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Effects of *Lacticaseibacillus paracasei* Shirota Supplementation on Growth Performance, Intestinal Health, and Fecal AFB_1 Metabolite in AFB_1-Exposed Rats

(Kesan Pemberian Lacticaseibacillus paracasei Shirota terhadap Prestasi Pertumbuhan, Kesihatan Usus dan Metabolit AFB_1 di dalam Najis Tikus Terdedah AFB_1)

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Abstract

Aflatoxin B_1 (AFB_1) is a toxin produced by Aspergillus species of fungi. Findings in the literature has shown the potential of probiotic treatment to alleviate AFB_1 toxicity. This study explores the effects of Lacticaseibacillus paracasei Shirota (LcS) supplementation on the growth performance, intestinal health, and excretion of faecal AFB_1 metabolite of AFB_1-exposed rats. Thirty-two male Sprague Dawley rats were divided into control, AFB_1, AFB1+LcS and LcS groups. AFB_1 was given at a complete dosage of 25 µg AFB_1/kg body weight, while LcS supplementation at 2×10^9 CFU/mL per day for four weeks. The average body weight of the AFB1 group showed no significant increase from week 2 to 4, while other groups had an increment throughout the study. The food intake of the AFB_1 and AFB_1+LcS groups had significantly reduced throughout the treatment period. AFB_1 exposure caused several changes in the histomorphometry parameters but was normalised with LcS supplementation. The AFB_1 group showed mild to moderate inflammation in all intestinal parts, whereas only mild inflammation was observed in the jejunum and ileum of the AFB_1+LcS group. Faecal Bifidobacterium spp. counts showed an increment in three groups, while the AFB_1 group showed a significant reduction. The faecal AFB_1 in the AFB_1 group was significantly lower than in the AFB_1+LcS group. In conclusion, AFB_1 affected growth performance and intestinal health, and wherein the effects were alleviated by LcS supplementation. Further investigation on intestinal permeability and serum and urinary AFB_1 level is suggested to understand the mechanism of probiotic-AFB_1 interaction in alleviating AFB_1 toxicity.

Keywords: Aflatoxin B_1; Probiotics; Lacticaseibacillus paracasei Shirota; Intestinal health

Abstrak

Aflatoksin B_1 (AFB_1) ialah toksin yang dihasilkan oleh spesies kulat Aspergillus. Penemuan dalam literatur telah menunjukkan potensi rawatan probiotik untuk mengurangkan ketoksikan AFB_1. Kajian ini meneroka kesan pemberian Lacticaseibacillus paracasei Shirota (LcS) terhadap prestasi pertumbuhan, kesihatan usus, dan perkumuhan metabolit AFB_1 di dalam najis tikus yang terdedah kepada AFB_1. Tiga puluh dua ekor tikus Sprague Dawley jantan dibahagikan kepada kumpulan kawalan, AFB_1, AFB_1+LcS dan LcS. AFB_1 diberikan pada dos lengkap 25 µg AFB_1/kg berat badan, manakala pemberian LcS pada 2×10^9 CFU/mL sehari

selama empat minggu. Purata berat badan kumpulan AFB_1 tidak menunjukkan peningkatan yang signifikan dari minggu 2 hingga 4, manakala kumpulan lain mengalami peningkatan sepanjang kajian. Selain itu, kumpulan AFB_1 dan AFB_1+LcS mempunyai pengurangan pengambilan makanan yang signifikan sepanjang rawatan. Pendedahan AFB_1 menyebabkan beberapa perubahan dalam parameter histomorfometri tetapi telah dinormalisasi dengan pemberian LcS. Selain itu, semua bahagian usus kumpulan AFB_1 menunjukkan keradangan ringan hingga sederhana, manakala hanya keradangan ringan diperhatikan dalam jejunum dan ileum kumpulan AFB_1+LcS. Kiraan Bifidobacterium spp. di dalam najis menunjukkan peningkatan dalam tiga kumpulan, sebaliknya kumpulan AFB_1 menunjukkan pengurangan yang signifikan. Tambahan pula, AFB_1 di dalam najis bagi kumpulan AFB_1 adalah jauh lebih rendah daripada kumpulan AFB_1+LcS. Kesimpulannya, AFB_1 menjejaskan prestasi pertumbuhan dan kesihatan usus, di mana kesannya telah dikurangkan dengan pemberian LcS. Penyiasatan lanjut terhadap kebolehtelapan usus dan paras AFB_1 di dalam serum dan urin dicadangkan untuk memahami mekanisme interaksi probiotik-AFB_1 untuk mengurangkan ketoksikan AFB_1.

Kata kunci: Aflatoxin B1; Probiotik; Lacticaseibacillus paracasei Shirota; Kesihatan usus

INTRODUCTION

Aspergillus paraciticus and *Aspergillus flavus* are fungi that produce poisonous compounds named mycotoxins. Several mycotoxins such as aflatoxins, fumonisins, ochratoxin A, patulin, zearalenone, nivalenol/deoxynivalenol, trichothecenes and ergot alkaloids are known to affect human health and livestock (WHO 2018). The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 (AFB_1) as Group 1 carcinogens. Besides, AFB_1 is linked to the occurrence of liver cancer in humans (IARC 2002). Aflatoxicosis is a foodborne disease associated with aflatoxin exposure, known to affect the organs, especially the liver negatively. Fever, vomiting, and abdominal pain are among the common symptoms experienced by infected hosts (Kumar et al. 2017).

Previous studies have shown the effect of AFB_1 exposure on the development of intestinal tumour-like growth (Liew et al. 2018; Nurul-Adilah et al. 2018) and changes in intestinal morphometry (Galarza-Seeber et al. 2016; Wang et al. 2018). Meanwhile, human AFB_1 exposure has been reported mostly in developing countries, where the screening and detection for fungus and the harvesting and storage practices of food commodities are not as strict as in the developed nations (CDC 2012). Several countries, such as northwest India in 1974, Malaysia in 1988, and Kenya in 1982, 2004 and 2005, had reported deaths due to the consumption of aflatoxin-contaminated foods (Lye et al. 1995; Abraham et al. 2012).

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (FAO/WHO 2001), have been reported to be able to alleviate the harmful effects of AFB_1 exposure. Probiotics can bind to AFB_1 (Hernandez-Mendoza et al. 2009; Liew et al. 2018), reduce the level of AFB_1 and its biomarkers in serum (Hernandez-Mendoza et al. 2009; Nikbakht Nasrabadi et al. 2013), and increase the excretion of AFB_1 and its metabolites via urine (Huang et al. 2017; Liew et al. 2018; Nurul-Adilah et al. 2018) and faeces (Ahlberg et al. 2015). Other than that, the supplementation of probiotics can potentially restore gut microbiota, reverse intestinal dysbiosis and reduce the risk of developing liver cancer (Liew & Mohd-Redzwan 2018).

