SM	2 806.4	1 929.9^{a}	1.46^{b}
YCW 0.1%	2 847.4	1 929.9^{a}	1.48^{b}
YCW 0.2%	2 878.1	1 938.1^{a}	1.49^{b}
***P*-value**			
Challenge	0.014	0.001	0.035
Additive	0.423	0.010	0.004
Challenge × additive	0.154	0.001	0.001

None = no additive; SM = salinomycin; YCW = yeast cell wall (Alimaya CatchMyco; Alimaya, Neuillyen-Donjon, France)
$^{a–c}$Means sharing the same superscripts are not significantly different from each other at ($P < 0.05$)

Jejunal histomorphology

The morphology samples from jejunum were studied after the coccidial challenge, and the results are presented in Table 4. The VH and VH : CD ratios of challenged birds were significantly lower than in non-challenged birds. On the other hand, the challenged birds had higher CD ($P = 0.001$), villous tip width ($P = 0.01$), villous base width ($P = 0.004$), villous surface area ($P = 0.04$) and muscle thickness ($P = 0.05$) than non-challenged birds. Both additives significantly increased VH compared to the control group. Birds fed 0.1% and 0.2% YCW had lower CD and higher VH : CD ratio when compared to anticoccidial and control groups ($P = 0.001$). The birds in the control group had significantly increased villous tip width in comparison to the additives. However, birds fed 0.1% and 0.2% YCW had significantly decreased both villous base width and villous surface area when compared to the control group. Birds fed 0.1% YCW had significantly decreased jejunal muscle thickness relative to anticoccidial and control groups.

Factor interactions were observed for VH, CD, VH : CD ratio, villous tip and base width, villous surface area and muscle thickness. Challenged birds fed the control diet had significantly lower VH when compared to all treatments. The higher CD was recorded for non-challenged birds fed a control diet. Challenged birds fed 0.1% and 0.2% YCW were not different from non-challenged birds from the control, anticoccidial and YCW treatments and had a significantly lower CD than challenged birds fed the anticoccidial. The challenged birds fed YCW had significantly higher VH : CD compared to challenged birds fed both anticoccidial and control diets and were not different from non-challenged treatments. The villous tip width of non-challenged birds fed the control diet was higher than in challenged and non-challenged treatments. The villous base width of challenged birds fed YCW was lower than in challenged birds fed anticoccidial ($P = 0.001$). Challenged birds fed the control diet had a significantly increased villous surface area when compared to all challenged and non-challenged treatments, except the birds fed anticoccidial. Under challenge conditions, birds which received the control diet had increased muscle thickness ($P = 0.001$) with respect to the challenged and non-challenged treatments. The muscle thickness of non-challenged birds fed YCW was decreased compared to non-challenged birds fed anticoccidial.

Histomorphology of bursa of Fabricius

The histomorphology of the bursa of Fabricius was studied after the coccidial challenge at day 24, and the data are presented in Table 5. Challenged birds had significantly higher follicle length than non-challenged birds. The follicle length and follicle area of birds fed 0.1% YCW ($P = 0.036$) and 0.2% YCW ($P = 0.02$) were consistently lower than in the control group.

Significant interactions were observed for follicle length and follicle area. Challenged control birds showed higher ($P = 0.001$) follicle length in comparison to all challenged and non-challenged treatments. The follicle length of challenged birds

https://doi.org/10.17221/24/2023-CJAS

Table 4. Jejunal morphology of broiler chickens fed different diets (day 24)

