FumDSB can alleviate the inflammatory response induced by fumonisin B₁ in growing pigs

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ABSTRACT
Fumonisin B₁ (FB₁) has the highest natural contamination rate among all fumonisin analogs and can inhibit food intake and weight gain of pigs. Under laboratory conditions, carboxylesterase FumDSB has a high FB₁ degradation rate and excellent pH and thermal stability. The present study sought to estimate the effects of FumDSB on growing pigs from the perspective of a brain–intestinal axis. Twenty-four growing pigs of similar weight were divided into Control, FB₁ (5 mg FB₁/kg feed), and FumDSB (5 mg FB₁/kg and 0.1% FumDSB in the feed) groups. After 42 days of feeding, hypothalamus and jejunum samples were collected for quantitative real-time fluorescence, western blotting, and immunohistochemistry. The results showed that FB₁ consumption can destruct the tissue structure of hypothalamus and jejunum, affect the expression and distribution of several appetite-related neuropeptides and inflammatory cytokines, thereby inducing neuroinflammatory responses and affecting food intake and weight gain. However, these anorexia effects and inflammatory responses are alleviated when FumDSB is added to the feed. In short, FumDSB can alleviate the inflammatory response induced by FB₁ in growing pigs.

ARTICLE HISTORY
Received 25 May 2022
Accepted 4 July 2022

KEYWORDS
Fumonisin B₁; carboxylesterase; brain–gut regulation; inflammatory cytokines; anorexia

Introduction
Fumonisins are water-soluble secondary metabolites produced by Fusarium spp., primarily Fusarium verticillioides, Fusarium moniliforme, and Fusarium proliferatum, and are widely found in corn, wheat, sorghum, and related processing by-products worldwide (Rodrigues and Naehrer 2012). To date, 28 types of fumonisins have been isolated and can be divided into four major groups: A, B, C, and P (Ponce-García et al. 2018). Fumonisin B (FB) analogs, including FB₁, FB₂, and FB₃, have the highest levels of contamination in nature, particularly FB₁, which represents 70%–80% of the naturally occurring amount (Kamle et al. 2019). Owing to its high contamination rate and high toxicity, fumonisin toxicological research has been focused on FB₁. According to previous reports, the toxic effects of FB₁ primarily include neurotoxicity, immunotoxicity, reproductive toxicity, mammalian cytotoxicity, and organ toxicity (Scott 2012; Pitt and Miller 2017; Sousa et al. 2022). Acute-induced poisoning owing to higher doses of FB₁ is extremely rare in today’s modern farming conditions. Nevertheless, several negative effects have been observed following the intake of lower FB₁, with the inhibitory effect of FB₁ on feed intake and weight gain gaining remarkable attention (Prodanov-Radulovic et al. 2014; Truby et al. 2020).

Appetite has long been recognized to be controlled by bidirectional communication between the brain and gut. Multiple signaling molecules can be expressed in the central nervous system (CNS), thereby promoting communication between the nervous system and gastrointestinal tract and regulating immune functions in the intestine directly and indirectly (Clemmensen et al. 2017). Inflammatory cytokines, mainly
interleukin (IL), tumor necrosis factor (TNF), and interferon (IFN), are released after the activation of innate immune defense response by pathogens or other harmful substances (Griffiths et al. 2009; Guillemot-Legris and Muccioli 2017). These cytokines can then act directly or indirectly on the hypothalamus and other nerve centers, thereby reducing appetite and interfering with gastrointestinal functions by regulating the secretion of neuropeptides, such as agouti-related peptide (AgRP), melanocortin-4 receptor (MC4R), Signal Transducer and Activator of Transcription 3 (STAT3), and pro-opiomelanocortin (POMC) (Dent et al. 2012). Appetite suppression caused by cytokines may be the main cause of cachexia in chronic diseases and part of the body’s acute response to various immune stimuli (Paulsen et al. 2017; Avila-Carrasco et al. 2019). Several in vivo and in vitro studies have revealed that FB1 can interfere with the expression of inflammatory cytokines in animals, further causing inflammation reactions and reducing immune function (Wu et al. 2014; Li et al. 2020). Meanwhile, fumonisins can damage the nervous system (Domijan 2012; Rudyk et al. 2020). Although decreased appetite caused by FB1 may be regulated by the brain and gastrointestinal tract, few studies have sought to assess the relationship between anorexia caused by FB1 and the two organs closely related to appetite: the hypothalamus and jejunum.