This study aims to determine the use of probiotic as a biological adsorbent of AFB_1 in alleviating AFB_1 toxicity effects. Indeed, the determination of faecal AFB_1 metabolites and faecal *Lactobacillus* spp., *Bifidobacterium* spp. and *Lacticaseibacillus paracasei* Shirota (LcS) profile would give deeper insight on the role of probiotic-aflatoxin complex formation in reducing the absorption of AFB_1. Due to lack of conclusive evidence, the present study explores the histomorphometry of the small intestine and colon especially in AFB_1-exposed rats, in which the histomorphometric data would reflect the integrity of the intestinal architecture. Finally, the rats' body weight and food consumption are assessed to demonstrate the toxicity effects of AFB_1 effects on the intestinal absorption and appetite regulation, thereby enhance the comprehension on the health effects associated with AFB_1 exposure.

MATERIALS AND METHODS

Animal treatment protocol

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) Universiti Putra Malaysia (UPM/IACUC/AUP-R083/2018). A total of 32 male Sprague Dawley rats (Alchemy Supplies Sdn. Bhd.,

Malaysia) aged from 7-8 weeks were divided equally (n = 8) into four groups; control, AFB_1, AFB_1+LcS, and LcS (Charan & Kantharia 2013; Nurul-Adilah et al. 2018). AFB_1 was given at a complete dosage of 25 μg AFB_1/ kg body weight, equivalent to the amount of toxin commonly found in AFB_1-contaminated food (Nurul-Adilah et al. 2018). The LcS was given to the rats at 2×10^9 CFU/mL, the minimum concentration reported to remove 50 % aflatoxin significantly (Nikbakht Nasrabadi et al. 2013). After completing the 4-weeks treatment, all rats were injected mixture of Ketamine (75 mg/kg body weight) and Xylazine (5 mg/kg body weight) via intraperitoneal injection, euthanized, and blood and organs were collected. A 4-weeks intervention was chosen based on the methodology proposed by others (Nurul-Adilah et al. 2018, Liew et al. 2018) as the duration is capable of inducing aflatoxicosis. Food intake was calculated by subtracting the initial food given with the leftovers. The body weight of rats was taken weekly and analysed at four points of time, excluding the period of acclimatisation.

Preparation of LcS culture

A probiotic drink containing LcS (purchased from a local store in Serdang, Selangor, Malaysia) was the source of live LcS bacteria. The 16s RNA bacterial sequence was analysed by the First BASE Laboratories Sdn. Bhd. (Seri Kembangan, Malaysia) to confirm the identity of the bacteria (Liew et al. 2022). One hundred microliters (100 μL) were taken from the probiotic drink and spread onto the De Man, Rogosa and Sharpe (MRS) agar (GranuCult™, Sigma-Aldrich). The agar was incubated for 48 hours at 37 ℃ aerobically. Then, one colony of LcS was transferred into MRS broth and further incubated for 24 hours at 37℃ in an incubator shaker at 250 rev/min. The growth of LcS was monitored optically every two hours at 600 nm.

The colony forming unit (CFU) was determined by transferring 100 μL of the broth onto MRS agar and spreading it evenly. The CFU was calculated using the following formula:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated}}$$

The CFU/mL was used in expressing the CFU of LcS culture prepared for the supplementation, while CFU/g was used in expressing the CFU of the *Lactobacillus* spp., *Bifidobacterium* spp. and LcS counts in faecal samples. The LcS supplementation was given daily to the rats in ABB_1+LcS and LcS groups at 2×10^9 CFU/mL via oral gavage.

Preparation of AFB_1 dosage

The AFB_1 (R-Biopharm, Darmstadt, Germany) stock was prepared by dissolving 25 μg AFB_1 in 10 mL PBS solution. An approximately 0.9 μg in 0.35 mL of AFB_1 was given daily for 4 weeks to each rat in the AFB_1 and AFB_1+LcS groups (Oghenesuvwe et al. 2014; Pandy, 2020) via oral gavage to get a complete dosage of 25 μg AFB1/kg of body weight.

Samples collection

Pooled faecal samples were collected at two points: at baseline (Day 0) and the end of animal treatment (Day 28). Samples for bacteria culture were stored in 50 % (v/v) glycerol solution (Nikbakht Nasrabadi et al. 2013) and kept in the freezer at -80 ℃ until further analysis. Meanwhile, the samples for analysis of faecal AFB_1 were stored immediately at -80 ℃ after collection.

The small intestine and colon samples were collected and cut accordingly (Vdoviaková et al. 2016), and treated with 10 % formalin solution, for 3 days at room temperature (37 ℃) (Nurul-Adilah et al. 2018).

Histomorphometry of the duodenum, jejunum, ileum, and colon

The tissue samples in formalin solution were washed a few times with 80–95 % ethanol, followed by dehydration in absolute ethanol. Then, the samples were cleared using xylene and embedded in paraffin. Subsequently, the tissues were cut into sections of 4 μm thickness. Lastly, the sections were stained with H&E for qualitative histological analysis (Nurul-Adilah et al. 2018).

The digital images of the samples were captured with a microscope equipped with a camera. Then, the images were analysed using ImageJ software (Wilson et al. 2018) for the villus height, villus width, crypt depth, surface area and villus height to crypt depth ratio (Galarza-Seeber et al. 2016).

Preparation of agar plates and faecal bacteria count

The MRS agar (GranuCult™, Sigma-Aldrich), Bifidobacterium agar (HiMedia Laboratories) and lactitol-LBS-vancomycin (LLV) agar (Yuki et al. 1999) were used to determine *Lactobacillus* spp., *Bifidobacterium* spp. and LcS counts, respectively. The bacterial content in faecal samples was determined by standard bacteria culture (Cappucino & Sherman 2010). The pooled faecal samples were diluted in sterile peptone water using serial dilution method to a total of five dilutions for each sample. The bacteria culture was conducted using spread plate technique on three different selective agars as stated previously. Each sample was repeated in triplicate and expressed in CFU/g. Later, the values were transformed into log CFU/g for further statistical analysis.

Determination of faecal AFB_1

AFB_1 was extracted from faecal samples based on a previous study (Firmin et al. 2011) and concentrated with the immunoaffinity column (IAC) (R-Biopharm RhÔne Ltd), before the metabolite was analysed using an enzyme immunoassay, RIDASCREEN®FAST Aflatoxin (Art. No.: R5202, R-Biopharm, Darmstadt, Germany).

Statistical analysis

Body weight, food intake, faecal *Lactobacillus* spp. and faecal *Bifidobacterium* spp. counts were determined by using one-way repeated measure ANOVA with post-hoc test (Tukey) and pairwise comparison test, whereas the morphometric data were analysed using one-way ANOVA. The comparison between faecal AFB_1 and LcS counts were determined using an independent sample t-test. All data were expressed as means and standard deviation (mean ± SD), and significance was assessed with $p < 0.05$. The histological analysis for inflammation and tumour-like growth were reported as descriptive results. Statistical analysis was conducted using IBM SPSS Statistics software (Version 26).

RESULT AND DISCUSSION

Changes in rats' body weight

The difference in body weight can be observed when comparing the AFB_1 group with other groups. Although there were no significant differences ($p > 0.05$) between the groups in week 3 and week 4, a slower increment of body weight was observed in the AFB_1 group compared to others. In addition, there was no significant increase ($p > 0.05$) in body weight from week 2 to week 4 within the AFB_1 group. Some rats in the AFB_1 group experienced weight loss during weeks 2, 3, and 4 of the trial, which significantly reduced the average weight growth of the rats in that group (Figure 1).

