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FUMONISIN B SERIES' IMPLICATIONS ON BIOMEMBRANES' FATTY ACID COMPOSITIONS OF SPECIFIC TISSUES FROM RATS, SWINE AND RABBITS

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QUOTES

"Pursue knowledge from cradle to grave"

Prophet Muhammad ﷺ (570-632)

"Knowledge is not simply acquired; one must actively pursue it"

Malik ibn Anas (711-795)

"Discovery is seeing the same thing as everyone, yet thinking differently"

Albert Szent-Györgyi (1893-1996)

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACL	Average chain length
ALA	α-linolenic acid
AP	Aminopentol backbone
ATP	Adenosine triphosphate
BW	Body weight
CAT	Catalase
CCT	Cytidine 5′-triphosphate:phosphocholine cytidylyltransfease
CDP	Cytidine 5′-diphosphocholine
CerS	Ceramide synthase
CHOL	Cholesterol
CTP	Cytidine 5′-triphosphate
D5D	Δ5-desaturase
D6D	Δ6-desaturase
Da	Dalton
DAG	Diacylglycerol
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
ELEM	Equine leukoencephalomalacia
ELOVL	Elongase gene
EPA	eicosapentaenoic acid
ER	Endoplasmic reticulum
EFSA	European Food Safety Authority
FAD	Fatty acid desaturase enzyme
FADS	Fatty acid desaturase gene
FAO	Food and Agriculture Organization of the United Nations
FB_1	Fumonisin B1
FBs	Fumonisin B series
FFA	Free fatty acid

FUM	Fumonisin
GI	Gastrointestinal
GIT	Gastrointestinal tract
GPI	Glycosyl-phosphatidylinositol
GPx	Glutathione peroxidase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HFB	Hydrolyzed fumonisin B
IARC	International Agency for Research on Cancer
LA	Linoleic acid
LOAEL	Lowest observed adverse effect level
LXR	Liver-X-Receptor
MAM	Mitochondrial-associated membranes
MDA	Malonaldehyde
MUFA	Monounsaturated fatty acids
ΣMUFA	Sum of monounsaturated fatty acids
MW	Molecular weight
n3	Omega-3 fatty acid
Σn3	Sum of omega-3 fatty acids
n6	Omega-6 fatty acid
Σn6	Sum of omega-6 fatty acids
Σn6: Σn3	Omega-6 to omega-3 ratio
Na^+/K^+ ATPase	An enzyme, sodium-potassium pump
NAPE	*N*-acyl-phosphatidylethanolamine
NOAEL	No observed adverse effect level
Nrf2	Nuclear factor erythroid-2
NTP	National Toxicology Program
$O_2^{\cdot -}$	Superoxide anion
$HO^{\bullet}$	Hydroxyl radical
Orm/ORMDL	Family of proteins
PA	Phosphatidic acid

PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine *N*-methyltransferase
pHFB	Partially hydrolyzed fumonisin B
PI	Phosphatidylinositol
PLA2	Phospholipase A2
PLC	Phospholipase C
PLD	Phospholipase D
PPE	Porcine pulmonary edema
PS	Phosphatidylserine
PSD	Phosphatidylserine decarboxylase
PSS	Phosphatidylserine synthase
PUFA	Polyunsaturated fatty acids
ΣPUFA	Sum of polyunsaturated fatty acids
RLEM	Rabbit leukoencephalomalacia
ROS	Reactive oxygen species
Sa	Sphinganine
SFA	Saturated fatty acid
ΣSFA	Sum of saturated fatty acids
SCD	Stearoyl-CoA desaturase
SM	Sphingomyelin
Sn	Stereospecific numbering
So	Sphingosine
SOD	Superoxide dismutase
SREBP	Sterol regulatory element-binding protein
TBARS	Thiobarbituric acid reactive substance
TCA	Tricarboxylic acid
TDI	Tolerable daily intake
UFA	Unsaturated fatty acid
UI	Unsaturation index
ZEN	Zearalenone

1. INTRODUCTION

Fungi or molds can be considered beneficial to mankind, animals, and the environment by playing essential roles in biotechnological industries such as food, beverages, and pharmaceuticals, as well as in the nutrient cycling processes (Read and Perez-Moreno, 2003; Shankar and Sharma, 2022). However, a large number of natural secondary metabolic products are produced through multiple pathways by toxigenic fungal species, which may not be essential for their viability. These secondary metabolites are undesirable substances with low molecular weights (MW ~700 Da) that cause a wide range of deleterious effects on vertebrates and plants, either directly or indirectly (through transformation), leading to mycotoxicoses (Bennett and Klich, 2003; Turner *et al.*, 2009). According to the available literature, worldwide mycotoxin contamination in foods and livestock feedstuffs is reported to be above 60% (Eskola *et al.*, 2020; Gruber-Dorninger *et al.*, 2019; Jalilzadeh-Amin *et al.*, 2023; Patriarca and Fernández Pinto, 2017; Santos Pereira *et al.*, 2019; Streit *et al.*, 2013). Hence, these works in the literature raise uncertainty regarding the earlier cited estimation of the Food and Agriculture Organization of the United Nations (FAO) (Boutrif and Canet, 1998), which stated that 25% of the world's agricultural products are contaminated.

Mycotoxins were discovered earlier, in the medieval period, as demonstrated by the recognition of ergot, which implicated a toxicological disease referred to as St. Anthony's fire (Bennett and Klich, 2003). Upon then, these compounds have received a great deal of attention, with a focus on their identification and biological impacts. To date, around 500,000 secondary metabolic products have been indexed in databases, among which 15,600 are derived from fungi (Bills and Gloer, 2016). Notably, a collection of various genera of fungi have been identified to contribute to these metabolites; however, specific genera like *Aspergilli*, *Fusaria*, *Penicillium*, *Alternaria*, and *Claviceps* have received increased attention, primarily due to their acknowledged mycotoxigenic activities (El-Sayed *et al.*, 2022; Pandey *et al.*, 2023). *Fusarium* species are highly prevalent in soil and food/feed crops, where they produce fusariotoxins, a family of secondary toxic metabolites produced by the genus *Fusarium*, notably including trichothecenes (such as deoxynivalenol (DON) and T-2 toxin), fumonisins (FUMs), zearalenone (ZEN), emerging mycotoxins (e.g., enniatins, beauvericin, fusaproliferin, and fusaric acid), and other mycotoxins. DON, ZEN, FUMs, and T-2 are apparently the most prevalent and studied mycotoxins worldwide, demonstrating 70%, 59%, 57%, and 15% prevalence risk, respectively, with an overall mycotoxin detection rate of 99% among all samples (dsm-firmenich, 2023). The existence of fusariotoxins has been observed to take a few molecular

structural appearances, including free, masked (plant material conjugated), and modified/transformed forms (modified either chemically or biologically via hydroxylation, hydrolysis, or thermal treatment) incorporating derivatives of acetyl, conjugated forms such as sulfate, sulfonate, and glucuronide, products that have undergone isomerization, and substances that have been hydrolyzed (Berthiller *et al.*, 2013; Ekwomadu *et al.*, 2021; Rychlik *et al.*, 2014). Therefore, these toxins vary in their structures and, consequently, their toxic active molecules/groups which define their modes of action and target animal species and within those organs/tissues.

FUMs represent a category of mycotoxins that are non-fluorescent and primarily synthesized by specific fungi, predominantly *Fusarium* species. Considerable concerns have been expressed worldwide, emphasizing its high prevalence and toxic effects (evident and potential) in plants, animals, and humans (Rheeder *et al.*, 2002). A large number of FUM isomers have been identified to date that vary in their degree of toxicity. FUM B_1 (FB_1) is, in fact, the most toxic structure of FUMs, displaying numerous adverse effects on vertebrates (Voss and Riley, 2013). This phenomenon of FUM toxicity is academically referred to as the "fumonisin paradox", a term coined to describe the riddle status surrounding the diminished bioavailability of FUM (3-5%) compared to its potent toxic effect (Shier, 2000). Among the dynamic ranges of toxicity caused by FUM is the disruption of cellular membranes. This perturbation is primarily attributed to its interference with sphingolipid metabolism, resulting in competition with ceramide synthase (CerS), a key enzyme involved in the metabolism of sphingolipids. This mechanism displays specific features, including the proportional increase of sphinganine (Sa) and, to a lesser extent, liberated sphingosine (So) (Riley and Merrill, 2019). The disruption of sphingolipid metabolism induced by FUMs is crucial, leading to modifications in the composition of membrane lipids and, consequently, the disintegration of cell membranes (Burger *et al.*, 2018). This disruption may have far-reaching consequences and possibly affect most of the major metabolic pathways that determine the sustainability of growth and the death of cells. Furthermore, the manipulation of membrane lipids induced by FUMs could also reveal these lipids as potential biomarkers of the toxicity of FUMs as well as the potential presence of histological lesions in different organs. Thus, this thesis is primarily aimed at assessing the effects of FUM B (FBs) on the fatty acids of membrane lipids. The plausible FBs-dose- and time-dependent effects of responsive long-chain fatty acids were also assessed, which may contribute to an in-risk assessment approach.

2. LITERATURE REVIEW

2.1. Biological membranes

Biological membranes (biomembranes) are essential elements of cells (both prokaryotic and eukaryotic cells) (Harayama and Riezman, 2018; Watson, 2015), and are mainly located on the outer surface and surrounding cell organelles; thus, their contents are distinguished from those in the environments around them. Despite this isolative feature, membranes permit, to some extent, an exchange process by which molecules transit in and out of the cell/organelle. This movement appears to occur in a selective manner to modulate a wide range of complex operations, such as intercellular communication, proliferation, differentiation, secretion, migration, invasion, and phagocytosis. The accomplishment of these biological processes demands the necessity of different structures, and thus, biomembranes are characterized by heterogeneity, varying from one cell/tissue to another and displaying distinct structures across cells and organelles. From a structural perspective, the plasma membrane exhibits asymmetrical bilayers (Janmey and Kinnunen, 2006), at which each leaflet provides certain functions. In the scientific community, these diverse structures have attracted a respected number of researchers to investigate their dynamic roles in cellular homeostasis, response to external elements, and modulation of disease progression or inhibition.

The diversity of membranes is attributed to various molecules situated within membranes (including lipids, proteins, and carbohydrates) and their collective interaction to produce conjugated compounds that vary in their properties compared to their individual parent structures. Notably, the membrane lipid matrix constitutes a major proportion of the membrane matrix and is composed of various structures exhibiting amphipathic properties. The fatty acid composition of membranes has been implicated in the modulation of various biological processes (Gelderblom *et al.*, 2002); thus, these components have received remarkable attention over the past decades. In this regard, investigating membrane fatty acids would provide an in-depth understanding of numerous related cellular events, alike normal and pathophysiological events.

2.1.1. Glycerophospholipids

Glycerophospholipids, also referred to simply as phospholipids, are fundamentally lipids that contain phosphorus (see Figure 1). They are primarily located in biomembranes and represent 50–60 mol% of the overall membrane lipid matrix (van Meer *et al.*, 2008). Their earliest identification was a landmark by Vauquelin (1811), who determined phosphorus from a brain lipid extract, making them intriguing compounds to investigate. Structurally, phospholipids resemble a diacylglycerol backbone (DAG), but

at the *sn*-3 position, an additional polar phosphorus group (possibly free or bound to another moiety) is attached. This additional group implies different physicochemical properties compared to those of DAG. For instance, the head group features hydrophilic properties, thus contributing to the overall amphipathic properties of phospholipids, a crucial factor determining the interactivity between membrane constituents and phospholipids. Over the past century, an enormous number of various phospholipids that can vary in structure within the same cell have been identified (van Meer *et al.*, 2008). This diversity emphasizes the profound functionality exhibited by these structures, which immensely contributes (directly and indirectly) to the sustainability of various metabolic pathways. Notably, under normal conditions, depending on the head group, phospholipids tend to occupy a defined leaflet of the bilayer. In this section, a selective discussion including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) has been carried out, aligning with the investigations performed in the dissertation.

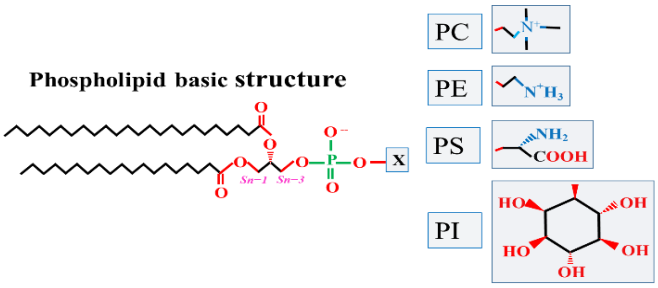


Figure 1. The molecular structures of different phospholipids have been adapted from the PubChem database (an open database for the public, available at https://pubchem.ncbi.nlm.nih.gov, accessed on 31 May 2024). Abbreviation: *Sn*, stereospecific numbering in the glycerol; ⁝ and ▲, chiral carbon centers.

2.1.1.1. Phosphatidylcholine

PC, also known as lecithin, was the first isolated phospholipid in 1850, with choline (a source of the methyl group) contributing to the formation of its polar head (Zeisel, 2012). Herein, it is very self-evident that the PC structure is not entirely endogenous, as choline is an essential nutrient for mammals. PC is a ubiquitous compound present in all cell membranes, ranging from prokaryotic cells (e.g., bacteria) to eukaryotic cells (i.e., cells of plants and animals). Structurally, PC exhibits two major linkage types in tissues: diacyl-PC (ester bond; most abundant in eukaryotes) and alkyl-PC (featuring an ether bond) (Lassègue *et al.*, 1993). In addition, a rare PC isomer is alkenyl-PC (vinyl ether bond), which is otherwise known as choline plasmalogen and plasmanylcholine. This lipid typically comprises two fatty acids linked to glycerol through ether and ester bonds at *sn*-1 and *sn*-2, respectively (Bozelli *et al.*, 2021; Koivuniemi, 2017).

PC represents the most abundant phospholipid class (making up about 50% of all phospholipids in the bilayers), especially in the lung surfactant, where dipalmitoyl-PC predominates (Goss *et al.*, 2013; Robertson, 1983). As the main bound phospholipid of the membrane bilayer, PC occupies the outer sheet (Li *et al.*, 2016). Notably, about 80 to 90% of the lipids in the outer leaflet of the plasma membrane are PCs. The ER is the primary site for PC synthesis and fundamentally involves numerous enzymes. However, one of the most important enzymes in this pathway is nuclear/cytosolic cytidine 5′-triphosphate (CTP):phosphocholine cytidylyl transferase (PCT), which is also commonly known as CCT (Sarri *et al.*, 2011; Vance and Choy, 1979). This enzyme is essential for the rate-limiting step of the cytidine 5′-diphosphocholine (CDP-choline, citicoline, or Kennedy) pathway (Kennedy, 1956). This CDP moiety is involved not only in PC biosynthesis (Cornell and Ridgway, 2015) but also in all other phospholipids, apart from phosphatidic acid (PA), depending on which moiety replaces choline. A distinctive pathway for PC biosynthesis exclusively takes place in the liver, where PC is generated from PE via sequential methylation (Vance, 1996), facilitated by the phosphatidylethanolamine *N*-methyltransferase (PEMT) that is found in the mitochondrial-associated membranes (MAMs).

PCs are believed to have relatively neutral molecular properties (having positive and negative charges but lacking a net charge), and their predominance plays an essential role in maintaining biomembrane integrity and functionality. Unlike other phospholipids, PC does not exhibit negative charge repulsion. PC serves as a precursor for sphingomyelin (SM) due to its choline molecule (Dawson, 2015). In addition, it acts as a precursor for other polar lipids, such as PA, lysophosphatidylcholines (LysoPCs), PS, and PAF. PC plays a crucial part in cell signaling processes and impacts the concentration of circulating lipoproteins (Cui and Houweling, 2002; Kanno *et al.*, 2007). Furthermore, it is integral to membrane trafficking and molecular transportation. LysoPC composed of docosahexaenoic acid (C22:6 n3, or DHA) at the *sn*-2 position has been demonstrated to be more effective than liberal DHA in crossing the blood-brain barrier (Lagarde *et al.*, 2016).

2.1.1.2. Phosphatidylethanolamine

Following PC in discovery and abundancy (15–25%) is PE, formerly known as “cephalin” (Patel and Witt, 2017; Thudichum, 1884). In neural tissues, PE can reach even higher levels, up to 45% (Vance and Tasseva, 2013), underscoring its essential role in neural tissue. In mammalian cells, PE tends to accumulate more in mitochondrial membranes and is exclusively localized in the cytosolic leaflet of the plasma membrane, in contrast to PC (Li *et al.*, 2016). This localization proposes a distinction in their structure and functions as well. The structure of PE involves the esterification of the phosphatidyl

group to the hydroxyl group of an amino group (namely, ethanolamine), resulting in a small reactive head group. PE does not form a bilayer independently (on its own) but exhibits an inverted hexagonal phase. This class of lipids features various linkages, including diacyl, alkyl, and alkenyl configurations. Ethanolamine plasmalogens, also known as plasmenylethanolamines, are more abundant than plasmenylcholine in many tissue types, except for the heart and smooth muscle (Bozelli *et al.*, 2021).

In eukaryotes, the biosynthesis of PE is an outcome of multiple pathways, notably the *de novo* synthesis of PE through CDP-ethanolamine (Kennedy, 1956) and the salvage pathway involving the decarboxylation of PS by phosphatidylserine decarboxylase (PSD) in the mitochondria (Borkenhagen *et al.*, 1961). Additional pathways involved in the remodeling of PE, which are also identified in bacteria and plants, include the following: (1) the base-exchange pathway between PE and PS (Sundler *et al.*, 1974); (2) the degradation of sphingosine-P via sphingosine-P lyase (Van Veldhoven *et al.*, 2000); and (3) the reacylation of Lyso-PE at MAMs (Riekhof *et al.*, 2007). Notably, despite the structural resemblance of PE and PC, PE exhibits distinct chemical and biological properties. PE stands apart from PC due to its smaller head group, which manifests less affinity for water. Consequently, PE can undergo compact aggregation and display heightened thermostability (Bouchet *et al.*, 2009; McIntosh, 1980). These attributes significantly contribute to membrane rigidity, making PE an indispensable component of membrane architecture, permeability, and fluidity.

In terms of membrane rigidity, PE often mimics the behavior of CHOL, particularly in insects (Dawaliby *et al.*, 2016). Considering these findings, the PC/PE ratio is likely to exert a substantial influence on the functionality, fluidity, stability (van der Veen *et al.*, 2017), and permeability of the membrane. Furthermore, PE plays a vital role in maintaining membrane integrity and participating in cellular signaling. Studies have revealed that PE is involved in various processes, including membrane-to-membrane fusion (Kreutzberger *et al.*, 2017), DAG generation through the involvement of phospholipase C (PLC), and the modification of membrane proteins through the mediation of reactive aldehydes (Pohl and Jovanovic, 2019). PE has also been observed to induce negative curvature in biomembranes (Strandberg *et al.*, 2012), which is primarily attributed to its diminutive conical head group. In addition, PE serves as a precursor for various other lipids, including *N*-acyl-phosphatidylethanolamine (NAPE), which serves as a crucial precursor during the biosynthesis of certain essential biological compounds in the brain (for example anandamide) (Jin *et al.*, 2007; Tsuboi *et al.*, 2018).

2.1.1.3. Phosphatidylserine

Folch and Schneider identified serine in cephalin components in 1941 (Folch and Schneider, 1941), marking the beginning of the discovery of PS. PS is a minor class of phospholipids in mammalian cells (2–15% of total phospholipids that tends to accumulate within the cerebral cortex. PS is not merely a component of the lipid bilayer; its present in various cellular organelles, underscoring its biological importance for the activities of these organelles, particularly the mitochondria and ER. The role of PS extends beyond this, as it serves as a substrate to sustain the production of PE. This highlights the complex interplay between these phospholipids and their mutual contributions. From a chemical perspective, PS exhibits strong reactivity to divalent metals, attributable to its unique moiety, the negatively charged head group derived from serine. However, serine is not the only distinctive feature that sets PS apart from other phospholipids; it also possesses diacyl isomers that are notably saturated at the *sn*-2 position (Vance, 2018).

In contrast to plants (Gardner and Hampton, 1999), yeasts, and prokaryotes (Kanfer and Kennedy, 1964), mammalian cells lack the *de novo* CDP-DAG biosynthetic pathway for PS biosynthesis. The production of PS in mammalian cells transpires both in the MAM and in the cytosol of the ER and is facilitated by calcium-dependent base exchange. This pathway is catalyzed by PS synthase-1 and -2 (PSS1 and PSS2, respectively), utilizing PC (catalyzed by PSS2) and PE (catalyzed by PSS2) as the primary precursors at both sites (Hübscher *et al.*, 1959). Subsequent to its production, a fraction of the PS translocates to the plasma membrane via passive diffusion. This lipid primarily localizes to the cytosolic leaflet of the plasma membrane (van Meer, 2011), although its migration to the outer leaflet is notable during programmed cell death (Fadok *et al.*, 1992) and cancer progression (Vallabhapurapu *et al.*, 2015).

The externalization of PS on the cell's outer layer serves as a molecular signal, prompting neighboring cells, including macrophages, to engulf and phagocytose the dying cell. Beyond this role, PS plays a multifaceted biological role in cellular functions. It contributes to the recognition and communication mechanisms between cells. The presence of PS is crucial during PE biosynthesis, as it acts as a source pool (Borkenhagen *et al.*, 1961). Furthermore, PS has been observed to interact with SLs, resulting in elevated interdigitation under the influence of CHOL (Skotland and Sandvig, 2019). PS is also vital for the maintenance of plasma membrane integrity within mammalian cells, as it modulates membrane fluidity and permeability, which are essential for the regular function of membrane-bound proteins.

PS has been implicated in the activation of protein kinase, prothrombinase, and neuroinflammation signaling pathways, and is an essential element of lipid-calcium-phosphate complexes (Lentz, 2003; Ma *et al.*, 2022; Merolli and Santin, 2009; Naeini *et al.*, 2020). Consequently, PS facilitates a range of membrane-bound signaling processes, including apoptosis, activation of enzymes, immune regulation, coagulation cascades, and mineral deposition during bone regeneration.

2.1.1.4. Phosphatidylinositol

Despite the early discovery of phosphatidylinositol (PI) (Anderson, 1930), its structure (1′-myo-inositol is linked to PA) has remained unelucidated for almost 3 decades (Pizer and Ballou, 1959). PI, an anionic phospholipid, features a distinctive inositol head group, characterized by a hexa-hydroxy ring consisting of six carbon atoms. This refers to the fact that the PI structure is composed of a ring, distinguishing it from other phospholipids such as PC, PE, and PS. However, numerous various structures have been identified, majorly depending on the phosphorylation process and its carbon site on the inositol. In this regard, PI is the simplest phosphoinositide and typically exists in a high proportion compared to other phosphoinositides. Furthermore, this lipid represents an elementary structure that can undergo phosphorylation to generate seven different derivates (Dickson and Hille, 2019). These derivatives vary in their abundance across organisms; for instance, in addition to PI, eukaryotes have been regarded as being dominated by PI4P and PI5P. The number of PI derivatives indicates the position of the site on the inositol where the addition of phosphorus occurred. In eukaryotes, the phosphorylation of positions 2 and 6 of PI is impeded due to steric hindrance. PI can constitute up to 10% of total phospholipids and is ubiquitously present in the cytosol of all cellular membranes and certain organelles (e.g., the ER and Golgi apparatus) (D'Souza and Epand, 2014; Payrastre *et al.*, 2001). The PI of eukaryotic organisms is primarily biosynthesized from PA via a *de novo* pathway and is catalyzed by CDP-DAG synthase (which serves as a rate-limiting enzyme (Blunsom and Cockcroft, 2020)) and CDP-DAG myo-inositol 3-phosphatidyltransferase (Daniels and Palmer, 1980). The primary role of these enzymes is to facilitate the sequential reactions during the production pathway, which involves the formation of intermediates and intermediate attachment to myo-inositol, respectively. Notably, PA is not the only available substrate for PI *de novo* production in mammals. For example, glucose-6-phosphate can be utilized during *de novo* pathways, underscoring the intricate potential interactions between various metabolic pathways of distinct molecules. There are three other marked biosynthetic routes for PI production, but these are not yet

reported in eukaryotes; instead, they exist in plants and prokaryotes. The most recent discovery in these noneukaryotic domains was reported a decade ago (Jorge *et al.*, 2015).

Though the initial discovery of PI occurred nearly a century ago, our understanding of the biological functions of PI has markedly advanced over the past three decades. PI is not merely a component of bilayer lipids; it is involved in various metabolic processes (Balla, 2013). Its significance extends to the brain, where it serves critical functions. In addition, it serves as the primary pool of the C20:4 n6 (AA) fatty acyl chain in animal cells, frequently occupying the *sn*-2 position (Anderson *et al.*, 2016; Lee *et al.*, 2016). This specific acyl chain is of paramount importance for the biosynthesis of eicosanoids, including prostaglandins (D'Souza and Epand, 2014; Yui *et al.*, 2015), a group of bioactive lipid metabolites involved in signaling related to cellular inflammation and stress. The cleavage mechanism by which AA is cleaved from PI involves the enzyme phospholipase A2 (PLA2), which transforms PI into LysoPI. Consequently, an accumulation of LysoPI indicates heightened PLA2 activity, implying metabolic alterations and, potentially, the progression of cancer (Piñeiro and Falasca, 2012).

Furthermore, PI contributes to the major signaling pathway, DAG, in mammalian cells, a process catalyzed by the enzymes PLA2 and PLC. Therefore, there is a wide range of dynamic functionalities of PI, including, for instance, its role in intracellular signaling, inflammation, and the formation of glycosyl bridges. The latter role displays remarkable importance concerning the adhesion of proteins, specifically those known as glycosyl-phosphatidylinositol (GPI)-anchored proteins, to the surface of the cellular membrane (Ferguson and Williams, 1988). It has been reported that PI can engage in regulating protein activities at the cellular interface. Various phosphoinositides, including PI3P, PI4P, PI5P, PI(4,5)P2 (likely to accumulate highly in membranes), PI(3,4)P2, PI(3,5)P2, and PI(3,4,5)P3, which accumulate at incredibly low concentrations (1%), significantly contribute to membrane organization. An in-depth discussion has been provided by Posor *et al.* (2022). In particular, the scheme of phospholipase D (PLD) is linked to the companionship of PI(4,5)P2. PLD activity comes across as being indirectly affecting cellular signaling via its product, PA.

2.2. Incorporation of fatty acids into lipids of biomembranes

Fatty acids are the fundamental building blocks of the biomembrane matrix, which is also composed of other complex molecules. Fatty acids typically exist in two forms: saturated and unsaturated monocarboxylic acids (SFA and UFA, correspondingly), both of which are characterized by a terminal carboxyl (-COOH) group and a terminal methyl ($-CH_3$) group, and are likewise designated as carbon 1 (Δ) and omega (ω or n), respectively. Over the past century, various nomenclature systems have

been proposed, including trivial, systematic, Δ^x, n − x, and lipid numbers (Fahy *et al.*, 2011; Liebisch *et al.*, 2020). The trivial nomenclature, though prevalent, lacks systematic patterns. In contrast, systematic nomenclature adheres to a more regular and structured approach based on the nomenclature of parent hydrocarbons. This process involves adding the suffix "oic" to the hydrocarbon name after removing the terminal "e". This nomenclature also involves the identification of the position of the first double bond from (n), with the series of fatty acids being named accordingly (e.g., n3, n6, n7, and n9 series). These distinctions among n-fatty acids lead to variations in their properties, consequently influencing the structure and function of biomembranes (Shaikh and Edidin, 2006).

Fatty acids can either be derived from the diet or biosynthesized within the cytosol and ER through an indigenous pathway known as *de novo* fatty acid synthesis, followed by elongation and possibly desaturation (see Figure 2). This synthesis is a complex process subjected to several factors, including species, transcription genes, dietary composition, age, sex, stored lipids, and both endogenous (metabolic and interactive molecules) and exogenous (environmental) factors. Many genes regulate the synthesis of fatty acids, which can vary among distinct species. In eukaryotic organisms, nearly 5% of the overall genes are responsible for most lipid structures (Sud *et al.*, 2007). Noteworthy, mammals lack enzymes to catalyze the introduction of an additional double bond (after the Δ9 position) on oleic acid, referring to their *de novo* impotence in generating polyunsaturated fatty acids (PUFAs). Essential fatty acids (linoleic acid (C18:2 n6, or LA) and α-linolenic acid (C18:3 n3, or ALA)) are fatty acids that cannot be generated via the mammalian endogenous pathway; thus, they must be obtained from dietary sources (Nakamura and Nara, 2004). This highlights that these dietary fatty acids can provide a pool of media that contributes to the generation of elongated PUFAs.

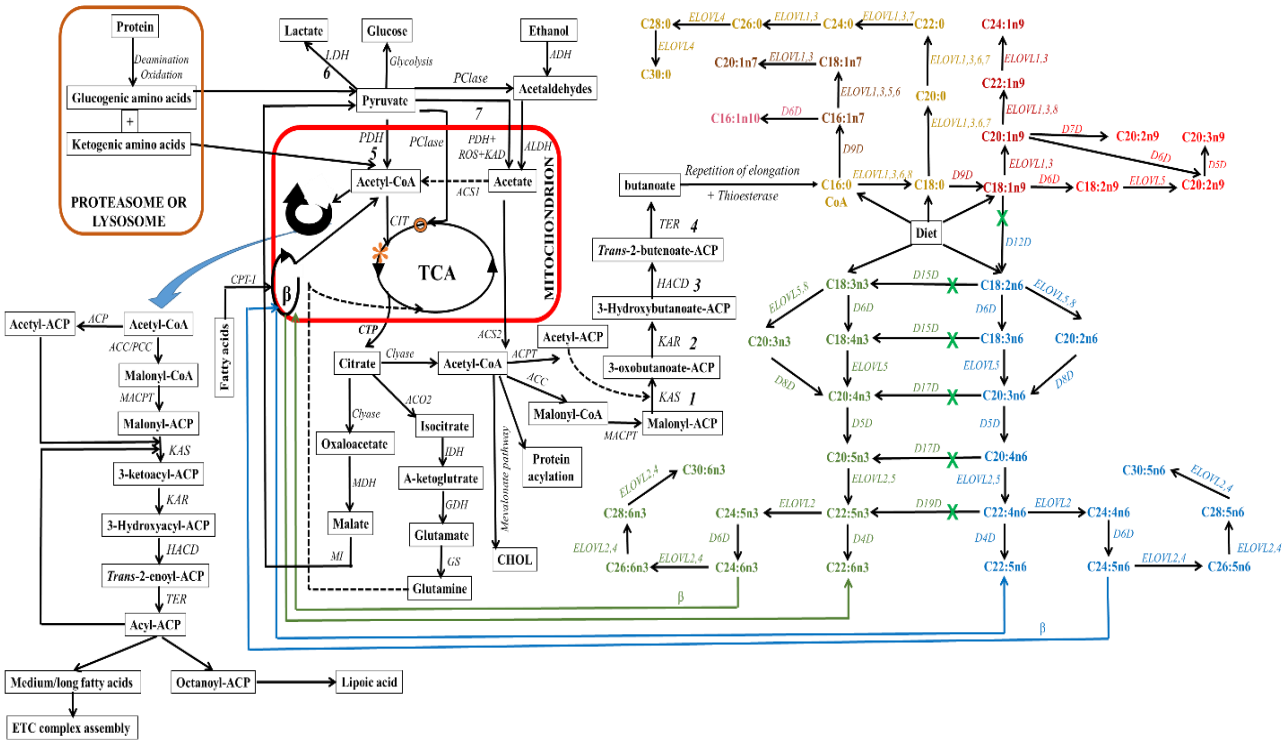


Figure 2. Schematic depiction of eukaryotic fatty acid biosynthesis and modification pathways, with emphasis on enzyme involvement (in italics). In this scheme, mitochondria are denoted by the red box, while proteasome/ribosome components are indicated by the orange box. However, light orange text delineates the elongation pathway for diverse saturated fatty acids. Within the diagram, both green and blue hues, accompanied by corresponding-colored arrows, elucidating the discrete pathways for n3- and n6-fatty acid synthesis, respectively. On the other hand, text with color spectrum transitioning from pink to red designates the pathways for the synthesis of various monounsaturated fatty acids. The green "X" indicates the unattainability of this pathway in mammals, particularly higher eukaryotes, owing to the absence of a specific enzyme. ELOVL8 is a fish-specific elongase. Abbreviations: 1, condensation; 2, reduction; 3, dehydrogenation; 4, reduction; 5, aerobic conditions; 6, hypoxia or anaerobic conditions; 7, aerobic conditions; ACC, acetyl-CoA carboxylase; ACP, acyl carrier protein; ACPT, acyl carrier protein transacylase; ACS1, acetyl-CoA synthetases-1; ACS2, acetyl-CoA synthetases-2; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ACO2, aconitase; β, beta oxidation; CHOL, cholesterol; CIT, citrate synthase; Clyase, citrate lyase; CPT-I, carnitine-palmitoyl transferase-I; CTP, citrate transporter protein, EAR, enoyl-ACP reductase; ETC, electron transport chain; GDH, glutamate dehydrogenase; GS, glutamine synthetase; HACD, β-hydroxyacyl-ACP dehydrase; IDH, isocitrate dehydrogenase; KAD, keto acid dehydrogenase; KAR, β-ketoacyl-ACP reductase; KAS, β-ketoacyl-ACP synthetase; MACPT, malonyl-CoA:ACP transacylase; MDH, malate dehydrogenase, MI, malic enzyme, PCase, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; PDH, pyruvate dehydrogenase; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; TER, trans-enoyl-ACP reductase; O, oxaloacetate; *, citric acid).

All living organisms, in order to adapt and sustain viability, require a mass of biological processes, whereby the *de novo* synthesis and incorporation of fatty acids into biomembranes are part of this framework. Apparently, archaea emerge as the only domain that is not restricted by this metabolic

framework (López-Lara and Soto, 2019). In this respect, instead of fatty acids, it utilizes isoprenoids. This section briefly explains the mechanism by which fatty acids are incorporated into phospholipids. The integration of fatty acids into phospholipids appears to occur via two dynamic processes. First, fatty acids are acylated to the structures of phosphatides through the glycerol-3-phosphate enzyme during biosynthesis. The second is based on a remodeling program for an existing phospholipid (Kent, 1995; Yamashita *et al.*, 2014), whose emergent configurations vary in their properties from those prior to the remodeling process (see Figure 3). In addition to the remodeling, structural diversity can be attributed to several factors, including the selection of *sn*-positions for certain fatty acids, the substrate preferences of biosynthetic enzymes, and the dynamics of the lipid remodeling program. Coleman *et al.* (2002) and Shindou *et al.* (2009) reported the marked affinity of specific enzymes for specific fatty acids. By way of illustration, acyl-CoA synthetase long-chain family members 3 and 4 manifest predilections for arachidonic acid (C20:4 n6, or AA) and eicosapentaenoic acid (C20:5 n3, or EPA), while 1-acylglycerol-3-phosphate O-acyltransferase-α favors myristic acid, palmitic acid (C16:0), and LA. Moreover, 1-acylglycerol-3-phosphate O-acyltransferase-β evinces an affinity for AA.

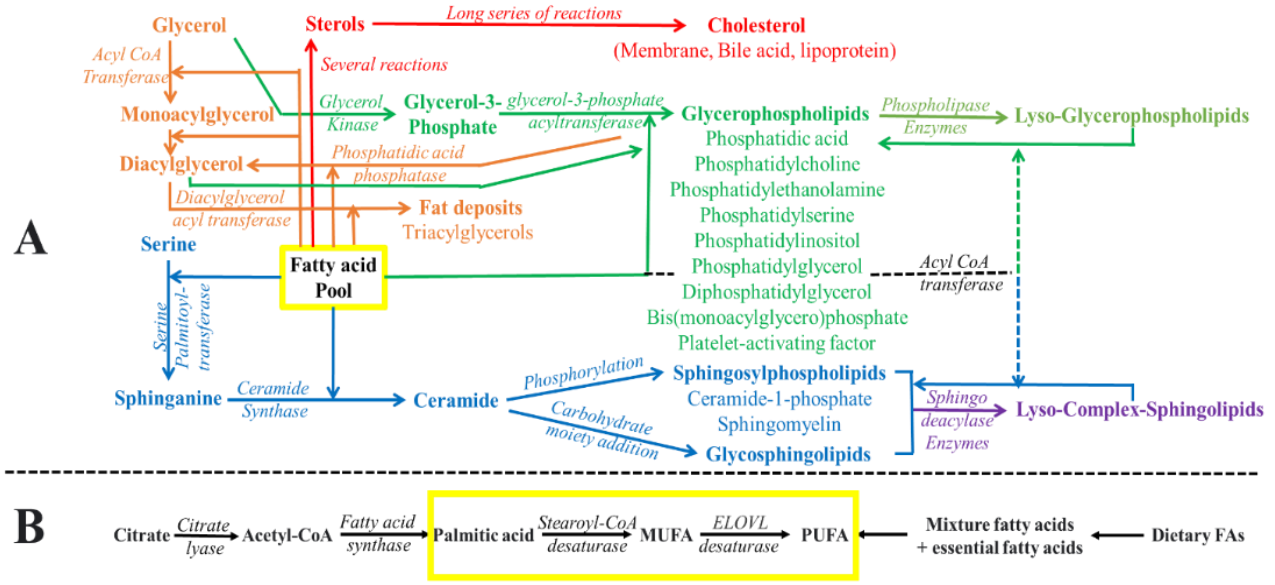


Figure 3. Schematic representation of (**A**) fatty acids incorporated into various lipids and (**B**) the origins of fatty acid pool formation (the *de novo* pathway and dietary sources). The depiction employs color coding to symbolize distinct metabolic pathways. The yellow box designates the fatty acid pool, signifying its integration into diverse membrane lipids. The brown shade denotes processes related to non-membrane and non-polar lipid formation. The red color represents the metabolic routes responsible for sterol production. Additionally, the green color signifies the integration of fatty acids into various phospholipids, while the blue hue corresponds to the incorporation of fatty acids into diverse sphingolipids.

In the context of lipid remodeling, the replacement of fatty acids within existing phospholipids plays a leading role. As an example, the incorporation of C20 fatty acids generally takes place post-*de novo* synthesis, necessitating the use of the remodeling pathway (MacDonald and Sprecher, 1991). Hence, this process involves the conversion of one distinct phospholipid into another (Vance, 2018), highlighting the importance of the Lands cycle in this operation. The Lands cycle is the cornerstone of remodeling (Lands, 2000; Lands and Merkl, 1963), due to its immense contribution to the restructuring process. In this pathway, two enzymes play major roles that contrast each other. As an illustration, the cleavage (deacylation) of fatty acids is catalyzed via the activity of phospholipase enzymes, while in contrast, acyltransferases catalyze the acylation of the desired fatty acids. CoA is needed in this operation, facilitating the reallocation of fatty acids from phospholipids to lysophosphatidates. Other remodeling pathways, such as the CoA-independent transacylation pathway and the direct transacylation pathway, have been recognized to favor PUFAs such as AA, EPA, and docosahexaenoic acid (C22:6 n3, or DHA). It is necessary to understand that distinct tissues vary in their modularity across these remodeling pathways (MacDonald and Sprecher, 1991), as comprehensively reviewed by Yamashita *et al.* (2014).

The translocation of phospholipids across bilayers involves numerous lipid-transporting proteins that express different activities, such as in the case of flippase, floppase, and scramblase. These proteins contribute significantly to the structure of biomembranes. While the flippase regulates the translocation from the outer to the inner leaflet, the activity of the floppase operates in the opposite direction. Notably, these enzymes (flippase and floppase) require adenosine triphosphate (ATP), which is unlike the case of scramblase. The latter enzyme is distinct from both flippase and floppase by its ability to function bidirectionally. Comprehensive insights into these membrane fatty acid transporters have been reviewed by Glatz *et al.* (2010) and Samovski *et al.* (2023).

2.3. Fatty acid profile of phospholipids

Commonly, in phospholipids, SFAs (largely palmitic acid or stearic acid (C18:0)) and UFAs tend to occupy the first and second glycerol stereospecific positions of phospholipids, respectively. However, this is not a constant state, as in some instances, identical fatty acids occupy these *sn*-positions (Hanahan *et al.*, 1960; Tattrie, 1959), although a slight mismatch would occur due to the pending characteristic of *sn*-2, which displays a perpendicular shape on the plane of the membrane (Büldt *et al.*, 1979; Pearson and Pascher, 1979). Typically, differences in the fatty acids of phospholipids are not restricted to the hydrocarbon chain length but may also include distinctions in the number, position, and adjustment of

double bonds. In mammals, it is likely that a relatively low proportion of *trans*-double bonds will be detected compared to bacteria (Gillan *et al.*, 1981), while *cis*-double bonds are copious in mammalian cells.

Commonly, the fatty acid composition of PCs is typically determined postsynthesis, with various events, such as deacylation and reacylation, occurring during their remodeling. These events, which are part of the Lands cycle, can also impact the structure of other phospholipids due to homeostatic mechanisms or metabolic implications (O'Donnell, 2022). Generally, the fatty acid composition of PCs varies across species and cell types (Kuksis *et al.*, 1969; Kuksis and Marai, 1967; Nakanishi *et al.*, 2010; O'Brien and Rouser, 1964; Wood and Harlow, 1969; Yabuuchi and O'Brien, 1968). SFAs like palmitic or stearic acids are typically abundant at the *sn*-1 position, while C18 unsaturated chains or longer PUFAs such as AA and DHA are more prevalent at the *sn*-2 position.