Monogastric animals are generally more vulnerable to mycotoxins as they lack microbiota that degrade mycotoxins (Li et al. 2017). Compared to other farmed livestock, pigs are more susceptible to FB1 in their diet owing to the high-proportion of corn in feed formulations and typical gastrointestinal characteristics of monogastric animals (Jakić et al. 2019). Besides causing liver lesions and pulmonary edema, FB1 consumption by pigs reduces feed conversion efficiency and weight gain, induces intestinal dysfunction and inflammatory responses, and increases intestinal susceptibility to infection (Bracarense et al. 2012; Pierron et al. 2016). As many health problems in growing and fattening pigs are directly or indirectly related to the high contamination rate of mycotoxins in the complete feed, scientists and farmers have been interested in identifying effective methods to reduce or eliminate the toxicity of mycotoxins. Multiple physical and chemical methods have been employed in practical applications to destroy the nutritional content of feed and reduce the palatability, thereby affecting the production performance of livestock (Wu et al. 2021). Biodegradation, which uses living microorganisms or biological enzymes to convert mycotoxins into low-toxicity or non-toxicity metabolites, has a higher conversion efficiency and safety (Chen et al. 2020; Li et al. 2021a).

To date, numerous living bacteria or biological enzymes have been confirmed to reduce or eliminate the toxic effects of FB1 (Alberts et al. 2019; Zhao et al. 2019). FumDSB, a novel carboxylesterase derived from Sphingomonas, can degrade toxic FB1 to non-toxic hydrolyzed fumonisin B1 (HFB1) by removing the two tricarboxylic acid (TCA) side chains of FB1, thereby reducing FB1 toxicity. Under laboratory conditions, the rate of degradation of FB1 by pure FumDSB can reach 100% (Li et al. 2021b). FumDSB can theoretically perform biochemical functions under animal physiological conditions. Thus, to explore the effects and safety of FumDSB in vivo, further animal experiments must be conducted. The present study aimed to determine the effects of FumDSB on neuropeptides and inflammatory cytokines related to appetite regulation and their safety on growing pigs.

Material and methods

Information of degrading enzyme FumDSB

FumDSB (Patent No: ZL201811171697.3) is a novel carboxylesterase capable of degrading fumonisins, which is derived from Sphingomonas. FumDSB gene encodes 511 amino acids and a stop codon, thereinto, the first 16 amino acids are signal peptides. Therefore, the theoretical molecular weight of pure FumDSB is 54 kDa. Specific sequence and preparation information has been reported by Li et al. (2021b).

Materials and supplies

FB1 standards with a chromatographic purity of 98% used in this study were purchased from...
Triplebond Co. (Guelph, ON, Canada). Trizol (Lot.15596026) and chloroform (Lot.13200) were purchased from Thermo Fisher (Massachusetts, USA), isopropanol (Lot.I112018) and anhydrous ethanol (Lot.E111991) were purchased from ALADDIN (Shanghai, China). Sodium citrate buffer (Lot. C1032) were purchased from Solarbio (Beijing, China), and Immunohistochemistry Two-Step Kits (PV-9000) were obtained from ZSBIO (Beijing, China). In this study, the target proteins IL-2, IL-6, IL-8, TNF-α, POMC, and nuclear transcription factor-κB (NF-κB) were purchased from Proteintech Co. (Hubei, China), whereas phosphate-NF-κB was purchased from Abcam Co. (Cambridge, Massachusetts, UK). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were acquired from Proteintech (Hubei, China). In addition, the Fusion imaging system was supplied by Fusion FX (Vilber, Paris, France), whereas Image-Pro Plus 6.0 software was supplied by Media Cybernetics (Silver Spring, MD, USA).

