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EVALUATION OF FUMONISINS EXPOSURE THROUGH STRUCTURAL AND FUNCTIONAL CHANGES IN THE GASTROINTESTINAL TRACT OF PIGS

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List of Abbreviations

15-ADON	15-acetlydeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
AAs	Amino acids
AFB_1	Aflatoxin B_1
AFs	Aflatoxins
AID	Apparent ileal digestibility
AJs	Adherens junctions
ALB	Albumin
ALKP	Alkaline phosphatase
ALT	Alanine trsansaminase
ANOVA	Analysis of variance
APCs	Antigen-presenting cells
AST	Aspartate transaminase
ATTD	Apparent total tract digestibility
BEAs	Beauvericins
BW	Body weight
cd	Cluster of differentiation
CD	Crypt depth
CerS	Ceramide synthase
CE-SSCP	Capillary electrophoresis single-stranded conformation polymorphism
CF	Crude fibre
CK	Creatine kinase
CLDN	Claudin
CP	Crude protein
DDGS	Distillers dried grains and solubles
DE	Digestible energy
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
EDc	Energy digestibility coefficient
EE	Ether extract
EFSA	European Food Safety Authority
ELEM	Equine leukoencephalomalacia
ENNs	Enniatins

ETEC	Enterotoxigenic *Escherichia coli*
EU	European Union
ExPEC	Extraintestinal pathogenic *Escherichia coli*
FAO	Food and Agriculture Organization
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FB_3	Fumonisin B_3
FI	Feed intake
FUMs	Fumonisins
FUSX	Fusarenon X
GALT	Gut-associated lymphoid tissues
GC	Gas chromatography
GE	Gross energy
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
GIM	Gastrointestinal microbiota
GIT	Gastrointestinal tract
GSH	Glutathione
GSHPx	Glutathione peroxidase
HDL	High-density lipoprotein
HRP	Horse radish peroxidase
Hsp70	Heat shock protein 70
IARC	International Agency for Research in Cancer
IECs	Intestinal epithelial cells
IL	Interleukin
IPEC	Intestinal porcine epithelial cell line
Isc	Short-circuit current
JAMs	Junctional adhesion molecules
LAB	Lactic acid bacteria
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LOD	Limit of detection
MAPKs	Mitogen- activated protein kinases
MATE-KC	Hungarian University of Agriculture and Life Sciences, Kaposvári Campus

MDA	Malondialdehyde
ME	Metabolizable energy
ME_m	Maintenance energy requirement
MHC-II	Major histocompatibility complex class-II
MLNs	Mesentric lymph nodes
MPS	Mononuclear phagocyte system
NIV	Nivalenol
NO	Nitric oxide
OCLN	Occludin
OTA	Ochratoxin
OTUs	Operational taxonomic units
PP	Peyer's patches
PPE	Porcine pulmonary oedema
PVTC	Post-valve T caecum
QIIME	Quantitative insights into microbial ecology
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
Sa	Sphinganine
SCFAs	Short-chain fatty acids
SCOOP	Scientific Cooperation
SMC	Spleen mononuclear cells
So	Sphingosine
TCTs	Trichothecenes
TEER	Transepithelial electrical resistance
TiO_2	Titanium dioxide
TJs	Tight junctions
TP	Total protein
UC	Ussing chamber
VFAs	Volatile fatty acids
VH	Villus height
WHO	World Health Organization
YCWE	Yeast cell wall enzyme
ZEN	Zearalenone

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1. INTRODUCTION

1.1. Research background

The harm posed by mycotoxins to human and animal health has unquestionably been since ancient times, and it will only get worse as the demand for the world's food supply rises in response to the expansion of the world's population (Marasas *et al.,* 1987). Mycotoxins are prevalent in a wide range of crops, and can cause various, very different clinical diseases in both humans and animals due to their remarkable variations in chemical structure (Kendra and Dyer, 2007). About 25% of the cereals produced worldwide, according to the Food and Agriculture Organization of the United Nations (FAO), are mycotoxin-contaminated (Rice and Ross, 1994). The most important mycotoxins that have received significant attention include aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (TCTs), zearalenone (ZEN) and fumonisins (FUMs).

The *Fusarium verticillioides* and *F. proliferatum* are the main plant pathogens that produce FUMs and commonly infect maize and maize-based products. The commonest occurring types of FUMs present in most collected field samples are fumonisin B_1 (FB_1), B_2 (FB_2), B_3 (FB_3) and B4 (FB_4) in approximate proportions of 70-80%, 15-25%, 3-8% and 1-2%, respectively (Szecsi *et al.,* 2010). Among these types, FB_1 is the most abundant in nature and toxic (Lerda, 2017). Fumonisin B_1 (FB_1) was isolated in 1988 by Gelderblom *et al.* (1988) and chemically characterized by Bezuidenhout *et al.* (1988). The structural resemblance of FB_1 to sphingosine (So; the major long-chain base backbone of cellular sphingolipids) has been established as a competitive inhibitor of sphinganine (Sa) and the enzyme sphingosine N-acyltransferase – also known as ceramide synthase (CerS) – has been established as the principal pathway of FB_1 toxicity in most studied species. This enzyme suppression disrupts sphingolipid metabolism, resulting in increased Sa and So in the serum and tissues of animals, as well as a reduction in complex sphingolipids. Thus, the Sa/So ratio has been used across several animal species as an early biomarker of FB_1- induced toxicity. Additionally, clinical signs induced by FB_1 are species-specific and vary depending on the primary target organ (Smith, 2018). In equines, FB_1 causes equine leukoencephalomalacia (ELEM); in pigs, the porcine pulmonary oedema (PPE) (da Rocha *et al.,* 2014); in rodents, hepatotoxic effects, and cancer (Gelderblom *et al.,* 1988; Gelderblom *et al.,* 1991) and nephrotoxic effects (Voss *et al.,* 1993) have been described. In humans, oesophageal cancer and neural tube defects have been linked with the consumption of FB_1 diets in some regions of the world (Chilaka *et al.,* 2017; Ortiz *et al.,* 2015). The

International Agency for Research on Cancer has since categorized FB_1 as a category 2B carcinogen (IARC, 2002).

Gastrointestinal (GI) health is in an excellent state when the diet, mucosa, and commensal microbiota are functioning harmoniously (Conway, 1994). The mucosa is made up of the digestive epithelium, which has a distinctive structure, the gut-associated lymphoid tissue (GALT), and the mucus that coats the epithelium. Within the gastrointestinal tract (GIT), the GALT, microbiota, mucus layer, and host epithelium interact to establish a complex and dynamic balance that supports the digestive system's effective functioning. Therefore, a proposed definition of gut health is "a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal are not constrained by intestinal dysfunction" (Celi *et al.,* 2019). Dietary composition (ingredients, nutrients, and additives) can critically impact the development and function of the digestive system, as well as the immune system and the microbiota since ingested nutrients play a substantial role in the development and functioning of the GIT (Conway, 1994). This is an indication that if feed is contaminated with mycotoxins, it can have a crucial impact on the overall functioning of the GIT and eventually, on the health, well-being, and overall performance of the animal.

Indeed, the post- absorptive effects brought on by FB_1 have been extensively studied leaving a notable gap on the GIT functions. Intriguingly, in monogastric animal species like the pig, FB_1 absorption rate is only 1 to 6 per cent (Bouhet sand Oswald, 2007). And together with the enterohepatic recycling role of the gut, the GIT is recurrently exposed to much higher levels of FB_1 relative to other tissues (Grenier *et al.,* 2015). Some studies have evaluated the potentially deleterious effects of FB_1 on GIT function and have reported immunomodulation (Swamy *et al.,* 2003), intestinal barrier dysfunctions (Bouhet and Oswald, 2007), and a reduction in nutritients digestion and absorption efficiency in the gut (Gbore and Egbunike, 2007; Lessard *et al.,* 2009; Gbore and Akele, 2010). Moreso, studies on the potential effects on the microbiota are limited. Other mycotoxins such as AFB_1 (Zhou *et al.,* 2019), deoxynivalenol (DON) (Wang *et al.,* 2019), ZEN (Li *et al.,* 2018), and TCTs (Gratz *et al.,* 2017) have been shown to have deleterious influence on gut microbiota composition. In a few studies, the presence of FB_1 (purified form; 0.5 mg/kg BW) in the diets acted as a predisposing factor for the increased invasion of *Escherichia. coli* colonization in the intestines of piglets (Oswald *et al.,* 2003). Burel *et al.* (2013) found that continuous exposure to 11.8 ppm FUMs (FB_1 + FB_2) reduced the faecal microbiota profiles which subsequently resulted in an imbalance of the microbiota of pigs. Later, Mateos *et al.* (2018) highlighted distinct effects of FB_1 on the faecal microbial

distribution of weaned piglets following 22 days of exposure to a diet contaminated with 12 mg/kg FB_1. In a much recent study, Zhang *et al.* (2021) highlighted a significant shift in the microbial flora of BALB/c mice exposed to increasing levels of dietary FB_1 while Yu *et al*. (2022) found a significant shift in the microbiota of broilers exposed to either naturally contaminated FUMs (FB_1, FB_2, FB_3) or hydrolyzed FUMs diet.

Hypothesis

It is well documented that during the period of nursery, the metabolism of a young pig is very active, and the gut microbiota is in a dynamically developing stage. The GI epithelium is highly stimulated at this stage; there is rapid protein turnover rate, and mycotoxins specifically target this tissue. In light of this, the incidence of mycotoxin contamination could exacerbate the incidence of unwellness and would eventually lower effective performance at a later stage of the pig's life. Further, maize – the most commonly affected commodity by FUMs – is a typical component of piglets in the second phase of nursery and as such, a clear indication of their susceptibility to FUMs intoxication.

Therefore, we hypothesized that following ingestion of either 15 or 30 mg/kg dietary FUMs (FB_1 + FB_2 + FB_3) by nursery pigs for 21 days, a series of perturbations in the normal functioning of the whole GIT may occur. Noteworthy, exceeding the European Union (EU) recommended threshold for FB_1 in the complete diet for pigs (max. 5 mg/kg of finished feed) was considered so that any potential repercussions would be clear during the 21- day- long trial. Further, these concentrations have been reported to fall within a range where FUMs occasionally occur (between 10 and 40 mg/kg feed) (Grenier and Applegate, 2013). Also, given the high percentage of maize in their diet and therefore highly at risk as well, the further objective was to subject fattening pigs to a much higher dietary dose of 40 mg/kg FUMs (FB_1 + FB_2 + FB_3) to ascertain the potential disturbance caused to the apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AAs).

1.2. Research objectives

Our research objective on one hand, was to evaluate the potential complex toxic effects induced by dietary FUMs on the overall GI functioning including the health status and nutrient balance of young pigs and on the other hand, to study the impact of dietary FUMs contamination on amino acid digestibility in fattening pigs. Specifically, the impact of different levels of dietary FUMs (15 or 30 mg/kg diet) intoxication was investigated on dietary energy and nutrient digestibility and mineral balance, digestive enzymes activity and intestinal morphology, antioxidant parameters, heat shock protein 70 (Hsp70) activity, serum biochemical parameters,

histological and histopathological examinations of internal organs and, caecal fermentation products as well as intestinal and faecal microbiota composition of nursery pigs in a 3- week-long feeding trial.

A further objective was to ascertain the potential effects that a higher dose of FUMs (40 mg/kg diet) would have on the AID of CP and AAs in fattening pigs during either a short (7 days) or long (21 days) period following intoxication.

2. LITERATURE REVIEW

2.1. Occurrence and toxicity of *Fusarium* mycotoxins

Bennett and Bentley (1989) defined mycotoxins as "metabolic intermediates or products found as differentiation products in restricted taxonomic groups, not essential to growth and life of the producing organism and biosynthesized from one or more general metabolites by a wider variety of pathways than is available in general metabolism". These intermediates are further described as harmful secondary metabolites of moulds that are capable of exerting detrimental effects on the health of humans and animals, either directly or via biological transformation (Beardall and Miller, 1994). Mould and fungus growth have been found in roughly 25% of the world's nuts, cereals, and rice, according to research by FAO and the World Health Organization (WHO) (Pandya and Arade, 2016). Unfortunately, it is almost impossible to avoid mycotoxins' occurrence regardless of the best agricultural management and post-harvest practices utilized. Therefore, while some studies are working to enhance methods for identifying mycotoxins in samples, others are attempting to alter or eliminate their existence to lessen their toxicity.

Mycotoxin-producing fungi that are frequently and constantly problematic and cause contamination to several agricultural products are those of the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, and *Claviceps* (Marin *et al.*, 2013). The most significant mycotoxins identified in food commodities include: AFs, produced by *Aspergillus* species; OTA, produced by both *Aspergillus* and *Penicillium*; TCTs [(type A: HT-2 and T-2 toxin, and type B: DON), ZEN, FB_1 and FB_2, and fusaproliferin, moniliformin, beauvericin, and enniatins (emerging mycotoxins) produced mainly by *Fusarium* species; ergot alkaloids produced by *Claviceps*; and altenuene, alternariol, alternariolmethyl ether, altertoxin, and tenuazonic acid produced by *Alternaria* species (Bottalico and Logrieco, 1998; Paster and Barkai-Golan, 2008).

A recent report on a survey conducted over 10 years collected about 74,830 samples from 100 countries to analyze the global occurrence of AFB_1, FUMs, ZEN, OTA, DON, and T-2 toxin. In the outcome (Table 1), at least one mycotoxin wass found in 88% of the samples tested for the presence of ≥ 3 mycotoxins. Deoxynivalenol (DON), FUMs, and ZEN, which were found in 64%, 60%, and 45% of all samples respectively, were the most common *Fusarium* mycotoxins. On the other hand, AFB_1, T-2, and OTA were detected in 23%, 19%, and 15% of the samples, respectively. The greatest median concentrations were found in FUMs and DON, at 723 μg/kg and 388 μg/kg, respectively (Gruber-Dorninger *et al.*, 2019).

Table 1. Global occurrence of significant mycotoxins in different commodities (adapted from Gruber-Dorninger *et al.,* 2019)

Mycotoxin	n[1]	Positive samples[2]		Median of positives (µg/kg)	1st Quartile of positives (µg/kg)	3rd Quartile of positives (µg/kg)	Maximum (µg/kg)
		n[1]	%				
				All samples			
AFB_1	51,475	11,941	23	4	2	17	10,918
FUMs[3]	46,477	27,890	60	723	240	1,858	290, 517
ZEN	61,413	27,559	45	55	25	147	105,000
DON	59,107	37,940	64	388	200	885	84,860
OTA	32,271	4,858	15	3	2	7	2,000
T-2 Toxin	27,850	5,289	19	22	8	40	3,051
				Complete feed			
AFB_1	16,563	4,251	26	6	2	23	10,918
FUMs[3]	16,285	11,825	73	555	198	1,297	290,517
ZEN	19,171	10,676	56	41	20	102	9,432
DON	18,649	13,004	70	294	134	600	32,893
OTA	11,990	2,801	23	3	2	6	1,582
T-2 Toxin	9,884	2,246	23	10	4	22	1,300
				Maize			
AFB_1	15,889	3,835	24	4	1	22	6,105
FUMs[3]	12,965	10,397	80	1300	520	2,940	218,883
ZEN	15,860	7,002	44	77	33	217	16,495
DON	12,660	8,486	67	520	260	1,240	51,374
OTA	6,388	334	5	3	2	14	889
T-2 Toxin	6,087	727	12	25	11	53	978
				Maize DDGS			
AFB_1	320	62	19	11	4	20	340
FUMs[3]	329	256	78	814	398	1,870	26,828
ZEN	368	275	75	102	60	237	2,896
DON	381	316	83	1490	574	2,579	84,860
OTA	280	62	22	4	2	11	53
T-2 Toxin	52	3	6	40	35	43	46
				Maize silage			
AFB_1	3,104	188	6	2	1	4	342
FUMs[3]	3,010	1,114	37	138	45	416	7,090
ZEN	3,735	1,508	40	84	34	201	6,239
DON	4,206	2,588	62	474	219	1,092	34,861
OTA	2,830	161	6	3	2	6	69
T-2 Toxin	1,800	58	3	20	10	51	685

AFB_1= aflatoxin B_1, DON= deoxynivalenol, FUMs= fumonisins, OTA= ochratoxin, ZEN= zearalenone. [1] sample number, [2] positive samples are defined as > limit of detection excluding aflatoxins below 0.5 ng/ g and other mycotoxins below 1 ng/ g, [3] sum of fumonisins B_1, B_2 and B_3

Table 1. Global occurrence of significant mycotoxins in different commodities ***continued***

				Soybean grains			
AFB_1	916	186	20	1	1	2	74
FUMs[3]	794	135	17	68	29	223	7,023
ZEN	1,024	364	36	43	26	71	4,336
DON	975	284	29	416	160	640	5,500
OTA	718	86	12	3	2	7	46
T-2 Toxin	557	102	18	29	23	37	317
				Soybean meal			
AFB_1	1,692	490	29	2	1	4	109
FUMs[3]	1,475	336	24	104	31	290	7,210
ZEN	1,767	1,072	61	47	33	83	3,720
DON	802	247	31	119	25	424	5,600
OTA	606	82	14	4	2	10	141
T-2 Toxin	975	324	33	33	25	44	754
				Wheat			
AFB_1	2,210	221	10	1	1	3	161
FUMs[3]	2,219	304	14	117	31	246	28,278
ZEN	4,925	1,624	33	34	20	75	23,278
DON	5,949	3,866	65	369	218	865	49,307
OTA	1,973	172	9	3	2	5	364
T-2 Toxin	1,993	439	22	25	13	35	1,300
				Barley			
AFB_1	727	64	9	1	1	2	120
FUMs[3]	776	65	8	53	17	366	10,485
ZEN	3,129	637	20	25	20	58	8,952
DON	4,046	2,468	61	359	234	750	35,000
OTA	730	46	6	3	2	9	150
T-2 Toxin	1,225	272	22	26	9	51	404
				Rice			
AFB_1	205	63	31	5	2	14	113
FUMs[3]	244	49	20	142	63	382	6,895
ZEN	220	74	34	60	34	107	1,530
DON	226	60	27	266	87	436	3,859
OTA	230	32	14	3	2	5	20
T-2 Toxin	54	5	9	9	8	26	30

AFB_1= aflatoxin B_1, DON= deoxynivalenol, FUMs= fumonisins, OTA= ochratoxin, ZEN= zearalenone. [1] sample number, [2] positive samples are defined as > limit of detection excluding aflatoxins below 0.5 ng/ g and other mycotoxins below 1 ng/ g, [3] sum of fumonisins B_1, B_2 and B_3

The *Fusarium* genera of phytopathogenic fungi produce cereal crop diseases that are hard to control (e.g. *Fusarium* head blight in wheat and *Fusarium* ear rot in maize). They are of the group hyphomycetes, a subgroup of ascomycetes (Desjardins, 2006). Of the more than 145 different *Fusarium* species discovered, a report suggests that one-seventh are harmful toxin producers (Moss and Thrane, 2004). The important kinds of *Fusarium* mycotoxins are the TCTs (HT-2, T-2 and DON), ZEN and FUMs. On a global scale, cereal grains and animal feed may be prone to multiple contaminations with TCTs, ZEN and FUMs (Placinta *et al.,* 1999). These mycotoxins mostly contaminate cereals like wheat, barley, oats, and maize.

Additionally, their predominance varies according to the type of species, geographic location and the environmental conditions that favour their growth and production (Lee *et al.,* 2015; Logrieco *et al.,* 2002). In 2003, the Scientific Cooperation (SCOOP) of different European countries undertook a task by reporting the responses of thirteen participating countries on how populations of these countries responded to fusario-mycotoxins exposure. The task entailed a total of 16 fusario-mycotoxins and almost 45000 total analyses. The task outcome showed higher percentages of 57% and 46% for DON and FUMs, respectively. The common food commodities most heavily contaminated with these mycotoxins reported in the same survey were maize and wheat (Gareis, 2003).

In Table 2, the occurrence of *Fusarium* mycotoxins in certain regions of the world and their allowed limits in various food/feed commodities (Ji *et al.*, 2019).

Table 2. Allowable limits of *Fusarium* mycotoxins in food and feeds in certain countries and regions (adapted from Ji *et al.*, 2019)

Commodity	Type of *Fusarium* mycotoxin	Country	Applicable products	Limit (μg/kg)
Food	DON	China	Cereals and their products	1000
		EU	Raw durum and oats, wet-milled corn	1750
			Unprocessed cereals other than hard wheat, oats, and corn	1250
			Cereal that can be consumed directly and cornflakes less than or equal to 500 μm in size	750
			Bread, snacks, desserts, and breakfast cereals	500
			Cereal-based foods for infants and young children	200
		America	Wheat for food milling	2000
			Final products made using edible wheat	1000
		Canada	Unpurified soft wheat in China	2000
			Soft wheat flour (adult food)	1200
			Soft wheat flour (baby food)	600
		Armenia	Wheat	700
			Barley	1000
		Belarus	Wheat	700
			Baby food	Prohibited
		Bulgaria	Grain and products made from grain for direct consumption or as processed food ingredients	1000
			Cereals which will be stored or subjected to further physical processing prior to consumption	2000
			Corn and corn products	1000
		Cuba	Imported cereals	300
		Cyprus	Grain	1200
		Serbia	Raw maize	1750
	ZEN	China	Wheat and flour	60
			Maize and maize flour (slag and slice)	60
		EU	Processed cereals for infants and young children	20
			Bread and breakfast cereals	50
			Grain products that can be eaten directly	75
			Maize, maize snacks, and maize breakfast cereals that can be eaten directly	100

Commodity	Type of *Fusarium* mycotoxin	Country	Applicable products	Limit (μg/kg)
			Maize flakes larger than 500 μm in size	200
			Maize flakes less than or equal to 500 μm in size	300
			Maize treated via wet grinding	350
			Refined maize oil	400
		Armenia	All foods	1000
		Austria	Wheat, rye, and hard wheat	60
		Belarus	Barley, wheat, and maize	1000
			Baby foods	Prohibited
		Bulgaria	Grain and processed grain products for direct consumption or for use as processed food ingredients	200
			Maize and maize products	200
		Chile	All foods	200
		Columbia	Sorghum	1000
		France	Grain and grain products	50
	FUMs	EU	Maize-based baby foods	200
			Maize snacks and maize breakfast cereals	800
			Maize, maize snacks, and maize breakfast cereals that can be eaten directly	1000
			Maize flakes larger than 500 μm in size	1400
			Maize flakes less than or equal to 500 μm in size	2000
			Maize treated via wet grinding	4000
		America	Edible maize	2000
	FB_1 and FB_2	Bulgaria	Maize and maize products	1000
	FB_1	Cuba	Maize and rice	1000
	FB_1	France	Grain and grain products	1000
	T-2	China	Distiller's dried grain with maize solubles for feed	100
			Formulated feeds for pigs and poultry	1000
		Armenia	All foods	100
		Belarus	Cereal, flour, and shelled oats	100
			Infant food	Prohibited
		Bulgaria	Grain and grain products for direct consumption and for use as processed food ingredients	100

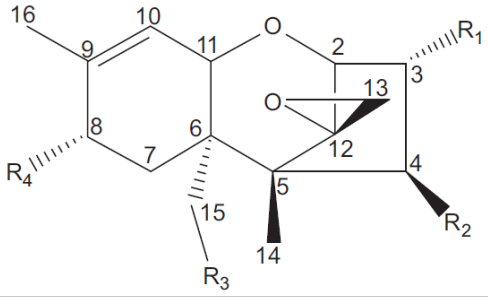
Commodity	Type of *Fusarium* mycotoxin	Country	Applicable products	Limit (µg/kg)
Feed	DON	China	Formulated feeds for pigs, calves, and lactating animals	1000
			Formulated feeds for cattle and poultry	3000
		Austria	Pannage	500
			Feed for fattening poultry	1500
			Feeds for breeding poultry and laying fowl	1000
			Feeds for beef cattle	1000
		Canada	Feeds for livestock and poultry	5000
			Feeds for pigs, calves, and cows	1000
		Cuba	All feedstuffs	300
		Cyprus	All feedstuffs except coarse grain	7000
			Complete feeds for pigs	1000
			Complete feeds for poultry and fattening calves	5000
			Complete feeds for other animals	3000
		Serbia	Feeds	8000
	ZEN	China	Feeds and distiller's dried grain with maize solubles	500
		Austria	Feeds for breeding swine	50
		Canada	Feeds for gilts and sows	3000
		Cyprus	Feedstuffs	2000
			Complete feeds for piglets	1000
			Complete feeds for all pigs except piglets	1500
	T-2	Canada	Feeds for pigs and poultry	1000
	HT-2	Canada	Feeds for livestock and poultry	100

DON= deoxynivalenol, EU= European Union, FUMs= fumonisins, FB_1= fumonisin B_1, FB_2= fumonisin B_2, OTA= ochratoxin, ZEN= zearalenone

The succeeding chapters delve into the prevalence and toxicity of the various *Fusarium* mycotoxins earlier briefly discussed. Furthermore, two significant emerging kinds namely enniatins and beauvericin have been discussed. These so-called emerging mycotoxins have the potency to induce clinical disorders and yet, no limits are set by global food/feed safety bodies for their levels of consumption (Bertero *et al.,* 2020). The FUMs group of fusario- mycotoxin is the main focus and so detailed further, per the various aspects of GIT functions for the rest of this literature.

2.1.1. Trichothecenes

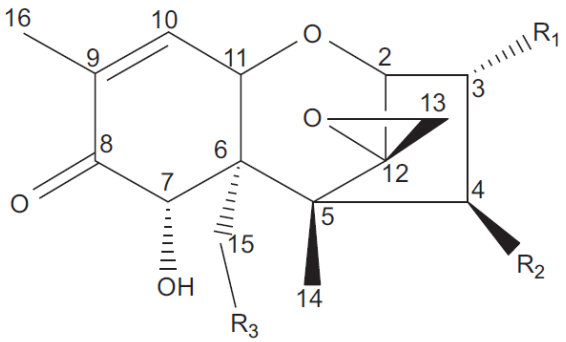
Trichothecenes (TCTs) are mainly produced by *Fusarium graminearum* albeit other several fungi are reported to be producers of TCTs as well. The common food commodities normally contaminated by TCTs are wheat, barley, oats, rye, and rice (Yazar and Omurtag, 2008). About 170 TCTs have been found and classified as kinds of A, B, C, and D based on differences in the functional hydroxyl and acetoxy side groups since their discovery. Type A and B are widely known to be of preliminary importance. Type A is usually represented by HT-2 and T-2 (Figure 1), with the latter being highly toxic (Grove, 1988).



Mycotoxin	R_1	R_2	R_3	R_4
HT2	OH	OH	OAc	$OCOCH_2CH(CH_3)_2$
T2	OH	OAc	OAc	$OCOCH_2CH(CH_3)_2$
MAS	OH	OH	OAc	H
DAS	OH	OAc	OAc	H

Figure 1. Chemical structures of Type A trichothecenes; DAS= Diacetoxyscirpenol, HT2= HT-2 toxin, MAS= monoacetoxyscirpenol, T2= T-2 toxin (Asam *et al.*, 2017)

Typical congeners of type B TCTs are nivalenol (NIV), DON, fusarenon X (FUSX), 3-acetyldeoxynivalenol (3-ADON) and 15-acetlydeoxynivalenol (15-ADON) representing the acetylated derivatives (Figure 2; Asam *et al.*, 2017).



Compound	R_1	R_2	R_3
NIV	OH	OH	OH
FUSX	OH	OAc	OH
DON	OH	H	OH
3-ADON	OAc	H	OH
15-ADON	OH	H	OAc

Figure 2. Chemical structures of Type B trichothecenes; NIV= Nivalenol, DON= deoxynivalenol, fusarenon X= FUSX, 3-acetyldeoxynivalenol= 3-ADON, 15- acetydeoxynivalenol= 15-ADON (Asam *et al.*, 2017)

In relevance, compared to the other TCTs, relatively a lot of data are reported on the occurrence of DON in feed and foodstuff and its toxicology is relatively well investigated like that of T-2 and HT-2. The type B TCTs has a carbonyl group at the C-8 position, while the type-A TCTs are either not oxidized, hydroxylated, or esterified at this position (Ueno, 1984).

Deoxynivalenol (DON) which is a type A is chemically described as 12, 13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-one ($C_{15}H20O6$, MW: 296.32, CAS 51481-10-8), crystallizes as colourless needles and is stable at 120 °C or moderately at 180 °C. It is soluble in water and some polar solvents (e.g., aqueous methanol, acetonitrile, and ethyl acetate; EFSA, 2004). Practically, DON is of outstanding importance among the B-type TCTs because of its frequent and ubiquitous occurrence at levels high enough to cause adverse effects in pigs (EFSA, 2004). The name “vomitoxin” has been coined for DON because of its strong emetic effects after consumption. It is transported to the brain, where it interferes with dopaminergic receptors (Vesonder *et al.*, 1973). Vital studies ascertaining emesis, growth and food/feed intake in various species and response to a varied dose of DON have been reported (Pestka and Smolinski, 2005).

Generally, TCTs are highly toxic at the subcellular, cellular, and organic system levels. They are also capable of inhibiting protein synthesis. In dairy cattle, swine, broilers and rats, exposure to TCTs results in disturbances in plasma glucose level, reduced blood cell and leukocyte count, weight loss, and alimentary toxic aleukia in humans as well as pathological symptoms in the liver and stomach. On the basic level, feed rejection as a result of TCTs

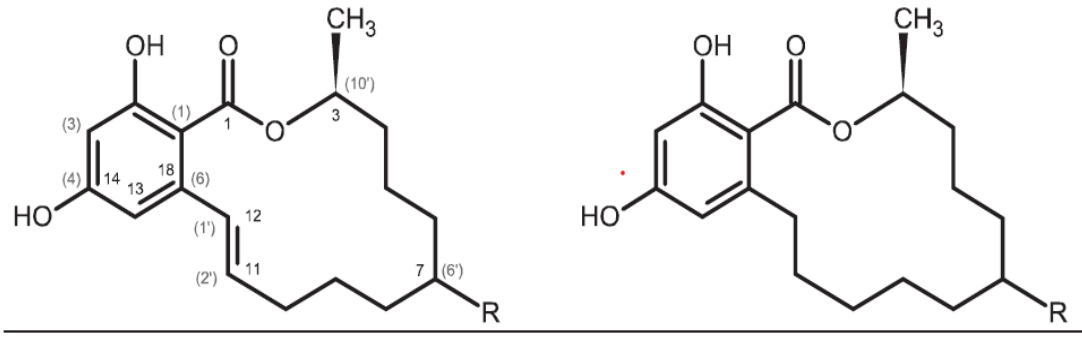
exposure is common, which subsequently limits the development of other signs. Animals tend to loathe eating and, in some cases, excessive salivation and emesis occur (Adhikari *et al.*, 2017).

The mechanism involved in T-2 and DON toxicity is primarily through oxidative stress-mediated deoxyribonucleic acid (DNA) damage and apoptosis (Zain, 2011). It has been reported by Kolf-Clauw *et al.* (2013) that in animal model experiments, TCTs can trigger necrotic lesions in the GIT. Exposure to TCTs in different animal species like rats, swine and poultry resulted in shortened intestinal villi height (VH) because of the apoptotic pathway induced by the mycotoxins which leads to nutrient malabsorption (Alizadeh *et al.*, 2015). Exposure of swine and rats to T-2 toxin for 7 days was sufficient to induce a substantial increase in the aerobic bacteria count in the intestine of both animal models (Tenk *et al.*, 1982). Although TCTs have serious negative impact on bacterial populations, the exact mechanism by which this happens is still not well understood (Liew and Mohd-Redzwan, 2018).

2.1.2. Zearalenone

The formation of ZEN (Figure 3) is favoured by high humidity and fluctuating temperatures, followed by low temperatures. Unfortunately, these conditions often occur in temperate regions during autumn harvest with high moisture content in late autumn and winter (Haschek *et al.*, 2013). The *Fusarium* species responsible for ZEN production are *Fusarium graminearum* and *F. culmorum*, a non-steroidal estrogenic [(member of 6-(100-hydroxy-60-oxo-trans-10-undecenyl)-b-resorcylic acid lactone (b-resorcylic acid lactones)] fusariotoxin biosynthesized through a polyketide pathway (Richard, 2007). The name "zearalenone" was coined from maize (*Zea mays*) and its chemical structure is an unsaturated ketone ("en-one") (Urry *et al.*, 1966).

The frequent co-occurrence of ZEN with FUMs and DON indicates that these fusariotoxins may be involved in a wide range of synergistic and additive interactions. Zearalenone (ZEN) has been linked to scabby grain toxicosis that occurred in Japan, China, Australia, and the USA, with symptoms including nausea, vomiting, and diarrhoea (Liao *et al.*, 2009).



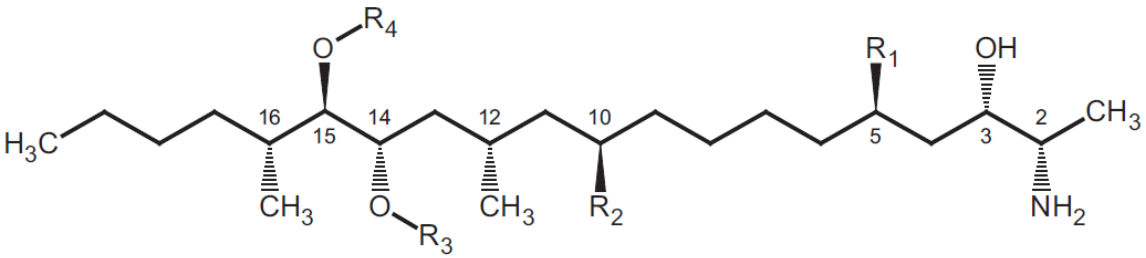
Compound	R		R
zearalenone (ZEN)	=O	zearalanone (ZAN)	=O
α-zearalenol (α-ZEL)	····OH	α-zearalanol (α-ZAL)	····OH
β-zearalenol (β-ZEL)	—OH	β-zearalanol (β-ZAL)	—OH

Figure 3. Chemical structures of zearalenone and zearalenols (Asam *et al.,* 2017)

Toxicological studies of ZEN revealed its effects on the reproductive system, including an enlarged uterus, altered reproductive tract, decreased fertility, as well as an abnormal level of progesterone and estradiol. Apart from this, the ingestion of ZEN during pregnancy reduced fetal weight and the survival rate of the embryo (Zhang *et al.*, 2014). Zearalenone (ZEN) has a structure that allows it to bind to the mammalian estrogen receptor, albeit with a lower affinity compared to the natural-occurring estrogens (Hueza *et al.,* 2014). Aside from these typical clinical disorders, reports suggest that ZEN results in hepatotoxic, haematotoxic, immunotoxic and genotoxic effects as well (Zhou *et al.*, 2017). Further, in the GIT, ZEN has been reported to be involved in several disorders although not hugely detrimental since the reproductive organ is the main target of ZEN insults. The transepithelial electrical resistance (TEER) approach revealed that ZEN triggered cell death without compromising cell integrity in investigations utilizing intestinal epithelial cells. However, its metabolites; α- and β-zearanol decreased cell integrity and this concluded the line of thought that ZEN and its metabolites acted differently in the gut (Marin *et al.*, 2015). In another study, Abassi *et al.* (2016) demonstrated that ZEN enhanced cell proliferation, increased colony formation and fastened cell migration of colon carcinoma cell line HCT116. Moreover, ZEN was also shown to downregulate the expression of tumour-suppressor genes (PCDH11X, DKK1, and TC5313860) in intestinal cells, indicating that gene expression modification was the cause of ZEN's carcinogenic effects (Taranu *et al.*, 2015). Nevertheless, consumption of ZEN contaminated diets by swine did not result in any intestinal disorientation in VH, mucosa thickness, and goblet cell number (Lewczuk *et al.*, 2016).