The fatty acid composition of PEs is highly dependent on the particular cell, tissue, and physiological conditions. In medias/tissues like chicken eggs, rat liver, and brain, palmitic and stearic acids tend to occupy the *sn*-1 position, while AA, oleic acid (C18:1 n9), and DHA are more common at the *sn*-2 position (Holub and Kuksis, 1969; Nakanishi *et al.*, 2010; Wood and Harlow, 1969). Notably, PEs in the erythrocyte membrane exhibit a greater tendency for the accumulation/recruitment of PUFAs compared to PC (Doğru Pekiner, 2002). Consequently, PE in this context contains more PUFAs, primarily AA and DHA, at the *sn*-2 position, despite its diacyl structure being similar to that of PCs.

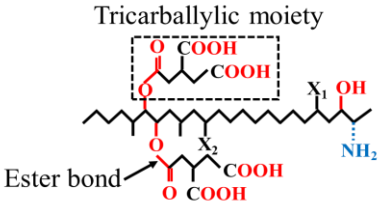
The composition of fatty acids in PS differs depending on the tissue type. Nonetheless, lipid remodeling and selective insertion of fatty acids are common processes that occur along the biosynthetic pathway. Therefore, the fatty acid composition of newly synthesized PS differs from that of its precursors, PE and PC. When PS was isolated from rat liver and cow brain and analyzed regiospecifically (Wood and Harlow, 1969; Yabuuchi and O'Brien, 1968), it was observed that stearic acid was more abundant at the *sn*-1 position, while the proportion of palmitic acid was lower there. These data underscore the distinct fatty acid incorporation pattern exhibited by PS in comparison to that exhibited by PC and PE. Furthermore, the acylation of PS at the *sn*-2 position has been shown to be tissue-specific, with high proportions of AA and DHA in the livers of rats and bovine brains, respectively. Similar findings regarding DHA in the brain regions of mice and rats have been reported by Hamilton *et al.* (2000) and Kim *et al.* (2014). However, stearic acid was the most abundant fatty acid at the *sn*-1 position in these cases. Remarkably, the incorporation of these UFAs into PS appears to be more extensive than in the case of PC,

as revealed by these studies. The fatty acid composition of PS at its *sn*-positions plays a crucial role in determining its functional properties to varying degrees (Clark *et al.*, 2013; Skotland and Sandvig, 2019). In mammalian cells, the composition of PI is characterized by the presence of stearic acid and AA at the *sn*-1 and *sn*-2 positions, respectively, as consistently demonstrated in assorted studies (Holub *et al.*, 1970; Nakanishi *et al.*, 2010; Thompson and MacDonald, 1975; Wood and Harlow, 1969). These two fatty acids collectively account for a substantial portion of PI-side chains, typically ranging from 50% to 80% in the brain and liver. Additionally, oleic acid is frequently recognized as the third most abundant fatty acid in the rat brain, while palmitic acid and DHA exhibit lower levels of acylation in this phospholipid class. The abundance of oleic acid depends on the tissue, as it is the most quantified fatty acid in the brain of a rat, followed by stearic and palmitic acids (Ulmann *et al.*, 2001). It is possible that multiple factors contribute to the recorded variability in PI structure, which may include genetic and dietary variations. In general, PI exhibits a strong preference for AA in its acyl composition. These remodeling processes can lead to the deacylation of preexisting PI, resulting in the formation of lyso-PI via the action of phospholipases. The incorporation of AA into lyso-PI is facilitated by lysophosphatidylinositol acyltransferase 1 (Gijón *et al.*, 2008).

2.4. Fumonisins

FUMs are a group containing numerous mycotoxins that share specific features. These toxins are mainly produced by *Fusarium* species from the *Liseola* section, and are primarily synthesized by *Fusarium verticillioides* (previously known as *Fusarium moniliforme*) and *Fusarium proliferatum*. This highlights the widespread status of these species around the globe. However, other non-*Fusarium* species (as an example, *Aspergillus niger*, *Aspergillus welwitschiae*, *Aspergillus awamori*, and *Tolypocladium* species) have also been shown to contribute to the overall production of FUMs. Distinctively, compared to *Fusarium* species, non-*Fusarium* species displayed the production of unique structures. As an illustration, FB_2, FB_4, and FB_6, have been identified in maize, peanuts, grapes, and onions (Frisvad *et al.*, 2007; Månsson *et al.*, 2010; Mogensen *et al.*, 2010; Norred, 1993; Perrone *et al.*, 2011; Rheeder *et al.*, 2002). FUM was first isolated in 1988 by the Gelderblom Research Group (Gelderblom *et al.*, 1988) at PROMEC, in the Republic of South Africa, from the *Fusarium verticillioides* MRC 826 fungal strain. Upon its isolation, its structure was rapidly elucidated (Bezuidenhout *et al.*, 1988). From a chemical perspective, FUMs are polyketide-derived mycotoxins that are composed of a long hydrocarbon chain with aminopolyols and two tricarballylic acid (TCA) side chains. These TCA side chains are typically esterified at the C-14 and C-15 hydroxy groups,

resulting in the formation of a relatively stable diester. Due to their polyhydric alcohol component and amino group, they structurally resemble a free sphingoid, primarily disrupting the entire process of sphingolipid metabolism within cells. Almost one hundred distinct structures of FUM have been detected and identified to date (Angeli *et al.*, 2022; Bartók *et al.*, 2006, 2010, 2014). The distinctions between these compounds are based on variations in the nitrogen functional group and the extent of the carbon backbone, resulting in 7 groups, including A, B, C, D, P, P_y, and L_a. Among these groups, fumonisin B (including FB_1, FB_2, FB_3, FB_4, FB_5, and FB_6) is the most renowned group of FUMs (see Figure 4), due to its high prevalence and substantial degree of toxicity, especially FB_1. This group has ten chiral centers, raising the possibility of over a thousand isomers in theory. FB_1, a potent mycotoxin with the empirical formula $C_{34}H_{59}NO_{15}$, naturally occurs in a variety of isomeric forms; according to Bartók *et al.* (2010), there are 28 isomers identified in addition to the original structure. The cytotoxic potential of FB_1 is fundamentally linked to its primary amino group. This is substantiated by evidence demonstrating that the acetylation process, which converts FA_1 to FB_1, results in a reduction in its cytotoxic properties (Stockmann-Juvala and Savolainen, 2008).



FB type	B_1	B_2	B_3	B_4
X_1	-OH	-OH	-H	-H
X_2	-OH	-H	-OH	-H

Figure 4. Type B fumonisin chemical structures, mainly those produced by *Fusarium* species.

FUMs are globally distributed and commonly contaminate various foods and feeds. However, their contamination levels vary across the globe (see Figure 5); however, FUM levels between 925–3052 µg/kg feed/feed-stuffs have been reported to occur within Europe (Marquardt and Madhyastha, 2015), which is below the tolerable limits recommended by the EU for livestock animals (European Commission, 2006). These findings are likely similar to those reported for various maize-based food products within the Hungarian market (Zentai *et al.*, 2019), revealing a lower daily intake than the established tolerable daily intake (TDI) of 1 µg FB_1/kg body weight (BW)/day by the European Food Safety Authority (EFSA) (Knutsen *et al.*, 2018a). As aforementioned, the FB group is the most prevalent, toxic, and studied group within FUMs. In particular, FB_1 toxin typically constitutes 70% of the overall FBs produced by *Fusarium verticillioides*, while the presence of FB_2 and FB_3 is relatively low, at 15–25% and 3–8%, respectively (Nelson *et al.*, 1993; Rheeder *et al.*, 2002). Maize and its base products are identified as the targeted crop/products for FUMs, emphasizing that contamination can

occur alike prior to and post-harvest. However, they have also been detected in other cereals, including barley, wheat, sorghum, and rice, as well as in other agricultural products like beans, soybeans, asparagus spears, and figs (Gao *et al.*, 2023; Karbancıoglu-Güler and Heperkan, 2009; Wong and Jeffries, 2006). Interestingly, the presence of FUMs in various water sources has been detected (Jevtić *et al.*, 2023), offering a new perspective for conducting exposure risk assessments.

The water-soluble nature (polarity), thermal stability (up to 150 °C), and potential transformation of these mycotoxins present a consequential challenge regarding their complete eradication during the food production processes. Consequently, there is an imperative need for the implementation of effective management strategies during the production phase. Within the EU, a variety of strategies are employed to reduce and detoxify FUMs in food and feedstuffs. These strategies include the use of genetically resistant crops, physicochemical treatments, biological antioxidants, competitive microorganisms, binders, and degradation processes (Gao *et al.*, 2023; Qu *et al.*, 2022; Santiago *et al.*, 2020). However, the reduction in FUM chemical structure and toxicity is contingent on the processing conditions and food matrix composition, which determine structural alterations and conjugate formation by interacting with food and feed components such as sugars and proteins (Falavigna *et al.*, 2012; Humpf and Voss, 2004; Schaarschmidt and Fauhl-Hassek, 2019). The interaction between FUMs and other food/feed components leads to the formation of hidden/masked configurations, making the quantification of total FUMs challenging using traditional targeted analytical approaches.

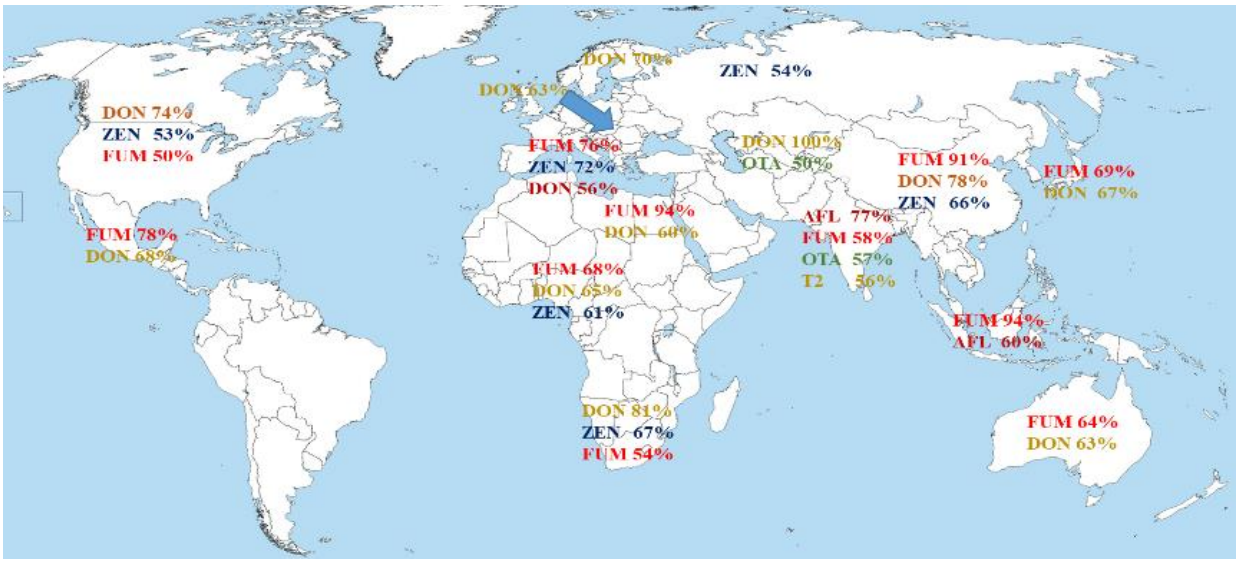


Figure 5. Mycotoxin prevalence worldwide from January to September 2023. The presented data considers only mycotoxin risks above 50%. The data is adapted from DSM database (available at https://www.dsm.com, accessed on 31 May 2024).

Upon ingestion by animals, the majority of FUMs (> 90%) remain unmetabolized and are excreted through faeces, whereas a small portion has been reported to be excreted through urine (< 1%) (Fodor *et al.*, 2008; Norred, 1993; Prelusky *et al.*, 1994; Schelstraete *et al.*, 2020; Schertz *et al.*, 2018; Shephard *et al.*, 1995). Notably, only FB_1 has been detected in human urine (Riley *et al.*, 2012), suggesting that the intake, bioavailability, and/or excretion of other FB isomers through urine are relatively lower than these of FB_1. Within the gastrointestinal tract (GIT), a small portion of ingested FUM can undergo bacterial enzymatic biotransformation (Grenier *et al.*, 2017; Gurung *et al.*, 1999; Schertz *et al.*, 2018), yielding partially hydrolyzed (lacking a single TCA moiety: $pHFB_1$ and $pHFB_2$) and hydrolyzed FUMs (lacking TCA moieties: HFB_1). Produced hydrolyzed FUMs have been shown to serve as a pool for CerS, resulting in the generation of *N*-acylated HFB_1 metabolites (Merrill *et al.*, 1993; Seiferlein *et al.*, 2007). *N*-acylated derivatives of FB_1 have been detected and quantified in rat liver and kidney upon intraperitoneal exposure to 0.5, 1, or 2 mg FB_1/kg BW for five consecutive days (Harrer *et al.*, 2015). Biologically, these *N*-acylated metabolites are potentially more toxic than their parents' FB_1 and HFB_1, which is attributed to their high adsorption and distribution rates, strong affinities to biological compounds, and cytotoxic potencies (Csenki *et al.*, 2023; Harrer *et al.*, 2013). In addition, only reported in bovines that further enzymatic biotransformation can occur in hepatic tissue (Spotti *et al.*, 2001). On the other hand, a small portion of the absorbed FUMs (3-5%) can undergo rapid clearance from plasma within a few hours following exposure. The liver and kidney have been observed to retain most of the absorbed FUMs (Prelusky *et al.*, 1996), although non-possible and/or negligible carry-over process has been reported in meat, milk, and eggs (Fodor *et al.*, 2008; Knutsen *et al.*, 2018b; Voss *et al.*, 2007).

2.5. Fumonisin toxicity across species

As a result of the high prevalence of FUMs, a threat to livestock and humans can be expected through accessing their food and feed chains. Despite the fact that FB_1 is often considered the most toxic within the FUM group, the risk evaluation conducted by EFSA underscores that the toxicological intensity and efficacy of FB_1 are relatively similar to those of FB_2 and FB_3 but superior to those of their transformed derivatives (Knutsen *et al.*, 2018b). To date, only FB_1 and FB_2 levels in animal feed have been regulated within the EU (European Commission, 2006). In this regard, exposure to FUMs (above the recommended levels) may lead to various pathophysiological symptoms and mycotoxicosis, depending on the species, exposure dose and period, animal sex and age, physiological status of the animals, ratio and purity of the FUM isomers, and body counter-regulation processes.

In mankind, the intake of a *Fusarium*-contaminated diet, or FUMs, has been shown to trigger or potentially cause various consequences. These potential consequences can be collectively summarized as growth retardation, neurotoxicity, hepatotoxicity, nephrotoxicity, embryotoxicity, GIT dysfunction, and potential increases in the risk of neural tube defects and cancer (Hou *et al.*, 2021; Marasas *et al.*, 2004; Minervini *et al.*, 2014; Voss and Riley, 2013; Yu *et al.*, 2020), although there is no clear evidence regarding some of the toxic impacts listed here. Notably, the direct involvement of FUM in cancer has not yet been fully established, resulting in FUM being categorized as a Group 2B substance (IARC, 2002), a categorization that is carried out by the International Agency for Research on Cancer (IARC). When assessing animals, numerous *in vivo* and *in vitro* investigations have endorsed the distinctive toxicity of FB_1, which varies across species and organs. The genus *Equus* is possibly the most sensitive species compared to other animals. Exposure to FB and FA isomers has been shown to cause equine leukoencephalomalacia (ELEM) (Riley *et al.*, 1997), cardiotoxicity, and hepatotoxicity (Riley *et al.*, 1997; Smith *et al.*, 2002). Within the framework of this dissertation, this section focuses mainly on the toxicological effects of FUMs on animals such as rats, swine, and rabbits.

2.5.1. Toxicity in rats

Among the various animals studied, rodents, including rats, are often the most extensively researched (both *in vitro* and *in vivo*), underscoring their reliability and suitability in toxicological investigations as well as their valuable contributions to advancements in the biomedical domain. This high preference for incorporation can be attributed to their diverse physiological parameters, high sensitivity, rapid reproduction rate, and minimal maintenance cost. Historically, toxicological reports regarding the exposure of FUMs in rats predated the discovery of FUM (Marasas *et al.*, 1984; Voss *et al.*, 1989). These reports underline the association between the intake of contaminated maize and the development of hepatotoxicity, nephrotoxicity, and carcinogenic effects and, in certain cases, elevated mortality rates. Following the discovery of FUMs, the toxicological impact of FUMs, particularly FBs, on rodent tissues was found to be strain- and sex-dependent. As a model, in BDIX strain male rats, the primary target of FB_1 is the liver, whereas the kidney is the main target in Sprague-Dawley, Fischer 344N, and RIVM:WU strain rats. The liver, rather than the kidney, is more vulnerable to FB_1 toxicity in female mice than in male mice (Joint FAO/WHO, 2002). These hepato- and nephrotoxic effects have also been underscored in the form of disruption of serum enzymes and histomorphological modifications (Domijan *et al.*, 2008; Pozzi *et al.*, 2001; Szabó *et al.*, 2018;

Tolleson *et al.*, 1996). Hepatotoxicity and nephrotoxicity in rats can potentially escalate cancer development. However, tumor progression within hepatic and renal tissues appears to be contingent upon long-term exposure to pure FB_1 (Howard *et al.*, 2001). In this regard, the aggressive nature of renal carcinomas induced by FB_1 in Fischer 344N male rats remains ambiguous, although FB_1 is recognized as a highly malignant variant of renal tubule tumors. It is important to highlight that FB_1 has not been proven to be directly genotoxic (Bondy *et al.*, 2012), suggesting that secondary toxicity routes, such as the modulation of apoptosis and proliferation, are involved in cancer induction. Based on toxicity findings in rats, the kidney can be regarded as the most targeted organ for FB exposure, followed by the liver. In a study conducted by Voss *et al.* (1995) and the assessment of the National Toxicology Program (NTP, 2001), the no observed adverse effect level (NOAEL) for the liver and kidneys was established to be 0.25 and 0.2 mg FB_1/kg BW/day, respectively. Noteworthy, these NOAEL values may not be valid in cases of different tissues and exposure to other FUM isomers. The toxic effects of FB_1 on rats appear to be more pronounced compared to its derivatives, especially on the ratio between sphingoid bases (Hahn *et al.*, 2015).

In rats, the toxic effects of FUMs extend beyond the liver and kidneys. A study by Lim *et al.* (1996) suggested that the esophagus of rats is a further targeted tissue, exhibiting a high rate of cell proliferation under FUM exposure. Embryotoxicity without fetal deformities post-FB_1 exposure has also been implicated in Syrian hamsters (Penner *et al.*, 1998) and rats as a result of FB_1 exposure, underscoring variations in free sphingoid base concentrations and myelination (specialized myelin layers that envelop the axons) in multiple cerebral regions of maturing rats (Kwon *et al.*, 1997). Rats exposed to FB_1 were also shown to develop neurotoxicity (Banczerowski-Pelyhe *et al.*, 2002), a consequence of a reduction in conduction velocity and spinal reflexes, as well as a decrease in the development of myenteric neurons. The damage that occurs in the spleen due to FB_1 exposure has been documented by Atroshi *et al.* (1999), suggesting that FB_1 has an immunotoxic effect. In 2002, Theumer *et al.* reported the *in vivo* and *in vitro* immunotoxic effects of FB_1 on spleen, small intestine, and macrophages of rats. Unlike in pigs, the rat lung does not seem to be a major target organ of FBs; however, Salam *et al.* (2012) reported that FB_1-induced histopathological lesions, including edema, within the lungs of male rats in a dose- and time-dependent manner. In addition to these effects, FUMs, specifically FB_1 and FB_2, have been shown to disrupt mineral metabolism in rats (Rudyk *et al.*, 2019). This disruption is a consequence of hepatotoxicity induced by these toxins, which subsequently leads to alterations in bone metabolism and homeostasis.

2.5.2. Toxicity in swine

Swine is regarded as the most pertinent and sensitive animal model, possessing a digestive system that closely mirrors that of humans (Guilloteau *et al.*, 2010). Swine are among the most vulnerable species, with a NOAEL of 1 mg FBs/kg feed (Zomborszky-Kovács *et al.*, 2002) and a minimum lowest observed adverse effect level (LOAEL) of 0.148 mg FBs/kg BW/day (Ali *et al.*, 2021; Terciolo *et al.*, 2019). This necessitates the re-evaluation of the European maximum limit of FBs (5 mg FB_{1+2}/kg) in swine feed and the LOAEL established by Knutsen *et al.* (2018b) and Schrenk *et al.* (2022) (0.2 mg FB_1/kg BW/day). FUM toxicity has been reported to affect various porcine tissues (brain, heart, lung, liver, kidney, pancreas, intestines, spleen, reproductive system, and lymphatic system, according to Dilkin *et al.* (2003), Gbore (2009, 2010), Gbore and Egbunike (2008), Marin *et al.* (2006) and Terciolo *et al.* (2019)), with the lung, liver, heart, and pancreas being the most targeted and researched organs. Similar to that in horses, the consumption of *Fusarium verticillioides*-contaminated feed and FB_1 has been shown to induce cardiotoxic effects and trigger porcine pulmonary edema (PPE) and hydrothorax (Haschek *et al.*, 2001), which are associated with high blood vessel permeability. Notably, the risk of PPE increases substantially when pigs develop respiratory infections (Kovács *et al.*, 2016; Pósa *et al.*, 2013). The literature exhibits variability in findings, potentially due to factors such as animal genetics, age, sex, toxin type and purity ratio, presence of secondary infection, exposure dose and duration, administration method, housing conditions, experimental practices prior to sample collection, and undetermined factors such as masked mycotoxins and co-occurrence with other mycotoxins. According to the available literature, PPE was observed under 3-6 mg FBs/kg feed exposure for 4 weeks (Souto *et al.*, 2015), while hepatotoxicity was noted at doses above 6 mg FBs/kg feed (Grenier *et al.*, 2013). In addition to these effects, FB_1 has been shown to induce toxicity within the GI system both *in vivo* and *in vitro*, leading to histological, immunological, microbial, and nutrient digestibility alterations (Oswald *et al.*, 2003; Pierron *et al.*, 2016; Zeebone *et al.*, 2020). These toxic effects of FBs may subsequently impair pig performance, distort blood biochemical data, cause abortion in pregnant sows (plausibly a consequence of fetal anoxia), and can result in mortality; however, mortality has only been observed at high (> 50 and 100 mg/kg feed) FB exposures (Fodor *et al.*, 2005). Unlike ZEN, FUMs are non-specific mycotoxins that target the boar reproductive system; however, Gbore (2009) reported deteriorations in semen quality upon FB_1 exposure.

2.5.3. Toxicity in rabbits

In addition to rats, the application of rabbits in toxicological studies is pronounced, a preference attributed to their valuable reproductive capabilities (rapid cycle), ease of various measurements (Kachlek *et al.*, 2016), and modest maintenance cost. Exposure to FUM in rabbits has evidently been implicated in the development of various toxicological issues. These toxicological patterns tend to be similar to those reported in other animal species. Bucci and Howard (1996) reported the first instance of neurotoxicity in rabbits, identifying leukoencephalomalacia and brain hemorrhage in pregnant rabbits exposed to FB_1. Apparently, rabbits are so far the only animal species to exhibit leukoencephalomalacia (RLEM) besides horses (ELEM). However, the majority of the literature on rabbits has investigated hematological and reproductive parameters (alike male and female), demonstrating the potency of FBs to alter both of these parameters (Ewuola, 2009; Ewuola and Egbunike, 2010a, 2010b; Gbore and Akele, 2010; Orsi *et al.*, 2007, 2009; Szabó *et al.*, 2014). In relation to blood biochemical components, the available data on serum nitrogenous compounds following FB_1 exposure reveal inconsistency in outcomes, suggesting contradictory effects (Gbore and Akele, 2010; Orsi *et al.*, 2007, 2009). However, the notion of a plausible potential decrease in serum proteins by FUMs appears logical since proteins exhibit immense interactions with lipids, a typical target fraction disrupted by FUM. Moreover, as in other species, the onset of hepatotoxicity and nephrotoxicity has typically been reported in weaned and pregnant rabbits upon exposure to FBs (Hafner *et al.*, 2016; Kovács *et al.*, 2003), with the latter study indicating that the fetal liver and kidneys are the target organs. Similar findings regarding FB_1 toxicity in male rabbit kidneys have also been reported by Gumprecht *et al.* (1995) and Orsi *et al.* (2007). Dysfunction of hepatic and renal tissues implies the modulation of biochemical data, including enzyme activities. Notably, the increase in erythrocyte Na^+/K^+ ATPase activity caused by FBs has been proven to occur in rabbits (Szabó *et al.*, 2014), but not in other species. The GI system also projected sensitivity to FB_1. Ewuola (2009) reported a marked effect on the rabbit GIT, highlighting histomorphological modifications. Based on the aforementioned studies (Ewuola, 2009; Ewuola and Egbunike, 2010a; Gbore and Akele, 2010) and their findings, the LOAEL for FB_1 has been determined to be 5 mg FB_1/kg of diet, which corresponds to 0.2 mg FB_1/kg BW/day.

2.6. Toxic effects of fumonisins on cells via membrane lipids

Numerous toxic effects of FUM have been described in the preceding sections. Notably, the toxic effects of FUMs are not confined to themselves. They are capable of interacting (additively,

synergistically, and antagonistically) with other mycotoxins (such as aflatoxin, DON, ZEN, and T-2) to generate distinct toxic effects compared to those of FUMs alone (Alassane-Kpembi *et al.*, 2017; Chen *et al.*, 2023; Grenier and Oswald, 2011; Kifer *et al.*, 2020; Kócsó *et al.*, 2021; Szabó *et al.*, 2017; Szabó-Fodor *et al.*, 2019). The toxicological consequences of FUM observed in animals are considered to be the result of multiple biochemical pathways. FUMs influence cellular regulatory sites, apparently through perturbations of lipid metabolism. For example, the effects of FUM are evidently striking in various animal species (such as rats, swine, and rabbits), as manifested by their increased serum lipid metabolites, namely, CHOL and/or low-density lipoproteins (Ali *et al.*, 2021; Dilkin *et al.*, 2010; Fodor *et al.*, 2015; Gbore and Egbunike, 2009; Gelderblom *et al.*, 1997; Gumprecht *et al.*, 1998; Kouadio *et al.*, 2013; Loiseau *et al.*, 2015; Terciolo *et al.*, 2019; Voss *et al.*, 1995; Voss *et al.*, 1993). These effects were not related to serum lipids, as proportional increases in liver CHOL have also been reported in rats, in alike *in vivo* (Burger *et al.*, 2007, 2018; Gelderblom *et al.*, 2002; Riedel *et al.*, 2015) and *in vitro* (Riedel *et al.*, 2016). However, serum CHOL does not always respond to FUMs, with some findings recording no alteration in its concentrations. This is mostly attributed to the variability in study design, including species, strains, toxin purity, dose paradigm, period of exposure, and administration methods. FBs have also been reported to increase the concentration of serum triglycerides in male and female rats exposed to 150 mg FB_1/kg feed for 28 days (Bondy *et al.*, 1996; Voss *et al.*, 1993). These modifications mostly corresponded to high FB_1 doses. When piglets were exposed to a relatively low dose of FB_1 (below 20 mg/kg diet), the proportion of serum triglycerides was unaltered (Ali *et al.*, 2019; Loiseau *et al.*, 2015). Generally, the disruption of triglyceride metabolism occurs outside of the serum, as Gelderblom *et al.* (1996a, 2002) reported the effect of FB_1 on the disruption of the fatty acid composition of triglycerides in the liver. However, this has not been proven regarding their fatty acid composition in the serum, liver, lungs, or kidneys of piglets fed a diet contaminated with FBs (Ali *et al.*, 2019), but compositional alterations in membrane fatty acids within the liver, lungs, and kidneys were observed. These findings suggest that the composition and structure of membrane lipids are more susceptible to FBs than are nonpolar lipids. In the following section, the mechanisms of action that interfere with membrane lipids and major proportional modifications in membrane lipids are discussed.

2.6.1. Underlying mechanisms of membrane disruption

Multiple mechanisms have been identified and proposed to contribute to the toxicity of FUMs. These mechanisms operate within the context of sphingolipid metabolism, oxidative stress, enzyme

activities, gene expression, deoxyribonucleic acid (DNA) integrity, the role of iron, ER function, and the tumor necrosis factor signaling pathway. This thesis focuses only on those mechanisms that specifically interfere with membrane lipid metabolism. However, a detailed discussion has been published on a recent review paper by Ali and Szabó (2024).

2.6.1.1. Disruption of sphingolipid metabolism

Sphingolipids constitute a minor component of membrane lipids, typically ranging from 10%–20% of all lipids present in cells (van Meer *et al.*, 2008). These lipids exist in various structures, ranging from simple to complex. However, they all share a similar long-chain sphingoid base backbone with a fatty acid linked via an amide bond. This backbone and the type of bond make them distinct membrane lipids, although some of their structures have been proven to possess phosphorus and choline. A further distinctive feature is the non-hydrolysable amide bond, which causes the attached fatty acid to be somewhat fixed and not subject to cleavage. This dissertation does not include a detailed discussion on the variant structures of sphingolipids and their functions; however, this has been published in a review paper that is part of this thesis (Ali and Szabó, 2023).

The toxicity induced by FUMs in animals fundamentally stems from the disruption of sphingolipid metabolism (Wang *et al.*, 1991), primarily suggesting the inhibition of CerS (also known as sphinganine/sphingosine *N*-acyltransferase), an essential enzyme for the acylation of fatty acids into Sa and So, which is the route for the biosynthesis of ceramide. This effect of FUM on CerS can be described as the "initial/direct effect", whereas compositional distortion in other sphingolipids can be depicted as "secondary/indirect effects". The inhibition of CerS has been attributed to structural resemblance with the substrates (Sa and So) of CerS, thus resulting in the classification of FUMs as sphinganine-analog mycotoxins (Chen *et al.*, 2020). In the presence of CerS, the aminopentol backbone (AP) within FUM is known to suppress the enzyme's activity and compete with sphingoid bases for enzyme binding, leading to the production of *N*-acylated AP analogs. Merrill *et al.* (2001) reported that *N*-acylated-APs exhibit a toxicity level that is 10 times greater than that of FUM or APs in HT-29 cells in an *in vitro* model. Furthermore, the TCA moiety of FUM acts competitively with fatty acyl-CoA. Hence, the inhibition of CerS has been shown to elevate the tissue concentrations of sphingoid bases (majorly Sa and, to a lesser extent, So) and their phosphate metabolites, leading to shifts in the ratios of Sa:So and Sa:Sa-1-phosphate (to understand the mechanism of disruption, please see Figure 6). These proposed alterations were subsequently confirmed by earlier studies by Desai *et al.* (2002), Enongene *et al.* (2000), Riley *et al.* (1993, 1994), Riley and Voss (2006), Wang *et al.* (1999)

and Yoo *et al.* (1992), and reported to consequently disrupt membrane lipid constituents (Riley *et al.*, 2001). When sphingolipid metabolites are altered, a specific signal is produced in cells. For instance, sphingoid bases and ceramide tend to express apoptotic signals, whereas sphingoid-1-phosphate analogs express survival signals (Riley and Merrill, 2019; Stockmann-Juvala and Savolainen, 2008; Voss *et al.*, 2007). Nonetheless, identifying the precise signaling roles of sphingoid bases is potentially complex, given their diverse signals and extensive interactions with a multitude of cellular molecules, including CHOL, phospholipids, and proteins (Katoh, 2004; Merrill, 2011; Natarajan *et al.*, 1994; Riley *et al.*, 1996; Zupancic *et al.*, 2014).

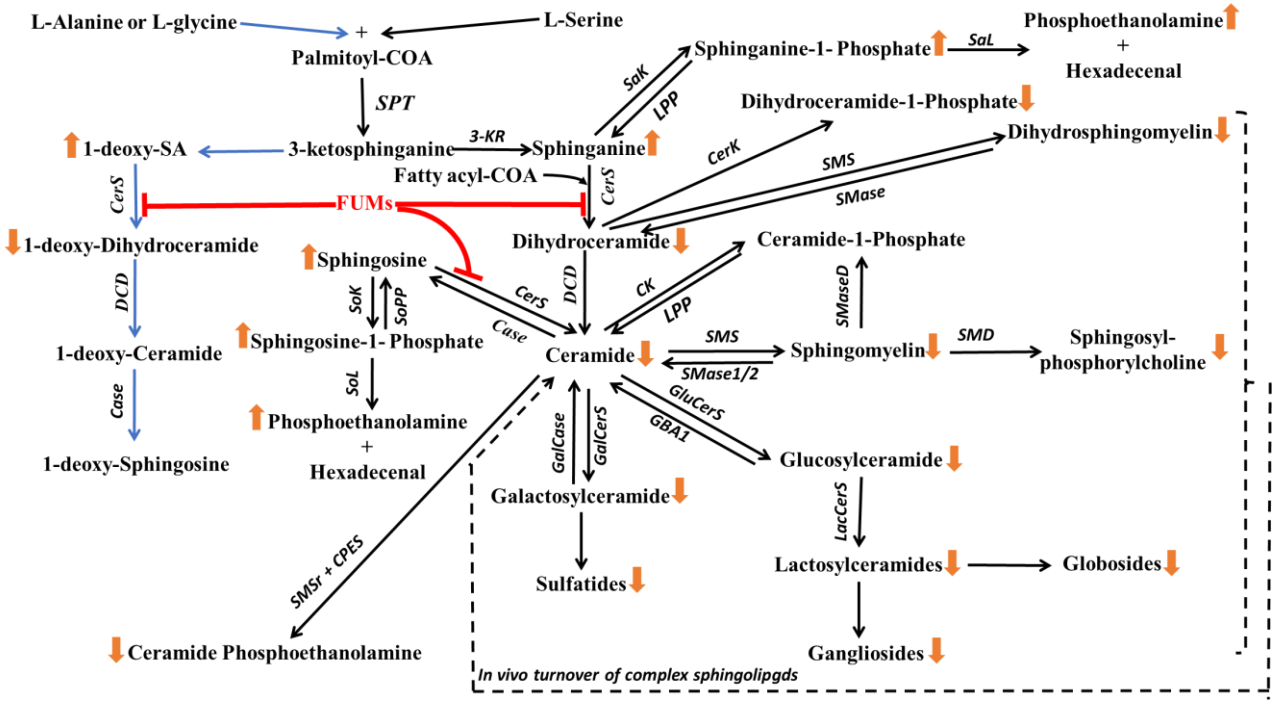


Figure 6. Schematic representation of the *de novo* biosynthesis pathway of major complex sphingolipids, and the interference of FUMs with their metabolism. The metabolic pathway of deoxy-sphingolipids is denoted by blue arrows, whereas the metabolic pathway of non-deoxy-sphingolipids is represented by black arrows. Orange arrows indicate proportional elevation (the tip towards up) or depletion (the tip towards down). Abbreviations: 3-KR, 3-ketosphinanine reductase; Case, ceramidase; CerK, ceramide kinase; CerS, ceramide synthase; CPES, ceramide phosphoethanolamine synthase; DCD, dihydroceramide desaturase; FUMs, fumonisins; GalCase, galactosylceramidase; GalCerS, galactosylceramide synthase; GBA1, acid β-glucosidase; GluCerS, Glucosylceramide synthase; LacCerS, lactosylceramide synthase; LPP, lipid phosphate phosphatase; Sak, sphinganine kinase; SaL, sphinganine lyase; SMaseD; sphingomyelinaseD; SMD, sphingomyelin deacylase; SMS, sphingomyelin synthase; SMS1/2, sphingomyelin-1 or -2; SMSr, sphingomyelin synthase related proteins; Sok, sphingosine kinase; SoL, sphingosine lyase; SoPP, sphingosine phosphate phosphatase; SPT, serine palmitoyltransferase.

It appears that sphingolipid distortion by FUMs modify the overall membrane lipid composition via multiple distinct mechanisms: 1) by acting as precursors for long-chain aldehydes and various phospholipids; 2) by modifying the fatty acyl side chain of complex sphingolipids; 3) by modifying the complex interaction between sphingolipids and membrane lipids (for example, CHOL and phospholipids), thereby affecting membrane integrity and cellular signaling; and 4) by inducing toxicity in specific species associated with specific changes in membrane lipids (biomarkers), implicating the intricate interaction between multiple pathways. Additional pathways, which may be triggered by an imbalance in sphingolipid metabolism and lead indirectly to alterations in membrane lipids, are discussed in the following sections.

The ER is the primary site for Sa production; however, it is crucial to emphasize that free So does not originate from the *de novo* pathway. Instead, it is produced through the salvage pathway, which consists of the hydrolysis of ceramide (facilitated by ceramidase) driven by the *in vivo* turnover of complex sphingolipids. Although this process is natural, a high turnover rate is likely associated with the loss of epithelial integrity as well as the inhibition of cell growth and adhesion (Desai *et al.*, 2002). In cases of high accumulation of sphingoid bases, a metabolic shift to phosphorylation of these bases may occur, an event that is likely coupled with the proportional elevation of 1-deoxy-SA in the livers and kidneys of rats and mice (Voss *et al.*, 2009; Zitomer *et al.*, 2009). These authors proposed the involvement of these deoxy analogs in FUM toxicity, although further investigations are needed to comprehend the biological roles of these analogs. Kinases specific to Sa and So have the ability to phosphorylate these compounds, leading to the creation of their corresponding 1-phosphate derivatives (Hannun and Obeid, 2008; Merrill, 2002). This event is a highly resistant step against the apoptotic mode of action of FUMs (Sharma *et al.*, 2004). The phosphorylation process is reversible, with sphingoid-1-phosphate being capable of dephosphorylation via sphingoid-1-phosphate phosphatases. In situations of high accumulation, these sphingoid-1-phosphate derivatives act as substrates for the synthesis of phospholipids. For example, the production of fatty acid aldehydes and ethanolamine phosphate has been reported to potentially be driven by the cleavage activity of sphingoid phosphate lyase on sphingoid-1-phosphate. These generated products are later integrated into PEs (Merrill, 2002; Riley *et al.*, 1996; Stoffel *et al.*, 1974). These findings pertain to the direct disruption of sphingolipid metabolism, particularly through FUMs, which affect the proportions and composition of membrane phospholipids.

Though ceramides are not directly incorporated into membranes, they serve as precursors for the construction of vital membrane pieces known as complex sphingolipids like SMs and glycosphingolipids. Therefore, the typical mode of action of FUMs, which typically involves the suppression of CerS, could result in reduced production of ceramides (Tolleson *et al.*, 1999; Yoo *et al.*, 1996) and their complex sphingolipids. A proportional depletion in these lipids due to FUMs may lead to profound modifications in cell membrane integrity and cell signaling (Riley *et al.*, 2001; Riley and Merrill, 2019). Complex sphingolipids are characterized by long and very long SFA chains (16–24 carbon chains in most mammalian cells and 34 carbon chains in mammalian germ cells) (O'Brien and Rouser, 1964; Robinson *et al.*, 1992; Skotland and Sandvig, 2022). Consequently, any decrease in the production of complex sphingolipids, such as in the case of FUMs, could directly alter the overall composition of biomembranes. For instance, a remarkable reduction in SM, particularly SM-d18:1/16:0, SM-d18:0/18:0, SM-d18:1/18:0, and SM-d18:1/24:1, in porcine liver due to FB_1 exposure was reported by Loiseau *et al.* (2015). The same authors reported alterations in ceramide fatty species in the livers and lungs of piglets. Szabó *et al.* (2017), and Szabó *et al.* (2016a) suggested that the decrease in arachidic acid (C20:0) and behenic acid (C22:0) in rat hepatocellular membranes could be attributed to the FB_1 mode of action on ceramide. In support of this proposal, Zitomer *et al.* (2009) reported modifications in the proportions of behenic, lignoceric (C24:0), and nervonic (C24:1 n9) acids in ceramide and 1-deoxydihydroceramide ^{13}C bases of LY-B-LCB1 cells exposed to FB_1.

The interaction of complex sphingolipids, particularly SM, with membrane cholesterol (CHOL) and phospholipids plays a crucial role in determining the final composition of membrane lipids. According to Skotland and Sandvig (2019), PS interacts with sphingolipids, leading to increased interdigitation, a process that is enhanced by the presence of CHOL. SM significantly contributes to lipid raft formation by interacting with CHOL to create membrane microdomains, where approximately 70% of total cellular SM is found (Prinetti *et al.*, 2001). The interaction between SM and CHOL is facilitated by the unique characteristics of SM molecules, such as their extended saturated chains and their ability to donate and accept hydrogen. Modifications in these lipid fractions could have a profound impact on membrane leaflet interdigitation and cell membrane integrity. Such as CHOL facilitates lipid raft creation by interacting with various phospholipids, particularly demonstrating a preferential interaction with those highly incorporating SFAs (Niu and Litman, 2002). In this context, changes in the interaction between sphingolipids and CHOL could imply changes in CHOL's interaction with phospholipids, thereby altering the integrity and physicochemical properties of the membrane.