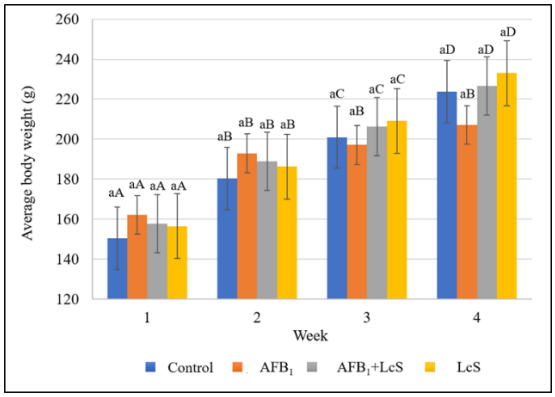

Figure 1 The average body weight of rats in four groups (n = 8). Values with different lowercase superscript (a) indicate significant different ($p < 0.05$) between groups at the same time period. Values with different uppercase superscript (A, B, C, D) indicate significant different ($p < 0.05$) within each group across different time periods from baseline to end. End. Error bars indicate standard deviation.

The AFB_1 exposure impacted the body weight of the rats in the present study, but the average body weight did not significantly decrease, in contrast to earlier studies (Liew et al. 2018; Nurul-Adilah et al. 2018; Nikbakht Nasrabadi et al. 2013). It is clear from the literature that rats exposed to AFB_1 gained much less body weight than those in the control groups, whether fed a normal diet (Liew et al. 2018; Nikbakht Nasrabadi et al. 2013) or a high-protein diet (Nurul-Adilah et al. 2018). This can be due to impaired digestive enzyme activities, which result in the malabsorption of nutrients (Liew et al. 2018) and the change in the metabolic processes such as tricarboxylic acid (TCA) cycle, glucose, and fatty acid synthesis. The supplementation of LcS (Liew et al. 2018; Nurul-Adilah et al. 2018; Nikbakht Nasrabadi et al. 2013) and other probiotics (El-Nezami et al. 2000) in rats exposed with AFB_1, as in the AFB_1+LcS group of the present study showed a substantial weight gain, similar to the trend observed in the control and LcS groups. This is due to the binding capacity of probiotics towards AFB_1 (Liew et al. 2018; Hernandez-Mendoza et al. 2009; Nikbakht Nasrabadi et al. 2013; Awad et al. 2009) which can reduce AFB_1 absorption and damage towards the intestine (Awad et al. 2009), subsequently mitigate the adverse effects of AFB_1 toxicity on gut health.

The effects of AFB_1-exposure and LcS supplementation on food intake

The AFB_1 group showed a significant reduction ($p < 0.05$) in average food intake throughout the study. A significant reduction ($p < 0.05$) was also observed in the AFB_1+LcS group from Week 1 to Week 3. However, there was a significant increase ($p < 0.05$) in food intake of this group from Week 3 to Week 4, unlike the AFB_1 group which remains to decrease until the end of the study. Other than that, the LcS group had a significant increase ($p < 0.05$) in food intake throughout the study, and only the control group showed no significant changes over the 4-week period (Figure 2).

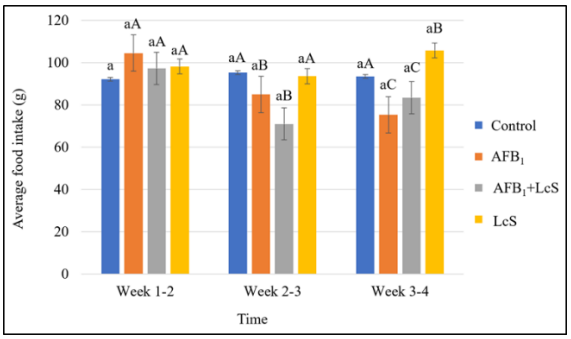

Figure 2 The average food intake of rats for each group (n = 8). Values with different lowercase superscript (a) indicate significant different ($p < 0.05$) between groups at the same time period. Values with different uppercase superscript (A, B, C) indicate significant different ($p < 0.05$) within each group across different time periods from baseline to end. End. Error bars indicate standard deviation.

AFB_1 was associated with its neurodegenerative effect, affecting the expressions of neuropeptide EM66 and its precursors, SgII, which plays an important role in regulating appetite in rats exposed repeatedly to AFB_1 (Trebak et al. 2015). Besides, the binding of AFB_1 towards DNA and RNA produces aflatoxin B2α, a compound that can reduce enzyme activity required for digestion and absorption by reacting to amino groups of functional protein (Pandey & Chauhan 2007). This may explain the reduced body weight and

food intake of AFB_1-exposed rats. Similar to previous findings, AFB_1 ingestion reduced food intake in chicken (Pandey & Chauhan 2007) and pigs (Rustemeyer et al. 2010). Other hormones that might be affected by AFB_1 exposure are leptin, insulin, adiponectin, resistin, nesfatin-1, adropin, omentin-1, GLP-1, GLP-2 and glucagon (Cabral et al. 2020), even though the findings in existing literature are inconclusive. As reported previously, the binding capacity of LcS towards AFB_1 was able to reduce the AFB_1 absorption, thus preventing any intestinal damage (Awad et al. 2009), toxin translocation (Schoultz & Keita 2020), nutrients malabsorption and metabolism (Nikbakht Nasrabadi et al. 2013), as well as the alteration of hormones, enzymes or neuropeptides production related to appetite, digestion and absorption (Cabral et al. 2020; Trebak et al. 2015; Pandey & Chauhan 2007). Indeed, these were reflected in a higher average food intake in the LcS and AFB_1+LcS groups in comparison with the AFB_1 group, as observed in the present study.

Morphometric analysis of the duodenum, jejunum, and ileum

As shown in Table 1, the villus heights of the duodenum and ileum of the AFB_1 group were significantly reduced ($p < 0.05$) compared to the control group. Meanwhile, the AFB_1+LcS group showed improvement in villus height measurements in the duodenum compared to the AFB_1 group. The villus height of the ileum of the AFB_1+LcS group was slightly higher than that of the AFB_1 group, however, there was no significant difference ($p > 0.05$) between the two groups. The crypt depth of the AFB_1 group was also lower at the duodenum compared to AFB_1+LcS. The ileal crypt depth of the AFB_1 group was significantly lower ($p < 0.05$) in comparison to the AFB_1+LcS group. However, there were no significant differences ($p > 0.05$) in villus height and crypt depth in the jejunum of rats in all groups.

The villus width and surface area of the duodenum did not show any significant changes ($p > 0.05$) with exposure to AFB_1 and supplementation of LcS compared to the control group. Besides, the villus width of the jejunum in the AFB_1 group was significantly higher ($p < 0.05$), leading to a much bigger surface area than the control group. In the ileum, no significant differences ($p > 0.05$) were found between all groups. However, the surface areas of the AFB_1 and AFB_1+LcS groups were smaller, as the villus height of these groups were significantly reduced ($p < 0.05$) compared to the control and LcS groups. The significant increase ($p < 0.05$) in villus width observed in the jejunum of the AFB_1 group might be contributed by the accumulation of inflammatory cells in the villi.