Treatment means	Villi height (µm)	Crypt depth (µm)	VH:CD	Width villus tip (µm)	Width villus base (µm)	villus surface area (mm^2)	Jejunal muscle thickness (µm)
No challenge none	944.8^{c}	183.3^{c}	5.55^{a}	240.5^{a}	247.3^{a}	0.230^{a}	204.5^{d}
No challenge SM	1 108.6^{a}	210.8^{c}	5.43^{a}	162.8^{b}	168.9^{b}	0.185^{abc}	265.3^{b}
No challenge YCW 0.1%	1 058.9^{ab}	166.5^{c}	6.49^{a}	141.8^{b}	177.1^{ab}	0.169^{bc}	187.9^{d}
No challenge YCW 0.2%	1 028.7^{ab}	182.9^{c}	6.17^{a}	132.7^{b}	142.4^{b}	0.143^{c}	214.2^{cd}
Challenge none	761.9^{d}	402.4^{a}	2.00^{c}	178.1^{b}	207.3^{ab}	0.146^{c}	330.3^{a}
Challenge SM	1 007.7^{bc}	310.9^{b}	3.82^{b}	182.4^{b}	242.3^{a}	0.211^{ab}	262.1^{cb}
Challenge YCW 0.1%	1 037.6^{ab}	187.8^{c}	5.88^{a}	160.7^{b}	159.3^{b}	0.165^{bc}	236.7^{cbd}
Challenge YCW 0.2%	1 013.9^{bc}	191.6^{c}	5.45^{a}	164.8^{b}	155.9^{b}	0.163^{bc}	213.8^{cd}
Pooled SEM	9.49	7.72	0.170	5.88	7.23	0.060	5.29
Main effects							
Challenge							
None	1 067.5^{a}	188.9^{b}	5.98^{a}	146.7^{b}	162.3^{b}	0.146^{b}	226.1^{b}
Eimeria	1 017.7^{b}	255.9^{a}	4.66^{b}	173.5^{a}	205.7^{a}	0.191^{a}	248.1^{a}
Additive							
None	888.5^{b}	250.7^{a}	4.43^{b}	221.3^{a}	235.0^{a}	0.204^{a}	243.2^{ab}
SM	1 064.2^{a}	254.8^{a}	4.72^{b}	171.4^{b}	201.2^{ab}	0.196^{ab}	263.9^{a}
YCW 0.1%	1 051.1^{a}	172.1^{b}	6.28^{a}	148.7^{b}	170.6^{bc}	0.168^{cb}	205.7^{c}
YCW 0.2%	1 026.3^{a}	184.4^{b}	6.05^{a}	138.1^{b}	144.6^{c}	0.146^{c}	214.1^{bc}
***P*-value**							
Challenge	0.007	0.001	0.001	0.010	0.004	0.040	0.054
Additive	0.001	0.001	0.001	0.001	0.001	0.002	0.001
Challenge × additive	0.001	0.001	0.001	0.002	0.001	0.002	0.001

None = no additive; SM = salinomycin; VH:CD = villous height to crypt depth ratio; YCW = yeast cell wall (Alimaya Catch-Myco; Alimaya, Neuillyen-Donjon, France)

$^{a–d}$Means sharing the same superscripts are not significantly different from each other at ($P < 0.05$)

fed 0.1% and 0.2 YCW was not significantly different compared to non-challenged treatments. The Follicle area of challenged control birds was significantly increased compared to all other treatments (challenged and non-challenged).

Serum biochemistry

Serum biochemical results are presented in Table 6. Challenged birds had significantly increased ALT and AST in comparison to non-challenged birds. Birds which received 0.2% YCW had an increased ($P = 0.05$) globulin concentration when compared to birds with 0.1% YCW.

Significant interactions were observed for cholesterol, ALT, AST, and total protein. The highest cholesterol concentrations were recorded for challenged control birds. Challenged YCW birds were not significantly different when compared to all non-challenged treatments. Challenged control birds had significantly increased ALT and AST concentrations compared to all non-challenged birds. However, challenged birds which received YCW had a reduced ($P = 0.001$) serum AST concentration compared to challenged control birds. Moreover, challenged birds which received 0.1% YCW had a significantly reduced serum ATL concentration when compared to the challenged control. Non-challenged birds, which received 0.2% YCW, had significantly increased total protein when compared to non-challenged anticoccidial and 0.1% YCW treatments.