2.1.3. Fumonisins

The FUMs group of *Fusarium* mycotoxins are majorly produced by *Fusarium verticillioides* and *F. proliferatum* and infect chiefly maize and its based products. In regions characterized by temperate-warm conditions, a high incidence of FUMs contamination occurs (Gil-Serna *et al.*, 2013). The A, B, C, and P series are among the four extant series formed from the 28 existing FUMs, with the B-series being the most important. The B series have FB_1, FB, FB_3 and FB_4 (Figure 4) as the commonly occurring congeners with FB_1 being widely discussed due to its profound toxicity (Lerda, 2017). Chemical structure of FB_1 is an O14, O15- diester of 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyicosane with two molecules of propane-1,2,3-tricarboxylic acid (Bezuidenhout *et al.*, 1988). Although bearing the same structure, there are some differences. The FB_2 lacks the C-10 hydroxy group (Bezuidenhout *et al.*, 1988), FB_3 lacks the C-5 hydroxy group (Plattner *et al.*, 1992) and FB_4 lacks both (Cawood *et al.*, 1991).



Compound	R_1	R_2	R_3	R_4
FB1	OH	OH	TCA	TCA
FB2	OH	H	TCA	TCA
FB3	H	OH	TCA	TCA
FB4	H	H	TCA	TCA

Figure 4. Chemical structures of fumonisins; FB_1, FB_2, FB_3 and FB_4 (Asam *et al.*, 2017)

Epidemiological studies report that in humans, consumption of elevated levels of FB_1 diet can be related to the high incidence of human oesophagal cancer (Gelderblom and Marasas, 2012) and some neural tube defects in some parts of the world where maize is the main staple food crop and therefore, maize- based diets are mostly consumed (Marasas *et al.*, 2004). Following this recognition, the International Agency for Research in Cancer (IARC) has described FB_1 as possibly carcinogenic, hence, Class 2B classification (IARC, 2002). In animals, FB_1 toxicity is peculiar to, and varies by animal species; from equine leukoencephalomalacia (ELEM) in horses (Marasas *et al.*, 1988) to nephrotoxicity in rabbits, rats, and lambs, as well as hepatotoxicity in all species studied (Bolger *et al.*, 2001). Pigs have been shown to develop pulmonary oedema (PPE) (Harrison *et al.*, 1990) and hydrothorax (Haschek *et al.*, 2001). It is worth noting that, very little of FB_1 is absorbed from the gut, metabolized very slowly, and excreted quickly. This mechanism of toxicity is in direct opposition to its profound toxic

consequences and has subsequently been dubbed the "fumonisin paradox" (Shier, 2000). There are several plausible explanations for the "fumonisin paradox" including modification of the molecule in plants, fungi or animals (biologically modified), or also during storage and upon processing (chemically modified). Fumonisins (FUMs) can also form complexes with matrix compounds (e.g. starch or proteins), which can be absorbed from the GIT after didegstion. All these modifications affect the analytical outcome, thus making the exposure assessment more uncertain (EFSA, 2018).

In monogastric animals like the pig, FB_1 absorption rate is only about 1 – 6 % of the total FUMs consumed (Bouhet and Oswald, 2007). Pigs fed with FB_1- containing feed or culture material at doses ≥ 92 ppm or ≥ 16 mg/kg body weight/ day (BW/ d) developed lethal PPE in 4-7 days, according to reports (Harrison *et al.,* 1990; Colvin *et al.,* 1993; Motelin *et al.,* 1994; Gumprecht *et al.,* 1998). In addition, lethal PPE has been reported to occur within 7 days after daily intravenous administration of FB_1 (Haschek *et al.*, 2001). When FB_1 exposure was consistent over a period of 8 weeks, level as low as 1 mg FB_1/ kg feed had produced proliferation of the connective tissue (fibrosis) in the lung (Zomborszky-Kovács *et al.,* 2002).

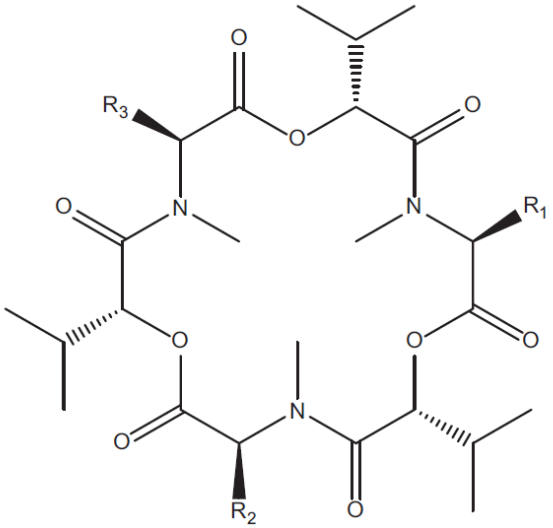
Generally, the cellular mechanism behind FB_1- induced toxicity is its ability to inhibit the activity of the key enzyme involved in sphingolipid metabolism – CerS, due to the structural analogy to the sphingoid bases So and Sa. This distortion leads to intracellular accumulation of So and Sa in the body and ultimately results in disturbances of cellular processes such as cell growth, differentiation, morphology, permeability, and apoptosis (Riley *et al.,* 2001). Indeed, FB_1 toxicity has been well proven to disrupt cellular processes and cause a wide range of diseases in studied species, as previously described. However, multiple investigations have lately revealed that FB_1 is progressively targeting the GIT. Various reports from *in vivo* and *in vitro* studies suggest that FB_1 compromise intestinal processes of nutrients digestion and absorption (Gbore and Egbunike, 2007; Gbore, 2010) and weakens gut barrier integrity (Bouhet and Oswald, 2007; Loiseau *et al.,* 2007; Lallès *et al.,* 2009; Lessard *et al.*, 2009; Bracarense *et al.,* 2012; Romero *et al.,* 2016) as well as disturbs the microbiota of the GIT (Kim *et al.,* 2019; Mateos *et al.,* 2018). The effects of FB_1 on different areas of GIT functioning are discussed in further detail under Chapter 2.2.

2.1.4. Emerging *Fusarium* mycotoxins

The so-called emerging *Fusarium* mycotoxins are reported to exist in substantial amounts and contaminate most cereals and cereal by-products. They include enniatins (ENNs), beauvericins (BEAs), fusaproliferin and moniliformin and these toxins are reported to be potentially toxic

like the major known fusariotoxins (Asam *et al.,* 2017). Regretfully, there are no known regulations about their intakes nor are they tested for during sample collection and mycotoxins testing in agricultural commodities. Among these fusariotoxins, ENNs and BEAs have caught significant interest in mycotoxins research.

Enniatins (ENN) was isolated by Gäumann *et al.* (1947) from a mycelium called *Fusarium orthoceras* var. *enniatinum* and henceforth, the name enniatin was coined. Beauvericin (BEA) on the other hand, was isolated from *Beauveria bassiana*, thus, the name beauvericin was derived (Hamill *et al.,* 1969). They are both cyclic hexadepsipeptides that consist of three N-methylated amino acids and three D-2- hydroxy isovaleric acids, which alternatingly form the branched chain by ester and peptide bonds. Among the 27 different ENNs described, ENN B, B1, A1, and A are the most frequent occurring congeners. Enniatin B and A consist of three valine and isoleucine units, respectively, while ENN B1 and ENN A1 contain two different AAs. Enniatin B1 (ENN B1) is built up from one isoleucine and two valine units and *vice versa*. Enniatin A1 (ENN A1) consists of one valine and two isoleucine units. In contrast to ENNs, BEA is composed of three phenylalanine residues (Desjardins, 2006; Gäumann *et al.*, 1947; Hamill *et al.*, 1969) (Figure 5). In processed and unprocessed grains collected in Europe, ENN B is frequently detected in amounts ranging from a few µg/kg to over mg/kg (Jestoi, 2008).



Compound	R_1	R_2	R_3
ENN B	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$
ENN B1	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$
ENN A1	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$
ENN A	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$
BEA	$CH_2C_6H_5$	$CH_2C_6H_5$	$CH_2C_6H_5$

Figure 5. Chemical structures of enniatins (ENN) and beauvericin (BEA) (Asam *et al.*, 2017)

Several studies are providing evidence of the presence of these emerging mycotoxins in many foods and feed commodities including their toxic mechanisms (Juan *et al.,* 2013). Point

noteworthy, co-contamination of mycotoxins in agricultural products is not uncommon and continues to pose a huge threat to food/feed safety and health of humans and animals. And with the advent of emerging fusariotoxins like ENNs and BEAs possibly co-existing with the major mycotoxins in food/feed commodities, this presents a dire concern for human and animal health since the extent of their toxicity could be magnified.

2.1.5. Co-occurrence of mycotoxins

Through the ingestion of contaminated diets, humans and animals may be exposed to a single mycotoxin or a variety of them due to their diverse and complicated chemical structures and pervasive existence. Mycotoxins and their metabolites can cause severe acute poisoning, which can result in death, as well as long-term negative health effects, depending on how much is taken. Research on individual mycotoxins is actually fairly prevalent, as was covered in the preceding chapters. It should be noted that most fungal strains produce more than one form of mycotoxin and thus crops can become infected with multiple mycotoxins. Additionally, because it is usual to feed animals a variety of grains, there is a higher chance that they will be exposed to various mycotoxins at once. Therefore, co-contamination of agricultural products with multiple mycotoxins is frequently discussed (Grenier and Oswald, 2011).

For further reading, frequently occurring mixtures of mycotoxins in different plant products have been reported for OTA and citrinin (Pohland *et al.,* 1992; Vrabcheva *et al.,* 2000); OTA and ZEN (Halabi *et al.,* 1998); OTA and penicillic acid (Stoev *et al.,* 2001); OTA and AFB_1 (Sedmikova *et al.,* 2001); patulin and citrinin (Martins *et al.,* 2002); FB_1 and moniliformin (Gutema *et al.,* 2000); AFB_1, FB_1, ZEN, DON, and nivalenol (Sardjono *et al.,* 1998; Gonzalez *et al.,* 1999; Eskola *et al.,* 2001); DON, nivalenol, diacetoxyscirpenol, T-2, HT-2, and other TCTs (Eskola *et al.,* 2001; Pronk *et al.,* 2002).

2.2. Response of the gastrointestinal tract to fumonisins

Maintaining optimal gut health is a vital feature of animal nutrition. The gut must undergo maturation to digest and absorb nutrients from food, develop defenses against pathogenic bacteria, get rid of exogenous toxins, and harbour commensal microbiota and dietary antigens throughout the foetal and postnatal period. As the body's initial epithelial barrier, the intestine's epithelium must also regulate the flow of foreign substances that aid in the development of the region's immune system (Cader and Kaser, 2013). Therefore, any form of destruction to the normal functioning of the GIT can be consequential to poor animal performance and health. As mentioned earlier, the poor absorption rate of FB_1 in monogastric animal species implies that the gut epithelium is exposed to high concentrations of ingested FUMs. The following

chapters discuss the potential effects of FUMs on the digestive and absorptive processes, gut barrier and immune functions, and the gut microbiota of the GIT.

2.2.1. Fumonisins and the digestive and absorptive processes of the gastrointestinal tract

The composition of feed for farm animals is essential for intestinal processes of digestion and absorption and to cover the maintenance and production. The gut serves as both the recipient and barrier to ingested food/feed components and noxious substances that may be present. In birds and mammalian small intestines, crypts are responsible for the turnover of villus cells while villi are responsible for the digestion (brush border enzymes) and absorption of nutrients. A high villus is an indicator of a substantial absorption surface while flat crypts mean normal villus cell turnover. Both are worthwhile, and this is designated by a high villus to crypt depth (CD) ratio (VH: CD) (Gao *et al.,* 2008). Cells from the intestinal crypts are responsible for enterocyte renewal and CD is positively related to the proliferative rate that can be measured by Ki-67 staining (Willing and Van Kessel, 2007).

Interference of mycotoxins with key processes of digestion and absorption often is the result of impaired intestinal functions (Grenier and Applegate, 2013) which in the long run, can harm the animals' growth, performance, and productivity. Following FUMs challenge, Rauber *et al.* (2013) found morphologic changes in the small intestine of broilers receiving a 100 or 200 mg/kg FB_1 diet for 28 days, and this was exhibited by the significant reduction in VH and VH: CD ratio, but no changes in CD. In addition, a 42-day trial involving weanling pigs exposed to 30 mg/kg FB_1 resulted in intestinal villous fusion and atrophy (Piva *et al.,* 2005). Furthermore, it was demonstrated that ingestion of 6 ppm of FB_1 induced morphological and histological alterations in the intestine, with atrophy and fusion of the villi, decreased VH and cell proliferation in the jejunum, and reduced numbers of goblet cells and lymphocytes (Lallès *et al.,* 2009; Bracarense *et al.,* 2012). Anther study reported a reduction in VH which in turn, reduced the percentage of cells positive to Ki-67 staining when nursery piglets were exposed to a combination of 180 μg/kg AFs, 9 mg/kg FB_1 and 1 mg/kg DON for 48 days. The authors of the study explained this out-turn as the mycotoxins' potential to impair crypt cell proliferation (Kim *et al.,* 2019). Interestingly, Lessard *et al.* (2009) found a rather increment in ileal VH following a 9-day exposure of piglets to an FB_1- rich diet which, the authors could not explain.

The Ussing Chamber (UC) is used to study epithelial tissue integrity and permeability electro-physiologically i.e., transepithelial electrical potential or short-circuit current (Isc). The Isc is induced by sodium absorption (Na^+) and chloride (Cl^-) ion secretion. The Isc measurement is

a remarkable indicator of the transport of sugar or amino acid, as many nutrients are transported by carrier systems and are normally transported with Na^+ (Grenier and Applegate, 2013). Lessard *et al.* (2009) in their work, demonstrated how FB_1 could modulate some aspects of jejunal absorptive and secretory physiology without necessarily altering epithelial barrier function. The authors found a delayed increase in basal Isc of jejunal mucosa in UC for FB_1-treated pigs relative to the control pigs. This was explained by the increase in spontaneous trans-mucosal net ion transport, for example by Na^+ and (or) Cl^- channel processes (Li *et al.*, 2004).

When the digestibility of dietary nutrients is impaired, it can result in inefficient breakdown and absorption of essential dietary components. Again, Lessard *et al.* (2009) highlighted a significant reduction of alkaline phosphatase (ALKP) and aminopeptidase N enzyme activity and suggested that this may have interfered with the digestion of proteins and peptides in the FB_1- rich (*in vitro* culture of the high- FB_1- producing *F. verticillioides* strain NRRL 34281) extract- treated group. Grenier *et al.* (2013) supported that these changes could be a key factor attributable to villi morphology. No details of this phenomenon were demonstrated in their work, however. Moreover, it was theorized that the intake of FB_1- rich extract may result in alterations in the regulation of Na- dependent glucose absorption and the basal and induced secretory properties of the intestinal mucosa. Although no alterations were evident in the barrier function of the pigs, the authors did not rule out such an occurrence should the exposure period be longer than 9 days (Lessard *et al.*, 2009). Due to its distinctive ability to inhibit sphingolipid metabolism, FB_1 alters the function of the cell membrane and lipid packing (Ferrante *et al.*, 2002). Exposure of pigs to FB_1 (1.5 mg/kg BW) for 7 days resulted in a significant increase in the concentration of Sa and So and a decrease in the total glycolipid content as well as the alteration in the jejunal glycolipid composition, whereas no changes were observed in the duodenum and ileum (Loiseau *et al.*, 2007).

2.2.2. Fumonisins and the barrier functions of the gastrointestinal tract

The epithelium lines the entire length of the GIT, and it is essential to provide barrier function to the gut. It consists of a thin layer of cells that lines the lumen of the intestine and contains enterocytes, lymphoid, enteroendocrine, and goblet cells at the villi, and the Paneth cells under the crypts (Fink and Koo, 2016). The epithelial layer acts as a barrier to noxious substances such as pathogens, toxins, and antigens that have been ingested. The epithelial cells are bridged with desmosomes, tight junctions (TJs), and adherens junctions (AJs). The mechanical linkage of adjacent cells is the responsibility of AJs and desmosomes. Whereas the TJs control the intercellular space and regulate selective paracellular ionic solute transport (Capaldo *et al.*,

2014). The TJs are composed of the transmembrane and cytoplasmic scaffolding proteins. At the apical-lateral membrane of the epithelial cells, transmembrane TJs proteins such as occludin (OCLN), claudin (CLDN), junctional adhesion molecules (JAMs) and tricellulin form a parallel barrier (Chiba *et al.,* 2008; Schneeberger and Lynch, 2004). A distortion to this parallel barrier is consequential to a defective gut barrier integrity.

To investigate the integrity of the epithelial barrier in both *in vitro* and *ex vivo* assays, the TEER is the frequent marker utilized. This instrumentation follows the cycle of cell differentiation and, standard values for a completed non-permeable barrier are established based on individual devices and sizes inserted (Akbari *et al.,* 2017). Direct approaches include the use of paracellular flux probes and the assessment of the expression of TJs proteins together with histological approaches that illuminate alterations in the intestinal architecture, and epithelial cell damage (Bischoff *et al.,* 2014). Impairment of the intestinal barrier integrity induced by FB_1 has been shown in different *in vitro*, *ex vivo* and *in vivo* studies, some of which are summarized in Table 3.

Table 3. A summary of the effect of fumonisins on intestinal barrier integrity (adapted from (Grenier and Applegate, 2013)

Experimental model	Dosage	Exposure period	Effect on barrier function	Reference
Caco-2 cells	1–100, µM	7 days	A reduction in TEER values. Decrease in transcript level of CLDN3, CLDN4 and OCLN.	Romero *et al.,* 2016
IPEC-1 Cells	50–200, µM	16 days	A reduction in TEER values. Increase in permeability of FB_1.	Loiseau *et al.,* 2007
IPEC-1 Cells	20–200, µM	4 hours	Increase in translocation of pathogenic *Escherichia coli* (strain 28C).	Bouhet and Oswald, 2007
IPEC-1 Cells	50–500, µM	28 days	A reduction in TEER values.	Bouhet *et al.,* 2004
Porcine jejunal explants	10, µM	2 hours	Increase in TEER values. Increase in permeability of HRP.	Lallès *et al.,* 2009
Piglets	3, mg/kgfeed	5 weeks	Decrease in protein expression of OCLN in the ileum.	Bracarense *et al.,* 2012
Piglets	0.5, mg/kg BW	7 days	Increase in translocation of pathogenic *Escherichia coli* (strain 28CNalr).	Oswald *et al.,* 2003

CLDN= Claudin, HRP= Horse radish peroxidase, IPEC= Intestinal porcine epithelial cell line, OCLN= Ocluddin, TEER= Transepithelial electrical resistance

Sphingolipids and lipid rafts play a major role in establishing and maintaining TJs (Lambert *et al.,* 2007). Indeed, FB_1 alters the intestinal barrier function by influencing sphingolipid metabolism, as demonstrated by an increase in the number of free sphingoid bases, a depletion of glycolipids in the plasma membrane and an increase in trans-epithelial flux (Bouhet *et al.,* 2004; Loiseau *et al.,* 2007). Yamazoe *et al*. (2017) reported an accumulation of Sa altered glycoprotein distribution in the jejunum which caused an increase in the transepithelial passage of FB_1. The increase in intestinal permeability, in turn, promotes the translocation of pathogenic bacteria (Kelly *et al.,* 2015). In contrast, although Burel *et al*. (2013) found a change in the ratio of So and Sa in specific pathogen- free piglets receiving 11.8 ppm FB_1, which, considering the above findings can alter intestinal functions, the extent of change of the sphingoid bases observed in their work was described insufficient to induce any abnormalities in gut barrier function. Similarly, despite the effects observed for immune and oxidative stress markers, Kim *et al.* (2019) observed no noticeable effects on the TJs proteins OCLN, CLDN, or zona occludens-1 in piglets receiving a combination of AFs, FB_1 and DON in the concentrations of 180 µg/ kg, 9 mg/kg, and 1 mg/kg respectively.

2.2.3. Fumonisins and the immune functions of the gastrointestinal tract

The intestinal epithelium cells and the cells of the gut-associated lymphoid tissue (GALT) are the principal targets of ingested dietary pollutants. Several components are involved in the innate defence system of the intestine, including mucosal secretions (mucous layer, lysozymes, antibacterial peptides, etc.), the intercelluar TJs, the phagocytic cells (macrophage, neutrophils), the mast cells, the glycoconjugates express on the epithelia cells that mimic receptor bacteria, and intestinal motility. The adaptive defence system of the GALT consists of the lymphoid tissues associated with the intestine [Peyer's patches (PP) and mesenteric lymph nodes (MLNs)], which act as inducers of mucosal immune responses) and of a more diffuse zones, the lamina propria being the effector of the local immune response. The PP, MLNs and lamina propria contain the antigen presenting cells (APCs) and B and T lymphocytes which all participate in the local immune activity (Le Huërou-Luron and Ferret-Bernard, 2015). Through the absorption of antigen in the lamina propria, maturation and migration to GALT, and interaction with T cells in these regions, the APCs are crucial in bridging innate and acquired immune responses. Along the length of the digestive system, simultaneous early and quick responses are generated locally by mucus, intraepithelial immune cells, and intestinal epithelial cells (IECs), which serve as vital sentinels and shields (Grenier *et al.,* 2013).

It is known that some mycotoxins with more clear-cut effects can inhibit protein synthesis (Creppy, 2002). Thus, the impacts of mycotoxins are particularly susceptible to intestinal and immunological cells, as well as other cells that rapidly multiplies or synthesize proteins. Deoxynivalenol (DON) is particularly widely studied for its ability to impair macrophages, B and T lymphocytes, and natural killer cells in various ways (Maresca, 2013). With regards to FB_1, the fundamental toxic cellular pathway which is the inhibition of CerS activity has been shown to suppress T-dependent immune response [cluster of differentiation (cd) 3, cd4, cd8, cd45], which inhibits DNA synthesis, also modifying T-lymphocyte surface antigen expression (Martinova, 1998). Piva *et al.* (2005) revealed severe infiltration of lymphocytes and monocytes; moderate infiltration of eosinophils and the presence of submucosal nodular lymphoid aggregates in weanling piglets exposed to a 30 ppm dose of FB_1 for 42 days. In another study, Devriendt *et al.* (2009) showed that FB_1 can reduce the induction of an antigen-specific intestinal immune response following oral F4 fimbriae (which is the surface protein of Enterotoxigenic *Escherichia coli*, ETEC) immunization. The authors indicated how key steps involved in immune response were altered in the intestines of FB_1- exposed piglets. They highlighted impairment in the T- cell stimulatory capacity and attributed this to the effects on APCs. The ineffectiveness of MHC-II (Major Histocompatibility Complex Class-II), cd80/86, and IL-12p40 expression might explain the low response of intestinal APCs from FB_1- exposed animals to F4 stimulation. Out-turn of this was the ineffectiveness of APCs to communicate and stimulate intestinal T- cells which ultimately may have resulted in defective production of specific immunoglobulin. Furthermore, it is evidenced that extraintestinal pathogenic *Escherichia coli* (ExPEC) under normal conditions can persist in the large intestine of pigs but can colonize the gut and translocate to internal organs following a defective immune response (Oswald *et al.,* 2003). Thus, the impaired immune response observed after FB_1 exposure in the work of Devriendt *et al*. (2009) may be attributable to the translocation of ExPEC to the lungs, liver, and spleen.

2.2.4. Fumonisins and the gastrointestinal microbiota

The extensive efforts over the years to investigate the ecology of the gastrointestinal microbiota (GIM) are undoubtedly due to the tremendous benefits these organisms confer on their hosts (Luckey, 1972). The gut microbiome over time changes gradually, shifting from aerobes in the neonate to strict anaerobes in adults and finally climaxing (Kim *et al.*, 2011). The microbes serve as a natural defence against pathogen colonization and guarantee good animal health (Zoetendal *et al.,* 2004). Valuable studies have shown that the GIM could influence many metabolic steps and affect many aspects of host physiology, including nutritional status and

stress responses (Lankelma *et al.,* 2015). An important factor in maintaining animal health is an adequate composition of the intestinal microbiota, as well as the quantitative and qualitative integrity of the gut ecosystem. Data on the effects of mycotoxins on the GIM are reported to be lacking. For the most part, FB_1 has been gaining considerable interest and yet, not much has been reported on its effects on the commensal bacteria harbouring the gut.

Bacterial counting of aerobic and anaerobic cultivable indicators can be used to determine the impact of mycotoxins on the microbiota. In a growing pig, for instance, the GIT is colonized by a highly diverse consortium and about 90% of the bacterial community Operational Taxonomic Units (OTUs) are of the phyla Firmicutes and Bacteroidetes; dominated by the Lactobacillaceae, Lachnospiraceae, Ruminococcaceae and Prevotellaceae families and, *Lactobacillus, Prevotella* and *Blautia* genera (Mateos *et al.,* 2018). Utilizing the capillary electrophoresis single-stranded conformation polymorphism (CE-SSCP), Burel *et al.* (2013) highlighted that chronic exposure to 11.8 ppm of FUMs (FB_1 + FB_2) transiently affected the balance of the digestive microbiota of pigs. The authors showed that FUMs could reduce the faecal microbiota's single-stranded conformation polymorphism profiles of treated animals compared to untreated animals. In addition, after the co-contamination of FB_1 and Salmonella, the authors described what happened next as transient, but occurred faster and with more intensity than that seen in the exposed group.

In a 4-week experiment to investigate the dynamic effects of FB_1 (12 mg/kg diet) exposure to young weaned pigs' faecal microbiota, Mateos *et al.* (2018) discovered pronounced effects of FB_1 on faecal microbiota and, it occurred as early as 15 days following exposure and peaked at 22 days. After 29 days, the effect was alleviated albeit differences existed in the taxa relative abundance in both the treated and control groups. In addition, there was a significant increment or abundance of *Lactobacillus*, which was also the case of an *in vitro* study to investigate the interaction between FB_1 and caecal microorganisms in pigs (Dang *et al.,* 2017). Recently, some researchers found *Lactobacillus brevis, L. plantarum, L. pentosus* and some yeasts can degrade FB_1 (Zhao *et al.,* 2016). Zhao *et al.* (2016) explained the underlying mechanism of *Lactobacillus* to remove FB_1 as a process of physical adsorption involving differing constituents of the cell wall. Further with the aspect of degradation, a much recent work carried out demonstrated an increase of Lactobacillaceae family proportion in the intestinal microbiome of mycotoxin fed (AFs, FB_1 and DON) pigs as well as an increase of gram-positive bacteria (known to effectively degrade DON) such as *Turicibacter sanguinis* and *Clostridium sp.* when yeast cell wall enzyme (YCWE) was added to the diets of the pigs (Kim *et al.,* 2019). These observations were explained as a sign of induced adaptation of the microbiome and the

pigs themselves to better handle mycotoxin disturbance. Although this latter study involved a combination of FB_1 and two other major mycotoxins (AFB_1 and DON), it still provides a decent amount of evidence that FB_1 potentially modifies the host's control of its symbiotic microbial community.

Summary

A popular quote has been heard often by Prof. Tom Scott of the University of Saskatchewan in Canada – "mycotoxins, they are everywhere" (Broom, 2015). This concise information illustrates the ongoing struggle farmers and nutritionists face every day to reduce the detrimental effects of mycotoxin contamination regardless of the well established permissible levels in the various feed/food. As was previously indicated, exposure to FUMs – one of the important mycotoxins of toxicological studies – in humans and animals has a wide range of detrimental consequences that continue to occur and also in unanticipated ways. Over the years, the majority of studies describing the toxicity caused by FUMs have centered on the post-absorptive abnormalities. In fact, based on the well established documents of organ-specific effects (liver, kidneys and in certain specific conditions; brain and lung) across the various animal species examined following FUMs intoxication, this is expected. Nevertheless, it is worth noting that ingested toxins such as FUMs and their negative consequences on animals are unquestionably connected through the gut. Further, FUMs absorption in the gut varies and happens to be poorly absorbed in the livestock species in question like pigs. A very clear indication that the gut epithelium is exposed more to ingested FUMs. The literature has endeavoured to report the available studies pertaining to FUMs toxic effects on the GIT of the various animal species with frequent emphasis on swine. However, there is still a shortage on the effects on the gut microbiota of swine. This implies that the potential effects of FUMs on the many integral components of gut health and functions should be regularly examined, much like those of other mycotoxins that are frequently addressed in the context of gut health, such as AFB_1 and DON. Additionally, the literature has briefly discussed the significance of the effect of the co-occurrence of mycotoxins in animal feed as well as other newly emerging mycotoxins that have received less attention.

In conclusion, the adverse effect of *Fusarium* mycotoxins including FUMs in mostly GIT functions of livestock animals in particular, pigs, remains an integral aspect of mycotoxicology. And the findings of this study seek to contribute to the body of evidence supporting the detrimental effects of the presence of FUMs in pig feed.

3. MATERIALS AND METHODS

3.1. Nursery pigs' study

3.1.1. Animals, housing and feeding

The experiment was carried out according to the regulations of the Hungarian Animal Protection Act. The allowance number for the studies was SOI/31/00308-10/2017 (date of approval: 28 February 2017, by the Hungarian National Scientific Ethical Committee on Animal Experimentation and issued on 27 March 2017 by the Somogy County Government Office, Department of Food Chain Safety and Animal Health).

In mycotoxin toxicity studies, males are frequently used compared to females perhaps, due to their more constant hormone levels and are thus less variable compared to females. Thus, the study was carried out with a total of eighteen (18) male Danbred weaned pigs at five weeks old and averaging 13.5 ± 1.3 kg. The experiment took place at the Department of Farm Animal Nutriton at the Hungarian University of Agriculture and Life Sciences, Kaposvári Campus (MATE-KC). The pigs were kept in individual metabolic cages (80 x 80 cm) at the experimental unit and were allowed a 2-week physiological acclimatization period. At exactly 7 weeks of age, the pigs were alloted to the 3 different diets; a control group fed a diet that contained no fungal culture of FUMs [(FB_1 + FB_2 + FB_3); G1], a group fed with 15 mg/kg (G2) and another group fed with 30 mg/kg FUMs (G3) – contaminated diets (n = 6) for 21 days.

Feed was of commercial origin and contained primarily a mixed ration of maize, wheat, extracted soybean meal, barley, distillers dried grains and solubles (DDGS), rapeseed, sunflower meal, animal fat, additives, mineral and vitamin premix. Titanium dioxide (TiO_2; 0.5%) was added to the feed as an indigestible marker to evaluate the apparent total tract digestibility (ATTD) of nutrients. The feed was restrictedly fed and provided twice a day in two equal portions. The daily feed ration was adjusted as an amount that covers 2.5 times the maintenance energy requirement (ME_m= 450 kJ/ $BW^{0.75}$/ d). The would-be leftover feed was measured back every day. The analyzed nutrient content of the feed is given in Table 4. Drinking water was made available *ad libitum*.

Table 4. Analyzed nutrient content of the experimental feed

Analyzed nutrient content	
Item, g/kg	
Crude protein	172
Ether extract	42
Crude fibre	38
Ash	47
Starch	418
Calcium	6.13
Phosphorus	5.2
Sodium	1.52
Potassium	4.07
Magnesium	1.74
Item, mg/kg	
Zinc	189
Copper	20
Digestible Energy*, MJ/kg	13.7
Metabolisable Energy*, MJ/kg	13.2
Gross Energy*, MJ/kg	16.9

* Declared by the producer

Pigs were weighed individually with gram precision at the beginning of the trial and at weekly intervals and, their health status was monitored every day. The daily feed intake (FI) for each week was calculated based on the weekly BW of the pigs (Table 5). The room temperature was adjusted according to the needs of the nursery pigs and the diarrhoea score was monitored throughout the trial. At the end of the experiment period, pigs were euthanized by exsanguination after sedation (Euthanyl-Pentobarbital Sodium, 400 mg/mL, Dechra Veterinary Products, Shrewsbury, UK). Blood samples were taken during exsanguination into heparin tubes (20 IU/mL whole blood) and was centrifuged for 10 min at 1000 × g (SIGMA 3-30KS refrigerated centrifuge, Osterode am Harz, Germany) for plasma separation. The kidney, liver, lung and small intestinal secions were weighed and immediately stored at -80 °C for their further prospective analysis.

Table 5. Daily feed ration adjusted as as an amount that covers 2.5 times the maintenance energy requirement of nursery pigs fed varied levels of dietary fumonisins

Daily feed intake, g	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
Week 1	524	555	562
Week 2	694	700	668
Week 3	800	800	800

3.1.2. Mycotoxin and experimental diets preparation

The fungal strain *Fusarium verticillioides* (MRC 826) was inoculated on pre-soaked, sterile maize kernels, in a form of spore suspension. Fungal culture was produced according to (Fodor *et al.*, 2006a). The final FUMs (FB_1 + FB_2 + FB_3) concentrations were 2000-4000 mg/kg in the air-dried culture material harvested in different batches. The fungal culture was mixed into the ration of the experimental animals to provide feed concentration of 15 and 30 mg/kg a daily FUMs (FB_1 + FB_2 + FB_3). The diet fed to the control group did not contain detectable amounts of FUMs. In the diets, the absence of FUMs co-occurrence with DON, ZEN, and T-2 toxin was also confirmed, in which the analyzed diets did not contain detectable concentrations (below the limit of detection; 0.053, 0.005, and 0.011 mg/kg for DON, ZEN, and T-2 toxin, respectively).

Concentrations of mycotoxins in prepared samples were determined with a Shimadzu 2020 LCMS system (Shimadzu, Kyoto, Japan). To obtain high-resolution chromatographic separation, a XB-C18 Kinetex analytical column (100 × 2.1 mm, 2.6 μm; Phenomenex) was used with a 0.25 mL/min flow rate (injected sample volume: 10 μL). The gradient elution was performed employing eluents A (0.2 % formic acid + 0.005 M ammonium formate) and B (methanol + 0.005 M ammonium formate), using the following gradient programme: 0.0–1.0min 10 % eluent B, 1.0–13.0 min linear increase of eluent B to 100 %, 13.0–16.0 min 100 % eluent B, 16.0–17.0 linear decrease of eluent B to 10 %, and 17.0–20.0 min 10 % eluent B. Three different mass per charge ratio (m/z) values were used for each mycotoxin (i.e., 1 for quantification and 2 for confirmation of the detected mycotoxin), as shown in Table 6 and the mycotoxins concentration used for the experimental diets follows in Table 7 (ELKH-Mycotoxins in the Food Chain Research Lab's own method, unpublished).

Table 6. Mass per charge ratio (m/z) values used for five different mycotoxins in LCMS system

Mycotoxins	Quantification (m/z)	Confirmation (m/z)
Fumonisin B_1	722.4 (+)	760.3 (+), 720.3 (-)
Fumonisin B_2	706.4 (+)	744.3 (+), 704.4 (-)
Deoxynivalenol	335.0 (+)	297.0 (+), 340.9 (-)
Zearalenone	317.0 (-)	357.0 (+), 319.0 (+)
T-2 toxin	505.1 (+)	484.2 (+), 589.0 (+)

(+) or (-) indicates polarity of the ionic charge

Table 7. Level of total fumonisins used for the formulation of contaminated diet, and limit of detection of fumonisins and other mycotoxins in experimental feed

Mycotoxins	LOD, mg/kg	Control	15 mg/kg FUMs diet	30 mg/kg FUMs diet
FB_1	0.031	nd		
FB_2	0.051	nd	15.4*	29.75*
FB_3	-	nd		
ZEN	0.005	nd	nd	nd
DON	0.053	nd	nd	nd
T-2 toxin	0.011	nd	nd	nd

* = FB_1 + FB_2 + FB_3, FB_1= fumonisin B_1, FB_2= fumonisin B_2, FB_3= fumonisin B_3, DON= deoxynivalenol, ZEN= zearalenone, LOD= limit of detection, nd= not detected

3.1.2. Metabolic study

3.1.2.1. Collection of urine and faeces, handling, and preparation of samples

From day 17 to 21 of the trial, faecal and urine samples were collected separately, weighed, and immediately frozen at -18 °C. Approximately 200 g of faeces were collected 4 times a day. For the balance study, urine samples from each day were combined and strained through glass wool to remove any particulate matter or debris. To effectively trap ammonia, urine was collected over sulphuric acid and maintained at a pH of 2 or lower.

