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Saccharomyces Cerevisiae Cell Wall Dietary Supplementation on the Performance and Intestinal Mucosa Development and Integrity of Broiler Chickens Vaccinated Against Coccidiosis

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■**Keywords**

Coccidiosis, vaccine, broiler, intestinal mucosa, morphometrics, Saccharomyces cerevisiae.

ABSTRACT

This study was carried out to verify if *Saccharomyces cerevisiae* cell wall (SCCW) dietary supplementation (0.2%) was capable of protecting the intestinal mucosa of broiler chickens vaccinated against coccidiosis. Body weight gain, feed intake, feed conversion and intestinal mucosa morphometric parameters and epithelial loss were evaluated. In the experiment,400 day-old male chicks were distributed according to a completely randomized design in a 2x2 factorial arrangement. The following treatments were applied: T1 - no vaccination/ no SCCW supplementation; T2 – no vaccination/SCCW supplementation; T3 – vaccination/no SCCW supplementation; and T4 – vaccination/SCCW supplementation to four replicates of 25 birds each. Birds were vaccinated on the first day of age using a spray vaccine (Coccivac B®, Coopers), containing E. acervulina, E. maxima, E. mivati and E. tenella. S. cerevisiae cell wall was supplied from the first day of age. Live performance, intestinal morphometric parameters and epithelial loss were evaluated at 14, 21 and 28 days of age. Performance was affected by vaccination only at 21-days of age, when body weight gain was reduced in the vaccinated birds, but no body weight difference was observed on day 28. Vaccine also increased the crypt depth ($p<0.05$) in the duodenum and jejunum, suggesting a high cell activity in the crypt:villus transition area to maintain the epithelial cell turnover. Villi number/area (103,269 μm^2) was not affected ($p>0.05$) by vaccine or cell wall supplementation, and epithelial loss was more pronounced in the duodenum and jejunum. In conclusion, the findings of this study suggest that S. cerevisiae cell wall supplementation may be an useful management tool to maintain the intestinal integrity of broilers vaccinated against coccidiosis.

INTRODUCTION

Coccidiosis in one of the most expensive diseases affecting the poultry industry, as treatment and prophylaxis costs are high and it also may severely impair flock performance. Coccidiosis causes intestinal lesions, affecting nutrient digestion and absorption (Maiorka *et al.*, 2002) and therefore, poor feed utilization adversely affecting growth performance and flock uniformity.

The use of antibiotics is banned in many countries and vaccines are the most interesting alternative tools to control coccidiosis (Vermeulen *et al.*, 2001). Vaccination eliminates the need of feed withdrawal times, as it is the case when anticoccidial drugs are utilized. However, the vaccine may cause intestinal mucosa lesions, since the principle of vaccination is based on the multiplication of oocysts in the mucosa and the development of natural immunity against Eimeria (Long *et al.*, 1986).

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It has been shown that nutrients or additives may stimulate intestinal mucosa epithelial growth (Maiorka *et al.*, 2002). Most of these substances (e.g., glutamine), when added to the diet, increase villus height and crypt depth, clearly indicating high mitotic activity (Maiorka *et al.*, 2000; Murakami *et al.*, 2007; Soltan, 2009). Other substances acting in the intestinal lumen may also improve bird performance possibly by reducing pathogenic bacteria numbers in the gut microbiota. For instance, mannan oligosacharides (MOS), which are derived from Saccharomyces cerevisiae outer cell wall, improve broiler performance at an early age (Tucker el al., 2003; Yang *et al.*, 2005), but does not show any clear positive effect on the intestinal mucosa morphology and enzyme activities at later ages (Yang *et al.*, 2007). Santin *et al.* (2001) previously reported an increase in villus height in the intestine of broilers fed Saccharomyces cerevisiae cell wall. The main effect of MOS seems to be related to the mannose-based receptors found in the intestine, since the mannose present in the mannan oligosacharide molecule may act as an analog receptor for the pathogenic bacteria that express type-1 fimbrae, preventing these bacteria to adhere to the intestinal mucosa, consequently improving intestinal health.