DISCUSSION

Coccidiosis is still the main problem in poultry production, with great economic losses, causing gastrointestinal tract damage, shedding oocysts to the surrounding environment and performance reduction, including WG, FCR, livability and feed intake (Martins et al. 2022). The coccidiosis challenge

https://doi.org/10.17221/24/2023-CJAS

Table 5. Bursa of Fabricius histomorphology of broiler chickens fed different diets (day 24)

Treatment means	Follicle length (mm)	Follicle width (mm)	Follicle area (mm^2)
No challenge none	5.02^{bc}	2.99	12.9^{b}
No challenge SM	4.56^{c}	3.02	11.6^{b}
No challenge YCW 0.1%	4.52^{c}	2.94	10.9^{b}
No challenge YCW 0.2%	4.50^{c}	2.95	10.6^{b}
Challenge none	6.58^{a}	3.99	21.5^{a}
Challenge SM	5.61^{b}	3.60	16.5^{b}
Challenge YCW 0.1%	4.88^{bc}	2.82	11.1^{b}
Challenge YCW 0.2%	4.98^{bc}	2.71	11.0^{b}
Pooled SEM	0.155	0.117	0.860
Main effects			
Challenge			
None	4.53^{b}	2.97	11.1
Eimeria	5.16^{a}	3.04	12.9
Additive			
None	5.80^{a}	3.490	17.2^{a}
SM	5.09^{ab}	3.312	14.1^{ab}
YCW 0.1%	4.74^{b}	2.879	11.0^{b}
YCW 0.2%	4.70^{b}	2.831	10.8^{b}
***P*-value**			
Challenge	0.024	0.663	0.066
Additive	0.036	0.120	0.020
Challenge × additive	0.001	0.065	0.002

None = no additive; SM = salinomycin; YCW = yeast cell wall (Alimaya CatchMyco; Alimaya, Neuillyen-Donjon, France)
$^{a–c}$Means sharing the same superscripts are not significantly different from each other at ($P < 0.05$)

of this study was successfully induced, as shown by the depression of FCR, and WG, bloody diarrhoea and increased *Eimeria* oocysts output. After *Eimeria tenella* inoculation, the output of oocysts in the challenged control group was significantly increased on day 7 and then gradually decreased till day 14. As a potential solution to this problem, the efficacy of YCW was assessed for their capability to reduce the severity of experimentally challenged coccidiosis in broiler chickens.

Using YCW from *Saccharomyces cerevisiae* yeast in broiler chicken diet has increased in the last decade. This experiment showed that the birds fed YCW and anticoccidial, respectively, had significantly increased WG and decreased FCR when compared to the control diet at days 24 and 35. Similar results have been reported by Pascual et al. (2020), who showed that dietary supplementation of YCW improved FCR at day 44. Gomez-Verduzco et al. (2009) suggested that dietary supplementation of YCW in broiler diets significantly improved WG and FCR under natural exposure to *Eimeria* spp. This was not the case in previous studies in which YCW showed no positive effect on both WG and FCR of broiler chickens (Shanmugasundaram et al. 2013; Sedghi et al. 2022). The improvement of performance observed in birds fed different levels of YCW, either challenged or non-challenged, in the current study could be well explained as a result of a reduced *Eimeria* infection and subsequent improvements in gut health, which contributed to improved digestion and absorption of nutrients. Gomez-Verduzco et al. (2009) demonstrated that YCW has the ability to modulate the immune system by increasing humoral and cellular immune systems, reducing parasite excretion in infected animals and increasing mucosal IgA secretions. Reports indicated that YWC enhances macrophage response in animals (Che et al. 2012). Immune cell activation produces anti-inflammation cytokine expressions, for example, IL-10 and IL-1, which are produced by active macrophages. It has been reported that inflammatory cytokine IL-1 and macrophage nitric oxide production increased in broiler chickens fed YCW, which can be expected to reduce the pathogenesis of infection (Shanmugasundaram et al. 2013; Sedghi et al. 2022). Furthermore, YCW supplementation has a trophic effect on gut health. The results of this study showed YCW improved jejunal histomorphology. Gut health has major effects on digestion, absorption and metabolism of nutrients, which may positively improve performance.

