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INFLUENCE OF *LEVILACTOBACILLUS BREVIS* ON GROWTH PERFORMANCE OF WEANED PIGLETS UNDER FUMONISIN B_1 CHALLENGES

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ABSTRACT

Piglets are highly susceptible to fumonisin B_1 (FB_1) toxicity. The study aimed to investigate the influence of probiotic Levilactobacillus brevis (L. brevis) on growth performance, body weight gain (BWG) and feed conversion ratio (FCR) of weaned piglets under FB_1 challenges. Twenty-six piglets with an initial body weight of 11.03 ± 1.32 kg were randomly assigned to four dietary treatment groups for 28 days: control (C, commercial diet), control feed supplemented with individual probiotic L. brevis (Lb), 50 ppm FB_1 -contaminated control diet (F), and 50 ppm FB_1-contaminated control diet supplemented with L. brevis (FLb). Individual FI was recorded daily while body weight was weekly. Statistical analysis was conducted using the R program (R 4.4.1) to test for normality, then T-test and Wilcoxon tests depending on the distribution. Results showed no significant difference in the FI of different groups, but pigs in F group had somewhat lower gain and higher FCR than the pigs in C treatment ($p > 0.05$). A combination of L. brevis with FB_1 showed a significant reduction in BWG and an increase in FCR at the end of the supplemental period compared to all treatments ($p < 0.05$) suggesting a complex interaction. These are preliminary findings, further histological, biochemical and microbiota analysis will elucidate the mechanisms underlying the complex interactions affecting growth performance.

INTRODUCTION

Fumonisins are produced by *Fusarium verticillioides* and *Fusarium proliferatum* pathogens with fumonisin B_1 (FB_1) having been reported as 70–80% most common type of fumonisin, collected in field samples and the most toxic (Szécsi et al., 2010). The FBs

toxicity mechanism is associated with the free amino group and the tricarballylic acid side groups; therefore, their removal can significantly reduce cytotoxicity and phytotoxicity (Voss et al., 2007). Detoxification or elimination of mycotoxins by technological treatments is complex due to their heat-chemical stability, their resistance to storage and processing conditions (Temba et al., 2016).

Probiotics are live, non-toxigenic, non-pathogenic and fermentative microorganisms with health-promoting characteristics which when administered in adequate amounts in commercial products can confer health benefits to the consuming animal (Dalié et al., 2010). They include; *Saccharomyces cerevisiae*, *Bifidobacterium*, *Lactobacillus* and *Bacillus* species (Dalié et al., 2010). Studies have reported that the cell surface of probiotics has toxin binding ability (Sangsila et al., 2016). For instance, the lactic acid bacteria (LAB) and yeasts particularly *Saccharomyces* genus, can remove mycotoxins through biodegradation or surface adsorption (Dalié et al., 2010). The cell wall structure of LAB consists of thick, multilayered peptidoglycan sacculi with glycopolymers including; S-layer proteins, teichoic acids and polysaccharides (Chapot-Chartier and Kulakauskas, 2014). The amino acid sequence of the peptide bridges of the peptidoglycan and the negatively charged functional groups of S-layer proteins is reported to enhance the binding efficiency of the LAB species (Niderkorn et al., 2009). Piglets are highly vulnerable to mycotoxins due to the high percentage of cereals in their diet. In most cases, their feed is based on corn as it has low to nearly no anti-nutritional compound levels. Additionally, piglets are more sensitive as they have a limited gastrointestinal microbiome, reduced capacity of hepatic enzymes for detoxification, and immature immune system and the developing intestinal epithelium has higher gut permeability. Several studies have evaluated the potentially deleterious attributes of growth performance effects of FB_1 in pigs and have reported intestinal barrier dysfunctions (Bouhet & Oswald, 2007), a reduction in nutrient digestion and absorption efficiency in the gut (Lessard et al., 2009).

Recently, it has been reported that biological control of FB_1 using lactic acid bacteria is a promising approach because of their microbial antagonist effect on the toxin (Khalil et al., 2015; Niderkorn et al., 2009). However, *Levilactobacillus brevis* has been less studied and limited data is available on their mycotoxin binding ability, than other LAB probiotic strains. The study therefore investigates the influence of *L. brevis* (AT-2076) on the growth performance of weaned piglets under FB_1 exposure.