Feed, animals, and sampling

After 1 g FB₁ standard was completely dissolved in 1 L of ethyl acetate, the mixed liquor was sprayed uniformly on the 1 kg of talc carrier and left for 12 h in order to volatilize the ethyl acetate. Talc carrier containing FB₁ was mixed into normal corn meal to prepare toxic corn meal containing 10 mg/kg FB₁, which was used to replace the corn meal used in the experimental feed formula.

The basic diet employed in the present animal feeding experiment was prepared according to recommendations by the NRC (1998). Table 1 lists the composition and nutrients in the pig feed.

| Ingredients (100%) | Nutrient levels (%)² |
|--------------------|----------------------|
| Corn flour 66.0    | Crude protein 17.4    |
| Soybean flour 21.0 | Calcium 0.78         |
| Millfeed 5.0       | Total phosphorus 0.52 |
| Extruded soybean 4.0 | Methionine + Cysteine 0.60 |
| Premix¹ 4.0        | Lysine 0.96          |
| Total 100          | ME (MJ/kg) 12.79     |

¹The premix provided the following in the diet (per kg): VA 12, 000 IU; VB 1.0 mg; VB₃ 3.7 mg; VB₆ 3 mg; VB₁₂ 0.02 mg; VO₃ 2, 000 IU; VE 40 mg; Niacin 15 mg; folic acid 0.6 mg; Choline 250 mg; Mn 40 mg; Fe 100 mg; Ca 2.1 g; Zn 100 mg; Cu 180 mg; I 0.30 mg; Se 0.30 mg; Co 1.0 mg.
²ME (Metabolizable energy) was a calculated value, while the others were measured values.

Histomorphological observation by hematoxylin and eosin (HE) staining

Tissue samples were removed from the 4% paraformaldehyde fixative solution and washed with running water. Thereafter, the samples were dehydrated in a processing box using a graded series of solutions with increasing ethanol concentrations. After treatment with xylene to
become transparent, these tissue samples were embedded in paraffin blocks. The tissue slices were stained with hematoxylin and eosin, and then dehydrated with ethanol and xylene. Finally, the histomorphology of the tissue samples was observed using a microscope.

**mRNA expression detected by quantitative real-time PCR (qRT-PCR)**

Frozen samples were extracted with Trizol, and total RNA was isolated and purified using chloroform, isopropanol, and anhydrous ethanol. The integrity of the extracted RNA was checked by agarose gel electrophoresis. After it was measured with an ultra-micro UV spectrophotometer, the concentration of RNA was diluted to 800–1,000 μg/mL for a subsequent reverse transcription test. GAPDH and β-actin, which served as double internal references, were used to verify the target gene products. Shanghai Sangon Biotech Co. constructed the primer sequences (Shanghai, China), which are shown in **Table 2**. More detailed steps were performed according to Wang et al. (2021).

**Protein expression detected by Western blot**

In this study, GAPDH (1:2,000) and β-actin (1:2,000) were used as an internal reference protein. The specific steps for western blot were performed according to the previous protocols described by Wang et al. (2021). Finally, the Fusion imaging system was applied to capture images, and Image-Pro Plus 6.0 software was used to analyse the optical density.

**Protein distribution observed by immunohistochemistry (IHC)**

Slides were pre-treated similarly to those prepped for HE staining. Xylene was used to deparaffinize the tissue slices, and gradient alcohol was used to downward dewaxing and benzene removal. Antigen was then restored using sodium citrate buffer, and endogenous peroxidase activity was blocked with 3% H2O2. In this experiment, primary monoclonal mouse antibodies against IL-2 (1:150), IL-6 (1:200), IL-8 (1:150), TNF-α (1:300), POMC (1:200), and phospho-NF-κB (1:500) were used. After incubating samples with the primary antibody overnight at 4°C, the secondary antibody was incubated using the Immunohistochemistry Two-Step Kit. The slices were photographed and examined under a microscope after being redyed with hematoxylin, dehydrated with a series of solutions with an increasing alcohol gradient, and sealed with neutral resin. Finally, Image-Pro Plus 6.0 software was used to quantitatively analyse the intensity of the positive reaction.

**Data statistics**

SPSS 21 statistics were used to analyse all test data in this study (IBM Co., New York, USA). One-way analysis of variance and least significant difference test were performed to compare the mean values of individual groups. Data are presented as means ± standard deviation. Statistically significant differences and extremely significant differences were indicated using \( p < 0.05 \) and \( p < 0.01 \), respectively.