This study also demonstrated that the coccidiosis challenge caused dramatic changes in jejunal histomorphology. After coccidiosis inoculation, challenged birds had significantly decreased VH and VH : CD and increased CD depth, villous base and tip width, muscle thickness and villous surface area compared with non-challenged birds. This was in agreement with the findings of Hussein et al. (2021), who also reported that coccidial challenge reduced VH, VH : CD and increased CD ratio villous base and tip width, and jejunum muscle thickness. The higher villous base and tip width caused increased surface area and villous abnormalities. The challenged birds fed YCW had increased VH, reduced CD and increased VH : CD ratio compared to the challenged control with no additives. This was in accordance

https://doi.org/10.17221/24/2023-CJAS

Table 6. Serum biochemistry of broiler chickens fed different diets (day 24)

Treatment means	Cholesterol (mmol/l)	ALT (IU/l)	AST (IU/l)	Protein (g/l)	Globulin (g/l)
No challenge none	91.3^{bc}	2.43^{c}	158.3^{c}	2.53^{ab}	1.48
No challenge SM	86.0^{c}	2.30^{c}	135.8^{c}	1.80^{b}	1.09
No challenge YCW 0.1%	92.3^{bc}	2.48^{c}	142.8^{c}	1.96^{b}	1.21
No challenge YCW 0.2%	110.5^{bc}	2.38^{c}	13.5^{c}	3.02^{a}	1.86
Challenge none	141.3^{a}	4.70^{a}	244.3^{a}	2.35^{ab}	1.34
Challenge SM	119.8^{ab}	3.80^{ab}	212.8^{ab}	2.57^{ab}	1.51
Challenge YCW 0.1%	103.5^{bc}	2.55^{bc}	147.3^{c}	1.99^{b}	1.12
Challenge YCW 0.2%	104.0^{bc}	3.80^{ab}	179.3^{bc}	2.41^{ab}	1.52
Pooled SEM	4.10	0.199	8.21	0.103	0.072
Main effects					
Challenge					
None	96.3	2.38^{b}	138.0^{b}	2.26	1.38
Eimeria	109.1	3.38^{a}	179.8^{a}	2.33	1.38
Additive					
None	116.3	3.56	201.3	2.44	1.41^{ab}
SM	102.8	3.05	174.3	2.18	1.30^{ab}
YCW 0.1%	97.9	2.51	145.0	1.98	1.16^{b}
YCW 0.2%	107.3	3.09	157.4	2.72	1.69^{a}
***P*-value**					
Challenge	0.072	0.01	0.004	0.800	0.900
Additive	0.455	0.335	0.078	0.056	0.051
Challenge × additive	0.004	0.001	0.001	0.042	0.104

ALT = alanine transaminase; AST = aspartate aminotransferase; None = no additive; SM = salinomycin; YCW = yeast cell wall (Alimaya CatchMyco; Alimaya, Neuillyen-Donjon, France)

$^{a–c}$Means sharing the same superscripts are not significantly different from each other at ($P < 0.05$)

with the findings of Sedghi et al. (2022), who showed that YCW improved VH, CD and VH : CD ratio in broiler chickens. Alkhulaifi et al. (2022) noticed that the birds fed YCW had increased VH and better intestinal health under the *C. perfringens* challenge. The MOS or β-glucan as components of YCW could reduce colonising and adhering of pathogens in the intestine, enhancing intestine goblet cells account, thus improving intestinal morphology and growth performance (Agazzi et al. 2020).