3.1.2.2. Laboratory analysis

All laboratory analyses were done at the Chemistry Laboratory of MATE-KC. According to methods described in the Association of Official Analytical Chemists (AOAC, 2000), the chemical compositions of the diets and faecal samples were analyzed and used to calculate for the ATTD of CP, ether extract (EE), crude fibre (CF), starch, ash, calcium (Ca), phosphorus (P) and the trace minerals.

The TiO_2 was analyzed by measuring 150 mg of sample and a Foss Se + K_2SO_4 catalyst pill in a digestion tube after which 25 ml sulfuric acid and 6 ml H_2O_2 were added. The mixture was digested at 400 °C for 4 hours. After cooling, the mixture was transferred to a 100 ml volumetric flask and was filled up with water. A 10 ml sample was transferred to a test tube and 1 ml of colour reagent (mixture of sulfuric acid, phosphoric acid, and hydrogen peroxide) was added and was left to stand for one hour. It was measured with a spectrophotometer at 410 nm wavelength and was compared to a standard curve of different dilutions of TiO_2 prepared similarly (Lab's own method, unpublished).

The gross energy (GE) content of the feeds, faecal, and urine samples were determined by analyzing duplicate samples using IKA-Calorimeter C4000 adiabatic bomb calorimeter with

benzoic acid used as a standard. The urinary energy was determined by a modified method described (Lammers *et al.*, 2008). A total of 16.0 g of filtered subsample urine was added to 4.0 g of dried cellulose and subsequently freeze-dried for 24 h. The energy content of cellulose was also determined, and urinary energy was calculated by subtracting the energy in cellulose from the energy in the samples containing both urine and cellulose. The trial outflow is shown in Figure 6 below.

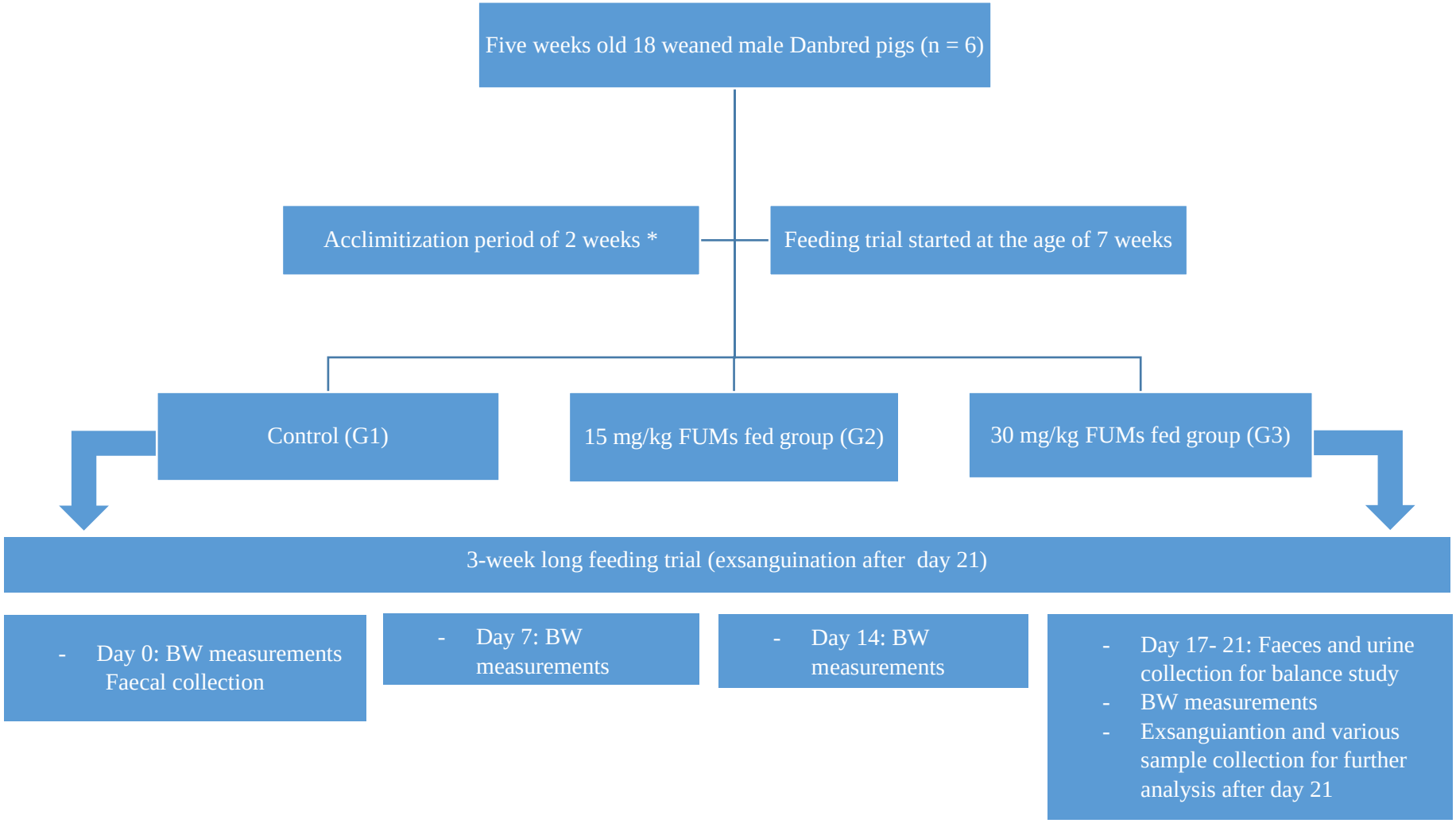


Figure 6. General outflow of the feeding trial. * Acclimatization period was to ensure that the gut microbiota was relatively stable

3.1.2.3. Calculations

Apparent total tract digestibility

Apparent total tract digestibility (ATTD; g/g) of CP, EE, CF, starch, and ash, as well as the minerals, Ca, P, magnesium (Mg), potassium (K), sodium (Na), copper (Cu) and zinc (Zn), was calculated using the TiO_2 concentration in faeces and feed calculated in dry matter (DM) basis and using Equation 1:

***Equation 1*:** Apparent total tract digestibility of nutrients and minerals

$$ATTD, g/g = 100 - (100 * \frac{Marker\ in\ feed * Nutrient\ or\ mineral\ in\ feces}{Marker\ in\ feces * Nutrient\ or\ mineral\ in\ feed})$$

Mineral retention

Retention of nitrogen (N), P, Ca, Mg, K, Na, Cu, and Zn was calculated using Equation 2:

***Equation 2**:* Retention of N and minerals

$$MR = Mi - Mof - Mou$$

where *MR* is the mineral retention (g/ 5d), *Mi* is dietary mineral intake (g/ 5d), *Mof* (g or mg/ 5d) is mineral output via faeces (g or mg/ 5d) and *Mou* (g or mg/ 5d) is mineral output via urine (g), respectively. The faecal mineral output was calculated as mineral intake (g or mg) * (1 – digestibility rate of the mineral), while urine mineral output was calculated from the amount of urine excreted multiplied by the mineral content in the urine.

Digestible and metabolizable energy

Dietary digestible energy content was calculated using Equation 3:

***Equation 3**:* Digestible energy content

$$DE = GE * EDc$$

Where *DE* is the dietary digestible energy (MJ/kg), *GE* is the gross energy content of the feed (MJ/kg) and *EDc* is the energy digestibility coefficient of the feed, respectively.

Dietary metabolizable energy content was calculated using the Equation 4:

Equation 4: Metabolizable energy content

$$ME = (DEi - Eu)/FI$$

Where *ME* is the dietary metabolizable energy (MJ/ kg), *DEi* is digestible energy intake (MJ/ 5d), *Eu* is the energy excreted via urine (MJ/ 5d) and *FI* is the feed intake (kg/ 5d), respectively.

3.1.3. Determination of gastrointestinal tract function

3.1.3.1. Determination of enzymes' activity

Pancreatic samples and individual segments of the small intestine were first put in Eppendorf or in plastic bags (depending on the size and consistency) and then CO_2 was blown into the Eppendorf or plastic bag and immediately closed. After this procedure, the samples were kept in a freezer at -80 °C until analyzed. Contents of the small intestine and pancreas were diluted with a phosphate buffer (pH 7) (Marounek *et al.,* 1995). Lactase and maltase activities were determined in the duodenum, jejunum, and ileum of the small intestine. Amylase, protease, and lipase activities were determined in the pancreas. Dry matter (DM) was determined by heating at 105 °C for 24 h.

Amylase determination

The activity of amylase was assayed according to Kopečný and Bartoš (1990) using soluble starch at a concentration of 4 mg/ml. For incubation, 1 ml of the substrate and 1 ml of digesta were diluted, mixed, and incubated at 38 °C for 1 h. The process was terminated by adding 1 ml of 0.3 M $Ba(OH)_2$ and 1 ml of 0.3 M $ZnSO_4$. The precipitate was then removed by centrifugation (4000 g, 10 min) (Marounek *et al.,* 1995). Reducing sugars were determined in the supernatant (Somogyi, 1952). Calibration was done with glucose and calibration standards were prepared in distilled water ranging from 0-0.50 μmol/ml. Next, centrifuged samples were prepared at an appropriate dilution and 1 ml of standard/sample was added to a separate tube. The blank tube consisted of 1 ml distilled water. Next, a copper reagent was added to each tube, mixed, and placed in boiling water for 10 min. Tubes were then cooled to room temperature and 1 ml of arsenomolybdate reagent (Nelson Reagent) was added to each tube. Absorbance was measured at 520 nm. Activities were related to 1 ml of the original digesta and per mg of dry matter. Enzymatic activity was expressed as mg sugar per h per DM of digesta.

Protease determination

Samples were diluted with a phosphate buffer (pH 7.5) (Marounek *et al.,* 1995). Azocasein solution was prepared at a concentration of 4 mg/ml in 0.1 M potassium phosphate buffer (pH 7.5). Prepared for each sample were four plastic 10 ml polypropylene tubes containing: 1) azocasein solution; 2) azocasein solution and 25% trichloroacetic acid; 3) 0.1 M potassium phosphate buffer (pH 7.5); 4) 0.1 M potassium phosphate buffer (pH 7.5) + 1 ml 25%

trichloroacetic acid. At t = 0 digesta samples were added to all tubes. After 1 h incubation at 39 °C, 25% trichloroacetic acid was added to Tube (1) and Tube (3). Tubes were then transferred to iced water and centrifuged at 4000 g. The supernatant was removed into another tube with 0.5 M NaOH. Absorbance was measured at 440 nm. Result absorbance was calculated from four values A1-A2-A3+A4. Calibration was marked by a 0.2 mg/ml solution of azocasein. Proteolytic activity was expressed as mg azocasein hydrolyzed per h per dry matter of digesta (Hoffmann *et al.*, 2010).

Lipase determination

The activity of lipase was measured by a modified method of (Bier, 1955). In brief, samples were incubated with 2.5 mM Tris-HCl buffer containing 0.8 ml of 0.025 M $CaCl_2$, 0.1 ml of 0.2 M Na_2HPO_4 and 1 ml of emulsified tributyrin. The reaction mixture was incubated for 1 h at 37 °C. Liberated butyrate was determined by gas chromatography (GC) (Labio a.s., Czech Republic) using a 15 m capillary column. Parameters of GC analysis were the temperature of injection and detector (200 °C); total pressure (50 kPa), O_2 (120 ml/min), make up (20 ml/min), temperature 1 was 75 °C, 25 s and 5 °C/min, temperature 2 was 80 °C, 80 s and 5 °C/min, temperature 3 was 120 °C, 4 s and 20 °C/min and temperature 4 was 160 °C 180 s, and finally, injection volume at 1 µl.

Maltase and lactase determination

Maltase and lactase activities were assayed according to (Kopečný and Bartoš, 1990) using maltose or lactose at a concentration of 4 mg/ml. 1 ml of the substrate and 1 ml of digesta were diluted, mixed, and incubated at 38 °C for 1 h. The process was terminated by adding 1 ml of 0.3 M $Ba(OH)_2$ and 1 ml of 0.3 M $ZnSO_4$. The precipitate was then removed by centrifugation (4000 g, 10 min) (Marounek *et al.*, 1995). The D-glucose was assayed using a glucose assay reagent kit. Calibration was made with glucose standard solution (D-glucose in a concentration of 1 mg/ml in 0.1% benzoic acid) (Sigma Aldrich). The standards for calibration were prepared in a concentration of 20–80 µg/ml. For reaction, 1 ml of sample was used with a blank reagent/calibration standard. The reaction was initiated by adding 2 ml of assay reagent to the first tube and mixing. Glucose oxidase/peroxidase reagent (Sigma Aldrich) was prepared by dissolving the capsule in 39.2 ml of deionized water. O-Dianisidine Reagent (Sigma Aldrich) was reconstituted with 1 ml of deionized water. Next, the assay reagent was prepared by mixing 0.8 ml of the o-dianisidine reagent with 39.2 ml of the glucose oxidase/peroxidase reagent. Tubes were then incubated for 30 min at 37 °C. The reaction was stopped by adding 2 ml of 12 M H_2SO_4. Tubes were carefully mixed, and absorbance was measured against a blank

reagent at 540 nm. Maltase and lactase activity was expressed as mg glucose per h per DM of digesta.

All enzyme activity measurements were performed in cooperation with Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences, Prague (Czech Republic).

3.1.3.2. Histology and histopathology

Approximately 3 cm long segments of the small intestine were collected from the duodenum (approximately 6 cm distal from the pylorus), jejunum (from the middle part) and ileum (approximately 6 cm proximal to the ileocecal junction). Formalin-fixed (10% buffered formalin; pH, 7.2) samples were embedded in paraffin and sectioned in transverse. After deparaffinization and dehydration, tissues were processed by standard histologic techniques for the production of 4 μm thick sections which were then stained with hematoxylin and eosin (H.E.). The structure of the mucosa was observed using an ocular micrometre to examine the orientation of the villus height and crypt depth. Measurement of VH and CD was done by taking cross-sections of 5 randomly selected villi. The ratio of the villus to the crypt was estimated by dividing VH by CD.

Following macroscopic external and internal examination of the organs, the liver, kidney, lung, and small intestine were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin-eosin (H.E.) and examined by light microscopy. Individual animals from each group were examined and the main pathological alterations were described and scored according to the extent and severity as follows:

- = no alteration / changes
1 = slight / small scale/ few
2 = medium degree / medium scale / medium number
3 = pronounced / extensive / numerous

These examinations were performed by Autopsy Public Claims Company Limited, Budapest, Hungary.

3.1.3.3. Short-chain fatty acids determination

About 3 g of caecal digesta was homogenized with 4.5 ml metaphosphoric acid (4.16%), then centrifuged at 10,000 g for 10 min and filtrated. The concentration of SCFAs was measured with GC (Shimadzu GC 2010, Japan). A Nukol 30 m x 0.25 mm x 0.25 μm capillar column

(Supelco, Bellefonte, PA, USA) was used for separation and flame ionization detector. The split ratio was 1:50, 1 µl was the injected volume, carrier gas was helium at 0.84 ml/min. Detector conditions: air 400 ml/min, hydrogen 47 ml/min, temperature: injector 250 °C, detector 250 °C, column 150 °C. 2-etil-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) was used as internal standard.

3.1.3.4. Microbiota analysis

For the intestinal microbiota analysis, the intestine was placed on a sterilized autoclave bag, on which each intestinal section was excised. Before excision, the 10 cm intestinal sections were sealed at both ends of the region with sterile bundles and the sealed intestinal section was placed in a sterile Petri dish. At the next stage of sampling, one end of the intestinal section was cut with a sterile scalpel and the contents were dropped into a sterile urine collection vessel. From each of the shaken samples, 0.2-0.2 g was measured with a sterile spatula into 4 sterile 1.5 ml Eppendorf tubes, and approximately 0.2 g was measured into a fifth Eppendorf tube, into which 200 µl of 50% glycerol had been previously measured into. The 4-4 samples were stored primarily as duplicates for metagenomic use, while the 5th was stored specifically for microbiological purposes. Samples sin Eppendorf tubes and residual samples in the urine storage container were immediately placed in a -20 °C freezer after which they were stored long- term at -70 °C after sampling was completed.

Amplicon sequencing of the V3-V4 region of the 16S rRNA gene was performed by LGC Genomics GmbH (Berlin, Germany). Library preparation and sequencing were performed using an Illumina MiSeq platform with v3 chemistry. The DNA fragments were amplified using amplification primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') (Klindworth *et al.*, 2013). The primers also contained the Illumina sequencing adapter sequence and a unique barcode index. Resulting amplicons were sequenced using the Illumina MiSeq v3 600-cycle kit to provide paired-end read lengths of 2 x 300 bp. Demultiplexing of all libraries for each sequencing lane were attained using the Illumina bcl2fastq 2.17.1.14 software (Illumina Inc., San Diego, USA). The combination of forward and reverse reads were carried out using the BBMerge 34.48 tool (Bushnell *et al.*, 2017). The mothur software package [(v1.35.1, (Schloss *et al.*, 2009)] was used for pre-processing and OTUs picking from Illumina amplicon sequencing data by clustering at the 97 % identity level. Creation of relative abundance taxonomical tables in an Excel format for was performed with QIIME 1.9.0. (LGC Genomics GmbH, Berlin, Germany)(Caporaso *et al.*, 2010).

Analyses were performed in cooperation with the Institute of Genetics and Biotechnology of MATE (Gödöllő, Hungary).

3.1.4. Blood serum biochemistry and antioxidant parameters determination

Fresh blood samples were taken during exsanguination into heparin tubes (20 IU/mL whole blood). Samples were centrifuged for 10 min at 1000 × g (SIGMA 3-30KS refrigerated centrifuge, Osterode am Harz, Germany) for plasma separation. The concentration of plasma total protein (TP), albumin (ALB), creatinine concentrations, the activities of the alanine aminotransferases (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase and alkaline phosphatase (ALKP) were determined using Roche Hitachi 912 Chemistry Analyzer (Hitachi, Tokyo, Japan) and commercial diagnostic reagent kits (Diagnosticum Ltd., Budapest, Hungary) at the Vet-Med Laboratory, Budapest (Hungary).

For the determination of lipid peroxidation and antioxidant status, samples were stored at -80 °C until analysis. Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) level with the 2-thiobarbituric acid method in cell hemolysate (Botsoglou *et al.,* 1994). The concentration of reduced glutathione (GSH) was measured by Sedlak and Lindsay (1968) and the activity of glutathione peroxidase (GSHPx) according to Lawrence and Burk (1978).

3.1.5. Western blot analysis of Hsp70 activity

The western blot analysis of Hsp70 activity in the kidney and liver was done according to a method described by Kócsó *et al*. (2018). In summary, kidney and liver tissue samples were homogenized in 500 µl lysis buffer (1% NP40, 1% sodium deoxycholate, 0.1% SDS, 15 mM NaCl, 10 mM phosphate buffer, 2 mM EDTA, 2 mg/ml aprotinin, 0.5 mg/ml leupeptin, 2 mM sodium vanadate, 20 mM NaF, 0.5 mM DTT, 1 mM PMSF) for 3 min. Next, the cell lysate was centrifuged (13,000 rpm, 30 min, 4 °C) and the pellet was collected. The total protein concentration of the samples was determined with the BCA™ Protein Assay KIT (Thermo-Fisher, Budapest, Hungary). Thirty to 35 µg protein/sample quantities were applied to 10% SDS polyacrylamide gels [30% Acrylamide/Bis-acrylamide, 1.5 M Tris (pH 8.8), 1.0 M Tris (pH 6.8), 100 g/L SDS, 100 g/L APS, TEMED] and transferred to nitrocellulose (0.45 µm) membranes. The membranes were washed for 3×5 minutes with TBS-T (1×TBS pH 7.6, 0.1% Tween 20), then blocked in phosphate-buffered saline (10 × PBS) containing 5% non-fat dried milk powder, 1% BSA and 0.1% Tween 20. Subsequently, the membranes were incubated with the primary anti-Hsp70 antibodies (1:1000; Sigma, Budapest) at 4 °C for 12 h. As an internal control, anti-β-actin antibodies (1:10,000; Sigma, Budapest, Hungary) were used. After another 3×5-min washing with TBS-T (pH 7.5), secondary antibodies conjugated with HRP were used

in 1:500 dilution (Biomarker, Budapest, Hungary) to quantify the binding of the primary antibodies. After a repeated 3×5-min washing with TBS-T, the light emission of the blotted proteins was ensured using a Westernbright Enhanced Chemiluminescent HRP substrate detection system (Biomedica, Budapest, Hungary), and the proteins were detected either on CL-Xposure clear-blue X-ray films (Protein Simple, Santa Clara, CA, USA).

3.1.6. Statistical analysis

Statistical analyses of the digestibility and mineral retention, intestinal morphology, enzyme activity, antioxidant parameters, Hsp70 activity, SCFAs and serum biochemical measurements were performed using the SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Results were subjected to a one-way analysis of variance (ANOVA). In case of a significant treatment effect, the intergroup differences were checked by a Tukey post-hoc test. A *P* value < 0.05 was described as significant (SPSS, 2012).

For the microbiota statistical analysis, creation of relative abundance taxonomical tables in an Excel format for was performed with QIIME 1.9.0. (LGC Genomics GmbH, Berlin, Germany) (Caporaso *et al.*, 2010). Relative abundance data generally did not follow a normal distribution, as assessed by the Shapiro-Wilk test (Ju and Zhang, 2015). Differential abundance testing in the duodenum, jejunum, and ileum content, and faeces of the three treatment groups [Control (G1), 15 mg/kg FUMs (G2) and 30 mg/kg FUMs (G3)] was performed by the non-parametric Kruskal-Wallis test using IBM SPSS Statistics 27.0 software (SPSS Inc., Chicago, IL, USA (Hazra and Gogtay, 2016; Weiss *et al.*, 2017; Xia and Sun, 2017). A difference was considered significant at $P < 0.05$. The bacterial composition of day 0 and day 21 faecal samples in the three treatment groups was compared using the Wilcoxon Signed-Rank test (Ju and Zhang, 2015; Hazra and Gogtay, 2016; Chen *et al.*, 2017).

3.2. Cannulated-fattening pigs' study

3.2.1. Experimental design, conditions and diet preparation

The experiment was carried out according to the regulations of the Hungarian Animal Protection Act. The allowance number for the studies was SOI/31/00997-7/2018.

A total of ten Danbred breed fattening pigs (average BW of 65.5 ± 4.1 kg) fitted with a Post-valve T- cecum (PVTC) cannula as described by Leeuwen *et al*. (1991) were enrolled in the study. Pigs were kept in individual crates (200 x 120 cm) located in the Experimental Animal Unit of MATE-KC. A maize-soybean-based diet of commercial origin was used as the basal diet (feed composition is listed in Table 8). The pigs were then randomly assigned to 2 different diets; a control diet that contained no FUMs nor any trace of other mycotoxins (tested and

confirmed) and a 40 mg/kg FUMs contaminated diet (n= 5 animals/group). Feed was offered that covers 2.5 times the maintenance energy requirement (ME_m = 450 kJ/ $BW^{0.75}$/ d). Control pigs and treated pigs consumed average of 2,021 and 2,007 g feed/ d, respectively. The study had a 2 x 2 factorial design, the independent variables were the treatments (control or 40 mg/kg FUMs diet) and the exposure time [7 days (short- exposure) or 21 days (long exposure)]. The pigs were weighed individually at the beginning of the trial and at the end of the trial and, their health status was monitored every day. The room temperature was adjusted according to the breeder's guidelines.

Table 8. Analyzed nutrient and feed composition of the experimental feed for cannulated-fattening pigs

Feed composition		Analyzed nutrient content of feed	
Feed component	*g/kg*	*Nutrient*	*g/kg*
Maize	612.35	Crude protein	180.9
Soybean meal	247	Ether extract	34.7
Barley	101	Crude fibre	24.9
Sunflower oil	40	Ash	55.3
Limestone	13	Starch	518.9
Monocalcium phosphate	11.1	Calcium	7.7
Vitamin and mineral premix	5.0	Phosphorus	5.8
Salt	4.0		
L-lysine HCL	1.3		
DL-methionine	1.0		

Preparation of the FUMs used for the fattening pigs followed the same procedure as that of the nursery pigs and described earlier (Fodor *et al.*, 2006a). The fungal culture concentrations were 25.57 mg/g FB_1, 6.17 mg/g FB_2 and 3.01 mg/g FB_3. The LOD for FB_1 and FB_2 were 0.031 and 0.051 mg/kgrespectively; LOD for FB_3 was not measured. Diet fed to the control group did not contain detectable amounts of FUMs or other mycotoxins. The FUMs level used to prepare the contaminated diet is listed in Table 9.

Table 9. Fuminisin level of *in vitro*- produced fungal culture, the experimental diets and limit of detection of mycotoxis measured

Mycotoxins	LOD, mg/kg	Fungal cuture, mg/g	Control diet	40 mg/kg FUMs diet
FB_1	0.031	25.57	nd	30.37
FB_2	0.051	6.17	nd	7.12
FB_3	-	3.01	nd	3.1
ZEN	0.005	-	nd	nd
DON	0.053	-	nd	nd
T-2 toxin	0.011	-	nd	nd

FB_1= fumonisin B_1, FB_2= fumonisin B_2, FB_3= fumonisin B_3, ZEN= zearalenone, DON= deoxynivalenol, LOD= limit of detection, nd= not detected

3.2.2. Digestibility trial

The placement of the post-valve T caecum (PVTC) cannula was done in anesthesia according to a method by Leeuwen *et al.* (1991). A projection perpendicular to the ileum was utilized to separate the caecum's corpus and apex using an intestinal clamp. The caecum was transsected between the intestinal clamp and the string after a purse string suture was placed in the portion of the caecum that needed to be preserved (distance to the clamp was 0.5 cm). The caecum was removed and a large T-cannula was used in its stead. After the caecum was cut, the flange of the cannula was introduced in the large intestine and the cannula aperture was positioned in front of the ileocaecal valve. The flange was fixed to the intestinal wall by tightening a pre-placed purse-string suture. The cannula was then exteriorized through an incision in the body wall, fixed externally by mounting a ring of silicone rubber and closed with a silicone rubber stop. The digesta from the ileum then flows into the colon after the cannula is closed. When the ileocaecal valve is opened, it protrudes into the cannula's aperture, allowing the digesta to flow straight into it and be collected using plastic bags.

Feed was offered twice daily (at 07:30 h and 15:30 h) and in equal proportions. Ileal digesta samples were collected (08:00 h to 20:00 h) in the durable plastic bags around the PVTC cannula secured with rubber bands. After day 6 of feeding experimental diets, and for 3 consecutive days from then on i.e., on days 7, 8, and 9, the first collection period of ileal digesta began and was labelled as the ‘short-term’ exposure period. After day 20 of feeding experimental diets and for 3 consecutive days as well, i.e., on days 21, 22, and 23, the second ileal digesta collection period began and was labelled as the ‘long-term’ exposure period. Collection of the digesta samples from each animal was a matter of constantly checking whether the plastic bags were filled with enough ileal digesta, i.e., not too small, and not too much that could spill out of the bags. The trial outflow is presented in Figure 7 below.

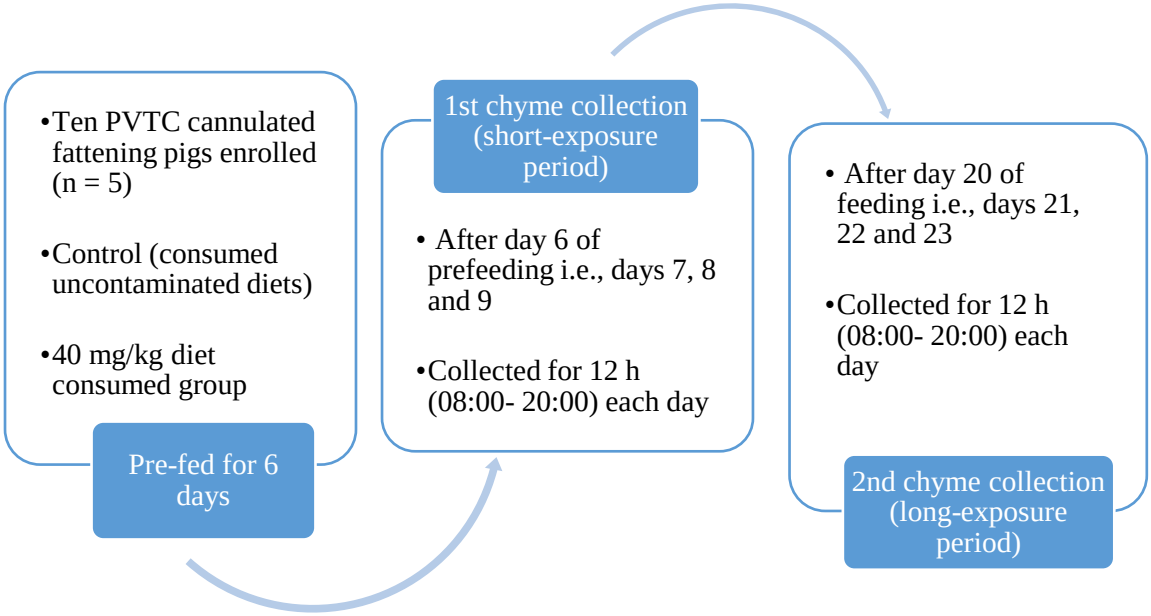


Figure 7. Feeding and digesta collection outflow of fattening pigs trial

The collected ileal digesta samples were weighed and immediately stored at -20 °C to prevent bacterial degradation of the AAs. At the end of the experiment, the samples were freeze-dried and ground before the analyses. Pigs were euthanized by exsanguination after sedation (intramuscular injection of zolazepam and tiletamine (2.5 mg/kg, Zoletil, Virbac), xylazine (3 mg/kg, CP-Xylazin 2%, CP-Pharma Handelsgesellschaft) and azaperone (6 mg/kg, Stresnil, Janssen-Cilag GmBH, Germany) at the end of the experiment.

3.2.3. Laboratory analysis

Chemical analysis of samples was done in cooperation with the laboratory of MATE–KC. The CP and the AAs– aspartic, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, ammonia, and arginine were measured using MSZ EN ISO 13903:2005 standardization procedure. Nitrogen was determined with the Kjeldahl method (AOAC, 2000). Titanium dioxide (TiO_2) was analyzed by the same procedure as described earlier under the nursery pigs' laboratory analysis. Table 10 shows the analyzed AAs of the feed.

Table 10. Analyzed amino acids content of the fattening pigs' feed

Amino acid	g/100g sample
Aspartic acid	1.65
Threonine	0.66
Serine	0.84
Glutamic acid	3.33
Proline	1.12
Glycine	0.69
Alanine	0.89
Cystine	0.26
Valine	0.76
Methionine	0.28
Isoleucine	0.65
Leucine	1.43
Tyrosine	0.45
Phenylalanine	0.80
Histidine	0.42
Lysine	0.95
Ammonia	0.30
Arginine	0.96

3.2.4. Calculation and statistical analysis

Apparent ileal digestibility of the CP and AAs was calculated using the TiO_2 concentration in ileal digesta samples and feed with Equation 5:

Equation 5: Apparent ileal digestibility of CP and AAs

$$AID, g/g = \left[1 - \left(\frac{\%\ TiO_2\ in\ feed\ *\ \%\ CP\ or\ AA\ in\ ileal\ digesta}{\%\ TiO_2\ in\ ileal\ digesta\ *\ \%\ CP\ or\ AA\ in\ feed}\right)\right]$$

where;

AID is the apparent ileal digestibility; TiO_2 is titanium dioxide, CP is the crude protein, and AA is the amino acid in question.

Statistical analyses were performed using the SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Results were subjected to a two-way ANOVA of the SPSS version 20.0 software using FUM dose (i= 2; 0 or 40 mg FUMs/ kg feed) and duration (j= 2; short vs long term exposure) as fixed factors. A *P* value of < 0.05 was considered significant (SPSS, 2012).

4. RESULTS

4.1. Effect of dietary fumonisins on nutrient digestibility, mineral retention, energy utilization, intestinal morphology, health indicators and microbiota of nursery pigs

4.1.1. Growth rate and clinical signs

Given the present dosages of dietary FUMs used (15 or 30 mg/kg diet), no mortality nor diseases were observed during the 21 day exposure of nursery pigs to treatments. We observed episodes of diarrhoea in the animals during the 2 week acclimatization period but this was only transitional since the animals exhibited complete recovery before the intoxication period began.

There was no significant ($P > 0.05$) difference in the overall growth performance of pigs although a numerical reduction in the total weight gain (existing already in the first week) of pigs fed FUMs contaminated diet could be detected. The pigs were fed restrictively and there was no feed refusal, thus, the total feed consumption remained statistically ($P > 0.05$) unchanged throughout the 21- day long trial. Relative weights of kidney, liver, lungs, and pancreas remained unaffected either ($P > 0.05$) at the end of the trial (Table 11).

Table 11. Effect of varied levels of dietary fumonisins on growth and relative organ weights in nursery pigs (data are means ± standard deviation (SD) of 6 individuals/group)

Groups	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
Growth performance, kg			
Cumulative FI	19.76 ± 2.1	20.45 ± 1.4	20.38 ± 1.4
Initial BW	12.98 ± 1.7	13.80 ± 1.2	13.80 ± 1.1
Week 1 WG	2.88 ± 0.4	2.51 ± 0.3	2.43 ± 0.4
Week 2 WG	3.28 ± 0.2	3.23 ± 0.2	2.97 ± 0.5
Week 3 WG	3.43 ± 0.2	3.48 ± 0.3	3.11 ± 0.9
Total WG	9.60 ± 0.5	9.22 ± 0.6	8.50 ± 1.5
Relative organ weights (expressed in % BW)			
Liver	2.61 ± 0.2	2.44 ± 0.3	2.39 ± 0.5
Lung	1.07 ± 0.2	1.1 ± 0.2	1.0 ± 0.2
Kidney	0.39 ± 0.0	0.38 ± 0.0	0.35 ± 0.1
Pancreas	0.27 ± 0.0	0.23 ± 0.0	0.24 ± 0.0

FI= Feed intake, BW= Bodyweight, WG= Weight gain. No different lowercase letters denotes no significant difference at $P < 0.05$

4.1.2. Nutrients apparent total tract digestibility

Table 12 presents the results of the ATTD of nutrients. The ATTD values of DM, CP and starch remained unchanged in all groups. Although the ATTD value of EE was lower for animals that received the contaminated diets compared to the control animals, the difference was not significant. The ATTD values for CF, ash, Ca and P were significantly reduced in G2 or G3 ($P < 0.05$) as compared to G1.