Despite the relatively lower abundance of sphingolipids, they have been shown to possess vital biological functions, such as sustaining the physical properties of biomembranes, functioning as secondary messengers, acting as ligands for cell membrane receptors, and playing a pivotal role in facilitating communication between cells and their external environment (Breslow, 2013). Therefore, modifications in the proportion of sphingolipids could strongly affect cell functions and overall survival. For example, toxicological reports have indicated their potential/involvement in altering gene expression, oxidative stress, intercellular communication, cell adhesion, proliferation, and the apoptosis rate. These induced biological events have been implicated in the development of pathophysiology and diseases, including cancer (Hannun and Obeid, 2018; Lee *et al.*, 2023). Considering that the proposed biochemical mechanisms of action involve alterations in *de novo* biosynthetic pathways, nutritional factors (sphingolipid composition) may substantially contribute to the potency of FUM and its observed toxic effects in animals. The interaction between dietary sphingolipids and blood-circulating lipids has been established (reviewed by Calzada *et al.* (2023) and Norris *et al.* (2019)). Furthermore, numerous investigations have aimed to establish associations between diseases and lipid biomarkers (Afshinnia *et al.*, 2018; McGranaghan *et al.*, 2021; Teixeira *et al.*, 2023; Tian *et al.*, 2022; Wei *et al.*, 2024; Zhao *et al.*, 2014). In the case of FUMs, certain organ toxicities and diseases have been observed to be species-specific. This might suggest a potential biomarker linked with these diseases, such as oleic acid and respiratory diseases (Lopez *et al.*, 2014). These lipid biomarkers often express a survival or apoptotic signal, depending on the tissue/organ where lipids accumulate.

2.6.1.2. Oxidative stress and lipid peroxidation

It is likely that liberated mitochondrial electrons interact with molecular oxygen to produce reactive oxygen species (ROS). ROS are typically composed of multiple compounds, the most recognized and prevalent of which are hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and superoxide radical ($O_2^{\cdot-}$) (Averill-Bates, 2023; Ma, 2013). Under normal metabolic conditions, ROS manifest valuable roles, mainly by providing systematic protection to cells. The redox system has been demonstrated to control the level of ROS within cells, a mechanism that ends by neutralizing ROS. This neutralization process is known to exhibit complexity, as indicated by the multiple enzymes and genes involved. The enzymes engaged in this operation are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with the antioxidant compound glutathione (GSH) (Wang *et al.*, 2016). However, an increase in ROS under metabolic dysfunction and disorders might not be compromised by the redox system, leading to an adverse condition referred to as oxidative stress. The

consequences of this process may include damage to cellular macromolecules, including lipids (Ma, 2013; Trachootham *et al.*, 2008).

The augmentation of oxidative stress by FUMs appears to not yet be fully understood, probably involving numerous factors. Remarkably, FUM does not induce direct oxidative stress; rather, it is a consequence of disruption of sphingolipid metabolism (second messengers involved in survival and apoptotic signals), deregulation of Ca^{2+} uptake, disruption of mitochondrial-associated membranes, dysfunction of mitochondria (inhibition of Complex 1), induction of cytotoxicity on DNA and protein synthesis, and disintegration of membranes such as lipid rafts (Arumugam *et al.*, 2019; Bratic and Larsson, 2013; Domijan and Abramov, 2011; Hammerschmidt and Brüning, 2022; Zigdon *et al.*, 2013). Elevated levels of ROS due to FB_1 exposure have been reported in *in vitro* and *in vivo* models (reviewed by da Silva *et al.* (2018) and Wang *et al.* (2016)), as well as to modify the Keap system-nuclear factor erythroid-2 (keap-Nrf2) pathway to trigger antioxidant transcriptions (Arumugam *et al.*, 2019), as demonstrated in Figure 7. The transformation of Keap-Nrf2 usually entails the adjustment of the cysteine thiols in both Keap1 and Nrf2, which is believed to modify the structure of Keap1–Nrf2–Cul3 complex, thereby obstructing the ubiquitination of Nrf2 (Lee and Hu, 2020; Yu and Xiao, 2021).

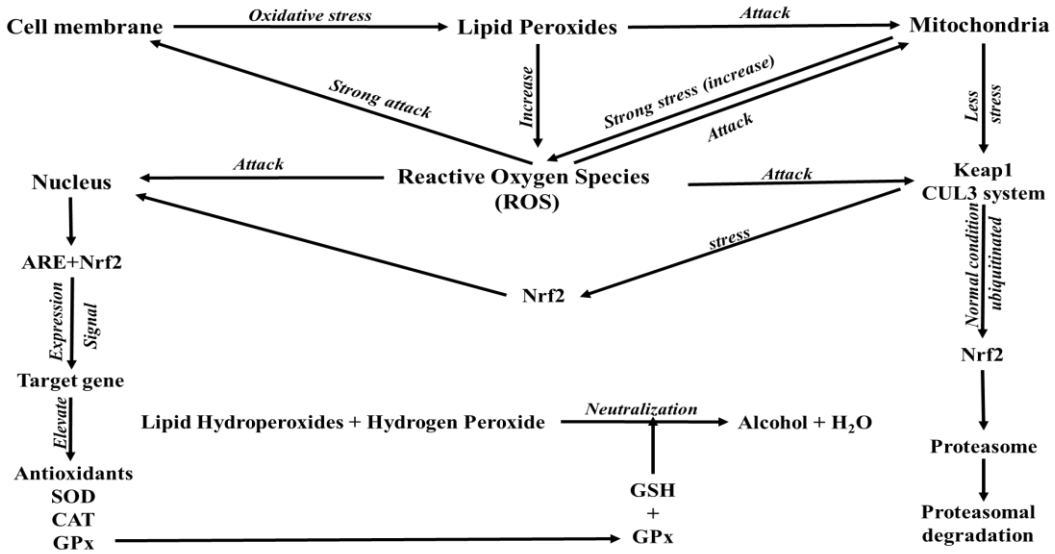


Figure 7. A diagrammatic depiction of the relationship between ROS production and biomembranes, as well as the role of redox system in neutralization of lipid hydroperoxides. Abbreviations: ARE, antioxidant response element; CAT, catalase; GPx, glutathione peroxidase; GSH, glutathione; Nrf2, Nuclear factor E2-related factor 2; SOD, superoxide dismutase.

A consequence of ROS elevation is the induction of H^+ removal reactions from membrane lipids (primarily lipids consisting of a high number of double bonds), an event known as "lipid peroxidation" (Hassan *et al.*, 2015; Stockmann-Juvala and Savolainen, 2008). The domain of lipid peroxidation research has been growing rapidly during the last decade (Ayala *et al.*, 2014), which is attributed to its serious adverse effects that can lead to the generation of lipid-peroxyl radicals and lipid hydroperoxides, which aggravate the destruction of membrane structures as well as macromolecules (including proteins and DNA). These events can possibly be biologically assessed through investigating the reduced activity of GSH, alterations in the expression of SOD, CAT, and GPx, and proportional elevation of the generated malonaldehyde (MDA) (Abbès *et al.*, 2016; Abel and Gelderblom, 1998; Ali *et al.*, 2019; Domijan *et al.*, 2007; Fodor *et al.*, 2015; Kócsó *et al.*, 2021; Poersch *et al.*, 2014; Szabó *et al.*, 2018). To align with the thesis's perspective, this section primarily focuses on *in vivo* model reports.

Primarily focusing on the effects of FUMs on the liver, most of the available *in vivo* data on lipid peroxidation and membrane lipids were obtained from rat models, followed by rabbits, with a single report on swine. Abel and Gelderblom (1998) reported the ability of FB_1 to induce oxidative stress in an *in vivo* model. These authors reported increased levels of thiobarbituric acid reactive substance (TBARS) as a result of exposure to 250 and 500 mg/kg FB_1 in the feed for 21 days. This was perhaps the first hint suggesting that FB_1 sensitizes hepatocytes towards oxidative stress. Similar findings were reported three years later in the rat liver by Gelderblom *et al.* (2001), who proposed that the hepatotoxic effects and augmented lipid peroxidation play a crucial role in initiating cancer. The consequence of these events is damage to cellular and microsomal membranes. Although these studies employed high doses and prolonged exposure designs, the augmentation of lipid peroxidation in rat liver was noticed in a study (Szabó *et al.*, 2016a) characterized by a lower toxin level and a shorter exposure period (50 mg FB_1/kg dose for 5 days). These authors reported an increase in the level of MDA and a decrease in the activity of GSH. High hepatic ROS generation by FB_1 has also been reported in rabbits fed 10 mg/kg diet for 4 weeks (Szabó *et al.*, 2016b), although the authors reported a minimal rate of lipid peroxidation. In piglets, 20 mg FBs/kg feed for 10 days resulted in the elevation of the lipid peroxidation end product (MDA) and antioxidant biomarkers in the liver (Ali *et al.*, 2019). Those studies by Ali *et al.* (2019), Szabó *et al.* (2016a, 2016b) proposed the potential involvement of lipid peroxidation in shaping the overall fatty acid profile of liver plasma and mitochondrial membranes, remarkably through decreasing proportions of UFAs. It is well known that PUFAs are

more prone to ROS than other types of fatty acids. According to Holman (1954), DHA is 8- and 320-fold more sensitive to ROS than are LA and oleic acid, respectively.

The kidney has not been intensively investigated within the area of potential oxidative stress induced by FUMs in relation to modifications in membrane lipids. When the kidney was inspected, FB_1 was shown not to exert lipid peroxidation in rats and piglets, despite alterations in membrane polar lipids being observed (Ali *et al.*, 2021; Szabó *et al.*, 2017). These studies were based on the EU-permitted level (5 mg/kg diet) as well as a shorter exposure period. However, Ali *et al.* (2019) found no indicator of oxidative stress within the renal tissue of piglets fed 20 mg FBs/kg feed for 10 days. On the other hand, after the GIT, the blood is the second body medium exposed to FUMs. Regrettably, no data are yet available concerning the association between oxidative stress induced by FUM and alterations in the membrane lipids of erythrocytes or in reproductive system organs. Nevertheless, 5 mg FBs/kg feed failed to augment oxidative stress in plasma and hemolyzed erythrocytes in piglets and rabbits (Ali *et al.*, 2021; Szabó *et al.*, 2016b), but the membrane lipid composition was not determined. In this regard, future studies should focus on filling this gap in the data.

2.6.1.3. Alterations in enzyme activities associated with lipid metabolism

The impact of FUM on lipid metabolism is very clear in the case of the inhibition of *N*-acyl transferase (ceramide synthase, or CerS), which has six isoforms in mammalian cells (Loiseau *et al.*, 2015; Wang *et al.*, 2016). Notably, each isoform exhibits affinity for a specific length of fatty acyl-CoA; therefore, they are crucial determinants of the ceramide fatty acid composition (Laviad *et al.*, 2008) and its overall concentration. Although FUM is theoretically capable of inhibiting all isoforms of CerS, selective interactions with specific isoforms have been reported to be tissue-specific. In piglets gavaged with 1.5 mg FB_1/kg BW/day for 9 days (Loiseau *et al.*, 2015), FB_1 upregulated the expression of genes encoding activities of 2- and 4-isoforms of CerS in the lungs. On the other hand, CerS-1 in the liver was more susceptible to FB_1. The observed modulation in acylated ceramide fatty acids suggests a plausible interaction between this toxin and the isoforms of CerS, as well as the distribution ratio of their activities. For instance, the 6-isoform of CerS regulates the acylation of fatty acids with C18 to C20 carbon chains, and the acylation of C14 to C16 fatty acids is linked with alike CerS-5 and -6 (Mullen *et al.*, 2012). These findings underscore the natural role of the selectivity of each enzyme for fatty acids. Among CerS enzymes, CerS-2 is predominant, and its activity appears to be marked in FUM-targeted tissues such as the lungs, liver, and kidneys. Therefore, its inhibition by FBs is particularly interesting due to its relationship with the low accumulation rate of ceramide in cells,

notably those with 20 to 26 fatty acids (Riley and Merrill, 2019; Mullen *et al.*, 2012; Laviad *et al.*, 2008), indicating the role of this enzyme in their acylation. In addition, the inhibition of this CerS isoform would project an aggregation of ceramides with short-chain fatty acids, which is suggested to be a compensatory mechanism in mice (Pewzner-Jung *et al.*, 2010). Upon incorporation into complex sphingolipids, these short-chain fatty acids can impact the physicochemical properties of membranes as well as the interactions between membrane lipids. Remaining with the sphingolipids, FUM has been proven to elevate the concentrations of sphingoid bases and their 1-phosphate derivatives. This indicates the ongoing activity of palmitoyltransferase after exposure to FUMs, an event promoted by Orm/ORMDL proteins (Davis *et al.*, 2019; He *et al.*, 2006), and a shift in the activity of sphingoid kinase. As mentioned in the previous section, an imbalance in sphingolipid accumulation can trigger their hydrolysis via sphingoid-lyase to produce fatty aldehydes and ethanolamine phosphate, which can possibly be incorporated into membrane lipids, particularly into PE. This refers to an imbalance in the proportion of membrane lipids.

Remarkably, the available literature lacks sufficient empirical data to support the notion that FUM directly disrupts the activities of enzymes related to phospholipid fatty acid metabolism. Therefore, at the present time, the plausible route is an indirect mechanism, although this is not yet fully comprehended. Basically, the major enzymes involved in *de novo* lipid metabolism are elongases (extending the chain of fatty acids, which is encoded by ELOVL genes), stearoyl-CoA desaturase (SCD: introduces a double bond on an SFA), and fatty acid desaturase enzymes (FAD: adds a double bond on a monounsaturated fatty acid (MUFA)) (Jump, 2009; Nakamura and Nara, 2004; Paton and Ntambi, 2009). Although the potential of FB_1 interference with phospholipase D (PLD) has been reported earlier in yeasts by Wu *et al.* (1995), the alteration of FAD by FUMs is the first reported alteration in this area. In an *in vivo* rat model exploring the liver microsomal fraction, a dose-dependent decrease in the activity of Δ6-desaturase (D6D) was observed upon FB_1 exposure (Gelderblom *et al.*, 2002). It is important to understand that this enzyme activity was determined analytically, whereas the following proposed enzymes were determined mathematically by calculating the ratio between the fatty acids related to the enzymatic activity precursor/product. In this context, subsequent research has built upon the observation of a decrease in Δ5-desaturase (D5D) activity, which was initially identified through the measurement of lipid biomarkers, specifically the 20:3n6/20:4n6 ratio. This finding has been corroborated in various studies using *in vivo* and *in vitro* models of FUM exposure in swine and rats, including those conducted by Ali *et al.* (2019, 2021) and

Burger *et al.* (2007, 2018). Desaturase enzymes, especially D6D, are known to be influenced by ER stress (Teng *et al.*, 2015), a typical toxic condition of FUM exposure expressed by an imbalance in sphingolipid metabolism. Thus, Riley and Merrill (2019) proposed that dysfunction of the ER is a factor resulting in the changing activities of D6D. The same mechanism can potentially be involved in altering the activity of SCD since it is anchored to the ER membrane (Paton and Ntambi, 2009). Nevertheless, this domain of research requires further studies to comprehend other events leading to desaturase alterations.

Although there are considerable data supporting the notion that FUMs interfere with the activity of delta-desaturase enzymes, the impact of FUMs on elongases is not explicitly delineated in the literature. However, the compositional disruption of medium- and long-SFAs within the membranes of rats' livers and piglets' livers and kidneys has been reported to be a consequence of FUM exposure (Ali *et al.*, 2019, 2021; Burger *et al.*, 2007, 2018). Apparently, it is incredibly challenging to specify the modification of elongase activities in these studies due to the complexity of events occurring simultaneously. Given the integral roles of SCD, delta-desaturase, and elongase enzymes in fatty acid metabolism, it is likely that FUMs influence the activity of elongase enzymes. However, this hypothesis necessitates further empirical investigation for validation.

2.6.1.4. Modulation of gene signaling involved in lipid metabolism

While the potential of fumonisins to cause genetic harm remains a contentious topic, it is widely accepted that they do not directly interact with DNA to cause genotoxicity. This recognition is based on empirical data from both *in vivo* and *in vitro* studies (Bondy *et al.*, 2012; Gelderblom and Marasas, 2012; IARC, 2002). However, genotoxic alterations were observed upon FUM exposure in rat liver, according to both *in vivo* and *in vitro* studies (Domijan *et al.*, 2008; Knasmüller *et al.*, 1997), revealing indirect reactivity between DNA and FUMs. Indeed, DNA damage caused by FB_1 has been observed to be dose- and time-dependent (Domijan *et al.*, 2008). Notably, the genotoxic effect of FUM is not yet fully understood, with many proposals suggesting that oxidative stress is a likely factor. For example, Sahu *et al.* (1998) studied whether FB_1 affects nuclear membranes and DNA in rats' livers and confirmed the induction of nucleolemmal lipid peroxidation and DNA damage. Thus, the authors hypothesized that peroxyl radicals and hydroxyl radicals are responsible for direct DNA damage. Though this appears to be a possible factor, Domijan *et al.* (2007) reported that DNA lesions developed earlier than the activation of the redox antioxidant system, suggesting that an imbalance in sphingolipid metabolism plays role in DNA damage.

Focusing on the effect of FUMs on sphingolipids leads to a compositional imbalance in their respective structures. Consequently, it is plausible that the comprehensive impact of fumonisins encompasses a multitude of biochemical processes. The metabolism of sphingolipids involves the inhibition of their enzymes, notably CerS, by FUMs. The six CerS isoforms are regulated by six genes found on different chromosomes (Pewzner-Jung *et al.*, 2006) that are susceptible to FUM, as demonstrated earlier in the lungs and livers of piglets exposed to FB_1 (Loiseau *et al.*, 2015), leading to alterations in fatty acid chains in ceramide and consequently SM. A similar approach can be established for desaturase enzymes, in which fatty acid desaturase gene 2 (FADS-2, which regulates the activity of D6D) has been reported to be altered due to FB_1 exposure (Gelderblom *et al.*, 2002) and its toxic effect on the ER (Teng *et al.*, 2015). However, the impact of FUMs on other FADSs, such as FADS1, FADS-3, and FADS-6, remains unexplored, underscoring the need for further research in these areas. The focus of these investigations should be to examine both direct and indirect influences, such as the consequences of specific sphingolipid accumulation or removal on lipid-gene signals and the interplay between sphingolipid genes and other lipid genes, which modify gene signals associated with lipid metabolism.

Remarkably, the lipogenesis process within mammalian cells is regulated by alike endogenous gene matrix and exogenous factors. For instance, the transcription of liver lipid genes is susceptible to alterations caused by PUFA accumulation (Blake and Clarke, 1990; Jump *et al.*, 1996). It is known that FUMs exert modifications on fatty acid compositions, suggesting that they consequently affect lipogenesis and membrane lipid structures. The Liver-X-Receptor (LXR), a member of the nuclear receptor superfamily, is predominantly found in the livers of humans and rodents (Gronemeyer *et al.*, 2004). It has been identified as a key player in CHOL and lipid metabolism, influencing the function of sterol regulatory element-binding protein-1 (SREBP-1c), FAS, and SCD-1 (Son and Paton, 2020). The transcription of LXR has been shown to play an important role in the livers of mice exposed to 10 mg FB_1/kg BW/day for 28 days (Régnier *et al.*, 2019). These authors proposed that LXR plays a crucial protective role against FB_1 toxicity, whereby its knockout may exacerbate alterations in lipogenesis homeostasis and FUM toxicoses.

2.6.2. Fumonisins-induced modification to the membrane lipid profile

Historically, membrane fatty acids have been shown to provide a general image of cell metabolism and disease progression, thus establishing them as reliable biomarkers. In this respect, lipidic biomarkers vary across pathophysiological conditions, depending on the animal, investigated tissue,

and severity of the condition. In the case of FUMs, the fatty acid composition of membrane lipids provides data that can potentially be incorporated into understanding the toxicity mechanism and cancer induction by FUMs. This section highlights major studies correlating FUMs with the disruption of membrane lipids.

2.6.2.1. Liver

The toxicity of FUM to membranes is well documented in the available literature on various species models, both *in vivo* and *in vitro*. Indeed, the liver appears to be the most investigated tissue concerning the modulation of membrane lipids by FUMs, with the most data obtained from rats compared to swine and rabbits. These data primarily focused on the fatty acids of the phospholipidome and specific phospholipid subclasses like PC, PE, PS, and PI. Remarkably, FBs exhibited potency in modulating the fatty acids of these membrane fractions (Ali *et al.*, 2019, 2021; Burger *et al.*, 2007, 2018; Gelderblom *et al.*, 1996a, 1997, 2001, 2002; Riedel *et al.*, 2015, 2016; Szabó *et al.*, 2016a, 2016b, 2017), whereas the destabilization of membrane fatty acids was related to the magnitude of the toxicity induced by this toxin. Thus, numerous underlying events have been proposed to aggravate the observed outcomes, primarily underscoring the involvement of sphingolipid metabolism, D6D, and lipid peroxidation (Gelderblom *et al.*, 2002; Riedel *et al.*, 2016; Szabó *et al.*, 2016a). Furthermore, the majority of these studies aimed to elucidate the role of lipid metabolism in cancer progression.

Gelderblom *et al.* (1996a) were the first to study the toxic effect of FB_1 on PC and PE in primary rat hepatocytes, revealing an elevation in total n6 fatty acids (Σn6) in PC and some of its respective fatty acids, with PE showing resistance in its fatty acid composition. Following these findings, the authors used a similar approach but in an *in vivo* model of rat liver (Gelderblom *et al.*, 1997), including drastic acute short-term exposure and prolonged chronic exposure. It is important to mention here that the findings of this study revealed results distinct from those of the former report, indicating potential contradictions in findings between *in vitro* and *in vivo* models. In the *in vivo* model, an FB_1 dose (500 mg/kg feed) resulted in a drastic proportional decrease in SM. Furthermore, in both settings of the *in vivo* model, PE was markedly increased, revealing compositional fatty acid modifications, primarily marked by the elevated proportions of LA, although acute exposure was more pronounced due to decreased EPA and Σn6 in PCs. Acute exposure also increased the proportion of EPA, whereas chronic exposure increased the overall n3 fatty acid (Σn3) content of PE, which subsequently decreased the Σn6: Σn3 ratio. These modifications in n6 and n3 fatty acids allowed the authors to conduct a further experiment to examine the potential alteration of enzymes involved in lipid

metabolism, particularly D6D and D5D (Gelderblom *et al.*, 2002). The outcomes of this investigation were promising, revealing that FB_1 can modify (decrease) the activities of D6D (determined analytically) and D5D (determined by the elevated specific lipid biomarker: 20:3n6/20:4n6 ratio). Notably, the activity of D6D decreased in a dose-dependent manner in response to FB_1. Furthermore, liver membrane CHOL and phospholipid fractions (PC, PE, PS, and PI) responded to FB_1 > 50 mg/kg diet, although the extent of the response varied among the fractions: PC and PE > PS and PI. In this study, the most pronounced proportional modifications of fatty acids were the elevation in total saturation (ΣSFA), monounsaturation (ΣMUFA), and Σn6 (including some of their respective fatty acids), while n3 fatty acids exhibited decreases due to the inhibition of D6D. These alterations were again confirmed by Burger *et al.* (2007), who supported the role of desaturase inhibition in FB_1 toxicity and reported an increase in CHOL and PE levels, an elevation in the CHOL/phospholipid ratio, a reduction in the PC/PE ratio, an elevation in ΣMUFA, and an increase in the LA proportion. The authors reported that PE varies in its extent of accumulation among liver cellular and subcellular membranes, with a high tendency observed in the microsomal, mitochondrial, and plasma membranes. SM exhibited, to some extent, a similar pattern: a strong decreasing tendency in the mitochondrial and nuclear fractions. However, this study also indicated different patterns regarding the Σn6: Σn3 ratio and total polyunsaturation (ΣPUFA), both of which decreased. Thus, the ratio between ΣPUFAs and SFAs exhibited a decrease in the PE fraction. The authors also observed a greater accumulation of AA in PE than in PC across various subcellular membranes, which led to a decrease in the AA-PC/PE ratio. The authors emphasized this decrease to shift into the prostanoid synthesis of the E2 series. AA has been shown to be involved in the regulation of mitochondria (interacting with the mitochondrial electron transport chain to induce a strong signal toward reactive oxygen species generation), and its metabolites influence cellular proliferation and apoptosis (Cocco *et al.*, 1999; Wang *et al.*, 2021).

Based on these findings, further investigations were carried out to confirm these modifications and their biological consequences in cancer, focusing merely on the modulation of PE and PC. For example, in an *in vivo* rat liver model, Riedel *et al.* (2015) reported findings similar to those of Burger *et al.* (2007) in the rat liver (*in vivo* study), which were combined with the modulation of membrane structure and fluidity. These authors proposed that these alterations are a consequence of carcinogenic stimulation. However, these modifications are contingent on the tissue/cell type. In an *in vitro* design, Chang liver cells were more resistant to FB_1 exposure than were primary rat hepatocytes. This observation was based on several lipid indicators, including a decrease in the PE/PC ratio and an

increase in CHOL and SM. Furthermore, Chang cells exhibited a high SFA composition within the PC and PE fractions, demonstrating more rigid membranes. The authors indicated that these changes modulate cell growth arrest, a mechanism to inhibit the potential carcinogenic effect of FB_1. Burger *et al.* (2018) reported distinct proportional alterations in the membrane lipids of primary rat hepatocytes compared to those reported in Chang cells. The originally asymmetrical hepatic subcellular membrane structures lost their integrity as a key result of FB_1, as the membrane microdomains were compositionally modified and consequently affected the lipid raft situation. In this study, the fatty acid composition of PE was more susceptible to FB_1 than to PC, resulting in an increase in the concentrations of n6 fatty acids and their respective sums while decreasing Σn3 due to a proportional reduction in EPA and DHA. This overall structural modification of membrane rafts is coupled with the alteration of signaling mechanisms, ultimately modulating cell regulation towards survival and/or cancer progression. Notably, those reported changes corresponded to a drastic cancer-promoting effect of FB_1 (250 mg/kg feed in *in vivo* models and 250 µM in *in vitro* models) (Gelderblom *et al.*, 1996b). Similar findings, including a decrease in n3 fatty acids, in the rat liver have also been reported to be induced under exposure to a low dose of FB_1 (Szabó *et al.*, 2016a), 50 mg FB_1/kg feed for 5 days. The authors implicated the role of lipid peroxidation (increased MDA in intoxicated rat liver) in compromising these n3 PUFAs. These authors also assessed the effect of lower doses of FB_1, DON, and ZEN (individually and combined) on rat liver membrane lipids (Szabó *et al.*, 2017), in which FB_1 alone could not modulate the disruption of membrane lipids, although hepato-histopathological findings were observed (Szabó-Fodor *et al.*, 2019). However, the effect of FB_1 was pronounced under the co-occurrence of DON, indicating the potential additive interaction between these fusariotoxins. It is important to understand that a relatively lower FB_1 dose than the established NOAEL for rat liver (1.25 mg/kg BW/day, according to EFSA (2005)) was applied in this study, which would likely explain the non-observed effect of FB_1 on the fatty acid profile of membrane lipids.

According to the aforementioned information, all these findings primarily corresponded to FB_1 exposure alone, while the co-occurrence effect of other FUMs was not determined. In addition, the above investigations were specific to the rat liver. Few data are available on the livers of other species, including three reports on swine and only one report on rabbit. However, neither the above-mentioned nor the following reports have investigated the dose- and time-dependent response of membrane fatty acids to FBs. In swine, hepatic membrane lipids exhibit a decrease in Σn3, whereas a high dose of FBs

can also compromise n6 fatty acids (Ali *et al.*, 2019, 2021). In the study by Ali *et al.* (2019), compositional modifications in membrane fatty acids were more pronounced than those in triglycerides and were attributed to histomorphological changes. Interestingly, a dose in line with the EU-permitted FB level (5 mg FB_{1+2}/kg feed) for 21 days was able to induce compositional alterations in porcine hepatic cell membranes, even before the induction of lipid peroxidation (Ali *et al.*, 2021). This pattern of findings appears to be similar to that of Domijan *et al.* (2007), where DNA fragmentation was observed before the induction of oxidative stress. Ali *et al.* (2021) also observed aggravated modifications in membrane lipids due to the co-occurrence of other *Fusarium* mycotoxins, namely DON and ZEN, which reflects the same pattern observed in rats (Szabó *et al.*, 2017). A further study on piglets investigated the effects of FB_1 on the proportion of SM and its fatty acids in porcine liver (Loiseau *et al.*, 2015). This study confirmed that long and very long MUFAs in SM are more susceptible to FB_1, revealing a reduction in their levels. The authors also reported modifications in the fatty acids of ceramides stemming from the selective affinity of FB_1 for specific isoforms of CerS. When examining the liver of rabbits, the plasma membrane of this tissue exhibited resistance to FB_1 (minimal modulation of membrane lipids (Szabó *et al.*, 2016b)) compared to its effect in swine. However, the hepatic subcellular lipids (mitochondria) revealed organelle stress, as indicated by elevated oleic acid and ΣMUFA, and decreased arachidonic, EPA, and ΣPUFA. This study also assessed the multi-toxic effects of FB_1 and T-2, which apparently exhibited antagonistic-like interactions. Overall, modifications in the membrane lipids of livers from swine and rabbits appear similar to those reported in rats, with some minimal discrepancies. These differences can be attributed to multiple factors, including genotype, applied dose of FBs, administration method, period of exposure, and the potential co-occurrence of other mycotoxins.

2.6.2.2. Kidneys

Nephrotoxicity has been reported to develop after exposure to FBs in male and female rats (Bucci *et al.*, 1998; Szabó *et al.*, 2018; Voss *et al.*, 1995), swine (Terciolo *et al.*, 2019), and rabbits (Gumprecht *et al.*, 1995; Kovács *et al.*, 2003; Orsi *et al.*, 2007). Despite the fact that the rat has been the most investigated animal model related to fatty acid modulation by FUMs, and its kidneys have been identified as the target organ (Voss *et al.*, 1995) for FB_1 toxicity, the available data are likely minimal in rats and swine (only based on *in vivo* models), while no data are available on rabbits and other animal species.

In a study by Szabó *et al.* (2018), rats were gavaged with 150 µg FB_1/animal/day (a dose lower than the established NOAEL of 0.2 mg FB_1/kg BW/day for rat kidneys) for 14 days. There were no marked modifications in the fatty acid proportions of renal total phospholipids. This observation is logical since no marked nephro-histomorphological alterations were proven (Szabó-Fodor *et al.*, 2019). However, unlike in the case of the rat liver, FB_1 had an antagonistic effect on renal membrane lipids in the presence of ZEN.

Focusing on swine, piglets were the subject of two studies. Remarkably, the outcomes of these studies reflect discrepancies between low and high doses of FBs. While 20 mg of FB_1 did not alter the composition of triglycerides or membrane lipids in piglet kidneys (Ali *et al.*, 2019), 5 mg of FBs showed modulation of renal membrane lipids (Ali *et al.*, 2021). According to the latter report, modifications in the fatty acid composition of renal total phospholipids included an elevation in ΣMUFA and its ratio to ΣPUFA, which was accompanied by a decrease in Σn3 and ΣSFA. Therefore, alterations in the fatty acid composition of kidney total phospholipids mirror those observed in the hepatocellular membranes of rats and swine. Notably, the outcomes of both studies on piglets were somewhat dependent on the development of histological lesions within the kidney, in which Ali *et al.* (2019) found no marked lesions in the kidney.

2.6.2.3. Erythrocytes

While the GIT is the first system exposed to FUMs, the blood represents the second medium. Elevated blood vessel permeability has been correlated with the progression of PPE, an atypical case of FB_1 toxicity in swine (Haschek *et al.*, 2001). The blood is characterized by a rapid elevation in the Sa-to-So ratio, a biomarker for assessing FUM exposure (Riley *et al.*, 2015). This elevation in free sphingoid bases plays a crucial role in hematotoxicity. As an illustration, the accumulation of Sa has been implicated in modulating intracellular ions (e.g., Ca^{2+}, Na^{+}, and K^{+}) and inducing apoptosis in leukocytes. It is well known that Na^{+}/K^{+} ATPase regulates the concentrations of these ions via the sodium-potassium pump within cells. The operation of Na^{+}/K^{+} ATPase is regulated by modulators such as those outside and inside the cell (Kazennov *et al.*, 1998). Furthermore, it has functional associations with several components of the membrane, especially systems that transport ions, proteins in the membrane, and the fatty acid composition of membrane phospholipids (Cohen *et al.*, 2005; Wu *et al.*, 2004). Thus, investigating membrane lipid composition would provide insight into the potential fatty acids involved in the regulation of the Na^{+}/K^{+} ATPase, which has been demonstrated to be just an enzyme activity increase by FB_1 in rabbit erythrocytes (Szabó *et al.*, 2014).

Although the authors proposed that the decrease in ceramide content caused by FB_1 (10 mg/kg feed for 28 days) increased the activity of Na^+/K^+ ATPase, alterations in fatty acid levels in erythrocyte membranes were also proven. Multiple proportional decreases in long PUFAs were observed after 14 days of FB_1 exposure. Remarkably, these findings reflect similarities to those reported in the liver and kidney. Furthermore, the interaction between FB_1 and T-2 in erythrocyte lipids was also antagonistic, similar to the observation in rat liver by Szabó *et al.* (2016a).

2.6.2.4. Reproductive system

FUMs are not typically considered mycotoxins that target the reproductive system; however, numerous studies have reported their toxic effects on both males and females. Many academic studies have linked FB_1 to decreased fertility and induced fetal toxicity in rodents (Gbore *et al.*, 2012; Lumsangkul *et al.*, 2019). This toxin was not only toxic to females, as it has also been reported to trigger reproductive toxicity in boars (Gbore, 2009; Gbore and Egbunike, 2008). In male rabbits, long-term exposure to low doses of FB_1 (0.13, 5, and 7.5 mg/kg feed for 196 days) has been associated with mild to moderate histopathological lesions in the testes, including degeneration of Sertoli cells (Ewuola, 2009). This toxicity is likely to alter the process of spermatogenesis, which is mainly marked by a striking decrease in sperm reserves in the testis, caput, corpus, and caudal epididymis (Ewuola and Egbunike, 2010b). Moreover, growing rabbits exposed to FB_1 above 7.5 mg/kg diet exhibited late puberty (Ewuola and Egbunike, 2010a), along with a decline in sperm quality characterized by severe abnormalities and reduced motility and viability. Similar findings were underscored due to FB_1 exposure in an *in vitro* model of equine spermatozoa (Minervini *et al.*, 2010). Interestingly, a low dose of 5 mg FB_1/kg feed was found to alter spermatogenesis in male rabbits, whereby this effect was exacerbated when DON and ZEN were also present (Fodor *et al.*, 2015), indicating an additive or less than additive effect. Given the previously mentioned evidence of the toxic effects of FB_1 on the male reproductive system, it is plausible that FB_1 could affect the membrane lipids of the testis and spermia. Regrettably, none of the studies that investigated the effects of FUMs on the genital system, for males and females, have included lipid data. Therefore, conducting experiments in this direction could provide further insight into the toxicological effects of FUMs.

2.7. Aims of the studies

Lipid compositional alterations, elevated/augmented oxidative stress, and sodium-potassium pump activity modifications (via an altered membrane lipid composition) have been proposed as outcomes of FB_1 exposure, as previously elucidated. Such modifications typically have far-reaching, profound

consequences, such as the disintegration of membrane integrity, leading cells to lose their functionality. To date, a few *in vitro* studies have investigated the disruption of membrane lipids by FB_1; however, these studies reported relatively drastic effects of toxin doses known to trigger cancer. These studies unutilized rat hepatocytes and cancer cells in their design. Further *in vivo* studies employing the same toxin doses are also available, reporting, to some extent, discrepancies between *in vitro* and *in vivo* models in rat liver. It is obvious that the rat model was the most utilized, whereas scarce investigations have been carried out on swine (liver, kidney, and lung) and rabbits (erythrocytes). Therefore, the existing literature might be considered somewhat inadequate, especially concerning swine and rabbits. Thus, further studies are necessary to confirm the reported alterations. In addition, there is a noticeable gap in the approach to dose- and time-dependent effects. Even though various dose paradigms were employed in the previously mentioned studies, no dose-response relationship was reported in relation to membrane fatty acids. Moreover, multiple studies have reported the effect of FB_1 on the male genital system, but no report has yet determined the magnitude of its toxicity on the fatty acid composition of the membranes of male reproductive tissues. Thus, it would be intriguing to investigate the following points in a sequential experimental plan, which are directly related to the dissertation of the doctorate:

1. Evaluation of the potential disruption caused by FBs to cellular membranes across different tissues and species (*in vivo* models), with a primary focus on cellular integrity.
2. Examination of the consequences of FBs on rats, swine, and rabbits, primarily focusing on the dose- and time-dependent response of fatty acids.
3. Investigation of the influence of FBs on the cation flux enzymatic regulation of erythrocytes in swine.
4. Determination of the potential induction of lipid peroxidation by FBs and its contribution to the final composition of membrane fatty acids.

3. MATERIALS AND METHODS

The comprehensive details of the materials and methodologies utilized in each study are included in the corresponding articles in Chapter 4. To avoid redundancy, this chapter does not repeat the descriptions of the materials and methods. Instead, it features a table (Table 1) that elucidates the core concept of each study along with the measurements and assessments employed.

Table 1. Overview of investigations into fatty acid composition alterations in various animal tissues: Present conditions and parameters assessed

Animal/tissue/Parameter	Study 1	Study 2	Study 3	Study 4
Rat	✓	-	-	-
Pig	-	✓	✓	-
Rabbit	-	-	-	✓
Liver	✓	-	✓	-
Kidney	✓	-	-	-
Lung	-	-	✓	-
Erythrocytes		✓	-	-
Testis	-	-	-	✓
Sperm cells	-	-	-	✓
Pure FB_1	✓	-	-	-
FB_1+FB_2+FB_3	-	✓	✓	✓
Interpretationally administration	✓	-	-	-
Oral administration	-	✓	✓	✓
Total phospholipid	-	✓	-	✓
Phosphatidylcholine	✓	-	✓	-
Phosphatidylethanolamine	✓	-	✓	-
Phosphatidylserine	✓	-	✓	-
Phosphatidylinositol	✓	-	✓	-
Dose-dependence	✓	✓	✓	✓
Time-dependent	✓	-	-	-
Oxidative capacity	✓	✓	✓	✓
Na^+/K^+ ATPase	-	✓	-	-
Sperm quality	-	-	-	✓
Correlation	✓	✓	✓	✓
Histological assessment	✓	-	✓	✓
Biochemical assessment	✓	-	✓	-

4. PUBLICATIONS INCLUDED IN THE THESIS

4.1. Fumonisin B1 induced compositional modifications of the renal and hepatic membrane lipids in rats – Dose and exposure time dependence

Title	Fumonisin B1 induced compositional modifications of the renal and hepatic membrane lipids in rats – dose and exposure time dependence
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Fumonisin B_1 induced compositional modifications of the renal and hepatic membrane lipids in rats – Dose and exposure time dependence

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ABSTRACT

Male Wistar rats were intraperitoneally dosed with fumonisin B1 (FB_1; 0, 20, 50 and 100 mg kg^{-1} dietary dose equivalent) for 5 & 10 days to assess dose- and time-dependent effects on renal and hepatic phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) fatty acid (FA) profiles. Renal PC showed increasing FA saturation (SAT) after 5 days; after 10 days polyunsaturation (PUFA) decreased markedly (Σ n3 (total n3), Σ n6, PUFA, unsaturation index (UI) and average FA chain length (ACL)), mostly with linear dose response. In the PI FAs similar changes were observed, decreasing monounsaturated FA, PUFA, UI and ACL (5 & 10 days), while the PE fraction was responsive in Σ n6 (↓) and SAT (↑), but only after 5 days (without dose response for both PI & PE). Liver PC exhibited increasing saturation (C16:0), decreasing polyunsaturation (C20:3 n6 [dihomo-γ-linolenic acid, DGLA]; C20:3 n3); the PI FA profile showed similar alterations after 5 days. PC & PI FA failed to respond in a dose-dependent manner to FB_1. In PE FA profile DGLA decreased, with a decrease of the total n6 FA proportion and dose-dependent increase of n3 FAs. Results revealed expressed renal sensitivity, supporting our earlier published results in terms of oxidative stress and histopathological modifications.

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Introduction

Fumonisins are mycotoxins (fungal secondary metabolites) produced by *Fusarium verticillioides* and *Fusarium proliferatum*. The 28 fumonisin (FUM) analogues characterised since 1988 can be divided into four main groups identified as the fumonisin A, B, C, and P series (Rheeder et al. 2002), from which toxicologically most risky/hazardous are the B analogues, FB_1 being the most well-known mycotoxin in this series.

In vivo studies on the toxicity of FB_1 indicate that kidneys and liver are the main target organs, particularly in rodents, providing species-specific effects (Bertero et al. 2018). All fumonisins are highly polar and water-soluble compounds. Structurally, the fumonisin backbone resembles the sphingoid bases sphinganine (Sa) and sphingosine (So) especially with the amino and hydroxy groups in positions C2 and C3, thus the key event is fumonisin-mediated inhibition of ceramide synthases (CerS) (Burger et al. 2007). Inhibition of CerS results in the disruption of sphingolipid metabolism and, as a consequence, alterations in other lipid pathways.

As a connexion to this critical point, multiple cellular or subcellular membrane-lipid-oriented *in vivo* studies (Gelderblom et al. 2002: microsomal membranes; Burger et al. 2007: microsomes, nuclei, mitochondria and plasma membrane, Burger et al. 2018: lipid rafts) have been focused on the harmful effects of FB_1.

The kidney definitely undergoes damage, since FB_1 is water-soluble and is eliminated in high part via the urine. Acute nephrotoxicity is rare, but repeated exposure leads to tubular epithelial injury and singe cell degeneration. Studies in rodents indicated dose-dependent relationship between biochemical alterations in sphingolipid metabolism and FB_1 exposure and a close correlation with the extent and severity of FB_1-induced histopathological modifications in the target organs of FB_1 toxicity and carcinogenicity (Riley et al. 1994; Gelderblom et al. 1996; Szabó et al.

