https://doi.org/10.17221/237/2020-CJAS

Broiler chick performance using *Saccharomyces cerevisiae* yeast cell wall as an anti-mycotoxin additive

Vinícius Machado dos Santos[1], Gabriel da Silva Oliveira[2], Cristina Amorim Ribeiro de Lima[3], Fernando Augusto Curvello[4]*

[1]*Laboratory of Poultry Science, Federal Institute of Brasília – Campus Planaltina, Brasília, Brazil*
[2]*Faculty of Agronomy and Veterinary Medicine (FAV), University of Brasília, Brasília, Brazil*
[3]*Department of Nutrition and Pasture, Federal Rural University of Rio de Janeiro, Seropédica, Brazil*
[4]*Department of Animal Production, Federal Rural University of Rio de Janeiro, Seropédica, Brazil*

**Corresponding author: vinicius.santos@ifb.edu.br*

Citation: dos Santos V.M., Oliveira G.S., de Lima C.A.R., Curvello F.A. (2021): Broiler chick performance using *Saccharomyces cerevisiae* yeast cell wall as an anti-mycotoxin additive. Czech J. Anim. Sci., 66: 65–72.

Abstract: The objective of this study was to evaluate the effects of using the *Saccharomyces cerevisiae* yeast cell wall (YCW) as an aflatoxin B_1 (AFB_1) adsorbent in broiler chicken feed on performance and carcass characteristics. The present study used a randomized complete block with four treatments in a 2 (with or without AFB_1) × 2 (with or without YCW) factorial design. No interaction effect ($P > 0.05$) between AFB_1 and YCW was found on the studied performance variables. The addition of YCW to the diets stimulated the feed intake of chickens during 1–21 days of age. However, YCW did not significantly increase ($P > 0.05$) weight gain nor did it change feed conversion. The presence of AFB_1 in the diet did not affect ($P > 0.05$) performance parameters. The addition of YCW to the feed containing AFB_1 significantly increased ($P < 0.05$) the post-fasting live weight (781.12 g), chilled carcass weight (554.41 g), and leg weight (163.34 g) compared to feed without AFB_1 and YCW (764.84 g; 533.41 g; 161.88 g), feed with only YCW (764.22 g; 546.87 g; 159.34 g), and feed with only AFB_1 (735.41 g; 510.56 g; 152.75 g). In conclusion, YCW effectively reduced some of the deleterious effects of AFB_1 in broilers.

Keywords: adsorbent; aflatoxin; organ weight; poultry; poultry farming; poultry feed

When produced under inadequate conditions, i.e., without the establishment of and compliance with good agricultural practices, maize, the main energy component of poultry feed, exhibits a compromised nutritional value. In tropical and subtropical countries, fungal development is favoured by factors such as temperature and humidity (Peraica et al. 1999). The growth of fungi in grains can occur at any stage of production, from the field to storage, especially when fungi find highly nutritious substrates (Wilson et al. 2002). During fungal growth, toxic compounds called mycotoxins are produced by the secondary metabolism of several fungi (Pittet et al. 1998).

Among existing mycotoxins, aflatoxin, which is produced by fungi of the genus *Aspergillus*, is often present in maize grains (Miller 1995). Aflatoxin causes considerable losses in the quantitative and qualitative value of feeds, compromising feed balance (Lopes et al. 1988). When ingested, aflatoxin affects several organs such as liver and organs associated with the immune system, leading to immunosuppression (Thaxton et al. 1974; Khan and Zahoor 2014; Salem et al. 2018) and causing negative effects on zootechnical performance and economic losses to the producer. Another problem generated by the ingestion of aflatoxin lies in the fact that the meat produced becomes a vehicle

https://doi.org/10.17221/237/2020-CJAS

of waste potentially toxic to human health (Jonker et al. 1999).

Several methods have been studied to reduce the toxic effects caused by aflatoxins on poultry. The use of adsorbents in feed is a method used to control mycotoxicosis. The *Saccharomyces cerevisiae* yeast cell wall (YCW) is an organic substance that has been used as an adsorbent of mycotoxins in animal feed (Santin et al. 2003). Positive *in vitro* results match the results of *in vivo* studies, where YCW was able to minimize the toxicity of aflatoxins, demonstrating the beneficial effects of its addition to broiler feed (Celyk et al. 2003; Santin et al. 2003; Oliveira et al. 2015).