Previous studies showed inconclusive findings on the morphometric measurements in relation to aflatoxin exposure (Galarza-Seeber et al. 2016). There were no significant changes in villus height of AFB_1-exposed broiler chickens, while a significant reduction in the villus width and crypt depth was reported with probiotic yeast supplementation (Povolini et al. 2019). Other studies reported a decrease in villi height of jejunum with aflatoxin exposure compared to the control group of broiler chicks (Jahanian et al. 2016) and an increase in villus height with AFB_1 exposure (Applegate et al. 2009; Zhang et al., 2014). However, the literature also suggests that supplementing probiotics in animal models is known to improve intestinal morphology. There was a significant increase in villus height, crypt depth and villus width in the duodenum, ileum and cecum (Galosi et al. 2021), a significantly higher villus height, width and surface area in duodenum and jejunum of one-day-old chicken (Sobolewska et al. 2017), and a significant increase in villus

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Table 1 Histomorphometric data of duodenum, jejunum, and ileum (n = 8)

	Mean ± SD				
	Villus height (µm)	**Crypt depth (µm)**	**Villus width (µm)**	**Surface area (mm^2)**	**VH:CD ratio**
Duodenum					
Control	267.95 ± 27.16^{a}	104.93 ± 10.16^{a}	74.21 ± 4.54^{a}	62.46 ± 7.34^{a}	2.58 ± 0.39^{a}
AFB_1	208.64 ± 23.18^{b}	80.69 ± 10.28^{b}	79.79 ± 11.84^{a}	52.74 ± 12.62^{a}	2.62 ± 0.45^{ab}
AFB_1+LcS	246.33 ± 26.73^{a}	94.32 ± 16.52^{ab}	72.30 ± 14.00^{a}	56.16 ± 13.64^{a}	2.70 ± 0.60^{ab}
LcS	250.31 ± 15.81^{a}	79.48 ± 7.58^{b}	67.11 ± 21.79^{a}	53.40 ± 20.40^{a}	3.17 ± 0.33^{b}
Jejunum					
Control	203.33 ± 9.68^{a}	79.38 ± 8.35^{a}	67.69 ± 10.02^{a}	43.19 ± 6.67^{a}	2.59 ± 0.30^{a}
AFB_1	200.33 ± 14.71^{a}	80.99 ± 5.99^{a}	80.40 ± 9.67^{b}	50.88 ± 9.25^{a}	2.49 ± 0.26^{a}
AFB_1+LcS	190.52 ± 7.78^{a}	78.43 ± 11.61^{a}	69.58 ± 6.44^{ab}	41.61 ± 3.68^{a}	2.48 ± 0.42^{a}
LcS	188.66 ± 16.50^{a}	75.04 ± 10.47^{a}	67.26 ± 11.18^{a}	40.24 ± 9.35^{b}	2.58 ± 0.55^{a}
Ileum					
Control	264.67 ± 49.02^{a}	99.58 ± 14.78^{ac}	73.52 ± 13.26^{a}	61.28 ± 18.33^{ab}	2.74 ± 0.82^{ab}
AFB_1	203.16 ± 15.43^{b}	82.22 ± 13.99^{d}	73.26 ± 9.61^{a}	46.83 ± 5.45^{b}	2.54 ± 0.52^{ac}
AFB_1+LcS	209.10 ± 32.19^{b}	112.81 ± 8.77^{ab}	75.54 ± 10.53^{a}	49.64 ± 10.35^{ab}	1.88 ± 0.40^{c}
LcS	265.54 ± 14.84^{a}	93.53 ± 6.92^{c}	78.07 ± 13.70^{a}	65.20 ± 12.51^{a}	2.86 ± 0.32^{ab}

The values with different superscript letters (a, b, c, d) indicate significant different ($p < 0.05$). VH:CD ratio is the ratio between villus height and crypt depth of the same sample.

height and villus height to crypt depth ratio, while the crypt depth measurement in the ileum was significant reduced (Awad et al. 2008). The contradicted findings from others and this study might be due to some notable differences in the treatment protocol conducted. The study by Galosi et al. (2021) uses a multi-strain probiotic supplement at 2×10^{11} CFU/L, while Sobolewska et al. (2017) uses synbiotic, a mixture of probiotic and prebiotic in one-day old chicken. Despite the findings of this study contradicting some literature suggesting that probiotic treatment enhances intestinal morphology, it is evident that LcS supplementation in AFB_1-exposed rats alleviates morphological damage to the intestine, albeit without necessarily improving its morphology compared to the control group. Hence, to enhance intestinal morphology through probiotic supplementation in the future, researchers should consider various factors such as dosage, animal's developmental phase or age, and the combination of probiotics or prebiotics utilised.

A healthy small intestine is essential for proper physiology, including nutrient absorption, immune function, and gut microbiota balance. The epithelial barrier, which is comprised of epithelial intercellular junctions, would be altered by alterations in intestinal morphometry. This important structure is responsible for maintaining the intestinal permeability and integrity intact (Bhat et al. 2019; Ducatelle et al. 2018). The atrophy of intestinal structure, especially the villus, would affect the absorptive capability of the intestine (Feng et al. 2017), as it will influence the surface for absorption, the overall nutrients transport system and the expression of the enzyme on the brush border membrane (Awad et al. 2009). In this study, the improvement observed in the AFB_1+LcS group compared to the AFB_1 group in term of villus height and crypt depth in the duodenum and ileum, underscore the beneficial effects of LcS supplementation in mitigating AFB_1 exposure, and capable of maintaining the proper intestinal physiology.

Presence of inflammation in the duodenum, ileum, and colon of AFB_1-exposed rats

In contrast to the control group, the AFB_1 group displayed signs of inflammation in all parts of the intestine [Figure 3(A) – (C)] and colon [Figure 3 (D)]. In particular, the jejunum samples [Figure 3 (B)] demonstrated moderate inflammation characterised by dense, highly accumulated pink lymphocyte stains. On the other hand, only mild inflammation was observed in the jejunum and ileum of the AFB_1+LcS group. This observation may indicate the anti-inflammatory activity of LcS against AFB_1 on the intestine. The control and LcS groups showed no sign of inflammation in all parts of the intestines observed. Previous studies have reported similar findings on the accumulation of inflammatory cells (Povolini et al. 2019; Jin et al. 2021), as well as the occurrence of congested blood vessels (Akinrinde et al. 2019) and oedema (Povolini et al. 2019) in small intestines of AFB_1-exposed animals. An increase in pro-inflammatory biomarkers such as toll-like receptor 4 (TLR4), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), thioredoxin-interacting protein (TXNIP), nucleotide-binding domain-like receptor protein 3 (NLRP3), and interleukin-18 (IL-18) were reported in AFB_1-exposed ducks (Jin et al. 2021).