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2018b). In Sprague–Dawley rats fed FB_1, concentrations of free sphingoid bases were found to be significantly higher in the kidney as compared to the liver, consistent with its greater susceptibility (Riley et al. 1994). However, besides the demonstration of FB_1-mediated ceramide synthesis inhibition (Wang et al. 1991), there is only a hint that FB_1 modifies the cellular FA profile, namely the anionic tricarballylic acids of FB_1 interfere with binding of fatty acyl-CoA (Merrill et al. 2001). More recently we published results from an *in vivo* rat study in which FB_1 (and other fusariotoxins) were shown to modulate the fatty acid profile of the renal total phospholipids (Szabó et al. 2018a), but neither dose response, nor exposure time dependence has been tested so far in the kidney, in the lipid fractions.

The hepatic effects, specifically in rats, are characterised with the marked proportional modification of the lipid fractions sphingomyelin, PE, PC, and ultimately total phospholipids (Gelderblom et al. 1997). Authors interpreted the fatty acid (FA) compositional modifications (disturbance of the n6/n3 FA balance) within the individual fractions as a risk factor affecting hepatocyte cell proliferation. The same theory has been tested by Riedel et al. (2016), describing FB_1-induced increase of the PE fraction in the rat liver, with increasing C16:0 levels and decreasing polyunsaturation. The altered lipid phenotype has been evaluated as a plausible growth stimulus for cancer promoters (Riedel et al. 2016), leading ultimately to the formation of hepatocyte nodules. The factors leading to the exertion of the possible carcinogenic potential (group 2B) are multiple, to which membrane lipid peroxidation must as well be added (Sahu et al. 1998; Szabó et al. 2016). An important addition to the cited *in vivo* results is that, *in vitro*, in primary hepatocytes, FB_1 has been proven to modify PE FA composition, PC/PE ratio, and ultimately the lipid raft function in primary rat hepatocytes (Burger et al. 2018). This effect is a further addition to the cytotoxic and pro-apoptotic character of FB_1 (Burger et al. 2007).

This study was intended to exactly test the dose- and time-dependent modification of the renal and hepatic PC, PI and PE fatty acid profiles of rats exposed to FB_1 at a relatively low level.

Material and methods

Animals and feeding

Adult male Wistar Crl:WI BR rats (8 weeks of age at the beginning) were enrolled in the study and were kept in metabolic cages (Tecniplast, Castronno, Italy) individually. The animals (n = 6 in each group, total n = 48) were fed Ssniff R/M-Z + H feed (Ssniff GmbH, Soest, Germany). The rats were kept in a 12-h light and 12-h dark daily rhythm, at 20°C in a rodent room, with a relative air humidity of 50%. Feed was offered *ad libitum*, and feed intake was measured daily.

Increasing concentrations of FB_1 (0, 20, 50 and 100 mg kg^{-1} diet, expressed as feed dose equivalent) were tested in a short and then a longer-term experiment (5 and 10 days, respectively). The pure mycotoxin was purchased from Merck-Sigma-Aldrich (Schnelldorf, Germany), and stock solutions were prepared with sterile physiological salt solution. The solutions contained the daily toxin dose in exactly 1 ml, and this solution was administered as a single intraperitoneal dose. For the control animals (C), 1 ml of sterile physiological salt solution was dosed.

Mycotoxin treatment was set as follows: 36 µg per animal day^{-1} (approx. 120 µg kg^{-1} BW day^{-1}), 90 µg per animal day^{-1} (approx. 300 µg kg^{-1} BW day^{-1}) and 180 µg per animal day^{-1} (approx. 600 µg kg^{-1} BW day^{-1}). Calculating with the average feed intake of 30 g per animal day^{-1} and the absorption ratio of the toxin (Martinez-Larranaga et al. 1999), the intraperitoneal (i.p.) administration represented the following dietary exposures: approx. 20, 50 and 100 mg kg^{-1} dietary equivalent for FB_1.

The two, basically similar treatments lasted for 5 (n = 24) and 10 days (n = 24), respectively, only the exposure time being different. On day 6 and 11, after taking blood from the retro-orbital plexus, the animals were sacrificed by cervical dislocation and were immediately dissected.

The experimental protocol was authorised by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office, under the permission number SOI/31/00308-10/2017.

Tissue lipid analysis

Kidney and liver samples (after frozen storage at −20°C) were homogenised (IKA T25 Digital Ultra

Turrax, Staufen, Germany) in 20-fold volume of chloroform:methanol (2:1) and total lipid content (complex lipids) was extracted according to Folch et al. (1957). Solvents were ultrapure-grade (Merck Sigma-Aldrich, Schnelldorf, Germany) and 0.01% butylated hydroxytoluene was added to prevent fatty acid oxidation.

For the separation of phospholipid classes, thin layer chromatography (TLC) was used. Extracted complex lipids were spotted onto pre-dried (110°C, 2 h) 20 × 20 cm TLC plates (Sigma Cat. No.: 99570). Separation was performed in one dimension, using the eluent mixture of chloroform: methanol:water (25:10:1), developing the plate until the top, in an all-glass, covered, TLC chamber (Christie 2003). Primuline spray (5 mg in 100 ml of acetone:water (80:20, Merck-Sigma Cat. No.: 206865)) was used to stain lipid spots, and detection was performed under ultraviolet light (365 nm). To identify PL classes, certified reference materials were used as follows: L-α-phosphatidylcholine (Merck-Sigma Cat. No.: P3556), L-α-phosphatidylinositol (from Glycine max, Merck-Sigma Cat. No. P6636) and L-α-phosphatidylethanolamine (Merck-Sigma Cat. No.: P7943).

Identified fractions were scrapped off from the plates and were extracted 3 times into the TLC eluent mixture. Subsequently, solvent was evaporated entirely and lipids were trans-methylated with an acid-catalysed method, using 1% H_2SO_4 in methanol (Christie 2003). Fatty acid methyl esters were extracted into 150 µL ultrapure n-hexane for gas chromatography, which was performed on a GC-MS-QP2010 Plus equipment (AOC 20i automatic injector), equipped with a Phenomenex Zebron ZB-WAX Capillary GC column (30 m, 0.25 mm ID, 0.25-µm film, Phenomenex Inc., Torrance, CA, USA). Characteristic operating conditions were: injector temperature: 270°C, helium flow: 28 cm sec^{-1}. The oven temperature was programmed from 80°C to 205°C: 2.5°C min^{-1}, 5 min at 205°C, from 205°C to 250°C 10°C min^{-1} and 5 min at 210°C. The makeup gas was nitrogen. To identify individual FA, an authentic external FA standard (37 Component FAME Mix, Sigma Cat. No.: CRM47885) was used. Fatty acid results were expressed as weight % of total fatty acid methyl esters. The unsaturation index (UI) was calculated to express the number of double bonds in 100 FA chains.

Statistical analysis

Statistical analyses were performed using IBM SPSS 20.0 (2012) software. Data processing and the mathematical-statistical calculations were performed using one-way analysis of variance (ANOVA). The grouping variable was the mycotoxin dose (df = 3), while exposure times (5 and 10 days) were handled separately.

Linear regression model was used to test dose-dependent fitting in case of the fatty acid components and calculated parameters. Results were only interpreted if the linear regression model fitting gave an R square value over 0.6; this fitting was tested exclusively in cases where ANOVA provided inter-group differences.

In the statistical analyses, differences between groups were considered significant when P values were <0.05.

Results

Kidney

Kidney phosphatidylcholine – 5 days

Inter-group differences. The kidney PC fraction provided marked toxin dose-associated response (Table 1). In the 5-day treatment myristic (C14:0) and palmitic (C16:0) acids showed proportional increases, resulting in the similarly increasing level of total FA saturation. In contrast, from within the polyunsaturated FAs, C20:3 n6 (dihomo-α-linolenic, DGLA), C20:4 n6, C20:5 n3, C22:5 n3 and C22:6 n3 decreased, as compared to the control. As a result, Σ n3 and Σ n6 FA proportions, as well as the total polyunsaturation (PUFA), unsaturation index (UI) and the average FA chain length decreased in all intoxicated groups as compared to the control.

Dose response. Cases, where the linear fitting was acceptable, are summarised in Table 7. These were C16:0, C20:4 n6, C22:6 n3, PUFA, Σ n3, Σ n6, UI and ACL.

Table 1. The renal phosphatidylcholine fatty acid profile of the rats after 5 and 10 days of FB1 exposure.

Group	Control		20 mg kg $^{-1}$		50 mg kg $^{-1}$		100 mg kg $^{-1}$	
Fatty acid	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Kidney phosphatidylcholine - 5-day exposure								
C14:0	0.37 ± 0.03	**a**	0.62 ± 0.10	**b**	0.59 ± 0.09	**b**	0.58 ± 0.13	**b**
C15:0	0.23 ± 0.02		0.33 ± 0.07		0.24 ± 0.04		0.25 ± 0.12	
C16:0	31.3 ± 1.15	**a**	35.0 ± 2.22	**b**	36.0 ± 1.13	**b**	36.9 ± 0.84	**b**
C16:1 n7	0.24 ± 0.09		0.24 ± 0.08		0.34 ± 0.11		0.27 ± 0.13	
C18:0	35.7 ± 2.99	**a**	36.8 ± 2.30	**ab**	40.1 ± 2.25	**bc**	40.6 ± 1.70	**c**
C18:1 n9c	3.12 ± 0.52		3.44 ± 0.43		3.41 ± 0.32		2.98 ± 0.52	
C18:2 n6c	6.09 ± 0.99	**b**	5.94 ± 0.81	**b**	5.01 ± 0.47	**ab**	4.64 ± 0.72	**a**
C18:3 n6	0.05 ± 0.01		0.05 ± 0.02		0.04 ± 0.01		0.05 ± 0.00	
C18:3 n3	0.05 ± 0.01	**a**	0.09 ± 0.02	**b**	0.06 ± 0.01	**ab**	0.06 ± 0.01	**a**
C20:0	0.26 ± 0.04		0.30 ± 0.04		0.31 ± 0.03		0.30 ± 0.03	
C20:1 n9	0.11 ± 0.04	**b**	0.07 ± 0.02	**ab**	0.09 ± 0.02	**ab**	0.06 ± 0.02	**a**
C20:2 n6	0.19 ± 0.06		0.21 ± 0.06		0.18 ± 0.09		0.15 ± 0.04	
C20:3 n6	0.72 ± 0.16	**b**	0.53 ± 0.13	**a**	0.41 ± 0.04	**a**	0.39 ± 0.11	**a**
C20:4 n6	19.9 ± 2.30	**b**	15.2 ± 2.85	**a**	12.2 ± 2.28	**a**	11.8 ± 1.05	**a**
C20:3 n3	0.02 ± 0.00		0.02 ± 0.01		0.02 ± 0.00		0.01 ± 0.00	
C20:5 n3	0.11 ± 0.02	**ab**	0.14 ± 0.04	**b**	0.10 ± 0.02	**a**	0.08 ± 0.02	**a**
C22:0	0.06 ± 0.01		0.06 ± 0.02		0.06 ± 0.01		0.05 ± 0.01	
C22:5 n3	0.23 ± 0.03	**b**	0.18 ± 0.03	**a**	0.15 ± 0.04	**a**	0.14 ± 0.02	**a**
C22:6 n3	1.15 ± 0.14	**c**	0.75 ± 0.11	**b**	0.50 ± 0.12	**a**	0.57 ± 0.04	**a**
C24:0	0.09 ± 0.02		0.07 ± 0.06		0.06 ± 0.01		0.06 ± 0.03	
Σ saturated	68.0 ± 4.12	**a**	73.2 ± 3.92	**ab**	77.5 ± 3.14	**bc**	78.8 ± 2.35	**c**
Σ unsaturated	32.0 ± 4.12	**c**	26.8 ± 3.92	**bc**	22.5 ± 3.14	**ab**	21.2 ± 2.35	**a**
MUFA	3.48 ± 0.59		3.76 ± 0.48		3.83 ± 0.41		3.31 ± 0.61	
PUFA	28.5 ± 3.55	**c**	23.0 ± 3.73	**b**	18.7 ± 2.81	**ab**	17.9 ± 1.81	**a**
Σ n6	26.8 ± 3.34	**c**	21.7 ± 3.56	**b**	17.7 ± 2.64	**ab**	16.9 ± 1.72	**a**
Σ n3	1.55 ± 0.19	**c**	1.17 ± 0.18	**b**	0.83 ± 0.15	**a**	0.87 ± 0.06	**a**
n6 / n3	17.4 ± 0.88	**a**	18.6 ± 2.02	**ab**	21.3 ± 1.03	**c**	19.4 ± 0.90	**bc**
odd chain	0.23 ± 0.02		0.33 ± 0.07		0.24 ± 0.04		0.25 ± 0.12	
UI	106.9 ± 13.01	**c**	84.8 ± 13.8	**b**	68.9 ± 11.0	**ab**	66.2 ± 6.43	**a**
ACL	17.8 ± 0.08	**b**	17.6 ± 0.11	**a**	17.5 ± 0.07	**a**	17.5 ± 0.04	**a**
Kidney phosphatidylcholine - 10-day exposure								
C14:0	0.27 ± 0.08		0.25 ± 0.14		0.25 ± 0.11		0.25 ± 0.02	
C15:0	29.1 ± 1.32	**a**	32.8 ± 0.79	**ab**	34.0 ± 4.89	**b**	31.6 ± 0.57	**a**
C16:0	0.30 ± 0.07	**a**	0.33 ± 0.16	**a**	0.64 ± 0.12	**b**	0.72 ± 0.14	**b**
C16:1 n7	0.41 ± 0.04		0.39 ± 0.04		0.28 ± 0.18		0.35 ± 0.07	
C18:0	34.2 ± 1.73		36.6 ± 2.08		30.8 ± 7.48		34.2 ± 1.86	
C18:1 n9c	3.84 ± 0.31	**a**	4.95 ± 0.81	**a**	7.58 ± 1.02	**b**	7.49 ± 0.99	**b**
C18:2 n6c	5.64 ± 0.52		5.33 ± 0.60		4.66 ± 2.29		5.97 ± 0.65	
C18:3 n6	0.08 ± 0.01		0.04 ± 0.01		0.07 ± 0.05		0.07 ± 0.02	
C18:3 n3	0.05 ± 0.01		0.07 ± 0.02		0.09 ± 0.04		0.09 ± 0.01	
C20:0	0.28 ± 0.03		0.36 ± 0.04		0.38 ± 0.12		0.36 ± 0.04	
C20:1 n9	0.10 ± 0.03	**a**	0.18 ± 0.03	**b**	0.32 ± 0.08	**c**	0.32 ± 0.04	**c**
C20:2 n6	0.28 ± 0.06	**a**	0.42 ± 0.06	**ab**	0.50 ± 0.18	**b**	0.52 ± 0.06	**b**
C20:3 n6	1.14 ± 0.19	**b**	0.47 ± 0.10	**a**	0.43 ± 0.13	**a**	0.36 ± 0.06	**a**
C20:4 n6	22.0 ± 2.35	**b**	15.7 ± 0.99	**a**	17.8 ± 2.89	**a**	15.6 ± 1.05	**a**
C20:3 n3	0.04 ± 0.02		0.02 ± 0.00		0.03 ±		0.02 ± 0.01	
C20:5 n3	0.12 ± 0.03	**b**	0.10 ± 0.03	**ab**	0.10 ± 0.03	**ab**	0.07 ± 0.02	**a**
C22:0	0.06 ± 0.01		0.08 ± 0.03		0.08 ± 0.03		0.08 ± 0.01	
C22:5 n3	0.24 ± 0.02	**a**	0.24 ± 0.03	**a**	0.33 ± 0.04	**b**	0.34 ± 0.07	**b**
C22:6 n3	1.29 ± 0.18	**b**	0.88 ± 0.08	**a**	1.04 ± 0.15	**ab**	0.99 ± 0.19	**a**
C24:0	0.16 ± 0.09		0.12 ± 0.04		0.10 ± 0.03		0.11 ± 0.02	
Σ saturated	64.8 ± 3.13	**a**	71.2 ± 2.15	**b**	66.8 ± 2.35	**a**	67.9 ± 2.41	**ab**
Σ unsaturated	35.2 ± 3.13	**b**	28.8 ± 2.15	**a**	33.2 ± 2.35	**b**	32.1 ± 2.41	**ab**
MUFA	4.34 ± 0.33	**a**	5.51 ± 0.80	**a**	8.19 ± 1.25	**b**	8.16 ± 0.93	**b**
PUFA	30.9 ± 3.08	**b**	23.3 ± 1.60	**a**	25.0 ± 1.28	**a**	24.0 ± 1.68	**a**
Σ n6	28.8 ± 2.84	**a**	21.6 ± 1.48	**a**	23.0 ± 0.96	**a**	22.0 ± 1.47	**b**
Σ n3	1.74 ± 0.22	**b**	1.30 ± 0.11	**a**	1.56 ± 0.22	**ab**	1.49 ± 0.26	**ab**
n6 / n3	16.6 ± 0.74		16.6 ± 1.28		14.9 ± 1.69		15.0 ± 1.96	
odd chain	29.1 ± 1.32	**a**	32.8 ± 0.79	**ab**	34.0 ± 4.89	**b**	31.6 ± 0.57	**ab**
UI	117.6 ± 11.76	**b**	88.7 ± 5.91	**a**	99.8 ± 10.05	**a**	92.9 ± 6.76	**a**
ACL	17.6 ± 0.10	**b**	17.4 ± 0.04	**a**	17.4 ± 0.09	**a**	17.4 ± 0.04	**a**

[a,b]Different uppercase letters indicate significant difference of the mean values at $p < 0.05$.

Kidney phosphatidylcholine – 10 days

Inter-group differences. Fatty acids where the mycotoxin exposure caused inter-group differences were gondoic acid (C20:1 n9) and, totally similar to the 5-day exposure, DGLA and arachidonic acid (C20:4 n6, AA). Control *vs.* toxin treated group differences were detected moreover for UI and ACL (Table 1).

Dose response. When testing for linear dose response, palmitic, oleic, gondoic acids, DGLA and total monounsaturation (MUFA) were providing linear results (Table 7). All r-square values were markedly higher in this treatment (i.e. 10-day exposure), as compared to the 5-day case (Table 7).

Kidney phosphatidylinositol – 5 days

Inter-group differences. In the renal PI FA profile, increasing saturated FA proportions were detected (myristic and stearic acids), as compared to the control (Table 2). Moreover, this increasing saturation was paired with decreasing monounsaturation, polyunsaturation, UI and ACL, as a result of the mycotoxin treatment.

Dose response. The marked decrease of unsaturation was underscored with the linear regression results, namely stearic acid provided an accurate fitting with positive slope, while the opposite was proven for PUFA (Table 7).

Kidney phosphatidylinositol – 10 days

Inter-group differences. The mycotoxin treatment associated differences were proven for stearic acid and consequently for the total saturation level (Table 2). Moreover, linoleic acid (LA), DGLA, PUFA, Σ n6 FA and UI decreased markedly, as compared to the control.

Dose response. From all FAs tested for a possible dose response the only one providing a mycotoxin-related proportional, linear depletion was DGLA.

Kidney phosphatidylethanolamine FA profile – 5 days

Inter-group differences. There were two marked processes attained in this lipid fraction: the determinant depletion of the n6 FAs (C18:2 n6 and C20:3 n6), and the increasing saturation (Σ saturated FA), as compared to the control (Table 3).

Kidney phosphatidylethanolamine FA profile – 10 days

Inter-group differences. Two saturated fatty acids, arachidic and behenic acids (C20:0 & C22:0), showed significant proportional increase as a result of mycotoxin dosage (Table 3).

Liver

Liver phosphatidylcholine – 5 days

Inter-group differences. As compared to the control, palmitic acid proportion increased in all intoxicated groups, while DGLA, eicosatrienoic acid (C20:3 n3) and ACL proportions decreased (Table 4).

Liver phosphatidylcholine – 10 days

Inter-group differences. In a control *vs.* mycotoxin treated form, there were only three compounds/groups with elevated levels: oleic acid (C18:1 n9), docosahexaenoic acid (C22:6 n3) and the Σ n3. In contrast, FB_1 administration depleted the DGLA and eicosatrienoic acid proportions (Table 4).

Liver phosphatidylinositol – 5 days

Inter-group differences. The comparison between control and treated groups revealed an increasing level of palmitic acid, in all toxin-fed animals. In contrast, decreasing proportions were found for palmitoleic, oleic, linoleic acids and DGLA, along with the total n6 FA and the PUFA level (Table 5).

Liver phosphatidyl-inositol – 10 days

Inter-group differences. Minimal mycotoxin-associated effects were detected in this fraction. As compared to the control, gondoic acid proportion increased, but only unsaturation index and ACL were different ($50\ mg\ kg^{-1} < 100\ mg\ kg^{-1}$) (Table 5).

Liver phosphatidylethanolamine FA profile – 5 days

Inter-group differences. In the FA profile of this PL class, the proportion of DGLA decreased markedly, as compared to the control (Table 6).

Liver phosphatidylethanolamine FA profile – 10 days

Inter-group differences. In this setting, the proportion of linoleic acid, and as a consequence, the total n6 FA proportion decreased, as compared to the control. In the n3 FAs EPA decreased and the total n3 proportion increased in the toxin treated group, as compared to the control (Table 6).

Table 2. The renal phosphatidyl-inositol fatty acid profile of the rats after 5 and 10 days of FB1 exposure.

Group	Control		20 mg kg $^{-1}$		50 mg kg $^{-1}$		100 mg kg $^{-1}$	
Fatty acid	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Kidney phosphatidyl-inositol - 5-day exposure								
C14:0	0.57 ± 0.09	**a**	0.79 ± 0.07	**b**	0.77 ± 0.05	**b**	0.73 ± 0.12	**b**
C15:0	0.14 ± 0.01		0.19 ± 0.06		0.16 ± 0.03		0.16 ± 0.03	
C16:0	33.2 ± 3.85	**a**	36.6 ± 1.51	**ab**	38.4 ± 0.53	**b**	39.3 ± 0.86	**b**
C16:1 n7	0.37 ± 0.18		0.24 ± 0.13		0.29 ± 0.08		0.28 ± 0.03	
C18:0	45.2 ± 3.53	**a**	47.0 ± 2.91	**ab**	49.7 ± 1.07	**b**	50.1 ± 1.37	**b**
C18:1 n9c	1.33 ± 0.62		0.89 ± 0.21		0.76 ± 0.13		0.87 ± 0.41	
C18:2 n6c	12.3 ± 6.26		11.58 ± 3.48		7.58 ± 0.88		6.57 ± 1.38	
C18:3 n6	0.03 ± 0.00		0.04 ± 0.01		±		±	
C18:3 n3	0.06 ± 0.04		0.08 ± 0.03		0.06 ± 0.02		0.04 ± 0.02	
C20:0	0.30 ± 0.15		0.39 ± 0.03		0.40 ± 0.01		0.41 ± 0.01	
C20:1 n9	0.20 ± 0.03		0.00 ±		0.23 ±		±	
C20:2 n6	0.23 ± 0.09	**b**	0.23 ± 0.07	**b**	0.17 ± 0.05	**ab**	0.12 ± 0.04	**a**
C20:3 n6	0.37 ± 0.15	**b**	0.31 ± 0.12	**ab**	0.20 ± 0.04	**a**	0.17 ± 0.06	**a**
C20:4 n6	4.98 ± 7.91		1.09 ± 0.41		1.03 ± 0.47		0.91 ± 0.29	
C20:3 n3	0.02 ± 0.01		0.03 ± 0.01					
C20:5 n3	0.05 ± 0.06		0.06 ± 0.01					
C22:0	0.06 ± 0.01		0.07 ± 0.02		0.07 ± 0.02		0.07 ± 0.01	
C22:5 n3	0.13 ± 0.08	**b**	0.09 ± 0.03	**ab**	0.05 ± 0.01	**a**	0.05 ± 0.01	**a**
C22:6 n3	0.43 ± 0.51		0.21 ± 0.07		0.12 ± 0.05		0.12 ± 0.02	
C24:0	0.09 ± 0.04		0.08 ± 0.04		0.08 ± 0.02		0.09 ± 0.02	
Σ saturated	79.6 ± 7.10	**a**	85.2 ± 4.32	**ab**	89.7 ± 1.42	**b**	90.9 ± 1.71	**b**
Σ unsaturated	20.4 ± 7.11	**b**	14.8 ± 4.32	**ab**	10.3 ± 1.42	**a**	9.13 ± 1.71	**a**
MUFA	1.90 ± 0.60	**b**	1.13 ± 0.27	**a**	1.09 ± 0.20	**a**	1.16 ± 0.41	**a**
PUFA	18.5 ± 6.67	**b**	13.6 ± 4.16	**ab**	9.21 ± 1.26	**a**	7.97 ± 1.59	**a**
Σ n6	17.6 ± 6.17	**b**	13.0 ± 3.98	**ab**	8.81 ± 1.20	**a**	7.64 ± 1.55	**a**
Σ n3	0.67 ± 0.62		0.41 ± 0.13		0.23 ± 0.05		0.21 ± 0.03	
n6 / n3	34.4 ± 10.62		32.1 ± 3.21		38.7 ± 6.41		37.0 ± 8.42	
odd chain	0.14 ± 0.01		0.19 ± 0.06		0.16 ± 0.03		0.16 ± 0.03	
UI	51.6 ± 29.30	**b**	32.2 ± 9.77	**ab**	22.5 ± 3.63	**a**	19.8 ± 3.72	**a**
ACL	17.5 ± 0.25	**b**	17.3 ± 0.04	**ab**	17.2 ± 0.02	**a**	17.2 ± 0.03	**a**
Kidney phosphatidyl-inositol - 10-day exposure								
C14:0	0.21 ± 0.05		0.23 ± 0.04		0.19 ± 0.02		0.22 ± 0.06	
C15:0	32.7 ± 1.69	**a**	36.3 ± 1.18	**b**	34.7 ± 0.46	**b**	35.8 ± 1.11	**b**
C16:0	0.62 ± 0.16		0.48 ± 0.15		0.65 ± 0.26		0.61 ± 0.24	
C16:1 n7	0.32 ± 0.04	**ab**	0.34 ± 0.02	**ab**	0.29 ± 0.06	**a**	0.36 ± 0.04	**b**
C18:0	43.5 ± 2.26	**a**	48.0 ± 1.93	**b**	47.3 ± 1.15	**b**	48.8 ± 2.76	**b**
C18:1 n9c	1.71 ± 0.20	**a**	1.74 ± 0.42	**ab**	2.58 ± 0.27	**c**	2.47 ± 0.77	**bc**
C18:2 n6c	15.3 ± 2.51	**b**	8.41 ± 1.31	**a**	9.44 ± 0.75	**a**	6.99 ± 1.92	**a**
C18:3 n6	0.05 ± 0.02		0.04 ±		±		±	
C18:3 n3	0.08 ± 0.03		0.08 ± 0.03		0.09 ± 0.02		0.10 ± 0.03	
C20:0	0.37 ± 0.04	**a**	0.42 ± 0.05	**ab**	0.42 ± 0.02	**ab**	0.43 ± 0.02	**b**
C20:1 n9	0.25 ± 0.07	**ab**	0.20 ± 0.09	**a**	0.20 ± 0.12	**a**	0.38 ± 0.09	**b**
C20:2 n6	0.36 ± 0.07	**b**	0.27 ± 0.07	**ab**	0.28 ± 0.03	**ab**	0.26 ± 0.06	**a**
C20:3 n6	0.69 ± 0.12	**b**	0.16 ± 0.08	**a**	0.14 ± 0.02	**a**	0.11 ± 0.06	**a**
C20:4 n6	2.36 ± 1.30		2.00 ± 1.45		2.25 ± 0.39		2.19 ± 0.85	
C20:3 n3	0.07 ± 0.01						0.04 ± 0.01	
C20:5 n3	0.03 ± 0.01		0.02 ± 0.03		0.02 ± 0.03		0.03 ± 0.01	
C22:0	0.08 ± 0.01		0.08 ± 0.01		0.08 ± 0.02		0.08 ± 0.01	
C22:5 n3	0.14 ± 0.07		0.07 ± 0.02		0.12 ± 0.06		0.10 ± 0.05	
C22:6 n3	0.43 ± 0.13	**b**	0.22 ± 0.10	**a**	0.35 ± 0.09	**ab**	0.28 ± 0.13	**ab**
C24:0	0.15 ± 0.03		0.12 ± 0.03		0.12 ± 0.03		0.13 ± 0.05	
Σ saturated	78.3 ± 3.74	**a**	86.5 ± 2.82	**b**	84.2 ± 1.31	**b**	86.8 ± 3.45	**b**
Σ unsaturated	21.7 ± 3.74	**b**	13.5 ± 2.82	**a**	15.8 ± 1.31	**a**	13.2 ± 3.45	**a**
MUFA	2.27 ± 0.22	**a**	2.28 ± 0.45	**a**	3.07 ± 0.28	**b**	3.20 ± 0.77	**b**
PUFA	19.5 ± 3.71	**b**	11.2 ± 2.39	**a**	12.7 ± 1.09	**a**	10.0 ± 2.69	**a**
Σ n6	18.4 ± 3.49	**b**	10.6 ± 2.23	**a**	11.8 ± 0.98	**a**	9.29 ± 2.47	**a**
Σ n3	0.68 ± 0.20	**b**	0.37 ± 0.14	**a**	0.57 ± 0.13	**ab**	0.46 ± 0.19	**ab**
n6 / n3	28.0 ± 3.92		30.1 ± 7.10		21.6 ± 4.67		22.0 ± 5.19	
odd chain	32.7 ± 1.69	**a**	36.3 ± 1.18	**b**	34.7 ± 0.46	**b**	35.8 ± 1.11	**b**
UI	48.9 ± 10.37	**b**	30.1 ± 8.33	**a**	35.0 ± 3.37	**a**	29.2 ± 8.08	**a**
ACL	17.1 ± 0.08	**b**	16.9 ± 0.07	**a**	17.0 ± 0.02	**ab**	16.9 ± 0.05	**a**

[a,b]: different uppercase letters indicate significant difference of the mean values at $p < 0.05$.

Table 3. The renal phosphatidyl-ethanolamine fatty acid profile of the rats after 5 and 10 days of FB1 exposure.

Group	Control		20 mg kg $^{-1}$		50 mg kg $^{-1}$		100 mg kg $^{-1}$	
Fatty acid	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Kidney phosphatidyl-ethanolamine - 5-day exposure								
C14:0	0.40 ± 0.14	**a**	0.50 ± 0.08	**ab**	0.58 ± 0.07	**b**	0.48 ± 0.06	**ab**
C15:0	0.11 ± 0.02	**a**	0.15 ± 0.03	**ab**	0.16 ± 0.04	**b**	0.11 ± 0.02	**a**
C16:0	26.1 ± 5.96	**a**	26.8 ± 2.89	**ab**	29.9 ± 1.21	**ab**	31.3 ± 1.56	**b**
C16:1 n7	0.28 ± 0.17		0.23 ± 0.09		0.21 ± 0.04		0.20 ± 0.04	
C18:0	41.7 ± 4.45		43.0 ± 2.53		45.0 ± 1.41		46.2 ± 1.66	
C18:1 n9c	2.62 ± 1.20		2.78 ± 0.47		2.33 ± 0.21		2.03 ± 0.39	
C18:2 n6c	4.92 ± 2.96	**b**	2.36 ± 0.62	**a**	2.06 ± 0.20	**a**	1.81 ± 0.26	**a**
C18:3 n6	0.04 ± 0.00		0.03 ± 0.01		0.02 ± 0.01		0.03 ± 0.02	
C18:3 n3	0.05 ± 0.03		0.04 ± 0.02		0.03 ± 0.01		0.03 ± 0.01	
C20:0	0.30 ± 0.07	**a**	0.32 ± 0.03	**ab**	0.35 ± 0.02	**b**	0.34 ± 0.02	**b**
C20:1 n9	0.17 ± 0.04	**b**	0.05 ± 0.02	**a**	0.06 ± 0.02	**a**	0.05 ± 0.02	**a**
C20:2 n6	0.10 ± 0.10	**b**	0.06 ± 0.04	**ab**	0.07 ± 0.01	**ab**	0.06 ± 0.02	**a**
C20:3 n6	0.28 ± 0.08	**b**	0.16 ± 0.05	**a**	0.12 ± 0.04	**a**	0.11 ± 0.04	**a**
C20:4 n6	20.9 ± 10.6		21.5 ± 4.08		17.4 ± 2.11		15.9 ± 2.28	
C20:3 n3					0.02 ± 0.02		0.03 ± 0.01	
C20:5 n3	0.16 ± 0.03	**a**	0.19 ± 0.04	**b**	0.15 ± 0.02	**ab**	0.13 ± 0.04	**a**
C22:0	0.05 ± 0.01		0.05 ± 0.01		0.05 ± 0.01		0.05 ± 0.01	
C22:5 n3	0.24 ± 0.09		0.23 ± 0.04		0.19 ± 0.03		0.16 ± 0.04	
C22:6 n3	1.51 ± 0.72	**b**	1.29 ± 0.29	**ab**	1.11 ± 0.16	**ab**	0.95 ± 0.17	**a**
C24:0	0.05 ± 0.02		0.05 ± 0.01		0.06 ± 0.02		0.05 ± 0.01	
Σ saturated	68.7 ± 10.5	**a**	71.0 ± 5.52	**b**	76.3 ± 2.70	**b**	78.5 ± 3.17	**b**
Σ unsaturated	31.3 ± 10.5		29.0 ± 5.52		23.7 ± 2.70		21.5 ± 3.17	
MUFA	3.07 ± 1.28		3.05 ± 0.48		2.60 ± 0.23		2.27 ± 0.43	
PUFA	28.2 ± 9.29	**b**	25.9 ± 5.06	**a**	21.1 ± 2.50	**a**	19.2 ± 2.76	**a**
Σ n6	26.2 ± 8.49	**b**	24.1 ± 4.67	**ab**	19.6 ± 2.31	**ab**	17.9 ± 2.51	**a**
Σ n3	1.93 ± 0.89		1.76 ± 0.38		1.48 ± 0.20		1.27 ± 0.23	
n6 / n3	18.8 ± 14.6		13.8 ± 1.19		13.3 ± 0.94		14.2 ± 0.77	
odd chain	0.11 ± 0.02	**a**	0.15 ± 0.03	**ab**	0.16 ± 0.04	**b**	0.11 ± 0.02	**a**
UI	108.9 ± 44.1		104.6 ± 20.1		85.3 ± 10.1		77.4 ± 11.4	
ACL	18.0 ± 0.37		17.9 ± 0.16		17.8 ± 0.08		17.7 ± 0.09	
Kidney phosphatidyl-ethanolamine - 10-day exposure								
C14:0	0.13 ± 0.02		0.17 ± 0.04		0.12 ± 0.01		0.14 ± 0.06	
C15:0	21.5 ± 1.40	**a**	25.9 ± 1.79	**b**	23.6 ± 0.73	**ab**	24.4 ± 1.95	**b**
C16:0	0.29 ± 0.06		0.31 ± 0.13		0.31 ± 0.12		0.31 ± 0.12	
C16:1 n7	0.36 ± 0.02	**b**	0.36 ± 0.02	**b**	0.27 ± 0.03	**a**	0.34 ± 0.03	**b**
C18:0	37.5 ± 1.69		40.8 ± 2.27		38.8 ± 0.88		40.4 ± 2.75	
C18:1 n9c	3.41 ± 0.52	**ab**	2.99 ± 0.85	**a**	4.44 ± 0.24	**b**	3.65 ± 0.96	**ab**
C18:2 n6c	2.83 ± 0.34		2.21 ± 0.47		2.17 ± 0.17		2.62 ± 0.73	
C18:3 n6	0.05 ± 0.01		0.04 ± 0.02		0.03 ± 0.01		0.04 ± 0.02	
C18:3 n3	0.03 ± 0.01		0.05 ± 0.02		0.05 ± 0.02		0.05 ± 0.02	
C20:0	0.29 ± 0.02	**a**	0.37 ± 0.02	**c**	0.34 ± 0.01	**b**	0.37 ± 0.02	**c**
C20:1 n9	0.18 ± 0.05	**a**	0.17 ± 0.07	**a**	0.25 ± 0.04	**ab**	0.33 ± 0.04	**b**
C20:2 n6	0.11 ± 0.03		0.11 ± 0.05		0.17 ± 0.04		0.17 ± 0.04	
C20:3 n6	0.39 ± 0.05		0.19 ± 0.06		0.14 ± 0.04		2.92 ± 6.80	
C20:4 n6	30.1 ± 2.73	**c**	23.4 ± 2.47	**ab**	26.1 ± 0.87	**bc**	21.3 ± 4.10	**a**
C20:3 n3	0.03 ± 0.01				0.02 ± 0.01		0.03 ± 0.00	
C20:5 n3	0.18 ± 0.06	**b**	0.15 ± 0.05	**ab**	0.13 ± 0.04	**ab**	0.10 ± 0.03	**a**
C22:0	0.05 ± 0.01	**a**	0.07 ± 0.01	**b**	0.07 ± 0.01	**b**	0.07 ± 0.01	**b**
C22:5 n3	0.28 ± 0.03	**a**	0.32 ± 0.07	**ab**	0.42 ± 0.06	**b**	0.41 ± 0.13	**ab**
C22:6 n3	1.87 ± 0.16		1.73 ± 0.16		2.10 ± 0.27		1.88 ± 0.34	
C24:0	0.07 ± 0.02		0.09 ± 0.01		0.07 ± 0.02		0.08 ± 0.03	
Σ saturated	60.2 ± 3.02	**a**	68.3 ± 3.78	**b**	63.8 ± 1.48	**ab**	66.2 ± 4.64	**b**
Σ unsaturated	39.8 ± 3.02	**b**	31.7 ± 3.78	**a**	36.2 ± 1.48	**ab**	33.8 ± 4.64	**a**
MUFA	3.95 ± 0.53	**ab**	3.52 ± 0.85	**a**	4.96 ± 0.23	**b**	4.33 ± 0.97	**ab**
PUFA	35.8 ± 3.10	**b**	28.2 ± 3.23	**a**	31.3 ± 1.26	**ab**	29.4 ± 4.53	**a**
Σ n6	33.3 ± 2.90	**b**	25.8 ± 2.95	**a**	28.4 ± 1.01	**ab**	26.8 ± 4.59	**a**
Σ n3	2.41 ± 0.22		2.25 ± 0.27		2.71 ± 0.31		2.45 ± 0.47	
n6 / n3	13.9 ± 0.56		11.5 ± 0.52		10.6 ± 0.98		11.5 ± 4.05	
odd chain	21.5 ± 1.40	**a**	25.9 ± 1.79	**b**	23.6 ± 0.73	**ab**	24.4 ± 1.95	**a**
UI	145.1 ± 12.29	**b**	115.4 ± 12.78	**a**	130.0 ± 5.48	**ab**	117.8 ± 14.4	**a**
ACL	18.0 ± 0.10	**b**	17.7 ± 0.11	**a**	17.9 ± 0.05	**ab**	17.8 ± 0.14	**a**

[a,b]: different uppercase letters indicate significant difference of the mean values at $p < 0.05$.

Table 4. The hepatic phosphatidylcholine fatty acid profile of the rats after 5 and 10 days of FB1 exposure.

