

EFFICACY OF YEAST CELL WALLS IN COUNTERACTING ADVERSE FUMONISIN B1 EFFECTS ON IN-VITRO RUMEN FERMENTATION

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Abstract

The efficacy of yeast cell walls at counteracting the adverse effects of fumonisin B1 (FB1) on in-vitro rumen fermentation was determined. The experiment was conducted in a completely randomized design and divided into 4 treatments covering diet alone (control), diet with 0.5 mg/kg FB1 added, diet with 0.5% yeast cell wall added, and diet with both 0.5 mg/kg FB1 and 0.5% yeast cell wall added. FB1 significantly reduced gas production performance as well as *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens* proliferation, but increased ammonia nitrogen concentration ($P<0.05$) compared to the control. By contrast, an elevated *F. succinogenes* population was a response to yeast cell wall supplementation ($P<0.05$), which did not affect *B. fibrisolvens* copy numbers. Moreover, both yeast cell wall and FB1 did not influence the *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, volatile fatty acid concentrations, or the *Ruminococcus albus* population. The findings indicated that FB1 had negative effects on gas production, ammonia concentration, and major fibrolytic bacteria population *in vitro*. Nonetheless, yeast cell wall possessed beneficial properties, alleviating adverse FB1 effects on fibrolytic bacteria.

Keywords: Yeast cell wall, Fumonisin B1, mycotoxin, fermentation, fibrolytic bacteria

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Introduction

Fumonisin (FUM) are fungal secondary metabolites consisting of sphingosine and sphinganine (sphingolipid intermediates)-like and polyalcohol structures (Wang *et al.*, 1991; Tardieu *et al.*, 2006) that are produced primarily by *Fusarium verticillioides* (formerly known as *F. moniliforme*) and *F. proliferatum* (Caloni *et al.*, 2000; Heidtmann-Bemvenuti *et al.*, 2011). FUM is commonly known as a carcinogen (Jouany *et al.*, 2009; Zain, 2011), a neurotoxin (Domijan, 2012), and for its harmful effects on growth, reproduction, immunity (Osweiler *et al.*, 1993), and milk production (Sahu *et al.*, 2004). Recently, FUM contamination in feedstuffs and feed has been documented globally and caused serious health concerns (Kim *et al.*, 2014; Rodrigues *et al.*, 2011; Rodrigues and Naehrer, 2012). Among diverse homologous FUM, FB1 was the most abundant (70% of FUM) and commonly contaminating animal feed (Jouany *et al.*, 2009; Kim *et al.*, 2014; Zain, 2011). In addition, FB1 and FB2 mixture could be found up to 98% in cattle feed, ranging from 0.042 to 2.990 mg/kg (Kim *et al.*, 2014). Generally, ruminants were relatively less susceptible to mycotoxins, especially FB1. FUMs were tolerant to rumen microbial degradation, while other mycotoxins were rendered somewhat less toxic by the ruminal ecosystem (Abidin and Khatoon, 2012). Thus, FB1 was mainly excreted (60-90%) as un-metabolized FB1 (Caloni *et al.*, 2000) or FB1-hydrolysed metabolites (aminopolyols and aminopentol) in feces (Prelusky *et al.*, 1996; Rice and Ross, 1994; Smith and Thakur, 1996).

Recently, *Saccharomyces cerevisiae*-derived yeast cell wall has been proven beneficial to promoting gut health and growth (Al-Masri *et al.* 2011), reducing mycotoxin interference on nutrient digestibility (Kim *et al.*, 2019), improving digestibility (Lei *et al.*, 2013; Tao *et al.*, 2015), and decontaminating mycotoxins (Keller *et al.*, 2015). As anti-FB1 additives, both *S. cerevisiae* viable cells and cell walls have been introduced with the adsorption capacity to reduce the gastrointestinal absorption (Bueno *et al.*, 2005; Keller *et al.*, 2015). However, most published literature has

focused on *S. cerevisiae* viable cells and FUM mixtures, with no extant literature clarifying the relationships between yeast cell walls, homologous FB1, and rumen microbial fermentation. Therefore, the objective of this study was to determine the efficacy of yeast cell wall supplementation to counteract the adverse effects of FB1 on microbial populations and rumen fermentation using an *in vitro* gas production technique.