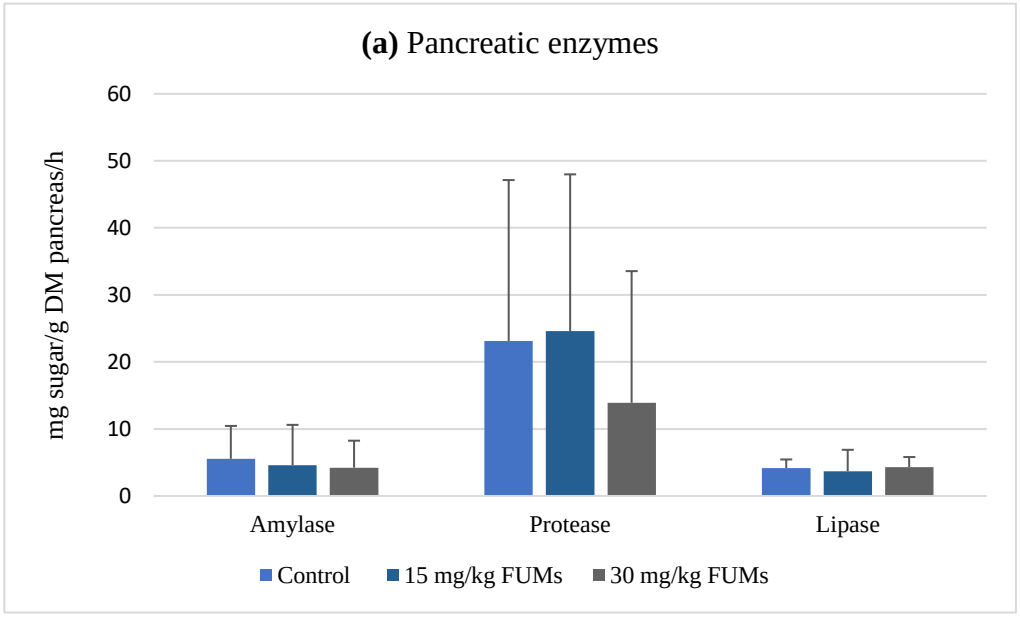
Table 12. Effect of varied levels of dietary fumonisins on apparent total tract digestibility (%) of nutrients in nursery pigs (data are means ± standard deviation (SD) of 6 individuals/group)

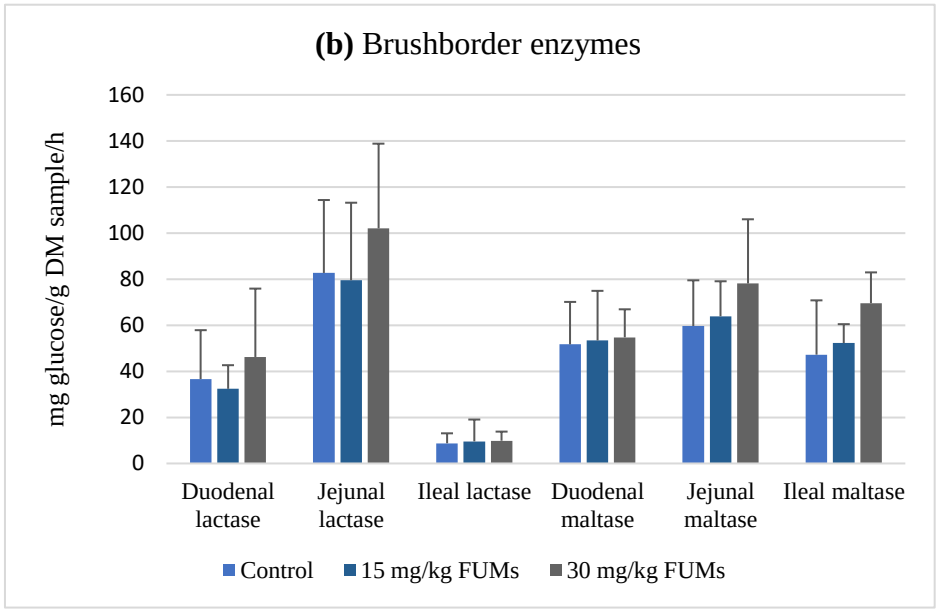
ATTD, %	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
Dry matter	84.8 ± 1.0	83.6 ± 0.5	83.8 ± 1.1
Crude protein	83.8 ± 2.1	83.3 ± 1.4	82.3 ± 1.2
Ether extract	64.3 ± 7.3	56.2 ± 8.6	57.1 ± 9.6
Crude fiber	44.8 ± 1.4 **b**	40.5 ± 2.5 **a**	40.6 ± 3.0 **a**
Starch	99.1 ± 0.2	98.9 ± 0.1	99.1 ± 0.2
Ash	58.6 ± 3.3 **b**	52.6 ± 1.7 **a**	53.6 ± 1.6 **a**
Calcium	65.4 ± 6.6 **b**	56.3 ± 3.4 **a**	53.9 ± 6.9 **a**
Phosphorus	67.5 ± 3.5 **b**	62.6 ± 1.5 **a**	60.4 ± 3.6 **a**

ATTD= apparent total tract digestibility. Different lowercase letter indexes, a or b, without common letter in the same row differ significantly at $P < 0.05$

4.1.3. Digestive enzymes activity and villus architecture

The effect of dietary FUMs on digestive enzymes and villus architecture are presented in Figure 8 (Labels a-c). According to the statistical analysis, feeding either 15 or 30 mg/kg FUMscontaminated diets had no significant impact ($P > 0.05$) on the activities of pancreatic enzymes (Label a) or brush border enzymes (Label b) of the pigs following dietary exposure of 21 days compared to the non-contaminated diet fed counterparts. Similarly, there was no remarkable alteration in VH, CD or VH: CD ($P > 0.05$) along the length of the small intestine (Label c).





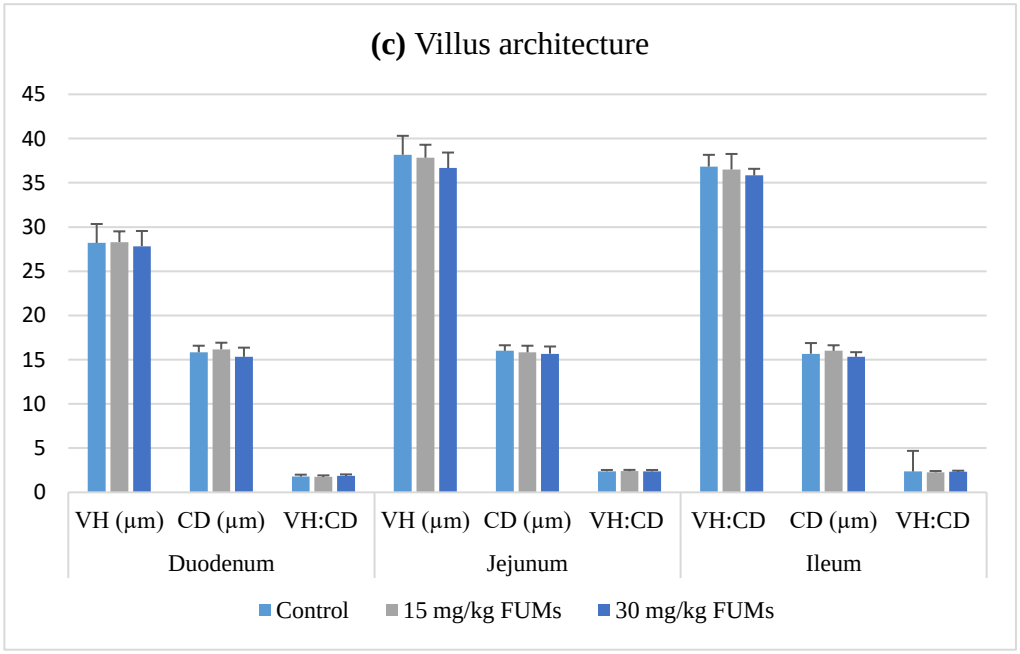


Figure 8. Effect of varied levels of dietary fumonisins on **(a)** pancreatic enzymes' activity, **(b)** brush border enzymes' activity and **(c)** intestinal architecture. No significant differences between treatments were observed for all

4.1.4. Balance study and energy utilization

In Table 13, the results of digestibility and retention of macrominerals are shown. The ATTD (%) for both Ca and P was lower in FUMs treated animals compared to controls. The Ca excretion via faeces was significantly higher ($P < 0.05$) in G3 as compared to the G1 or G2. Significant ($P < 0.05$) but the opposite trend was observed for Ca excretion via urine, thus, Ca retention did not statistically differ and was similar (between 11.7 and 12.8 in 5 days) in all treatments. Phosphorus (P) excretion through faeces was notably high ($P < 0.05$) in the two intoxicated groups as compared to the control group. However, P excretion through urine was lower in pigs at G2 or G3 than in G1 treatment ($P < 0.05$). Regardless of the numerical variations that could be observed in faecal and urinal outputs, the ATTD and relative retention rates of N, K, Na and Mg were not influenced ($P > 0.05$) by dietary treatments.

Among microminerals analyzed in the study, only Zn and Cu had reduced ATTD rates and further exhibited poor relative retention in the intoxicated groups compared to the control group (Table 14). Fumonisins (FUMs) exposure increased the faecal Zn excretion and resulted in negative ATTD rates for G2 and G3. The G1 group had a Zn ATTD rate of 20.6% while ATTD of –13.7% and –15.4% were obtained for either the G2 or G3, respectively. Although numerical variations could be detected in the faecal output of Cu, statistical analysis did not prove any significant ($P > 0.05$) differences within the groups. Cu ATTD obtained in G2 or G3 were 5.8% or 11.9% respectively, and were significantly lower ($P = 0.001$), as compared to G1 which was relatively high i.e., 20.3%. Further, the urinary and faecal output of Cu was unaffected ($P > 0.05$) by treatments. However, relative retention of Cu was notably low in G2 (0.05) and G3 (0.11) while it remained high in G1 (0.19) ($P = 0.001$).

Table 13. Effect of varied levels of dietary fumonisins on apparent total tract digestibility (%) and retention (mg/ 5d) of macrominerals in nursery pigs (data are means ± standard deviation (SD) of 6 individuals/group)

Groups	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
ATTD (%) of DM	84.8 ± 1.0	83.6 ± 0.5	83.8 ± 1.1
Nitrogen, g/ 5d			
Intake	105.3 ± 0.0	105.3 ± 0.0	104.5 ± 1.9
ATTD (%)	83.5 ± 2.1	82.8 ± 0.9	82.4 ± 2.2
Output in faeces	17.4 ± 2.2	18.1 ± 0.9	18.3 ± 2.1
Output in urine	22.8 ± 8.6	18.9 ± 10.4	12.4 ± 4.3
Retention	68.1 ± 7.1	68.3 ± 10.5	73.8 ± 4.4
Retained: intake	0.65 ± 0.07	0.65 ± 0.10	0.71 ± 0.04
Calcium, g/ 5d			
Intake	23.9 ± 0.0	23.9 ± 0.0	23.7 ± 0.4
ATTD (%)	65.3 ± 6.6 **b**	56.3 ± 3.4 **a**	54.0 ± 9.4 **a**
Output in faeces	8.3 ±1.6 **a**	10.5 ± 0.8 **ab**	11.6 ± 2.0 **b**
Output in urine	2.8 ±1.7 **b**	1.4 ± 0.7 **ab**	1.1 ± 0.5 **a**
Retention	12.8 ± 2.6	12.1 ± 0.7	11.7 ±1.4
Retained: intake	0.54 ± 0.1	0.51 ± 0.0	0.49 ± 0.1
Phosphorus, g/ 5d			
Intake	20.28 ± 0.0	20.28 ± 0.0	20.13 ± 0.4
ATTD (%)	67.5 ± 3.5 **b**	62.6 ± 1.5 **a**	60.0 ± 3.6 **a**
Output in faeces	6.6 ± 0.7 **a**	7.6 ± 0.3 **b**	7.9 ± 0.6 **b**
Output in urine	1.4 ± 0.8 **b**	0.6 ± 0.2 **a**	1.1 ± 0.4 **ab**
Retention	12.3 ±1.2	12.2 ± 0.5	11.1 ± 0.9
Retained: intake	0.61 ± 0.1	0.60 ± 0.0	0.55 ± 0.0
Potassium, g/ 5d			
Intake	15.87 ± 0.0	15.87 ± 0.0	15.76 ± 0.3
ATTD (%)	81.7 ± 2.9	78.1 ± 5.6	82.4 ± 2.2
Output in faeces	2.9 ± 0.5	3.5 ± 0.9	2.8 ± 0.3
Output in urine	11.0 ± 3.2	9.9 ± 0.3	9.4 ± 3.6
Retention	2.0 ± 3.4	2.5 ± 2.5	3.6 ± 3.2
Retained: intake	0.13 ± 0.2	0.16 ± 0.2	0.23 ± 0.2
Sodium, g/ 5d			
Intake	5.93 ± 0.0	5.93 ± 0.0	5.88 ± 0.1
ATTD (%)	89.1 ± 4.1	92.0 ± 2.9	90.8 ± 1.7
Output in faeces	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.3
Output in urine	2.7 ± 0.3	2.0 ± 0.8	2.7 ± 0.8
Retention	2.6 ± 0.4	3.4 ± 0.9	2.5 ± 0.7
Retained: intake	0.44 ± 0.1	0.58 ± 0.2	0.42 ± 0.1
Magnesium, g/ 5d			
Intake	6.79 ± 0.0	6.79 ± 0.0	6.74 ± 0.1
ATTD (%)	22.9 ± 5.8	22.1 ± 4.3	23.6 ± 7.4
Output in faeces	5.2 ± 0.4	5.3 ± 0.3	5.3 ± 0.3
Output in urine	0.9 ± 0.2	0.9 ± 0.3	0.7 ± 0.2
Retention	0.6 ± 0.4	0.6 ± 0.3	0.9 ± 0.5
Retained: intake	0.10 ± 0.1	0.09 ± 0.0	0.13 ± 0.1

ATTD= apparent total tract digestibility, DM= Dry matter. Different lowercase letter indexes, a or b, without common letter in the same row differ significantly at $P < 0.05$

Table 14. Effect of varied levels of dietary fumonisins on apparent total tract digestibility (%) and retention (mg/ 5d) of microminerals in nursery pigs (data are means ± standard deviation (SD) of 6 individuals/group)

Groups	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
ATTD (%) of DM	84.8 ± 1.0	83.6 ± 0.5	83.8 ± 1.1
Zinc, mg/ 5d			
Intake	737.1 ± 0.0	737.1 ± 0.0	731.7 ± 0.01
ATTD (%)	20.6 ± 1.7 **b**	- 13.7 ± 7.7 **a**	- 15.4 ± 8.3 **a**
Output in faeces	585.2 ± 86.4 **a**	837.9 ± 56.6 **b**	844.9± 72.5 **b**
Output in urine	15.1 ± 3.2	16.7 ± 8.5	12.9 ± 8.0
Retention	136.8 ± 88.7 **b**	- 117.6 ± 51.4 **a**	- 126.1 ± 61.3 **a**
Retained: intake	0.19 ± 0.1 **b**	- 0.16 ± 0.1 **a**	- 0.17± 0.1 **a**
Copper, mg/ 5d			
Intake	78.0 ± 0.0	78.0 ± 0.0	77.4 ± 0.0
ATTD (%)	20.3 ± 6.3 **b**	5.8 ± 3.9 **a**	11.9 ± 3.9 **a**
Output in faeces	62.1 ± 4.9	97.2 ± 58.1	68.2 ± 3.5
Output in urine	0.7 ± 2.7	0.7 ± 2.7	0.9 ± 2.1
Retention	15.2 ± 4.9 **b**	3.9 ± 2.8 **a**	8.4 ± 3.2 **a**
Retained: intake	0.19 ± 0.1 **b**	0.05 ± 0.0 **a**	0.11 ± 0.0 **a**

ATTD= apparent total tract digestibility, DM= dry matter. Different lowercase letter indexes, a or b, without common letter in the same row differ significantly at $P < 0.05$

Energy utilization by pigs fed the 3 different dietary levels of FUMs is presented in Table 15. The GE intake, energy excreted via faeces, energy excreted via urine and ME content of the feed remained unchanged statistically ($P > 0.05$), unlike DE content. The DE content was significantly lower ($P < 0.05$) in 15 mg/kg FUMs contaminated feed compared to the the control.

Table 15. Effect of varied levels of dietary fumonisins on energy utilization in nursery pigs (data are means ± standard deviation (SD) of 6 individuals/group)

Nutrients	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
Gross energy intake, MJ	65.9	65.9	65.4
Energy excretion via feces, MJ	11.1	11.9	11.8
Energy excretion via urine, MJ	2.1	2.1	1.6
DE content of the feed, MJ/kg	14.1 **b**	13.85 **a**	13.93 **ab**
ME content of the feed, MJ/kg	13.5	13.3	13.4

DE= digestible energy, ME= metabolizable energy. Different lowercase letter indexes, a or b, without common letter in the same row differ significantly at $P < 0.05$

4.1.5. Caecal fermentation by-products and microbiota distribution

All the caecal fermentation outputs (formic acid, acetic acid, propionic acid and valeric acid) analyzed in the study remained unaffected by dietary FUMs ($P > 0.05$) (Figure 9).

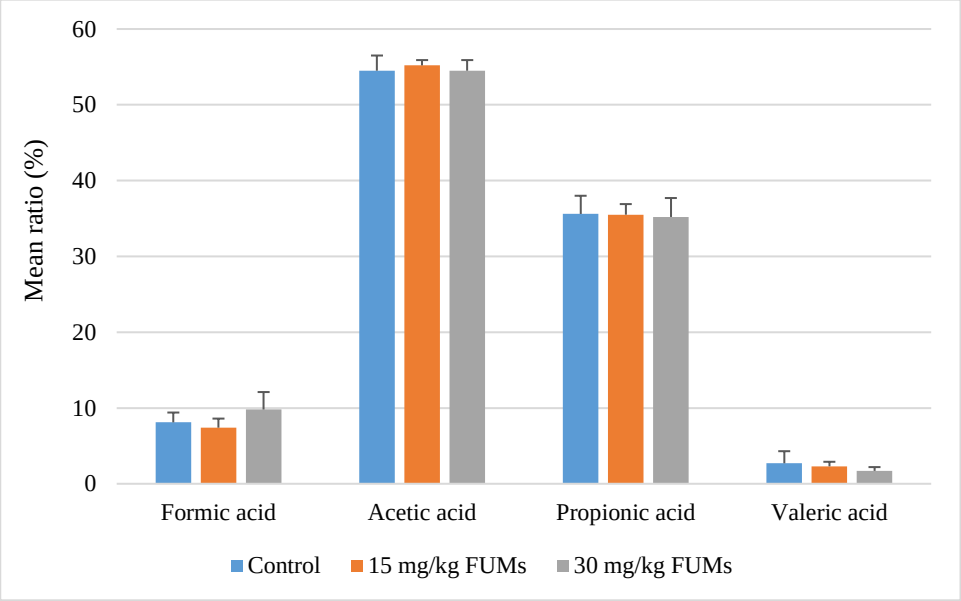


Figure 9. Cecal fermentation output of nursery pigs fed varied levels of dietary fumonisins for 21 days. No significant differences were observed for all

Due to the highly complex nature of the gut microbiota, our analysis focused on identifying bacterial species with an altered abundance following treatment. Figure 10 shows phylum-level bacterial composition in the duodenum, jejunum, ileum, and faeces (days 0 and 21) of control animals (G1). The most abundant phylum in all intestinal sections and in faeces was the *Firmicutes*. In the faeces at day 0, *Firmicutes* and *Bacteroidetes* dominated and were followed by the *Proteobacteria* and *Spirochaetae*. In the faeces at day 21, *Firmicutes* increased ($P = 0.03$) while *Bacteroidetes* declined ($P = 0.03$) compared to day 0. Overall, in all examined intestinal sections *Firmicutes* dominated, where in the duodenum *Firmicutes* had 84.9% mean relative abundance while *Actinobacteria* and *Proteobacteria* followed in close succession with 5.8% and 4.8%, respectively. In the jejunum, *Tenericutes* was the second most abundant phylum (12.46%), followed by *Actinobacteria* and *Proteobacteria* (2.87% and 1.24%, respectively). In the ileum, however, *Proteobacteria* (3.1%) was the second most abundant, followed by *Tenericutes* (2.2%).

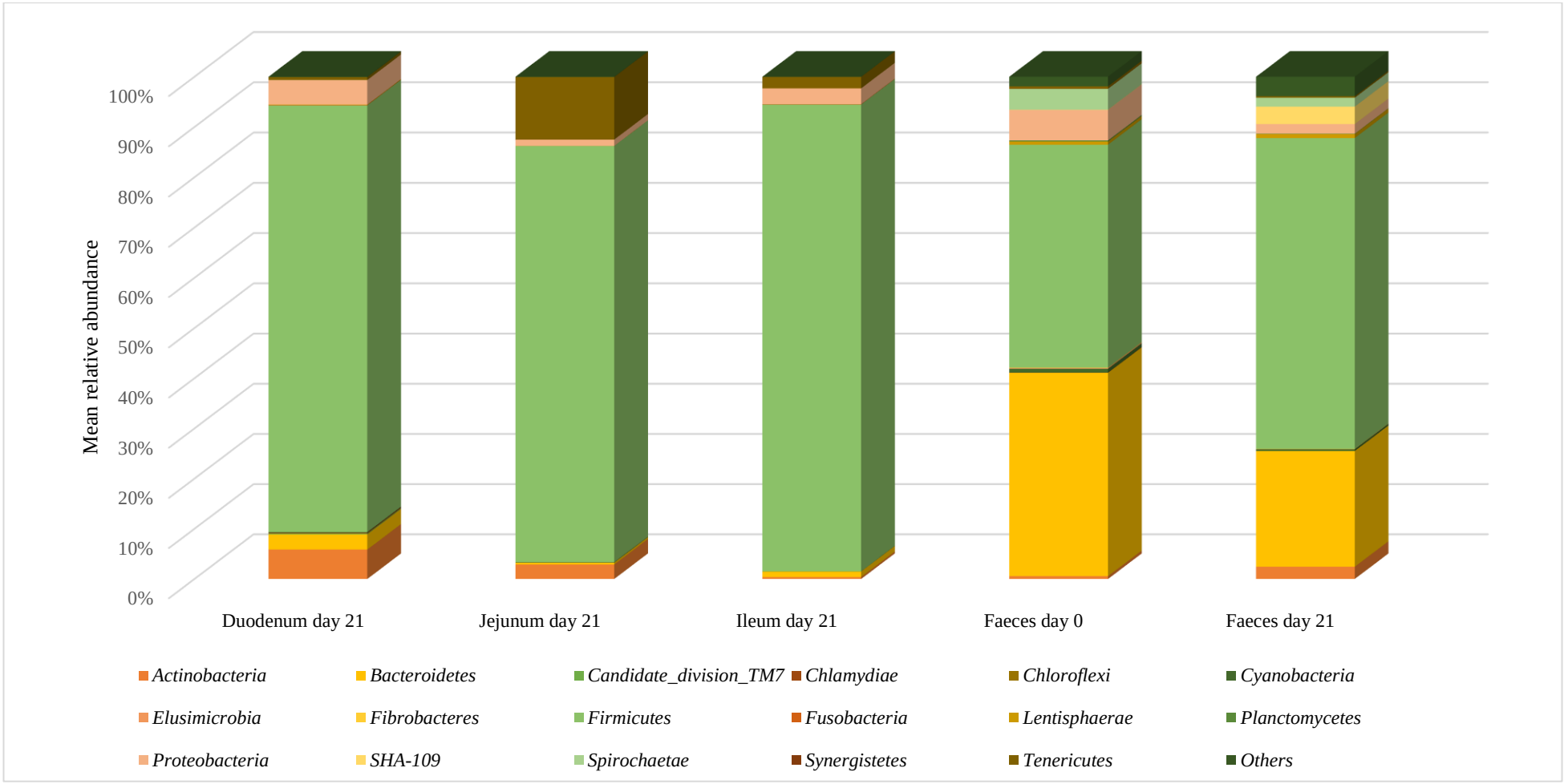


Figure 10. Gut longitudinal distribution of significant phyla in control group (G1) of nursery pigs (data is mean of 6 pigs)

As mentioned earlier, our analysis focused on identifying bacterial taxa that were altered after FUMs treatment. Overall, between the control group and the FUMs fed treatment group (G2 and/or G3), a total of 13 significant changes were found at the genus level. The faecal samples showed the highest number of significant differences ($P < 0.05$) in the relative abundances of eight genera. In the duodenum, jejunum, and ileum, three, one and one genera/genus showed a statistically altered (increased or decreased) abundance in response to FUMs treatment, respectively (Table 16). In the duodenum, *Alloprevotella* (G1 vs. G3 comparison; $P = 0.013$), *Campylobacter* (G1 vs. G3, G2 vs. G3 comparison; $P = 0.016, 0.012$), and *Lachnospiraceae*: *Incertae Sedis* (G1 vs. G3; G2 vs. G3 comparison $P = 0.009, 0.035$, respectively) showed a significant reduction. Likewise, *Turicibacter* in the jejunum and *Clostridium sensu stricto 1* in the ileum both decreased in a G1 vs. G3 comparison with P values of 0.001 and 0.009, respectively. In the faecal samples, there was a significant increase in the relative abundances of the genera *Solobacterium*, *Faecalibacterium*, *Anaerofilum*, *Ruminococcus*, *Subdoligranulum*, *Pseudobutyrivibrio*, *Coprococcus* and *Roseburia* in a G1 vs. G3 and/or in a G2 vs. G3 comparison ($P < 0.05$). Some selected significant genera including *Alloprevotella*, *Campylobacter*, *Clostridium sensu stricto 1* and *Pseudobutyrivibrio* bar graph representations are given in Figure 11 (a-d, respectively).

Table 16. Colonization pattern observed in animals (6 pigs/ group) fed either a control diet (G1), and 15 or 30 mg/kg FUMs (G2 and G3, respectively) contaminated diets for 21 days

Sample type	Significant changes in relative abundance	Group effect	*P*-value
Duodenum	Bacteroidales: Prevotellaceae: *Alloprevotella*	G1G3 ↓	0.013
Duodenum	Campylobacteraceae: *Campylobacter*	G1G3, G2G3 ↓	0.016, 0.012
Duodenum	Firmicutes: Clostridiales: Lachnospiraceae: *Incertae Sedis*	G1G3, G2G3 ↓	0.009, 0.035
Jejunum	Firmicutes: Erysipelotrichaceae: *Turicibacter*	G1G3 ↓	0.001
Ileum	Clostridiales: Clostridiaceae: *Clostridium sensu stricto 1*	G1G3 ↓	0.009
Faeces	Firmicutes: Erysipelotrichaceae: *Solobacterium*	G1G3, G2G3 ↑	0.040, 0.003
Faeces	Firmicutes: Clostridiales: Ruminococcaceae: *Faecalibacterium*	G2G3 ↑	0.020
Faeces	Firmicutes: Clostridiales: Ruminococcaceae: *Anaerofilum*	G1G3, G2G3 ↑	0.027, 0.008
Faeces	Firmicutes: Clostridiales: Ruminococcaceae: *Ruminococcus*	G2G3 ↑	0.011
Faeces	Firmicutes: Clostridiales: Ruminococcaceae: *Subdoligranulum*	G2G3 ↑	0.015
Faeces	Firmicutes: Clostridiales: Lachnospiraceae: *Pseudobutyrivibrio*	G1G3, G2G3 ↑	0.031, 0.027
Faeces	Firmicutes: Clostridiales: Lachnospiraceae: *Coprococcus*	G2G3 ↑	0.04
Faeces	Firmicutes: Clostridiales: Lachnospiraceae: *Roseburia*	G2G3 ↑	0.011

Symbols are the following= ↑ indicates an increase, ↓ indicates a decrease between the groups, G1= control group, G2= 15 mg/kg FUMs dosed group, G3= 30 mg/kg FUMs dosed group

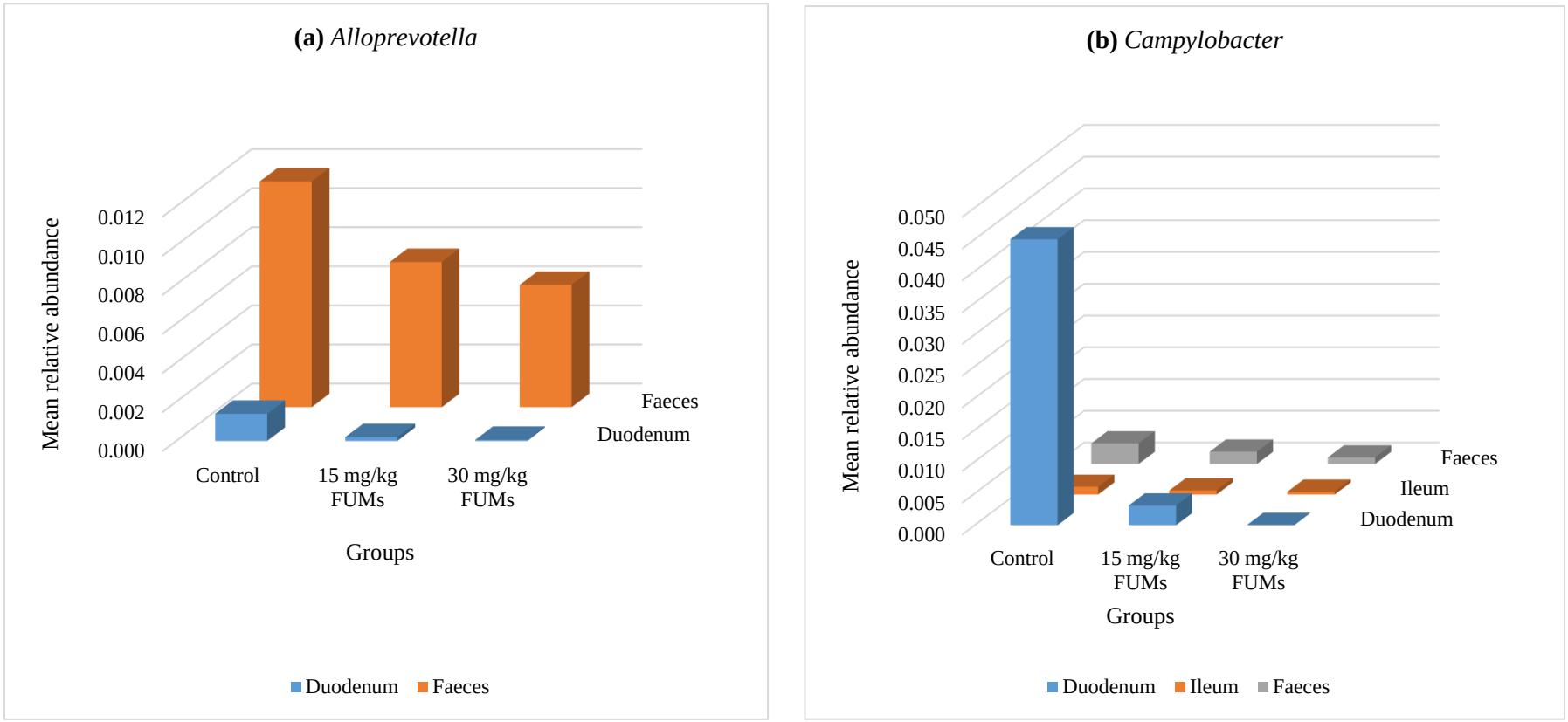
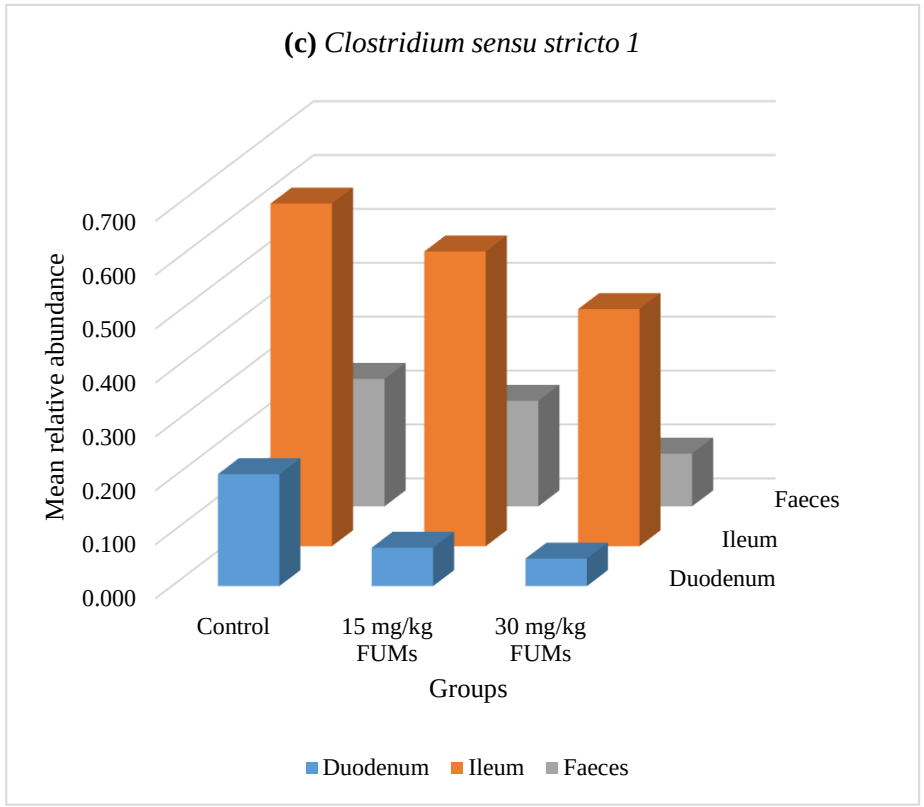


Figure 11. Bar graphs representation of significant differences in **(a)** *Alloprevotella* in duodenum and faeces and **(b)** *Campylobacter* in ileum, duodenum and faeces of nursery pigs exposed to varied levels of dietary fumonisins for 21 days



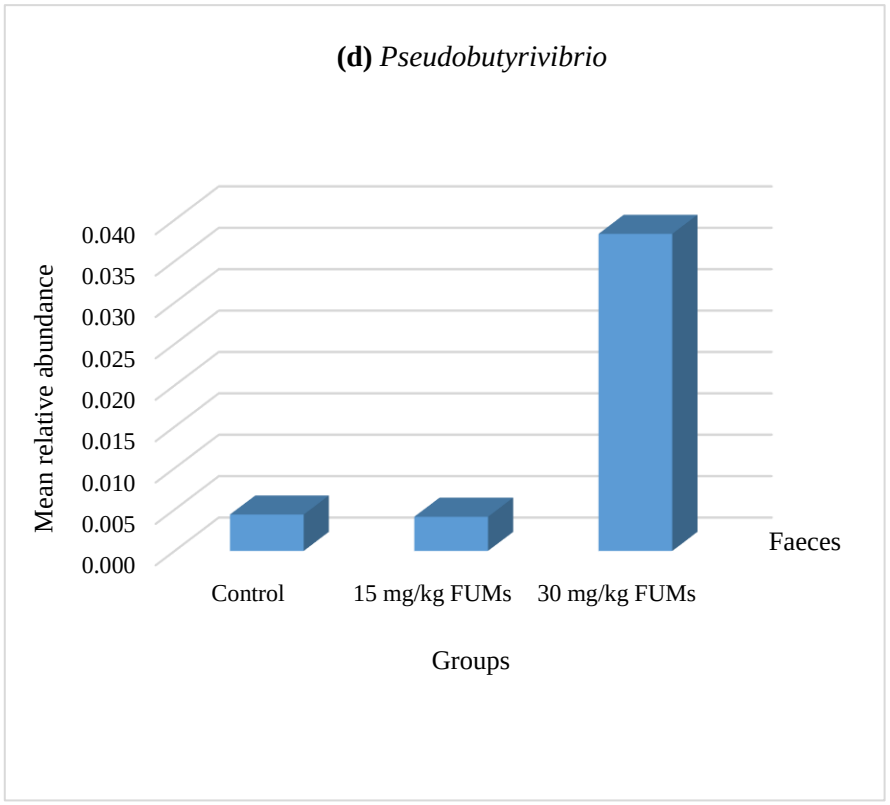


Figure 11. Bar graph representation of significant differences in **(c)** *Clostridium sensu stricto 1* in duodenum, ileum and faeces and **(d)** *Pseudobutyrivibrio* in faeces of nursery pigs exposed to varied levels of dietary fumonisins for 21 days

Although *Lactobacillus* relative abundances were not significantly different between the three treatment groups within a particular intestinal segment (Figure 11), Kruskal-Wallis tests demonstrated that *Lactobacillus* abundances were markedly higher ($P < 0.01$) in the proximal part of the intestinal tract (i.e., in the duodenum) compared to the faeces in all treatment groups. Moreover, Pearson correlation analyses showed that *Lactobacillus* relative abundances correlated between the duodenum and ileum (r = 0.86, $P < 0.01$), and between the ileum and faeces (r = 0.67, $P < 0.05$) among the three examined groups (Figure 12).