Group	Control		20 mg kg $^{-1}$		50 mg kg $^{-1}$		100 mg kg $^{-1}$	
Fatty acid	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Liver phosphatidylcholine - 5-day exposure								
C14:0	0.35 ± 0.02	**a**	0.45 ± 0.06	**b**	0.43 ± 0.09	**ab**	0.52 ± 0.05	**b**
C15:0	0.22 ± 0.02		0.23 ± 0.04		0.24 ± 0.04		0.24 ± 0.05	
C16:0	24.7 ± 1.25	**a**	27.7 ± 1.46	**b**	29.1 ± 1.25	**b**	29.4 ± 1.85	**b**
C16:1 n7	0.45 ± 0.14	**a**	0.38 ± 0.09	**a**	0.47 ± 0.06	**ab**	0.62 ± 0.10	**b**
C18:0	34.2 ± 2.15		35.2 ± 2.59		34.9 ± 2.36		33.5 ± 2.86	
C18:1 n9c	1.88 ± 0.17	**a**	2.12 ± 0.32	**ab**	2.80 ± 0.89	**b**	2.88 ± 0.27	**b**
C18:2 n6c	9.72 ± 0.67		9.74 ± 1.60		9.20 ± 1.14		10.5 ± 0.54	
C18:3 n6	0.12 ± 0.03		0.13 ± 0.03		0.12 ± 0.04		0.13 ± 0.04	
C18:3 n3	0.06 ± 0.01	**a**	0.08 ± 0.02	**b**	0.08 ± 0.01	**ab**	0.09 ± 0.01	**b**
C20:0	0.15 ± 0.02	**a**	0.18 ± 0.03	**ab**	0.19 ± 0.03	**b**	0.16 ± 0.02	**ab**
C20:1 n9	0.08 ± 0.03		0.07 ± 0.01		0.05 ± 0.01		0.06 ± 0.02	
C20:2 n6	0.32 ± 0.10	**b**	0.24 ± 0.09	**ab**	0.20 ± 0.04	**a**	0.20 ± 0.03	**a**
C20:3 n6	0.52 ± 0.09	**b**	0.25 ± 0.04	**a**	0.24 ± 0.07	**a**	0.23 ± 0.06	**a**
C20:4 n6	22.8 ± 1.90	**b**	20.1 ± 2.11	**ab**	18.2 ± 1.54	**ab**	17.2 ± 5.42	**a**
C20:3 n3	0.02 ± 0.00	**b**	0.02 ± 0.00	**a**	0.02 ± 0.01	**a**	0.02 ± 0.01	**a**
C20:5 n3	0.16 ± 0.04	**b**	0.08 ± 0.02	**a**	0.06 ± 0.02	**a**	0.06 ± 0.01	**a**
C22:0	0.03 ± 0.01		0.03 ± 0.00		0.03 ± 0.00		0.03 ± 0.01	
C22:5 n3	0.70 ± 0.20	**c**	0.37 ± 0.07	**a**	0.46 ± 0.06	**ab**	0.58 ± 0.10	**bc**
C22:6 n3	3.48 ± 0.65	**b**	2.55 ± 0.35	**a**	3.13 ± 0.51	**ab**	3.50 ± 0.51	**b**
C24:0	0.03 ± 0.01		0.03 ± 0.01		0.03 ± 0.01		0.03 ± 0.01	
Σ saturated	59.7 ± 2.96		63.9 ± 3.63		65.0 ± 2.70		63.9 ± 4.63	
Σ unsaturated	40.3 ± 2.96		36.1 ± 3.63		35.0 ± 2.70		36.1 ± 4.63	
MUFA	2.40 ± 0.31	**a**	2.56 ± 0.38	**ab**	3.33 ± 0.93	**bc**	3.56 ± 0.35	**c**
PUFA	37.9 ± 2.84	**b**	33.5 ± 3.43	**ab**	31.7 ± 2.29	**a**	32.5 ± 4.70	**ab**
Σ n6	33.2 ± 2.18	**b**	30.2 ± 3.19	**ab**	27.7 ± 1.95	**a**	28.1 ± 5.21	**ab**
Σ n3	4.41 ± 0.79	**b**	3.10 ± 0.40	**a**	3.74 ± 0.51	**ab**	4.24 ± 0.55	**b**
n6 / n3	7.66 ± 1.01	**a**	9.83 ± 1.21	**b**	7.49 ± 0.81	**a**	6.82 ± 1.83	**a**
odd chain	0.22 ± 0.02		0.23 ± 0.04		0.24 ± 0.04		0.24 ± 0.05	
UI	141.1 ± 11.9	**b**	121.7 ± 11.78	**ab**	117.5 ± 9.55	**a**	119.2 ± 18.18	**a**
ACL	18.1 ± 0.09	**b**	17.9 ± 0.08	**a**	17.9 ± 0.07	**a**	17.9 ± 0.12	**a**
Liver phosphatidylcholine - 10-day exposure								
C14:0	0.24 ± 0.02		0.29 ± 0.08		0.26 ± 0.12		0.27 ± 0.09	
C15:0	25.2 ± 1.41		26.6 ± 2.71		27.1 ± 0.85		27.1 ± 1.47	
C16:0	0.75 ± 0.27		0.72 ± 0.30		0.60 ± 0.19		0.46 ± 0.13	
C16:1 n7	0.53 ± 0.01		0.50 ± 0.14		0.44 ± 0.19		0.46 ± 0.17	
C18:0	31.8 ± 1.65		31.8 ± 2.57		31.3 ± 1.55		29.8 ± 1.72	
C18:1 n9c	2.29 ± 0.23	**a**	3.46 ± 0.78	**b**	4.01 ± 0.66	**b**	3.76 ± 0.59	**b**
C18:2 n6c	9.25 ± 0.55		8.35 ± 0.51		8.26 ± 1.13		9.44 ± 1.27	
C18:3 n6	0.13 ± 0.02		0.12 ± 0.08		0.16 ± 0.05		0.13 ± 0.08	
C18:3 n3	0.05 ± 0.01	**a**	0.08 ± 0.02	**b**	0.07 ± 0.02	**ab**	0.08 ± 0.01	**b**
C20:0	0.14 ± 0.02	**a**	0.17 ± 0.02	**ab**	0.19 ± 0.01	**b**	0.16 ± 0.03	**ab**
C20:1 n9	0.10 ± 0.03	**a**	0.14 ± 0.02	**b**	0.12 ± 0.03	**ab**	0.09 ± 0.03	**a**
C20:2 n6	0.34 ± 0.05		0.34 ± 0.11		0.25 ± 0.14		0.25 ± 0.11	
C20:3 n6	0.80 ± 0.34	**b**	0.44 ± 0.15	**a**	0.44 ± 0.14	**a**	0.42 ± 0.14	**a**
C20:4 n6	23.1 ± 1.63	**b**	19.7 ± 6.67	**a**	19.8 ± 1.20	**a**	20.1 ± 1.81	**a**
C20:3 n3	0.03 ± 0.01		0.02 ± 0.01		0.02 ± 0.01		0.03 ± 0.01	
C20:5 n3	0.16 ± 0.07	**b**	0.08 ± 0.03	**a**	0.07 ± 0.02	**a**	0.06 ± 0.01	**a**
C22:0	0.03 ± 0.00		0.03 ± 0.01		0.04 ± 0.01		0.04 ± 0.01	
C22:5 n3	0.75 ± 0.14		0.88 ± 0.28		0.94 ± 0.15		1.03 ± 0.17	
C22:6 n3	3.90 ± 0.71	**a**	5.72 ± 1.00	**b**	5.42 ± 0.47	**b**	5.74 ± 0.55	**b**
C24:0	0.03 ± 0.00	**a**	0.03 ± 0.01	**ab**	0.04 ± 0.01	**ab**	0.06 ± 0.03	**b**
Σ saturated	58.5 ± 2.84		60.1 ± 5.24		60.0 ± 2.09		58.4 ± 2.76	
Σ unsaturated	41.5 ± 2.84		39.9 ± 5.24		40.0 ± 2.09		41.6 ± 2.76	
MUFA	2.92 ± 0.24	**a**	4.10 ± 0.90	**ab**	4.57 ± 0.78	**b**	4.32 ± 0.75	**b**
PUFA	38.5 ± 2.61		35.7 ± 5.84		35.4 ± 1.41		37.3 ± 2.70	
Σ n6	33.3 ± 1.75		28.6 ± 6.63		28.7 ± 1.38		30.1 ± 2.14	
Σ n3	4.90 ± 0.83	**a**	6.79 ± 1.22	**b**	6.52 ± 0.55	**b**	6.94 ± 0.67	**b**
n6 / n3	6.92 ± 0.90	**b**	4.43 ± 1.53	**a**	4.43 ± 0.52	**a**	4.35 ± 0.30	**a**
odd chain	25.2 ± 1.41		26.6 ± 2.71		27.1 ± 0.85		27.1 ± 1.47	
UI	145.6 ± 11.86		141.5 ± 21.90		140.5 ± 6.15		145.9 ± 10.5	
ACL	17.9 ± 0.11		17.8 ± 0.19		17.8 ± 0.04		17.83 ± 0.09	

[a,b]: different uppercase letters indicate significant difference of the mean values at $p < 0.05$.

Table 5. The hepatic phosphatidyl-inositol fatty acid profile of the rats after 5 and 10 days of FB1 exposure.

Group	Control		20 mg kg $^{-1}$		50 mg kg $^{-1}$		100 mg kg^{-1}	
Fatty acid	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Liver phosphatidyl-inositol - 5-day exposure								
C12:0	0.03 ± 0.01	**a**	0.06 ± 0.01	**b**	0.08 ± 0.02	**ab**	0.07 ± 0.05	**b**
C14:0	0.62 ± 0.03		0.72 ± 0.04		0.61 ± 0.10		0.73 ± 0.10	
C15:0	0.15 ± 0.02		0.15 ± 0.01		0.15 ± 0.02		0.15 ± 0.03	
C16:0	34.6 ± 0.73	**a**	36.3 ± 1.30	**b**	36.29 ± 0.42	**b**	37.4 ± 0.68	**b**
C16:1 n7	0.55 ± 0.14	**b**	0.30 ± 0.11	**a**	0.30 ± 0.10	**a**	0.35 ± 0.10	**a**
C18:0	45.8 ± 1.85	**a**	48.6 ± 2.36	**ab**	50.03 ± 2.90	**b**	47.4 ± 0.88	**ab**
C18:1 n9c	1.11 ± 0.19	**b**	0.75 ± 0.23	**a**	0.59 ± 0.14	**a**	0.68 ± 0.11	**a**
C18:2 n6c	14.7 ± 2.07	**b**	10.9 ± 3.00	**a**	9.85 ± 2.23	**a**	10.9 ± 0.93	**a**
C18:3 n6	0.04 ± 0.02		0.02 ± 0.01		0.02 ± 0.01		0.03 ±	
C18:3 n3	0.10 ± 0.03		0.08 ± 0.02		0.08 ± 0.02		0.10 ± 0.00	
C20:0	0.38 ± 0.01	**a**	0.40 ± 0.03	**ab**	0.41 ± 0.03	**b**	0.38 ± 0.01	**ab**
C20:1 n9	0.05 ± 0.08	**a**	0.12 ± 0.12	**ab**	0.24 ± 0.04	**b**	0.24 ± 0.05	**b**
C20:2 n6	0.26 ± 0.04		0.24 ± 0.07		0.20 ± 0.04		0.21 ± 0.05	
C20:3 n6	0.24 ± 0.05	**b**	0.11 ± 0.03	**a**	0.11 ± 0.06	**a**	0.09 ± 0.03	**a**
C20:4 n6	0.97 ± 0.32		0.91 ± 0.45		0.72 ± 0.30		0.84 ± 0.17	
C20:3 n3	0.05 ± 0.01		0.04 ± 0.02		0.04 ± 0.01		0.04 ± 0.01	
C20:5 n3	0.01 ±		0.01 ± 0.01					
C22:0	0.05 ± 0.01		0.06 ± 0.01		0.06 ± 0.01		0.05 ± 0.01	
C22:5 n3	0.07 ± 0.04		0.05 ± 0.02		0.05 ± 0.02		0.07 ± 0.02	
C22:6 n3	0.23 ± 0.06		0.23 ± 0.08		0.26 ± 0.09		0.31 ± 0.06	
C24:0	0.07 ± 0.02	**b**	0.05 ± 0.01	**ab**	0.05 ± 0.01	**ab**	0.05 ± 0.01	**a**
Σ saturated	81.7 ± 2.52	**a**	86.3 ± 3.72	**b**	87.67 ± 2.66	**b**	86.3 ± 0.95	**b**
Σ unsaturated	18.3 ± 2.52	**b**	13.7 ± 3.72	**a**	12.33 ± 2.66	**a**	13.7 ± 0.95	**a**
MUFA	1.69 ± 0.30		1.13 ± 0.37		1.00 ± 0.24		1.19 ± 0.29	
PUFA	16.6 ± 2.24	**b**	12.6 ± 3.46	**a**	11.33 ± 2.47	**a**	12.5 ± 0.83	**a**
Σ n6	15.9 ± 2.15	**b**	11.9 ± 3.32	**a**	10.70 ± 2.37	**a**	11.8 ± 0.87	**a**
Σ n3	0.44 ± 0.09		0.40 ± 0.11		0.42 ± 0.11		0.51 ± 0.07	
n6 / n3	37.0 ± 8.22		30.0 ± 5.88		25.87 ± 5.69		23.7 ± 4.16	
odd chain	0.15 ± 0.02		0.15 ± 0.01		0.15 ± 0.02		0.15 ± 0.03	
UI	38.4 ± 5.21	**b**	29.4 ± 8.12	**ab**	26.53 ± 5.64	**a**	29.6 ± 1.76	**ab**
ACL	17.3 ± 0.02	**b**	17.3 ± 0.04	**ab**	17.29 ± 0.02	**ab**	17.3 ± 0.02	**a**
Liver phosphatidyl-inositol - 10-day exposure								
C12:0	0.68 ± 0.15		0.75 ± 0.05		0.71 ± 0.05		0.63 ± 0.05	
C14:0	0.19 ± 0.06		0.22 ± 0.03		0.19 ± 0.01		0.19 ± 0.04	
C15:0	37.2 ± 2.67	**b**	35.7 ± 0.46	**ab**	36.4 ± 0.57	**b**	33.8 ± 1.43	**a**
C16:0	0.38 ± 0.12		0.47 ± 0.28		0.39 ± 0.06		0.48 ± 0.09	
C16:1 n7	0.35 ± 0.04		0.34 ± 0.02		0.34 ± 0.03		0.35 ± 0.04	
C18:0	48.5 ± 3.52	**ab**	46.4 ± 0.95	**ab**	49.4 ± 0.77	**b**	45.5 ± 2.30	**a**
C18:1 n9c	1.04 ± 0.34	**a**	1.31 ± 0.16	**ab**	1.42 ± 0.15	**ab**	1.61 ± 0.40	**b**
C18:2 n6c	6.60 ± 5.79	**a**	11.6 ± 0.58	**ab**	8.06 ± 0.93	**ab**	12.8 ± 2.48	**b**
C18:3 n6	0.01 ± 0.02		0.04 ± 0.01		0.03 ± 0.02		0.04 ± 0.05	
C18:3 n3	0.08 ± 0.03	**a**	0.10 ± 0.02	**ab**	0.11 ± 0.03	**ab**	0.14 ± 0.03	**b**
C20:0	0.41 ± 0.02	**ab**	0.41 ± 0.04	**ab**	0.45 ± 0.02	**b**	0.39 ± 0.03	**a**
C20:1 n9	0.19 ± 0.09	**a**	0.30 ± 0.03	**b**	0.32 ± 0.06	**b**	0.15 ± 0.05	**a**
C20:2 n6	0.20 ± 0.12	**a**	0.32 ± 0.05	**b**	0.23 ± 0.04	**ab**	0.35 ± 0.05	**b**
C20:3 n6	0.18 ± 0.12		0.18 ± 0.05		0.10 ± 0.02		0.15 ± 0.08	
C20:4 n6	2.53 ± 1.21	**b**	1.01 ± 0.43	**a**	0.95 ± 0.24	**a**	1.64 ± 1.03	**ab**
C20:3 n3	0.03 ± 0.01		0.05 ± 0.01		0.04 ± 0.01		0.04 ± 0.00	
C20:5 n3	0.05 ± 0.02		0.02 ± 0.01		0.03 ± 0.02		0.03 ± 0.01	
C22:0	0.07 ± 0.02		0.06 ± 0.01		0.07 ± 0.01		0.06 ± 0.01	
C22:5 n3	0.20 ± 0.08		0.11 ± 0.07		0.11 ± 0.03		0.20 ± 0.10	
C22:6 n3	0.94 ± 0.55	**ab**	0.55 ± 0.27	**a**	0.53 ± 0.18	**a**	1.36 ± 0.77	**b**
C24:0	0.09 ± 0.02	**b**	0.07 ± 0.02	**ab**	0.06 ± 0.01	**a**	0.07 ± 0.01	**ab**
Σ saturated	87.7 ± 5.88	**b**	84.1 ± 1.04	**ab**	87.8 ± 1.27	**b**	81.2 ± 3.72	**a**
Σ unsaturated	12.3 ± 5.88	**a**	15.9 ± 1.04	**ab**	12.2 ± 1.27	**a**	18.8 ± 3.72	**b**
MUFA	1.58 ± 0.30	**a**	1.95 ± 0.16	**ab**	2.07 ± 0.12	**b**	2.12 ± 0.40	**b**
PUFA	10.8 ± 5.67	**a**	13.9 ± 0.93	**ab**	10.2 ± 1.19	**a**	16.7 ± 3.65	**b**
Σ n6	9.32 ± 5.59	**a**	12.8 ± 0.78	**ab**	9.14 ± 1.08	**a**	14.6 ± 2.89	**b**
Σ n3	1.24 ± 0.63		0.84 ± 0.33		0.79 ± 0.21		1.72 ± 0.89	
n6 / n3	10.7 ± 10.59		17.2 ± 6.33		12.3 ± 2.87		9.90 ± 3.32	
odd chain	37.2 ± 2.67		35.7 ± 0.46		36.4 ± 0.57		33.8 ± 1.43	
UI	32.9 ± 12.29	**ab**	34.8 ± 3.77	**ab**	27.0 ± 3.45	**a**	45.2 ± 12.1	**b**
ACL	16.9 ± 0.10	**ab**	16.9 ± 0.02	**ab**	16.9 ± 0.03	**a**	17.0 ± 0.09	**b**

[a,b]: different uppercase letters indicate significant difference of the mean values at $p < 0.05$.

Table 6. The hepatic phosphatidyl-ethanolamine fatty acid profile of the rats after 5 and 10 days of FB1 exposure.

Group	Control		20 mg kg $^{-1}$		50 mg kg $^{-1}$		100 mg kg $^{-1}$	
Fatty acid	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Liver phosphatidyl-ethanolamine - 5-day exposure								
C14:0	0.47 ± 0.32		0.47 ± 0.07		0.40 ± 0.07		0.40 ± 0.04	
C15:0	0.13 ± 0.03	**ab**	0.14 ± 0.02	**ab**	0.16 ± 0.03	**b**	0.12 ± 0.02	**a**
C16:0	29.1 ± 0.92		31.1 ± 2.75		30.4 ± 1.46		29.9 ± 0.34	
C16:1 n7	0.38 ± 0.47		0.12 ± 0.07		0.13 ± 0.03		0.18 ± 0.04	
C18:0	36.0 ± 5.58	**a**	42.5 ± 4.52	**b**	41.5 ± 2.14	**ab**	39.9 ± 1.17	**ab**
C18:1 n9c	5.13 ± 8.40		1.55 ± 0.37		1.57 ± 0.31		1.73 ± 0.24	
C18:2 n6c	6.24 ± 2.24		5.56 ± 0.98		4.84 ± 0.70		5.41 ± 0.61	
C18:3 n6	0.06 ± 0.02		0.06 ± 0.02		0.05 ± 0.02		0.04 ± 0.01	
C18:3 n3	0.17 ± 0.19		0.09 ± 0.02		0.08 ± 0.02		0.09 ± 0.03	
C20:0	0.23 ± 0.03		0.27 ± 0.05		0.27 ± 0.04		0.24 ± 0.01	
C20:1 n9	0.16 ± 0.18		0.05 ± 0.02		0.06 ± 0.02		0.10 ± 0.05	
C20:2 n6	0.23 ± 0.13		0.17 ± 0.08		0.15 ± 0.02		0.15 ± 0.03	
C20:3 n6	0.24 ± 0.06	**b**	0.12 ± 0.02	**a**	0.13 ± 0.03	**a**	0.11 ± 0.03	**a**
C20:4 n6	15.0 ± 5.12		12.1 ± 5.48		12.8 ± 1.59		13.2 ± 0.73	
C20:3 n3	0.07 ±		0.01 ± 0.00		±		±	
C20:5 n3	0.15 ± 0.05	**b**	0.09 ± 0.03	**ab**	0.08 ± 0.03	**a**	0.08 ± 0.02	**a**
C22:0	0.04 ± 0.02		0.04 ± 0.01		0.03 ± 0.01		0.03 ± 0.00	
C22:5 n3	1.27 ± 0.55	**b**	0.72 ± 0.15	**a**	0.93 ± 0.15	**ab**	1.06 ± 0.15	**ab**
C22:6 n3	4.92 ± 1.83	**a**	4.71 ± 0.82	**a**	6.33 ± 0.94	**ab**	7.21 ± 0.48	**b**
C24:0	0.03 ± 0.01		0.03 ± 0.01		0.04 ± 0.01		0.03 ± 0.01	
Σ saturated	66.1 ± 5.35	**a**	74.7 ± 7.31	**b**	72.8 ± 3.20	**ab**	70.7 ± 1.27	**ab**
Σ unsaturated	33.9 ± 5.35	**b**	25.3 ± 7.31	**a**	27.2 ± 3.21	**ab**	29.3 ± 1.27	**ab**
MUFA	5.68 ± 9.04		1.72 ± 0.43		1.76 ± 0.31		2.01 ± 0.21	
PUFA	28.3 ± 5.48		23.6 ± 6.99		25.4 ± 3.04		27.3 ± 1.21	
Σ n6	21.5 ± 3.59		17.8 ± 6.35		17.8 ± 2.06		18.7 ± 1.24	
Σ n3	6.53 ± 2.17	**ab**	5.62 ± 0.93	**a**	7.43 ± 1.07	**ab**	8.43 ± 0.53	**b**
n6 / n3	3.57 ± 1.10	**b**	3.14 ± 1.08	**ab**	2.42 ± 0.21	**ab**	2.23 ± 0.23	**a**
odd chain	0.13 ± 0.03	**ab**	0.14 ± 0.02	**ab**	0.16 ± 0.03	**b**	0.12 ± 0.02	**a**
UI	116.5 ± 21.5		94.7 ± 27.6		106.8 ± 13.21		115.5 ± 4.33	
ACL	18.0 ± 0.22		17.8 ± 0.19		17.9 ± 0.10		18.0 ± 0.02	
Liver phosphatidyl-ethanolamine - 10-day exposure								
C14:0	0.14 ± 0.02		0.17 ± 0.02		0.16 ± 0.04		0.15 ± 0.05	
C15:0	27.3 ± 1.35		26.3 ± 0.82		26.3 ± 1.31		25.2 ± 1.67	
C16:0	0.52 ± 0.38	**b**	0.25 ± 0.11	**ab**	0.28 ± 0.08	**ab**	0.19 ± 0.06	**a**
C16:1 n7	0.49 ± 0.04		0.48 ± 0.09		0.43 ± 0.16		0.47 ± 0.16	
C18:0	34.6 ± 2.13		34.8 ± 1.28		37.5 ± 1.55		35.2 ± 2.13	
C18:1 n9c	1.66 ± 0.19	**a**	2.71 ± 0.54	**b**	2.84 ± 0.59	**b**	2.23 ± 0.53	**ab**
C18:2 n6c	8.43 ± 3.48	**b**	4.81 ± 0.75	**a**	4.50 ± 0.89	**a**	4.16 ± 0.89	**a**
C18:3 n6	0.06 ± 0.01		0.09 ± 0.02		0.09 ± 0.03		0.06 ± 0.03	
C18:3 n3	0.09 ± 0.03		0.11 ± 0.04		0.10 ± 0.05		0.07 ± 0.02	
C20:0	0.20 ± 0.02	**a**	0.23 ± 0.02	**a**	0.28 ± 0.03	**b**	0.21 ± 0.03	**a**
C20:1 n9	0.12 ± 0.04	**ab**	0.16 ± 0.01	**bc**	0.18 ± 0.04	**c**	0.08 ± 0.04	**a**
C20:2 n6	0.23 ± 0.15		0.27 ± 0.08		0.23 ± 0.08		0.18 ± 0.10	
C20:3 n6	0.37 ± 0.18		0.27 ± 0.14		0.19 ± 0.07		0.23 ± 0.13	
C20:4 n6	17.6 ± 1.10	**b**	16.2 ± 1.68	**b**	13.7 ± 1.05	**a**	15.8 ± 1.46	**ab**
C20:3 n3	0.02 ± 0.01		0.02 ± 0.01		0.02 ± 0.01		±	
C20:5 n3	0.18 ± 0.04	**b**	0.10 ± 0.03	**a**	0.09 ± 0.03	**a**	0.10 ± 0.02	**a**
C22:0	0.03 ± 0.01	**a**	0.03 ± 0.01	**a**	0.05 ± 0.00	**b**	0.04 ± 0.01	**ab**
C22:5 n3	1.43 ± 0.28		1.51 ± 0.27		1.52 ± 0.22		1.78 ± 0.34	
C22:6 n3	6.11 ± 0.99	**a**	11.0 ± 2.13	**b**	11.1 ± 1.54	**b**	13.5 ± 1.62	**b**
C24:0	0.04 ± 0.01		0.04 ± 0.01		0.05 ± 0.01		0.04 ± 0.01	
Σ saturated	63.2 ± 3.14		62.3 ± 2.03		65.0 ± 2.72		61.4 ± 3.72	
Σ unsaturated	36.8 ± 3.14		37.7 ± 2.03		35.0 ± 2.72		38.6 ± 3.72	
MUFA	2.26 ± 0.23	**a**	3.35 ± 0.55	**b**	3.45 ± 0.55	**b**	2.78 ± 0.64	**ab**
PUFA	34.5 ± 2.94		34.4 ± 1.60		31.5 ± 2.43		35.8 ± 3.53	
Σ n6	26.4 ± 2.58	**b**	21.4 ± 1.85	**a**	18.5 ± 1.74	**a**	20.2 ± 2.28	**a**
Σ n3	7.83 ± 1.15	**a**	12.7 ± 2.26	**b**	12.8 ± 1.71	**b**	15.4 ± 1.87	**b**
n6 / n3	3.43 ± 0.60	**b**	1.74 ± 0.42	**a**	1.46 ± 0.23	**a**	1.32 ± 0.18	**a**
odd chain	27.3 ± 1.35		26.3 ± 0.82		26.3 ± 1.31		25.2 ± 1.67	
UI	136.2 ± 9.01	**a**	153.8 ± 8.81	**ab**	143.5 ± 11.2	**a**	165.7 ± 15.3	**b**
ACL	17.8 ± 0.08	**a**	18.0 ± 0.07	**bc**	18.0 ± 0.10	**ab**	18.1 ± 0.13	**c**

[a,b]: different uppercase letters indicate significant difference of the mean values at $p < 0.05$.

Table 7. Characteristics of the linear dose response equations in the kidney and the liver lipid fractions (only cases with an R^2 value exceeding 0.6 are shown).

Kidney PC			5 days				10 days	
	Compound	Slope	Constant	R^2	Compound	Slope	Constant	R^2
	C16:0	30.3	1.78	0.635	C16:0	0.156	0.108	0.639
	C20:4 n6	-2.74	21.6	0.639	C18:1 n9	1.359	2.565	0.721
	C22:6 n3	-0.198	1.24	0.672	C20:1 n9	0.082	0.027	0.733
	PUFA	-3.63	31.1	0.639	C20:3 n6	-0.238	1.193	0.628
	Σ n3	-3.38	29.2	0.633	MUFA	1.413	3.02	0.719
	Σ n6	-0.236	1.69	0.689				
	UI	-13.8	116.8	0.641				
	ACL	-0.106	17.9	0.656				
Kidney PI			5 days				10 days	
	Compound	Slope	Constant	R^2	Compound	Slope	Constant	R^2
	C18:0	1.728	43.7	0.645	C20:3 n6	-0.175	0.715	0.616
	PUFA	-3.6	21.3	0.725				
Liver PE			5 days				10 days	
					Compound	Slope	Constant	R^2
					Σ n3	2.23	6.66	0.617

Dose response. Linear fitting was only attained with reliable level for total n3 (R^2 = 0.616, slope = 2.23, C = 6.66) (Table 7).

Discussion

Our hypothesis was based on the key effect of FB_1 mycotoxin: FB_1 disrupts cell lipid metabolism via the inhibition of sphinganine N-acyltransferase (Wang et al. 1991) leading to cell deregulation (membrane damaging effect). However, there are few reports referring to the detailed membrane lipid compositional consequences of FB_1 intoxication in rats (and other monogastric mammals).

Kidney

Results reported so far on the membrane disruptor nature of FB_1 in terms of kidney are mostly associated with indirect effects, namely modifications via mycotoxin-induced oxidative stress. In our recent study (Szabó et al. 2018b) we reported FB_1-associated influence on the kidney absolute and relative weight in rats, as well as a strong depletion of renal (cortical) reduced glutathione and an enzymatic adaptation (glutathione peroxidase); similar results have been published by Kang and Alexander (1996). However, besides the onset of oxidative stress, we did not report, and as far as we are aware, no other study is available on plausible, renal fatty acid compositional effects of FB_1. There exists a very robust knowledge on the structure-based sphingolipid modification of FB_1 (Riley et al. 2001; Dellafiora et al. 2018); anyway, primates and rodents liver *in vivo*, and hepatocytes *in vitro* were the most frequent targets of investigation (Voss et al. 1996). Meanwhile, liver has been more intensely studied, our (Fodor et al. 2006; weaned piglets) and other (Shephard et al. 1992; Norred et al. 1993; Voss et al. 1996) results provided evidence that urinary excretion and short time renal tissue presence is a vital problem in rats. Though in pigs and rabbits, liver seems to be the primary target, in rats this is the kidney (Voss et al. 2001; Szabó et al. 2018b). The FB_1 induced nephrotoxic effect has been proven by local apoptosis, mostly in the tubular epithelial cells. This process may progress to lesions extending deeper into the medulla. This leads to cell loss, and disturbance in the replacement, leading ultimately to possible carcinogenesis (Hannun and Obeid 2008).

The fumonisin B_1-associated intoxication is clearly nephrotoxic, but since rats are providing more sensitive reaction, more markedly in the males (Voss et al. 2001), most literature on other species has been excluded.

Kidney phosphatidylcholine FAs

Indeed, fumonisin B_1-related literature on renal PC FA profiles is strongly limited; nephropathies matched with oxidative stress may provide plausible similarity. A recent lipidomic study on aristolochic acid-induced nephropathy was used to

identify possible target metabolites from the total of 69 compounds identified from the renal lipidome (Zhao et al. 2015). Reactive metabolites in the PC family were partly in agreement with our findings.

Though a lipidomic approach is generally more specifically targeted on intact molecules, and we only measured FA residues, marked similarity has been found in some instances.

The most important FA undergoing FB_1-induced proportional modification was DHA (↓). Zhao et al. (2015) reported the following PC22:6 molecular species to be reactive onward aristolochic acid treatment: PC16:0/22:6n3, PC18:3n3/22:6n3 and PC16:1/22:6n3. Since DHA is the most reductive n3 FA in the entire profile, its sensitivity and proportional decrease in the PC fraction refers to the onset of oxidative stress in the kidney cortex, as proven in our companion study (Szabó et al. 2018a). The carcinogenic aristolochic acid treatment modified PC22:6 molecular species containing C16:0, C16:1n7 and C18:3n3. From these latter, we detected proportional modification in case of C16:0, mostly associated with increasing saturation of the PC fraction. Another important saturated fatty acid providing agreement with Zhao et al. (2015) was myristic acid (C14:0), which is co-occurring with DPA (C22:5 n3), providing as well decreasing proportion in both studies. The crucial role of DHA (and DPA) in the kidney has been underscored by a further study of Zhao et al. (2014), wherein rats with chronic kidney disease (CKD) the decreasing renal DHA, DPA and palmitic acid (not in the separated PC fraction) was associated with the illness and oppositely: the normal kidney function was associated with increasing proportions of these metabolites.

On the other hand, arachidonic acid (C20:4 n6, AA) has as well been found to be depleted from the PC fraction. AA is cleaved from PC (from position *sn*-2) by phospholipase A_2 (PLA_2) to enter the synthesis of n6 FA-derived eicosanoids. Lower presence of AA maybe thus reasoned by the up-regulated eicosanoid synthesis (Zhao et al. 2014); meanwhile, the proportional decrease of EPA, DPA and DHA may refer to the progression of n3 FA-derived eicosanoid synthesis. As a strongly similar process, EPA and DHA have been shown to undergo proportional decrease in rats in chronic kidney disease (Zhao et al. 2014). Another explanation for the depletion of n3 FAs maybe that PLA_2 activity increased lysoPC amounts (as supported by the results of Iwase et al. 2008 in diabetic nephropathy), which was excluded from our scope and analysis.

Summarising the findings, it seems that rat FB_1 intoxication in terms of renal cortical PC FA profile is associated with proportional modifications mostly occurring in the n6 and n3 long-chain PUFA and similar to those reported in kidney diseases of different aetiologies.

Kidney phosphatidylinositol FA composition

As far as the authors are aware, there is limited information regarding the PI FA compositional alterations in intoxicated model animals' kidney. Focusing on the molecular species of interest (PI fatty acids) in pathological states, one of the most relevant results were published in case of colorectal cancer, namely the specific accumulation of arachidonic acid-containing phosphatidylinositol molecular species (PI18:0/20:4n6) (Hiraide et al. 2016). Arachidonic acid changes were not detected in our study in the PI fraction, but in both exposures (5 & 10 days) the proportion of the saturated FA residue (stearic acid) increased as a result of toxicosis.

Indeed, the PI FA-species specific occurrence is less characteristic for different pathological states, i.e. FAs do not act as indicators in different pathological states. The most frequently occurring PI molecular species in a healthy state is 1-stearoyl-2-arachidonyl PI (PI18:0/20:4n6), and this species is not the most characteristic product of *de novo* PI synthesis (D'Souza and Epand 2014). According to D'Souza and Epand (2014), the FA profile (in particular the relative presence of arachidonoyl (AA) and docosahexaenoyl (DHA) chains) of the PI lipid fraction is the rate-limiting factor in the formation of bioactive, inflammatory or anti-inflammatory lipid compounds. AA is liberated from the *sn*-2 position and is the root source of oxygenated derivatives, namely eicosanoids, while DHA is the precursor of D-resolvins (Weylandt et al. 2012). What we have found was the decrease of DHA in the 10-day treatment. Though we did not measure DHA-derived bioactive lipid components, the renal

concentration of cytotoxic malondialdehyde increased in this treatment (Szabó et al. 2018b). We thus suppose two processes during short-term FB_1 exposure to be attainable in parallel. The first is the specific proportional increase of the stearoyl residue, most probable due to the specific cleavage of n6 and n3 series PUFA (PUFA↓ proven in both exposures). As second, this loss in unsaturation of the PI fraction is partially channelled towards the synthesis of lipid peroxidation products (MDA↑, Szabó et al. 2018b). In contrast, the role and importance of AA, as highlighted by the relevant literature has not been proven in our study.

Kidney phosphatidylethanolamine FAs

The FA residues mostly affected by the FB_1 dosage (directly or via the lowered feed intake and the associated n6 FA imbalance) were n6 (linoleic acid, LA ↓ & DGLA ↓), and the total n6 FA proportion. In addition, none of the individual saturated FAs showed marked proportional modification, while the total saturation increased.

Zhao et al. (2013), when seeking biomarkers in case of chronic renal failure in rats, highlighted LA as a possible biomarker. The markedly down-regulated LA level was explained by a disturbance in FA metabolism, more specifically an impaired n6 FA metabolism. LA being essential, its uptake was checked in our study – feed intake decreased, relative kidney weight increased in the 10-day setting. Focusing strictly on the PE FA metabolites, PE18:4/22:4, PE20:3/18:1, PE20:0/18:0 and PE22:5/20:3 were published to be altered in aristolochic acid-induced nephropathy (Zhao et al. 2015). The latter is similar to our result, namely the depletion of DGLA from the PE fraction. According to Vance (2013), PLA_2 catalyzes conversion of phosphatidylethanolamine cell membrane component to lysophosphatidylethanolamine (LPE) by removing one of its fatty acid groups. Thus, activation of PLA_2 can account for elevation of LPE and depression of PE in the renal tissue.

In the study of Zhao et al. (2015), renal tissue FA concentration increased significantly due to intoxication. Indeed, we did not analyse FA profile quantitatively, but saturated FA proportion increased markedly in the PE fraction. According to Zhao et al. (2015), renal failure is coupled with a drastic impairment of the FA oxidation in the renal tubular epithelial cells (as reported also by Szabó et al. 2018b). The impairment of FA oxidation leads to compromised mitochondrial ATP generation, and ultimately to the functional failure of tubular epithelial cells, as confirmed in our companion study, by means of histology (Szabó et al. 2018b). In that study, tubular degeneration and necrosis and the detachment of tubular epithelial cells was found up to the dose of 50 mg kg^{-1}, while at 100 mg kg^{-1}, in well-defined areas, the dilatation of tubule groups was found, sometimes the internal epithelium was atrophic or fully absent; in their lumen, hyalin cones were developed (Szabó et al. 2018b). This fact, the functional failure, is supported by our recent finding, the accretion of total-saturated fatty acids and the PUFA depletion. The delivery of FAs requires L-carnitine, mostly with a palmitoyl-, stearoyl- or oleoyl-side chain (Indiveri et al. 2011); thus, these FA residues are likewise essential for the mitochondrial FA oxidation process and subsequent ATP generation. We thus assume that increased saturation, and in the 10-day setting decreased UI and ACL, may be associated with compromised renal epithelial cell function (via lower FA transport capacity) (as histologically supported by Szabó et al. 2018b).

According to Yang et al. (2019), renal failure (as induced by ochratoxin A) is primarily attributed to the disturbance of FA oxidation (mitochondrial dysfunction), which is coupled with FA-associated lipotoxicity. The most relevant finding in the murine renal PE lipidome was the decrease of the PE16:0/20:4n6, PE18:2n6/20:4n6 and PE16:0/18:2n6 species, as a result of ochratoxin A intoxication. Along with these, the amount of decomposition products, namely LysoPE16:0 and LysoPE18:0 increased. The results are in general agreement with our present findings (decrease of n6 FAs, in particular C18:2 n6), though we did not handle PE and LysoPE fractions separately. Anyway, Yang et al. (2019) attributed the FA-specific findings to multiple factors: mycotoxin-induced progressive lipid peroxidation and as well to changes in the regulation of energy metabolism. The most impressive finding was that energy metabolism regulation (and its modification) is closely related to ceramide accumulation

(Raucci et al. 2010), which is the key event in case of a FB_1 intoxication (Zitomer et al. 2009).

Liver

Within mammals, in the rodents, kidney and liver have been proven to be primary target organs for FB_1 toxicosis, in a species-specific manner. Burger et al. (2007) tested systematically, whether the FB_1 induced modifications of cellular membrane structure are involved in the promotion of liver cancer. Authors analysed the cholesterol, phosphatidylcholine, phosphatidylethanolamine and sphingolipid fractions for FA profile, in multiple subcellular fractions.

The novel points and the *differentia specifica* of this study were that we applied markedly lower FB_1 concentration (20–50–100 mg kg^{-1} vs. 250 mg kg^{-1} dietary dose) and we intended to additively analyse the PI fractions' reactions. The involvement of PE resembled the inner cellular membrane leaflet, and has been implemented as a fraction that is proportionally modified by the FB_1 moiety incorporated into the cell, while PI and PC were mostly interpreted as characteristic fractions for the outer leaflet.

Liver phosphatidylcholine FAs

When comparing our results to those in the relevant literature, it is important to note that palmitate proportion was increased in the PC FA profile after 5 days (but no increase was found after 10 days at any FB_1 concentrations), agreeing with the microsomal results of Burger et al. (2007). Interestingly, Burger et al. (2007) did not find a similar result in the mitochondrial, plasma and nucleus membrane, and we were not able to prove it after 10 days of intoxication. This change along with the lowering of the average chain length (ACL) and the unsaturation index (UI) points towards a less fluid membrane structure (PUFA↓), which has been compensated for partially by the monounsaturated FA accretion (MUFA↑), when considering the increasing doses (5 & 10 days as well for MUFA). Burger et al. (2007) interpreted these modifications as a means of membrane compositional adaptation, primarily for the maintenance of cellular homoeostasis.

Additionally, the characteristic change reported for C20:4 n6 (AA), namely a decrease, has been proven also in this present study, in the 5-day exposure. AA proportional decrease due to the increasing toxin load might be associated with its role in primary hepatocyte nodule formation (Abel et al. 2001). Besides the fact that AA is prone to lipid peroxidation, AA metabolism has been proven to be disrupted by FB_1, since prostaglandin E2 inhibits the mitoinhibitory effect of FB_1 (Gelderblom et al. 1999). These authors declared that the FB_1-induced disruption of the n6 FA metabolic pathway, and even that of prostaglandin synthesis is likely to be an important event in the mitoinhibitory effect of FB_1 on growth factor responses. Thus, we suppose that in our case, in the 5-day exposure, the short interval produced results similar to those above, namely a rapid AA depletion, mostly by the highest FB_1 dose. This was matched with the proportional decrease of DGLA. Interestingly, the 10-day setting was only characterised with the decreasing DGLA proportion, while AA showed the opposite change, most specifically at higher FB_1 doses, a proportional increase. We may thus suppose that the disruption or modification of the metabolism of AA (and the precursor n6 compounds) is a transient effect of FB_1 in the PC fraction. Indeed, this has been underscored by the opposite results in the 10-day case, but, additionally, this reaction is especially characteristic for the higher FB_1 doses. From our dataset, it seems that high (50–100 mg kg^{-1}) FB_1 dietary dose is strongly and selectively compromising the metabolism of n6 FAs (DGLA & AA), while that of the n3 FAs was not systematically affected. In our related report (Szabó et al. 2018b) we found antioxidant adaptation in the 5-day setting (transient glutathione peroxidase activity increase with the elevation of the FB_1 dose), but this was not proven in the longer setting. Additionally, the liver malondialdehyde concentration proves a higher rate of *in vivo* lipid peroxidation in the short (5-day) setting, referring to the onset of (and a possible contribution to the n6 FA metabolism disturbance) oxidative stress, but only temporarily. The above statement is further corroborated with the results of Gelderblom et al. (2002), reporting a n6 FA increase in the

microsomal PC of rats after 21 days, when feeding 100 and 250 mg kg^{-1} FB_1.

A further marked change was the increase of MUFAs in the PC fraction, with its most important component, oleic acid (C18:1 n9, OA), in both exposures. According to Burger et al. (2007), increased OA proportion is associated with the disruption of the Δ6 desaturase enzyme, augmenting the up-regulation of Δ9 desaturation (resulting in OA enrichment of the membranes). Gelderblom et al. (2002) reported a similar OA recruitment in the rat hepatic microsomal membranes, as a result of 10 and 50 mg kg^{-1} dietary FB_1 dose over a period of 21 days. Gelderblom et al. (2002) explained the MUFA (OA) increase in the PC fraction as a process to counteract the impact of other PUFAs in the maintenance of membrane homoeostasis and fluidity. We share this idea, but it is important to add that MUFAs are as well increased in the short term (5-day), and even in the mid-term (10-day), and in latter case, n6 FAs did not provide marked proportional modification. On the contrary, dose associated increase of MUFA was coupled with a similar increase of the Σ n3 FAs. Thus, we also suppose a lipid peroxidation-lowering effect of OA and MUFA, since n3 FAs provide eightfold relative peroxidative sensitivity, as compared to OA or other MUFAs (Hulbert 2005). Our related study on the rate of hepatic lipid peroxidation (Szabó et al. 2018ab) supported this hypothesis, since malondialdehyde concentration was only elevated significantly in the 10-day treatment, where the Σ n3 FA increment was as well significant, if comparing control *vs.* 20 mg kg^{-1}.