**Results**

**Growing and economic parameter**

As shown in **Table 3**, when FB1 and FumDSB were added to the feed, the values of ADFI and ADG decreased significantly \( (p < 0.05) \) compared with those of the control, whereas F/G increased significantly \( (p < 0.05) \). Notably, when FumDSB

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**Table 2.** Primer sequences of the related genes used for qRT-PCR.

| Target gene | GenBank no. | Primer sequence (5′–3′) |
|-------------|-------------|------------------------|
| IL-2-F      | NM_213861.1 | CCCTTGACATCTAGGCAAAC   |
| IL-2-R      | NM_214399.1 | ACAGCAGTTAATGCTCAATCA   |
| IL-6-F      | NM_213867.1 | TTCAAGGTCAGTACGCGGA   |
| IL-6-R      | NM_213867.1 | ACCTGTGGCTCCACTTCAA   |
| TNF-α-F     | NM_000594.4 | GCCCTTCCACCAAGCTTTC   |
| TNF-α-R     | NM_000594.4 | AGGTGGCCCTCTGATGCA   |
| IFN-γ-F     | NM_213948.1 | AGCTTTCAGCTTGGTGA   |
| IFN-γ-R     | NM_213948.1 | TGGTCTCGTTGATGCTG   |
| NF-κB-F     | NM_001114281.1 | GCTATAACGCTGGTGACG   |
| NF-κB-R     | NM_001114281.1 | CCGCAATGAGGAGAAATCT   |
| STAT3-F     | NM_001044580.1 | TTGCAGTTGCTCCATCTC   |
| STAT3-R     | NM_001044580.1 | CACCTGACCCACGTCGGA   |
| AgRP-F      | NM_001011693.1 | AGGAGCTGACAGCAGGAGG   |
| AgRP-R      | NM_001011693.1 | GTGGCGCTGTGCGGAAAG   |
| POMC-F      | NM_213858.1 | GAAGCTGGCCTGCTGAGG   |
| POMC-R      | NM_213858.1 | TTTAACAGACGAGACGAGG   |
| MC4R-F      | NM_0005912.3 | GAAACAAAACCCGACACCG   |
| MC4R-R      | NM_0005912.3 | TCGAGTGAAACGAGATTGGG   |
| GAPDH-F     | NM_001206359.1 | TGGACAAGCTTCCGGTCTCC   |
| GAPDH-R     | NM_001206359.1 | TGGACAAGCTTCCGGTCTCC   |
was added as a detoxifying enzyme, the values of ADFI and ADG were significantly higher than those of pigs in the FB1 group ($p < 0.05$).

### Histomorphological observation by HE staining

Figure 1 show the representative morphological photographs of the hypothalamus stained with HE. Compared with the control, slight morphological abnormalities in the hypothalamus of pigs were observed, such as increased perivascular space, increased numbers of glial cells, and loose interstitial edema. The various injuries were relieved after addition of FumDSB as a degrading enzyme for FB1.

**Figure 1.** Typical hypothalamic morphology pictures in the three groups. Ctrl, FB1 and FumDSB refer to the different treatments. 40×, 100×, and 200× represent the microscopy magnifications used for microscopy. Parts of the abnormalities are indicated by black arrows.

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**Figure 2.** show the representative morphological photographs of the jejunum stained with HE. In the control group, the villi of the small intestine were found to be arranged neatly; villi epithelium and intestinal gland structure were intact. However, the jejunum’s intestinal villi epithelium was fragmented and showed histological lesions after FB1 administration. Inflammatory cell infiltration was

| Group       | Initial Weight (kg) | Final Weight (kg) | ADG (g)      | ADFI (kg) | F/G    |
|-------------|---------------------|-------------------|--------------|-----------|--------|
| Control     | 20.68 ± 1.21        | 49.75 ± 3.26a     | 692.26 ± 55.40a | 1.66 ± 0.15a | 2.39 ± 0.27a |
| FB1         | 20.80 ± 1.35        | 45.81 ± 3.61c     | 595.54 ± 57.27c | 1.56 ± 0.23c | 2.62 ± 0.42a |
| FumDSB      | 20.45 ± 0.75        | 47.53 ± 2.58b     | 644.76 ± 51.70b | 1.62 ± 0.21b | 2.50 ± 0.26b |

The values with different small letters in the same column differ significantly ($p < 0.05$).