Materials and Methods

Experimental design and treatments

This experiment was conducted in a completely randomized design (CRD) using *in vitro* rumen fermentation. The experiment was divided into four treatments comprising diet alone (control), diet with 0.5 µg/mg FB1 added, diet with 0.5% yeast cell wall (*S. cerevisiae*) added, and diet with both FB1 at 0.5 mg/kg and 0.5% yeast cell wall added. The basal diet consisted of concentrate and rice straw as a roughage source (40:60 ratio), that were oven dried at 60°C for 48 h before being ground to pass through a 1-mm screen and added to 40-ml bottles (200 mg each) for further incubation. The sample incubation was conducted in quintuplicate (5 replications per treatment).

FB1 Preparation and determination

Commercially available FB1 (Trilogy Analytical Laboratories, Catalog number: TSL-202, Washington, DC, USA) was prepared as a 5 mg/l of FB1 solution using sterile deionized water. The FB1 concentration was then determined by a commercial cELISA kit (R-Biopharm, Darmstadt, Germany) according to Rodríguez-Cervantes *et al.* (2013).

Rumen and Substrate Inoculums

Rumen fluid and particulate matter were collected from three dairy cows. They were individually penned, had free access to water, and were fed with rice straw as a roughage source *ad libitum* for a week before rumen

fluid sampling by a suction pump. The rumen fluid samples were then readily processed for the *in vitro* fermentation according to the technique described by Makkar *et al.* (1995). The rumen inoculum was transferred into each sample bottle and incubated in a hot air oven at 39°C for 72 h.

Sample Collection and Analysis

Basal diet was analyzed for chemical compositions (dry matter, crude protein (CP), ether extract (EE), and ash) using the procedure of the Association of Official Analytical Chemists (AOAC, 1997) and neutral detergent fiber (NDF) as well as for acid detergent fiber (ADF; Van Soest *et al.*, 1991).

The gas production kinetics were recorded at 1, 2, 4, 6, 8, 12, 24, 48, and 72 h. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:

$$Y = a + b (1 - e^{(ct)})$$

where *a* equals the gas production from the immediately soluble fraction, *b* stands for the gas production from the insoluble fraction, *c* represents the gas production rate constant for the insoluble fraction, *t* represents the incubation time, (*a*+*b*) stands for the potential

extent of gas production, and *y* equals the gas production at *t*. At 24 and 48 h post inoculation, a set of samples was determined *in vitro* true digestibility according to Van Soest and Robertson (1985). Effective gas production potential (EP) was also calculated according to Ørskov and McDonald (1979):

$$EP = a + [bc/(k + c)]$$

where *k* = 0.05.

Ruminal Fermentation

Ammonia Nitrogen, Volatile Fatty Acids, and Bacterial Population

After 24 and 48 incubations, the ruminal inoculums were collected and then analyzed for ammonia nitrogen (NH₃-N) and volatile fatty acids (VFAs). Briefly, 30 mL of each ruminal inoculum fluid were mixed with 1 M H₂SO₄ and subsequently centrifuged at 15,000× *g* for 15 min. Samples were then analyzed separately for NH₃-N using the micro-Kjeldahl methods (AOAC, 1990) and for VFAs with high performance liquid chromatography (model RF-10AXmuGiL; Shimadzu, Japan) according to the protocol described by Mathew *et al.* (1997).

The ruminal inoculums were also used for the quantitation of targeted fibrolytic bacteria including *F. succinogenes*, *Ruminococcus*

Table 1. Ingredients and chemical compositions of the basal diet

Items	Concentrate	Rice straw
Ingredients (% of dry matter)		
Cassava chip	45.0	
Soybean meal	17.0	
Maize	10.0	
Palm kernel meal	8.0	
Rice bran	5.0	
Soybean hulls	5.0	
Molasses	6.7	
Mineral premix	0.5	
Urea	1.8	
Salt	0.5	
Sulfur	0.5	
Chemical compositions		
Dry matter (DM, %)	91.9	94.3
	----- % of dry matter-----	
Organic matter (OM)	83.2	83
Ash	7.8	12.5
Crude protein (CP)	22.1	2.50
Ether extract (EE)	3.90	2.10
Neutral detergent fiber (NDF)	21.1	81.4
Acid detergent fiber (ADF)	11.7	50.8
Acid detergent lignin (ADL)	3.6	5.9

albus and *B. fibrisolvens* via quantitative PCR and fluorescence detection of SYBR green mix by allowing reactions with cycling parameters (Table 2) as described by Wongnen *et al.* (2017). The community DNA of the selected bacteria were prepared from 500- μ l rumen fluid using the repeated bead beating plus column method (Yu and Morrison, 2004) and measuring the genomic DNA concentration by BioDrop (Isogen Life Science, USA) thereafter. The species specific 16sDNA gene primers used in this experiment were confirmed using in-silico techniques with the Basic Local Alignment Search Tool program in the Gene-Bank Database of the National Center for Biotechnology Information by our colleagues (Suphrap *et al.*, 2017; Wongnen, *et al.*, 2017).