Elevated production of pro-inflammatory cytokines and the release of free radicals resulting from AFB_1 exposure are expected to induce oxidative stress, potentially compromising the proper physiological functions of the immune system over time (Jin et al. 2021; Dey et al. 2021). The NF-κB signalling pathway is

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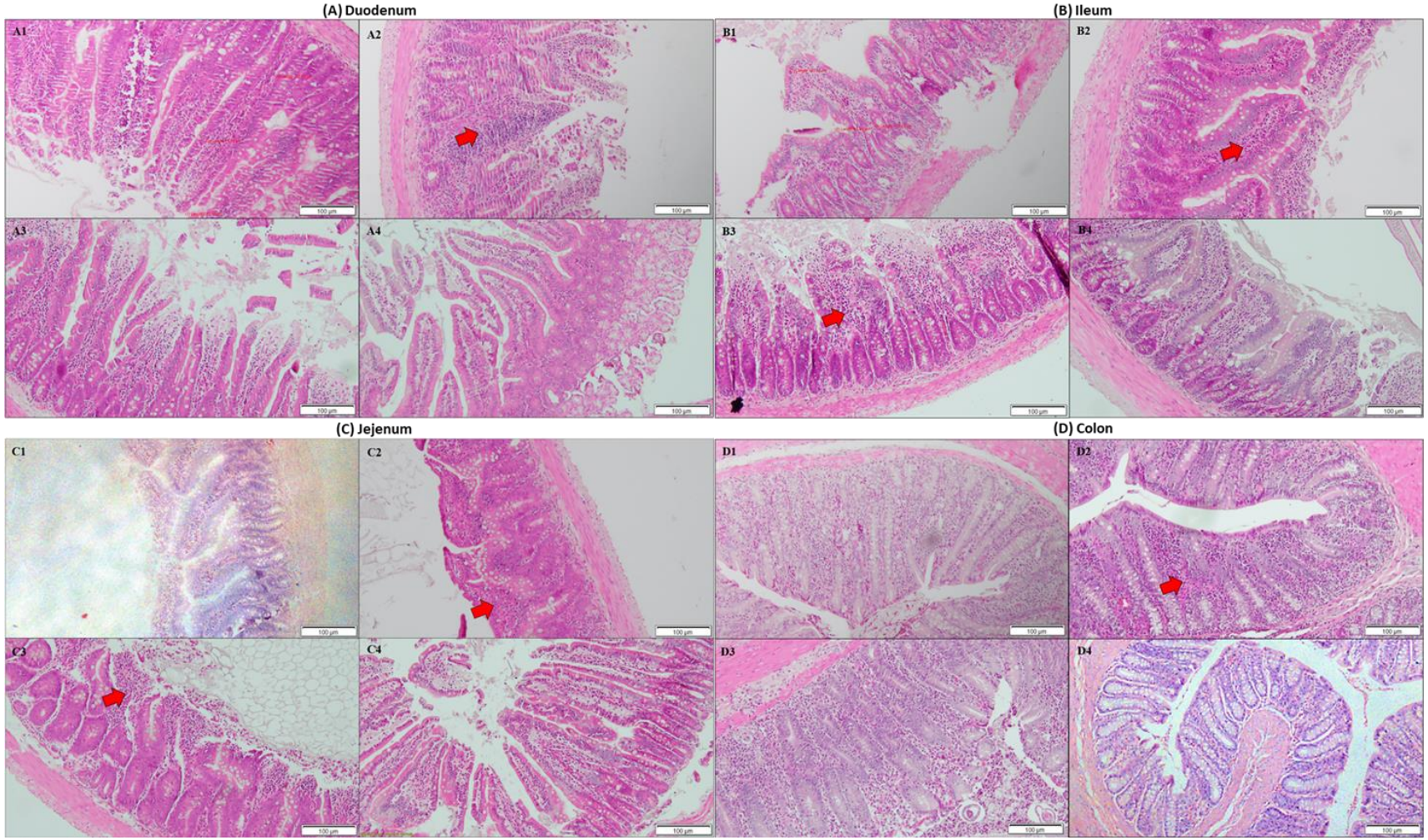

Figure 3. H&E staining of the duodenum (A), jejunum (B), ileum (C) and colon (D). 1: Control group, 2: AFB_1 group, 3: AFB_1+LcS group, 4: LcS group. The arrow indicates inflammation. In the duodenum, mild lymphocyte accumulation (inflammation) was observed in the AFB_1 group, and no inflammation was found in other groups. In the jejunum and ileum, moderate lymphocyte accumulation (inflammation) was observed in the AFB_1 group, while the AFB_1+LcS group showed mild accumulation. The control and LcS groups did not show any inflammation. In the colon, moderate lymphocyte accumulation (inflammation) was observed in the AFB_1 group. There was no inflammation found in other groups.

one of the inflammatory signalling pathways activated by AFB_1-induced oxidative stress (Jin et al. 2021), triggering the production of primarily TNF-α and interleukin-1 (IL-1) that are associated with irritable bowel diseases (IBD), rheumatoid arthritis, chronic obstructive pulmonary disease (COPD) and asthma (Lawrence 2009).

Absence of tumour-like growth in the duodenum, jejunum, ileum and colon in AFB_1-exposed rats

There was no tumour-like growth observed in AFB_1-exposed rats in the present study. This finding contradicts the previous study (Nurul-Adilah et al., 2018), which reported the presence of carcinoma in both the small intestine and colon. Only a few studies have reported AFB_1 toxicity and tumour growth in intestinal tissue, particularly the small intestine. There were reports on AFB_1 exposure on the progression of colorectal cancer (Carvajal-Moreno 2017; Cullen et al. 1987), which were dose-dependent (Cullen et al. 1987), tumour progression in the colon (Ibrahim 2013), and the occurrence of intestinal adenocarcinoma, but none were observed in the lower dosage of AFB_1 (Cullen et al. 1987).

Changes in *Lactobacillus* spp. and *Bifidobacterium* spp. faecal count on selective agar media

There were significant increases ($p < 0.05$) in faecal *Lactobacillus* spp. count from baseline to the end for the control and LcS groups, while there were no significant differences ($p > 0.05$) in the other two groups. On the other hand, there was a significant reduction ($p < 0.05$) in *Bifidobacterium* spp. count in the AFB_1 groups compared to the other groups (Figure 4).

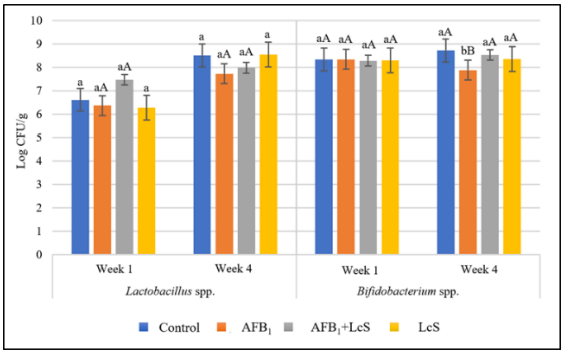

Figure 4 The comparison of rats' faecal *Lactobacillus* spp. and *Bifidobacterium* spp. counts. Values with different lowercase superscript (a, b) indicate significant different ($p < 0.05$) between groups at the same time period. Values with different uppercase superscript (A, B) indicate significant different ($p < 0.05$) within each group across different time periods from baseline to end. Time period; 1) Week 1: Baseline, and 2) Week 4: End. Error bars indicate standard deviations.