**Table 3.** Production performance of growing pigs in the three groups (mean ± SD, n = 8).

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**Figure 1** and **Figure 2** refer to the representative morphological photographs of the hypothalamus and jejunum stained with HE, respectively. The microscopic magnifications used are 40×, 100×, and 200×.
also observed in the intestinal glands and nearby connective tissues. Furthermore, compared with that of the FB1 group, the small intestine villi of the FumDSB group had more integrity, accompanied by a reduced number of inflammatory cells.

**mRNA expression of neuropeptides detected by qRT-PCR**

The qRT-PCR results indicated that the mRNA expression levels of MC4R, POMC, and STAT3 in the hypothalamus and jejunum samples were increased after the intake of FB1 and significantly higher than those in the control group ($p < 0.05$). In contrast, the expression level of AgRP was lower ($p < 0.05$). Of note, the MC4R expression levels were increased by 25-fold in the hypothalamus relative to that of the control, but only by 3.97-fold in the jejunum. The expression levels of MC4R, POMC, and STAT3 in the hypothalamus and jejunum were significantly lower in the FumDSB group than those in the FB1 group ($p < 0.05$); however, there was no significant difference in the expression of AgRP between the hypothalamus and jejunum ($p > 0.05$). Additionally, compared with the control group, except for no significant difference in POMC expression in the hypothalamus and STAT3 expression in the jejunum, the expression of other neuropeptides were significantly higher in the FumDSB group (Figure 3).

**mRNA expression of inflammatory cytokines detected by qRT-PCR**

The mRNA expression trends of inflammatory cytokines in the hypothalamus and jejunum...
were not identical, according to the qRT-PCR data (Figure 4). Compared with those in the control group and FumDSB group, the expressions levels of IL-6, IL-8, TNF-α, and NF-κB in the hypothalamus of the FB1 group were significantly increased ($p < 0.05$). Meanwhile, the expression levels of IL-6, TNF-α, and NF-κB in the FumDSB group were not significantly different from those in the control group, except for IL-8, which was significantly increased ($p < 0.01$). IL-2 mRNA expression was not detected in the hypothalamus. Similarly, the expressions levels of IL-2, IL-6, and TNF-α in the jejunum in the FB1 group and FumDSB group were significantly higher than those in the control group ($p < 0.05$); compared with those in the FB1 group, the expression levels of IL-2 and IL-6 in the FumDSB group were significantly lower, whereas that of TNF-α was significantly higher ($p < 0.05$). There was no significant difference in IL-8 expression among the three groups ($p > 0.05$).

**Protein expression of inflammatory cytokines in hypothalamus detected by Western blot**

The protein expression levels of IL-2, IL-6, IL-8, TNF-α, NF-κB, and POMC were increased significantly after the addition of FB1 ($p < 0.05$) compared to levels in the control. However, when FumDSB was added as a degrading enzyme, the protein expression levels of several inflammatory cytokines were significantly lowered compared to levels in the FB1 group ($p < 0.05$), except that of IL-6 ($p > 0.05$). Additionally, the expression levels of IL-2, IL-8, TNF-α, NF-κB, and POMC were not significantly different from those found in the control group, although the expression of IL-2, IL-8, and TNF-α showed an upward trend (Figure 5).
Distribution of inflammatory cytokines in hypothalamus observed by IHC

IHC results revealed that the immunopositive signals for IL-2, IL-6, IL-8, TNF-α, and POMC were detected in the hypothalamus of growing pigs. The IL-2 positive reaction spots were clustered near the neuronal cells. Following FB1 ingestion, a strong positive reaction of IL-2 was observed, characterized by positive yellowish-brown substances that were uneven in size and irregular in shape and mainly distributed as circles and rings. After adding FumDSB, the positive reaction showed a weaker trend (Supplementary Figure 1).