The high amount of yeast generated as a by-product in the alcohol and beer industries increases interest in the use of its derivatives as functional additives in broiler feed. The objective of this study was to evaluate the effect of the use of YCW as an aflatoxin B_1 (AFB_1) adsorbent in broiler chicken feed on performance and carcass characteristics. We hypothesized that low concentrations of AFB_1 reduce broiler chicken performance at 21 days and that YCW reduces the negative effect of AFB_1 on broiler chicken performance.

MATERIAL AND METHODS

All procedures relating to the use of live birds in this study were approved by the Animal Care and Use Committee of the Federal Rural University of Rio de Janeiro (UFRRJ). In addition, unnecessary discomfort to the birds was also avoided by using proper housing and handling techniques.

Experimental procedures

A total of 288 one-day-old male Cobb broiler chicks were housed and distributed in a randomized block experimental design, with four treatments arranged in a 2 (without AFB_1; with AFB_1) × 2 (without YCW; with YCW) factorial arrangement (Table 1).

The one-day-old chicks were individually weighed (average weight of 42.34 g) and randomly distributed into eight metal battery cages consisting of four floors, with each floor measuring 0.98 × 0.50 × 0.21 m (length, width, and height, respectively). Each battery was occupied only by chicks receiving the same treatment. Each block encompassed one floor, with two replicates per block, eight replicates per experimental group, and nine birds per replicate.

Table 1. Aflatoxin B_1 (AFB_1) levels and *Saccharomyces cerevisiae* yeast cell wall (YCW) concentrations in the experimental diets

Experimental groups	AFB_1 (mg/kg of feed)	YCW (g/kg of feed)
1	0	0
2	0	2
3	1	0
4	1	2

Each cage had a nipple drinker and a 0.50-m trough feeder. In the first week of rearing, cup drinkers and feeder trays were used. To warm the birds, a 60-W incandescent lamp was installed in each cage. The lighting programme was 24 h (natural and artificial) on the first day and 23 h from the second day until slaughter, and to ensure desirable environmental conditions for the birds, two fans were installed in the shed together with nebulizers. The average temperature throughout the study was 29.6 °C. These data were monitored by analogue thermometers.

The aflatoxin used for this assay was AFB_1, and the adsorbent was a complex of polysaccharides from YCW. Aflatoxin B_1 was produced in the laboratory from the controlled fermentation of the fungal strain *Aspergillus flavus* RC 2053 in polished rice, forming the AFB_1 core structure, according to the techniques described by Shotwell et al. (1966), with modifications. The obtained AFB_1 was autoclaved, dried at 50 °C in an oven, and ground. The mycotoxin was extracted by the method described by Soares and Rodriguez-Amaya (1989) with modifications and quantified by high-performance liquid chromatography (HPLC).

The HPLC system was equipped with a pump, an automatic injector, and a fluorescence detector. The compounds used in the mobile phase were methanol, acetonitrile, and water (27 : 8 : 65 vol/vol/vol) degassed in an ultrasonic bath. The column (150 × 4.6 mm, particles of 5 µm) was maintained at 45 °C with a flow rate of 0.4 ml/minute. The injection volume was 20 µl. The excitation wavelength was 360 nm, and the emission wavelength was 440 nm. In the feeds used, AFB_1 was incorporated at a ratio of 1 ppm (1 mg AFB_1/kg feed). After

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obtaining a homogeneous mixture, a sample of each feed was collected for extraction and quantification by HPLC to confirm the AFB_1 concentration in the experimental feed.

The adsorbent tested was purchased from a commercial market and consisted of a complex of non-starch polysaccharides obtained from YCW purification. The adsorbent had the following chemical composition: protein, 28%; phosphorus, 1%; beta glucans, 23%; mannans, 21%; fat, 20%; ash, 4%; and dry matter, 95%. The product had a cream-to-gold colour, a typical yeast odour, and no evidence of impurities, and according to the manufacturer, the product is free of antibiotics, heavy metals, chemical residues, and microbial contaminants.

The feed used was manufactured especially for the experiment (Table 2), and its composition met the minimal requirements recommended by the manual for the strain used in this experiment (Cobb 500 manual 2008, www.cobb-vantress.com/assets/Cobb-Files/99b0cf062c/61bd2490-56d1-11e9-bfbd-7963ec6b06e5.pdf). The feed was analysed to ensure that it was free of any mycotoxins. Aflatoxin B_1 and the adsorbent were added to the feed according to the treatments and mixed for 20 min in a Y-mixer (Cirelli, Descalvado, Brazil). The birds had access to water and feed *ad libitum* and were vaccinated against Marek's disease, Newcastle disease, and Gumboro disease.