The presence of mycotoxin within the gut can disrupt the gut microbiota by diminishing beneficial bacteria and increasing harmful strains (Liew & Mohd-Redzwan 2018; Wang et al. 2015). Interestingly, in this study, there were no significant changes in faecal *Lactobacillus* spp. of AFB_1-exposed rats, while there was a significant reduction in *Bifidobacterium* spp. count in the AFB_1 groups. These findings align with the previous study, suggesting a dose-dependent relationship (He et al. 2018). The significant reduction of *Bifidobacterium spp.* counts in AFB_1-exposed rats might suggest either caused by its direct impact on intestinal dysbiosis (Ducatelle et al. 2018) or by the changes in serum cortisol levels due to stress (Aizawa et al. 2019) associated with AFB_1 exposure.

Presence of AFB_1 and LcS in faeces of AFB_1-exposed rats

Faecal samples of the AFB_1+LcS group were found to have significantly higher ($p < 0.05$) AFB_1 concentration compared to the AFB_1 group (Table 2), and the AFB_1+LcS group had significantly higher ($p < 0.05$) LcS recovered on LLV agar compared to the LcS group (Table 3).

Table 2 Faecal AFB_1 metabolite in AFB_1+LcS and AFB1 groups

Groups	Mean ± SD (log CFU/g)	*t*-value	*p*-value
AFB_1+ LcS	8.75 ± 0.05	5.092	0.007
LcS	7.79 ± 0.32		

The independent sample t-test of pooled samples of 8 rats per group for the AFB_1+LcS and AFB_1 group. Triplicate analysis was conducted from pooled samples of each group. The means and standard deviations (SD) indicate the measurement of faecal AFB_1. Means are significantly different at $p < 0.05$.

Table 3 LcS recovered from faecal samples of rats in AFB_1+LcS and LcS groups on LLV Agar

Groups	Mean ± SD (µg/L)	*t*-value	*p*-value
AFB_1+ LcS	45.48 ± 0.23	77.319	0.000
AFB_1	32.21 ± 0.19		

The independent sample t-test of pooled samples of 8 rats per group for the AFB_1+LcS and AFB_1 group. Triplicate analysis was conducted from pooled samples of each group. The means and standard deviations (SD) indicate the measurement of faecal LcS count. Means are significantly different at $p < 0.05$.

Previous studies have reported significantly higher serum AFB_1 in AFB_1-induced rats as compared to those supplemented with LcS (Liew et al. 2018; Nikbakht Nasrabadi et al. 2013), which indicates a higher absorption of AFB_1 in the absence of probiotics. The formation of a probiotic-aflatoxin complex in the gut, which is subsequently excreted together in faeces illustrates the interaction between bacteria and aflatoxin (Ahlberg et al. 2015). The detection of AFB_1 and its metabolites in faecal and urine samples of AFB_1-exposed animals indicates the level of unabsorbed AFB_1 (Gratz et al. 2006). AFB_1 metabolites such as AFM_1 (Nikbakht Nasrabadi et al. 2013) and AFB-N^7guanine (Huang et al. 2017) were also reduced in the presence of probiotic supplementation. In this study, the higher concentration of AFB_1 and LcS counts recovered from faecal samples in the AFB_1+LcS group compared to the AFB_1 group suggests an enhanced excretion of AFB_1 via faecal route, facilitated by the formation of a probiotic-aflatoxin complex. As a result, intestinal histomorphometry is preserved against damage caused by AFB_1, ensuring its proper functioning.

The probiotic-aflatoxin complex formation mainly depends on the probiotics' binding efficiency. The viable LcS, cell wall fragments and heat-treated LcS were reported to have 98, 97 and 81 % binding efficiency towards AFB_1 *in vitro* (Liew et al. 2018). Furthermore, eight different *Lactobacillus casei* strains were tested, and the percentage of AFB_1 bound was from 10 to 50 % (Hernandez-Mendoza et al. 2009). In another study, *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii* spp. *shermanii* JS reduced AFB_1 absorption in chicken duodenum at one-minute exposure, with 70, 37 and 63 % efficiency, respectively (El-Nezami et al. 2000). In previous studies, probiotics have been employed as supplements due to their ability to bind aflatoxin, aiming to alleviate the detrimental effects of AFB_1 exposure. This study delves deeper into the binding efficiency of LcS, elucidating it through LcS counts recovered from faecal samples. Moreover, the AFB_1+LcS group exhibited higher faecal AFB_1 concentration compared to the LcS group, further underscoring this binding mechanism.

CONCLUSION

The present study shows that with LcS supplementation, the harmful effects of AFB_1 exposure can be alleviated to a degree that is almost similar to the non-exposed rats. The AFB_1+LcS group shows an increase in average body weight throughout the study, while the average food intake showed a significant increase at Week 3 to Week 4, a rebound after experiencing a significant reduction from Week 1 to Week

2 in comparison to the AFB_1 only group. Other than that, the H&E staining of the small intestine and colon only shows a mild to no inflammation with LcS supplementation, whereas a mild to moderate inflammation in the absence of any probiotic supplementation. The histomorphometry of small intestine also shows no significant differences in almost all measurements in the duodenum, jejunum and ileum of the AFB_1+LcS group in comparison to the control group, which indicates the role of LcS in maintaining the integrity of the small intestine. The findings on the increased levels of faecal AFB_1 concentration and elevated counts of faecal LcS in the AFB_1+LcS group provides explanation and reinforcement for the proposed mechanism of AFB_1-LcS binding in the intestine and its subsequent excretion via faecal route. For a deeper comprehension of how LcS supplementation influences AFB_1 excretion, further investigation into aspects such as intestinal permeability, serum and urinary AFB_1 levels, and similar factors is warranted.

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REFERENCES

Abraham, A., Al-Khaldi, S., Assimon, S. A. Beaudry, C., Benner, R. A., Bennet, R., Binet, R., Cahill, S. M., Burkhardt, W., Chen, Y., Day, J., Deeds, J., DeGrasse, S., DePaola, A., Feng, P., Foley, S., Fry Jr., F. S., Granade, H. R., Hait, J., … Ziobro, G. 2012. Bad Bug Book, Handbook of Foodborne Pathogenic Microorganisms, and Natural Toxins. (K. Lampel, S. Al-Khaldi, & S. M. Cahill, Eds.) (2nd Ed.). Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA). FDA.