Statistical Analysis

The data were statistically analyzed according to CRD by ANOVA using the general linear model procedure (SAS, 1998). Duncan's New Multiple Range Test was applied for the treatment mean comparisons (Steel and Torrie, 1980). P-values lower than 0.05 were considered significant.

Results and Discussion

The Chemical Composition of Basal Diet

The chemical compositions of concentrate and rice straw are listed in Table 1. In particular, the rice straw contained 2.5% CP, 2.1% EE, 83.0% OM, 81.4% NDF, 50.8 % ADF and 5.9% ADL of dry matter, respectively, which was in accordance with a previous report (Kang and Wanapat, 2013) and contained 2.3% CP, 87.4% OM, 74.1% NDF, and 58.5 % ADF of dry matter basis. The concentrate was also formulated to contain 22.1% CP, 2.1% EE, 83.0% OM, 21.1 % NDF, 11.7 % ADF, and 3.6% ADL of dry matter as shown in Table 1.

Gas Production Kinetics and *in vitro* Digestibility

The resulting gas production from the immediately soluble fraction and insoluble fraction as well as the gas production rate constant for the insoluble fraction were significant among treatments ($P < 0.01$, Table 3; Figure 1). The potential extent of gas production was similar between treatments (Table 3, $P = 0.15$), but EP was significantly different between treatments (Table 3, $P < 0.01$).

Table 2. Primer sequences used to quantify the targeted fibrolytic bacteria by qPCR

Target bacterial	Primer sequences (5'-3')	Annealing Temperature (°C)	Product size (bp)
<i>B. fibrisolvens</i>	F- AAAGCTCTATCAGCAGGGAA R- GTAAATCCGGATAACACTTG	55.2	126
<i>R. albus</i>	F- GCCTACTGGGCTTTAACTGA R- CCCACACCTAGTAATCATCG	50.8	103
<i>F. succinogenes</i>	F- CAACCCACGTTTCAGTT R- TGTGTAGCCCAGGATGTAA	56.9	113

F = Forward, R – Reverse, (Suphrap *et al.*, 2017)

Table 3. Effects of Fumonisin B1 (FB1) and yeast cell wall on *in vitro* gas production of dietary treatments

Items	Treatments				SEM	P-value
	T1	T2	T3	T4		
Gas production						
A	2.2 ^a	0.94 ^b	0.94 ^b	0.89 ^b	0.19	<0.01
B	61.0 ^c	61.6 ^{bc}	64.0 ^a	63.0 ^b	0.42	0.02
C	0.047 ^a	0.041 ^b	0.043 ^b	0.042 ^b	<0.01	<0.01
D	63.2	62.6	64.9	63.9	0.39	0.15
Effective gas production potential (EP)						
	31.9 ^a	28.3 ^c	30.5 ^b	29.6 ^{bc}	0.39	<0.01

^{a,b,c}Means in the same row with different superscript differ ($P < 0.05$). A = the gas production from the immediately soluble fraction, B = the gas production from the insoluble fraction, C = the gas production rate constant for the insoluble fraction, D = the potential extent of gas production, $EP = A + [BC/(k + C)]$, where $k = 0.05$ (Ørskov and McDonald, 1979), T1 = Control group (diet), T2 = Diet with 0.5 μ g/mg FB1 added, T3 = Diet with 0.5% yeast cell wall added, T4 = Diet with 0.5 μ g/mg FB1 and 0.5% yeast cell wall added.

The gas production from the immediately soluble fraction and the gas production rate constant for the insoluble fraction of the diet plus FB1 and yeast cell wall samples were significantly lower than the control ($P < 0.01$). The results indicated that FB1 and yeast cell wall affected the rumen fermentation. In particular, FB1 interfered with ruminal bacteria activity as gases normally produced by the rumen microbes.

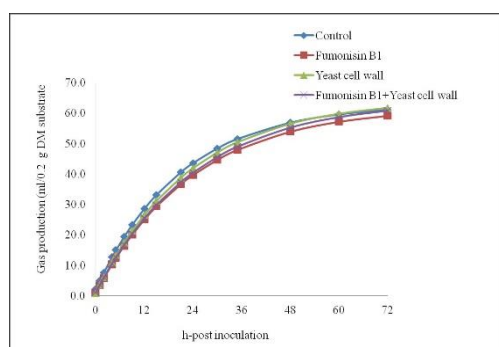


Figure 1. Effect of Fumonisin B1 (FB1) and yeast cell wall on gas production.
T1 = Control group (diet), T2 = Diet with 0.5 µg/mg FB1 added, T3 = Diet with 0.5% yeast cell wall added, T4 = Diet with 0.5 µg/mg FB1 and 0.5% yeast cell wall added.