MATERIALS AND METHODS

The experiment was carried out according to the regulations of the Hungarian Animal Protection Act, the license was issued by Somogy County Governor's Office (SO/31/00764-10/2023). Twenty-six castrated male Danbred weaned pigs at 4 weeks of age, weighing an average of 11.03 ± 1.32 kg were allowed a 2-week physiological acclimatization period. The pigs were kept in individual metabolic cages (80 x 80 cm) at the experimental animal unit of the Department of Farm Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Kaposvár Campus. At 6 weeks of age, the pigs were randomly assigned in a 2 x 2 factorial design to four dietary treatment groups (n = 6 or 7) for 28 days: control (C, commercial diet), control feed supplemented with

individual probiotic *L. brevis* AT-2076 (Lb; Pertovics et al., 2019), 50 ppm FB_1 -contaminated control diet (F), and 50 ppm FB_1-contaminated control diet supplemented with *L. brevis* AT-2076 (FLb). The commercial diet was a mixed ration of corn, soybean meal, sunflower oil, barley, additives, mineral and vitamin premix. The pigs were weighed individually with gram precision at the beginning of the trial and weekly intervals. The pigs were fed twice a day on equal proportions, 13.4 MJ/kg ME per kg of feed offered as an amount that provided 3 times the maintenance energy while water was provided *ad libitum*. The feed refusal was measured and reported daily to calculate the feed intake and feed conversion. The health status of the pigs was monitored daily throughout the trial period. The room temperature was adjusted according to the needs of the nursery pigs.

The fungal strain *Fusarium verticillioides* (MRC 826) was used for fumonisin production. The finely ground fungal culture was mixed into the ration of the experimental animals to provide a daily feed concentration of 50 ppm FBs. Statistical analysis of BWG, FI and FCR data was conducted using the R program (R 4.4.1) to test for normality. Results were subjected to parametric (T-test) in case of normal distribution and non-parametric (Wilcoxon tests) where the distribution was not normal. A *p*-value of ≤ 0.05 was regarded as significant.

RESULTS AND DISCUSSION

The effect of dietary treatments on the growth performance of piglets is shown in Table 1.

Table 1 Effects of *L. brevis* (AT-2076) on growth performance of weaned piglets under FB_1 exposure

Group	Treatments			
	C	F	Lb	FLb
Initial BW (kg)	10.99 ± 0.53[a]	11.00 ± 0.49[a]	10.67 ± 0.49[a]	11.47 ± 0.64[a]
Final BW (kg)	25.81 ± 9.32[a]	24.95± 8.52[a]	25.33 ± 8.21[a]	24.75 ± 7.64[a]
Total FI (kg)	17.62 ± 3.48[a]	17.24 ± 4.17[a]	17.35 ± 4.77[a]	17.82 ± 1.78[a]
Total WG (kg)	14.83 ± 0.61[a]	13.95 ± 0.4[ab]	14.66 ± 0.53[a]	13.28 ± 0.43[b]
Total FCR	1.20 ± 0.04[a]	1.24 ± 0.02[ab]	1.19 ± 0.03[a]	1.35 ± 0.04[b]

[ab] Means in a row with different superscripts are significantly different ($p < 0.05$), BW = Body weight, WG = Weight gain, FI = Feed intake, FCR = Feed conversion ratio, C = control, F = 50 ppm FB_1,Lb = individual *L. brevis* AT-2076, FLb = 50 ppm FB_1 plus *L. brevis* AT-2076

Across all treatment groups, there was a progressive and consistent increase in feed consumption showing a typical growth-related feeding behavior. The daily feed rations were defined on a BW basis, the insignificant ($p > 0.05$) treatment effect indicates that there was no feed refusal and neither the FB_1 exposure nor *L. brevis* supplementation significantly influenced the appetite of the animals and feed palatability. The restricted feeding ensured a standardized level of *L. brevis AT-2076* and FB_1 intoxication across treatment groups and allowed to evaluation of the treatment effects without interference from their unequal uptake by the piglets.