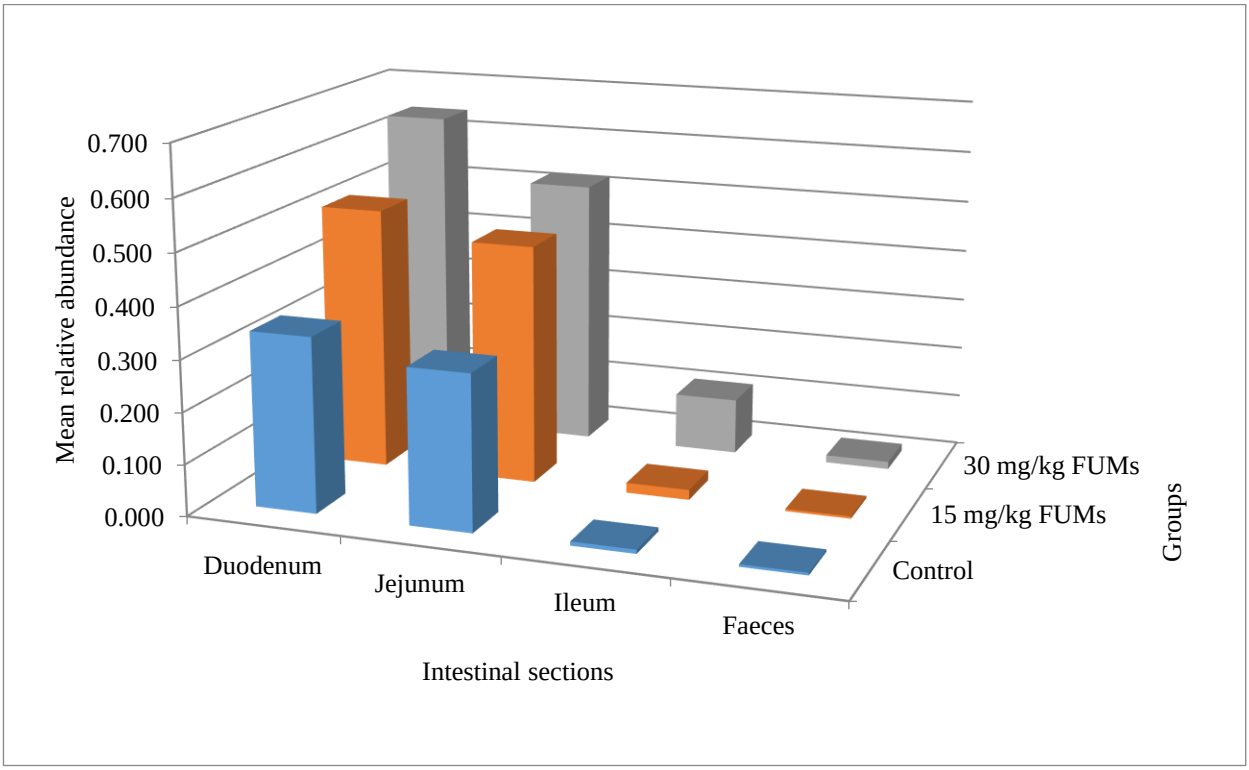


Figure 12. Mean relative abundance of *Lactobacillus* in the intestinal and faecal microbiota of nursery pigs

4.1.6. Serum biochemical measurements and antioxidant parameters analysis

Results on serum biochemical parameters as a response to different levels of dietary FUMs are summarized in Table 17. Feeding a control diet (G1), 15 mg/kg (G2) or 30 mg/kg (G3) FUMs contaminated diets altered some serum biochemical compounds. The blood TP concentration was significantly increased ($P = 0.01$) in G3 as opposed to G1 and G2. The AST, GGT and ALKP activities were all significantly elevated ($P < 0.05$) in G3 compared to G1; G2 was indifferent regarding GGT and ALKP levels. The CK was significantly elevated ($P = 0.02$) to about 3 folds in G3 compared to G1 and G2. There was also a notable rise ($P = 0.003$) in LDH activity in G3 compared to G1 or G2. Feeding pigs with a 30 mg/kg FUMs contaminated diet significantly elevated ($P < 0.05$) creatinine and cholesterol concentrations as well as Ca and

Mg levels compared to G1 or G2. When compared to the other two groups, G3 had a significantly declined GFR (P = 0.03). However, there was no dietary FUMs influence (P > 0.05) on the serum levels of ALB, ALT, urea, Fe, Na and Cl in all groups.

Table 17. Effect of varied levels of dietary fumonisins on blood serum biochemical parameters of nursery pigs (data are means ± standard deviation (SD) of 6 individuals/group)

Blood serum parameters	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)
Total protein, g/L	56.2 ± 2.7 **a**	56.8 ± 2.2 **a**	60.9 ± 2.8 **b**
Albumin, g/L	35.6 ± 3.6	34.4 ± 1.8	35.7 ± 3.8
AST, U/L	57.5 ± 18.2 **a**	87.8 ± 22.9 **a**	336.5 ± 269.6 **b**
ALT, U/L	65.7 ± 16.7	86.3 ± 22.89	100.8 ± 55.3
GGT, U/L	37 ± 7.0 **a**	53.2 ± 18.5 **ab**	122.8 ± 102.2 **b**
ALKP, U/L	270.7 ± 40.6 **a**	406 ± 212.3 **ab**	1356.5 ± 1384.6 **b**
CK, U/L	1382.5 ± 686.5 **a**	1351.5 ± 852.2 **a**	4449.8 ± 3315.5 **b**
LDH, U/L	1192 ± 165.7 **a**	1323.8 ± 212.7 **a**	2272.3 ± 693.7 **b**
GFR	90 ± 0.0 **a**	88.7 ± 3.3 **a**	76.4 ± 12.6 **b**
Urea, µmol/L	3.3 ± 0.6	4.3 ± 0.9	3.9 ± 0.1
Cholesterol, µmol/L	2.2 ± 0.1 **a**	3 ± 0.5 **a**	4.5 ± 0.9 **b**
Creatinine, µmol/L	88 ± 8.17 **a**	88 ± 8.17 **a**	102.3 ± 4.5 **b**
Ca, µmol/L	2.7 ± 0.13 **a**	2.7 ± 0.18 **ab**	2.9 ± 0.11 **b**
Mg, µmol/L	1.0 ± 0.12 **a**	1.0 ± 0.10 **a**	1.2 ± 0.12 **b**
Fe, µmol/L	26.1 ± 4.8	21.7 ± 3.4	24.1 ± 6.7
Na, µmol/L	147.3 ± 4.1	147 ± 2.6	143.5 ± 3.0
Cl, µmol/L	101.5 ± 5.7	116.8 ± 41.3	97.3 ± 2.9

ALKP= alkaline phosphatase, ALT= alanine transaminase, AST= aspartate transaminase, CK= creatine kinase, GGT= gamma-glutamyl transferase, GFR= glomerular filtration rate, LDH= lactate dehydrogenase. Different lowercase letter indexes, a or b, without common letter in the same row differ significantly at P < 0.05

Meanwhile, none of the antioxidant parameters, GSH and GSHPx and the end product of lipid peroxidation, MDA, determined in the lung, liver, kidney, or plasma exhibited a significant alteration in their activity levels or concentration (P > 0.05) of all animals (Figure 13, Labels a-d).

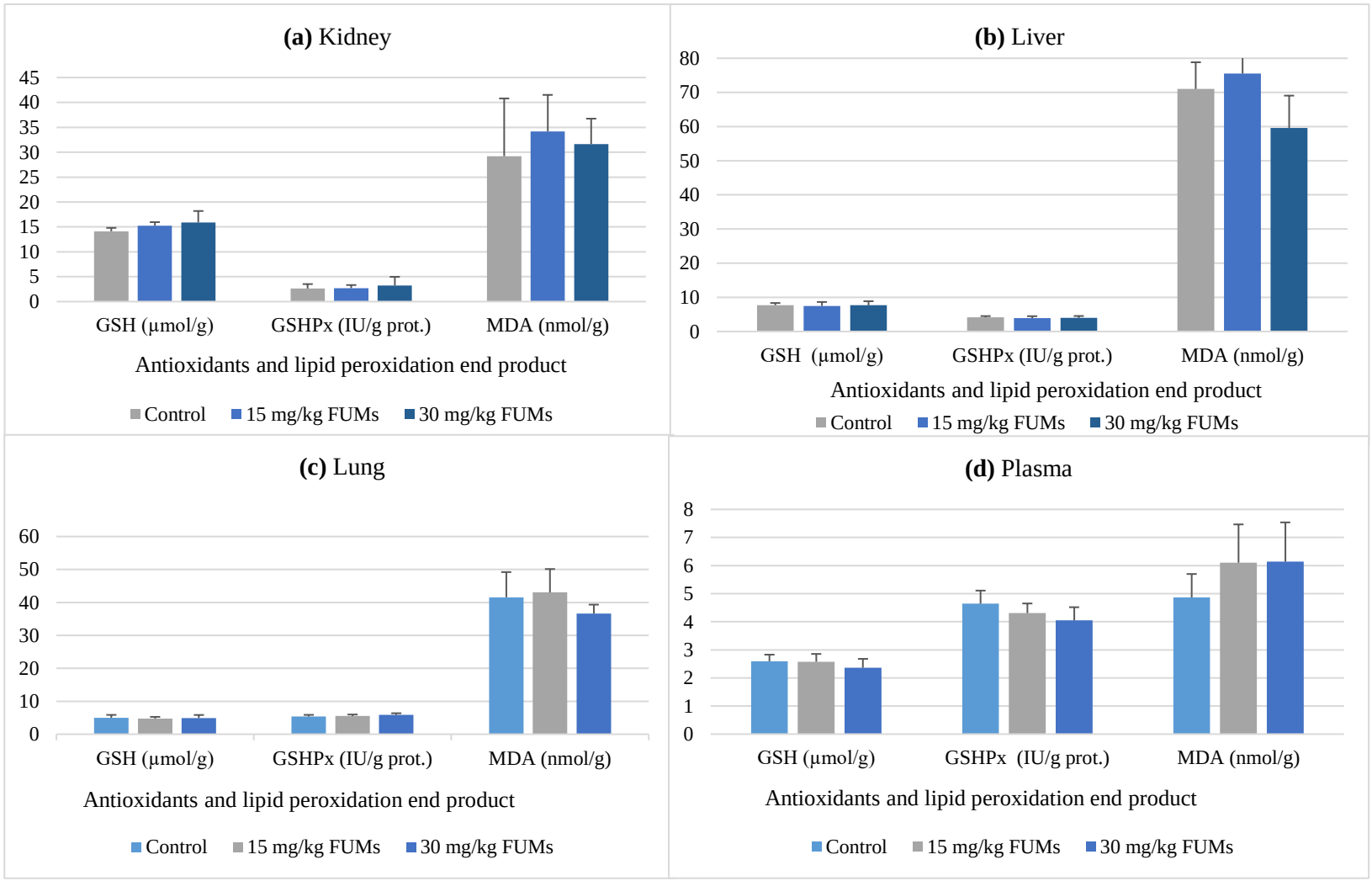


Figure 13. Effects of varied levels of dietary fumonins on glutathione (GSH) glutathione peroxidase (GSHPx) and the end product of lipid peroxidation malondialdehyde (MDA) in the **(a)** kidney **(b)** liver **(c)** lung and **(d)** blood plasma of nursery pigs. No significant difference were observed for all

Hsp70 Activity

The expression of Hsp70 in the kidney and liver is presented in Figure 14 (Labels a and b). The Hsp70 expression in the kidney (Label a) was not affected significantly by dietary treatments ($P > 0.05$). However, the liver Hsp70 expression (Label b) was significantly high ($P < 0.05$) in G3 relative to G1 or G2.

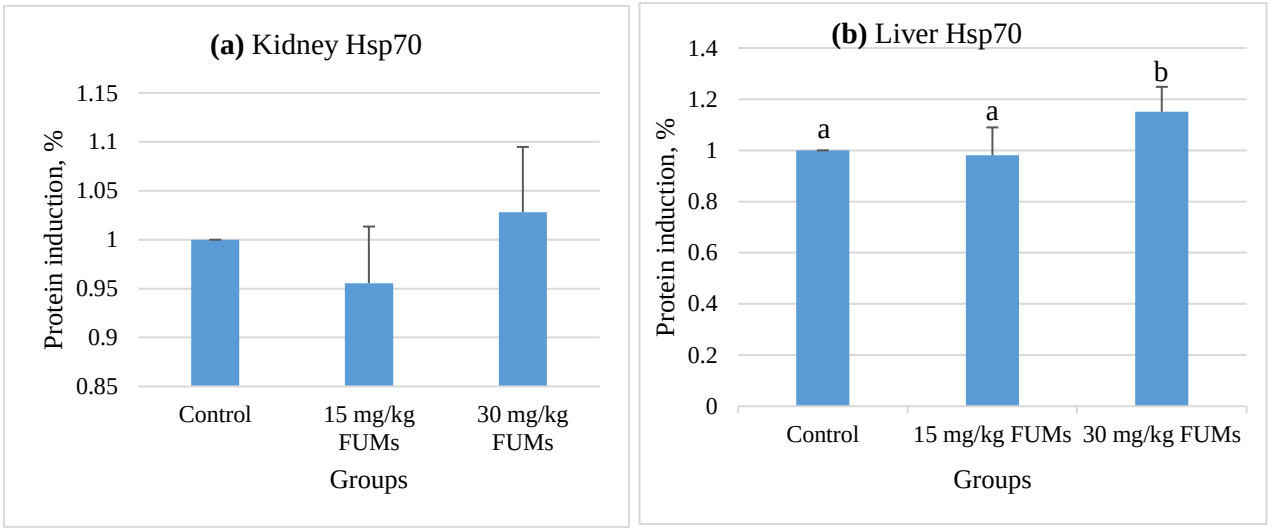
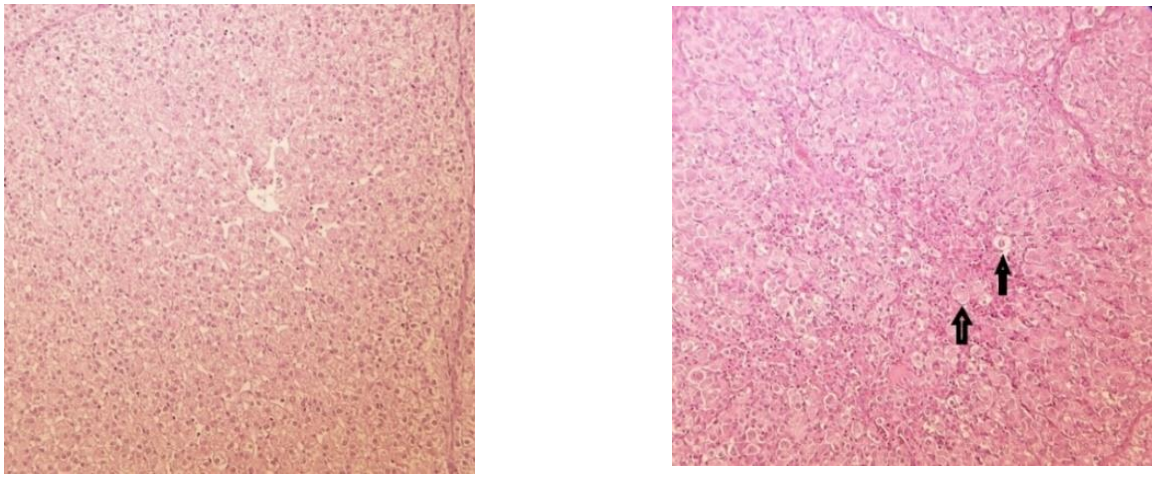


Figure 14. Effect of varied levels of dietary fumonisins on Hsp70 expression in the **(a)** kidney and **(b)** liver of nursery pigs. Different lowercase letter indexes, a or b, on each bar differ significantly at $P < 0.05$

4.1.7. Histopathological examinations

The total number of animals exhibiting a patholoical symptom according to the scoring as described earlier under the methodology of histology and histopathology are summarized and presented in Table 18. None of the control animals showed any sign of histological alterations examined in the liver Figure 15 (Panel a). Liver cells of animals exposed to FUMs exhibited decreased glycogen content. As can be observed in Figure 15 (Panel b), there was decreased microscopic vacuolization which is associated with high glycogen content in healthy control animals in sections stained with H.E. stain; scattered solitary liver cell deaths swelling and scattered focal proliferation of cells belonging to the mononuclear phagocyte system (MPS). Further, all animals receiving either 15 or 30 mg/kg FUMs diet exhibited some form of damage in the liver.



(a) **(b)**

Figure 15. Effect of varied levels of dietary fumonisins on the histology of liver. **(a)** healthy (control) pig liver. The cytoplasm of liver cells is vacuolated due to high glycogen content (faint staining) and **(b)** liver of a 30 mg/kg FUMs exposed pig after 21 days. Decreased glycogen content of the cytoplasm and more dead (detached, rounded, faintly stained) hepatocytes (↑) can be seen

In the kidneys, tubular epithelial detachment and, in this context, clusters of collapsed epithelial cells detached in the cavity of the drainage ducts of the marrow, as well as scattered inter-tubular foci of lymphohistiocytic infiltrations were observed in all animals fed the 30 mg/kg FUMs diet (G3) and not in either G1 or G2 groups. However, lymphohistiocytic infiltrates were present in one animal of each of the aforementioned groups (G1 or G2). Additionally, in both G2 and G3 groups, mild focal lymphohistiocytic infiltrates were seen interstitially, and mild focal fibrotic thickening of the pleural covering the lungs was observed. No signs of severe pulmonary oedema were observed, however. Further, mild diffuse lymphocytic and histiocytic infiltration (a physiological phenomenon associated with normal GALT) was observed in the small intestinal mucosa in all groups (G1, G2 and G3). In G3, a decrease in the number of lymphocytes (lymphocyte depletion) and a slight decrease in the diameter of the follicles could also be detected.

Table 18. Histopathological alterations observed in nursery pigs fed with varied levels of dietary fumonisins (data are means ± standard deviation (SD) of 6 individuals/group) for 21 days

Organ	Pathological examination	Control (G1)	15 mg/kg FUMs (G2)	30 mg/kg FUMs (G3)	
		number of observations			∑
Liver	- decrease in glycogen content of liver cells	0	6	6	12
	- Single liver cell death	0	6	6	12
	- Swelling of MPS cells	0	6	6	12
	- Proliferation of MPS cells	0	6	6	12
Kidney	- tubular epithelium detachment	0	0	6	6
	- lymphocytic infiltration	1	1	6	8
Lung	- interstitial lymphocytic infiltration	2	3	4	9
	- pleural fibrosis	0	2	3	5
Ileum	- GALT lymphocyte depletion	0	0	3	3

GALT= gut-associated lymphoid tissue, MPS= mononuclear phagocyte system. ∑= the total number of animals exhibiting the given alteration irrespective of dietary FUMs treatment

4.2. Cannulated-fattening pigs' study

In this study cannulated pigs were used to determine the AID of AAs in case of a short (7 days) and long- term (21 days) FUMs exposure. Feeding a 40 mg FUMs/ kg diet did not hamper the growth rate of the intoxicated group ($P > 0.05$) compared to the control animals (Table 19).

Table 19. Effect of dietary fumonisins on the body weight of fattening pigs in the trial

Item, kg	Control	40 mg/kg FUMs
Initial BW	67.3 ± 5.0	67.8 ± 3.7
Final BW	81.2 ± 5.7	83.1 ± 5.7

BW= body weight. No significant differences were observed

The main effect of duration (d), and the interaction of treatment (t) and duration (d) (t x d) tended to impact the AID of histidine ($P = 0.001$ and 0.047, respectively). On the other hand, only t x d effect was observed for arginine and tyrosine (($P = 0.003$ and 0.047, respectively) (Table 20).

Table 20. Effect of long or short-term exposure of dietary fumonisins on apparent ileal digestibility (AID) of dry matter, crude protein, and amino acids (AAs) in fattening pigs (data are means ± standard deviation (SD) of 5 individuals/group)

Duration, d	Short-term effect, 7 days		Long-term effect, 21 days		*P* -values		
Treatments, t	Control	40 mg/kg FUMs	Control	40 mg/kg FUMs	Treatment (t) effect	Duration (d) effect	Interaction (t x d)
AID of AAs, g/g							
Dry matter	0.7404 ± 0.013	0.7467 ± 0.011	0.7641 ± 0.027	0.7432 ± 0.007	0.29	0.16	0.06
Crude protein	0.7737 ± 0.011	0.7927 ± 0.017	0.7876 ± 0.007	0.7778 ± 0.022	0.58	0.95	0.099
Amino acids							
Arginine	0.8524 ± 0.007	0.8659 ± 0.005	0.8642 ± 0.009	0.8500 ± 0.007	0.93	0.58	**0.003**
Threonine	0.7032 ± 0.024	0.7094 ± 0.024	0.7082 ± 0.026	0.6866 ± 0.011	0.494	0.43	0.23
Valine	0.7668 ± 0.020	0.7734 ± 0.019	0.7614 ± 0.015	0.7553 ± 0.017	0.98	0.23	0.50
Phenylalanine	0.8031 ± 0.018	0.8149 ± 0.016	0.8128 ± 0.007	0.8048 ± 0.014	0.803	0.98	0.22
Methionine	0.8743 ± 0.020	0.8867 ± 0.013	0.8780 ± 0.005	0.8708 ± 0.012	0.71	0.39	0.18
Lysine	0.8394 ± 0.009	0.8426 ± 0.011	0.8593 ± 0.15	0.8508 ± 0.013	0.70	0.06	0.41
Histidine	0.7886 ± 0.020	0.8084 ± 0.015	0.8458 ± 0.008	0.8325 ± 0.009	0.67	**< 0.001**	**0.047**
Isoleucine	0.7934 ± 0.020	0.8062 ± 0.021	0.7973 ± 0.009	0.7945 ± 0.019	0.60	0.68	0.42
Leucine	0.8307 ± 0.024	0.8431 ± 0.019	0.8416 ± 0.008	0.8341 ± 0.018	0.80	0.92	0.31
Tyrosine	0.6958 ± 0.030	0.7130 ± 0.013	0.7260 ± 0.004	0.7075 ± 0.010	0.94	0.15	**0.047**
Alanine	0.7661 ± 0.016	0.7725 ± 0.015	0.7471 ± 0.018	0.7506 ± 0.25	0.63	0.07	0.89
Glutamic acid	0.8421 ± 0.013	0.8546 ± 0.006	0.8496 ± 0.008	0.8393 ± 0.015	0.86	0.53	0.08
Glycine	0.5863 ± 0.034	0.6169 ± 0.057	0.6308 ± 0.008	0.6070 ± 0.051	0.88	0.46	0.25
Cysteine	0.7184 ± 0.021	0.7368 ± 0.027	0.7559 ± 0.011	0.7342 ± 0.019	0.88	0.12	0.08
Aspartic acid	0.7708 ± 0.015	0.7771 ± 0.010	0.7782 ± 0.011	0.7686 ± 0.010	0.79	0.93	0.28
Proline	0.6874 ± 0.069	0.6811 ± 0.116	0.5993 ± 0.103	0.6891 ± 0.040	0.400	0.418	0.34
Serine	0.7724 ± 0.016	0.7802 ± 0.016	0.7906 ± 0.010	0.7760 ± 0.016	0.671	0.39	0.18

5. DISCUSSION

5.1. Effect of dietary fumonisins on growth performance of nursery and fattening pigs

In the present study, nursery pigs exposed to a 15 or 30 mg/kg FUMs nor cannulated fattening pigs exposed to a 40 mg/kg FUMs diet exhibited any growth stunt or morbidity following 21 days of feeding. The effects of FUMs on the growth performance of several animal species have been characterized as inconsistent in the literature, with BW loss or gain reduction not always occurring. In a report by the French Agency for Food Safety (AFSSA, 2009), FUMs intoxication below 25 mg/kg causes no apparent clinical changes – which partially agrees with our nursery pigs results – but at an exposure level of above 50 ppm, pigs exhibit poor growth performance and liver damage. In addition, acute exposure to FUMs contaminated diet (above 100 mg/kg of feed for 3 – 5 days) resulted in acute, but deadly PPE.

According to research, FUMs toxicity in pigs mainly affects animal performance and growth at dosages above 100 mg FB_1/ kg, with effects lasting four to eight weeks. In contrast, lower doses either have no effect at all or have minor effects (Knutsen *et al.,* 2018) which is consistent with our findings. Furthermore, in a study that was conducted very recently, piglets were fed diets containing concentrations ranging from low to high, i.e., 3.7, 8.1, or 12.2 mg FB_1/ kg diet, for a period of 28 days. The authors found no performance changes in the piglets (Terciolo *et al.,* 2019). Further, Tóth *et al.* (2000) observed severe PPE in pigs and yet, no growth depression or reduced feed intake when pigs were exposed to a dose of 40 ppm dietary FB_1. Although the dosage used in Tóth's work is the same dosage used in our fattening pigs' study, we did not investigate internal organ damage in the fattening pigs. Noteworthy, in the nursery pigs, we observed a mild form of PPE that was exhibited in some individual cases especially in the 30 mg/kg FUMs diet fed group (photo-documented).

5.2. Dietary fumonisins impair nutrient digestibility and mineral balance without hampering digestive enzyme activity or intestinal architecture

Despite no growth or health impairments observed in the nursery pigs, it is apparent that at either a dietary dose of 15 or 30 mg/kg FUMs fed diet, the nutritive value of the feed can be reduced as well as a remarkable reduction in ATTD of energy and some trace minerals. Indeed, a lower apparent digestibility is attributed to a reduction of nutrients hydrolysis and absorption, or increment in endogenous secretions, or both (Wilfart *et al.,* 2007). The decline in ATTD values

observed for CF, ash, Ca, P, and ash, as the dosage increased, suggests a potentially distorted absorptive function along the gut of the pigs. Mycotoxins have been reported to be capable of including non-specific GIT hypo-functionality in animals (Gbore *et al.,* 2010). The decline in CF digestibility signifies a possible shift in the composition of gut microbiota since dietary fibre is a key player in the state of existence of intestinal microbes. Even though the digestibility of EE did not differ statistically, the observed numeric decline in its digestibility values in both treatment groups suggests a possible interference in the lipid metabolism of the pigs. In any case, more investigation into this hypothesis is needed. Elsewhere, Gbore and Egbunike (2007) reported a reduction in CF and CP digestibility in a dose-response pattern when an increasing dosage of dietary FB_1 (0.2, 5.0, 10, and 15 mg/kg diet) was provided to growing pigs in a 6- month feeding trial.

Furthermore, the ATTD and retention rates of the trace minerals Cu and Zn were notably affected by treatments. This is the first study, as far as we are aware, to discuss the potential toxicity of FUMs on intestinal mineral metabolism. Digestibility of minerals reflects the dissolution and absorption of minerals from the gut lumen (Espinosa and Stein, 2021), however, during the digestion, minerals are transported not only from but to the gut lumen as well. As a result, the faecal collection method has certain drawbacks and only provides a crude estimate of mineral metabolism. It is still a frequent approach for determining mineral bioavailability when used in combination with urine collection (Patterson *et al.,* 2008). If we consider the significant reduction in apparent faecal ash digestibility i.e., 59%, 53% and 54% for the control, 15 and 30 mg/kg FUMs fed groups, respectively, we may propose an absorption defect of minerals or a higher outflow of minerals to the gut lumen. Indeed, previous works have demonstrated that an increase in faecal ash digestibility was a result of increased absorption of minerals in pigs (Jongbloed *et al.,* 2000; Kies *et al.,* 2005). Thus, we suspected the opposite out-turn of poor digestibility. Interestingly, this was reflected more in the trace minerals Cu and Zn. The fact that none of the macroelements' balance was affected by dietary FUMs suggests that Na, K, and Mg were perhaps, efficiently utilized by the nursery pigs regardless of the presence of dietary FUMs.

As this is the first report on whether FUMs toxicity affects mineral metabolism, to facilitate a better interpretation and discussion of our results, we used values obtained for the control group, henceforth termed "reference value/s", and compared them with values reported in the literature. Kornegay and Harper (1997) in a review, highlighted the range of digestibility coefficients and

retention rates of N, Ca, P, Mg, Na, K, Zn, and Cu at the various growth stages of swine. Comparatively, the reference values for the ATTD in the affected trace minerals (Cu and Zn) in the present study somewhat agree with the rates reported for young pigs in the review by Kornegay and Harper (1997). Reference values of 20.3% and 20.6% ATTD rates of Cu and Zn respectively reported presently, fall within a normal range of 8 to 25 % for Cu and 20 to 45 % for Zn (Kornegay and Harper, 1997). Yang (2019) recently reported an apparent digestibility of 25% for Cu and 31% for Zn in weaned piglets with a slightly higher initial BW (initial BW of 14.5 kg) than in the present study (initial BW of 13.5 kg). Thus, in comparison to the control, we believe that the significantly lower (Cu; 5.8% and 11.9% for 15 and 30 mg/kg fed groups, respectively) and negative (Zn; about -14% and -15% for 15 and 30 mg/kg fed groups, respectively) digestibility values indicate a compromised absorption and particularly for Zn, an accelerated endogenous loss as a result of FUMs toxicity.

Additionally, we compared the present retention values expressed as a percentage of the intake of the control pigs (reference values) to what has already been reported in the literature. A range of 5 to 40 % was reported by Apgar and Kornegay (1996) for relative Cu retention. Albeit all the presently reported values to fall within this range i.e., 19, 5 and 11 per cent for the control, 15 or 30 mg/kg- FUMs fed groups, respectively, it is clear that the control group had much better retention relative to treated groups. In addition, a range of 5 to 40 % was reported for Zn (Houdijk *et al.,* 1999; Rincker *et al.,* 2005) – while in the present report, a rate of 19% was calculated for the reference value (obtained in the control group); meanwhile, -16% and -17% were reported for the 15 and 30 mg/kg FUMs groups, respectively. In the literature, trace minerals have been described as having a harmonious interplay in various biological components as well as in the feed (Davis, 1980) and are controlled by the interaction between luminal absorption from, and endogenous secretion into the intestines (Windisch, 2002). Thus, the lower values obtained for ATTD and retention rates of Cu and Zn in FUMs exposed pigs may be an indication that excretion of considerable amounts of these minerals of endogenous origin was induced. While we might suggest that a higher maintenance requirement for these trace minerals should be prioritized when feed is contaminated by FUMs, this would not apply to the present findings because no deficiency symptoms as a result of a supposed limit in the bioavailability of the trace minerals, were exhibited by treated pigs.

In addition to the above, the compromised digestibility rates reported earlier for CF could be a potential contributor to the significant Zn and Cu deficit in the FUMs intoxicated animals. Crude fiber (CF) acts as a substrate for hindgut microbial fermentation to produce volatile fatty acids (VFAs) which are utilized by pigs as an energy source, and also facilitate and increase mineral solubilization in the lumen (Samal and Behura, 2015). Thus, the compromised ATTD of this nutrient in the presence of FUMs reflects poor fermentation that could limit the amount of VFAs and may interfere with the bioavailability of minerals. It has been reported that the reabsorption of trace minerals in the large intestine can be improved by supporting higher microbial activity and its VFAs production, since the lower pH limits the formation of the phytate complex in the hindgut (Brommage *et al.,* 1993). Phytase has a pH optimum at pH 4.5 and/or 5.5 depending on its origin (Dersjant-li *et al.,* 2015), thus, the higher VFAs production makes a better environment for the phytase activity. In the case of higher pH in the hindgut, the possibility of phytic acid complex formation is higher and limits the absorption of the minerals (Lopez *et al.,* 2002). Although mineral absorption in the hindgut is limited, it substantially aids an effective completion of the active transport mechanism in the small intestine. The present research only examined the main VFAs/ short-chain fatty acids (SCFAs) and not all VFAs, and these were not notably impacted by FUMs presence (discussed later). We are convinced that further investigation is needed to elucidate the mechanism behind the present findings.

Trace minerals' involvement in several physiological pathways makes their metabolism complex and challenging to understand (Suttle, 2010). Given the present findings, perhaps, the toxin may have supported antagonistic interactions and resulted in the lower digestibility and depressed retention rates of Zn and Cu. Generally, trace minerals are well known to have antagonistic interactions within themselves (Richards *et al.,* 2010), and antagonistic interaction between Zn and Cu is particularly profound in pig feeding (Jondreville *et al.,* 2003). Intestinal metallothioneins mediate this Zn/Cu antagonistic interaction (O'Dell, 1989). Zinc (Zn) stimulates the induction of these metalloproteins, which strongly bind Cu in mucosal cells. When Zn levels are too high, metallothionein builds up and obstructs Cu absorption in the intestinal absorption site (O'Dell, 1989). In our case, however, there was not an intended over-supply of Zn and therefore, we cannot conclude on this basis. Thus, we suspect that FUMs may have induced a much more complex mechanism that interfered with the absorptive capacity of the intestine such as the efflux of these minerals, their basic transport mechanisms and basic homeostatic regulations that influence their uptake in the intestinal lumen. We could have monitored these processes as well, but it was not

the intention of the present research. On that note, this provides an interesting field of research on the metabolism of trace elements in the presence of FUMs. Perhaps, a fine design with the inclusion of other parameters like tissues and organs mineral concentrations, an extension of the experiment period, toxin dosage, etc., to better understand trace minerals metabolism in the gut of pigs when FUMs is present in their feed.

Nitrogen (N) balance studies are carried out to assess the biological value of various feedstuffs and diets, to ascertain the protein requirements for various products, but it is also reliable method to ascertain the amount of ME in various feedstuffs and diets (Just *et al.*, 1982). Regarding the energy utilization, the N balance assessment and calculated ME in the present study indicate that FUMs did not have major interference in the dietary protein and energy utilization of the piglets and supporting this was the unaffected N digestibility as well. Wang *et al*. (2018) used approximately the same FUMs dose (30 mg/kg diet) in their trial; the proportion of maize naturally contaminated with FB_1 containing 73 ppm FB_1 and 0.07 ppm AFB_1 was 40% in nursery pigs' feed. The authors reported a slight adverse effect of the FUMs on ATTD of energy (agrees with the present results) and protein. Contrary to Jang *et al.* (2018), the authors demonstrated that nutrient digestibility was not affected by naturally contaminated maize (containing DON, FUMs, and ZEN). In line with our results, in a broiler chicken study, 20 mg/kg FUMs contamination did not shift either the AID of protein, the endogenous N loss or the energy digestibility (Jia *et al.*, 2020).

In addition, no architectural disorganization of villi along the small intestine and unperturbed enzymes' activity contradicts other works such as that of Ewuola *et al.* (2003), Piva *et al.* (2005) and Lessard *et al.* (2009). Piva *et al.* (2005) observed villous fusion and atrophy following 42 day dietary exposure of weanling pigs to 30 ppm FB_1. In the report of Lessard *et al.* (2009), the authors found that FB_1 induced intestinal lesions through disruption of villus architecture, enzyme activities and intestinal physiology when weanling pigs were administered an FB_1 rich extract corresponding to 1.5 mg FB_1/ kg BW for 9 days daily. Earlier work by Ewuola *et al.* (2003) had revealed progressive erosion of the epithelial lining of the small intestine resulting from chronic exposure to 1.69 − 1.90 mg FUMs/ kg feed in rabbits. Also, increasing wear-off of intestinal mucosa was induced by increasing levels of dietary FB_1 along with the GIT of pigs in a chronic study (Gbore, 2007). The unusual treatment-unaffected outcome of the present study on the intestinal architecture and enzyme activity remains to be elucidated.

5.3. Dietary fumonisins modulate some health markers

This aspect of the nursery pigs' trial aimed to ascertain how dietary FUMs modulate health indicators following 21 day- long exposure. Indeed, the presence of FUMs triggered some physiological disturbances in the nursery pigs.