The gas production from the insoluble fraction in the yeast cell wall supplement treatment was higher than the other treatments ($P < 0.05$). This result suggests that rumen microbes can digest yeast cell wall as an additional substrate (Oeztuerk *et al.*, 2016) because of the monoprotein and glucan portion of the cell wall (Lipke and Ovalle, 1988). Moreover, an increased bacterial population in

the rumen may involve the action of yeast cell wall. Therefore, yeast cell wall supplementation may alleviate FB1 effects and enhance the gas production from diet degradation (Lila *et al.*, 2004).

The effect of the yeast cell wall and FB1 on *in vitro* digestibility is shown in Table 4. No differences were found between treatments for *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) at 24 and 48 h post-inoculation, suggesting that FB1 did not affect microbial efficiency. The addition of yeast cell wall did not significantly affect IVDMD. In relation to a previous study, the underlying mechanism could not be clarified (Oeztuerk *et al.*, 2016).

Effect on VFA and NH₃-N concentrations

The results of the yeast cell wall and FB1 treatments on the VFAs and NH₃-N concentrations are shown in Table 5. The total VFAs, acetic acid (C2), propionic acid (C3), butyric acid (C4), and C2:C3 ratio were not affected by either yeast cell wall supplementation or FB1 after 24 and 48-h incubations when compared with the control ($P > 0.05$, Table 5). By contrast, the NH₃-N concentration was significantly higher in response to FB1 than the other groups at 24 and 48 h after incubation ($P < 0.05$). This could be explained by FB1 possibly interfering with microbial activities and hence, retarding the NH₃-N uptake by ruminal microbes. Theoretically, the optimal NH₃-N concentration for microbial growth and activity would be 5-25 mg% (Preston and Leng, 1987). Pengpeng and Tan (2013) also noted that an optimal ruminal NH₃-N concentration for microbial growth varying from 2.5 mM to 18 mM. In response to yeast cell wall supplementation, the NH₃-N

Table 4. Effects of Fumonisin B1 (FB1) and yeast cell wall on *in vitro* digestibility

Items	Treatments				SEM	P-value
	T1	T2	T3	T4		
<i>In vitro</i> dry matter digestibility (IVDMD), %						
24h	55.0	53.4	55.1	52.8	0.58	0.49
48h	62.6	65.6	63.8	63.6	0.55	0.48
<i>In vitro</i> organic matter digestibility (IVOMD), %						
24h	60.1	58.2	60.1	58.4	0.52	0.29
48h	67.6	70.5	69.5	68.8	0.51	0.21

^{a,b,c}Means in the same row with different superscript differ ($P < 0.05$), T1 = Control group (diet), T2 = Diet with 0.5 µg/mg FB1 added, T3 = Diet with 0.5% yeast cell wall added, T4 = Diet with 0.5 µg/mg FB1 and 0.5% yeast cell wall added.

Table 5. Effects of Fumonisin B1 (FB1) and yeast cell wall on volatile fatty acids and ammonia nitrogen

Items	Treatments				SEM	P-value
	T1	T2	T3	T4		
Total VFAs (mmol)						
24h	88.4	87.1	79.4	86.9	2.39	0.65
48h	92.9	98.4	98.3	92.8	1.61	0.50
Acetic acid (C₂, mol/100 mol)						
24h	49.6	48.7	50.2	51.2	1.13	0.94
48h	51.3	52.5	53.0	50.9	0.6	0.67
Propionic acid (C₃, mol/100 mol)						
24h	37.3	38.9	39.4	37.21	0.96	0.86
48h	37.3	36.8	35.4	38.0	0.55	0.50
Butyric acid (C₄, mol/100 mol)						
24h	13.1	12.4	10.4	11.7	0.43	0.10
48h	11.4	10.7	11.6	11.2	0.3	0.84
C₂:C₃ ratio						
24h	1.30	1.25	1.30	1.40	0.06	0.92
48h	1.38	1.43	1.50	1.34	0.04	0.54
NH₃-N, mg%						
24h	14.0 ^{bc}	17.0 ^a	12.2 ^c	15.8 ^{ab}	0.74	0.03
48h	18.8 ^b	20.0 ^a	18.2 ^b	18.2 ^b	0.93	0.01

^{a,b,c}Means in the same row with different superscript differ (P<0.05), T1 = Control group (diet), T2 = Diet with 0.5 µg/mg FB1 added, T3 = Diet with 0.5% yeast cell wall added, T4 = Diet with 0.5 µg/mg FB1 and 0.5% yeast cell wall added.