Similarly, the immunopositive signals of IL-6 were concentrated near the nerve cells, which were mainly colored in the cytoplasm. In these three treatments, the FB1 group had the most yellowish-brown positive cells and strongest positive responses, whereas the FumDSB group had a weaker positive response than the FB1 group (Supplementary Figure 2). Positive spots of IL-8 were mainly stained in the cytoplasm and cell membrane, with a small amount around the secretory cells. The number of positive brown cells with irregular shapes was largest in the FB1 group among the three groups. Meanwhile, the intensity of the positive reaction ranged from high to low in the FB1, FumDSB, and control groups (Supplementary Figure 3).

The positive substances of TNF-α primarily gathered around the nerve cells and aggregated into clusters, with very little staining in the nucleus. The FB1 group had the most positive cells and the strongest positive reaction, whereas the FumDSB group had the weakest positive reaction (Supplementary Figure 4). The immunopositive spots of POMC

**Figure 4:** mRNA expression of inflammatory cytokines in the hypothalamus (a) and jejunum (b) based on qRT-PCR (mean ± SD, n = 6). Control, FB1, and FumDSB indicate the different treatments. Different lowercase letters and capital letters indicate significant difference at p < 0.05 and p < 0.01, respectively.
were granular, and mainly concentrated near neurons. Furthermore, compared with that in the control and FumDSB groups, the intensity of the positive responses was higher in the FB1 group (Supplementary Figure 5). Similarly, the FB1 group had the strongest positive reaction of NF-κB among the three groups, whereas the FumDSB group had the weakest positive reaction (Supplementary Figure 6).

**Protein expression of inflammatory cytokines in jejunum detected by Western blot**

The result of western blotting (Figure 6) indicated that the protein expression levels of IL-2, IL-8, TNF-α, NF-κB, and POMC increased significantly in the FB1 group \((p < 0.05)\) compared with those in the control group, whereas that of NF-κB and POMC decreased significantly after the addition of FumDSB. The expression levels of NF-κB and POMC in the FumDSB group were also significantly higher than those found in the control group \((p < 0.05)\); however, the expression levels of IL-2, IL-8, and TNF-α were not significantly different from those of the control group. There was no significant difference between IL-6 expression among the three groups \((p > 0.05)\).

**Distribution of inflammatory cytokines in jejunum observed by IHC**

IHC revealed that immunopositive signals for IL-2, IL-6, IL-8, TNF-α, NF-κB, and POMC could be observed in the hypothalamus of growing “pigs.” Positive substances for IL-2 were primarily colored in the cytoplasm and cell membrane and distributed to the jejunal villous epithelium, lamina propria, and small intestinal gland cells. The degree of positive response in the FB1 and FumDSB groups was significantly higher than in the control group (Supplementary Figure 7). Positive IL-6 spots with irregular shapes were mainly found in patches in the small intestinal glands and surrounding connective tissue; however, no positive staining spots were observed on the jejunal villi. The degree of positive response was stronger in the FB1 and FumDSB groups than in the control group (Supplementary Figure 8). The positive spots for IL-8 were mainly found...
in the jejunal epithelial cells, but were also found near the small intestine gland; these positive spots were extremely rare in the control group. The FB₁ group had the strongest positive responses, and the FumDSB group had a weaker positive response than the FB₁ group (Supplementary Figure 9). TNF-α-positive cell sites were primarily found in the jejunal villous epithelial cells and small intestinal glandular cells, and were distributed in a circular or diffuse pattern near the small intestinal glands. In these three groups, the FB₁ group had the strongest positive response (Supplementary Figure 10). The positive sites of POMC were mainly concentrated around the epithelial cells at the top of jejunal villi, and the intensity of the positive responses was stronger in the FB₁ and FumDSB groups than that in the control group (Supplementary Figure 11). Meanwhile, the intensity of the positive reaction of NF-κB ranged from high to low in the FB₁, FumDSB, and control groups (Supplementary Figure 12).