Liver phosphatidylinositol FA composition

The phosphatidylinositol fraction of the rat liver, undergoing FB_1 intoxication has been analysed by Gelderblom et al. (2002). The primary effect of 250 mg kg^{-1} dietary FB_1 was the increase of the PI fraction (moiety) in the microsomes. The cited study tested 10-50-100-250 mg kg^{-1} FB_1, and the FA profile of PI was as well determined.

Our dataset provided decreased MUFA and Σ n6 FA levels, in the 5-day treatment, in parallel with the increasing FB_1 load. However, in the 10-day interval, the mentioned alterations were not proven, while C20:1 n9 increased in the 20 and 50 mg kg^{-1} groups, as compared to the control. It is important to mention that in this longer exposure the unsaturation index and the average FA chain length (UI and ACL) were significantly higher in the 100 mg kg^{-1} group, as compared to the 50 mg kg^{-1}.

In contrast, results of Gelderblom et al. (2002) suggest elevation of monounsaturation increased linoleic acid proportion, as well as increases in the Σ n6 and Σ n3 FAs. The basic difference between the two studies was that Gelderblom et al. (2002) exposed the rats to FB_1 for 21 days, we used 5 and 10 days only. This latter, quasi acute treatment was the one providing similarity to the findings of Gelderblom et al. (2002), while the short exposure led to markedly different results. The longer exposure, providing longer (ACL) and more unsaturated (UI) FAs echoes the increase of the PUFA components (n6 and n3 FAs), as described by Gelderblom et al. (2002). We thus suppose a rapid hepatic membrane lipid disintegration, as already published for rats (50 mg kg^{-1} for 5 days), where the total PL fraction was depleted in nearly all n3 PUFAs and a marked glutathione depletion was as well shown, without the activation of the antioxidant enzyme system (Szabó et al. 2016). It is highly plausible that during a longer exposure (10 or 21 days) enzymatic antioxidant adaptation is compensating the progression of lipid peroxidation, as reported in our recent study for glutathione peroxidase (Szabó et al. 2018b). In contrast, higher mycotoxin dose (50–100 mg kg^{-1}) and short exposure (5 days) are exerting drastic effect on the PI FA profile, most probably via a peroxidative route.

Liver phosphatidylethanolamine FA composition

The effective liver phosphatidylethanolamine (PE) reactivity to FB_1 intoxication has been published by Gelderblom et al. (2002), in rat microsomal membranes, reporting elevated PE concentration at 100 and 250 mg kg^{-1} FB_1 dietary dose.

What we found in the PE fraction was that its compositional reaction was likewise slow. In the 5-day treatment, only the highest dose (100 mg kg^{-1}) led to an increased docosahexaenoic acid (C22:6 n3, DHA) level, while all intoxicated groups showed decreased DGLA proportion, as compared to the control. Gelderblom et al.

(2002) and Burger et al. (2007) reported the increase of PE fraction absolutely (in quantity) and also its increasing ratio within the total PL fraction. This increasing abundance mathematically means increasing FA concentrations (even if the FA compositional pattern is unchanged), as proven by the authors. Gelderblom et al. (2002) described increasing concentration of MUFA, PUFA, n6 and n3 FAs at 100 and 250 mg kg^{-1} FB_1 dietary dose in the rat microsomal membrane PE fraction. This quasi-uniform FA level increase has not been found in our study at lower FB_1 levels. The only FA group providing a very clear (and statistically underscored linear) dose-dependent proportional increase was the Σ n3 FA. It is interesting that n3 FA metabolism was intensified, since the dietary precursor (C18:3 n3; ALA) was not proportionally modified. There are multiple desaturation (and elongation) steps involved in this metabolic pathway; it is highly plausible that Δ5 and Δ6 desaturase activity is changed as an effect of FB_1 (Gelderblom et al. 2002). On the contrary, the increasing proportion of Σ n3 FAs was matched with a decrease of the Σ n6 FAs in a manner in which their sum, the Σ PUFA level was unchanged. The results of Burger et al. (2007) are mostly similar to ours, since in the rat liver microsomal PE fraction – mostly due to the increase of C22:5 n3 and DHA – increased Σ n3 proportion was found, as a result of 21 days on 250 mg kg^{-1} FB_1. A robust explanation for the increasing n3 proportion is however still missing in case of the PE fraction (Burger et al. 2007), and according to Gelderblom et al. (2002), the clarification may require a targeted study on the phospholipid-metabolising enzymes. More specifically, this concerns enzymes which are involved in the cleavage of ARA, which has been proportionally decreased due to 50 mg kg^{-1} in the PE fraction (10 days). Gelderblom et al. (2002) published similar results in the hepatic microsomal PE, but the aetiology was less clear: lipid peroxidation and as well-impaired FA desaturation has been supposed, along with emphasising the role of AA in cancer promotion (Gelderblom et al. 2001).

Checking the FA indices used to estimate Δ6 and Δ5 desaturase activity, the only one providing inter-group difference (decreased value in the FB_1 treated groups) was the ratio of C18:2 n6/C18:3 n6 (LA/GLA; *data not shown)*, referring to Δ5 desaturation. Gelderblom et al. (2002) reported that the disturbance of Δ5 desaturation is a specific effect of FB_1, affecting the major PL classes. The authors generally explained this decrease as a shift in one of the components of the C18:2n6/C18:3n6 ratio, mostly the increase of GLA, or the increase of LA (Burger et al. 2007). We found the opposite, the decrease of LA, an essential FA. Due to the essential nature of LA, the decrease found must be a simple dietary effect (feed intake decreased; see: Szabó et al. 2018b), referring to the fact that at 20–50–100 mg kg^{-1} FB_1 did not act as a membrane synthesis disruptor/inhibitor at the level of PE FA profile. The proportional decrease of AA may as well be coupled to the decrease of its essential precursor, LA.

Conclusions

In conclusion, rat kidney has been found to be more reactive in terms of membrane lipid compositional modifications, induced by low dose and relatively short-term FB_1 exposure. The most responsive lipid fraction in the rat renal cortex was that of PC, providing perturbed n6, n3, and thus, PUFA balance (in a dose-dependent manner), which was partly proven as well for the PI. The rat liver was less sensitive towards FB_1, the PE fraction providing dose-response. Results were consonant with earlier (Szabó et al. 2018b) histopathological and peroxidation endpoint findings.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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4.2. Orally administered fumonisins affect porcine red cell membrane sodium pump activity and lipid profile without apparent oxidative damage

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Orally Administered Fumonisins Affect Porcine Red Cell Membrane Sodium Pump Activity and Lipid Profile without Apparent Oxidative Damage

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Abstract: Weaned piglets ($n = 3 \times 6$) were fed 0, 15 and 30 mg/kg diet fumonisin (FB_1, FB_2 and FB_3, i.e., FBs, a sphinganine analogue mycotoxin), from the age of 35 days for 21 days, to assess mycotoxin induced, dose-dependent changes in the red cells' membrane. Ouabain sensitive Na^+/K^+ ATPase activity was determined from lysed red cell membranes, membrane fatty acid (FA) profile was analysed, as well as antioxidant and lipid peroxidation endpoints. Final body weight was higher in the 30 mg/kg group (vs. control), even besides identical cumulative feed intake. After 3 weeks, there was a difference between control and the 30 mg/kg group in red cell membrane sodium pump activity; this change was dose-dependent (sig.: 0.036; $R^2 = 0.58$). Membrane FA profile was strongly saturated with non-systematic inter-group differences; pooled data provided negative correlation with sodium pump activity (all individual membrane n6 FAs). Intracellular antioxidants (reduced glutathione and glutathione peroxidase) and lipid peroxidation indicators (conj. dienes, trienes and malondialdehyde) were non-responsive. We suppose a ceramide synthesis inhibitor (FB_1) effect exerted onto the cell membrane, proven to be toxin dose-dependent and increasing sodium pump activity, with only indirect FA compositional correlations and lack of lipid peroxidation.

Keywords: fumonisins; red blood cell; sodium pump; membrane; fatty acids; oxidative stress; pig

Key Contribution: Weaned piglets exposed to fumonisins at 15 and 30 mg/kg diet provided markedly altered red blood cell Na^+/K^+ ATPase activity after 3 weeks, in a dose-dependent manner. This alteration was characteristically non-oxidative stress associated and membrane lipid fatty acid profile provided indirect correlations.

1. Introduction

Fumonisins are mycotoxins (fungal secondary metabolites) produced in the highest quantities by *Fusarium verticillioides* and *Fusarium proliferatum* mould strains, infecting food and feed cereals. The 28 fumonisin analogues characterized since 1988 can be divided into four main groups: series A, B, C and P [1], from which the B analogues are toxicologically the most hazardous, fumonisin B_1 (FB_1) being the most well-known and the most toxic in the latter series [2]. Fumonisin occurrence is very frequent in cereals and cereal products but primarily in corn; the prevalence was 78% in 2020

in the tested corn samples [3], representing the main farm animal feed component. Fumonisins are specifically harmful to pigs, leading to a typical porcine toxicosis syndrome named porcine pulmonary edema. Hepatic lesions consisting of apoptosis, necrosis and hepatocyte proliferation, besides elevated serum cholesterol concentration are the further consequences. In chronic studies, oesophageal plaques, hyperplastic hepatic nodules and right ventricular hypertrophy were found in pigs as well [4].

At a molecular level, fumonisin B_1 administration disrupts sphingolipid biosynthesis, with the greatest alterations in sphingosine and sphinganine concentrations in porcine kidney, liver, lung and heart [4,5]. FB_1 shows structural similarity to the cellular sphingolipids and inhibits ceramide synthase, leading to the accumulation of sphinganine and depletion of ceramide [6]. Fumonisin B_1 is so potent and so specific in this regard that it is referred to as a direct ceramide synthesis inhibitor [7]. In in vitro exposures, there is a quick increase in the free sphingoid base, sphinganine [8], while sphinganine acylation (with fatty acids) is also inhibited.

The ceramide synthesis inhibitor effect has thus been proven in vitro [8] and in vivo in numerous animal species and multiple tissue types [4]. According to a recent review [2], FB_1 has been shown to produce pleiotropic toxicities in animals, including neurotoxicity, hepatotoxicity and nephrotoxicity, and recently we proposed haematotoxicity [9]. Underlying mechanisms include disrupted sphingolipid metabolism, oxidative stress, activation of endoplasmic reticulum stress, modulation of autophagy and the alteration of DNA methylation [2].

These systematic effects have also consequences on the ion balance and its regulation. Haschek et al. (1992) [10] described in short-term cardiovascular studies decreased cardiac contractility, mean systemic arterial pressure, heart rate and cardiac output due to FB_1 and increased mean pulmonary arterial pressure, the changes being compatible with the inhibition of L-type calcium channels by increased sphingosine and/or sphinganine concentration. In 2014, we [9] described dramatically increased cation flux (Na^+, K^+) in FB_1 fed rabbits' erythrocytes, the result being fully consonant with those of Mays et al. (1995) [11], reporting that Na^+/K^+ ATPase sorting to the different membrane domains (in renal cells apically and baso-laterally) is modified by the inhibition of the sphingolipid synthesis, the typical FB_1 mode of action. Thus, FB_1 exerted its sodium pump modification effects definitely by altering the lipid synthesis, and not directly interfering with Na^+/K^+ ATPase enzyme itself.

Based on the recent results, and the continuously increasing fumonisin burden, we aimed to study whether weaned piglets are susceptible to a graded dietary FBs exposure (dose dependence), testing circulating red cell membrane FA composition, Na^+/K^+ ATPase activity and oxidative stress indicators, after in vivo exposure.

2. Results

2.1. Body Weight, Organ Masses

2.1.1. Inter-Group Differences and Dose Response

Final body weight (BW) was significantly higher in the 30 mg FBs/kg group, as compared to the control, but absolute organ mass mean values were not different among any of the groups, as compared with analysis of variance (Table 1). The relative kidney weight was the lowest in the 30 mg/kg group and significantly different from the control. Cumulative feed intake for the entire 21 days was identical in the three groups (Table 1). The only somatic trait that was found to be linearly related to mycotoxin dose was the final BW (sig.: 0.027; $R^2 = 0.27$).

Table 1. Somatic traits of the experimental and control piglet groups (n = 6/group; data are group means ± SD (standard deviation) of 6 individual data; different uppercase letters indicate significant difference of means at $p \leq 0.05$. Between group differences were compared with one-way ANOVA and LSD "post hoc" test; BW: bodyweight).

Group	Control	15 mg/kg	30 mg/kg
Somatic Traits	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
BW initial (g)	12,980 ± 1720	13,800 ± 1200	13,800 ± 1340
BW final (g)	21,467 ± 1735 a	23,067 ± 1454 ab	23,367 ± 629 b
cumulative feed intake (g)	19,759 ± 2102	20,450 ± 1352	20,382 ± 1352
lung (g)	227.2 ± 45.0	237.1 ± 43.0	253.0 ± 70.0
liver (g)	527.4 ± 42.1	587.2 ± 56.9	563.0 ± 96.2
kidney (g)	84.1 ± 2.53	86.2 ± 6.83	79.4 ± 10.6
pancreas (g)	50.0 ± 6.39	57.1 ± 7.62	56.5 ± 9.81
lung (% of BW)	1.06 ± 0.20	1.03 ± 0.15	1.09 ± 0.30
liver (% of BW)	2.48 ± 0.38	2.55 ± 0.23	2.42 ± 0.45
kindey (% of BW)	0.39 ± 0.03 b	0.37 ± 0.04 ab	0.34 ± 0.05 a
pancreas (% of BW)	0.23 ± 0.04	0.25 ± 0.04	0.24 ± 0.04

2.1.2. Sodium Pump Activity Correlations

The somatic traits determined did not provide any significant correlation (Pearson correlation) with the RBC sodium pump activity.

2.2. Red Cell Membrane Sodium Pump Activity and Dose Response

Inter-Group Differences and Dose Response

After 5 weeks of FBs feeding, there was a difference among the control and the 30 mg/kg groups in the red cell membrane sodium pump activity (Figure 1). The FBs feeding increased the sodium pump activity significantly only in the latter group, while the 15 mg/kg treatment provided intermediate data (control < 15 < 30), the latter without statistical significance. Testing the dose response of this alteration, the linear estimation was significant (y intercept = 52,982; slope = 35,340; sig.: 0.036; $R^2 = 0.58$).

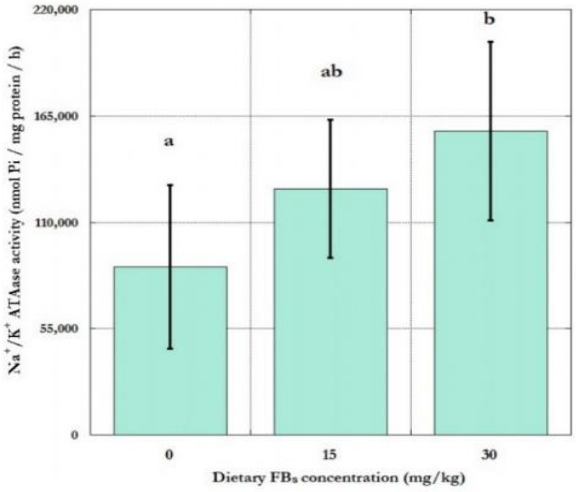

Figure 1. The total Na^+/K^+ ATPase activity of red cells of the experimental piglet groups (n = 6/group; columns represent group means ± SD of 6 individual data; different uppercase letters indicate significant difference of means at $p \leq 0.05$. Between group differences were compared with one-way ANOVA and LSD "post hoc" test).

2.3. Red Cell Fatty Acid Profile

The membrane fatty acid profile is summarized in Table 2, also showing inter-group differences and the dose-response data.

Table 2. The red cell membrane fatty acid profile in the three piglet groups (n = 6/group; data are group means ± SD of 6 individual data; different uppercase indicate significant difference of means at $p \leq 0.05$. Between group differences were compared with one-way ANOVA and LSD "post hoc" test; BW: bodyweight; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid).

Group	Control			15 mg/kg			30 mg/kg		
Red Cell Membrane FA Profile	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
C12:0	0.04	± 0.01	b	0.02	± 0.01	a	0.02	± 0.00	ab
C14:0	0.52	± 0.04		0.50	± 0.03		0.52	± 0.05	
C15:0	0.10	± 0.05		0.14	± 0.08		0.09	± 0.02	
C16:0	43.6	± 0.97	ab	42.6	± 1.69	a	44.5	± 1.33	b
C16:1n7	0.08	± 0.02		0.11	± 0.04		0.09	± 0.04	
C17:0	0.40	± 0.12		0.59	± 0.25		0.38	± 0.05	
C18:0	48.7	± 1.52		46.7	± 2.86		48.8	± 1.63	
C18:1n9c	3.90	± 1.64	ab	6.15	± 2.68	b	2.99	± 0.72	a
C18:1n7	0.23	± 0.10		0.33	± 0.18		0.17	± 0.03	
C18:2n6	0.96	± 0.70		1.54	± 1.29		0.37	± 0.11	
C18:3n3	0.02	± 0.00		0.02	± 0.01		0.03	± 0.00	
C20:0	0.45	± 0.03		0.43	± 0.05		0.43	± 0.03	
C20:1n9	0.02	± 0.00		0.02	± 0.01		0.02	± 0.00	
C20:2n6	0.02	± 0.01		0.02	± 0.01		0.01	± 0.01	
C20:3n6	0.02	± 0.01	ab	0.03	± 0.02	b	0.01	± 0.00	a
C21:0	0.03	± 0.02		0.03	± 0.02		0.04	± 0.02	
C20:4n6	0.25	± 0.17	ab	0.46	± 0.33	b	0.10	± 0.04	a
C22:0	0.07	± 0.01		0.06	± 0.01		0.06	± 0.00	
C22:6n3	0.15	± 0.05		0.18	± 0.04		0.15	± 0.06	
C24:1n9	0.08	± 0.08		0.09	± 0.08		0.08	± 0.07	
Σ saturated	93.1	± 0.91	ab	91.0	± 4.32	a	96.0	± 0.94	b
Σ unsaturated	6.92	± 0.91		8.95	± 4.32		3.96	± 0.94	
Σ MUFA	5.32	± 0.63	ab	6.70	± 2.92	b	3.34	± 0.78	a
Σ PUFA	1.40	± 0.87		2.25	± 1.68		0.63	± 0.17	
Σ n3	0.15	± 0.05		0.19	± 0.05		0.16	± 0.06	
Σ n6	1.25	± 0.88		2.06	± 1.64		0.49	± 0.16	
Σ n6/Σ n3	9.38	± 7.30		9.70	± 6.59		3.77	± 1.49	
Σ odd chain FA	0.50	± 0.17		0.72	± 0.33		0.47	± 0.06	

2.3.1. Inter-Group Differences and Dose Response

The proportion of C12:0 (lauric acid) was the highest in the control group and the lowest in the 15 mg/kg treatment, the 30 mg/kg showing intermediate values.

Clear toxin dose dependent difference in the fatty acid proportions was found for the following FAs: C18:1 n9 (oleic acid), C20:3 n6 (dihomo-γ-linolenic acid), C20:4 n6 (arachidonic acid) and total monounsaturation (MUFA), 15 mg/kg group showing higher data than the 30 mg/kg treatment. In all these instances the control group had intermediate proportional values. The only compound for which the 15 mg/kg group had the lowest proportion was C16:0 (palmitic acid), and as a direct consequence, the sum of saturated FAs. As a consequence of this non-linear alteration mode, well-fitting linear dose-response was not proven in any of the cases.

2.3.2. Sodium Pump Activity Correlations

The Pearson correlation between the membrane fatty acid proportions and the sodium pump activity values is given in Table 3, for the description of the inter-relationship between the variables.

Practically all individual n6 FAs provided negative correlation with the sodium pump activity, as well as the total polyunsaturated FAs (PUFA).

Table 3. Pearson correlation parameters between sodium pump activity and the fatty acid profile data. (Calculations were performed on pooled (i.e., 3 groups handled together as one, $n = 18$), individual data pairs were used for the analysis. Significance was set to $p \leq 0.05$).

Compound	Sig.	Pearson Corr. Coeff.
C18:2 n6	0.017	−0.671
C20:2 n6	0.001	−0.821
C20:3 n6	0.022	−0.65
C20:4 n6	0.023	−0.648
Σ PUFA	0.012	−0.697
Σ n6	0.014	−0.683

2.4. Red Cell Antioxidant Status and Lipid Peroxidation

Whole red cell homogenate reduced glutathione (GSH), glutathione peroxidase (GSHPx), conjugated diene and triene concentrations (CD and CT), and malondieldyde (MDA) concentration did not provide any inter-group differences (Figure 2), neither linear dose-response, nor any correlations with the sodium pump activity.

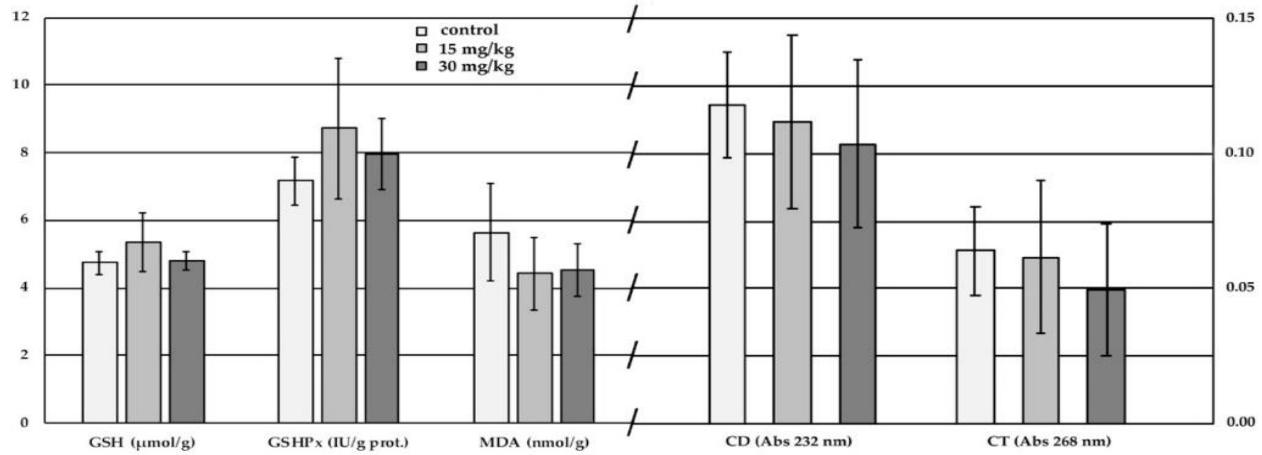

Figure 2. The antioxidant and lipid peroxidation traits of the red cells of the experimental piglet groups (lack of different uppercase index letters represent the lack of significant difference at $p < 0.05$). ($n = 6$/group; columns represent group means ± SD of 6 individual data. Between group differences were compared with one-way ANOVA and LSD "post hoc" test).

3. Discussion

3.1. Body Weight, Organ Masses

Inter-Group Differences and Dose Response

The FBs administration level was relatively high in this study, being definitely above the limit values for pigs [12]. The study planning aimed to reach or approach an intoxication status/niveau that is not mild, primarily to affect the cellular composition of the already circulating, ripe red cells [9,13] and to test possible mediator effect of plausible oxidative stress. Though the initial BW was equal in all groups, the highest fumonisin dose increased it, as compared to the control. This increase was not matched with the increase of any of the organ mass values (and was neither a result of increased feed intake, Table 1). In contrast, final BW provided linear fumonisin dose dependence (sig.: 0.027;

$R^2 = 0.27$, as tested on individual data-pairs). (Moreover, by testing weekly BW gain in the three groups separately, it was clearly visible that gain slowed down, and its standard deviation (as assessed with Levene's F test) increased markedly, in parallel (data not shown)).

Generally, FB_1 is responsible for the induction of either organ, or even body mass alterations. We have reported [14] that the liver mass decreased, kidney mass increased and BW increased due to 5 and 10 days of FB_1 exposure at 0, 20, 50 and 100 mg/kg dietary dose equivalent, though in other cases no alteration was found in rats [15]. Furthermore, in rabbits, liver mass was found to increase as a result of 10 mg FB_1/kg diet exposure for 4 weeks [16]. Specifically, for pigs, FB_1 has been reported as a growth inhibitor [17], most probably by damaging the barrier function of the intestinal epithelial cells [18]. In contrast, in an approximation of the limit values [12], histological symptoms in intestine and myocardium appeared at 3.7 mg FBs/kg and in the kidney at a higher dose (8.1 mg/kg feed), while at the highest feed dose (12.2 mg FBs/kg) all investigated organs showed histological modifications, primarily lesions. In the above study [12], 12.2 mg/kg diet did not lead to growth differences, nor the 20 mg FB_1/kg for 10 days could alter the performance, body weight and feed intake in another test [13]. Since our present study strongly exceeded the cited mycotoxin concentrations [12] and exposure period [13], we assumed that increased growth of the mostly intoxicated animals was attributable to some extent of slight edema [13], although absolute lung weight was not altered statistically. It is important to add that pulmonary edema was present in some individual cases, was photo-documented, but this was not a systematic finding. In this study, we did not confirm any alteration of the absolute organ weights, while total body mass increased. Ultimately, the daily and cumulative feed intake (Table 1) and feed conversion ratio were as well checked, not providing any inter-group differences (data not shown). Therefore, we assume that the applied dose did not compromise the piglet performance, at least under the examined period, whereas the recorded increase in body mass of the 30 mg/kg diet group is a slight increase that might be a result of non-systematic edema that not detected in all animals. Results of the biochemical analysis (higher enzymatic activities of LDH, γ-GT and CK for 30 mg/kg diet, data not shown) are indirectly indicating possible slight muscular hypertrophy, in which increase in body mass is the major consequence. This later assumption cannot be proven in this study since the muscular mass was not examined.

3.2. Red Cell Membrane Sodium Pump Activity and Dose Response

Similarly to our first report [9] on the disturbance of cation active transport as induced by FB_1, we found a strongly similar result now, but in a species never tested for this reaction before. Which fumonisin modified pathways/compounds are exactly involved in the dose-dependent of the RBC active cation transport has not been elucidated yet.

Early in vitro studies revealed that FBs are effective inhibitors of sphinganine (sphingosine) N-acyl transferase (ceramid synthases, CerS) [19]. By in vitro exposure, when cells are exposed to FB_1, there is a quick increase in the free sphingoid base, sphinganine, in tissues and body fluids [20], while sphinganine acylation is inhibited. Moreover, it has been added [21] that FB_1 inhibits as well the production of 1-deoxydihydroceramide, playing important roles in cellular level regulation.

The well-documented biological effect of FB_1 is thus a more complex inhibition of sphingolipid synthesis, leading to cellular ceramide level depletion (blocking the acylation by FAs). Evidence exists that ceramide effectively modulates Na^+/K^+ ATPase enzyme activity (and that of further sodium transporters), participating in its regulatory network in the renal cells' basolateral membrane [22]. Moreover, increased ceramide levels induce ouabain sensitive sodium pump inhibition [22] but indirectly; this happens in renal cells via the modulation of protein kinases A and C. Ceramide can have a crucial role in the regulation of the complex Na^+ transport apparatus (directly inhibiting furosemide sensitive Na^+-ATPase but indirectly inhibiting ouabain sensitive Na^+/K^+ ATPase), involving also effector proteins [22]. This supposed modulatory role (possessing most (~60%) of the Na^+/K^+ ATPase inhibitory activity) of ceramide onward Na^+/K^+ ATPase is a novel finding in the red cells. In the present

dataset, gained from a sustained FB_1 intoxication study, (FB_1 is known as an inhibitor of ceramide synthesis) strongly elevated RBC sodium pump activity was proven after 3 weeks of exposure.

Ceramide has the property to act as a regulator of multiple cellular processes, like cell proliferation and apoptosis, which are coupled respectively with decreasing and increasing the Na^+/K^+ ATPase activity (in nucleated cells) [23]. These antagonistic effects might be time-dependent, due to different signalling pathways and their different activation time intervals. Since apoptosis is a process characterized by cell volume shrinkage, and it has a direct influence on cellular ion exchange dynamics, those (more specifically K^+) are indeed triggering factors of apoptotic processes [24], as found in lymphocytes. This K^+ homeostasis modification is merely a result of Na^+/K^+ ATPase activity change [23]. Anyway, ouabain sensitive sodium pump is ubiquitous and has multiple triggering factors, like hormonal changes, substrate concentration, as well as the embedding membranes' physicochemical properties [25,26].

Specifically for human erythrocytes, the enzyme stimulatory effect of ceramide (for Ca^{2+}-ATPase) was presented [27]. An interesting property of this Ca^{2+}-ATPase that distinguishes it from other P-type ionic pumps is the multiplicity of its regulatory mechanisms [28]. Authors found that ceramide acts on the enzyme activity in its second messenger role and not by influencing membrane or caveola properties. HepG2 cells react to in vitro ceramide addition with increased Na^+/K^+ ATPase activity [23]. It seems that ceramide provides an enzyme activating effect on P-type ionic pumps, but the ceramide metabolite, sphingosine is in lack of this property; this effect has been reported to be time-dependent [23].

In summary, we found a speed-up of the most important cations' influx (Na^+) and efflux (K^+) in the porcine red cells, as induced by a ceramide-synthase inhibitor, and newly, providing inhibitor dose-dependence.

3.3. Red Cell Fatty Acid Profile

3.3.1. Inter-Group Differences, Dose Response and Sodium Pump Activity Correlations

The Na^+/K^+ ATPase inhibition/lowered activity is mostly characteristic for apoptosis, while activity increase is generally a sign of cell proliferation, as reported in hepatic cells [29–31]. We analysed mixed, circulating red cell populations and did not check haematology (neither polychromatic erythroblasts). While in our first study [9] FB_1 exerted clearly defined effects on the rabbit RBC membrane FA composition, here we mostly detected differences between the lower and the higher toxin doses. If control data are considered as a baseline, lower and higher FBs doses exerted likewise divergent effects on the FA profile. It is hard to explain this, since it looks like lower FB_1 dose has opposite effects than the higher. If focusing specifically on the divergent alterations provoked by the two doses (lower vs. higher), we detected a definitely lower niveau of membrane saturation (lower level of C16:0, higher C18:1 n9 and C20:4 n6, lower total saturation and higher total monounsaturation). These modifications are unequivocally referring to a more rigid physicochemical property of the cell membrane, associated with the higher FBs dose.

In summary, it rather seems that the biological compound, the cellular membrane itself, was an originally less sensitive system in terms of lipid composition towards lipid peroxidative damage. We assume that further studies are needed to clarify the contribution of FAs to the supposed regulatory role of Na^+/K^+ ATPase. Most probably, these studies need to separate lipid classes before FA analysis into more relevant sub-classes.

3.3.2. Sodium Pump Activity Correlations

The Pearson correlation between the membrane fatty acids and the sodium pump activity is given in Table 3. Nearly all individual n6 FAs proportional value provided negative correlation with the sodium pump activity, as well as the total polyunsaturated fatty acids (PUFA).

Since Na^+/K^+-ATPase is an intrinsic membrane protein, the physico-chemical properties of the membrane constituents should be an important determinant of enzyme activity [32]. Free fatty

acids, or those released from the membrane by phospholipase A_2 (PLA_2) tend to inhibit the Na^+/K^+ ATPase [32], but we did not detect drastic membrane PL disruption for the fatty acid containing lipids. Indeed, this was underscored by the membrane FA compositional results as well, since only mild changes were proven.

Though we measured whole cell membrane, data of Else et al. (2003) [33] in a different membrane model partly supported our data. Though we did not find any positive correlation with the long-chain, n-3 FAs [33], we provided evidence that nearly all n-6 FAs acted as sodium pump activity "slowing down agents" (Table 3). This is consonant with the cited results [33], but it is rather novel that in our, basically small dataset all n-6 series FA were negatively correlated with the sodium pump activity.

There is still a debate on the role of FAs in the regulation of sodium pump. Considering non-ester bound, long chain acids (C16:0, C18:1 n9 and C18:2 n6), those were reported to be very efficiently incorporated to the human erythrocyte membrane, but there, they still remained free fatty acids and did not alter the molecular activity of the enzyme [34]. In a next step, the same authors found [35] lower incorporation efficacy of phosphatidylcholine-FAs (PC-FA) but reported PC-FA dependent decrease (C12:0, C14:0 and C16:0) of the sodium pump. Results prove that the sodium pump in situ is sensitive to lipid fatty acid profile. Our dataset proved this as well, but it has to be added that our analytical approach was only assessing FA methyl esters liberated from an ester bond, thus excluding all free FAs [36].

In summary, we suppose that only a minor toxic effect was attainable in the circulating porcine red cell cohort; fatty acid proportions and enzyme activity data were only loosely related, meanwhile the entire dataset was proving some basic inter-relationship between red cell membrane composition and FA profile. Though only indirectly conceptualized, the basic effect of FBs onward the ceramide synthesis and its consequences seemed to be effectively proven in our data as well, in a species not tested yet in this aspect.

3.4. Red Cell Antioxidant Status and Lipid Peroxidation

Analysing early phase (conjugated diene and triene) and progressive (MDA) lipid peroxidation, as well as cellular antioxidant (reduced GSH) concentration and antioxidant enzyme (GSHPx) activity underscored the full lack of lipid peroxidation and antioxidant depletion in this study, in this tissue type. In our earlier relevant study [9], we did not check lipid peroxidation in the homogenized red cells.

Direct incorporation of FB_1 sensitizes PC bilayer to lipid peroxidation [37], but a metabolized form of FB_1 is not always found to be oxidative. In some of our earlier studies, we frequently tested FB_1 induced oxidative stress in red cell homogenate, but only in a very early approach, at 45 mg FB_1/kg feed dose for 10 days, piglets provided lower reduced glutathione content in the red blood cell haemolysate samples [38].

The erythrocyte is a unique biological structure containing generally high PUFA amounts, molecular oxygen and ferrous ions in the ligand state. For these reasons, it might be expected to be highly vulnerable to the main potential hazard of an aerobic environment [39]. Indeed, due to these cellular properties, the lack of nucleus and especially mitochondria, dramatic lipid peroxidation was not expected. The primary site of possible lipid peroxidation is thus the membrane. The general lack of peroxidative damage that was found here might be attributed to the overall high level of saturation of the porcine red cell membrane (Table 2).

In summary, the ghost cell membrane preparation seemed to result in a relatively high FA saturation, most probably preventing lipid peroxidation. This was in full agreement with the lack of lipid peroxidation and lack of alteration in the antioxidant defence system, as analysed in intact, whole red cells.

4. Conclusions

We postulate that red blood cell damage or functional modification is efficiently achieved by fumonisin Bs in piglets after only three weeks. The significantly elevated sodium pump activity is

with the highest plausibility induced by the ceramide synthesis inhibitor effect of FB_1. This mycotoxin leads to a characteristically non-oxidative stress associated state with markedly elevated sodium pump activity and complex membrane lipid fatty acid profile, providing relevant correlations for all n-6 fatty acids.

5. Materials and Methods

5.1. Experimental Design and Animals

Altogether 3 x 6 weaned Danbred piglets were enrolled in the study at the age of 35 days. After a 14-day adaptation (at the exact age of 49 days) period, the duration of the feeding trial was 21 days. One group was fed a piglet diet complemented with 15 mg/kg FB toxins (FB_1, FB_2 and FB_3 in a fungal culture), whereas another 30 mg/kg (the control was FBs free). Detailed diet composition is given in Table 4. Water was offered ad libitum. The piglets were caged individually in 80 × 80 cm area metabolic cages. The temperature of the trial room was controlled in accordance with the needs of weaned piglets. Bodyweight was measured individually. At the end of the trial, the piglets were euthanised by exsanguination after sedation (Euthanyl-Pentobarbital Sodium, 400 mg/mL, Dechra Veterinary Products, Shrewsbury, UK), and splanchnic organs and blood were sampled.

Table 4. Diet proximate composition of the piglets.

Crude protein (%)	17.50
Crude fat (%)	3.30
Crude fiber (%)	3.70
Crude ash (%)	5.00
Lysine (g/kg)	1.11
Methionine (g/kg)	0.37
Ca (g/kg)	0.65
P (g/kg)	0.50
Na (g/kg)	0.18
DE (MJ/kg)	14.70
ME (MJ/kg)	14.10

5.2. Feed Mycotoxin Contamination

The basic feed was of commercial origin. Feed was given twice a day, in two equal portions, and the amount of feed not consumed by the animals was measured back. A *Fusarium verticillioides* fungal culture of high FB_1 concentration (for production details see: [40], culture name: RL 596) was mixed into the ration of the experimental animals, so as to provide a daily FBs (FB_1+FB_2+FB_3) feed concentration of 15 and 30 mg/kg. The mycotoxin concentration of the control and the experimental feed was determined with LC-MS (Shimadzu, Kyoto, Japan). The limit of detection (LOD) for FB_1 was 3 μg/kg. The diet fed to the control group did not contain detectable amounts of FBs (the full absence of deoxinivalenol, zearalenone and T-2 toxin was as well controlled and confirmed).

Fumonisin B contents were determined with a LC-MS method, described in detail earlier [41].

5.3. Blood Sampling, Erythrocythe "Ghost" Preparation

Fresh venous blood was sampled into heparinized (20 IU/mL whole blood) tubes and was centrifuged for 10 min at 1000 g (SIGMA 3-30KS refrigerated centrifuge, Osterode am Harz, Germany). Plasma and the buffy coat were removed, and the erythrocyte bulk was washed 3 times with 10 volumes of TRIS-HCl (0.1 M; pH = 7.4) at 4 °C. After each wash, the buffy coat (and the washing medium as supernatant) was siphoned. Red blood cell (RBC) lysis was induced by ice-cold hypotonic TRIS-HCl solution (15 mM; pH = 7.4). Erythrocyte lysate was centrifuged at 15,000 g for 10 min at 4 °C repeatedly, until the washing medium was colorless (~7 times). Washing medium hemoglobin content was controlled with spectrophotometry at 418 nm in 10 mm path length optical cuvettes against

medium blank until 0.001 Abs value (Shimadzu UV160 spectrophotometer, Shimadzu, Kyoto, Japan). The original method was described by Shanmugasundaram et al. (1992) [42]. The protein content of the suspension was determined according to Lowry et al. (1951) [43], with bovine serum albumin as a standard (Shimadzu UV1900 spectrophotometer, Shimadzu, Kyoto, Japan). The RBC ghosts were stored frozen (−70 °C) until analysis.

5.4. Determination of the RBC Na^+/K^+ ATPase Activity

For the assay procedure, a quantity equal to 300 μg protein was used. The relatively high quantity was reasoned by the fact that the abundance of the enzyme is relatively low in the red cells [44]. RBC ghosts were pre-incubated (10 min at 37 °C) in an incubation medium (92 mM TRIS-HCl (pH 7.4), 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$ and 1 mM EDTA) [45]. The reaction was started by the addition of 6 mM vanadate free ATP (disodium salt, Merck-Sigma Aldrich A26209). Incubations (37 °C at 30 min) were performed in the presence (2 mM) and absence of ouabain, a specific Na^+/K^+ ATPase inhibitor (ouabain octahydrate, Merck-Sigma Aldrich O3125). The reaction was stopped by addition of ice-cold trichloroacetic acid at a final concentration of 5%, and samples were centrifuged at 5000 g for 10 min at 4 °C. The phosphate liberation was determined from the supernatant and was given as the difference of the Pi liberation without and with ouabain, in nmol Pi/mg protein/h. The liberated Pi was measured photometrically (Shimadzu UV1900 spectrophotometer, Shimadzu, Kyoto, Japan), according to Hurst (1964) [46], in 10 mm path length optical glass cuvettes (Hellma Optik GmbH, Jena, Germany). All assays were performed in triplicate, and blanks were included in each run to determine the endogenous phosphate concentration and the non-enzymatic ATP breakdown (i.e., Pi liberation). The amount of phosphate was read from the standard curve prepared using known concentrations of KH_2PO_4, according to Beltowski and Wojcicka (2002) [47].

To ascertain that the ATP concentration in the medium is reaching the level of enzyme saturation, a simple test was performed, namely, the doubling of the enzyme quantity doubled the apparent enzyme activity, while the doubling of the ATP concentration did not alter it.

5.5. Determination of the RBC Membrane Fatty Acid Composition

The RBC ghost moiety (i.e., porous, lysed cells) not used for enzyme assay was directly lipid-extracted according to the method of Folch et al. (1957) [48] and the gained complex lipids' (FA) composition was determined with gas chromatography (Shimadzu Nexis 2030, Kyoto, Japan), in the form of fatty acid methyl esters [36], after a separation on a Phenomenex Zebron ZB-Wax capillary column (30 m × 0.25 mm × 0.25 micrometer film, Phenomenex Inc., Torrance, CA, USA). The chromatographic evaluation was performed with the LabSolutions 5.93 software, using the PostRun module (Shimadzu, Kyoto, Japan) with manual peak integration. Fatty acid composition was expressed as weight % of total FA methyl esters. The identification of the FAs was performed based on the retention time of a CRM external standard (Supelco 37 Component FAME Mix, Merck-Sigma Aldrich, CRM47885).

5.6. Red Cell Antioxidant Status and Lipid Peroxidation

For the determination of lipid peroxidation and antioxidant status, the 3 × washed red blood cell samples (not lysed cells) were stored at −70 °C until analysis. Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) levels with 2-thiobarbituric acid method in cell hemolysate [49] and the determination of conjugated dienes (CD) and trienes (CT) according to the photometric method of AOAC (1984) [50]. The concentration of reduced glutathione (GSH) was measured by the method of Sedlak and Lindsay (1968) [51] and the activity of glutathione peroxidase (GSPHx) according to Lawrence and Burk (1978) [52].