Eimeria challenged birds had increased bursa of Fabricius follicles length and area when compared to non-challenged birds. This was in agreement with the findings of Hussein et al. (2021) and Beski (2023), who reported a negative effect of coccidiosis on bursal histomorphology. The bursa of Fabricius plays an important role in protecting poultry from infections with pathogenic microorganisms (Zhou et al. 2015). Coccidiosis, *E. tenella* infection, in chickens induces obvious harmful morphological changes in the bursa of Fabricius, which is a prolongation of the gastrointestinal tract. *E. tenella* infection also reduces resistance and antioxidant capability in poultry (Georgieva et al. 2006). The different stages of *Eimeria* life cycle cause influential inflammatory changes leading to fluid accumulation and subsequently cause enlargement of infected tissues, which could be an identification of early activation of the cellular immune system against pathogens (Nahed et al. 2022). However, in the current study, challenged birds fed YCW were able to eliminate the effect of coccidiosis on the bursa of Fabricius, in particular, on the follicle length and area. This result could be due to the efficacy of YCW in reducing coccidial infection. It was reported that feeding YCW under coccidiosis infection increased macrophage nitric oxide production, decreased faecal output of oocysts, reduced mRNA IL-10 content and increased IL-1 mRNA content (Shanmugasundaram et al. 2013).

https://doi.org/10.17221/24/2023-CJAS

Coccidial challenge caused dramatic changes in the serum concentrations of biochemical parameters. Challenged birds had an increased serum concentration of ALT and AST compared to non-challenged birds. This observation was in agreement with Hussein et al. (2021), who also reported that a coccidial challenge increased the serum concentration of AST and ALT after a coccidiosis challenge. Beski (2023) noticed that the challenged birds had significantly increased serum AST and ALT concentrations when compared to non-challenged birds. The higher ALT and AST serum concentrations in challenged birds could be due to possible liver damage. The generation of free radicals in different stages of coccidiosis could be a reason for liver damage (Srivastava et al. 2012). The ALT and AST enzymes are released immediately into the serum after hepatocellular damage, which is an indication of acute liver failure and inflammation (Senanayake et al. 2015). Hesabi Nameghi et al. (2019) reported that the birds infected with coccidiosis showed liver and epithelial lining of gut injuries. Additionally, intestinal mucosal damage and haemorrhage cause malabsorption of protein and other nutrients which increases protein catabolism in muscles, muscle degradation and AST and ALT levels (Williams 2005; El-Shazly et al. 2020). The results of this experiment also demonstrated that the challenged birds fed YCW had a significantly reduced serum concentration of ALT and AST. Similar results were reported by Hussein et al. (2021) and Kudupoje et al. (2022). In general, YCW as prebiotics prevents the colonisation of pathogenic bacteria in the gastrointestinal tract, lowering gut pH, stimulating the immune system and increasing beneficial bacteria in the gut (M'Sadeq et al. 2015). Furthermore, improved beneficial microflora in the gut could also inhibit the absorption of toxins and thus help to prevent liver disease and to decrease the levels of serum AST and ALT (He et al. 2019).

CONCLUSION

It can be concluded that coccidial challenge decreased broiler chickens' performance, VH, VH : CD ratio and increased jejunum CD, bursa of Fabricius follicle length, serum ALT, and serum AST. Supplementation of YCW was effective in mitigating a performance decline resulting from this challenge. The data also demonstrated that YCW improved gut integrity by increasing VH and VH : CD ratio and decreasing CD. Birds fed YCW had a reduced bursa of Fabricius follicle length compared to the control group. Challenged birds fed YCW had reduced ALT and AST concentrations in serum when compared to the challenged control. YCW appeared to be a valuable feed additive for controlling coccidiosis.

Conflict of interest

The author declares no conflict of interest.