### Discussion

#### Detoxification of FumDSB on FB₁

Despite a high level of FB₁ contamination in corn and other grains, cases of acute intoxication caused by a high dose of FB₁ are uncommon. Nevertheless, several negative effects have been observed after the long-term consumption of lower FB₁ doses, such as destroyed tissue morphology, decreased food consumption and weight gain, reduced production performance, reduced specific immune responses, and increased chances of infection (Burel et al. 2013; Chen et al. 2019). Thus, standards for the tolerance limits of fumonisins have been issued worldwide. Generally, except for ruminants and poultry, the minimum limits for FBs in compound feeds for pigs, rabbits, horses, and other types of animals and pets are below 5 mg/kg (Tardieu et al. 2019; Peillod et al. 2021). Reportedly, approximately 5 mg/kg of FB₁ can cause changes in neurochemical signaling molecules in the CNS and adverse

### Figure 6

Relative protein expression of inflammatory cytokines in the jejunum based on western blot analysis (mean ± SD, n = 6). The left side of the figure is the strip diagram of western blot. Ctrl, FB₁, and FumDSB + FB₁ refer to the different treatments, and each treatment includes three sample strips. P65 refers to phosphorylated NF-κB, which perform after phosphorylation into the nucleus. The right side of the figure refers to the gray value of western blot. Different lowercase letters and capital letters indicate significant difference at p < 0.05 and p < 0.01, respectively.
physiological responses (Jaksić et al. 2019). The concentration of FB1 used in this study was 5 mg/kg. Further, the addition of FB1 was found to reduce ADFI and ADG in growing pigs and destroy the integrity of the jejunal barrier. FB1 only had a slightly negative effect on the morphology of the hypothalamus in growing pigs. This result may be due to the inability of large quantities of FB1 to cross the special blood–brain barrier of mammals (Al-Jaal et al. 2019). Furthermore, the findings of this study indicate that the consumption of FB1 can interfere with the expression and distribution of several appetite-related neuropeptides factors and pro-inflammatory.

Owing to the potential threat of fumonisin contamination to human and animal health, researchers have sought to identify a safe and effective detoxification method. Compared with physical and chemical methods, enzymatic degradation of fumonisins is an ideal detoxification method owing to its faster degradation rate and edible safety (Kamle et al. 2019). FumDSB is a unique carboxylesterase derived from the Sphingomonadales bacterium (Li et al. 2021b). The toxicity of FB1 in the enzyme-catalyzed reaction was primarily related to the structure of two TCA side chains, which can participate in the simulation of ceramide synthase substrates (including sphingosine bases and fatty acyl-CoA) and suppress enzymatic activity through competitive inhibition (Merrill et al. 2001; Chen et al. 2018). The toxic effects of FB1 could be reduced by effectively removing the two TCA side chains of FB1 (Heinl et al. 2010; Zhao et al. 2019). The data LC - hybrid quadrupole time-of-flight mass spectrometry showed that FB1 can be degraded to HFB1 using FumDSB. Although HFB1 is also a type of ceramide synthase inhibitor, its effect was 10-fold weaker than that of FB1. Furthermore, cytotoxicity studies proved that the inflammatory reactions of porcine enterocyte and immune cells induced by FB1 were significantly higher than those induced by HFB1 obtained by enzymatic hydrolysis (Gu et al. 2019). In this study, FumDSB was added to the feed of growing pigs as a detoxification enzyme of FB1. According to the results, FumDSB can reduce the inflammatory response induced by the addition of FB1. This result might be due to the ability of FumDSB to degrade FB1 to HFB1, ultimately alleviating the toxicity of FB1. Combined with suitable pH and thermal stability, FumDSB can be used in food and feed processing as a detoxifying enzyme for FB1.