Following FUMs intoxication, the liver and kidney majorly end up with most of the toxic load. These toxic insults are manifested through up-regulation of serum biochemical parameters (Haschek *et al.,* 2001) and histopathological findings. The effects of FUMs on modifying blood biochemical parameters and suggesting internal organ damage are normally in a dose-response manner (Colvin *et al.,* 1993; Riley *et al.,* 1993; Schertz *et al.,* 2018). In the present results, 30 mg FUMs/ kg feed resulted in a significant elevation of the most sensitive enzyme to liver injury, AST (about 5 folds increase as compared to the control group) – an increment that exceeds the normal physiological range of 31-58 U/L (Kaneko *et al.,* 2008). The histopathology revealed notable damage in the liver (reduction in glycogen content and indicative of hepatocytes death). In addition, the significant increase in serum cholesterol levels corroborates the revelation of liver damage reported previously (Dilkin *et al.,* 2010; Fodor *et al.,* 2006b). In addition to a significant elevation in blood cholesterol in the work of Schertz *et al*. (2018) the authors reported no FUMs related impact on serum ALKP and AST levels after acute oral exposure of barrows to 3425 nmol FB_1/ kg BW and thus, partially agrees with our findings. The present work revealed a rise in the total serum protein levels while (Gbore and Egbunike, 2007) reported that boars fed diets containing ≥ 10.0 mg FB_1 /kg had significantly lower serum total protein and albumin which was attributed to altered protein metabolism. In our work, increase of ALB was nearly significant. This is a sign of intact hepatic protein synthesis. Hepatotoxicity was thus, proven by AST, but by far not by the proteins.

In the kidney, alterations were pronounced and frequent in animals receiving the 30 mg/kg FUMs diet. These alterations were indicative of progression of renal toxicity and confirmed by the significant decline in serum GFR, elevation in CK, and creatinine levels in the 30 mg/kg FUMs fed pigs. These alterations in the kidney may have shifted the levels of the electrolytes (Mg and Ca) as well.

Increased generation of reactive oxygen species (ROS) and lipid peroxidation following FB_1 intoxication implies that oxidative stress may be involved in FB_1- induced toxicity (Klarić *et al.,* 2007; Stockmann-Juvala *et al.,* 2004). Glutathione (GSH) is the most essential low-molecular-

weight antioxidant among the several antioxidant parameters operating in celsls. In the present study, none of the antioxidant parameters – (GSH, GSHPx) or lipidperoxidation indicating (MDA) parameters examined appeared to be affected by the presence of dietary FUMs. However, the ability of FB_1 to moderately mediate oxidative damage (Golli-bennour and Bacha, 2011) was attested through the elevated expression of Hsp70 expression of the liver of pigs fed the 30 mg/kg FUMs diet. Redox-sensitive signalling molecules such as mitogen-activated protein kinases (MAPKs) and heat shock proteins (Hsps) are activated when ROS are produced or the cellular redox status is perturbed (Rumora *et al.,* 2007). The 70 kDa Hsp or Hsp70, is the most prevalent sub-group of the Hsp families in mammalian cells, and the one most closely connected to cytoprotection against a range of noxious substances (Walter *et al.,* 1994). In partial agreement with our findings, FB_1 increased cellular GSH content and Hsps expression in rat liver while the exact opposite was observed in the kidney (Rumora *et al.,* 2007). Further, in slight agreement with the present outcome, Kócsó *et al.* (2018) reported an elevated expression of Hsp70 activity in the kidney and lung, but not the antioxidant parameters (GSH, GSHPx) or MDA of rats exposed to 50 mg/kg dietary FB_1 for 5 days. Given these observations, perhaps, the stimulus triggering the increased synthesis of Hsps was some factor not affecting the mitochondrial respiratory chain.

5.4. Dietary fumonisins alter the distribution of the intestinal and faecal microbiota without affecting the fermentation by-products

The efficient functioning of the gut microbiome is governed by the interplay of intrinsic and extrinsic factors (physiological state of the animal, feed and nutrients availability and their endogenous secretion into the gut lumen, immune status, housing, environmental conditions, etc.) (Che *et al.,* 2019; Gebhardt *et al.,* 2020; Kubasova *et al.,* 2018; Quan *et al.,* 2019). In addition, the concentration and balance of SCFAs indicate that the microbiota is doing well. Many various host, environmental, nutritional, and microbiological variables influence gut bacteria's ability to produce SCFAs. And the quantity and kinds of SCFAs generated in healthy individuals are mostly determined by substrate availability, bacterial species makeup of the microbiota, and intestinal transit time (Macfarlane and Macfarlane, 2003). Relevance to the present findings however, the absence of effect of treatments on the concentrations of the SCFAs (formic acid, acetic acid, propionic acid, and valeric acid) studied might be attributed to the fact that there was no feed rejection and hence no reduction in nutrients availability.

Consistent with the literature, the present study found that *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were the most common phyla found in all the pigs' intestinal tracts. Earlier works with pigs reported similar findings (Kim *et al.*, 2011; Looft *et al.*, 2012; Ramayo-Caldas *et al.*, 2016); a similar trend in humans (Qin *et al.*, 2010) and in broilers (Yu *et al.*, 2022). The reduction in the abundance of *Alloprevotella* (Fig. 11a) belonging to the Prevotellaceae family and order Bacteroidales is consistent with the findings of (Zhang *et al.* (2021). In an 8- week- long study involving BALB/c mice intragastrically exposed to 0.162, 0.486, 1.458, and 4.374 mg/kg FB_1 BW, the authors reported a marked reduction in *Alloprevotella* (Zhang *et al.*, 2021). Due to its capacity to create succinate and acetate, two compounds with anti-inflammatory characteristics and roles in strengthening the intestinal barrier (Downes *et al.*, 2013), this gram-negative bacterium is characterized as beneficial (Ren *et al.*, 2016). In addition, in a G1 vs. G3 comparison, there was a significant downward tendency for the Lachnospiraceae– *Incertae sedis* group and for the order Clostridiales in the duodenum and in the ileum, respectively. Butyric acid is produced by these bacteria according to reports (Oliphant and Allen-Vercoe, 2019; Vacca *et al.*, 2020), and its profound benefits include reducing intestinal inflammation and fortifying the intestine's response to dietary changes (Zhong *et al.*, 2019). As a result, a reduction in the number of these essential SCFAs producing bacteria, as seen in the current findings, points to a possible compromise of the pigs' intestinal integrity. Given the outcome of the present study, it is conceivable that the presence of FUMs in pigs' diet resulted in a negative impact on the proliferation of essential intestinal bacteria involved in gut barrier fortifying responses. Additionally, these observed alterations in the abundances of several crucial bacteria found in the intestines reinforce the notion that the gut is extremely vulnerable to damage posed by dietary FUMs.

The faecal bacterial flora after 21 days showed a dominance of the families Ruminococcaceae (14.1%) and Lachnospiraceae (17.8%). Further, the differentially abundant genera related to these two prominent families were *Solobacterium, Faecalibacterium, Anaerofilum, Ruminococcus, Subdoligranulum, Pseudobutyrivibrio, Coprococcus* and *Roseburia*. It has been reported that the gut microbiota of healthy piglets exhibited a larger number of Prevotellaceae, Lachnospiraceae, Ruminococcaceae, and Lactobacillaceae in comparison to diarrhoeic piglets (Dou *et al.*, 2017). A similar pattern was observed when pigs were fed a diet containing 12 mg/kg FB_1 for 0, 8, 15, 22 and 29 days. The abundance of the families Lactobacillaceae, Lachnospiraceae, Ruminococcaceae, and Prevotellaceae were all substantial, but Lachnospiraceae experienced a considerable reduction through time (Mateos *et al.*, 2018). In the same study, a significant increase of OTUs assigned to

Prevotella, *Treponema* and *Lactabacillus* and a significant decrease in the relative abundances of OTUs belonging to *Faecalibacterium*, *Prevotella*, *Mitsuokella*, *Roseburia*, *Ruminococcus*, and *Succinivibrio* was also reported. Intriguingly, several of these latter genera also had decreased relative abundances ($P < 0.05$) in a G1 vs. G2 comparison in our work, using a similar FUMs dose in G2 (i.e., 15 mg/kg feed) to that of Mateos *et al.* (12 mg/kg FB_1), with the exception of *Prevotella*, where this decrease was also significant ($P = 0.037$). Elsewhere, utilizing the SSCP faecal microbiota profiles, Burel *et al.* (2013) reported a transient imbalance of the microbiota of pigs chronically fed with an 11.8 ppm FB_1 diet during the first 4- weeks of exposure and then fluctuating until finally becoming similar again at the end of their trial. In addition, when the digestive tract was colonized further with Salmonella, the outcome was more devastating (Burel *et al.,* 2013). This is an indication that a slight or minor change in the microbiota paves way for the possible invasion of opportunistic pathogenic bacteria.

The genus *Lactobacillus* is the largest genus in lactic acid bacteria (LAB), and also the most predominant genus in the small intestine (De Angelis *et al.,* 2016). The increase in *Lactobacillus sp*. that was seen in the present study in a dose-response pattern, though statistically not significant, may have been brought on by this bacteria's quick response to counteract any potential negative effects of the presence of FUMs in the small intestine. The high relative abundance of Lactabacillaceae in the various sections of the small intestine (i.e., duodenum, jejunum and ileum) has been reported elsewhere (Gresse *et al.,* 2019). Similar to the current investigation, Moon *et al.* (2020) subjected pigs to DON (0.8 mg/kg) for 30 days and found a considerably higher number of Lactobacillaceae in the small intestine as opposed to the feces. In fact, it seems that *Lactobacillus* thrives most abundantly in the small intestine in response to FUMs or DON exposure. Additionally, we discovered a striking decrease in the *Campylobacter* genus (Fig. 11b), a member of the family Campylobacteraceae, which has been linked to post-weaning diarrhea in piglets (Li *et al.,* 2018). Perhaps a covert factor in suppressing the growth of *Campylobacter* notably in the duodenum of the treated pigs was the action of Lactobacillaceae. Even though this is just a hypothesis, it all serves to demonstrate how the microbiota composition can be altered while attempting to balance any possible adverse effects following exposure to FUMs. They therefore hold a great deal of significance for their hosts.

Increased shifts in *Lactobacillus sp.* following mycotoxins including FUMs exposure have been emphasized in other investigations as well. In their study, Mateos *et al.* (2018) reported a

remarkable increase of *Lactobacillus* which was also the case in an *in vitro* study to investigate the interaction between FB_1 and caecal microorganisms in pigs (Dang *et al.,* 2017). In the work of Dang *et al.* (2017), *Lactobacillus* and total bacteria increased, while anaerobic bacteria showed a considerable reduction. Elsewhere, after exposing both nursery and growing pigs to multi-toxins including FUMs, there was an increase in the Lactobacillacae family in the intestinal microbiome (Kim *et al.,* 2019). These outcomes may confirm the establishment that *Lactobacillus sp*. can reduce mycotoxin toxic activities by the extra-cellular binding to mycotoxins (De Angelis *et al.,* 2006; Zou *et al.,* 2012). The underlying mechanism was explained as a process of physical adsorption involving various components of the cell wall (Zhao *et al.,* 2016). The current discovery thus opens the door to future investigation into the many *Lactobacillus* strains that may be isolated and possibly employed as probiotics to enhance gut health in case of FUMs toxicosis.

Because of their capacity to complete tasks that individual strains or species cannot, microbial consortia have attracted increasing attention for their use in toxin biodegradation (Festa *et al.,* 2013). The current investigation identified three bacterial genera in the pig's gut such as *Pseudomonas* (of the family Pseudomonadaceae and order Pseudomonales), *Sphingomonas* (of the family Sphingomonadaceae and order Sphingomonadales), and *Acromobacter*, that may be able to break down FUMs. *Pseudomonas* showed an abundance trend of G1<G3 in all examined intestinal sections with a tendency for higher levels in the proximal part of the intestine than the distal part; *Sphingomonas* showed a notable abundance in the duodenum and jejunum of the G3 group only and lastly, *Achromobacter* was most abundant also in the duodenum and jejunum of the G3 group (Figures A-C can be found in the Appendix). Even while this result was not statistically significant ($P > 0.05$), it is conceivable that an intestinal consortium of microorganisms may work together to break down FUMs also in the intestine. In a partial agreement, from a used mushroom compost, Zhao *et al.* (2019) identified the SAAS79 FB_1-degrading bacterial consortium, which primarily included members of the *Pseudomonas*, *Comamonas*, *Delftia*, *Sphingobacterium*, and *Achromobacter* genera. The authors found that with a 90% degradation rate, SAAS79 could degrade FB_1 in 3 h into less harmful products and attributed this to a possible synergistic interaction between the bacterial consortia's species in the degradation process since no active single-degrader was found in the consortium (Zhao *et al.,* 2019).

5.5. Dietary fumonisins have small impact on apparent ileal amino acids digestibility in fattening pigs

Dietary amino acids (AAs) are required by animals primarily for maintenance, protein accretion, and production of biologically active compounds (e.g. hormones, enzymes, neurotransmitters). Because protein sources are the most expensive components in feed, the livestock industry to produce economical and high-quality products is dependent on the optimal use of dietary AAs (Wu *et al.*, 2014). The best possible use is also crucial for animal health because it may prevent liver and kidney damage and also save the environment by reducing nitrogen emissions. When it comes to nutrient absorption, immunological response, and growth efficiency, AAs are crucial metabolic intermediaries (Wu, 2009). Further, dietary AAs generated by animal cells perform regulatory functions in nutrient metabolism such as protein turnover and lipid synthesis and oxidation to promote lean tissue development and adipose tissue decrease (Blachier *et al.*, 2010). These processes also tend to be major targets for mycotoxins' harmful effects. Looking at the unanticipated ways FUMs have emerged to induce toxic insults, the present study examined the potentiality of FUMs to impede ileal digestibility of CP and AAs in fattening pigs.

As demonstrated by the findings of the present study, dietary FUMs did not obstruct the AID of CP regardless of the exposure time (7 vs 21 days). Elsewhere, when an increasing dose of dietary FB_1 (0.2, 5.0, 10, and 15 mg/kg) was fed to growing pigs in a 6- month trial, a noticeable reduction in EE and CP digestibility in a dose-response manner was highlighted (Gbore and Egbunike, 2007). Besides, in a sub-chronic investigation, a substantial drop in digestibility values of CP was confirmed in Wistar rats subjected to increasing amounts of FB_1 (0.2, 10, or 20 mg/kg diet), albeit a detrimental effect on growth performance was accompanied (Gbore *et al.*, 2010). In a much longer exposure feeding trial (6 months) feeding 10.0 or 15.0 mg FB_1/ kg diet, lower serum protein values were seen in pubertal boars which were subsequently attributed to an impairment of the protein metabolism in the pigs (Gbore, 2013). Although in this study there was no digestibility measurement, it was hypothesized that the exposure to the FB_1 diet over such a long period might have interfered with some physiological processes of digestion and absorption and thus, resulted in an inefficient use of dietary protein since protein synthesis is closely linked to the availability of dietary protein (Iyayi and Tewe, 1998).

Arginine is a central intestinal metabolite, both as a constituent of protein synthesis and together with threonine, glutamine, methionine, and cysteine, play a critical role in protecting gut barrier

function and maintaining gut mucosal immunity. This study shown that AID of arginine reduced over a period of 21 days compared to 7 days in the FUMs fed group (~ 0.8700 vs 0.8500 g/g AID rate at day 7 vs 21; $P = 0.003$) as opposed to the control group. Fumonisn B_1 (FB_1) is known to be capable of triggering mild triggering oxidative damage or apoptosis depending on the species and cell types (Stockmann-Juvala and Savolainen, 2008). Nitric oxide (NO) is known to play a crucial function in controlling the antioxidant defense system (Dai *et al.,* 2013). In the small intestine, arginine promotes the generation of NO within physiological limits (Rhoads and Wu, 2009). Accordingly, it has been hypothesized that a 1% supplementation of arginine aids in scavenging the excess ROS brought on by mycotoxin- contaminated feed, improving the balance between the production of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radical and the biological defense against the toxicity of these oxidants in growing pigs (Duan *et al.,* 2014). We could plausibly link the oxidative damage propensity of FUMs to the poor AID of arginine. To substantiate this supposition however, more research is needed. Histidine and tyrosine, in contrast to arginine, are AAs that are rarely examined in investigations of intestinal diseases (Liu *et al.,* 2017). As a result, with more investigation into the molecular and physiological workings that control the actions of such AAs, we will be able to make reliable judgments about how mycotoxins affect the digestibility of these AAs in the GIT.

To our knowledge, there have not been works highlighting the effects of solely FUMs in AAs digestibility. However, a decent number of similar works have been conducted but as co-contamination trials of chiefly *Fusarium* mycotoxins, including FUMs. Co-contamination of food/feed commodities with mycotoxins is increasingly becoming difficult to fully anticipate the detrimental effects of the combined toxicity of these toxins with the majority of mycotoxin mixes having additive, antagonistic or synergistic effects (Smith *et al.,* 2016). Thus, co-occurrence may aggravate the negative effects of mycotoxins and pose serious health risks to animals. In a study carried out by Jo *et al.* (2016) with the *Fusarium* mycotoxins DON and ZEN, the authors used equal levels i.e., 10 mg/kg of DON and ZEN to investigate their effects on the AID of CP and AAs in growing pigs. The study revealed a significant reduction in digestibility of lysine, threonine, valine, and tryptophan caused by DON, whereas ZEN effects were unremarkable. Although unclear about the results, the authors of the study suggested this turn-out to be a result of DON's peculiar intestinal disturbance processes and so, digestion and absorption of dietary components are negatively impacted in the process.

In a similar co-contamination trial, young pigs were exposed to a combination of aflatoxin (AFB_1) (0.62 ppb), ochratoxin (11.39 ppb), DON (3 ppm), and FB_1 (2 ppm) to assess the glutamate effect on ameliorating perturbation to the intestinal structure of the pigs. The authors found suppression of growth, impaired intestinal architecture, oxidative damage, and modification of the serum AAs profile in pigs that received the intoxicated diet compared to the toxin-free fed group (Duan *et al.*, 2014). Previous research has also shown that adding the combination of DON and FUMs (DON 5.0 mg/kg + FUMs 20 mg/kg) to the diets of poultry for 21 days resulted in a significant decrease in DM and ileal energy digestibility. Whereas DON, FUMs, or their combination, on the other hand, showed no effect on endogenous AAs loss or standardized CP and AAs digestibility (Jia *et al.*, 2020). These results slightly agree with the outcome of the present study.

6. CONCLUSIONS

1. The present study revealed the consequence of dietary fumonisins with regards to digestion of nutrients in the gastrointestinal tract. A dietary concentration of 15 or 30 mg/kg FUMs had adverse effects on nutrient digestibility and microminerals retention without affecting overall growth performance of nursery pigs following 21 days of exposure. This implies a decreased nutritional value of the mixed feed.
2. Fumonisins' peculiar effect on liver and kidney was attested in the present research. Hepatotoxic and nephrotoxic state as marked by the perturbations in serum AST, LDH, ALT, CK, and total cholesterol were observed in the study. Also, some histopathological alterations were seen in the liver and kidney which are indicative of organ injury induced by dietary fumonisins.
3. A high expression of Hsp70 activity in the liver of the group receiving the largest fumonisins could be interpreted as a sign of a possible counteraction to a potential induction of oxidative damage, even if none of the oxidative damage biomarkers demonstrated any effect.
4. The presence of dietary fumonisins in concentrations of 15 or 30 mg/kg diet did not affect the short-chain fatty acids concentrations. However, the faecal populations were shifted to the abundance of gut-health-promoting families such as Ruminococcaceae and Lachnospiraceae and their respective genera *Solobacterium, Faecalibacterium, Anaerofilum, Ruminococcus, Subdoligranulum, Pseudobutyrivibrio, Coprococcus*, and *Roseburia*. And although *Lactobacillus* was statistically unremarkable, the observed abundance trends present in the duodenum, ileum, and jejunum displayed a typical dose-response increment pattern, with the highest abundance in the duodenum and a decreasing trend in the distal direction of the intestine.
5. Following their exposure to a high concentration of 40 mg/kg FUMs for 7- or 21- days to assess the impact on crude protein and amino acid digestibility, the resulting effect of less impact revealed by this dosage implies the resilience of fattening pigs at such a growth stage and thus, their ability to manage dietary fumonisins better.

6. RECOMMENDATIONS

The current research looked at the impact of dietary fumonisins on structural and functional integrity of the gastrointestinal tract and microbiota in pigs. Some results such as the noticeable changes in nutritional digestibility, some health markers, and a putative shift in the microbial community were expected. The results on trace mineral metabolism in the nursery pigs were however vague and could be treated as a preliminary outcome. The following recommendations are thus, advised:

1. To better acknowledge the impact of fumonisins on mineral metabolism following our findings, a further trial should be carried out to ascertain the exact toxic mechanism induced by fumonisins in impeding absorptive processes in the GIT. Perhaps, a change of methodology, lengthening the exposure duration, dosage level manipulation, and the inclusion of other parameters such as tissues, bone or serum mineral concentration would be worth understanding the exact underlying mechanisms induced by dietary fumonisins.
2. The negligible but obvious abundance of *Lactobacillus sp*. opens the possibility for further research into the detoxification of dietary fumonisins by certain *Lactobacilli sp*.
3. The current results have emulated a situation in which fumonisins can result in unexplained production losses due to unclear clinical signs, although such doses are unusual in a commercial setting. The findings of this study should serve as a reminder of the value of continual animal feed evaluation in the animal production industry, particularly in light of the ongoing extreme weather events that are absolutely important factors in the development of mycotoxins.

7. NEW SCIENTIFIC RESULTS

1. Dietary fumonisins exposure at 15 or 30 mg/kg diet concentration depressed the apparent total tract diestibility of Ca and P, but not the Ca and P retention in nursery pigs in a not significant dose-dependent manner.
2. The retention of Cu and Zn in nursery pigs was impaired in case of fumonisins intoxication, which was attributed completely to the reduction in the digestibility of these two minerals.
3. Using amplicon sequencing of the 16 rRNA gene V3-V4 regions and the QIIME 1.9.0 pipeline, it was established that dietary dose of either 15 or 30 mg/kg fumonisins fed to nursery pigs resulted in a significant down-growth of some beneficial bacteria such as *Alloprevetolla, Campylobacter* and *Lachnospiraceae: Incertae Sedis* (in the duodenum), *Turicibacter* (in the jejunum) and *Clostridium sensu stricto 1* (in the ileum).
4. The faecal populations were shifted to the abundance of gut health promoting families such as Ruminococcaceae and Lachnospiraceae and their respective genera *Solobacterium, Faecalibacterium, Anaerofilum, Ruminococcus, Subdoligranulum, Pseudobutyrivibrio, Coprococcus,* and *Roseburia*.
5. Based on the observed abundance trends, *Lactobacillus sp*. present in the duodenum, ileum, and jejunum displayed a typical dose-response increment pattern, with the highest abundance in the duodenum and a decreasing trend in the distal direction of the intestine.
6. Dietary fumonisins in a concentration of 15 or 30 mg/kg diet did not affect the caecal fermentation outputs in nursery pigs.
7. Dietary dose of 40 mg/kg FUMs in 7- or 21- days exposure interval had a significant effect on the apparent ileal digestibility of arginine, histidine, and tyrosine. Although the permissible level of fumonisins is 5 mg/kg in swine feeds, this result indicates that gut functioning, at least the absorption of dietary crude protein and amino acids, is not impaired by a relatively high dose of fumonisins in fattening pigs.

8. SUMMARY

Fumonisins (FUMs) are one of the significant *Fusarium* mycotoxins of toxicological importance. The post-absorptive effects of FUMs are widely discussed leaving a remarkable gap in intestinal processes of digestion, absorption and the gut microbiota functions. Due to the comparatively poor absorption of FUMs by monogastric animal species like pigs, the GIT is more exposed to the toxin than any other tissue. The research therefore, subjected nursery pigs found to be more sensitive to mycotoxins exposure, to determine the potential effects on the overall GIT functioning including gut and faecal microbiota composition. And further, to investigate the apparent ileal digestibility of crude protein and amino acids in fattening pigs known also to be chiefly exposed to FUMs since their feed usually contains high amount of maize.

1. Effect of dietary 15 and 30 mg/kg FUMs (FB_1 + FB_2 + FB_3) on nutrient digestibility, mineral balance, intestinal and general health of nursery pigs

Their young stage of growth and comparatively increased vulnerability to *Fusarium* mycotoxins exposure makes a nursery pig an excellent model to study the toxic effects of FUMs in such a complex manner. The study enrolled eighteen nursery pigs of 7 weeks old to three dietary treatments; a control diet devoid of any traces of FUMs or other mycotoxins, a diet that contained 15 mg/kg or a diet that contained 30 mg/kg FUMs in fungal culture for 21 days. Titanium dioxide (0.5%) was added as an indigestible marker to the feeds, and faecal and urine samples were collected during the last 5 days (day 17 – 21) for the determination of apparent total tract digestibility (ATTD) of nutrients and a balanced study of nitrogen and minerals. The DE and ME content of the feeds were determined as well. Contents of the pancreas and small intestine including their sections were taken for the determination of digestive enzymes (amylase, protease, lipase, maltase, and lactase) activity and villus architecture (villus height, crypt depth and their ratio). For health investigations, serum biochemical measurements, antioxidant parameters (GSH and GSHPx), lipid peroxidation end product (MDA), Hsp70 activity, and histopathological examination of internal organs were performed. Caecal SCFAs concentration were determined as well as the composition of the gut and faecal microbiota were analyzed using amplicon sequencing of the 16S rRNA gene V3-V4 regions on Illumina MiSeq platform. Statistical analyses were performed with the SPSS version 27.0 software. Dietary FUMs exhibited no discernible impact on growth parameters, digestive enzymes, or intestinal architecture ($P > 0.05$) of all groups. There were no significant effects on GSH, GSHPx, MDA, or the concentration of caecal SCFAs. Further,

dietary FUMs did not affect ($P > 0.05$) the retention rate of Ca, K, Mg, Na, and P in all groups. However, the ATTD of CF, ash, Ca, P, Cu, and Zn was remarkably ($P < 0.05$) poor in pigs fed either 15 or 30 mg/kg FUMs contaminated feed compared to the control. Also, the retention of the aforementioned trace minerals was depressed as well. The DE content was significantly lower ($P < 0.05$) when the feed contained 15 mg/kg FUMs, but no statistically reliable treatment effect was confirmed for ME content. In addition, dietary FUMs substantially increased ($P < 0.05$) Hsp70 expression in the liver, as well as an increase in certain serum biomarkers suggestive of internal organ injuries, and histological alterations in the liver, kidney, lung and small intestine of groups treated with FUMs compared to the control group. Treatments shifted the microbial population in the intestine to lower levels of the families Prevotellaceae, Campylobacteraceae, Lachnospiraceae, Erysipelotrichaceae, and Clostridiaceae; and *Alloprevotella*, *Campylobacter*, *Incertae Sedis* (duodenum), *Turicibacter* (jejunum), *Clostridium sensu stricto 1* (ileum) genera compared to control. Faecal microbial composition had higher levels of Erysipelotrichaceae, Ruminococcaceae and Lachnospiraceae families and *Solobacterium*, *Faecalibacterium*, *Anaerofilum*, *Ruminococcus*, *Subdoligranulum, Pseudobutyrivibrio*, *Coprococcus*, and *Roseburia* genera. *Lactobacillus* was more abundant in the proximal part of the gut as opposed to its distal portion.

2. Effect of dietary 40 mg/kg FUMs (FB_1 + FB_2 + FB_3) on the apparent ileal digestibility (AID) of amino acids in Ffattening pigs' fattening pigs' study

Since their mixed feed contains higher proportions of maize as their major energy source, growing-finishing/ fattening pigs are invariably at risk of FUMs toxicity. Pigs at this growth stage have a fully developed digestive and immune system, making them resistant to FUMs toxicity better than younger pigs. Proteins constitute one of the most important nutritional components in pigs' diets, as well as a key component in various biological processes. The number of studies examining the effects of FUMs on the digestibility of AAs is limited. Thus, we enrolled ten Danbred fattening pigs with an initial BW of 67.5 ± 4.1 kg inserted with a PVTC cannula to study the likely effect of FUMs on AID of CP and AAs. The pigs were randomly divided into two: a control group fed a commercially prepared basal diet and a group fed a 40 mg/kg FUMs in fungal culture diet. Titanium dioxide (0.5%) was added as an indigestible marker to diets. During two separate periods i.e., on days 7-8-9 (as short term exposure) and 21-22-23 (as long term exposure), ileal digesta were collected for 3 consecutive days for the determination of AID of CP and the various AAs. Data were subjected to a two-way ANOVA of the SPSS version 20.0 software using FUMs dose

(i=2; 0 or 40 mg FUMs/kg feed) and duration (j=2; short vs long term exposure) as fixed factors. A dose of 40 mg/kg FUMs contaminated diet did not affect ($P > 0.05$) the overall body weight of pigs. According to our findings, a dietary intake of 40 mg/kg FUMs substantially interfered with the AID of arginine, histidine, and tyrosine ($P = 0.003$, 0.047, and 0.047, respectively) in terms of the dose and duration interaction effect. In addition, the main duration effect of the AID of histidine was significant ($P < 0.001$). It is, therefore, conceivable that a dietary dose of a 40 mg/kg FUMs contaminated diet does not drastically affect CP and AAs digestibility in fattening pigs for a period of 7 or 21 days.

Overall, it can be concluded that feeding nursery pigs with dietary FUMs at doses of 15 or 30 mg/kg affected the balance of Cu and Zn and significantly disturbed and altered the population of some important bacteria, especially those that promote intestinal health. In addition, dietary FUMs had a negative impact on the nutritional value of the mixed feed by reducing the ATTD rate of CF, ash, Ca and P. With regards to certain health indices, dietary FUMs promoted liver and kidney injuries in the nursery pigs. In addition, it may be argued that fattening pigs were more resistant to FUMs toxicity because a dietary dose of 40 mg/kg FUMs significantly affected only three of all the amino acids investigated i.e., histidine, arginine, and tyrosine.

9. ÖSSZEFOGLALÁS

A fumonizinek (FUM) a *Fusarium* penészgombák toxikológiai szempontból fontos másodlagos anyagcsere termékei (mikotoxinok). Míg hatásuk a szervezet általános egészségi állapotára, az általuk előidézett kórformák és azok molekuláris háttere viszonylag jól ismeretek, nagyon kevés kísérletes adat áll rendelkezésre az emésztőkészülékben kifejtett hatásukról, pl. táplálóanyagok emésztése, felszívódása, a bél mikrobióta összetételének és működésének megváltoztatása. Ugyanakkor, mivel a szájon át felvett toxin alig szívódik fel (4-6%-ban), a gasztrointesztinális traktus (GIT) a hatásának leginkább kitett szerv. A doktori munka célja ezért néhány jellemző parameter meghatározásán keresztül, a FUM-nek a GIT működésére kifejtett hatásának feltárása volt.

1. Gombatenyészettel takarmányba kevert 15 és 30 mg/kg FUM (FB_1 + FB_2 + FB_3) hatása egyes táplálóanyagok emészthetőségére és retenciójára, a szervezet általános és a bél egészségi állapotáraválasztott malacokban

A választott malacok a fajnál és koruknál fogva a fumonizinekre legérzékenyebb gazdasági állatok, ezért kiváló modellek a toxin hatásának megismerésére. A kísérletben 7 hetes malacokat három csoportba osztottuk (n= 6 / csoport): a kontroll állatok FUM mentes takarmányt fogyasztottak, míg a többi állat takarmányát 15, illetve 30 mg/tak.kg FUM-el egészítettük ki. Fel nem szívódó markerként titán-dioxidot használtunk (0,5%), az egyedileg tartott állatoktól bélsár és vizelet mintát gyűjtöttünk 5 napon keresztül, a táplálóanyagok látszólagos emészthetőségének (apparent total tract digestibility, ATTD) meghatározására. Meghatároztuk a takarmány emészthető (DE) és metabolizálható (ME) energiatartalmát is. A hasnyálmirigyből és a vékonybélből vett mintákból megmértük néhány emésztőenzim aktivitását (amiláz, proteázok, lipáz, maltáz és laktáz). A vékonybél 3 szakaszán lemértük a bélbolyhok magasságát (villus height, VH) és a kripták mélységét (crypt depth, CD), kiszámítottuk arányukat (VH/CD). Megmértük a fob klinikai kémiai paramétereket, az antioxidáns rendszer működését jelző glutation (GSH) koncentrációját, a glutation-peroxidáz (GSHPx) aktivitását, valamint a lipdperoxidációt jelző malodialdehid (MDA) tartalmat. Végeztünk kórszövettani vizsgálatokat, valamint Hsp70 mérést is egyes szervekből. Meghatároztuk a bél mikrobióta összetételét (16S rRNS metagenomikai szekvenálása) és működésének jellemzésére a vakbéltartalom illózsírsav koncentrációját.

A FUM-nek nem volt szignifikáns hatása az állatok növekedésére, az emésztőenzimek aktivitására, a bélboholy morfológiájára (VH/CD), a GSH, GSHPx és MDA paraméterekre,

valamint az illózsírsav-termelésre. Nem befolyásolta a Ca, K, Mg, Na és P retenciót sem. Ugyanakkor a bélsárban mért látszólagos emészthetőség (ATTD) jelentősen csökkent a nyersrost, a nyershamu, a Cu és a Zn esetében ($P < 0,05$) a 15 és a 30 ppm FUM-ot fogyasztó állatokban a kontrollokhoz képest. A Zn és a Cu retenciója is jelentősen romlott. A takarmány emészthető energia-tartalma (DE) 15 ppm FUM hatására szintén csökkent, míg az expozíció nem befolyásolta a metabolizálható energia-tartalmat (ME). A FUM megnövelte a Hsp70 expresszióját a májban, valamint egyes klinikai kémiai paraméterek is jelezték a máj és a vese károsodását. Ezeket a kórszövettan megerősítette.

A bél mikrobióta összetételét a FUM kezelés a következők szerint módosította: a *Solobacterium, Faecalibacterium, Anaerofilum, Ruminococcus, Subdoligranulum, Pseudobutyrivibrio, Coprococcus,* és *Roseburia* nemzettségekben növekedés, addig az *Alloprevotella, Campylobacter, Incertae Sedis, Turicibacter* és *Clostridium sensu stricto 1* nemzettségekben csökkenés volt tapasztalható.

2. Gombatenyészettel takarmányba kevert 40 mg/kg FUM (FB_1 + FB_2 + FB_3) hatása az aminosavak látszólagos ilealis emészthetőségére hízósertésekben

Mivel a sertések takarmánya nagyobb arányban tartalmaz energia-forrásként kukoricát, a hízósertések nagy FUM expozíció kockázatának vannak kitéve. Erre az időszakra ugyanakkor a sertések tápcsatornája és immunrendszere már kifejlett, így kevésbé érzékenyek a toxikus hatásokra, mint a választott malacok. A takarmányok fehérje és azon belül aminosav tartalma, a fehérje emészthetősége és az aminosavak hasznosulása különösen fontos. Arra vonatkozóan, hogy egyes mikotoxinok ezen tényezőket hogyan befolyásolják, alig található adat a szakirodalomban.

Kísérletünben Danbred hízósertésekbe (67,5 ± 4.1 kg kezdeti élősúlyban) sebészileg PVTC kanült ültettünk és a nyersfehérje, valamint az aminosavak látszólagos ileális emészthetőségét (apparent ileal digestibility, AID) határoztuk meg. Két kísérleti csoportot különítettünk el, kontroll (FUM mentes) és 40 mg/ tak.kg FUM-t tartalmazó takarmányt fogyasztó állatokból. Jelzőanyagként fel nem szívódó titán-dioxidot használtunk (0,5%). Két időszakban gyűjtöttük az ileális béltartalom mintákat, 3-3 egymást követő napon, egyszer 7, egyszer pedig 21 napos expozíciót követően. Az adatok elemzéséhez kéttényezős variancia-analízist végeztünk (ANOVA, SPSS version 20.0 software), a kezelés és az expozíciós idő hatását, valamint ezek interakcióját vizsgáltuk.