REFERENCES

Agazzi A, Perricone V, Omodei Zorini F, Sandrini S, Mariani E, Jiang XR, Ferrari A, Crestani M, Nguyen TX, Bontempo V, Domeneghini C, Savoini G. Dietary mannan oligosaccharides modulate gut inflammatory response and improve duodenal villi height in post-weaning piglets improving feed efficiency. Animals. 2020 Jul 28;10(8): 14 p.

Alkhulaifi MM, Alqhtani AH, Alharthi AS, Al Sulaiman AR, Abudabos AM. Influence of prebiotic yeast cell wall extracts on growth performance, carcase attributes, biochemical metabolites, and intestinal morphology and bacteriology of broiler chickens challenged with Salmonella typhimurium and Clostridium perfringens. Ital J Anim Sci. 2022 Dec 31;21(1):1190-9.

Aviagen. Ross 308: Ross broilers management handbook. Huntsville: Aviagen Inc; 2018. 147 p. Available from: http://eu.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf

Beski SSM. Anticoccidial effects of dietary chamomile against experimentally induced coccidiosis in broiler chicken. Czech J Anim Sci. 2023 Jan 19;68(1):30-43.

Blake DP, Knox J, Dehaeck B, Huntington B, Rathinam T, Ravipati V, Ayoade S, Gilbert W, Adebambo AO, Jatau ID, Raman M, Parker D, Rushton J, Tomley FM. Re-calculating the cost of coccidiosis in chickens. Vet Res. 2020 Sep 14;51(1): 14 p.

Che T, Johnson R, Kelley K, Dawson K, Moran C, Pettigrew J. Effects of mannan oligosaccharide on cytokine secretions by porcine alveolar macrophages and serum cytokine concentrations in nursery pigs. J Anim Sci. 2012 Feb 1;90(2):657-68.

El-Shazly KA, El-Latif AA, Abdo W, El-Morsey A, El-Aziz MIA, El-Mogazy H. The anticoccidial activity of the fluoroquinolone lomefloxacin against experimental Eimeria tenella infection in broiler chickens. Parasitol Res. 2020 Jun;119(6):1955-68.

https://doi.org/10.17221/24/2023-CJAS

Felici M, Tugnoli B, Piva A, Grilli E. In vitro assessment of anticoccidials: Methods and molecules. Animals. 2021 Jun 30;11(7): 19 p.

Georgieva NV, Koinarski V, Gadjeva V. Antioxidant status during the course of Eimeria tenella infection in broiler chickens. Vet J. 2006 Nov 1;172(3):488-92.

Gomez-Verduzco G, Cortes-Cuevas A, Lopez-Coello C, Avila-Gonzalez E, Nava GM. Dietary supplementation of mannan-oligosaccharide enhances neonatal immune responses in chickens during natural exposure to Eimeria spp. Acta Vet Scand. 2009 Mar 19;51(1):1-7.

He T, Long S, Mahfuz S, Wu D, Wang X, Wei X, Piao X. Effects of probiotics as antibiotics substitutes on growth performance, serum biochemical parameters, intestinal morphology, and barrier function of broilers. Animals. 2019 Nov 18;9(11): 10 p.

Hesabi Nameghi A, Edalatian O, Bakhshalinejad R. Effects of a blend of thyme, peppermint and eucalyptus essential oils on growth performance, serum lipid and hepatic enzyme indices, immune response and ileal morphology and microflora in broilers. J Anim Physiol Anim Nutr. 2019 Sep;103(5):1388-98.

Hussein SM, M'Sadeq SA, Beski SSM, Mahmood AL, Frankel TL. Different combinations of peppermint, chamomile and a yeast prebiotic have different impacts on production and severity of intestinal and bursal abnormalities of broilers challenged with coccidiosis. Ital J Anim Sci. 2021 Jun 15;20(1):1924-34.

Iji PA, Saki A, Tivey DR. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br Poult Sci. 2001 Sep;42(4):505-13.