The present study was conducted to study the effect of the addition of Saccharomyces cerevisiae cell wall (0.2%) in broiler feeds on the performance, morphometric parameters and epithelial loss in small intestine segments (duodenum, jejunum and ileum), when birds were vaccinated at one day of age against coccidiosis

MATERIALS AND METHODS

Birds and Diets

A total of 400 one-day-old male Cobb-500® broiler chickens were reared under thermoneutral conditions in two environmentally-controlled rooms and fed diets based on corn and soybean meal, containing with 22% crude protein (CP) and 2,950 kcal of metabolizable energy/kg, according Rostagno *et al* (2000). Sacchromyces cerevisiae cell wall (SCCW) was added to the diet at the level of 0.2% and the birds were fed ad libitum during the entire experimental period of 28 days (Table 1).

Vaccine

Birds were vaccinated at one day of age against coccidiosis via spray with a vaccine (Coccivac®-B, Coopers) containing E. acervuline, E. maxima, E. mivati, and E. tenella.

Table 1 – Ingredients and calculated nutritional composition of the experimental diets.

Ingredients (%)	Treatments	
	T1 and T3	T2 and T4
Corn	52.42	52.42
Soybean meal	39.72	39.72
Soybean oil	2.56	2.56
Dicalcium phosphate	1.61	1.61
CaCO3	1.40	1.40
NaCl	0.41	0.41
Supplement*	0.65	0.65
DL-methionine	0.23	0.23
Saccharomyces cerevisiae cell wall		0.20
Kaolin	1.00	0.80
Total	100.00	100.00
	Calculated values	
Metabolizable energy (kcal/kg)	2.900,00	
Crude protein (%)	22.00	
Calcium (%)	1.00	
Available phosphorus (%)	0.50	
Sodium (%)	0.20	
Methionine (%)	0.50	
Methionine+cystine (%)	0.88	
Lysine (%)	1.13	

*Mineral and vitamin supplement: amount added per kg of feed: Vit. A 7000 IU; Vit D3 1400 IU; Vit E 16.65 mg; Vit K 1.5 mg; Vit B1 0.6 mg; Vit B2 2.36 mg; Vit B6 0.6 mg; Vit B12 1.32 mcg; Biotin 0.15 mg; Choline 1.54 g; Pantothenic acid 9.32 mg; Niacin 30.12 mg; Folic acid 1.42 mg; Selenium 0.65 mg; Iodine 0.35 mg; Iron 57.72 mg; Copper 12.3 mg; Zinc 141.48 mg; Manganese 173 mg; Potassium 7.88 g; Sodium 1.80 g; Sulfur 0.72 g; Magnesium 0.90 g.

Experimental Protocol

The experiment was carried out according to a completely randomized experimental design in 2 x 2 factorial arrangement. The following treatments were applied: T1 - no vaccination/ no SCCW supplementation; T2 – no vaccination/SCCW supplementation; T3 – vaccine/no SCCW supplementation; and T4 – vaccine/ SCCW supplementation. Each treatment had four replicates of 25 birds each. Body weight gain (g), feed intake (g) and feed conversion (g/g) were recorded on days seven, 14, 21 and 28 for each treatment. On these days, after 12 hours of fasting, five birds per treatment were randomly selected for the analyses of intestinal morphometrics and mucosa integrity. Two centimeters of each intestinal segment (duodenum, jejunum, and ileum) were collected, fixed in Bouin solution for 24 hours, dehydrated in a standard alcohol-toluene sequence, and embedded in plastic paraffin (Histosec®,

with hematoxylin-eosin, according Behmer *et al.* (1976).Villus height (µm) and crypt depth (µm) were measured in each segment in 70 microscopic fields using an image-analysis system (Video Plan, Carl Zeiss, Germany). Villus:crypt ratio was also calculated for each intestinal segment.