**Inflammatory reactions and decrease in feed intake induced by FB1**

The hypothalamus is recognized as the primary area of the CNS responsible for regulating food intake and energy metabolism. Several studies suggest that changes in chemicals related to regulation (such as brain–gut peptides and cytokines) in the hypothalamus can induce neuroinflammatory responses, thereby leading to a loss of appetite (Griffiths et al. 2009; Hyun et al. 2019). The suppression of appetite caused by cytokinesis is regarded as part of the body’s acute response to various immune stimuli (Paulsen et al. 2017). As an organ related to appetite, the intestine is the first line of defense when FB1 invades the body, whereas the hypothalamus is the primary control center for appetite. Nevertheless, only few studies have investigated the relationship between feeding inhibition induced by FB1 and brain–gut regulation. MC4R is a critical signaling molecule involved in the regulation of food intake, body weight, and energy balance. Further studies suggest that AgRP and POMC, which are closely related to MC4R, also play key roles in controlling body weight, feed intake, and energy homeostasis (Baldini and Phelan 2019). According to the findings of this study, the addition of FB1 can increase the mRNA expression of MC4R and POMC (suppressing appetite) in the hypothalamus and jejunum while decreasing the expression of AgRP (promoting appetite). Notably, the expression of MC4R was most significantly changed in the hypothalamus, and was 25-fold higher than that of the normal control group. This result may be because appetite suppression mediated by MC4R primarily plays a role in the hypothalamus.

Previous studies proved that certain inflammatory factors (such as IL-1β, IL-6, IFN-γ, and TNF-α) can affect the expression of tight junction proteins in the blood–brain barrier through
multiple known signaling pathways, thereby disrupting normal physiological functions (Larochelle et al. 2011). Furthermore, inflammatory factors were found to affect astrocytes and microglia in the blood–brain barrier, destroying the barrier’s protective effect and promoting the role of inflammatory factors in the brain. NF-κB signal pathway was also activated when the inflammatory factor was increased. The increased expression of an inflammatory factor activated NF-κB, resulting in the formation of a positive feedback pathway that amplifies the inflammatory response (Shi et al. 2013). Previous studies revealed that FB1 can interfere with the expression of inflammatory cytokines in vivo and in vitro, thereby causing inflammation and reducing immune function in the body (Abbes et al. 2016; Chen et al. 2020). In the present study, the levels of IL-2, IL-6, IL-8, TNF-α, NF-κB, and POMC in the hypothalamus and jejunum were significantly higher after the addition of FB1 than the levels in the control. In brief, the negative effect of FB1 on appetite regulation was closely related to the higher expression of inflammatory cytokine in the hypothalamus and jejunum.

After being absorbed by the intestinal epithelial cell into the body, FB1 can induce inflammatory reactions, promote the production of pro-inflammatory cytokines, and then influence feeding behavior by activating the NF-κB pathway (Figure 7). As a common transcriptional factor, NF-κB can affect the secretion of inflammatory cytokines in cells after activation, thereby regulating the inflammatory response (He and Karin 2011; Ren et al. 2020). POMC acts as a link between NF-κB and MC4R and can combine with MC4R to suppress appetite. An increase in NF-κB can also promote the production of

![Pattern diagram of the intestinal inflammatory response induced by FB1](image.png)

**Figure 7.** Pattern diagram of the intestinal inflammatory response induced by FB1. After absorption into the body through the intestine, FB1 can induce the inflammatory response and then affect the feeding behavior by activating the NF-κB signal pathway.
POMC. Conversely, AgRP is an antagonist of MC4R and plays a role in promoting appetite (Griffiths et al. 2009). STAT3 can affect the signal transduction of MC4R and NF-κB, thereby participating in feeding regulation (Long et al. 2019). In summary, changes in several neuropeptides and inflammatory cytokines in the hypothalamus and jejunum can induce neuroinflammatory responses, thereby leading to a loss of appetite. In present study, the expression levels of appetite-related neuropeptides in the hypothalamus were more obvious than those in the jejunum; however, the inflammatory response was relatively weak, which may be related to the regulation path of the hypothalamus.

Conclusions

Taken together, the consumption of FB1 can destruct the tissue structure of hypothalamus and jejunum, interfere the expression and distribution of several appetite-related neuropeptides and inflammatory cytokines, thereby inducing neuroinflammatory responses and affecting food intake and weight gain. However, these anorexia effects and inflammatory responses are alleviated when FumDSB is added to the feed. In short, FumDSB can be used in food and feed processing as a detoxifying enzyme for FB1.

Acknowledgement

The authors thank our anonymous reviewers and our colleagues from the Animal Nutrition Laboratory for their valuable critiques and suggestions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the China Agriculture Research System of MOF and MARA [CARS-43-B-1], Shandong Double Tops Program (2019), Taishan Industry Leadership Project [TSCY20190107] and Key Research and Development Project of Shandong Province [2019GNC106007].

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