Kudupoje MB, Malathi V, Yiannikouris A. Impact of a natural fusarial multi-mycotoxin challenge on broiler chickens and mitigation properties provided by a yeast cell wall extract and a postbiotic yeast cell wall-based blend. Toxins. 2022 Apr 28;14(5): 26 p.

M'Sadeq SA, Wu S, Swick RA, Choct M. Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide. Anim Nutr. 2015 Mar 1; 1(1):1-11.

Martins RR, Silva LJG, Pereira A, Esteves A, Duarte SC, Pena A. Coccidiostats and poultry: A comprehensive review and current legislation. Foods. 2022 Sep 7;11(18): 20 p.

Nahed A, Abd El-Hack ME, Albaqami NM, Khafaga AF, Taha AE, Swelum AA, El-Saadony MT, Salem HM, El-Tahan AM, AbuQamar SF. Phytochemical control of poultry coccidiosis: A review. Poult Sci. 2022 Jan 1; 101(1): 22 p.

Pascual A, Pauletto M, Giantin M, Radaelli G, Ballarin C, Birolo M, Zomeno C, Dacasto M, Bortoletti M, Vascellari M, Xiccato G, Trocino A. Effect of dietary supplementation with yeast cell wall extracts on performance and gut response in broiler chickens. J Anim Sci Biotechnol. 2020 May 1;11: 11 p.

Peek HW, Landman WJ. Coccidiosis in poultry: Anticoccidial products, vaccines and other prevention strategies. Vet Q. 2011 Sep 1;31(3):143-61.

Qaid MM, Mansour L, Al-Garadi MA, Alqhtani AH, Al-abdullatif AA, Qasem MA, Murshed MA. Evaluation of the anticoccidial effect of traditional medicinal plants, Cinnamomum verum bark and Rumex nervosus leaves in experimentally infected broiler chickens with Eimeria tenella. Ital J Anim Sci. 2022 Dec 31;21(1):408-21.

Sedghi M, Mohammadi I, Sarrami Z, Ghasemi R, Azarfar A. Effects of a yeast cell wall product on the performance of broiler chickens and PGC-1α, TLR4, IL-10 and PPARγ genes expression. Ital J Anim Sci. 2022 Dec 31; 21(1):263-78.

Senanayake S, Ranasinghe J, Waduge R, Nizanantha K, Alexander P. Changes in the serum enzyme levels and liver lesions of broiler birds reared under different management conditions. Troo Agric Res. 2015 Nov 20; 26(4):584-95.

Shanmugasundaram R, Sifri M, Selvaraj RK. Effect of yeast cell product (CitriStim) supplementation on broiler performance and intestinal immune cell parameters during an experimental coccidial infection. Poult Sci. 2013 Feb 1; 92(2):358-63.

Srivastava P, Yadav N, Lella R, Schneider A, Jones A, Marlowe T, Lovett G, O'Loughlin K, Minderman H, Gogada R, Chandra D. Neem oil limonoids induces p53-independent apoptosis and autophagy. Carcinogenesis. 2012 Nov 1;33(11):2199-207.

Vandeplas S, Dubois Dauphin R, Beckers Y, Thonart P, Thewis A. Salmonella in chicken: Current and developing strategies to reduce contamination at farm level. J Food Prot. 2010 Apr;73(4):774-85.

Williams RB. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 2005 Jun 1; 34(3):159-80.

Yegani M, Korver DR. Factors affecting intestinal health in poultry. Poult Sci. 2008 Oct 1;87(10):2052-63.

Zhou BH, Liu LL, Liu J, Yuan FW, Tian EJ, Wang HW. Effect of diclazuril on the bursa of Fabricius morphology and SIgA expression in chickens infected with Eimeria tenella. Korean J Parasitol. 2015 Dec;53(6):675-82.

Received: February 14, 2023
Accepted: July 11, 2023
Published online: August 9, 2023