Evaluation of productive performance

To assess productive performance, feed intake, feed conversion, live weight, weight gain, and viability were evaluated from one to 21 days of age. The birds were weighed at the beginning and end of the experimental period to determine weight gain. Feed intake was calculated considering the feed provided minus the waste and leftovers in the feeders during the experimental period. In the event of death, the feed in the feeder was immediately weighed to calculate the corrected feed intake.

Subsequently, the feed conversion was calculated, i.e., the ratio of the average feed intake to the average weight gain of the birds from days 1 to 21. Viability (%) was calculated as the ratio between the number of live chickens at 21 days of age and the number of chicks initially housed.

Table 2. Bromatological and nutritional composition of the balanced feed

	Composition
Ingredients	
Corn (8% crude protein)	64.170
Soybean meal (46% crude protein)	28.900
Meat and bone meal	5.330
Ground salt	0.261
Calcitic limestone	0.430
Mineral mix[1]	0.200
Vitamin mix[2]	0.200
Sodium bicarbonate	0.144
DL-methionine	0.106
L-lysine HCl	0.259
Total	100.000
Nutrients	
Metabolizable energy (MJ/kg)	2.958
Crude protein (%)	21.000
Total phosphorus	0.681
Available phosphorus	0.450
Calcium	1.000
Digestible lysine	1.155
Total lysine	1.294
Digestible methionine	0.567
Methionine + digestible cystine	0.840
Methionine + total cystine (%)	0.935
Total methionine (%)	0.599
Digestible threonine (%)	0.664
Total threonine (%)	0.781
Total tryptophan (%)	0.240
Digestible tryptophan (%)	0.206
Sodium (%)	0.190
Potassium (%)	0.813
Chlorine (%)	0.290
Sodium + potassium – chlorine (mEq/kg)	208.924

[1]Provided per kg of diet: iron, 7 500 mg; copper, 2 250 mg; manganese, 15 000 mg; zinc, 15 000 mg; iodine, 250 mg; selenium, 62.5 mg; excipient *quantum satis*, 1 000 g

[2]Provided per kg of diet: vitamin A, 2 750 IU; vitamin D_3, 500 IU; vitamin E, 4 000 mg; vitamin K_3, 375 mg; vitamin B_1, 300 mg; vitamin B_2, 1 125 mg; vitamin B_6, 500 mg; vitamin B_{12}, 4 000 mg; niacin, 8 750 mg; calcium pantothenate, 2 500 mg; folic acid, 100 mg; biotin, 15 mg; L-methionine, 425 g; choline chloride, 125 g; colistin, 2 500 mg; and nicarbazine, 12 500 mg

https://doi.org/10.17221/237/2020-CJAS

Evaluation of the carcass characteristics

At the end of the performance test, at 21 days of age, the birds underwent solid fasting for 6 h before slaughter. To evaluate the carcass characteristics, 32 birds were used per treatment, totalling 128 birds. At 22 days of age, the birds were individually weighed, identified, and sacrificed by cervical dislocation. Subsequently, they were bled, scalded at 54 °C for 2 min, mechanically plucked, and manually eviscerated, removing the head, neck, and feet. After washing, the carcasses were hung for 5 min to remove excess water. The carcasses were weighed again to evaluate the hot carcass weight, packed in labelled plastic bags and cooled for 1 h in ice water.

Subsequently, they were transferred to a freezer at 5 °C for 24 h, from which they were removed for individual weighing and determination of the chilled carcass weight, followed by cutting and weighing of the breast, leg (thigh and drumstick), back, and wing. The absolute weights in grams of the viscera (heart, liver, and gizzard) and the organs of the immune system (spleen and bursa of Fabricius) and the total length of the intestine (cm) were also evaluated.

Statistical analysis

The collected data were subjected to analyses of variance using the general linear model procedure (PROC GLM) of the statistical analysis software SAS/STAT® Studio v3.8, University Edition (SAS Institute Inc., Cary, NC, USA), and means were compared using Tukey's test, with significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Behavioural signs and performance of broiler chickens (1–21 days)

In the present study, no abnormal behaviours of the birds were observed in the four treatment groups over the three weeks of the experiment. All birds were observed to be active and showed attraction to water and feed. However, these findings differ from those of Khan and Zahoor (2014), who reported that birds receiving AFB_1 for six weeks showed less attraction to feed and more attraction to water. Therefore, the time when birds are exposed to a mycotoxin must be considered because in the present study, due to the shorter time, differences in the behaviours of the birds could not be verified.