5.7. Statistical Analysis

For the comparison of the 3 group means (enzyme activity, initial and final bodyweight, fatty acid profile data within single rows) univariate (FBs concentration as grouping variable) analysis of variance (ANOVA) was used, with the LSD "post hoc" test for detailed inter-group differences. Pearson correlation was calculated between sodium pump activity and further biochemical variables, using individual data-pairs always. The extent of standard deviation was compared between groups with Levene's F test. For all tests, significance level was set to $p \leq 0.05$. IBM SPSS 20 for Windows (2010) [53] was used for the evaluation.

5.8. Ethical Issues

The experiments were 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: 27 March 2017).

Author Contributions: A.S. and M.K. conceived the study design, collected and analysed data, and wrote the manuscript. K.L. analysed fatty acids. M.M. and K.B. analysed antioxidants and lipid peroxidation. O.A. was involved in sample preparation, enzyme assay and corrected the manuscript. T.B. and L.H. performed mycotoxin analysis. All authors have read and agreed to the published version of the manuscript.

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4.3. Fumonisin B series mycotoxins' dose dependent effects on the porcine hepatic and pulmonary phospholipidome

Title	Fumonisin B series mycotoxins' dose dependent effects on the porcine hepatic and pulmonary phospholipidome
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Fumonisin B Series Mycotoxins' Dose Dependent Effects on the Porcine Hepatic and Pulmonary Phospholipidome

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Abstract: Male weaned piglets n = 6/group were fed Fumonisin B_{1+2+3} (FBs) mycotoxins at 0, 15, or 30 mg/kg diet for 3 weeks to assess the fatty acid (FA) composition of membrane lipid classes, lipid peroxidation, and histomorphological changes in the liver and lung. Growth performance and lipid peroxidation were unaltered, but histomorphological lesion scores increased in the liver. Linear dose–response was detected in liver phosphatidylcholines for C16:1n7, C18:1n9, and total monounsaturation and in lungs for C22:6n3, total n-3 and n-3:n-6, in pulmonary phosphatidylserines C20:0 and C24:0. Alterations associated with the highest FBs dose were detected in sphingomyelins (liver: total saturation ↓, total monounsaturation ↑), phosphatidylcholines (liver: total n-6 ↓, n-6:n-3 ↑; in lungs: total monounsaturation ↑, total polyunsaturation ↑), phosphatidylethanolamines (liver: total n-3 ↓; in lungs: total monounsaturation ↑ and n-6:n-3 ↑), phosphatidylserines (liver: n-6:n-3 ↑; in lungs: total saturation ↓, total polyunsatuartion ↑, and total n-6 and its ratio to n-3 ↑), and phosphatidylinositol (n-6:n-3 ↑; lungs: C22:1n9 ↑, C22:6n3 ↓, total saturation ↓, total monounsaturaion ↑). In conclusion, FBs exposures neither impaired growth nor induced substantial lipid peroxidation, but hepatotoxicity was proven with histopathological alterations at the applied exposure period and doses. FA results imply an enzymatic disturbance in FA metabolism, agreeing with earlier findings in rats.

Keywords: fumonisins; mycotoxin; lipid; swine; phospholipid; sphingomyelin; fatty acid; lipid peroxidation; liver; lung

Key Contribution: This work highlights the effects of fumonisin B series mycotoxins on the porcine liver and lung phospholipidome in a depth of the fatty acid profile, an approach not performed yet.

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1. Introduction

Mycotoxins are the secondary products that are generated from fungal metabolism. As of today, more than 400 metabolites have been identified; however, those that pose the greatest public health concerns have received the most attention. In a recent worldwide survey on mycotoxin presence in cereals, 100% of analyzed samples had 10 or more mycotoxins, of which 98% were infected by *Fusarium* genera [1,2]. Fumonisins, identified in 1988 [3], are mycotoxins that are mainly produced by *Fusarium* spp., namely *F. verticilloides* and *F. proliferatum*. Among the identified four groups (A, B, C, and P) of fumonisins, the B group (FBs: FB_1, FB_2, FB_3, FB_4, and FB_5^c) is the most studied due to its prevalence and health implications [4]. FBs have been detected in numerous cereal crops, although

maize is regarded to be the most prevalent type for fumonisins [5]. Hence, FBs may pose a worldwide hazard to humans and animals through accessing their food and feed chains.

The adverse effects of FBs on animals have been documented in numerous species, including horses, rats, mice, pigs, rabbits, cattle, sheep, chickens, ducks, and primates [6]. Commonly, despite FBs' low absorption rate [7], pigs are majorly exposed to FBs through the diet (composed of contaminated cereals, especially maize). Under FBs exposure, doses above and below EU established limits for pigs [8], histopathological abnormalities in the heart, lung, liver, kidney, and spleen have been recorded, whilst pulmonary edema (PPE) and cardiac dysfunction are the most frequent clinical signs in pigs [9]. Furthermore, FBs have been shown to disrupt the porcine intestinal barriers [10] and the membranes of erythrocytes by altering their lipid composition and Na^+/K^+ ATPase activity [11].

The structure of fumonisins is the driving force underlying the aforementioned adverse consequences. Fumonisins are similar to sphinganine (Sa) and thus disrupt sphingolipid metabolism, being ceramide synthase (CerS) inhibitors. It has been reported that FBs alter the ratio between sphingoid bases in a dose-dependent manner, resulting in a high sphinganine/sphingosine ratio (Sa/So) that modifies cell signaling, such as growth and differentiation [12]. In urine and serum, alteration in the Sa/So ratio has been established as a reference biomarker for FBs exposure, as has the sphinganine-1-phosphate (Sa-1-P) level, which is also an efficient biomarker. Other molecular modes of action, including oxidative stress, activation of endoplasmic reticulum stress, modulation of autophagy, and the alteration of DNA methylation, can be involved in the FBs' toxic effect on tissues and/or cell lines [13].

The literature indicates that FBs are harmful substances to the liver and lungs in pigs and mice [9,14]. However, the lungs and liver are the primary target organs for FBs in swine. FBs have been shown to trigger tumor necrosis factor-α expression in lungs of mice [14], to interfere with activities of specific CerS forms in porcine liver [15], and to quantitatively and qualitatively alter the porcine-hepatic and lung membrane lipids [15–17]. Thus, it is important to highlight that the available literature lacks data on the effects of FBs on the various bilayer-glycerophospholipid fractions and does not account for dose response influence. On the other side, few in vivo and in vitro (normal or carcinogenic lines) studies are available on FBs' effects on the rat hepato-membrane lipids, including data on glycerophospholipids with a major focus on phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions [18–24]. These studies on rat liver provided data on alterations in the microsomal polar lipids upon exposure to different high doses of FBs (below acute toxicity). FBs, particularly FB_1, can make the membranes highly susceptible to oxidation and promote the free radical-initiated lipid peroxidation. The higher relative oxygen diffusion–concentration products, as well as the increase of membrane permeability, may induce oxidative stress and cell damage [25].

Hence, this in vivo study mainly aimed to assess whether weaned piglets are sensitive to the oral FBs sub-acute exposure by investigating the dose-dependent effect of FBs on the cell membrane phospholipidome (namely, the sphingomyelin (SM), PC, PE, phosphatidylserine (PS), and phosphatidylinositol (PI)) from the liver and lungs. Furthermore, our study provides data on the histomorphological structure and lipid peroxidation status of the liver and lungs of weaned piglets.

2. Results

2.1. Animal Growth Performance

None of the FBs doses (15 and 30 mg/kg diet) were able to induce a remarkable modification in the animals' final body weight or gain, absolute and relative liver and lung weights, feed intake, or feed conversion efficiency (Table 1).

2.2. Sphingomyelin Fatty Acid Profile

Fatty acid profile of the **liver** sphingomyelin (SM) FAs is shown in Table 2. Among the detected saturated FAs, C12:0 (lauric acid), C20:0 (arachidic acid), and C22:0 (behenic

acid) proportions responded to the 30 mg FBs/kg diet exposure, leading to a proportional depletion in arachidic acid and an increase in lauric and behenic acids, relative to the control. In the calculated unsaturation index (UI) and average chain length (ACL), we found no significant difference between the treatments.

Table 1. The growth performance (in g), absolute and relative organ weights (g and %, respectively), and feed conversion efficiency of piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Parameter	Control	15 mg FBs	30 mg FBs
Initial body weight	12,980 ± 1720	13,800 ± 1200	13,800 ± 1080
Final body weight	21,467 ± 1735	23,067 ± 1454	23,367 ± 629
Body weight gain	8483 ± 2406	9267 ± 2240	9567 ± 1181
Cumulative feed intake	19,759 ± 2102	20,450 ± 1352	20,382 ± 1353
Feed conversion efficiency	2069 ± 299	2230 ± 246	2455 ± 389
Absolute liver weight	527.4 ± 42.1	587.2 ± 56.9	563.0 ± 69.2
Absolute lung weight	227.2 ± 45.0	237.1 ± 43.0	253.0 ± 70.0
Relative liver weight (%)	2.48 ± 0.38	2.55 ± 0.23	2.42 ± 0.45
Relative lung weight (%)	1.06 ± 0.20	1.03 ± 0.15	1.09 ± 0.30

Table 2. Fatty acid profiles of sphingomyelins from liver and lungs of the experimental piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Fatty Acid	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
C12:0	0.06 ± 0.04 ^{a}	0.09 ± 0.03 ^{ab}	0.10 ± 0.03 ^{b}	0.12 ± 0.09 ^{ab}	0.05 ± 0.05 ^{a}	0.20 ± 0.03 ^{b}
C14:0	0.69 ± 0.08	0.61 ± 0.16	0.61 ± 0.15	1.27 ± 0.34 ^{a}	1.36 ± 0.56 ^{ab}	1.96 ± 0.69 ^{b}
C16:0	34.7 ± 4.01	34.0 ± 3.87	30.7 ± 5.21	51.3 ± 2.77	47.3 ± 3.87	47.9 ± 6.09
C16:1n7	- ± -	- ± -	- ± -	0.34 ± 0.26	0.59 ± 0.32	0.60 ± 0.33
C18:0	29.1 ± 5.53	30.9 ± 2.05	26.3 ± 4.81	18.4 ± 1.73	16.1 ± 2.84	15.5 ± 2.84
C18:1n9c	5.27 ± 1.91	4.43 ± 1.71	5.5 ± 2.46	2.00 ± 1.29	2.59 ± 1.16	3.26 ± 1.45
C18:1n7	0.74 ± 0.35	0.57 ± 0.21	0.84 ± 0.34	0.41 ± 0.28	0.52 ± 0.28	0.63 ± 0.33
C18:2n6	3.26 ± 1.58	2.92 ± 1.22	3.95 ± 2.27	0.99 ± 0.71	1.26 ± 0.70	1.55 ± 0.87
C20:0	1.53 ± 0.25 ^{b}	1.46 ± 0.27 ^{b}	1.09 ± 0.36 ^{a}	1.91 ± 0.24	2.20 ± 0.54	1.90 ± 0.37
C20:1n9	- ± -	- ± -	- ± -	0.23 ± 0.05	0.20 ± 0.02	0.20 ± 0.05
C20:4n6	0.91 ± 0.60	1.36 ± 0.66	1.31 ± 0.54	0.76 ± 0.65	0.86 ± 0.44	1.12 ± 0.64
C22:0	1.60 ± 0.23 ^{a}	1.85 ± 0.27 ^{ab}	2.20 ± 0.58 ^{b}	2.95 ± 0.55	3.74 ± 1.08	3.38 ± 0.61
C22:1n9	9.49 ± 3.13	10.8 ± 3.76	11.4 ± 4.37	5.62 ± 1.55 ^{a}	6.72 ± 1.1 ^{ab}	8.97 ± 2.71 ^{b}
C23:0	1.42 ± 0.85	1.44 ± 0.36	1.73 ± 1.11	0.57 ± 0.07	0.73 ± 0.06	0.67 ± 0.24
C24:0	5.10 ± 3.58	4.95 ± 1.14	6.26 ± 2.77	4.57 ± 0.86 ^{b}	5.07 ± 1.07 ^{b}	3.28 ± 0.58 ^{a}
C24:1n9	3.77 ± 2.85	2.81 ± 0.95	4.71 ± 1.81	8.80 ± 2.01	10.8 ± 2.35	8.83 ± 2.00
saturation	72.8 ± 10.98	75.3 ± 4.90	68.6 ± 7.65	81.1 ± 2.75 ^{b}	76.6 ± 1.24 ^{a}	74.8 ± 3.39 ^{a}
unsaturation	23.4 ± 3.65	22.0 ± 3.74	25.7 ± 6.54	18.9 ± 2.75 ^{a}	23.4 ± 1.24 ^{b}	25.2 ± 3.39 ^{b}
monounsaturation	19.3 ± 3.81	17.7 ± 4.42	20.5 ± 7.81	17.3 ± 2.36 ^{a}	21.3 ± 1.51 ^{b}	22.5 ± 2.89 ^{b}
polyunsaturation	4.17 ± 1.77	4.28 ± 1.65	5.27 ± 2.79	1.62 ± 1.32	2.12 ± 1.13	2.67 ± 1.42
n-6	4.17 ± 1.77	4.28 ± 1.65	5.27 ± 2.79	1.62 ± 1.32	2.12 ± 1.13	2.67 ± 1.42
odd chain FA	1.42 ± 0.85	1.44 ± 0.36	1.73 ± 1.11	0.57 ± 0.07	0.73 ± 0.06	0.67 ± 0.24
unsaturation index	29.4 ± 4.5	29.0 ± 3.84	33.6 ± 6.65	21.8 ± 4.80 ^{a}	27.3 ± 2.50 ^{ab}	30.1 ± 5.54 ^{b}
average chain length	17.6 ± 1.59	17.9 ± 1.18	17.6 ± 1.52	18.1 ± 0.29	18.5 ± 0.34	18.3 ± 0.41

n-6, omega-6; FA, fatty acid; -, fatty acid below the detection limit or not possible to calculate due to a fatty acid limitation in samples; $^{a, b}$ values with different letters refer to a significant difference among the treatments ($p < 0.05$).

In the **lung** sphingomyelins (Table 2), in comparison to control, the highest dose of FBs increased the proportions of C14:0 (myristic acid) and C22:1n9 (erucic acid). In contrast, the C24:0 (lignoceric acid) proportion decreased in piglets fed 30 mg FBs/kg diet. For

the calculated indices, totals of unsaturation (UFA) and monounsaturation (MUFA) were decreased in animals fed the highest dose of FBs, as well as the UI.

Visualizations of the most pronounced FAs responsible for variations between phospholipid FA profiles are presented in Figures 1–3, as well as Figure 4, which expresses the proportional presence of each FA in the various lipid fractions, based on color intensity.

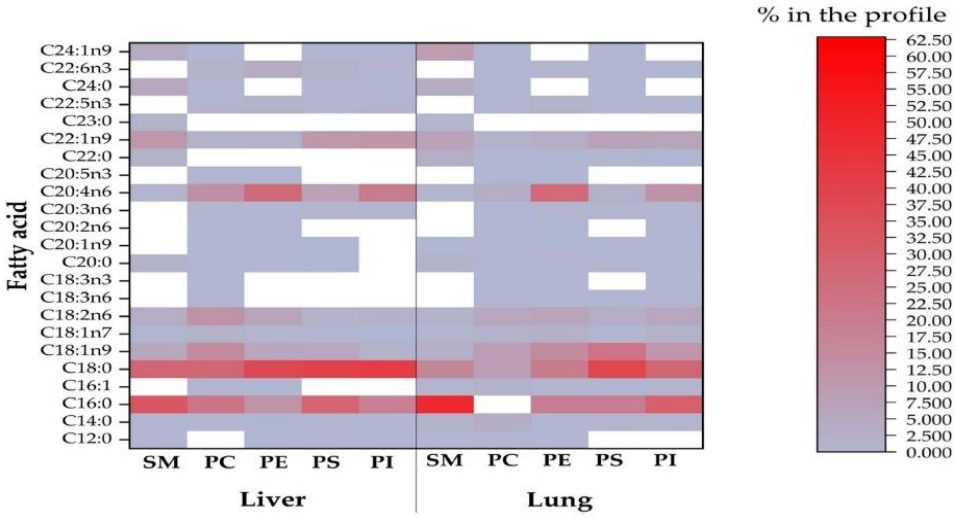

Figure 1. The compositional data of the FAs of all phospholipids from investigated liver and lungs (SM = sphingomyelin; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PS = phosphatidylserine; PI= phosphatidylinositol). The FA proportion increases with color intensity, whereas the white color represents FA methyl esters below the detection limit of the GC.

2.3. Phosphatidylcholine Fatty Acid Profile

Table 3 presents the FA profiles of phosphatidylcholine (PC). In the **liver**, all animals subjected to FBs exhibited a proportional increase in myristic acid. At the highest FBs exposure, C18:0 (stearic acid), C18:2n6 (linoleic acid, LA), C20:4n6 (arachidonic acid, AA), total polyunsaturation (PUFA) and overall omega-6 (n-6) FAs proportions decreased. Contrastingly, within the same group, proportions of C18:1n9 (oleic acid), C16:1n7 (palmitoleic acid), and total MUFA levels increased. In piglets fed 15 mg FBs/kg diet, an elevated proportion of C18:3n3 (α-linolenic, ALA) was detected, but lower proportions were detected in C22:5n3 (docosapentaenoic acid, DPA) and total n-3 FAs. None of the other calculated indices were different from the control.

In the **lung** PCs, FBs' exposures altered the FAs and their derived indices (Table 3). The proportions of C20:2n6 (eicosadienoic acid), DPA, C22:6n3 (docosahexaenoic acid, DHA), and the n-3 FAs total decreased in all intoxicated groups. In comparison with the control, proportions of lauric, myristic oleic and C22:1n9 (erucic acid) acids, total MUFA, and the ratio of n-6 to omega-3 FAs (n-6:n-3) were increased in lungs at 30 mg FBs/kg diet, but stearic acid and C18:1n7 (vaccenic acid) proportions decreased. Total saturation (SAT) decreased in the 15 mg FBs/kg group compared to the control and 30 mg FBs-setting, while the 15 mg/kg diet decreased proportions of C18:3n6 (γ-linolenic), C20:3n6 (dihomo-γ-linolenic acid), AA, total n-6 FAs, and UI, compared to control.

When linear dose-response was examined in the **liver PC** (Table 4), the marked increases of palmitoleic acid, oleic acid, and total MUFA were responsive with $R^2 > 0.6$. Linear fittings with reliable R^2 were attained for DHA, a total of n-3 FAs, and the ratio of n-6 to n-3 in the lung (Table 4).

Table 3. Fatty acid profile of phosphatidylcholines from liver and lungs of the experimental piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Fatty Acid	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
C12:0	0.01 ± -	- ± -	- ± -	0.02 ± 0.01 ^{ab}	0.01 ± 0.00 ^{a}	0.03 ± 0.01 ^{b}
C14:0	0.21 ± 0.02 ^{a}	0.30 ± 0.10 ^{b}	0.37 ± 0.05 ^{b}	2.99 ± 0.68 ^{a}	3.13 ± 0.41 ^{a}	4.08 ± 0.65 ^{b}
C16:0	22.0 ± 0.83	21.8 ± 1.99	23.9 ± 2.68	61.9 ± 1.28 ^{a}	66.1 ± 2.93 ^{b}	61.1 ± 1.58 ^{a}
C16:1n7	0.55 ± 0.06 ^{a}	0.64 ± 0.06 ^{b}	0.79 ± 0.08 ^{c}	1.98 ± 0.42	1.67 ± 0.59	2.27 ± 0.64
C18:0	28.1 ± 1.84 ^{b}	28.6 ± 1.51 ^{b}	26.2 ± 1.67 ^{a}	9.42 ± 0.88 ^{b}	9.42 ± 1.34 ^{b}	7.35 ± 1.12 ^{a}
C18:1n9	12.4 ± 1.89 ^{a}	14.1 ± 1.42 ^{a}	17.1 ± 1.12 ^{b}	8.68 ± 0.76 ^{a}	7.54 ± 1.26 ^{a}	9.99 ± 0.68 ^{b}
C18:1n7	1.96 ± 0.21	1.90 ± 0.30	1.95 ± 0.13	2.31 ± 0.21 ^{b}	1.75 ± 0.37 ^{a}	1.93 ± 0.27 ^{a}
C18:2n6	13.8 ± 0.96 ^{b}	14.0 ± 1.36 ^{b}	12.0 ± 1.04 ^{a}	5.60 ± 0.31 ^{ab}	4.73 ± 1.01 ^{a}	6.21 ± 1.21 ^{b}
C18:3n6	0.17 ± 0.04	0.19 ± 0.03	0.17 ± 0.02	0.13 ± 0.04 ^{b}	0.07 ± 0.02 ^{a}	0.09 ± 0.03 ^{ab}
C18:3n3	0.14 ± 0.03 ^{a}	0.17 ± 0.02 ^{b}	0.14 ± 0.02 ^{a}	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.02
C20:0	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.09 ± 0.03	0.10 ± 0.04	0.09 ± 0.01
C20:1n9	0.11 ± 0.02	0.12 ± 0.03	0.11 ± 0.03	0.15 ± 0.10	0.12 ± 0.03	0.13 ± 0.05
C20:2n6	0.18 ± 0.06	0.17 ± 0.05	0.13 ± 0.02	0.19 ± 0.05 ^{a}	0.12 ± 0.05 ^{b}	0.13 ± 0.02 ^{b}
C20:3n6	0.46 ± 0.12	0.49 ± 0.04	0.47 ± 0.05	0.44 ± 0.04 ^{b}	0.34 ± 0.08 ^{a}	0.44 ± 0.08 ^{b}
C20:4n6	15.2 ± 2.20 ^{b}	13.7 ± 1.35 ^{ab}	12.7 ± 2.69 ^{a}	3.95 ± 0.36 ^{b}	2.97 ± 0.63 ^{a}	3.49 ± 0.58 ^{ab}
C20:5n3	0.26 ± 0.09	0.29 ± 0.11	0.22 ± 0.04	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
C22:0	- ± -	- ± -	- ± -	0.07 ± 0.02	0.10 ± 0.04	0.08 ± 0.02
C22:1n9	1.19 ± 0.47	1.25 ± 0.67	1.15 ± 0.41	1.49 ± 0.17 ^{a}	1.23 ± 0.37 ^{a}	2.07 ± 0.41 ^{b}
C22:5n3	1.22 ± 0.23 ^{b}	0.91 ± 0.13 ^{a}	0.96 ± 0.24 ^{ab}	0.13 ± 0.02 ^{b}	0.10 ± 0.03 ^{a}	0.10 ± 0.02 ^{a}
C24:0	0.07 ± 0.03	0.05 ± 0.04	0.14 ± 0.06	0.08 ± 0.02 ^{ab}	0.14 ± 0.08 ^{b}	0.06 ± 0.02 ^{a}
C22:6n3	1.82 ± 0.60	1.20 ± 0.24	1.42 ± 0.69	0.16 ± 0.03 ^{b}	0.07 ± 0.04 ^{a}	0.04 ± 0.02 ^{a}
C24:1n9	- ± -	0.06 ± -	0.17 ± 0.10	0.11 ± 0.04	0.22 ± 0.12	0.22 ± 0.10
saturation	50.5 ± 2.11	50.9 ± 2.76	50.6 ± 4.25	74.6 ± 1.76 ^{a}	79.0 ± 3.77 ^{b}	72.8 ± 1.58 ^{a}
unsaturation	49.5 ± 2.11	49.0 ± 2.73	49.4 ± 4.25	25.4 ± 1.76 ^{b}	21.0 ± 3.77 ^{a}	27.2 ± 1.53 ^{b}
monounsaturation	16.2 ± 1.99 ^{a}	18.0 ± 1.83 ^{a}	21.1 ± 1.03 ^{b}	14.7 ± 1.15 ^{b}	12.5 ± 2.18 ^{a}	16.6 ± 0.57 ^{c}
polyunsaturation	33.3 ± 3.55 ^{b}	31.1 ± 2.73 ^{ab}	28.2 ± 4.23 ^{a}	10.7 ± 0.69 ^{b}	8.47 ± 1.75 ^{a}	10.6 ± 1.30 ^{b}
n-3	3.44 ± 0.75 ^{b}	2.52 ± 0.34 ^{a}	2.75 ± 0.96 ^{ab}	0.38 ± 0.04 ^{b}	0.25 ± 0.03 ^{a}	0.23 ± 0.03 ^{a}
n-6	29.9 ± 2.81 ^{b}	28.6 ± 2.58 ^{ab}	25.5 ± 3.39 ^{a}	10.3 ± 0.69 ^{b}	8.22 ± 1.74 ^{a}	10.4 ± 1.29 ^{b}
n-6:n-3	8.98 ± 1.75 ^{ab}	11.5 ± 1.49 ^{b}	9.8 ± 1.92 ^{a}	27.2 ± 3.91 ^{a}	33.29 ± 7.33 ^{a}	44.5 ± 7.01 ^{b}
unsaturation index	124 ± 12.5	115 ± 7.94	112 ± 16.9	45.6 ± 3.08 ^{b}	36.3 ± 6.71 ^{a}	45.7 ± 3.53 ^{b}
average chain length	18.0 ± 0.09	18.0 ± 0.06	17.9 ± 0.17	16.8 ± 0.05	16.7 ± 0.08	16.8 ± 0.08

n-3, omega-3; n-6, omega-6; n-6:n-3, ratio of omega-6 to omega-3; -, fatty acid below the detection limit or not possible to calculate due to a fatty acid limitation in samples; $^{a, b}$ values with different letters refer to a significant difference among the treatments ($p < 0.05$).

Table 4. Parameters of the linear dose response equations of fatty acids in the different phospholipid classes, obtained from liver and lung. Data represent only cases with R^2 above 0.6.

Liver				Lung			
Parameter	Slope	Constant	R^2	Parameter	Slope	Constant	R^2
Phosphatidylcholine				Phosphatidylcholine			
C16:1	−44.129	89.188	0.69	C22:6n3	31.164	−178.028	0.705
C18:1n9	−44.357	4.073	0.633	n-3	53.773	−133.946	0.667
monousaturation	−53.704	3.710	0.609	n-6:n-3	−20.943	1.027	0.594
				Phosphatidylserine			
				C22:0	40.488	−52.0917	0.782
				C24:0	40.566	−134.894	0.645

n-3, omega-3; n-6:n-3, omega-6 to omega-6 ratio.

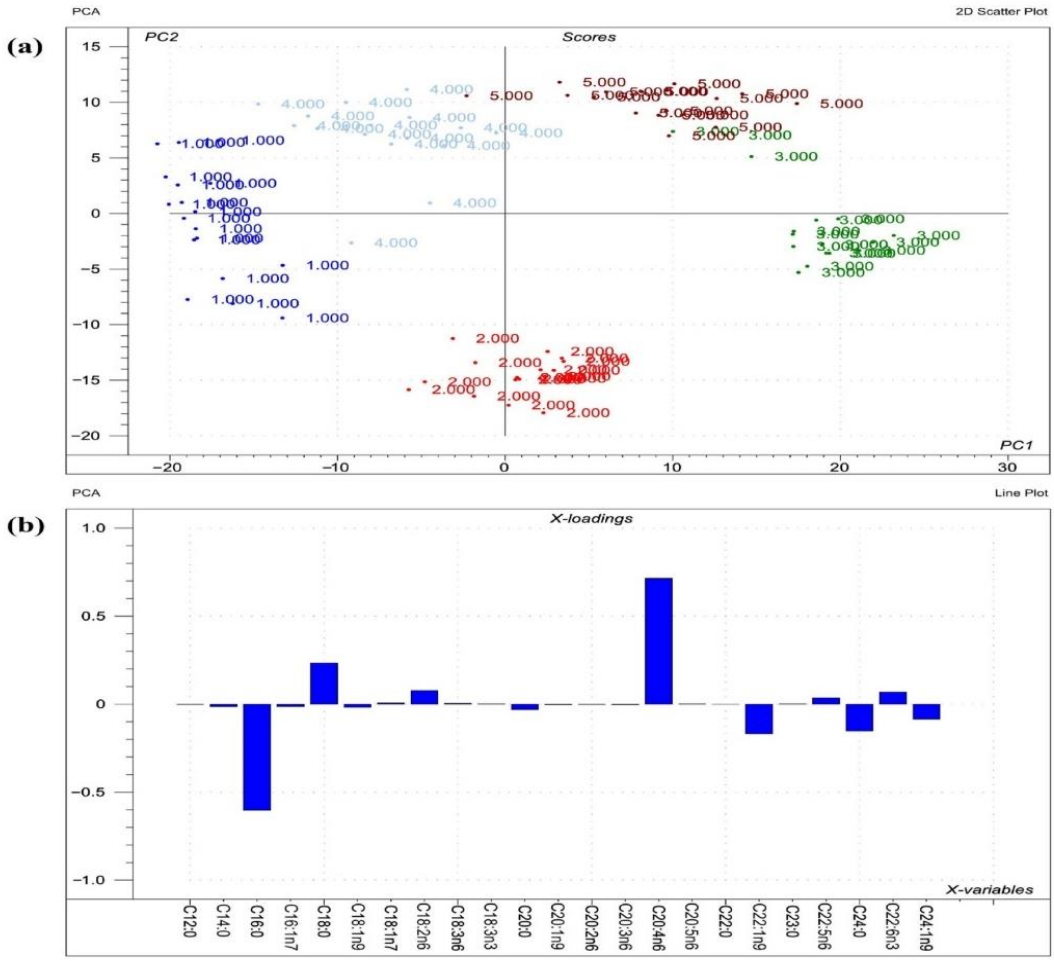

Figure 2. Results of PCA performed on the raw compositional data of the FAs of phospholipids from liver. (**a**) Score plot describes the orientation of the phospholipid classes from liver (1 = sphingomyelin; 2 = phosphatidylcholine; 3 = phosphatidylethanolamine; 4 = phosphatidylserine; 5 = phosphatidylinositol) in the plane of the first and second principal components (PC1 and PC2, respectively), where PC1 and PC2 are influenced by the multivariate data of FA of the organ phospholipids. PC1 and PC2 explain 57% and 28% of the total variance of FAs of the phospholipids, respectively. From the PCA, the polar FA pool of phospholipids from liver provided a perfect spatial separation of groups, referring to variation in their FA profiles. (**b**) Loading bar graph of the PC1 shows the contribution of the individual FAs of hepatic tissue to the newly developed latent variable; the higher the loading value, the greater is the impact of the respective FA's variance on the variance of PC1. From the loadings, the remarkable FAs that contributed to variance between organs are C20:4n6, C16:0, C18:0, C22:1n9, and C24:0.

2.4. Phosphatidylethanolamine Fatty Acid Profile

The FA profile of the **liver** phosphatidylethanolamine (PE) fraction can be seen in Table 5. None of the treatments had a marked effect on the saturated FAs or the total SAT. FBs-fed animals showed a higher proportion of palmitoleic acid, relative to control. For oleic acid, only the highest FBs-setting increased its proportion, although the total MUFA concentration was not altered. The total n-3 FAs and ACL were decreased in piglets fed 30 mg FBs/kg diet as compared to control.

Table 5. Fatty acid profile of phosphatidylethanolamines from liver and lungs of the experimental piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Fatty Acid	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
C12:0	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.07 ± 0.03 ^{ab}	0.03 ± 0.01 ^{a}	0.09 ± 0.03 ^{b}
C14:0	0.14 ± 0.03	0.14 ± 0.04	0.12 ± 0.02	0.30 ± 0.17	0.36 ± 0.24	0.40 ± 0.11
C16:0	11.9 ± 1.33	11.3 ± 1.52	11.5 ± 1.51	18.9 ± 2.85	19.3 ± 6.07	20.2 ± 3.23
C16:1n7	0.16 ± 0.03 ^{a}	0.20 ± 0.03 ^{b}	0.20 ± 0.04 ^{b}	0.41 ± 0.12 ^{a}	0.56 ± 0.10 ^{ab}	0.73 ± 0.24 ^{b}
C18:0	36.5 ± 1.24	37.2 ± 1.94	39.4 ± 5.11	21.7 ± 1.49	19.9 ± 0.79	20.7 ± 2.71
C18:1n9	5.49 ± 0.56 ^{a}	6.27 ± 1.33 ^{ab}	7.11 ± 1.08 ^{b}	14.3 ± 0.66	15.2 ± 1.01	15.3 ± 0.73
C18:1n7	1.06 ± 0.11	1.00 ± 0.23	1.01 ± 0.18	2.57 ± 0.46	2.72 ± 0.29	2.70 ± 0.20
C18:2n6	6.72 ± 0.80	7.19 ± 0.62	6.33 ± 0.83	5.47 ± 0.41 ^{a}	6.31 ± 0.49 ^{a}	7.36 ± 1.07 ^{b}
C18:3n6	- ± -	- ± -	- ± -	0.21 ± 0.17	0.19 ± 0.11	0.07 ± -
C18:3n3	- ± -	- ± -	- ± -	0.12 ± -	0.13 ± 0.06	0.12 ± 0.03
C20:0	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.04	0.16 ± 0.03	0.13 ± 0.01	0.16 ± 0.04
C20:1n9	0.15 ± 0.03	0.14 ± 0.03	0.12 ± 0.03	0.40 ± 0.04	0.39 ± 0.14	0.42 ± 0.08
C20:2n6	0.15 ± 0.04	0.11 ± 0.06	0.09 ± 0.04	0.17 ± 0.08	0.19 ± 0.13	0.16 ± 0.10
C20:3n6	0.33 ± 0.03	0.36 ± 0.07	0.33 ± 0.05	1.05 ± 0.08	1.21 ± 0.25	1.19 ± 0.19
C20:4n6	28.3 ± 1.84	27.9 ± 1.75	26.0 ± 3.04	28.3 ± 2.08	27.5 ± 3.94	24.0 ± 4.69
C20:5n3	0.26 ± 0.08 ^{ab}	0.36 ± 0.15 ^{b}	0.17 ± 0.06 ^{a}	0.23 ± 0.03	0.33 ± 0.11	0.28 ± 0.08
C22:0	- ± -	- ± -	- ± -	0.07 ± 0.01 ^{b}	0.04 ± 0.01 ^{a}	0.04 ± 0.01 ^{a}
C22:1n9	2.01 ± 0.36	2.04 ± 0.42	1.71 ± 0.47	3.06 ± 0.62 ^{a}	2.84 ± 0.42 ^{a}	4.14 ± 1.03 ^{b}
C22:5n3	2.29 ± 0.29	1.94 ± 0.15	1.90 ± 0.43	1.76 ± 0.18 ^{ab}	1.85 ± 0.41 ^{b}	1.48 ± 0.24 ^{a}
C22:6n3	4.46 ± 0.90	3.70 ± 0.88	3.65 ± 0.90	1.01 ± 0.33	1.01 ± 0.36	0.73 ± 0.34
saturation	48.6 ± 1.65	48.8 ± 2.44	51.0 ± 6.40	41.2 ± 1.98	39.7 ± 5.75	41.6 ± 5.00
unsaturation	51.4 ± 1.65	51.2 ± 2.45	48.5 ± 6.02	58.8 ± 1.98	60.3 ± 5.75	58.4 ± 5.00
monounsaturation	8.87 ± 0.75	9.65 ± 1.86	9.91 ± 1.23	20.69 ± 0.51 ^{a}	21.7 ± 1.20 ^{ab}	23.24 ± 1.76 ^{b}
polyunsaturation	42.5 ± 2.07	41.6 ± 2.44	38.5 ± 4.89	38.1 ± 1.94	38.5 ± 4.78	35.2 ± 4.07
n-3	7.01 ± 0.57 ^{b}	6.00 ± 0.83 ^{ab}	5.73 ± 1.03 ^{a}	3.02 ± 0.25	3.23 ± 0.51	2.53 ± 0.33
n-6	35.5 ± 1.63	35.6 ± 1.76	32.8 ± 4.02	35.1 ± 1.89	35.3 ± 4.41	32.7 ± 3.85
n-6:n-3	5.08 ± 0.30 ^{a}	6.01 ± 0.67 ^{b}	5.69 ± 0.56 ^{ab}	11.7 ± 1.09 ^{ab}	11.0 ± 1.25 ^{a}	13.0 ± 1.36 ^{b}
unsaturation index	176 ± 9.69	171 ± 10.1	160 ± 21.1	156 ± 8.56	156 ± 17.9	144 ± 18.1
average chain length	18.8 ± 0.17 ^{b}	18.7 ± 0.18 ^{ab}	18.4 ± 0.32 ^{a}	18.4 ± 0.11	18.4 ± 0.23	18.3 ± 0.19

n-3, omega-3; n-6, omega-6; n-6:n-3, ratio of omega-6 to omega-3, fatty acid; -, fatty acid below the detection limit or not possible to calculate due to a fatty acid limitation in samples; $^{a, b}$ values with different letters refer to a significant difference among the treatments ($p < 0.05$).

In the **lung** PE (Table 5), both applied FBs doses increased the behenic acid proportion; nevertheless, the overall SAT was not altered. The 30 mg FBs/kg diet increased proportions of palmitoleic acid, LA, erucic acid, and total MUFA as compared to control. We found no significant difference in the other calculated indices (totals of SAT, UFA, n-3, and n-6, as well as UI and ACL).

2.5. Phosphatidylserine Fatty Acid Profile

The phosphatidylserine (PS) FA composition results from the **liver** are shown in Table 6. Fatty acids where FBs-contaminated diets caused intergroup differences were lauric acid (increased), C24:1n9 (nervonic acid, increased), C20:1n9 (eicosenoic, decreased), and DPA (decreased). The n-6:n-3 ratio increased in the group with the highest FBs exposure, while none of the other presented calculated indices (total MUFA, total n-3 FAs, UI, and ACL) were altered.

The FA profile of **lung** PS (Table 6) showed proportional depletions in stearic, arachidic, behenic and lignoceric acids, as well as in DHA and total SAT, a result of FBs' exposures (15 and 30 mg/kg diet). In contrast, both FBs' treatments displayed high proportions of LA, dihomo-γ-linolenic, and arachidonic acids, and consequently for the total n-6 FAs and total PUFA. The total MUFA was not altered, although the proportion of erucic acid increased in the 30 mg/kg diet group.

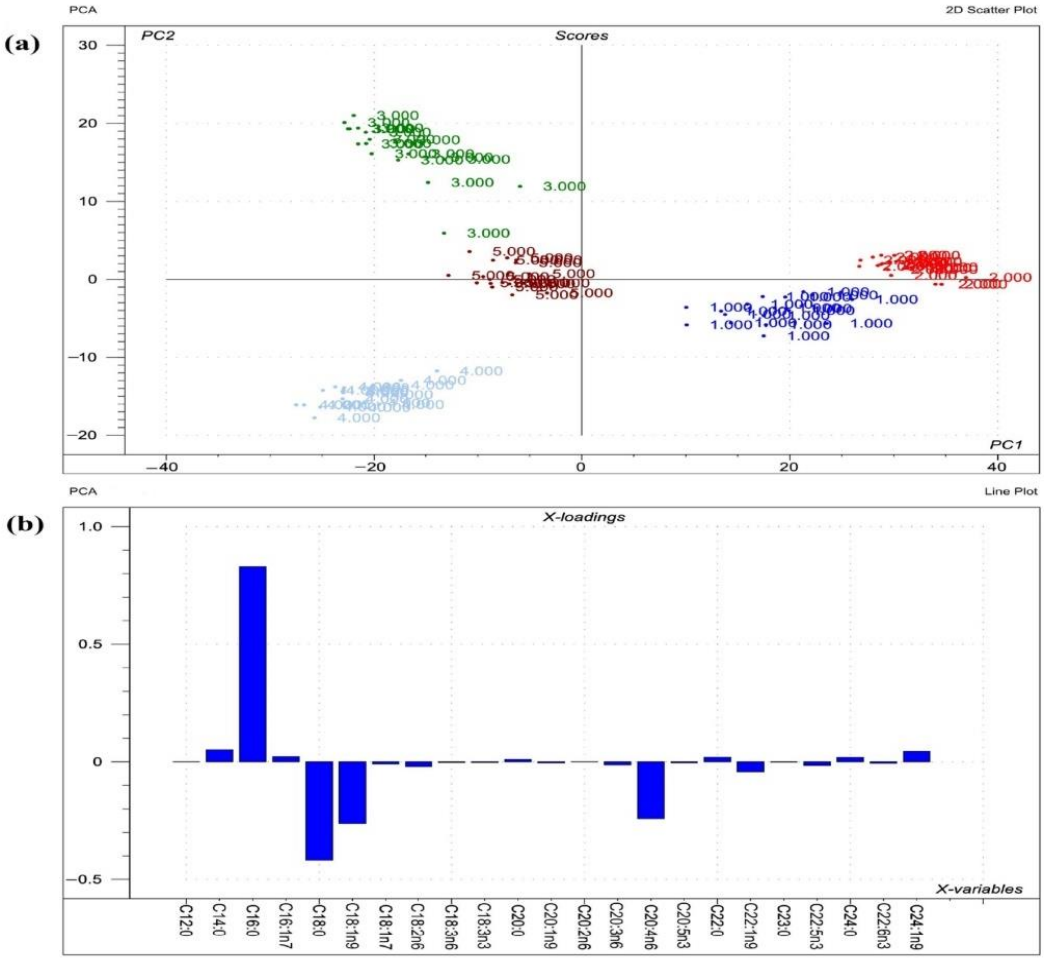

Figure 3. Results of PCA performed on the raw compositional data of the FAs of phospholipids from lungs. (**a**) Score plot depicts the orientation of the phospholipid classes from lung (1 = sphingomyelin; 2 = phosphatidylcholine; 3 = phosphatidylethanolamine; 4 = phosphatidylserine; 5 = phosphatidylinositol) in the plane of the first and second principal components (PC1 and PC2, respectively), where PC1 and PC2 are influenced by the multivariate data of FA of the organ phospholipids. PC1 and PC2 explain 73% and 18% of the total variance of FAs of the phospholipids, respectively. From the PCA, the polar FA pool of phospholipids from lung provided a perfect spatial separation of groups, referring to variation in their FA profiles; (**b**) Loading bar graph of the PC1 shows the contribution of the individual FAs of lung tissue to the newly developed latent variable: the higher the loading value, the greater is the impact of the respective FA's variance on the PC1 variance. From the loadings, the remarkable FAs that contributed to variance between organs are C16:0, C18:0, C18:1n9, and C20:4n6.