A 40 ppm-es dózisnak nem volt szignifikáns hatása az állatok termelési paramétereire. A nyersfehérje és az arginin, a hisztidin és a tirozin kivételével az egyes aminosavak emészthetőségét sem befolyásolta számottevően. A 21 napos expozíciót követően a treonin emészthetősége kisebb volt, mint a 7 napos terhelést követően.

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11.REFERENCES

Abassi, H., Ayed-Boussema, I., Shirley, S., Abid, S., Bacha, H. and Micheau, O. (2016): The mycotoxin zearalenone enhances cell proliferation, colony formation and promotes cell migration in the human colon carcinoma cell line HCT116. *Toxicology Letters*, *254*, 1–7.

Adhikari, M., Negi, B., Kaushik, N., Adhikari, A., Al-Khedhairy, A. A., Kaushik, N. K. and Choi, E. H. (2017): T-2 mycotoxin: Toxicological effects and decontamination strategies. *Oncotarget*, *8*(20), 33933.

Akbari, P., Braber, S., Varasteh, S., Alizadeh, A., Garssen, J. and Fink-Gremmels, J. (2017): The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins. *Archives of Toxicology*, *91*(3), 1007–1029. https://doi.org/10.1007/s00204-016-1794-8

Agence Française de Sécurité Sanitaire des Aliments (AFFSA) (2009): Évaluation des risques liés à la présence de mycotoxines dans les chaînes alimentaires humaine et animale. Rapport Final, 1–308.

Alizadeh, A. A., Aranda, V., Bardelli, A., Blanpain, C., Bock, C., Borowski, C., Caldas, C., Califano, A., Doherty, M. and Elsner, M. (2015): Toward understanding and exploiting tumor heterogeneity. *Nature Medicine*, *21*(8), 846–853.

Apgar, G. A. and Kornegay, E. T. (1996): Mineral balance of finishing pigs fed copper sulfate or a copper-lysine complex at growth-stimulating levels. *Journal of Animal Science*, *74*(7), 1594–1600.

Asam, S., Habler, K. and Rychlik, M. (2017): *Fusarium* mycotoxins in food. *Chemical Contaminants and Residues in Food*, 2nd Edition, 295–336. Elsevier Inc. https://doi.org/10.1016/B978-0-08-100674-0.00014-X

Association of Analytical Chemists (AOAC) (2000): Official Methods of Analysis. 17th Edition, Gaithersburg, MD, USA.

Beardall, J. M. and Miller, J. D. (1994): Diseases in humans with mycotoxins as possible causes. *Mycotoxins in grain: Compounds other than aflatoxin*, 487–539.

Bennett, J. W. and Bentley, R. (1989): What's in a name? – microbial secondary metabolism. *Advances in Applied Microbiology*, *34*, 1–28. Elsevier.

Bertero, A., Fossati, P., Tedesco, D. E. A. and Caloni, F. (2020): Beauvericin and enniatins: *In vitro* intestinal effects. *Toxins*, *12*(11), 686. https://doi.org/10.3390/toxins12110686

Bezuidenhout, S. C., Gelderblom, W. C. A., Gorst-Allman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G. and Vleggaar, R. (1988): Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *Journal of the Chemical Society, Chemical Communications*, *11*, 743–745.

Bier, M. (1955): Lipases: Methods in Enzymology. New York, *1*, 627–642.

Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.-D., Serino, M., Tilg, H., Watson, A. and Wells, J. M. (2014): Intestinal permeability–a new target for disease prevention and therapy. *BMC Gastroenterology*, *14*(1), 1–25.

Blachier, F., Lancha, A. H., Boutry, C. and Tomé, D. (2010): Alimentary proteins, amino acids

and cholesterolemia. *Amino Acids*, *38*(1), 15–22. https://doi.org/10.1007/s00726-009-0239-6

Bolger, M., Coker, R. D., DiNovi, M., Gaylor, D., Gelderblom, W., Olsen, M., Paster, N., Riley, R. T., Shephard, G. and Speijers, G. J. A. (2001): Fumonisins. *Safety Evaluation of Certain Mycotoxins in Food*, *47*, 103–297.

Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J. and Trakatellis, A. G. (1994): Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry*, *42*(9), 1931–1937.

Bottalico, A. and Logrieco, A. (1998): Toxigenic *Alternaria* species of economic importance. *Mycotoxins in Agriculture and Food Safety*, 83–126. CRC Press.

Bouhet, S., Hourcade, E., Loiseau, N., Fikry, A., Martinez, S., Roselli, M., Galtier, P., Mengheri, E. and Oswald, I. P. (2004): The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. *Toxicological Sciences*, *77*(1), 165–171. https://doi.org/10.1093/toxsci/kfh006

Bouhet, S. and Oswald, I. P. (2007): The intestine as a possible target for fumonisin toxicity. *Molecular Nutrition and Food Research*, *51*(8), 925–931. https://doi.org/10.1002/mnfr.200600266

Bracarense, A.-P. F. L., Lucioli, J., Grenier, B., Pacheco, G. D., Moll, W.-D., Schatzmayr, G. and Oswald, I. P. (2012): Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. *British Journal of Nutrition*, *107*(12), 1776–1786.

Brommage, R., Binacua, C., Antille, S. and Carrié, A.-L. (1993): Intestinal calcium absorption in rats is stimulated by dietary lactulose and other resistant sugars. *The Journal of Nutrition*, *123*(12), 2186–2194.

Broom, L. (2015): Mycotoxins and the intestine. *Animal Nutrition*, *1*(4), 262–265.

Burel, C., Tanguy, M., Guerre, P., Boilletot, E., Cariolet, R., Queguiner, M., Postollec, G., Pinton, P., Salvat, G., Oswald, I. P. and Fravalo, P. (2013): Effect of low dose of fumonisins on pig health: Immune status, intestinal microbiota and sensitivity to Salmonella. *Toxins*, *5*(4), 841–864. https://doi.org/10.3390/toxins504084

Bushnell, B., Rood, J. and Singer, E. (2017): BBMerge – Accurate paired shotgun read merging via overlap. *PLoS ONE*, *12*(10), 1–15. https://doi.org/10.1371/journal.pone.0185056

Cader, M. and Kaser, A. (2013): Recent advances in inflammatory bowel disease: Mucosal immune cells in intestinal inflammation. *Gut*, *62*(11), 1653–1664. https://doi.org/10.1136/gutjnl-2012-303955

Capaldo, C. T., Farkas, A. E. and Nusrat, A. (2014): Epithelial adhesive junctions. *F1000prime Reports*, *6*.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K. and Gordon, J. I. (2010): QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336.

Cawood, M. E., Gelderblom, W. C. A., Vleggaar, R., Behrend, Y., Thiel, P. G. and Marasas, W.

F. O. (1991): Isolation of the fumonisin mycotoxins: a quantitative approach. *Journal of Agricultural and Food Chemistry*, *39*(11), 1958–1962.

Celi, P., Verlhac, V., Pérez Calvo, E., Schmeisser, J. and Kluenter, A. M. (2019): Biomarkers of gastrointestinal functionality in animal nutrition and health. *Animal Feed Science and Technology*, *250*, 9–31. https://doi.org/10.1016/j.anifeedsci.2018.07.012

Che, D., Adams, S., Wei, C., Gui-Xin, Q., Atiba, E. M. and Hailong, J. (2019): Effects of *Astragalus membranaceus* fiber on growth performance, nutrient digestibility, microbial composition, VFA production, gut pH, and immunity of weaned pigs. *Microbiology Open*, *8*(5), 1–12. https://doi.org/10.1002/mbo3.712

Chen, L., Xu, Y., Chen, X., Fang, C., Zhao, L. and Chen, F. (2017): The maturing development of gut microbiota in commercial piglets during the weaning transition. *Frontiers in Microbiology*, *8*, 1–13. https://doi.org/10.3389/fmicb.2017.01688

Chiba, H., Osanai, M., Murata, M., Kojima, T. and Sawada, N. (2008): Transmembrane proteins of tight junctions. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1778*(3), 588–600.

Chilaka, C. A., De Boevre, M., Atanda, O. O. and De Saeger, S. (2017): The status of *Fusarium* mycotoxins in sub-Saharan Africa: A review of emerging trends and post-harvest mitigation strategies towards food control. *Toxins*, *9*(1). https://doi.org/10.3390/toxins9010019

Colvin, B. M., Cooley, A. J. and Beaver, R. W. (1993): Fumonisin toxicosis in swine: Clinical and pathologic findings. *Journal of Veterinary Diagnostic Investigation*, *5*(2), 232–241. https://doi.org/10.1177/104063879300500215

Conway, P. L. (1994): Function and regulation of the gastrointestinal microbiota of the pig. *Publication-European Association For Animal Production*, *80*, 231.

Creppy, E. E. (2002): Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters 127*(1-3), 19–28.

Dai, Z., Wu, Z., Yang, Y., Wang, J., Satterfield, M. C., Meininger, C. J., Bazer, F. W. and Wu, G. (2013): Nitric oxide and energy metabolism in mammals. *Biofactors*, *39*(4), 383–391.

Dang, H. A., Zsolnai, A., Kovacs, M., Bors, I., Bonai, A., Bota, B. and Szabó-Fodor, J. (2017): *In vitro* interaction between fumonisin B1 and the intestinal microflora of pigs. *Polish Journal of Microbiology*, *66*(2), 245–250.

Davis, G. K. (1980): Microelement interactions of zinc, copper, and iron in mammalian species. *Annals of the New York Academy of Sciences*, *355*(1), 130–139.

De Angelis, M., Calasso, M., Cavallo, N., Di Cagno, R. and Gobbetti, M. (2016): Functional proteomics within the genus *Lactobacillus*. *Proteomics*, *16*(6), 946–962. https://doi.org/https://doi.org/10.1002/pmic.201500117

De Angelis, M., Siragusa, S., Berloco, M., Caputo, L., Settanni, L., Alfonsi, G., Amerio, M., Grandi, A., Ragni, A. and Gobbetti, M. (2006): Selection of potential probiotic *Lactobacilli* from pig feces to be used as additives in pelleted feeding. *Research in Microbiology*, *157*(8), 792–801. https://doi.org/10.1016/j.resmic.2006.05.003

Dersjant-Li, Y., Awati, A., Schulze, H. and Partridge, G. (2015): Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing

factors. *Journal of the Science of Food and Agriculture*, *95*(5), 878–896.

Desjardins, A. E. (2006): *Fusarium* mycotoxins: Chemistry, genetics, and biology. *American Phytopathological Society* (APS Press).

Devriendt, B., Verdonck, F., Wache, Y., Bimczok, D., Oswald, I. P., Goddeeris, B. M. and Cox, E. (2009): The food contaminant fumonisin B1 reduces the maturation of porcine CD11R1 $^+ $ intestinal antigen presenting cells and antigen-specific immune responses, leading to a prolonged intestinal ETEC infection. *Veterinary Research*, *40*(4), 1–14.

Dou, S., Gadonna-Widehem, P., Rome, V., Hamoudi, D., Rhazi, L., Lakhal, L., Larcher, T., Bahi-Jaber, N., Pinon-Quintana, A., Guyonvarch, A., Huërou-Luron, I. L. E. and Abdennebi-Najar, L. (2017): Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. *PLoS ONE*, *12*(1), 1–20. https://doi.org/10.1371/journal.pone.0169851

Downes, J., Dewhirst, F. E., Tanner, A. C. and Wade, W. G. (2013): Description of *Alloprevotella rava gen*. nov., sp. nov., isolated from the human oral cavity, and reclassification of *Prevotella tannerae* Moore *et al*. 1994 as *Alloprevotella tannerae* gen. nov., comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *63*(4), 1214–1218.

Duan, J., Yin, J., Wu, M., Liao, P., Deng, D., Liu, G., Wen, Q., Wang, Y., Qiu, W., Liu, Y., Wu, X., Ren, W., Tan, B., Chen, M., Xiao, H., Wu, L., Li, T., Nyachoti, C. M., Adeola, O. and Yin, Y. (2014): Dietary glutamate supplementation ameliorates mycotoxin-induced abnormalities in the intestinal structure and expression of amino acid transporters in young pigs. *PLoS ONE*, *9*(11). https://doi.org/10.1371/journal.pone.0112357

Edite Bezerra da Rocha, M., Freire, F. da C. O., Erlan Feitosa Maia, F., Izabel Florindo Guedes, M. and Rondina, D. (2014): Mycotoxins and their effects on human and animal health. *Food Control*, *36*(1), 159–165. https://doi.org/10.1016/j.foodcont.2013.08.021

Eskola, M., Parikka IV, Pä. and Rizzo, A. (2001): Trichothecenes, ochratoxin A and zearalenone contamination and *Fusarium* infection in Finnish cereal samples in 1998. *Food Additives and Contaminants*, *18*(8), 707–718.

Espinosa, C. D. and Stein, H. H. (2021): Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status: A review. *Journal of Animal Science and Biotechnology*, *12*(1), 1–12. https://doi.org/10.1186/s40104-020-00533-3

European Food Safety Authority (EFSA). (2004): Report of the scientific panel on contaminants in the food chain on provisional findings on furan in food. *EFSA Journal*, *137*, 1–20.

European Food Safety Authority (EFSA). (2018): Appropriateness to set a group health-based guidance value for fumonisins and their modified forms. *EFSA Journal 16*(2), 5172. https://doi.org/10.2903/j.efsa.2018.5172

Ewuola, E. O., Ogunlade, J. T., Gbore, F. A., Salako, A. O., Idahor, K. O. and Egbunike, G. N. (2003): Performance evaluation and organ histology of rabbits fed *Fusarium verticillioides* culture material. *Tropical Animal Production and Investigations*, *6*, 111–119.

Ferrante, M. C., Meli, R., Mattace Raso, G., Esposito, E., Severino, L., Di Carlo, G. and Lucisano, A. (2002): Effect of fumonisin B1 on structure and function of macrophage plasma

membrane. *Toxicology Letters*, *129*, 181–187. https://doi.org/10.1016/S0378-4274(01)00476-3

Festa, S., Coppotelli, B. M. and Morelli, I. S. (2013): Bacterial diversity and functional interactions between bacterial strains from a phenanthrene-degrading consortium obtained from a chronically contaminated-soil. *International Biodeterioration and Biodegradation*, *85*, 42–51.

Fink, J. and Koo, B.-K. (2016): Clonal evolution of stem cells in the gastrointestinal tract. *Stem Cells, Pre-Neoplasia, and Early Cancer of the Upper Gastrointestinal Tract*, 11–25.

Fodor, J., Kametier, L. and Kovács, M. (2006a): Practical aspects of fumonisin production under laboratory conditions. *Mycotoxin Research*, *22*(4), 211–216. https://doi.org/10.1007/BF02946744

Fodor, J., Meyer, K., Riedlberger, M., Bauer, J., Horn, P., Kovács, F. and Kovács, M. (2006): Distribution and elimination of fumonisin analogues in weaned piglets after oral administration of Fusarium verticillioides fungal culture. *Food Additives and Contaminants*, *23*(5), 492–501. https://doi.org/10.1080/02652030500544964

Jestoi, M. (2008): Emerging *Fusarium* mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin– A review. *Critical Reviews in Food Science and Nutrition*, *48*(1), 21–49.

Gao, J., Zhang, H. J., Yu, S. H., Wu, S. G., Yoon, I., Quigley, J., Gao, Y. P. and Qi, G. H. (2008): Effects of yeast culture in broiler diets on performance and immunomodulatory functions. *Poultry Science*, *87*(7), 1377–1384.

Gareis, M. (2003): Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states. Report of experts participating in SCOOP task 3.2.10- Part A: Trichothecene, 13–235.

Gäumann, E., Roth, S., Ettlinger, L., Plattner, P. A. and Nager, U. (1947): Enniatin, ein neues, gegen mykobakterien wirksames antibiotikum. *Experientia*, *3*(5), 202–203. https://doi.org/10.1007/BF02163993

Gbore, F. (2010): Evaluation of subchronic dietary fumonisin B1 on nutrient digestibility and growth performance of rats Fumonisin toxicity in rats View project Health View project Evaluation of subchronic dietary fumonisin B 1 on nutrient digestibility and growth performance of rats. *African Journal of Biotechnology*, *9*(38), 6442–6447. http://www.academicjournals.org/AJB

Gbore, F. A. (2007): Effect of dietary fumonisin B on histomorphology and histopathology of organs of pubertal boars. *American-Eurasian Journal of Scientific Research*, *2*(2), 75–79.

Gbore, F. A. (2013): Protein profiles of serum, brain regions and hypophyses of pubertal boars fed diets containing fumonisin B1. *Ife Journal of Science*, *15*(1), 167–174.

Gbore, F. A. and Egbunike, G. N. (2007): Influence of dietary fumonisin B1 on nutrient utilization by growing pigs. *Livestock Research for Rural Development*, *19*(7), 93.

Gbore, F. A., Yinusa, R. I. and Salleh, B. (2010): Evaluation of subchronic dietary fumonisin B1 on nutrient digestibility and growth performance of rats. *African Journal of Biotechnology*, *9*(38), 6442–6447. https://doi.org/10.5897/AJB10.737

Gbore, F. A. and Akele, O. (2010): Growth performance, haematology and serum biochemistry of female rabbits (*Oryctolagus cuniculus*) fed dietary fumonisin. *Veterinarski Archive*, *80*(3), 431–443.

Gebhardt, J. T., Thomson, K. A., Woodworth, J. C., Dritz, S. S., Tokach, M. D., Derouchey, J. M., Goodband, R. D., Jones, C. K., Cochrane, R. A., Niederwerder, M. C., Fernando, S., Abbas, W. and Burkey, T. E. (2020): Effect of dietary medium-chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage. *Journal of Animal Science*, *98*(1), 1–11. https://doi.org/10.1093/jas/skz358

Gelderblom, W. C. A., Kriek, N. P. J., Marasas, W. F. O. and Thiel, P. G. (1991): Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B1, in rats. *Carcinogenesis*, *12*(7), 1247–1251. https://doi.org/10.1093/Carcin/12.7.1247

Gelderblom, W. C. A. and Marasas, W. F. O. (2012): Controversies in fumonisin mycotoxicology and risk assessment. *Human and Experimental Toxicology*, *31*(3), 215–235.

Gelderblom, W. C., Jaskiewicz, K., Marasas, W. F., Thiel, P. G., Horak, R. M., Vleggaar, R. and Kriek, N. (1988): Fumonisins- novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Applied and Environmental Microbiology*, *54*(7), 1806–1811.

Gil-Serna, J., Mateo, E. M., González-Jaén, M. T., Jiménez, M., Vázquez, C. and Patiño, B. (2013): Contamination of barley seeds with *Fusarium* species and their toxins in Spain: an integrated approach. *Food Additives and Contaminants: Part A*, *30*(2), 372–380.

Golli-bennour, E. El, and Bacha, H. (2011): Hsp70 expression as biomarkers of oxidative stress: Mycotoxins' exploration. *Toxicology*, *287*(1–3), 1–7. https://doi.org/10.1016/j.tox.2011.06.002

Gonzalez, H. H. L., Martinez, E. J., Pacin, A. M., Resnik, S. L. and Sydenham, E. W. (1999): Natural co-occurrence of fumonisins, deoxynivalenol, zearalenone and aflatoxins in field trial corn in Argentina. *Food Additives and Contaminants*, *16*(12), 565–569.

Gratz, S. W., Dinesh, R., Yoshinari, T., Holtrop, G., Richardson, A. J., Duncan, G., MacDonald, S., Lloyd, A. and Tarbin, J. (2017): Masked trichothecene and zearalenone mycotoxins withstand digestion and absorption in the upper GI tract but are efficiently hydrolyzed by human gut microbiota *in vitro*. *Molecular Nutrition and Food Research*, *61*(4), 1600680. https://doi.org/10.1002/mnfr.201600680

Grenier, B. and Applegate, T. J. (2013): Modulation of intestinal functions following mycotoxin ingestion: Meta-analysis of published experiments in animals. *Toxins 5*(2), 396–430. https://doi.org/10.3390/toxins5020396

Grenier, B., Bracarense, A. P. F., Schwartz, H. E., Lucioli, J., Cossalter, A. M., Moll, W. D., ... and Oswald, I. P. (2013): Biotransformation approaches to alleviate the effects induced by *Fusarium* mycotoxins in swine. *Journal of Agricultural and Food Chemistry*, *61*(27), 6711–6719.

Grenier, B. and Oswald, I. (2011): Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. *World Mycotoxin Journal*, *4*(3), 285–313.

Grenier, B., Schwartz-Zimmermann, H. E., Caha, S., Moll, W. D., Schatzmayr, G. and Applegate, T. J. (2015): Dose-dependent effects on sphingoid bases and cytokines in chickens fed diets prepared with *Fusarium verticillioides* culture material containing fumonisins. *Toxins*, *7*(4), 1253–1272. https://doi.org/10.3390/toxins7041253

Gresse, R., Durand, F. C., Dunière, L., Blanquet-Diot, S. and Forano, E. (2019): Microbiota composition and functional profiling throughout the gastrointestinal tract of commercial weaning piglets. *Microorganisms*, *7*(9). https://doi.org/10.3390/microorganisms7090343

Grove, J. F. (1988): Non-macrocyclic trichothecenes. *Natural Product Reports*, *5*(2), 187–209.

Gruber-Dorninger, C., Jenkins, T. and Schatzmayr, G. (2019): Global mycotoxin occurrence in feed: A ten-year survey. *Toxins*, *11*(7). https://doi.org/10.3390/toxins11070375

Gumprecht, L. A., Beasley, V. R., Weigel, R. M., Parker, H. M., Tumbleson, M. E., Bacon, C. W., Meredith, F. I. and Haschek, W. M. (1998): Development of fumonisin-induced hepatotoxicity and pulmonary edema in orally dosed swine: morphological and biochemical alterations. *Toxicologic Pathology*, *26*(6), 777–788.

Gutema, T., Munimbazi, C. and Bullerman, L. B. (2000): Occurrence of fumonisins and moniliformin in corn and corn-based food products of US origin. *Journal of Food Protection*, *63*(12), 1732–1737.

Halabi, K. S., Natour, R. M. and Tamimi, S. O. (1998): Individual and combined effects of chronic ochratoxin A and zearalenone mycotoxins on rat liver and kidney. *Arab Gulf Journal of Scientific Research*, *16*(2), 379–392.

Hamill, R. L., Higgens, C. E., Boaz, H. E. and Gorman, M. (1969): The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina*. *Tetrahedron Letters*, *10*(49), 4255–4258.

Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E. and Cole, J. R. (1990): Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. *Journal of Veterinary Diagnostic Investigation*, *2*(3), 217–221. https://doi.org/10.1177/104063879000200312

Haschek, W. M., Gumprecht, L. A., Smith, G., Tumbleson, M. E. and Constable, P. D. (2001): Fumonisin toxicosis in swine: An overview of porcine pulmonary edema and current perspectives. *Environmental Health Perspectives*, *109*(2), 251–257. https://doi.org/10.1289/ehp.01109s2251

Haschek, W. M., Rousseaux, C. G., Wallig, M. A., Bolon, B. and Ochoa, R. (2013): *Haschek and Rousseaux's Handbook of Toxicologic Pathology*. Academic Press, 2013.

Hazra, A. and Gogtay, N. (2016): Biostatistics series module 3: Comparing groups: Numerical variables. *Indian Journal of Dermatology*, *61*(3), 251–260. https://doi.org/10.4103/0019-5154.182416

Hoffmann, E. M., Selje-Assmann, N., Becker, K., Wallace, R. J. and Broderick, G. A. (2010): Screening for Anti-proteolytic Compounds BT - *In vitro* screening of plant resources for extra-nutritional attributes in ruminants: nuclear and related methodologies (P. E. Vercoe, H. P. S. Makkar, and A. C. Schlink (eds.); 55–86). Springer Netherlands. https://doi.org/10.1007/978-90-481-3297-3_5

Houdijk, J. G., Bosch, M. W., Tamminga, S., Verstegen, M. W., Berenpas, E. B. and Knoop, H. (1999): Apparent ileal and total-tract nutrient digestion by pigs as affected by dietary nondigestible oligosaccharides. *Journal of Animal Science, 77*(1), 148–158.

Hueza, I. M., Raspantini, P. C. F., Raspantini, L. E. R., Latorre, A. O. and Górniak, S. L. (2014): Zearalenone, an estrogenic mycotoxin, is an immunotoxic compound. *Toxins, 6*(3), 1080–1095.

SPSS. (2012): SPSS for Windows version 20. IBM SPSS statistics for Windows. Armonk (NY): IBM Corp.

International Agency for Research on Cancer (IARC). (2002): Fumonisin B1. IARC. *Monographs on the Evaluation of Carcinogenic Risk to Humans*, 82, 275–366.

Iyayi, E. A. and Tewe, O. O. (1998): Serum total protein, urea and creatinine levels as indices of quality of cassava diets for pigs. *Tropical Vet*erinary, *16*, 59–67.

Jang, Y. D., Escobar, C. S. and Lindemann, M. D. (2018): Effect of feeding a blend of naturally-contaminated corn on nutrient digestibility and feed preference in weanling pigs. *Journal of Animal Science*, 96, 123–123.

Ji, F., He, D., Olaniran, A. O., Mokoena, M. P., Xu, J. and Shi, J. (2019): Occurrence, toxicity, production and detection of *Fusarium* mycotoxin: A review. *Food Production, Processing and Nutrition, 1*(1), 1–14. https://doi.org/10.1186/s43014-019-0007-2

Jia, R., Liu, W., Zhao, L., Cao, L. and Shen, Z. (2020): Low doses of individual and combined deoxynivalenol and zearalenone in naturally moldy diets impair intestinal functions via inducing inflammation and disrupting epithelial barrier in the intestine of piglets. *Toxicology Letters*, *333*, 159–169. https://doi.org/10.1016/j.toxlet.2020.07.032

Jo, H., Kong, C., Song, M. and Kim, B. G. (2016): Effects of dietary deoxynivalenol and zearalenone on apparent ileal digestibility of amino acids in growing pigs. *Animal Feed Science and Technology*, *219*, 77–82. https://doi.org/10.1016/j.anifeedsci.2016.06.006

Jondreville, C., Revy, P. S. and Dourmad, J. Y. (2003): Dietary means to better control the environmental impact of copper and zinc by pigs from weaning to slaughter. *Livestock Production Science*, *84*(2), 147–156. https://doi.org/10.1016/j.livprodsci.2003.09.011

Jongbloed, A. W., Mroz, Z., Van Der Weij-Jongbloed, R. and Kemme, P. A. (2000): The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. *Livestock Production Science*, *67*(1-2), 113–122. https://doi.org/10.1016/S0301-6226(00)00179-2

Ju, F. and Zhang, T. (2015): 16S rRNA gene high-throughput sequencing data mining of microbial diversity and interactions. *Applied Microbiology and Biotechnology*, *99*(10), 4119–4129. https://doi.org/10.1007/s00253-015-6536-y

Juan, C., Mañes, J., Raiola, A. and Ritieni, A. (2013): Evaluation of beauvericin and enniatins in Italian cereal products and multicereal food by liquid chromatography coupled to triple quadrupole mass spectrometry. *Food Chemistry*, *140*(4), 755–762. https://doi.org/10.1016/j.foodchem.2012.08.021

Just, A., Fernandez, J. A. and Jorgensen, H. (1982): Nitrogen balance studies and nitrogen retention. *Physiologie Digestive Chez Le Porc*, *12*, 111–122.

Kaneko, J. J., Harvey, J. W. and Bruss, M. L. (2008): *Clinical Biochemistry of Domestic Animals*. Academic press. https://doi.org/10.1016/B978-0-12-370491-7.X0001-3

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G. and Hyland, N. P. (2015): Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in Cellular Neuroscience*, 392.

Kendra, D. F. and Dyer, R. B. (2007): Opportunities for biotechnology and policy regarding mycotoxin issues in international trade. *International Journal of Food Microbiology*, *119*(1–2), 147–151.

Kies, A. K., Gerrits, W. J. J., Schrama, J. W., Heetkamp, M. J. W., Van Der Linden, K. L., Zandstra, T. and Verstegen, M. W. A. (2005): Mineral absorption and excretion as affected by microbial phytase, and their effect on energy metabolism in young piglets. *Journal of Nutrition*, *135*(5), 1131–1138. https://doi.org/10.1093/jn/135.5.1131

Kim, H. B., Borewicz, K., White, B. A., Singer, R. S., Sreevatsan, S., Tu, Z. J. and Isaacson, R. E. (2011): Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. *Veterinary Microbiology*, *153*(1-2), 124–133. https://doi.org/10.1016/j.vetmic.2011.05.021

Kim, S. W., Holanda, D. M., Gao, X., Park, I. and Yiannikouris, A. (2019): Effcacy of a yeast cellwall extract to mitigate the effect of naturally co-occurring mycotoxins contaminating feed ingredients fed to young pigs: Impact on gut health, microbiome, and growth. *Toxins*, *11*(11). https://doi.org/10.3390/toxins11110633

Klarić, M. Š., Pepeljnjak, S., Domijan, A. and Petrik, J. (2007): Lipid peroxidation and glutathione levels in porcine kidney PK15 cells after individual and combined treatment with fumonisin B1, beauvericin and ochratoxin A. *Basic and Clinical Pharmacology and Toxicology*, *100*(3), 157–164.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F. O. (2013): Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), 1–11. https://doi.org/10.1093/nar/gks808

Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Nebbia, C. S., Petersen, A., Rose, M., Roudot, A. C., Schwerdtle, T., Vleminckx, C., Vollmer, G., … Oswald, I. P. (2018): Risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. *EFSA Journal*, *16*(5). https://doi.org/10.2903/j.efsa.2018.5242

Kócsó, D. J., Szabó-Fodor, J., Mézes, M., Balogh, K., Ferenczi, S., Szabó, A., Bóta, B. and Kovács, M. (2018): Fumonisin B 1 exposure increases Hsp70 expression in the lung and kidney of rats without inducing significant oxidative stress. *Acta Veterinaria Hungarica*, *66*(3), 394–407. https://doi.org/10.1556/004.2018.036

Kolf-Clauw, M., Sassahara, M., Lucioli, J., Rubira-Gerez, J., Alassane-Kpembi, I., Lyazhri, F., Borin, C. and Oswald, I. P. (2013): The emerging mycotoxin, enniatin B1, down-modulates the gastrointestinal toxicity of T-2 toxin *in vitro* on intestinal epithelial cells and *ex vivo* on intestinal explants. *Archives of Toxicology*, *87*(12), 2233–2241.

https://doi.org/10.1007/s00204-013-1067-8

Kopečný, J. and Bartoš, S. (1990): Activity of hydrolases in the gastrointestinal tract of goats. *Small Ruminant Research*, *3*(1), 25–35. https://doi.org/https://doi.org/10.1016/0921-4488(90)90028-5

Kornegay, E. T. and Harper, A. F. (1997): Environmental Nutrition: Nutrient Management Strategies to Reduce Nutrient Excretion of Swine. *Professional Animal Scientist*, *13*(3), 99–111. https://doi.org/10.15232/S1080-7446(15)31861-1

Kubasova, T., Davidova-Gerzova, L., Babak, V., Cejkova, D., Montagne, L., Le-Floc'h, N. and Rychlik, I. (2018): Effects of host genetics and environmental conditions on fecal microbiota composition of pigs. *PLoS ONE*, *13*(8), 1–11. https://doi.org/10.1371/journal.pone.0201901

Lallès, J.-P., Lessard, M. and Boudry, G. (2009): Intestinal barrier function is modulated by short-term exposure to fumonisin B1 in Ussing chambers. *Veterinary Research Communications*, *33*(8), 1039. https://doi.org/10.1007/s11259-009-9310-8

Lambert, D., Padfield, P. J., McLaughlin, J., Cannell, S. and O'Neill, C. A. (2007): Ochratoxin A displaces claudins from detergent resistant membrane microdomains. *Biochemical and Biophysical Research Communications*, *358*(2), 632–636.

Lammers, P. J., Kerr, B. J., Weber, T. E., Dozier, W. A., Kidd, M. T., Bregendahl, K. and Honeyman, M. S. (2008): Digestible and metabolizable energy of crude glycerol for growing pigs. *Journal of Animal Science*, *86*(3), 602–608. https://doi.org/10.2527/jas.2007-0453

Lankelma, J. M., Nieuwdorp, M., De Vos, W. M. and Wiersinga, W. J. (2015): The gut microbiota in internal medicine: Implications for health and disease. *The Netherlands Journal of Medicine*, *73*(2), 61–68.

Lawrence, R. A. and Burk, R. F. (1978): Species, tissue and subcellular distribution of non Se-dependent glutathione peroxidase activity. *The Journal of Nutrition*, *108*(2), 211–215. https://doi.org/10.1093/jn/108.2.211

Le Huërou-Luron, I. and Ferret-Bernard, S. (2015): Development of gut and gut-associated lymphoid tissues in piglets: role of maternal environment. *The Gestating and Lactating Sow*, 335–356.

Lee, T. Van Der, Zhang, H., Diepeningen, A. Van, and Waalwijk, C. (2015): Biogeography of *Fusarium graminearum* species complex and chemotypes: A review. *Food Additives and Contaminants: Part A*, *32*(4), 453–460. https://doi.org/10.1080/19440049.2014.984244

Leeuwen, P. van, Kleef, D. J. van, Kempen, G. J. M. van, Huisman, J. and Verstegen, M. W. A. (1991): The post valve T caecum cannulation technique an alternative method for chyme collection in pigs. *Journal of Animal Physiology and Animal Nutrition*, *65*, 183–193.

Lerda, D. (2017): Fumonisins in foods from Cordoba (Argentina), presence: mini review. *Toxicology Open Access*, *3*(2), 1–4.

Lessard, M., Boudry, G., Sève, B., Oswald, I. P. and Lallès, J. P. (2009): Intestinal physiology and peptidase activity in male pigs are modulated by consumption of corn culture extracts containing fumonisins. *Journal of Nutrition*, *139*(7), 1303–1307. https://doi.org/10.3945/jn.109.105023

Lewczuk, B., Przybylska-Gornowicz, B., Gajęcka, M., Targońska, K., Ziółkowska, N., Prusik, M. and Gajęcki, M. (2016): Histological structure of duodenum in gilts receiving low doses of zearalenone and deoxynivalenol in feed. *Experimental and Toxicologic Pathology*, *68*(2-3), 157–166.

Li, H., Sheppard, D. N. and Hug, M. J. (2004): Transepithelial electrical measurements with the Ussing Chamber. *Journal of Cystic Fibrosis: Official Journal of the European Cystic Fibrosis Society*, *3* (2), 123–126. https://doi.org/10.1016/j.jcf.2004.05.026

Li, P., Yang, S., Zhang, X., Huang, S., Wang, N., Wang, M., ... and He, J. (2018): Zearalenone changes the diversity and composition of caecum microbiota in weaned rabbit. *BioMed Research International*, *2018*, 10. https://doi.org/10.1155/2018/3623274

Liao, C. D., Chiueh, L. C. and Shih, D. C. (2009): Determination of zearalenone in cereals by high-performance liquid chromatography and liquid chromatography-electrospray tandem mass spectrometry. *Journal of Food and Drug Analysis*, *17*(1), 1.

Liew, W. P. P. and Mohd-Redzwan, S. (2018): Mycotoxin: Its impact on gut health and microbiota. *Frontiers in Cellular and Infection Microbiology 8,* 60. https://doi.org/10.3389/fcimb.2018.00060

Liu, Y., Wang, X., Hou, Y., Yin, Y., Qiu, Y., Wu, G. and Hu, C. A. A. (2017): Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. *Amino Acids*, *49*(8), 1277–1291. https://doi.org/10.1007/s00726-017-2450-1

Logrieco, A., Mule, G., Moretti, A. and Bottalico, A. (2002): Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. *Mycotoxins in Plant Disease*, 597–609. Springer, Dordrecht.