Scanning electron microscopy analysis of the intestinal epithelium was conducted in two-cm long samples collected from each segment (duodenum, jejunum, and ileum). The intestinal content was washed with saline solution buffered with 0.1 M phosphate, pH 7.4, and the tissue samples were fixed in 2% glutaraldehyde in phosphate buffer for 24 h at 4 °C. The tissue was subsequently washed in phosphate buffer and postfixed for 2 h in 1% osmium tetroxide. The samples were washed again with the same buffered solution and dehydrated in increasing ethanol series (30, 50, 70, 90, and 100% for 15 min each). Next, samples were dried in a critical-point drier with liquid carbon dioxide. The tissue was then placed in an appropriated specimen tray, covered with a 30-nm layer of gold, and observed under a scanning electron microscope (Jeol JSM 25SII model) operating at 15 kv. The average number of villi/segment was obtained by counting the number of villi in 6 areas measuring 103,269 µm^2 each.

Epithelial loss due vaccination was evaluated according Gomide Jr. *et al.* (2004), who classified mucosa damage in six-score scale: D0 – no apparent epithelial loss; D1 – little apical or enterocyte damage along the villi with normal extrusion cells; D3 – epithelial loss with connective tissue exposure in the apices of the villi; D4 – epithelial loss in the middle of the villus with connective tissue exposure; D5 – total epithelial loss with connective tissue exposure, and D6- complete damage of the epithelium and conjunctive tissue. Ten areas measuring 103,269 µm^2 were analyzed and villi were counted following the above classification. Data were expressed in percentage according the score of epithelial loss for each treatment. Intestinal mucosa which villi were classified as D0 and D1 were considered normal, and abnormal when villi were classified between D3 and D6.

Data were analyzed according to a completely randomized experimental design in a 2 x 2 factorial arrangement (vaccine and SCCW), with four replicates per treatment. Data were submitted to the GLM procedure of SAS (2000) software package, and means were compared using Tukey's test at 5% significance level.

RESULTS AND DISCUSSION

Growth performance

Live performance data are shown in Table 2. There was no statistical effect (p>0.05) of vaccination or yeast cell wall supplementation on body weight gain, feed conversion or feed intake of 7-d-old broilers. At 14 and 21 days,S. cerevisiae cell wall supplementation

Table 2 - Body weight gain (g), feed intake (g) and feed conversion ratio (g/g) during an experimental period of 28 days of broiler chickens that were fed or not 0.2% *Saccharomyces cerevisiae* cell wall in the diet and were vaccinated or not against coccidiosis.

		1 to 7 days			1 to 14 days			1 to 21 days			1 to 28 days		
		WG (g)	FI (g)	FCR (g/g)	WG (g)	FI (g)	FCR (g/g)	WG (g)	FI (g)	FCR (g/g)	WG (g)	FI (g)	FCR (g/g)
Vaccination	yes	121	160	1.31	379	549	1.44	759[b]	1100	1.45	1213	1800	1.48
	no	123	157	1.28	374	545	1.45	789[a]	1097	1.39	1231	1780	1.45
Dietary S.cerevisiae cell wall	yes	123	158	1.28	372	506[b]	1.36[b]	769	1066[b]	1.38[b]	1249	1756	1.40
	no	121	158	1.31	381	587[a]	1.54[a]	779	1131[a]	1.45[a]	1216	1733	1.42
p - values													
Vaccination(V)		0.55	0.11	0.21	0.53	0.50	0.80	0.03	0.85	0.08	0.42	0.37	0.21
S.cerevisiae cell wall (S)		0.14	0.85	0.35	0.21	<0.01	<0.01	0.46	0.00	0.04	0.56	0.29	0.12
V x S		0.82	0.67	0.68	0.42	0.35	0.23	0.83	0.95	0.90	0.26	0.22	0.77
CV%		2.90	2.31	4.24	3.62	2.27	4.06	3.27	3,39	4.33	3.59	2.45	3.73

[a,b]Means followed by different letters in the same column are statistically different according test of Tukey (p<0.05)
CV: Coefficient of variation

improved feed conversion (p<0.05), but had no effect (p>0.05) on body weight gain, which was lower at 21-days for the vaccinated compared with the non-vaccinated birds (p<0.05). However, at 28 days of age, the treatments did not influence (p>0.05) any observed of the evaluated performance parameters. Loddi (2003), studying the supplementation of MOS or MOS plus organic acidifier, did not find any statistical difference (p>0.05) in body weight gain, feed intake, or feed conversion in 42-d-old broilers. Yang *et al.* (2007) also did not report any positive effect of MOS on the performance of 35-d-old broilers. It should be stressed that all these experiments were carried out under experimental and hygienic conditions, suggesting very low challenge of pathogenic bacteria in the gut lumen.