Ahlberg, S. H., Joutsjoki, V., & Korhonen, H. J. 2015. Potential of lactic acid bacteria in aflatoxin risk mitigation. International Journal of Food Microbiology, 207, 87–102. doi: 10.1016/j.ijfoodmicro.2015.04.042

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Koga, N., Hattori, K., Ota, M., & Kunugi, H. 2019. Bifidobacterium and Lactobacillus counts in the gut microbiota of patients with bipolar disorder and healthy controls. Frontiers in Psychiatry, 10(JAN), 1–8. doi: 10.3389/fpsyt.2018.00730

Akinrinde, A. S., Adebiyi, O. E., & Asekun, A. 2019. Amelioration of Aflatoxin B1-induced gastrointestinal injuries by Eucalyptus oil in rats. Journal of Complementary and Integrative Medicine, 17(1), 1–11. doi: 10.1515/jcim-2019-0002

Applegate, T. J., Schatzmayr, G., Pricket, K., Troche, C., & Jiang, Z. 2009. Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poultry Science, 1235–1241. doi: 10.3382/ps.2008-00494

Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science, 88(1), 49–55. doi: 10.3382/ps.2008-00244

Awad, W., Ghareeb, K., & Böhm, J. 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing Enterococcus faecium and oligosaccharides. International Journal of Molecular Sciences, 9(11), 2205–2216. doi: 10.3390/ijms9112205

Bhat, A. A., Uppada, S., Achkar, I. W., Hashem, S., Yadav, S. K., Shanmugakonar, M., Al-Naemi, H. A., Haris, M., & Uddin, S. 2019. Tight junction proteins and signaling pathways in cancer and

inflammation: A functional crosstalk. Frontiers in Physiology, 10(JAN), 1–19. doi: 10.3389/fphys.2018.01942

Cabral, L. Q. T., Ximenez, J. A., Moreno, K. G. T., & Fernandes, R. 2020. Probiotics have minimal effects on appetite-related hormones in overweight or obese individuals: A systematic review of randomized controlled trials. Clinical Nutrition, 40(4), 1776–1787. doi: 10.1016/j.clnu.2020.10.028

Cappucino, J. G. & Sherman, N. 2010. Microbiology: A laboratory manual (7th Ed.). Pearson Education, Inc., San Francisco, CA.

Carvajal-Moreno, M. 2017. Do gastroenterologists consider aflatoxins as origin of digestive system cancers? Journal of Pharmacovigilance, 5(5). doi: 10.4172/2329-6887.1000242.

Centers for Disease Control and Prevention. 2012. CDC - Health Studies Program: Chemical Exposures - Aflatoxin. Centers for Disease Control and Prevention. https://www.cdc.gov/nceh/hsb/chemicals/aflatoxin.htm

Charan, J., & Kantharia, N. 2013. How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics, 4(4), 303–306. doi: 10.4103/0976-500X.119726

Cullen, J. M., Rueber, B. H., Hsieh, L. S., Hyde, D. M., & Hsieh, D. P. 1987. Carcinogenicity of Dietary Aflatoxin M1 in Male Fischer Rats Compared to Aflatoxin B1. Cancer Research, 47(7), 1913–1917.

Dey, D. K., Chang, S. N., & Kang, S. C. 2021. The inflammation response and risk associated with aflatoxin B1 contamination was minimized by insect peptide CopA3 treatment and act towards the beneficial health outcomes. Environmental Pollution, 268, 115713. doi: 10.1016/j.envpol.2020.115713

Ducatelle, R., Goossens, E., Meyer, F. De, Eeckhaut, V., Antonissen, G., Haesebrouck, F., & Immerseel, F. Van. 2018. Biomarkers for monitoring intestinal health in poultry: present status and future perspectives. Veterinary Research, 1–9. doi: 10.1186/s13567-018-0538-6

El-Nezami, H., Mykkanen, H., Kankaanpaa, P., Salminen, S., & Ahokas, J. 2000. Ability of Lactobacillus and Propionibacterium strains to remove aflatoxin B1 from the chicken duodenum. Journal of Food Protection, 63(4), 549–552. doi: 10.4315/0362-028x-63.4.549

Feng, G. D., He, J., Ao, X., & Chen, D. W. 2017. Effects of maize naturally contaminated with aflatoxin B1 on growth performance, intestinal morphology, and digestive physiology in ducks. Poultry Science, 96(6), 1948–1955. doi: 10.3382/ps/pew420

Firmin, S., Morgavi, D. P., Yiannikouris, A., & Boudra, H. 2011. Effectiveness of modified yeast cell wall extracts to reduce aflatoxin B1 absorption in dairy ewes. Journal of Dairy Science, 94(11), 5611–5619. doi: 10.3168/jds.2011-4446

Food and Agricultural Organization of the United Nations and World Health Organization 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. World Health Organization. http://www.who.int/foodsafety/publications/fs_manageme nt/en/probiotics.pdf

Galarza-Aeeber, R., Latorre, J. D., Bielke, L. R., Kuttappan, V. A., & Tellez, G. 2016. Leaky gut and mycotoxins: aflatoxin B1 does not increase gut permeability in broiler chickens. Frontiers in Veterinary Science, 3(February), 1–8. doi: 10.3389/fvets.2016.00010

Galosi, L., Desantis, S., Roncarati, A., Robino, P., Bellato, A., Nebbia, P., Ferrocino, I., Santamaria, N., Biagini, L., Filoni, L., Attili, A. R., & Rossi, G. 2021. Positive influence of a probiotic mixture on the intestinal morphology and microbiota of farmed guinea fowls (Numida meleagris). Frontiers in Veterinary Science, 8(October), 1–13. doi: 10.3389/fvets.2021.743899

Gratz, S., Ta, M., Juvonen, R. O., Viluksela, M., Turner, P. C., & Mykka, H. 2006. Lactobacillus rhamnosus Strain GG modulates intestinal absorption, faecal excretion, and toxicity of aflatoxin B1 in rats. Applied and Environmental Microbiology, 72(11), 7398–7400. doi: 10.1128/AEM.01348-06

He, L., Liu, Y., Guo, Y., Xiao, N., & Tan, Z. 2018. Influences of Aflatoxin B1 on main intestinal bacteria communities and

enzyme activities in mice. Toxin Reviews, 38(2), 121–126. doi: 10.1080/15569543.2018.1426611

Hernandez-Mendoza, A., Garcia, H. S., & Steele, J. L. 2009. Screening of Lactobacillus casei strains for their ability to bind aflatoxin B1. Food and Chemical Toxicology, 47(6), 1064–1068. doi: 10.1016/j.fct.2009.01.042

Huang, L., Duan, C., Zhao, Y., Gao, L., Niu, C., Xu, J. & Li, S. 2017. Reduction of aflatoxin B1 toxicity by Lactobacillus plantarum C88: A potential probiotic strain isolated from Chinese traditional fermented food "Tofu". PLoS ONE, 12(1), 1–16. doi: 10.1371/journal.pone.0170109

Ibrahim., A. A. E. 2013. Vitamin A downregulation Bcl-2 and TGF-a expression during colon cancer in AFB1-induced female rats. Journal of Natural Sciences Research, 3(5), 67-83.

International Agency for Research on Canceer (IARC) 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks in Humans, 82: 1–556. PMID:12687954.

Jahanian, E., Mahdavi, A. H., Asgary, S., & Jahanian, R. 2016. Effect of dietary supplementation of mannanoligosaccharides on growth performance, ileal microbial counts, and jejunal morphology in broiler chicksexposed to aflatoxins. Livestock Science, 190, 123–130. doi: 10.1016/j.livsci.2016.05.008

Jin, S., Yang, H., Jiao, Y., Pang, Q., Wang, Y., Wang, M., Shan, A., & Feng, X. 2021. Dietary curcumin alleviated acute ileum damage of ducks (Anas platyrhynchos) induced by AFB1 through regulating Nrf2-ARE and NF- κB signaling pathways. Foods, 10(6). doi: 10.3390/foods10061370

Kazemi, A., Noorbala, A. A., & Djafarian, K. (2020). Effect of probiotic and prebiotic versus placebo on appetite in patients with major depressive disorder: post hoc analysis of a randomised clinical trial. Journal of Human Nutrition and Dietetics, 33(1), 56–65.