No interaction effect ($P > 0.05$) between AFB_1 and YCW was found on the studied performance variables. The presence of AFB_1 in the diet did not affect ($P > 0.05$) the body weight, weight gain, feed intake, feed conversion, or viability of broiler chickens (Table 3). In contrast, Valdivia et al. (2001) observed that a diet containing 3 mg AFB_1/kg reduced weight gain and increased feed conversion at 21 days of exposure. However, these contradictory results may be associated with the difference in the level of aflatoxin present in the diet in both experiments; the level used in the present study did not affect post-hatching performance, as corroborated by Siloto et al. (2013), who observed that the presence of 1 mg AFB_1/kg of feed in the diet did not affect feed intake and body weight in layers. In addition, Manafi and Khosravinia (2013) also reported that the weight gain of broiler breeders was not affected by 500 µg of AFB_1/kg in the feed during eight experimental weeks and that no deaths occurred.

The addition of YCW to the chicken feed stimulated ($P = 0.001\,5$) feed intake from one to 21 days of age; however, this effect was not significantly reflected ($P > 0.05$) in live weight, weight gain, feed conversion, and viability (Table 3). The increase in feed intake with treatments with YCW may be related to the trophic effect caused by YCW polysaccharides on the digestive mucosa, which increases the villus height, especially dur-

Table 3. The body weight (BW), weight gain (WG), feed intake (FI), feed conversion (FC), and viability (VB) of broilers at 21 days of age according to treatment

Factors	BW (g)	WG (g)	FI (g)	FC	VB (%)
AFB_1 (mg/kg)					
0	732.87	690.57	1 121.16	1.604	98.08
1	754.13	711.13	1 134.04	1.609	97.14
YCW (g/kg)					
0	724.33	682.02	1 086.58[b]	1.598	97.53
2	762.67	720.28	1 168.62[a]	1.614	97.69

AFB_1 = aflatoxin B_1; YCW = *Saccharomyces cerevisiae* yeast cell wall

[a,b]Means with different superscript letters in columns differ significantly ($P < 0.05$)

https://doi.org/10.17221/237/2020-CJAS

ing the first seven days of life in chickens (Santin et al. 2001).

Carcass yields and characteristics of broiler chickens (1–21 days)

A significant interaction ($P < 0.05$) between AFB_1 and YCW was observed on post-fasting live weight (Figure 1A), chilled carcass weight (Figure 1B), and leg weight (Figure 1C). The addition of YCW to the feed containing AFB_1 significantly increased ($P < 0.05$) the post-fasting live weight (781.12 g), chilled carcass weight (554.41 g), and leg weight (163.34 g) compared to feed without AFB_1 and YCW (764.84 g; 533.41 g; 161.88 g), feed with only YCW (764.22 g; 546.87 g; 159.34 g), and feed with only AFB_1 (735.41 g; 510.56 g; 152.75 g). This result can be explained by the adsorbent action of YCW on AFB_1, reducing its bioavailability in the intestinal lumen and consequently decreasing its absorption (Davidson et al. 1987; Gonzalez Pereyra et al. 2014).

The carcass parameters for which no significant differences in the interactions between the studied factors were found were analysed separately (Table 4).

Table 4. Absolute weights of hot carcasses and breast, back, and wing cuts

Factors	Absolute weights (g)			
	hot carcass	breast	back	wing
AFB_1 (mg/kg)				
0	529.15	176.48[a]	141.90	60.04
1	524.35	168.06[b]	144.95	59.21
YCW (g/kg)				
0	512.51[b]	167.28[b]	136.39	57.89
2	540.98[a]	177.26[a]	140.47	61.37

AFB_1 = aflatoxin B_1; YCW = *Saccharomyces cerevisiae* yeast cell wall

[a,b]Means with different superscript letters in columns differ significantly ($P < 0.05$)

The presence of AFB_1 in the diet of the broilers significantly reduced the absolute weight of the breast ($P = 0.005\,3$; Table 4), but the absolute weight of the hot carcass and the absolute weight of the back and wings were not affected by AFB_1 ($P > 0.05$). Therefore, the reduction in post-fasting live weight and chilled carcass weight of broilers fed aflatoxin (Figure 1A and 1B) was reflected in the reduction in the absolute breast weight, showing a limitation to the maximum genetic potential of the broilers,