Table 3 - Villus height (µm), crypt depth (µm) and villus:crypt ratio (V/C) in the small intestine segments of 14-, 21-, and 28-d-old broiler chickens that were fed or not 0.2% *Saccharomyces cerevisiae* cell wall in the diet and were vaccinated or not against coccidiosis.

14 days										
		Duodenum			Jejunum			Ileum		
		Villus	Crypt	V/C	Villus	Crypt	V/C	Villus	Crpt	V/C
Vaccine	yes	1375	264[a]	5.20[b]	1074	226[a]	4.75	762	227	3.35
	no	1373	186[b]	7.38[a]	1024	180[b]	5.68	769	214	3.59
Dietary *S.cerevisiae* cell wall	yes	1354	218	6.21	1008	197	5.11	790	231	3.41
	no	1394	232	6.01	1096	214	5.12	738	209	3.53
				P values						
Vaccine (V)		0.97	<0.01	<0.01	0.48	<0.01	0.10	0.71	0.42	0.55
S. cerevisiae cell wall (S)		0.57	0.43	0.60	0.24	0.31	0.95	0.27	0.13	0.90
V x S		0.96	0.59	0.91	0.75	0.72	0.91	0.96	0.08	0.28
CV (%)		11.24	16.70	22.91	14.01	16.03	20.25	11.98	10.85	21.11
21 days										
		Duodenum			Jejunum			Ileum		
		Villus	Crypt	V/C	Villus	Crypt	V/C	Villus	Crypt	V/C
Vacine	yes	1465	377[a]	3.86[b]	1058[b]	305[a]	3.46[b]	888	228	3.96
	no	1598	210[b]	7.70[a]	1332[a]	196[b]	6.79[a]	904	209	4.48
Dietary *S.cerevisiae* cell wall	yes	1494	301	4.96	1266	256	4.94	909	238	3.81
	no	1559	295	5.28	1124	257	4.37	882	201	4.48
				P values						
Vaccine (V)		0.21	<0,01	<0,01	<0.01	<0.01	0.03	0.60	0.33	0.11
S. cerevisiae cell wall (S)		0.57	0.60	0.49	0.93	0.51	0.48	0.47	0.05	0.06
V x S		0.96	0.69	0.47	0.04	0.48	0.73	0.26	0.62	0.18
CV (%)		14.27	20.28	27.98	10.71	19.09	18.48	7.31	17.81	15.27
28 days										
		Duodenum			Jejunum			Ileum		
		Villus	Crypt	V/C	Villus	Crypt	V/C	Villus	Crypt	V/C
Vaccine	yes	1388	336	4.13	1230	306[a]	4.01	1027	226	4.54
	no	1587	351	4.52	1194	224[b]	5.33	1018	203	5.01
Dietary *S.cerevisiae* cell wall	yes	1545	344	4.58	1187	257	4.61	1007	208	4.84
	no	1434	344	4.42	1234	272	4.53	1046	226	4.56
				p - values						
Vaccine (V)		0.12	0.71	0.72	0.79	0.02	0.58	0.91	0.13	0.90
S. cerevisiae cell wall (S)		0.34	0.99	0.25	0.80	0.67	0.07	0.65	0.85	0.21
V x S		0.84	0.67	0.60	0.29	0.94	0.64	0.87	0.72	0.90
CV (%)		18.71	23.54	29.03	18.18	24.87	29.93	15.76	23.25	23.38

[a,b]Means followed by different letters in the same column are statistically different according test of Tukey (p<0.05)
CV: Coefficient of variation

Small intestine villus height and crypt depth

Villus height, crypt depth and villus:crypt ratio in the three evaluated intestinal segments (duodenum, jejunum and ileum) are shown in Table 3 according to treatments and bird age.