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., & Kang, S. G. 2017. Aflatoxins: A global concern for food safety, human health and their management. Frontiers in Microbiology, 7(January), 1–10. doi: 10.3389/fmicb.2016.02170

Lawrence, T. 2009. The Nuclear Factor NF-kB Pathway in Inflammation. Cold Spring Harbor Perspectives in Biology, 1(a001651), 1–10. doi: 10.1101/cshperspect.a001651

Liew, W.-P.-P., & Mohd-Redzwan, S. 2018. Mycotoxin: Its Impact on Gut Health and Microbiota. Frontiers in Cellular and Infection Microbiology, 8(February). doi: 10.3389/fcimb.2018.00060

Liew, W.-P.-P., Nurul-Adilah, Z., Than, L. T. L., & Mohd-Redzwan, S. 2018. The binding efficiency and interaction of Lactobacillus casei Shirota toward aflatoxin B1. Frontiers in Microbiology, 9(July), 1–12. doi: 10.3389/fmicb.2018.01503

Liew, W.-P.-P., Sabran, M.-R., Than, L.-T.-L. & Abd-Ghani, F. 2022. Metagenomic and proteomic approaches in elucidating aflatoxin B1 detoxification mechanisms of probiotic Lactobacillus casei Shirota towards intestine. Food and Chemical Toxicology. 160. 112808. doi: 10.1016/j.fct.2022.112808

Lye, M. S., Ghazali, A. A., Mohan, J., Alwin, N., and Nair, R. C. 1995. An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia. American Journal of Tropical Medicine and Hygiene. 53, 68– 72.

Nikbakht Nasrabadi, E., Jamaluddin, R., Abdul Mutalib, M. S., Khaza'ai, H., Khalesi, S., & Mohd-Redzwan, S. 2013. Reduction of aflatoxin level in aflatoxin-induced rats by the activity of probiotic Lactobacillus casei strain Shirota. Journal of Applied Microbiology, 114(5), 1507–1515. doi: 10.1111/jam.12148

Nurul-Adilah, Z., Liew, W.-P.-P., Mohd-Redzwan, S. & Amin, I. 2018. Effect of high protein diet and probiotic Lactobacillus casei Shirota supplementation in aflatoxin B1-induced rats. Biomed Research International, 23; 2018:9568351. doi: 10.1155/2018/9568351

Oghenesuvwe, E. E., Ekene, N. E. & Ajaghaku, D. L. 2014. Guidelines on dosage calculation and stock solution preparation in experimental animals'

studies. Journal of Natural Science Research. 4(18).

Pandey, I. & Chauhan, S. S. 2007. Studies on production performance and toxin residues in tissues and eggs of layer chickens fed on diets with various concentrations of aflatoxin AFB1. British Poultry Science. 48:6, 713-723. doi: 10.1080/00071660701713534

Pandy, V. 2020. A Simple Method for Animal Dose Calculation in Preclinical Research. EC Pharmacology and Toxicology. 8. 1-2.

Poloni, V., Magnoli, A., Fochesato, A., Cristofolini, A., Caverzan, M., Merkis, C. Montenegro, M., & Cavaglieri, L. 2019. A Saccharomyces cerevisiae RC016-based feed additive reduces liver toxicity, residual aflatoxin B1 levels and positively influences intestinal morphology in broiler chickens fed chronic aflatoxin B1-contaminated diets. Animal Nutrition, 6(1), 31– 38. doi: 10.1016/j.aninu.2019.11.006

Rustemeyer, S. M., Lamberson, W. R., Ledoux, D. R., Rottinghaus, G. E., Shaw, D. P., Cockrum, R. R., Kessler, K. L., Austin, K. J. & Cammack, K. M. 2010. Effects of dietary aflatoxin on the health and performance of growing barrows. Journal of Animal Science. 88. 3624-30. doi: 10.2527/jas.2009-2663

Schoultz, I., & Keita, Å. V. (2020). The intestinal barrier and current techniques for the assessment of gut permeability. Cells, 9(8), 1–30.

Sobolewska, A., Bogucka, J., Dankowiakowska, A., Elminowska-Wenda, G., Stadnicka, K., & Bednarczyk, M. 2017. The impact of synbiotic administration through in ovo technology on the microstructure of a broiler chicken small intestine tissue on the 1st and 42nd day of rearing. Journal of Animal Science and Biotechnology, 8(1), 1–8. doi: 10.1186/s40104-017-0193-1

Trebak, F., Alaoui, A., Alexandre, D., El, S., Anouar, Y., Chartrel, N., & Magoul, R. 2015. Neurotoxicology impact of aflatoxin B1 on hypothalamic neuropeptides regulating feeding behavior. Neurotoxicology, 49, 165–173. doi: 10.1016/j.neuro.2015.06.008

Vdoviaková, K., Petrovová, E., Maloveská, M., Krešáková, L., Teleky, J., Elias, M. Z. J., & Petrášová, D. 2016. Surgical anatomy of the gastrointestinal tract and its vasculature in the laboratory rat. Gastroenterology Research and Practice, 2016 (Article ID 2632368). doi: 10.1155/2016/2632368

Wang, F., Zuo, Z., Chen, K., Gao, C., & Yang, Z. 2018. Histopathological injuries, ultrastructural changes, and depressed TLR expression in the small intestine of broiler chickens with aflatoxin B1. Toxins, 10(131), 1–16. doi: 10.3390/toxins10040131

Wang, L., Llorente, C., Hartmann, P., Yang, A., Chen, P. & Schnabl, B. 2015. Methods to determine intestinal permeability and bacterial translocation during liver disease. Journal of Immunological Methods, (421), 44–53. doi: 10.1016/j.jim.2014.12.015

Wilson, F. D., Cummings, T. S., Barbosa, T. M., Williams, C. J., Gerard, P. D., & Peebles, E. D. 2018. Comparison of two methods for determination of intestinal villus to crypt ratios and documentation of early age-associated ratio changes in broiler chickens. Poultry Science, 97(5), 1757–1761. doi: 10.3382/ps/pex349

World Health Organization. 2018. Mycotoxins. World Health Organization. https://rb.gy/z9ldmw

Yuki, N., Watanabe, K., Mike, A., Tagami, Y., Tanaka, R., Ohwaki, M., & Morotomi, M. 1999. Survival of a probiotic, Lactobacillus casei strain Shirota, in the gastrointestinal tract: Selective isolation from faeces and identification using monoclonal antibodies. International Journal of Food Microbiology, 48, 51–57. doi: 10.1016/s0168-1605(99)00029-x

Zhang, S., Peng, X., Fang, J., Cui, H., Zuo, Z., & Chen, Z. 2014. Effects of aflatoxin B1 exposure and sodium selenite supplementation on the histology, cell proliferation, and cell cycle of jejunum in broilers. Biological Trace Element Research, 160(1), 32–40. doi: 10.1007/s12011-014-0009-5