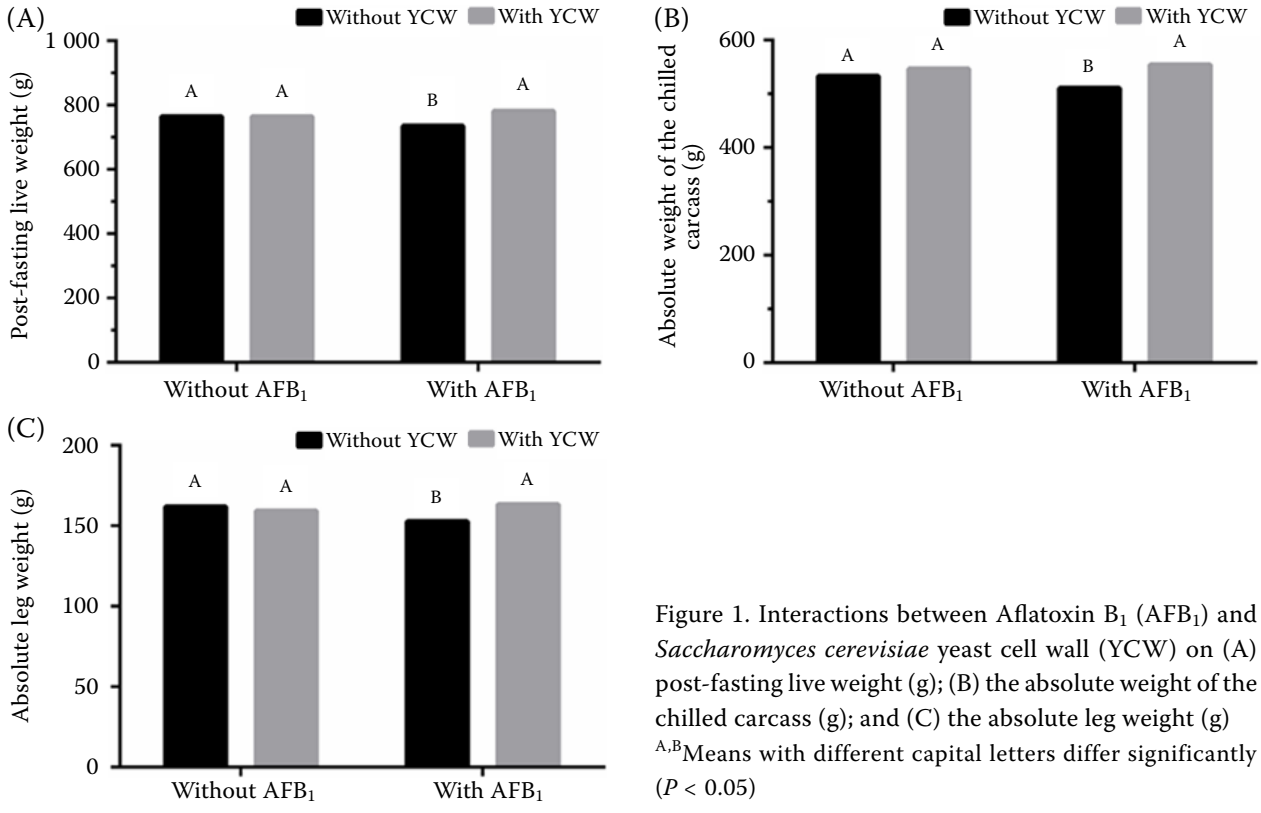

Figure 1. Interactions between Aflatoxin B_1 (AFB_1) and *Saccharomyces cerevisiae* yeast cell wall (YCW) on (A) post-fasting live weight (g); (B) the absolute weight of the chilled carcass (g); and (C) the absolute leg weight (g)

[A,B]Means with different capital letters differ significantly ($P < 0.05$)

https://doi.org/10.17221/237/2020-CJAS

especially the cut that is most valued by breeding programmes. However, the addition of YCW to the feeds promoted a significant increase ($P < 0.05$) in the hot carcass weight and absolute breast weight, but no significant effect ($P > 0.05$) on the absolute weight of the back and the wings was observed (Table 4), thereby demonstrating the beneficial effect of using YCW in animal feed.

Intestinal length and absolute weight of the viscera

A significant effect of the interaction between AFB_1 and YCW on the intestinal length was found ($P = 0.009\ 2$; Figure 2). The addition of YCW to the feed containing AFB_1 significantly reduced the length of the intestine (129.98 cm) compared to feed without AFB_1 and YCW (137.92 cm), feed with only YCW (138.96 cm), and feed with only AFB_1 (145.04 cm). This reduction can be considered beneficial because a decrease in the length of the intestine of broilers may result in more efficient absorption and use of nutrients (Miles et al. 2006).