Villus height was not affected (p>0.05) by coccidiosis vaccination or S. cerevisiae cell wall supplementation, except at 21 days of age, when villus height was reduced in the jejunum of the vaccinated animals (p<0.01). Santin *et al.* (2001) reported an effect of S. cerevisiae cell wall supplementation on villus height during the first week of life broilers, but that this effect was no longer detected after that age. Yang *et al.* (2007) also reported that the dietary supplementation of mannan oligosacharide (MOS) did not affect gut morphology of 35-d-old broilers. The effect of mannan oligosacharide present in the gut lumen on intestinal health seems to be dependent on the presence of mannose, since the pathogenic bacteria that express type-1 fimbrae attach to the mannose present in that molecule and are expelled from the gut. On the other hand, duodenum and jejunum crypt depth of 14- and 21-d-old broilers was affected (p<0.05) by vaccination, with higher values for vaccinated compared with non-vaccinated birds. The effect of vaccination on crypt depth was still observed in the jejunum at 28 days of age (p<0.05). Higher crypt dept suggests an increased mitotic activity in the crypt and, according to Pluske *et al.* (1997), this finding indicates high proliferative activity of the enterocytes in order to maintain adequate cell turnover in the epithelium (villus). The main effects on intestinal mucosa were restricted to duodenum and jejunum, since protozoa preferably proliferate at these sites.

An interaction between vaccine and S. cerevisiae cell wall dietary supplementation was observed villus height in the jejunum when broilers were 21 days old (Table 4). Data showed that the villi of broilers supplemented with yeast cell wall were higher (p<0.05), irrespective vaccination against coccidiosis.

Table 4 - Interaction between vaccination against coccidiosis and *S. cerevisae* cell wall dietary supplementation (0.2%) on jejunum villus height (µm) at 21 days of age.

		Vaccination	
		yes	no
Dietary S. cerevisae cell wall	yes	1263^{Ba}	984^{Bb}
	no	1401^{Aa}	1132^{Ab}

Means followed by the same capital letters in the same column and small letters in the same row are not different according the test of Tukey at 5%.probability level.

The number of villi/area (103,269 µm^2) was not affected (data not shown) by the treatments, irrespective of animal age. This finding suggests that the effect of vaccination and of the yeast cell wall dietary supplementation was not able to affect the number of villi, but only crypt depth and villus height at some broiler ages, as shown in Table 4.

The score of epithelial loss in the intestinal segments (Figures 1 to 3) revealed that most of the observed cell loss may be considered normal, since 90% of the losses were classified within scores 0 and 1, and were restricted to duodenum and jejunum. No epilthelial loss with scores 5 and 6 were detected, suggesting that vaccination did not severely damaged the intestinal epithelium. Scores 2 to 4 of epithelial loss of degrees were more frequent in the duodenum and ileum at 14 and 21 days of age. When S. cerevisiae cell wall was supplemented to the vaccinated broilers, there was a slight reduction in epithelial cell loss percentage. Since this study was based on subjective analysis, a more accurate methodology should be developed to confirm these results.

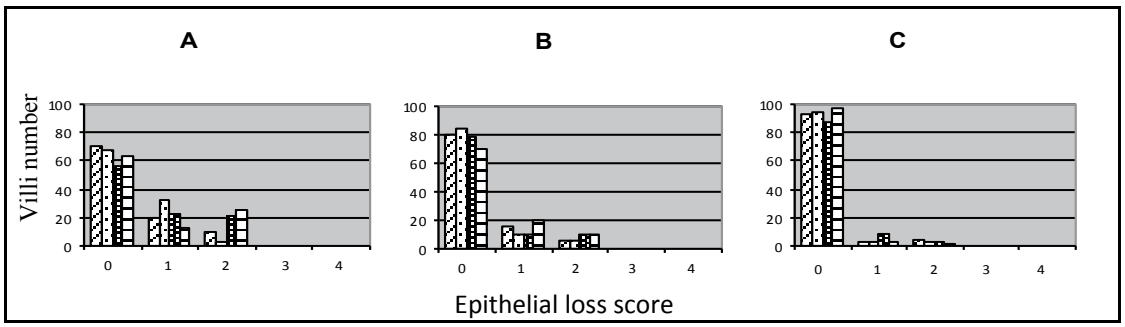

Figure 1- Epithelial loss in the duodenum (A), jejunum (B) and ileum (C) of 14-d-old broilers