Loiseau, N., Debrauwer, L., Sambou, T., Bouhet, S., Miller, J. D., Martin, P. G., Viadère, J. L., Pinton, P., Puel, O., Pineau, T., Tulliez, J., Galtier, P. and Oswald, I. P. (2007): Fumonisin B1 exposure and its selective effect on porcine jejunal segment: Sphingolipids, glycolipids and transepithelial passage disturbance. *Biochemical Pharmacology*, *74*(1), 144–152. https://doi.org/10.1016/j.bcp.2007.03.031

Looft, T., Johnson, T. A., Allen, H. K., Bayles, D. O., Alt, D. P., Stedtfeld, R. D., Sul, W. J., Stedtfeld, T. M., Chai, B., Cole, J. R., Hashsham, S. A., Tiedje, J. M. and Stanton, T. B. (2012): In-feed antibiotic effects on the swine intestinal microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(5), 1691–1696. https://doi.org/10.1073/pnas.1120238109

Lopez, H. W., Leenhardt, F., Coudray, C. and Remesy, C. (2002): Minerals and phytic acid interactions: is it a real problem for human nutrition? *International Journal of Food Science and Technology*, *37*(7), 727–739. https://doi.org/10.1046/j.1365-2621.2002.00618.x

Luckey, T. D. (1972): Introduction to intestinal microecology. *The American Journal of Clinical Nutrition*, *25*(12), 1292–1294. https://doi.org/10.1093/ajcn/25.12.1292

Macfarlane, S. and Macfarlane, G. (2003): Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society*, 62(1), 67–72. https://doi.org/10.1079/PNS2002207

Marasas, W. F., Kellerman, T. S., Gelderblom, W. C., Coetzer, J. A., Thiel, P. G. and van der Lugt, J. J. (1988): Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from

Fusarium moniliforme. *Onderstepoort Journal of Veterinary Research*, *55*(4), 197–203.

Marasas, W. F. O., Rabie, C. J., Lübben, A., Nelson, P. E., Toussoun, T. A. and Wyk, P. S. van. (1987): *Fusarium napiforme*, a new species from millet and sorghum in southern Africa. *Mycologia*, *79*(6), 910–914.

Marasas, W. F. O., Riley, R. T., Hendricks, K. A., Stevens, V. L., Sadler, T. W., Gelineau-van Waes, J., Missmer, S. A., Cabrera, J., Torres, O. and Gelderblom, W. C. A. (2004): Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *The Journal of Nutrition*, *134*(4), 711–716.

Marc Rhoads, J. and Wu, G. (2009): Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids*, *37*(1), 111–122.

Maresca, M. (2013): From the gut to the brain: Journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. *Toxins*, *5*(4), 784–820.

Marin, D. E., Motiu, M. and Taranu, I. (2015): Food contaminant zearalenone and its metabolites affect cytokine synthesis and intestinal epithelial integrity of porcine cells. *Toxins*, *7*(6), 1979–1988.

Marin, S., Ramos, A. J., Cano-Sancho, G. and Sanchis, V. (2013): Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, *60*, 218–237. https://doi.org/https://doi.org/10.1016/j.fct.2013.07.047

Marounek, M., Vovk, S. J. and Skřivanová, V. (1995): Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition*, *73*(3), 463–469. https://doi.org/10.1079/bjn19950048

Martinez Tuppia, C., Atanasova-Penichon, V., Chéreau, S., Ferrer, N., Marchegay, G., Savoie, J. and Richard-Forget, F. (2017): Yeast and bacteria from ensiled high moisture maize grains as potential mitigation agents of fumonisin B1. *Journal of the Science of Food and Agriculture*, *97*(8), 2443–2452.

Martinova, E. A. (1998): Influence of sphingolipids on T lymphocyte activation. *Biochemistry Biokhimiia*, *63*(1), 102–110.

Martins, M. L., Gimeno, A., Martins, H. M. and Bernardo, F. (2002): Co-occurrence of patulin and citrinin in Portuguese apples with rotten spots. *Food Additives and Contaminants*, *19*(6), 568–574.

Mateos, I., Combes, S., Pascal, G., Cauquil, L., Barilly, C., Cossalter, A. M., Laffitte, J., Botti, S., Pinton, P. and Oswald, I. P. (2018): Fumonisin-exposure impairs age-related ecological succession of bacterial species in weaned pig gut microbiota. *Toxins*, *10*(6), 1–14. https://doi.org/10.3390/toxins10060230

Moon, S. H., Koh, S. E., Oh, Y. and Cho, H. S. (2020): Exposure to low concentrations of mycotoxins triggers unique responses from the pig gut microbiome. *Korean Journal of Veterinary Service*, *43*(1), 39–44.

Moss, M. O. and Thrane, U. (2004): *Fusarium* taxonomy with relation to trichothecene

formation. *Toxicology Letters, 153*(1), 23–28.

Motelin, G. K., Haschek, W. M., Ness, D. K., Hall, W. F., Harlin, K. S., Schaeffer, D. J. and Beasley, V. R. (1994): Temporal and dose-response features in swine fed corn screenings contaminated with fumonisin mycotoxins. *Mycopathologia, 126*(1), 27–40. https://doi.org/10.1007/BF01371170

O'Dell, B. L. (1989): Mineral interactions relevant to nutrient requirements. *The Journal of Nutrition, 119*(12), 1832–1838. https://doi.org/10.1093/jn/119.12_Suppl.1832

Oliphant, K. and Allen-Vercoe, E. (2019): Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. *Microbiome, 7*(1), 1–15. https://doi.org/10.1186/s40168-019-0704-8

Ortiz, C. S., Richards, C., Terry, A., Parra, J. and Shim, W. B. (2015): Genetic variability and geographical distribution of mycotoxigenic *Fusarium verticillioides* strains isolated from maize fields in Texas. *Plant Pathology Journal, 31*(3), 203–211. https://doi.org/10.5423/PPJ.OA.02.2015.0020

Oswald, I. P., Desautels, C., Laffitte, J., Fournout, S., Peres, S. Y., Odin, M., Le Bars, P., Le Bars, J. and Fairbrother, J. M. (2003): Mycotoxin fumonisin B1 increases intestinal colonization by pathogenic *Escherichia coli* in pigs. *Applied and Environmental Microbiology, 69*(10), 5870–5874. https://doi.org/10.1128/AEM.69.10.5870-5874.2003

Pandya, J. P. and Arade, P. C. (2016): Mycotoxin: A devil of human, animal and crop health. *Advancements in Life Sciences, 5*, 3937–3941.

Paster, N. and Barkai-Golan, R. (2008): Mouldy fruits and vegetables as a source of mycotoxins: part 2. *World Mycotoxin Journal, 1*(4), 385–396. https://doi.org/10.3920/wmj2008.x044

Patterson, J. K., Lei, X. G. and Miller, D. D. (2008): The pig as an experimental model for elucidating the mechanisms governing dietary influence on mineral absorption. *Experimental Biology and Medicine, 233*(6), 651–664. https://doi.org/10.3181/0709-MR-262

Pestka, J. J. and Smolinski, A. T. (2005): Deoxynivalenol: toxicology and potential effects on humans. *Journal of Toxicology and Environmental Health, Part B, 8*(1), 39–69.

Piva, A., Casadei, G., Pagliuca, G., Cabassi, E., Galvano, F., Solfrizzo, M., ... and Diaz, D. E. (2005): Activated carbon does not prevent the toxicity of culture material containing fumonisin B1 when fed to weanling piglets. *Journal of Animal Science, 83*(8), 1939–1947.

Placinta, C. M., D'Mello, J. F. and Macdonald, A. M. C. (1999): A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed Science and Technology, 78*(1-2), 21–37.

Plattner, R. D., Weisleder, D., Shackelford, D. D., Peterson, R. and Powell, R. G. (1992): A new fumonisin from solid cultures of *Fusarium moniliforme*. *Mycopathologia, 117*(1), 23–28. https://doi.org/10.1007/BF00497275

Pohland, A. E., Nesheim, S. and Friedman, L. (1992): Ochratoxin A: A review (technical report). *Pure and Applied Chemistry, 64*(7), 1029–1046.

Pronk, M. E. J., Schothorst, R. C. and Van Egmond, H. P. (2002): Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3-and 15-acetyldeoxynivalenol;

A review of six trichothecenes. RIVM Report 388802024, Bilthoven, p. 75.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., … Zoetendal, E. (2010): A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65. https://doi.org/10.1038/nature08821

Quan, J., Cai, G., Yang, M., Zeng, Z., Ding, R., Wang, X., Zhuang, Z., Zhou, S., Li, S., Yang, H., Li, Z., Zheng, E., Huang, W., Yang, J. and Wu, Z. (2019): Exploring the fecal microbial composition and metagenomic functional capacities associated with feed efficiency in commercial DLY pigs. *Frontiers in Microbiology*, *10*, 1–12. https://doi.org/10.3389/fmicb.2019.00052

Ramayo-Caldas, Y., Mach, N., Lepage, P., Levenez, F., Denis, C., Lemonnier, G., Leplat, J. J., Billon, Y., Berri, M., Doré, J., Rogel-Gaillard, C. and Estellé, J. (2016): Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. *ISME Journal*, *10*(12), 2973–2977. https://doi.org/10.1038/ismej.2016.77

Rauber, R. H., Oliveira, M. S., Mallmann, A. O., Dilkin, P., Mallmann, C. A., Giacomini, L. Z. and Nascimento, V. P. (2013): Effects of fumonisin B1 on selected biological responses and performance of broiler chickens. *Pesquisa Veterinaria Brasileira*, *33*(9), 1081–1086. https://doi.org/10.1590/S0100-736X2013000900006

Ren, W., Chen, S., Zhang, L., Liu, G., Hussain, T., Hao, X., Yin, J., Duan, J., Tan, B., Wu, G., Bazer, F. W. and Yin, Y. (2016): Interferon tau affects mouse intestinal microbiota and expression of IL-17. *Mediators of Inflammation*, *2016*. https://doi.org/10.1155/2016/2839232

Rice, L. G. and Ross, P. F. (1994): Methods for detection and quantitation of fumonisins in corn, cereal products and animal excreta. *Journal of Food Protection*, *57*(6), 536–540.

Richard, J. L. (2007): Some major mycotoxins and their mycotoxicoses– An overview. *International Journal of Food Microbiology*, *119*(1), 3–10. https://doi.org/https://doi.org/10.1016/j.ijfoodmicro.2007.07.019

Richards, J. D., Zhao, J., Harrell, R. J., Atwell, C. A. and Dibner, J. J. (2010): Trace mineral nutrition in poultry and swine. *Asian-Australasian Journal of Animal Sciences*, *23*(11), 1527–1534.

Riley, R. T., An, N. H., Showker, J. L., Yoo, H. S., Norred, W. P., Chamberlain, W. J., Wang, E., Merrill, A. H., Motelin, G., Beasley, V. R. and Haschek, W. M. (1993): Alteration of tissue and serum sphinganine to sphingosine ratio: An early biomarker of exposure to fumonisin-containing feeds in pigs. *Toxicology and Applied Pharmacology*, *118*(1), 105–112). https://doi.org/10.1006/taap.1993.1015

Riley, R. T., Enongene, E., Voss, K. A., Norred, W. P., Meredith, F. I., Sharma, R. P., ... and Merrill Jr, A. H. (2001): Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environmental Health Perspectives*, *109*(2), 301–308.

Rincker, M. J., Hill, G. M., Link, J. E., Meyer, A. M. and Rowntree, J. E. (2005): Effects of dietary zinc and iron supplementation on mineral excretion, body composition, and mineral status of nursery pigs. *Journal of Animal Science*, *83*(12), 2762–2774.

Romero, A., Ares, I., Ramos, E., Castellano, V., Martínez, M., Anadón, A. and Martínez, M.

(2016): Mycotoxins modify the barrier function of Caco-2 cells through differential gene expression of specific claudin isoforms: Protective effect of illite mineral clay. *Toxicology*, *353–354*, 21–33. https://doi.org/10.1016/j.tox.2016.05.003

Rumora, L., Domijan, A. M., Grubišić, T. Ž. and Peraica, M. (2007): Mycotoxin fumonisin B1 alters cellular redox balance and signalling pathways in rat liver and kidney. *Toxicology*, *242*(1-3), 31–38. https://doi.org/10.1016/j.tox.2007.09.006

Samal, L. and Behura, N. C. (2015): Prebiotics: An emerging nutritional approach for improving gut health of livestock and poultry. *Asian Journal of Animal and Veterinary Advances*, *10*(11), 724–739. https://doi.org/10.3923/ajava.2015.724.739

Sardjono, A. N., Yamashita, A. and Yoshizawa, T. (1998): Natural co-occurrence of aflatoxins and *Fusarium* mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Additves and Contaminants*, *15*(4), 377–384.

Schertz, H., Dänicke, S., Frahm, J., Schatzmayr, D., Dohnal, I., Bichl, G., Schwartz-Zimmermann, H. E., Colicchia, S., Breves, G., Teifke, J. P. and Kluess, J. (2018): Biomarker evaluation and toxic effects of an acute oral and systemic fumonisin exposure of pigs with a special focus on dietary fumonisin esterase supplementation. *Toxins*, *10*(7): https://doi.org/10.3390/toxins10070296

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H. and Robinson, C. J. (2009): Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541.

Schneeberger, E. E. and Lynch, R. D. (2004): The tight junction: a multifunctional complex. *American Journal of Physiology-Cell Physiology*, *286*(6), C1213–C1228.

Sedlak, J. and Lindsay, R. H. (1968): Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, *25*, 192–205.

Sedmikova, M., Reisnerova, H., Dufkova, Z., Barta, I. and Jilek, F. (2001): Potential hazard of simultaneous occurrence of aflatoxin B1 and ochratoxin A. *Veterinarni Medicina-Praha-*, *46*(6), 169–174.

Shier, W. T. (2000): The fumonisin paradox: A review of research on oral bioavailability of fumonisin B1, a mycotoxin produced by *Fusarium moniliforme*. *Journal of Toxicology: Toxin Reviews*, *19*(2), 161–187. https://doi.org/10.1081/TXR-100100319

Smith, G. W. (2018): Fumonisins. *Veterinary Toxicology*, 1003–1018. Elsevier.

Smith, M. C., Madec, S., Coton, E. and Hymery, N. (2016): Natural co-occurrence of mycotoxins in foods and feeds and their *in vitro* combined toxicological effects. *Toxins*, *8*(4). https://doi.org/10.3390/toxins8040094

Somogyi, M. (1952): Notes on sugar determination. *Journal of Biological Chemistry*, *195*, 19–23.

Stiles, T. (1997): The revised OECD principles of good laboratory practice. A reflection upon the impact of the proposed changes on pre-clinical safety testing: Part 1: scope, definition of terms, responsibilities. *The Quality Assurance Journal for Pharmaceutical, Health and Environmental Professionals*, *2*(1), 13–18.

Stockmann-Juvala, H., Mikkola, J., Naarala, J., Loikkanen, J., Elovaara, E. and Savolainen, K. (2004): Oxidative stress induced by fumonisin B1 in continuous human and rodent neural cell cultures. *Free Radical Research*, *38*(9), 933–942.

Stockmann-Juvala, H. and Savolainen, K. (2008): A review of the toxic effects and mechanisms of action of fumonisin B1. *Human and Experimental Toxicology*, *27*(11), 799–809. https://doi.org/10.1177/0960327108099525

Stoev, S. D., Vitanov, S., Anguelov, G., Petkova-Bocharova, T. and Creppy, E. E. (2001): Experimental mycotoxic nephropathy in pigs provoked by a diet containing ochratoxin A and penicillic acid. *Veterinary Research Communications*, *25*(3), 205–223.

Suttle, N. F. (2010): The mineral nutrition of livestock, 4th Edition. Wallingford, Oxfordshire: CABI Publishing.

Swamy, H. V. L. N., Smith, T. K., MacDonald, E. J., Karrow, N. A., Woodward, B. and Boermans, H. J. (2003): Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological measurements of starter pigs, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *Journal of Animal Science*, *81*(11), 2792–2803. https://doi.org/10.2527/2003.81112792x

Szecsi, A., Szekeres, A., Bartok, T., Oros, G., Bartok, M. and Mesterhazy, A. (2010): Fumonisin B1-4-producing capacity of Hungarian *Fusarium verticillioides* isolates. *World Mycotoxin Journal*, *3*(1), 67–76.

Taranu, I., Braicu, C., Marin, D. E., Pistol, G. C., Motiu, M., Balacescu, L., Neagoe, I. B. and Burlacu, R. (2015): Exposure to zearalenone mycotoxin alters *in vitro* porcine intestinal epithelial cells by differential gene expression. *Toxicology Letters*, *232*(1), 310–325.

Tenk, I., Fodor, É. and Szathmáry, C. (1982): The effect of pure fusarium toxins (T-2, F-2, DAS) on the microflora of the gut and on plasma glucocorticoid levels in rat and swine. *Zentralblatt Für Bakteriologie, Mikrobiologie Und Hygiene. 1. Abt. Originale. A, Medizinische Mikrobiologie, Infektionskrankheiten Und Parasitologie*, *252*(3), 384–393. https://doi.org/https://doi.org/10.1016/S0174-3031(82)80013-9

Terciolo, C., Bracarense, A. P., Souto, P. C. M. C., Cossalter, A. M., Dopavogui, L., Loiseau, N., Oliveira, C. A. F., Pinton, P. and Oswald, I. P. (2019): Fumonisins at doses below EU regulatory limits induce histological alterations in piglets. *Toxins*, *11*(9). https://doi.org/10.3390/toxins11090548

Tóth, Á., Zomborszky-Kovács, M., Tornyos, G., Szalai, N. and Kübler, K. (2000): Effect of low doses of the mycotoxin fumonisin B1 on the body mass gain, feed intake and feed conversion rate of pigs. *Agriculture*, *6*, 149–151.

Ueno, Y. (1984): Toxicological features of T-2 toxin and related trichothecenes. *Toxicological Sciences*, *4*(2part2), 124–132. https://doi.org/10.1093/toxsci/4.2part2.124

Urry, W. H., Wehrmeister, H. L., Hodge, E. B. and Hidy, P. H. (1966): The structure of zearalenone. *Tetrahedron Letters*, *7*(27), 3109–3114. https://doi.org/10.1016/S0040-4039(01)99923-X

Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M. and De Angelis, M. (2020): The controversial role of human gut lachnospiraceae. *Microorganisms*, *8*(4), 1–25.

https://doi.org/10.3390/microorganisms8040573

Vesonder, R. F., Ciegler, A. and Jensen, A. H. (1973): Isolation of the emetic principle from *Fusarium*-infected corn. *Applied Microbiology*, *26*(6), 1008–1010.

Voss, K. A., Chamberlain, W. J., Bacon, C. W. and Norred, W. P. (1993): A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin B1. *Natural Toxins*, *1*(4), 222–228. https://doi.org/https://doi.org/10.1002/nt.2620010404

Vrabcheva, T., Usleber, E., Dietrich, R. and Märtlbauer, E. (2000): Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy. *Journal of Agricultural and Food Chemistry*, *48*(6), 2483–2488.

Walter, L., Rauh, F. and Günther, E. (1994): Comparative analysis of the three major histocompatibility complex-linked heat shock protein 70 (Hsp70) genes of the rat. *Immunogenetics*, *40*(5), 325–330. https://doi.org/10.1007/BF01246673

Wang, D., Thomas, A. and Lindemann, M. D. (2018): Supplementation of sodium bentonite clay did not alleviate the negative effect of fumonisin B1 contaminated corn on feed preference and nutrient digestibility in weanling pigs. *Journal of Animal Science*, *96*, 184.

Wang, S., Yang, J., Zhang, B., Zhang, L., Wu, K., Yang, A., Li, C., Wang, Y., Zhang, J. and Qi, D. (2019): Potential link between gut microbiota and deoxynivalenol-induced feed refusal in weaned piglets. *Journal of Agricultural and Food Chemistry*, *67*(17), 4976–4986. https://doi.org/10.1021/acs.jafc.9b01037

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R. and Knight, R. (2017): Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 1–18. https://doi.org/10.1186/s40168-017-0237-y

Wilfart, A., Montagne, L., Simmins, P. H., Van Milgen, J. and Noblet, J. (2007): Sites of nutrient digestion in growing pigs: Effect of dietary fiber. *Journal of Animal Science*, *85*(4), 976–983. https://doi.org/10.2527/jas.2006-431

Willing, B. P. and Van Kessel, A. G. (2007): Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. *Journal of Animal Science*, *85*(12), 3256–3266. https://doi.org/10.2527/jas.2007-0320

Windisch, W. (2002): Interaction of chemical species with biological regulation of the metabolism of essential trace elements. *Analytical and Bioanalytical Chemistry*, *372*(3), 421–425. https://doi.org/10.1007/s00216-001-1117-6

Wu, G. (2009): Amino acids: metabolism, functions, and nutrition. *Amino Acids*, *37*(1), 1–17.

Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J. and Wu, Z. (2014): Amino acid nutrition in animals: protein synthesis and beyond. *Annual Review of Animal Biosciences*, *2*(1), 387–417.

Xia, Y. and Sun, J. (2017): Hypothesis testing and statistical analysis of microbiome. *Genes and Diseases*, *4*(3), 138–148. https://doi.org/10.1016/j.gendis.2017.06.001

Yamazoe, Y., Koyama, N. and Kumagai, S. (2017): Possible role of phosphatidylcholine and sphingomyelin on fumonisin B1-mediated toxicity. *Food Safety*, *5*(3), 75–97.

https://doi.org/10.14252/foodsafetyfscj.2017004

Yang, Z. (2019): Determination of true micro-mineral digestibility and their endogenous losses in weanling pigs by the regression analysis (Doctoral dissertation, University of Guelph).

Yazar, S. and Omurtag, G. Z. (2008): Fumonisins, trichothecenes and zearalenone in cereals. *International Journal of Molecular Sciences*, *9*(11), 2062–2090.

Yu, S., Jia, B., Lin, H., Zhang, S., Yu, D., Liu, N. and Wu, A. (2022): Effects of fumonisin B and hydrolyzed fumonisin B on growth and intestinal microbiota in broilers. *Toxins*, *14*(3). https://doi.org/10.3390/toxins14030163

Zain, M. E. (2011): Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, *15*(2), 129–144. https://doi.org/10.1016/j.jscs.2010.06.006

Zhang, F., Chen, Z., Jiang, L., Chen, Z. and Sun, H. (2021): Response of fecal bacterial flora to the exposure of fumonisin b1 in balb/c mice. *Toxins*, *13*(9). https://doi.org/10.3390/toxins13090612

Zhang, Y., Jia, Z., Yin, S., Shan, A., Gao, R., Qu, Z., Liu, M. and Nie, S. (2014): Toxic effects of maternal zearalenone exposure on uterine capacity and fetal development in gestation rats. *Reproductive Sciences*, *21*(6), 743–753. https://doi.org/10.1177/1933719113512533

Zhao, H., Wang, X., Zhang, J., Zhang, J. and Zhang, B. (2016): The mechanism of *Lactobacillus* strains for their ability to remove fumonisins B1 and B2. *Food and Chemical Toxicology*, *97*, 40–46. https://doi.org/10.1016/j.fct.2016.08.028

Zhao, Z., Zhang, Y., Gong, A., Liu, N., Chen, S., Zhao, X., Li, X., Chen, L., Zhou, C. and Wang, J. (2019): Biodegradation of mycotoxin fumonisin B1 by a novel bacterial consortium SAAS79. *Applied Microbiology and Biotechnology*, *103*(17), 7129–7140. https://doi.org/10.1007/s00253-019-09979-6

Zhong, X., Zhang, Z., Wang, S., Cao, L., Zhou, L., Sun, A., Zhong, Z. and Nabben, M. (2019): Microbial-driven butyrate regulates jejunal homeostasis in piglets during the weaning stage. *Frontiers in Microbiology*, *10*, 1–18. https://doi.org/10.3389/fmicb.2018.03335

Zhou, H., George, S., Hay, C., Lee, J., Qian, H. and Sun, X. (2017): Individual and combined effects of aflatoxin B 1, deoxynivalenol and zearalenone on HepG2 and RAW 264.7 cell lines. *Food and Chemical Toxicology*: an international journal published for the *British Industrial Biological Research Association*, 103, 18–27. https://doi.org/10.1016/j.fct.2017.02.017

Zhou, J., Tang, L. and Wang, J. (2019): Chemosphere assessment of the adverse impacts of aflatoxin B1 on gut-microbiota dependent metabolism in F344 rats. *Chemosphere*, *217*, 618–628. https://doi.org/10.1016/j.chemosphere.2018.11.044

Zoetendal, E. G., Cheng, B., Koike, S. and Mackie, R. I. (2004): Molecular microbial ecology of the gastrointestinal tract: from phylogeny to function. *Current Issues in Intestinal Microbiology*, *5*(2), 31–48.

Zomborszky-Kovács, M., Vetési, F., Horn, P., Repa, I. and Kovács, F. (2002): Effects of Prolonged Exposure to Low-dose fumonisin B1 in pigs. *Journal of Veterinary Medicine, Series B*, *49*(4), 197–201. https://doi.org/10.1046/j.1439-0450.2002.00519.x

Zou, Y., Yang, Z. B., Yang, W. R., Jiang, S. Z., Zhang, G. G. and Chi, F. (2012): Effect of purified zearalenone on nutrient digestibility in broilers fed 2 levels of fumonisin from naturally contaminated corn (*Zea mays*). *Journal of Applied Poultry Research*, *21*(2), 251–258. https://doi.org/10.3382/japr.2011-00359

12.PUBLICATIONS AND PRESENTATIONS

List of publications relevant to the research

1. Zeebone, Y.Y., Kovács, M., Bóta, B. and Halas, V. (2022): The effect of dietary fumonisin exposure on apparent ileal digestibility of amino acids in fattening pigs. *Agriculture, 12*(10), 1720. https://doi.org/10.3390/agriculture12101720
2. Zeebone, Y. Y., Kovács, M., Bóta, B., Zdeněk, V., Taubner, T. and Halas, V. (2022): Dietary fumonisin may compromise the nutritive value of feed and distort copper and zinc digestibility and retention in weaned piglets. *Journal of Animal Physiology and Animal Nutrition,* 1–14. https://doi.org/10.1111/jpn.13724
3. Zeebone, Y.Y., Kovács, M., Bota, B., Balogh, K. and Halas, V. (2022): P164. Dietary fumonisins potentially modulate some health indicators in weaned piglets. *Animal Science Proceedings, 13*(2), 208.
4. Zeebone, Y.Y., Kovács, M., Bóta, B. and Halas, V. (2020): Effects of dietary fumonisins on nutrients digestibility in weanling pigs. *Acta Fytotechnica et Zootechnica, 23,* 23–28. https://doi.org/10.15414/afz.2020.23.mi-fpap.23-28
5. Zeebone, Y.Y., Kovács, M. and Halas, V. (2020): Effects of fumonisin B_1 on the gastrointestinal tract functionality. A review. (A fumonizin B1 hatása a tápcsatorna működésére. Irodalmi áttekintés). *Állattenyésztés és Takarmányozás, 69*(1), 53–65.

List of other publications

6. Kócsó, D.J., Ali, O., Kovács, M., Mézes, M., Balogh, K., Kachlek, M.L., Bóta, B., Zeebone, Y.Y. and Szabó, A. (2021): A preliminary study on changes in heat shock protein 70 levels induced by *Fusarium* mycotoxins in rats: in vivo study. *Mycotoxin Research, 37*(2), 141–148. https://doi.org/10.1007/s12550-021-00425-z
7. Szabó, A., Szabó-Fodor, J., Kachlek, M., Mézes, M., Balogh, K., Glávits, R., Ali, O., Zeebone, Y.Y. and Kovács, M. (2018): Dose and exposure time-dependent renal and hepatic effects of intraperitoneally administered fumonisin B_1 in rats. *Toxins, 10*(11), 465. https://doi.org/10.3390/toxins10110465
8. Boateng, M., Okai, D.B., Frimpong, Y.O. and Zeebone, Y.Y. (2015): Wet brewers' spent grains and wet brewers' spent yeast: problems associated with their usage and suggested solutions: a case study of the Ejisu-Juaben Municipality of Ghana. *Age, 2*(8), 6. Retrieved January 5, 2023, from http://www.lrrd.org/lrrd27/1/boat27005.htm

Oral presentations

1. Zeebone Y.Y., Kovács, M., Bóta, B. and Halas, V. **Effect of dietary fumonisin exposure on apparent ileal digestibility of amino acids in fattening pigs**. The 29th Animal Science Days International Symposium, September 13–17, 2021, Gödöllő, Hungary.
2. Zeebone, Y.Y., Kovács, M., Bóta, B. and Halas, V. **Effects of dietary fumonisins on nutrients digestibility in weanling pigs**. The 28th Animal Science Days International Symposium, September 23–25, 2020, Padova, Italy.
3. Zeebone Y.Y., Kovács, M. and Szabo-Fodor, J. **Dose dependent oxidative stress-mediated toxicity of fumonisin B_1 in rats**. The Scientific Student Conference in Applied Life Sciences, November 21, 2018, Faculty of Agricultural and Environmental Sciences, Hungarian University of Agriculture and Life Sciences (then Kaposvári Egyetem), Kaposvár, Hungary.

Poster presentations

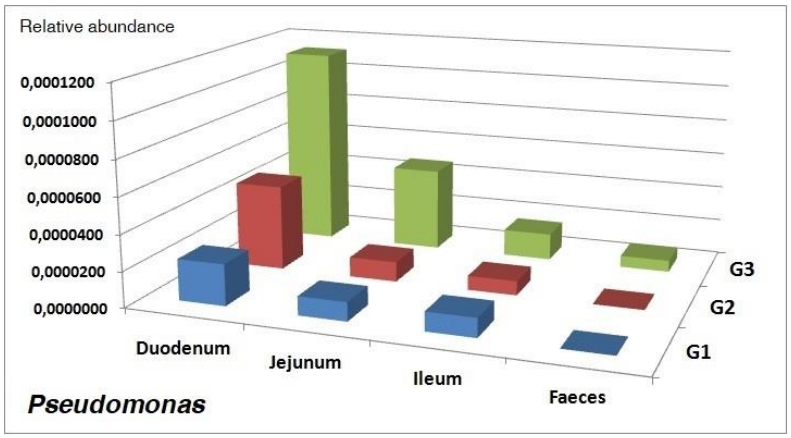
4. Zeebone, Y.Y., Kovács, M., Bóta, B., Libisch, B., Olasz, F. and Halas, V. **Potential effect of dietary fumonisins on the colonization of *Lactobacilli* in the gut and fecal microbiota of weaned pigs.** Hungarian University of Agriculture and Life Sciences 20th International Symposium on Animal Nutrition, KÁN University Days, September 29, 2022, Kaposvár, Hungary.
5. Zeebone, Y.Y., Kovács, M., Bota, B., Balogh, K. and Halas, V., 2022. **Dietary fumonisins potentially modulate some health indicators in weaned piglets**. The 15th International Symposium on Digestive Physiology of Pigs, May 17–20, 2022, Rotterdam, The Netherlands.

13. CURRICULUM VITAE

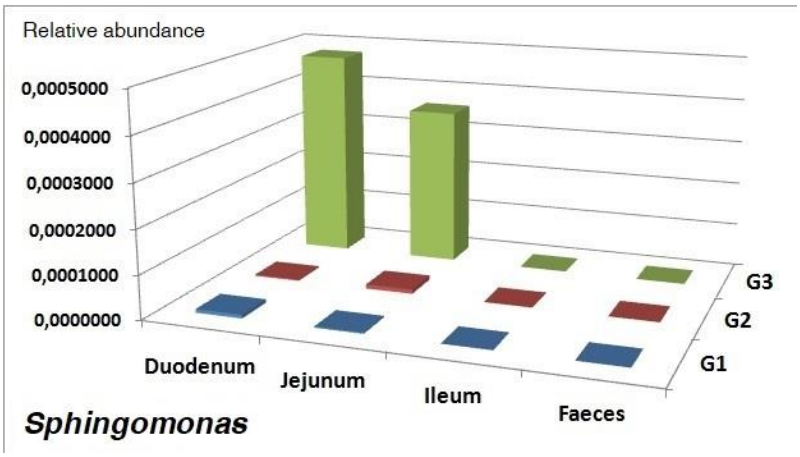
Yarsmin Yunus Zeebone graduated with a Bachelor's (BSc) degree in Agriculture (Animal Science major) in 2014 from the Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana. She served as a Teaching Assistant at KNUST's Department of Animal Science from 2014 to 2015. For a few months in 2016, she worked as a customer service assistant at the Abuakwa branch of the Ghana Water Company Limited in Kumasi, Ghana. Later that year, she was awarded a scholarship by the United Nations' Food and Agriculture Organization (FAO) and the Ministry of Agriculture of Hungary to pursue an MSc degree in Animal Nutrition and Feed Safety Engineering at the Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár Campus (then Kaposvári Egyetem). In May 2018, she received the Special Award of the Faculty's Scientific Student Conference in Applied Life Sciences Session held at MATE. After earning her MSc in Animal Nutrition and Feed Safety Engineering in June 2018, she continued her education at the same university– the Doctoral School of Animal Science– to pursue a PhD. Her doctoral dissertation focused on examining the effects of dietary fumonisins on gastrointestinal tract functions of pigs under the supervision of Prof. Dr. Melinda Kovács and Dr. Veronika Halas. Following the presentation of their paper entitled "Effects of dietary fumonisins on nutrients digestibility in weanling pigs," she was awarded the best presentation at the "Feeding and Nutrition Session" of the 28th Animal Science Days International Symposium held in Italy in September 2020. During her study, she worked as a research assistant with the Mycotoxins in the Food Chain Research Group (MTA-KE-SZIE, later: ELKH-MATE) where she assisted with the various experiments undertaken within a project. Additionally, she gave lectures and assisted with laboratory practical lessons in the "Nutritional Physiology" course to MSc students enrolled in the English-taught programme Animal Nutrition and Feed Safety Engineering.

14. APPENDIX

A



B



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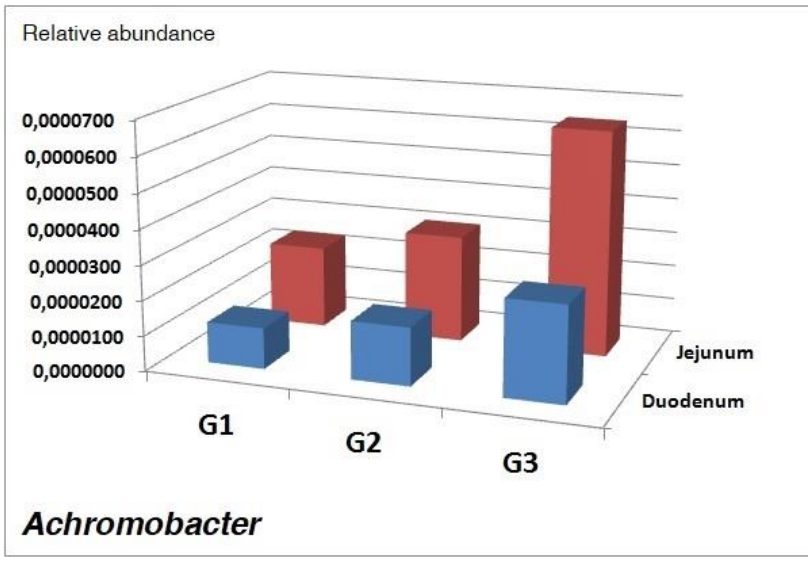

Picture documentation of mild forms of porcine pulmonary oedema as seen by fluids accumulation in the interstices of lungs of a weaned pig exposed to either 15 mg/kg another exposed to 30 mg/kg FUMs contaminated diet for 21