Piglets that received the control feed supplemented with individual probiotic *L. brevis AT-2076* (Lb), recorded numerically the lowest FCR, suggesting a slightly better feed and growth efficiency in toxin-free conditions, but it was not statistically proven ($p > 0.05$). In

a previous study Liu et al. (2015) confirmed an increased gain: feed and average daily gain in weaned piglets supplemented with 0.4 and 0.8 g/kg of *L. brevis.* The findings ascertain the probiotic's potential of *L. brevis* as a growth-promoting additive, but the effectiveness may depend on the specific strain. The F group that was exposed to FB1 alone increased somewhat the FCR indicating reduced nutrient utilization, however, again the effect was not confirmed by statistics ($p > 0.05$). The results may be explained by a reduced nutrient absorption efficiency in FB1-contaminated feed confirmed in in pigs (Lessard et al., 2009). Combining L. brevis AT-2076 and FB1 (FLb) group, resulted to a significant increase in FCR compared to all treatments (C vs FLb; F vs FLb; $p < 0.01$) (Lb vs FLb; $p < 0.05$), implying that the efficacy of L. brevis AT-2076 was compromised under FB1 exposure.

Piglets that were exposed to FB_1 alone (F) group reduced total BWG numerically ($p > 0.05$) 24.95 ± 8.52. Rao et al. (2020a) reported decreased average daily gain and gain: feed in nursery pigs that were exposed to fumonisin at a concentration of 60 mg/kg for 14 days and 30 mg/kg for 28 days respectively. Reduced growth performance is a potential deleterious effect of FBs toxicosis in pigs as it may reduce nutrient digestion and absorption efficiency in the gut (Lessard et al., 2009). The tendency was also similar in weekly results (data not shown). That is, an increase in FCR and lowest BWG was observed in the F group from day 0 to 21, with days 14 to 21 significantly different (C vs F; $p < 0.01$). Surprisingly, in the last week of the study (days 21 to 28), the F group demonstrated the lowest FCR and highest BWG suggesting that pigs might be able to adapt to FB_1 on prolonged exposure. In another study, nursery pigs exposed to 32.7-35.1 mg/kg fumonisin showed some recovery of growth performance between days 14 to 28 of the study which slightly matches our findings (Rao, et al., 2020b). However, the adaptive mechanism is unknown.

The combination of FB_1 and *L. brevis AT-2076* (FLb) group reported significant reductions in total BWG compared to all treatment groups (F vs FLb; $p<0.01$; C vs FLb and Lb vs FLb; $p < 0.05$) suggesting that *L. brevis* may exacerbate some toxic effects of FB_1 or it's beneficial properties may suppress under FB_1 exposure. The findings were inconsistent with other studies of *L. brevis* which have reported its protective effects against pathogens by slowing down the weight loss induced by *Yersinia enterocolitica* and *Salmonella typhimurium* in a mouse model (Shi et al., 2022, 2024). In another study, two probiotic strains of lactic acid bacteria were able to counteract the negative effect of FB_1 on body weight gain of Sprague-Dawley rats which were exposed to between 50 and 200 mg/kg FB_1 (Khalil et al., 2015). Therefore, the FLb group results highlighted a complex interaction between FB_1 toxicity and *L. brevis* in pigs.

Since the results were also assessed by week (data not shown), it was noted that the complex interaction in the FLb group showed between days 21 to 28. Although the mechanism behind the interaction remains unclear, FB_1 toxicity primarily disrupts sphingolipid metabolism which affects cellular signalling and intestinal barrier functions which impairs nutrient absorption (Bouhet & Oswald, 2007; Voss et al., 2007). Alternatively, L. *brevis* may increase the bioavailability of FB_1 metabolites (Niderkorn et al., 2009) or alter gut microbiota dynamics in ways that unintentionally amplify FB_1 toxicity

which in turn may have a negative influence on the growth performance of weaned piglets under FB_1 exposure.

CONCLUSION AND RECOMMENDATION

In conclusion, combining FB_1 with *L. brevis* (AT-2076) influenced more negatively the BWG and FCR than the toxin alone indicating reduced probiotic effectiveness under FB_1 exposure. The deleterious effects of individual FB_1 and complex interaction in a combination of FB_1 and *L. brevis* on FCR and BWG implies that the effects were either physiological or metabolic disruptions rather than behavioural as feed intake was consistent across treatments.

These are preliminary findings, thus biochemical, histological and microbiota analysis will help to understand the complex interaction and clarify the observed growth effects.

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