= no vaccination/no SCCW supplementation = no vaccination/ SCCW supplementation

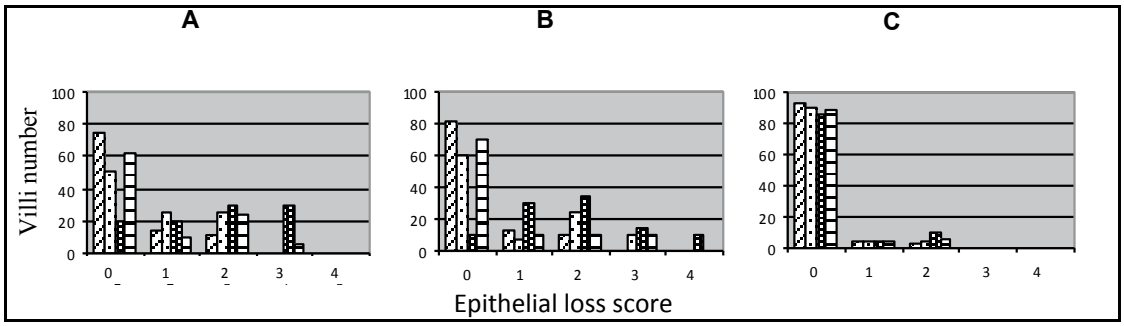

Figure 2. Epithelial loss in the duodenum (A), jejunum (B) and ileum (C) of 21-d-old broilers.

▨ = no vaccination/no SCCW supplementation ⁘ = no vaccination/ SCCW supplementation

= vaccination/no SCCW supplementation = vaccination/ SCCW supplementation

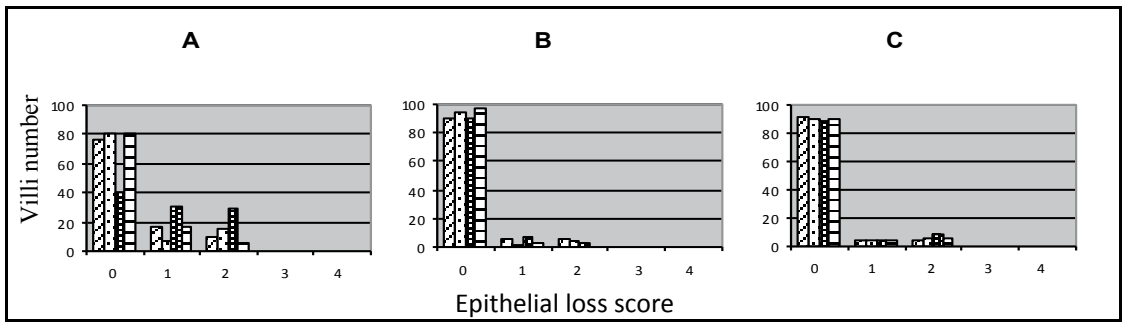

Figure 3. Epithelial loss in the duodenum (A), jejunum (B) and ileum (C) of 28-d-old broilers.

= no vaccination/no SCCW supplementation = no vaccination/ SCCW supplementation

The results of the present study showed that vaccination against coccidiosis (Coccivac®-B, Coopers) did not influence the performance of the broilers until 28 days of age. The vaccine affected, but not severely, the intestinal epithelium, especially the duodenum and the ileum, increasing their crypt depth, which suggests increased mitotic activity due to the need of maintaining adequate mucosa cell turnover in those segments. S. cerevisiae cell wall dietary supplementation did not influence bird performance, except for feed conversion ratio at 14 and 21 days; however, it reduced intestinal epithelial loss, suggesting that the use of S. cerevisiae cell wall may be an useful management tool to maintain intestinal integrity after coccidiosis vaccination.

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