Table 6. Effects of Fumonisin B1 and yeast cell wall on the fibrolytic bacterial population using qPCR

Items	Treatments				SEM	P-value
	T1	T2	T3	T4		
<i>Fibrobacter succinogenes</i> (×10 ⁷ copies/ml)	1.18 ^b	0.58 ^c	1.37 ^a	1.11 ^b	0.11	<0.01
<i>Ruminococcus albus</i> (×10 ⁶ copies/ml)	0.84	0.74	1.23	0.88	0.08	0.11
<i>Butyrivibrio fibrisolvens</i> (×10 ⁷ copies/ml)	1.62 ^a	1.48 ^b	1.64 ^a	1.58 ^a	0.02	0.02

^{a,b,c}Means in the same row with different superscript differ (P<0.05), T1 = Control group (diet), T2 = Diet with 0.5 µg/mg FB1 added, T3 = Diet with 0.5% yeast cell wall added, T4 = Diet with 0.5 µg/mg FB1 and 0.5% yeast cell wall added.

concentration was lower than the control in this trial. This was inconsistent with previous studies (higher, lower or no change), which may be due to differences in supplement patterns (single or multiple), yeast preparations, diet types, and/or experimental conditions (Oeztuerk *et al.*, 2016).

Rumen Bacterial Population

F. succinogenes, *R. albus*, and *R. fibrisolvens* were selected for this trial because they mainly involved fibrolytic activities in the rumen (Krause *et al.*, 2003; Morgavi *et al.*, 2013). The relationship between FB1 and yeast cell wall on the fibrolytic bacterial populations in the *in vitro* incubation is shown in Table 6. As a result, the *F. succinogenes* and *B. fibrisolvens* populations in the FB1-treated group were

significantly lower than in the control group (P<0.05). Among the targeted bacteria, *F. succinogenes* was highly affected by FB1 with an approximately 51% lower population than the control without any clarified mechanism. Conversely, the yeast cell supplement enhanced the *F. succinogenes* population by about 58% and 16% than the FB1-added and the control groups, respectively, which was similar to a previous live yeast model (Chaucheyras-Durand *et al.*, 2016). Our results demonstrated the advantage of yeast cell wall additive on the harmful effects of FB1 regarding the targeted bacteria. However, the yeast cell wall seemed to insignificantly promote *R. albus* growth with a 12% and 40% higher population than the FB1-contaminated and the control groups,

respectively. Furthermore, the yeast cell wall did not influence *B. fibrisolvens* abundance as compared with the control, which was in accordance with a previous report using live *S. cerevisiae* yeasts (Chaucheyras-Durand *et al.*, 2016). This may be due to the different growth requirements of *B. fibrisolvens*, in relation to either different physiologic states or strain diversity (Forster *et al.*, 1996; Willems *et al.*, 1996).

Detoxification

The detoxification efficiency of *S. cerevisiae* viable cells or cell wall against FB1 has been documented (Pizzolitto *et al.*, 2012; Chlebicz and Śliżewska, 2020). However, the research articles in relation to the FB1 detoxification effectiveness of *S. cerevisiae* cell walls were quite limited, as most studies focused on the binding activity of viable cells, which are theoretically higher than nonviable cells or yeast cell wall (Abidin and Khatoon, 2012; Pizzolitto *et al.*, 2012). As a potential feed additive supporting FUM removal, *S. cerevisiae* viable cells can detoxify FB1 and FB2 mixture in animal feed with an average 72% decrease of the initial concentration after 24 h of incubation (Chlebicz and Śliżewska, 2020). In agreement with previous reports, the FB1 concentration was about 44% and 50% lower in yeast cell wall supplemented samples after 24 and 48-h incubation in this model (data not shown). In contrast to the yeast viable cells, the cell wall structural integrity of nonviable cells might explain the impartiality of the difference. The fumonisin-microorganism interaction could also enhance detoxification activities (Pizzolitto *et al.*, 2012). At least, we have demonstrated that yeast cell walls could decrease the FB1 concentrations in animal feed *in vitro*.

Conclusions

This experiment demonstrated that FB1 had a negative impact on *in vitro* gas production performance, rumen fermentation and major fibrolytic bacteria populations. Nevertheless, yeast cell wall supplement could potentially

alleviate the adverse FB1 effects on the fibrolytic bacteria *in vitro*.

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