In contrast, the increase in the length of the intestine for birds that consumed diets with AFB_1 and without YCW may be due to the deleterious effect of the toxin on the intestinal villi, reducing the absorptive surface and leading to an increase in the length of the intestine to compensate for the reduced absorption surface (Yunus et al. 2011).

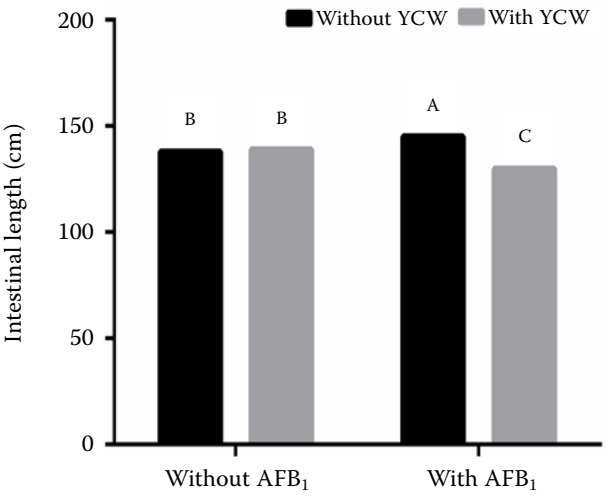

Figure 2. Interactions between aflatoxin B_1 (AFB_1) and *Saccharomyces cerevisiae* yeast cell wall (YCW) on intestinal length (cm)

$^{A–C}$Means with different capital letters differ significantly ($P < 0.05$)

No interaction effect ($P > 0.05$) between AFB_1 and YCW on the absolute weight of the organs was observed (Table 5). No significant differences ($P > 0.05$) in the absolute weights of liver, gizzard, heart, and spleen were noted when the aflatoxin factor was considered. The liver is the main target organ of action for aflatoxicosis (Kubena et al. 1990; Khan and Zahoor 2014; Salem et al. 2018), and although its absolute weight did not increase in the group that received only AFB_1, yellowish discoloration was observed in cases of aflatoxicosis exposure (Kumar and Balachandran 2009; Salem et al. 2018). In contrast, the tested AFB_1 concentration significantly reduced ($P = 0.025\ 2$) the absolute weight of the bursa of Fabricius. Other researchers have also reported a decrease in the weight of the bursa of Fabricius from birds receiving feed containing AFB_1 and ochratoxin A (Campbell et al. 1983; Gulfam et al. 2018). This decrease may be attributed to the reduced diameter of lymphoid follicles and the reduced number of lymphocytes (Bhatti et al. 2017). In this sense, changes in the weight of the bursa of Fabricius may reduce the immunological competence of birds (Kubena et al. 1990).

No significant effect ($P > 0.05$) of the addition of YCW to the feeds on the absolute weights of liver, gizzard, heart, and bursa of Fabricius was observed, but a significant increase ($P = 0.010\ 1$) in the absolute weight of the spleen was noted (Table 5). Increased spleen weight is known to indicate rapid

Table 5. Absolute weights of the liver, gizzard, heart, spleen, and bursa of Fabricius

Factors	Absolute weights (g)				
	liver	gizzard	heart	spleen	bursa of Fabricius
AFB_1 (mg/kg)					
0	18.95	18.37	3.49	0.60	1.90^{a}
1	18.75	18.49	3.45	0.56	1.61^{b}
YCW (g/kg)					
0	18.71	18.47	3.48	0.54^{b}	1.68
2	18.99	18.39	3.46	0.62^{a}	1.83

AFB_1 = aflatoxin B_1; YCW = *Saccharomyces cerevisiae* yeast cell wall

^{a,b}Means with different superscript letters in columns differ significantly ($P < 0.05$)

https://doi.org/10.17221/237/2020-CJAS

development of immune organs. Therefore, the addition of YCW to feed may promote the growth and development of this lymphoid organ and stimulate the immune response of birds (Ao et al. 2012).

CONCLUSIONS

The results obtained in the present study indicate that YCW effectively reduced some of the deleterious effects of AFB_1 in broilers, such as those on the absolute weights of spleen, cooled carcass and leg, post-fasting live weight, and intestinal length.

Conflict of interest

The authors declare no conflict of interest.

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Received: September 15, 2020
Accepted: December 18, 2020