As shown in Table 4, the FAs in the lungs with reliable linear fittings were behenic and lignoceric acids ($R^2 > 0.6$).

2.6. Phosphatidylinositol Fatty Acid Profile

For the liver phosphatidylinositol (PI) FA profile (Table 7), the comparison between control and FBs-treated groups revealed that the DPA, DHA, nervonic acid, and total n-3 FAs proportions decreased, and the n-6:n-3 ratio increased in all FBs-intoxicated piglets. The 15 mg FB/kg diet-associated differences were proven in lauric and stearic acids, and total SAT; the proportions were higher, while, within the same group, the proportions of erucic and total MUFA decreased.

Table 6. Fatty acid profiles of phosphatidylserines from liver and lungs of the experimental piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Fatty Acid	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
C12:0	0.02 ± 0.01 ^{a}	0.08 ± 0.02 ^{b}	0.09 ± 0.02 ^{b}	- ± -	- ± -	- ± -
C14:0	0.56 ± 0.13	0.66 ± 0.11	0.66 ± 0.09	0.39 ± 0.11	0.45 ± 0.18	0.58 ± 0.13
C16:0	26.8 ± 3.02	28.3 ± 4.08	29.2 ± 3.29	19.4 ± 2.49	18.8 ± 2.65	20.7 ± 3.29
C16:1n7	- ± -	- ± -	- ± -	0.18 ± 0.07	0.25 ± 0.12	0.32 ± 0.20
C18:0	41.8 ± 3.98	40.0 ± 2.54	38.1 ± 2.56	41.7 ± 1.98 ^{b}	38.5 ± 2.05 ^{a}	37.2 ± 1.83 ^{a}
C18:1n9	5.81 ± 1.39	4.41 ± 1.34	6.58 ± 2.25	23.2 ± 2.11 ^{ab}	24.6 ± 1.69 ^{b}	21.9 ± 1.62 ^{a}
C18:1n7	0.43 ± 0.11	0.46 ± 0.08	0.59 ± 0.16	1.15 ± 0.09	1.29 ± 0.11	1.18 ± 0.13
C18:2n6	1.73 ± 0.34	1.74 ± 0.28	2.08 ± 0.34	2.65 ± 0.25 ^{a}	3.57 ± 0.29 ^{b}	3.49 ± 0.78 ^{b}
C18:3n6	- ± -	- ± -	- ± -	0.16 ± 0.05 ^{a}	0.27 ± 0.10 ^{b}	0.10 ± 0.02 ^{a}
C20:0	0.32 ± 0.02	0.38 ± 0.09	0.41 ± 0.08	0.53 ± 0.06 ^{b}	0.44 ± 0.04 ^{a}	0.41 ± 0.06 ^{a}
C20:1n9	0.36 ± 0.05 ^{b}	0.23 ± 0.01 ^{a}	0.27 ± 0.05 ^{a}	0.37 ± 0.10	0.41 ± 0.03	0.40 ± 0.06
C20:3n6	0.43 ± 0.12	0.48 ± 0.10	0.57 ± 0.14	0.89 ± 0.10 ^{a}	1.21 ± 0.24 ^{b}	1.18 ± 0.17 ^{b}
C20:4n6	8.39 ± 1.82	6.96 ± 2.12	7.50 ± 1.23	2.24 ± 0.34 ^{a}	2.74 ± 0.40 ^{b}	2.69 ± 0.20 ^{b}
C22:0	- ± -	- ± -	- ± -	0.70 ± 0.17 ^{b}	0.52 ± 0.06 ^{a}	0.25 ± 0.04 ^{a}
C22:1n9	9.30 ± 1.54	11.7 ± 1.41	10.7 ± 3.00	5.46 ± 0.78 ^{a}	6.05 ± 1.12 ^{a}	8.79 ± 1.68 ^{b}
C22:5n3	0.84 ± 0.11 ^{b}	0.75 ± 0.16 ^{ab}	0.66 ± 0.11 ^{a}	0.35 ± 0.05	0.40 ± 0.04	0.35 ± 0.06
C24:0	0.13 ± 0.04	0.18 ± 0.07	- ± -	0.27 ± 0.04 ^{b}	0.16 ± 0.05 ^{a}	0.13 ± 0.04 ^{a}
C22:6n3	2.04 ± 0.71	1.58 ± 0.83	1.22 ± 0.75	0.17 ± 0.04 ^{b}	0.12 ± 0.01 ^{a}	0.12 ± 0.03 ^{a}
C24:1n9	0.08 ± 0.08 ^{a}	0.38 ± 0.12 ^{b}	0.28 ± 0.10 ^{b}	0.22 ± 0.05	0.27 ± 0.12	0.24 ± 0.04
saturation	69.7 ± 5.21	69.4 ± 5.64	68.5 ± 4.10	63.0 ± 2.59 ^{b}	58.8 ± 0.75 ^{a}	59.1 ± 2.32 ^{a}
unsaturation	27.5 ± 4.05	24.6 ± 6.17	28.2 ± 3.96	37.0 ± 2.59 ^{a}	41.2 ± 0.74 ^{b}	40.7 ± 2.56 ^{b}
monounsaturation	15.8 ± 2.30	14.8 ± 6.54	18.3 ± 2.61	30.6 ± 2.62	32.8 ± 0.90	32.8 ± 2.75
polyunsaturation	13.1 ± 2.29	11.5 ± 2.97	11.6 ± 2.34	6.45 ± 0.51 ^{a}	8.32 ± 0.88 ^{b}	7.86 ± 1.13 ^{b}
n-3	2.87 ± 0.72	2.33 ± 0.97	1.88 ± 0.84	0.53 ± 0.06	0.53 ± 0.04	0.46 ± 0.05
n-6	10.2 ± 1.65	9.18 ± 2.08	9.74 ± 1.59	5.92 ± 0.54 ^{a}	7.80 ± 0.86 ^{b}	7.39 ± 1.10 ^{b}
n-6:n-3	3.63 ± 0.52 ^{a}	4.16 ± 0.78 ^{ab}	5.98 ± 2.46 ^{b}	11.5 ± 2.02 ^{a}	14.9 ± 1.42 ^{b}	16.0 ± 1.96 ^{b}
unsaturation index	69.8 ± 11.7	60.8 ± 13.3	63.9 ± 11.6	50.7 ± 3.04	58.1 ± 2.36	47.4 ± 23.4
average chain length	17.9 ± 0.57	17.3 ± 1.86	17.8 ± 0.74	18.0 ± 0.07	18.0 ± 0.07	24.3 ± 15.3

n-3, omega-3; n-6, omega-6; n-6:n-3, ratio of omega-6 to omega-3, fatty acid; -, fatty acid below the detection limit or not possible to calculate due to a fatty acid limitation in samples; $^{a, b}$ values with different letters refer to a significant difference among the treatments ($p < 0.05$).

In the lung-PI (Table 7), administration of 30 FBs/kg diet increased the proportions of erucic acid and total MUFA, compared to control. In contrast, proportions of stearic acid, DHA, and total SAT decreased in piglets fed on the highest dose of FBs.

2.7. Antioxidants and Lipid Peroxidation

Table 8 shows the antioxidant and lipid peroxidation status of the liver and lungs. Regardless of the FBs dose applied and the investigated organ, no intergroup difference was detected in any of the investigated antioxidant parameters (reduced glutathione (GSH) content and glutathione peroxidase (GPx) activity) or in the end product of lipid peroxidation (thiobarbituric acid reactive substances (TBARS) expressed as malondialdehyde (MDA)).

2.8. Pathological Assessment

During the study, mortality did not occur. Based on necropsy data, only one animal in the 30 mg FBs/kg group showed pale liver. In the lungs, slight vasodilatation and hyperaemia in the mesenterium were observed in some piglets from control and FBs-treated animals. The histological assessment, expressed as a total lesion score, of the liver and lungs is shown in Figure 5. In liver, the total-lesion score responded positively to the applied FBs level, expressing low cellular glycogen, hepatocyte necrosis, as well as swelling and proliferation of the mononuclear phagocyte system (MPS). Furthermore, the liver provided a dose-dependent response, in which 15 and 30 mg FBs/kg diet expressed

mild and moderate intoxication, respectively. Despite the fact that lesions were found in the lungs (Figure 5a), no marked differences were found in the total lesion score among the groups (detected total scores were not above the mild toxicity level). The PPE was only recorded in a piglet fed on a 30 mg FBs/kg diet, in which the lung was expressed as heavy, swollen, pale, and doughy.

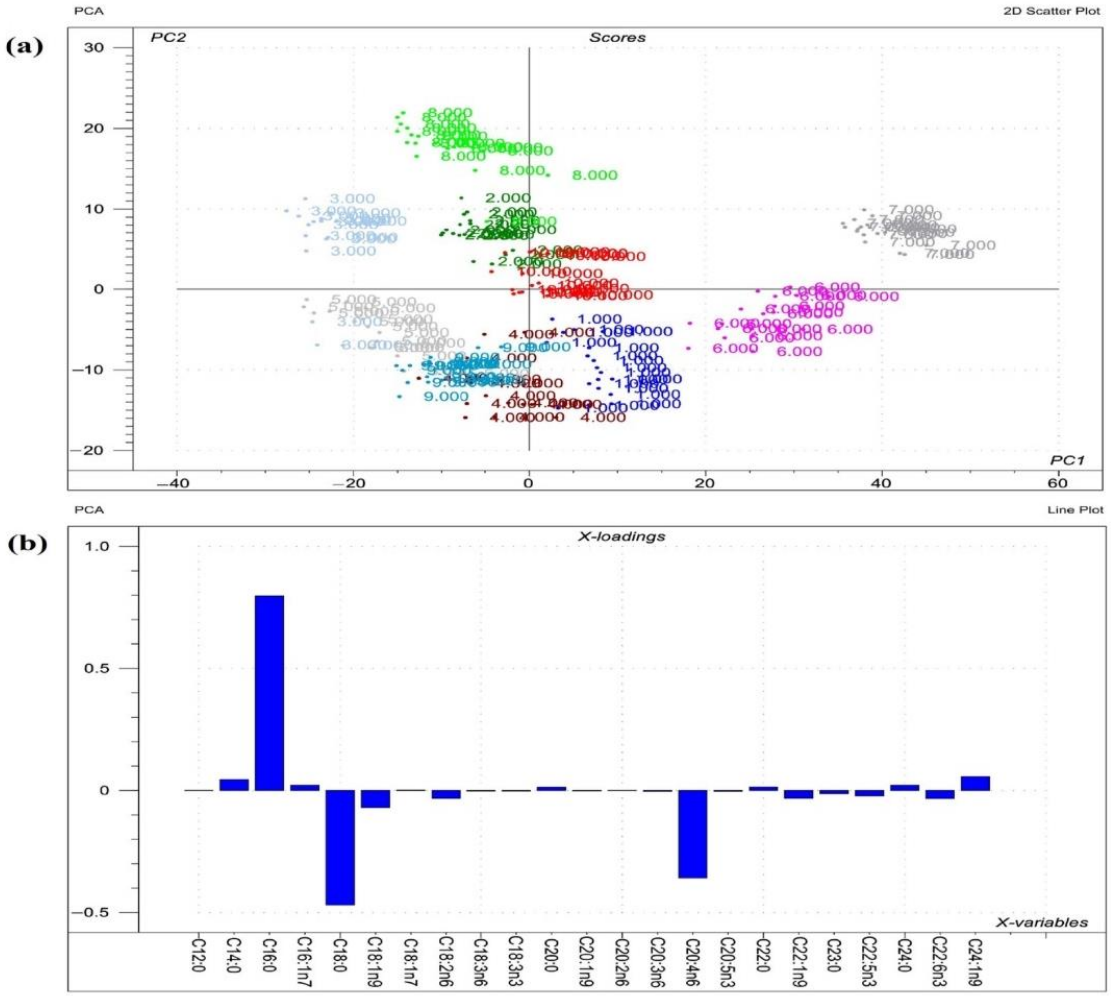

Figure 4. Results of the principal component analysis (PCA) performed on the raw compositional data of the fatty acids (FA)s) of phospholipids from organs. (**a**) Score plot depicts the orientation of the phospholipid classes from various organs (1 = liver sphingomyelin; 2 = liver phosphatidylcholine; 3 = liver phosphatidylethanolamine; 4 = liver phosphatidylserine; 5 = liver phosphatidylinositol; 6 = lung sphingomyelin; 7 = lung phosphatidylcholine; 8 = lung phosphatidylethanolamine; 9 = lung phosphatidylserine; 10 = lung phosphatidylinositol) in the plane of the first and second principal components (PC1 and PC2, respectively), where PC1 and PC2 are influenced by the multivariate data of FA of the organ phospholipids. PC1 and PC2 explain 64% and 17% of the total variance of the membrane FAs of the phospholipids, respectively. From the PCA, the polar FA pool of phospholipids from liver and lungs provided a perfect spatial separation of groups, referring to variation in their FA profiles; (**b**) Loading bar graph of the PC1 shows the contribution of the individual FAs from tissues to the newly developed latent variable; the higher the loading value, the greater is the impact of the respective FA's variance on the variance of PC1. From the loadings, the remarkable FAs that contributed to the variance between organs are C16:0, C18:0, C20:4n6, C18:1n9, and C24:1n9.

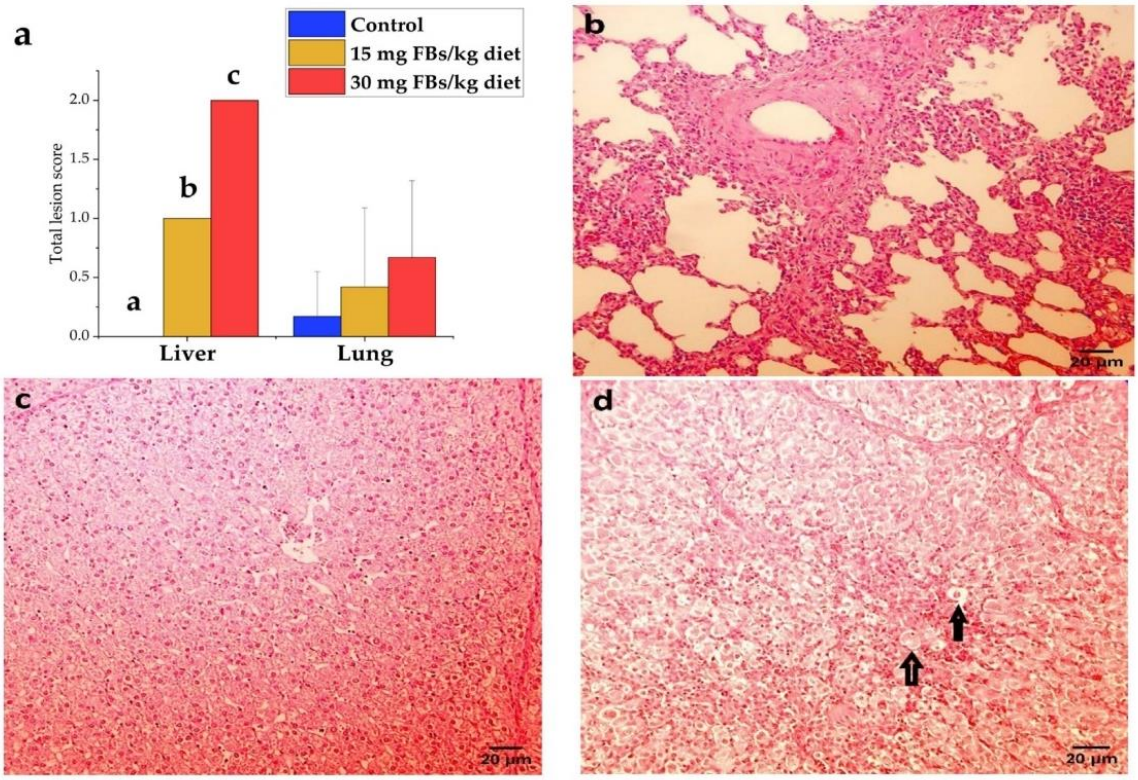

Figure 5. (**a**) Total lesion scores of liver and lungs recorded in experimental piglets (n = 6 animals/treatment, whereas columns represent means, and bars represent the standard deviation. The letters a, b, c above the bars indicate significant differences). (**b**) Lung of a healthy pig with mild lymphocytic and histiocytic infiltration in connective tissue (hematoxylin–eosin, 200 ×, scale bar = 20 µm). (**c**) A healthy piglet liver from control, where the cytoplasm of hepatocytes is finely granulated due to high glycogen content, resulting in an intense stain (hematoxylin–eosin, 200 ×, scale bar = 20 µm), although a PAS stain would be necessary to confirm our observation. (**d**) The liver of a highly FBs intoxicated piglet (30 mg/kg), where the glycogen content decreased in the hepatocytes' cytoplasm and a high frequency of necrotic (rounded, faintly stained) hepatocytes (↑) detected (hematoxylin–eosin, 200 ×, scale bar = 20 µm).

Table 7. Fatty acid profiles of phosphatidylinositols from liver and lungs of the experimental piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Fatty Acid	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
C12:0	0.08 ± 0.01 ^{a}	0.12 ± 0.02 ^{b}	0.09 ± 0.01 ^{ab}	- ± -	- ± -	- ± -
C14:0	0.38 ± 0.13	0.47 ± 0.11	0.36 ± 0.09	0.47 ± 0.06 ^{b}	0.38 ± 0.05 ^{a}	0.51 ± 0.05 ^{b}
C16:0	17.8 ± 1.96	21.4 ± 4.82	16.7 ± 4.36	31.0 ± 1.57	30.5 ± 1.94	29.7 ± 3.49
C16:1n7	- ± -	- ± -	- ± -	0.94 ± 0.44	1.23 ± 0.55	0.87 ± 0.15
C18:0	39.9 ± 2.54 ^{a}	44.7 ± 1.48 ^{b}	42.8 ± 3.34 ^{ab}	29.2 ± 2.15 ^{b}	27.2 ± 2.32 ^{ab}	26.6 ± 1.24 ^{a}
C18:1n9	1.74 ± 0.72	1.56 ± 0.35	1.88 ± 0.41	10.3 ± 1.68	12.2 ± 2.27	12.0 ± 0.91
C18:1n7	0.23 ± 0.08	0.27 ± 0.08	0.26 ± 0.03	1.81 ± 0.25	2.01 ± 0.27	1.88 ± 0.15
C18:2n6	2.24 ± 0.41	2.25 ± 0.34	1.91 ± 0.34	5.10 ± 0.97	5.75 ± 1.08	6.62 ± 2.46
C20:3n6	0.48 ± 0.05	0.51 ± 0.17	0.58 ± 0.07	0.42 ± 0.53	0.49 ± 0.29	0.04 ± -
C18:3n3	- ± -	- ± -	- ± -	0.28 ± 0.21	0.21 ± 0.11	0.14 ± 0.05
C20:0	- ± -	- ± -	- ± -	0.18 ± 0.09	0.15 ± 0.06	0.19 ± 0.02
C20:1n9	- ± -	- ± -	- ± -	0.23 ± 0.07	0.21 ± 0.05	0.22 ± 0.03

Table 7. *Cont.*

Fatty Acid	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
C20:2n6	- ± -	- ± -	- ± -	0.07 ± 0.01	0.05 ± 0.02	0.06 ± 0.04
C20:3n6	- ± -	- ± -	- ± -	0.64 ± 0.07	0.67 ± 0.13	0.66 ± 0.12
C20:4n6	19.7 ± 1.68	19.5 ± 4.83	22.4 ± 2.35	12.8 ± 1.12	13.1 ± 1.50	11.3 ± 1.73
C22:0	- ± -	- ± -	- ± -	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.05
C22:1n9	14.5 ± 5.42 ^{b}	8.01 ± 1.76 ^{a}	11.9 ± 5.67 ^{ab}	6.01 ± 1.84 ^{a}	5.39 ± 1.38 ^{a}	8.55 ± 1.22 ^{b}
C22:5n3	1.20 ± 0.30 ^{b}	0.64 ± 0.14 ^{a}	0.78 ± 0.34 ^{a}	0.41 ± 0.05	0.42 ± 0.07	0.40 ± 0.06
C24:0	0.19 ± 0.05	0.12 ± 0.02	0.11 ± 0.03	- ± -	- ± -	- ± -
C22:6n3	0.58 ± 0.14 ^{b}	0.36 ± 0.12 ^{a}	0.31 ± 0.16 ^{a}	0.19 ± 0.02 ^{b}	0.15 ± 0.04 ^{ab}	0.13 ± 0.08 ^{a}
C24:1n9	0.50 ± 0.25 ^{b}	0.17 ± 0.04 ^{a}	0.13 ± 0.04 ^{a}	- ± -	- ± -	- ± -
saturation	58.2 ± 3.91 ^{a}	66.8 ± 4.4 ^{b}	60.0 ± 7.75 ^{ab}	60.9 ± 2.25 ^{b}	58.3 ± 2.67 ^{ab}	56.9 ± 2.91 ^{a}
unsaturation	40.5 ± 4.40 ^{b}	33.1 ± 4.47 ^{a}	39.8 ± 7.59 ^{ab}	39.0 ± 2.27 ^{a}	41.7 ± 2.67 ^{ab}	42.9 ± 3.33 ^{b}
monounsaturation	16.9 ± 5.07 ^{b}	9.87 ± 1.92 ^{a}	13.9 ± 5.4 ^{ab}	19.3 ± 2.59 ^{a}	21.0 ± 2.09 ^{ab}	23.5 ± 1.43 ^{b}
polyunsaturation	23.6 ± 1.45	23.3 ± 5.48	25.9 ± 2.87	19.7 ± 0.85	20.7 ± 1.93	19.3 ± 3.41
n-3	1.67 ± 0.28 ^{b}	1.00 ± 0.22 ^{a}	0.96 ± 0.35 ^{a}	0.79 ± 0.18	0.78 ± 0.14	0.64 ± 0.09
n-6	21.9 ± 1.69	22.3 ± 5.27	24.9 ± 2.60	18.9 ± 0.75	19.9 ± 1.93	18.7 ± 3.35
n-6:n-3	13.5 ± 2.97 ^{a}	22.2 ± 1.41 ^{b}	28.6 ± 10.14 ^{b}	25.1 ± 5.61	26.1 ± 5.03	29.3 ± 4.28
unsaturation index	109 ± 3.85	99.1 ± 20.4	114 ± 15.3	75.6 ± 4.33	78.7 ± 6.48	73.7 ± 7.18
average chain length	18.5 ± 0.51	18.3 ± 0.18	18.6 ± 0.33	17.9 ± 0.11	17.9 ± 0.12	17.9 ± 0.15

n-3, omega-3; n-6, omega-6; n-6:n-3, ratio of omega-6 to omega-3, fatty acid; -, fatty acid below the detection limit or not possible to calculate due to a fatty acid limitation in samples; $^{a, b}$ values with different letters refer to a significant difference among the treatments ($p < 0.05$).

Table 8. The antioxidant enzymes and lipid peroxidation end product of liver and lungs of the experimental piglets (n = 6 animals/treatment). The results represent mean ± standard deviation (SD).

Parameter	Control	15 mg FBs	30 mg FBs	Control	15 mg FBs	30 mg FBs
	Liver			Lung		
GSH (micromol/g prot.)	7.71 ± 0.64	7.49 ± 1.14	7.67 ± 1.19	4.94 ± 0.93	4.76 ± 0.51	4.92 ± 0.93
GPx (U/g prot.)	4.15 ± 0.36	3.88 ± 0.60	4.00 ± 0.54	5.37 ± 0.51	5.53 ± 0.45	5.84 ± 0.53
MDA (nmol/g)	71.0 ± 7.82	65.9 ± 12.0	59.6 ± 9.45	41.6 ± 7.65	43.0 ± 7.09	36.6 ± 2.74

GSH, reduced glutathione; GPx, glutathione peroxidase; MDA, malondialdehyde.

3. Discussion

3.1. Animal Performance

Animal growth performance is generally a basic but very complex physiological trait that is influenced by the most adverse stimuli. In this study, higher FBs' doses (15 and 30 mg/kg diet) than the maximum proposed limit value for pigs (5 mg/kg diet) [26] were administered.

Despite the fact that FB_1 has been reported to impede pig growth [27] and induce anorexia [28–30], the highest FBs dose in this trial did not compromise growth (weights of the whole body and organs), feed intake, or feed conversion efficiency. Our insignificant finding of feed intake is not similar to those reported earlier [28–30]. We refer this inconsistency to the differences in experimental settings; applied dose, exposure period, genotype, age, and mycotoxin source form and purity. In regard to the growth perturbation, similar patterns to ours were reported in the studies of 12.2 mg FB_1 + FB_2/kg diet for 4 weeks [8]; 20 mg FB_1/kg for 10 days [17]; 7.2, 14.7, 21.9, 32.7, and 35.1 FB_1 + FB_2 mg/kg diet for 4 weeks [31]; 25.1 mg FB_1 + FB_2 + FB_3/kg diet for 42 days [32]; and 25.1 mg FB_1 + FB_2 + FB_3/kg diet for 42 days [33]. Our maximum FBs-dose settings (cal. 26.17 mg FBs/kg^{-1} of final body weight during the trial) and period of exposure are lower than those of [33]; consequently, no remarkable variation in animal performance was predicted.

It is important to highlight that the lung weight (abs. or rel.) was not affected, as well as the PPE was only documented in a single intoxicated piglet. Therefore, the unaffected

lung weight is a consequence of the absence of severe toxicity. There is a visible tendency in the toxin dose associated with lung weight (possibly indicating edema commencement) and body weight gain, but none was proven to be statistically significant. We keep the premise that prolonged exposure and a large group size (n > 6) will reveal minimal variances and lead to precise outcomes.

3.2. Sphingomyelins

The SM is the most abundant form of sphingolipids in cellular plasma membranes, accumulating primarily in the outer membrane leaflet. The biosynthesis of SMs is dependent on ceramide production, a key intermediate of sphingolipid metabolism and a major precursor of long-chain FA and complex sphingolipids. Relatively, the major components of its structure are rather long saturated chains (16:0, 18:0, and 24:0), although in mammalian spermatozoa, very long-chain (24:0 to 34:0) PUFA have been reported [34]. Our results are corroborated by the findings of [34], since we detected overall saturation levels exceeding 70 % in SMs from the liver and lungs.

Once ceramide is a precursor to SM synthesis, it is relevant to discuss the FBs' effects on ceramide. In mammals, ceramide synthesis is catalyzed by the ceramide synthase enzymes (CerS; not determined in this study), a family consisting of six isoforms varying in acyl chain length interval specificity [35]. In the liver, 30 mg FBs/kg feed elevated the proportions of lauric (approx. two-fold) and behenic acids (1.5-fold), while decreasing the proportion of arachidic acid without altering overall SAT. This confirms the findings of [15] in piglet liver, when 1.5 mg FB_1/kg body weight for 9 days increased the level of SM-d18:1/22:0. These patterns may be indicative of high ketoacyl-CoA synthase activity (markedly increased SM-C22:0/C20:0, data not shown) and/or inhibition of CerS activities in the liver due to FBs exposure. Remarkably, the upregulation of long and very long side-chained (such as C22:0) ceramide molecular species has been involved in apoptosis regulation, with CerS4 and CerS6 activities postulated to be implicated [36].

On a qualitative and quantitative scale, FBs' effects on lung SM were more pronounced. Total SAT dropped concurrently with myristic acid (although the lignoceric acid proportion declined, presumably implicating lower CerS3 activity), whereas erucic acid and the overall MUFA level increased proportionally. Indeed, it is difficult to hypothesize precise changes in the activities of CerS isoforms since their affinity for FB_1 is not uniform and the fraction and ratio across isoforms have not been determined. Similar findings have been reported for erucic acid [15]. It has been shown that erucic acid and MUFA in general have a protective effect against cytotoxicity, especially in cancer cell lines [19,37]. On the other hand, oleic acid has been linked with metabolic and inflammatory lung diseases [38]. Thus, we hypothesize that the large proportions of erucic and oleic acids in the lungs possibly resulted in diverse stimuli; a protective mechanism and an injury trigger, respectively.

Modifications in the hydrocarbon chain length lead to variations in the bio-physicochemical properties of compounds, although behavioral changes are not fully comprehended. FBs are known to negatively affect the biophysical properties of cellular membranes [19]. In the case of high chain asymmetry, mismatch compensation can occur, and one such effect in the lipid bilayer is the establishment of chain interdigitation [39], which has been demonstrated in SM [40] and asymmetric ceramides [41]. We thus suppose that modifications in the SM fatty chain lengths were related to the interaction of lipids in the bilayer, most likely via chain interdigitation, in order to preserve or alter the physicochemical properties of the cell membrane.

3.3. Phosphatidylcholines

In the PC fraction, 30 mg FBs-exposure increased the myristic acid proportion (approx. two-fold in the liver and lungs). In contrast, stearic acid diminished in liver and lung, which was concomitant with the increase of oleic acid. These findings are consistent with reported findings in piglet liver-total PL (in vivo by FBs exposure at EU permitted value for 21 days [16]) and rat liver-PC fraction (in vivo by i.p. FB_1 equal to 20, 50, and

100 mg/kg diet for days [18]; and 250 mg FB_1/kg diet for a 21-day period [22], and in vitro by primary hepatocytes exposed to 25, 75, 150, 250, and 500 μM FB_1 [21]). The low stearic acid proportions in the liver and lungs were most likely a consequence of the high activity of stearoyl co-enzyme A desaturase (SCD); a key enzyme mediating the desaturation of stearic acid [42]. We noticed high ratios of C18:0/C18:1n9 in the studied tissues, as well as C16:0/C16:1n7 in lung, indicating high SCD activities that likely exhibited antioxidant qualities against the FBs' cytotoxicity. However, controversial patterns reported the adverse effects of MUFA in subsequent studies [43,44]. The endogenous pulmonary surfactant is predominantly formed from the reuptake of degraded PC, whereas 10–14% is synthesized via de novo routes [45]. Modifications in the lung-PC-FAs can have a genuine impact on the lung state. Oleic acid has been found to trigger pulmonary injury and inflammation through disrupting the Na^+/K^+-ATPase, Na^+ channeling, membrane docking, and G-protein coupled receptor activities [38]. In our study, histological changes in the lung were minimal, suggestive of a potential role for elevated oleic acid and/or the low C18:0/C18:1n9 as early bio-indicators of the lung's cytotoxicity.

Due to elevated proportions of palmitoleic and oleic acids in the liver (in a dose–response manner), and oleic and erucic acids in the lungs, the total MUFA levels increased in the liver and lungs of the 30 mg FBs/kg diet group. These findings support the results of [16] in piglets when the liver total PLs' MUFA was increased, proposing a compensation mechanism for the depletion of total SAT that is responsible for membrane rigidity. In our case, the total MUFA markedly compromised both SAT and PUFA levels in the liver (altered SAT/MUFA and PUFA/MUFA, data not shown), and only PUFA in the lung (altered PUFA/MUFA). Rather than merely ensuring membrane rigidity maintenance, we assume MUFA elevation is likely to modify transmembrane signaling and the cell cycle. MUFA has been reported to suppress lipogenesis and enhance glucose sensitivity [46], but none of these were examined in this design.

The highest dose of FBs decreased overall levels of n-6 and n-3 FAs in PCs of the liver and lung, resulting in low and high n-6:n-3 ratios in the organs, respectively. These findings were mainly consequences of the depletion of LA and AA in the liver PCs, as well as the lung-PC's DPA and DHA in a dose response manner. Similar findings were reported [18,22] for AA, DPA, and DHA of PCs from different subcellular fractions of the rat liver membrane. Our liver-PC findings for AA, overall n-6 FAs, and its ratio to n-3 FAs seem to be inconsistent with those reported in piglets fed on a 20 mg FB_1/kg diet for 10 days [17] in the total PL pool from porcine liver. In our work, separated PC was the investigated fraction, not membrane polar lipids as a whole. Furthermore, we applied a longer exposure period as compared to [17]. The AA depletion in liver-PC indicates higher activity of the enzyme phospholipase-2 (PLA_2), most probably directing the substrate eicosanoids biosynthesis. PLA_2 was shown to hydrolyse AA and DHA preferentially [47,48].

Meanwhile AA is a precursor in eicosanoid biosynthesis, DHA is involved in the regulation of prostanoid production. The proportionate declines in lung-PC's DPA and DHA (ca. 3/4 proportional decrease) of the 30 mg FBs group largely indicated the progression of n-3 FA-derived prostanoid synthesis. The proportional decrease of DPA, DHA, and overall n-3 FAs by FBs has been reported in vitro and in vivo in the rat liver-PCs [20,22] and piglet liver-total PL [17], but has not yet been reported in the pig lungs. Thus, the novelty of this in vivo study is that we are reporting similar findings in the piglet lungs for the first time, in a dose-dependent manner, most likely due to the inhibition of rate-limiting delta-5- and delta-6-desatuarse enzymes (Δ5D and Δ6D) activities as reported in the rat liver [23]. Here, we are suggesting similar events in the porcine lung-PC fraction based on the marked findings in PC-C20:4n6/C20:3n6 (ΔD5, low in the highest FBs setting, data not shown), PC-C18:3n6/C18:2n6 (Δ6D, low in all FBs treated animals, data not shown), and PC-DPA and DHA (both depleted in lungs).

3.4. Phosphatidylethanolamines

PE, which accounts for 15–25% of mammalian cellular membrane lipids, is the second most abundant type of polar lipid in animal tissues. The efficacy of FBs in altering the rat liver-PE has been reported in numerous studies (in vivo and in vitro) on rats [18,21,22]. With respect to saturated FAs, unlike the finding in lung-PEs (FBs exposure decreased behenic acid), no change was detected in the liver-PE. In swine, effects of FBs on saturated FAs of the liver total PLs varied among studies; altered by FBs at the EU permitted limit for a 21-day period [16] and unaltered by 20 mg FB_1/kg diet for 9 days [17]. Indeed, the doses and exposure periods applied in these studies differ. In comparison to rat liver models that reported alterations in PE-FAs, we mainly relate our non-observed effect to possible species-specific effects of FBs, lower FBs' doses as compared to those of 250 mg FB_1/kg diet for 14 days [21] and 250 mg FB_1/kg diet for 21 days [22], and administration method and toxin purity as compared to applied intraperitoneally pure FB1 for 5 and 10 days [18].

In a similar manner to PC, proportions of monounsaturated FAs responded to FBs in a dose dependent manner, namely the palmitoleic (liver and lung (almost two-fold increase)), oleic (liver) and erucic (lung) acids. Similar findings have been reported in Chang cells [20]. Although monounsaturated FAs in liver-PE were responsive, their total sum was unaltered. Based on the available literature in swine, the MUFA findings on the total PL FA profile of porcine liver are controversial. A dose of 5 mg FBs/kg diet for 3 weeks increased MUFA level [16], whereas a 20 mg FB_1/kg diet for 10 days did not alter its level [17]. These patterns are likewise reflected by the specific FBs-exposure period on the total MUFA, rather than the applied dose. On the other hand, in vivo studies on the rat liver-PE fraction have documented the accumulation of MUFA level induced by FBs exposure [21–23] through the up-regulation of the delta-9-desaturase (Δ9D) enzyme. Remarkably, we noticed these MUFA increments were attained at greater FBs' doses (>100 mg/diet) than ours. Anyhow, our results revealed a high overall MUFA level in the lung, indicating the organ-specific response/sensitivity to FBs. Indeed, MUFA accumulation compromised the depletion of total PUFA, referring to modifications in cellular signaling and/or metabolism. The main compromised PUFA sub-group was the sum of n-3 FAs, which consequently increased the n-6:n-3 ratio, although no significant change was noticed in their individual FAs.

A further marked modification was the decrease of total n-3 FAs and average chain length in the liver of piglets exposed to FBs, but these alterations were not dose-dependent, resulting in a markedly higher n-6:n-3 ratio. Similar patterns have been reported in piglet liver PL [17] and rat liver-PE fraction [21,22]. This class of lipid consists of crucial FAs that are involved in signaling or communication pathways within and between cells through altering membrane protein function and gene expression [49]. In general, the level of n-3 FAs in cellular membrane depends on their levels in the diet and their competition with n-6 FAs [50,51]. In our case, the alteration in total n-3 FAs of liver-PE was independent of oxidative stress (Table 8) and feed intake (Table 2), implying alterations in the enzyme activities involving remodulation of the membrane lipid fractions. Unlike the PCs, PE resides in the cytofacial leaflet; hence, the FA ratios between polar lipids in the membranes play a crucial role in cellular signaling, metabolism, proliferation, and death. We noticed that, under FBs exposure, the different tissues displayed distinct responses in terms of FAs indices among membrane lipids. The total n-3-PC/PE was found to increase in the liver, but it decreased in the lungs (data not shown). These findings indicate that the organ-specific response mostly depends on the cytotoxicity level and the ratio between lipid fractions.

3.5. Phosphatidylserines

Ordinarily, PS mainly resides in the inner leaflet of the cellular membrane and is a minor class of total PLs (constituting 2–15% of the total membrane–lipid pool). To our knowledge, only one study has investigated the impact of FBs on the PS-FA profile of rat liver, but no data is yet available on PS from lungs of pigs exposed to FBs.

The liver dataset revealed no discernible FBs-effect on either total SAT or total MUFA of PS fraction. Similar patterns were reported [23] for SAT of liver-PS; however, FB_1-

doses of 50 and 100 mg/kg diet elevated the oleic acid proportion and total MUFA. In our hepatic and pulmonary results, total MUFA levels were not responsive. However, eicosenoic acid showed a marked proportional decrease along with the accumulation of nervonic acid (more than three-fold) in hepatocellular-PS, whereas in lung-PS, oleic acid decreased and erucic acid increased (1.5-fold more). Nervonic acid is produced from the carbon chain elongation of oleic acid by the cyclic addition of two carbon units provided by malonyl-CoA to the acyl chain [27]. Although nervonic acid is closely linked with the maintenance of nerve cells, it has also been observed to increase in patients with Alzheimer's disease, psychosis, depression disorder, and cardiovascular disease [52], warranting further investigations into its related diseases and mode of action. In the lungs, we assumed that the accumulated erucic acid in PS could protect against pulmonary infections (see Section 3.8). Erucic acid has recently been shown to protect rats' lungs from damage induced by the influenza A virus [53].

When we investigated the lung-PS FA profile, results indicated dose-dependent alterations in behenic and lignoceric acids (half proportional depletion). Stearic, arachidic, behenic, and lignoceric acids were the most responsive saturated FAs (all decreased), paralleling the low total SAT in intoxicated animals. Markedly, PUFA compromised the depletion of SAT (high PUFA/SAT), whereas contrastingly, no marked variation was detected in the SAT/MUFA. The depletion of SAT indirectly indicates that the FBs interfere with one or more of the four reactions of the elongation process in the endoplasmic reticulum and/or the insertion of an SAT type FA into position *sn-1* and *sn-2* of PS. The PS is a modulator of the membrane charge locality; as such, it is vital for neural transport and influences proteins involved in numerous metabolic processes (e.g., enzyme activation and apoptosis). PUFA accumulation in lung-PS was a result of the proportional increases in LA, dihomo-γ-linolenic, and arachidonic acids (n-6 FAs). Interestingly, the LA proportional increase by FB_1 exposure has been reported in rat liver-PS [23]. The n-6 FAs play vital roles in numerous cellular molecular pathways, such as inflammation, signal transduction, and cellular proliferation and apoptosis. Thus, we assume that alterations in the lung-PS have a profound impact on PS-related signaling events in the lung.

Our liver findings are relatively similar to those of [23], in which the n-6:n-3 FA decreased by FBs exposure, including extra the decrease of DPA proportion. Notably, similar patterns were noticed in lungs of intoxicated animals, associated with the proportional depletion of DHA. These patterns are independent of the lipid peroxidation process and dietary DHA intake (see Tables 1 and 8), speculating that alternative molecular events are responsible for these observations. The level of PS in bilayers has been found to be strongly influenced by its n-3 proportion within the membranes. PS accumulation is very profound in neural tissue with abundant DHA concentration [54]. In this design, the PC, PS, and PI fractions all showed a proportionate depletion in DHA, underpinning a possible depletion from the cellular-PS proportion. Anyhow, our data is neither quantitative nor lyso-PS has been analyzed; thus, further studies are essential to support that proposal.

3.6. Phosphatidylinositols

The PI represents 2–12% of total polar-lipids in mammalian cells and subcellular membranes. Analyzing the PI-FA profile, apart from a few proportional changes, it is clear that linear dose-dependent trends are absent, most likely a result of the applied doses and exposure period. Based on the literature, two studies investigated the impacts of FB_1 on the fatty profile of rat liver-PI [18,23].

Similar to the earlier report in rat liver [55], our piglet liver data revealed that stearic and arachidonic acids (together ca. 60% of the total PI-FAs pool) were the most abundant FAs in PI fraction. However, the lungs exhibited distinct patterns, with palmitic, stearic, arachidonic, and oleic acids being the most abundant FAs. Our dataset revealed that the proportions of PI-AA in the liver or lung remained unaffected, while the stearic acid proportion in the lung-PI decreased, depleting the total SAT level. Our AA finding is consistent with earlier results in the hepatic PI fraction of rats [18,23], but FBs' effects

on PI stearic acid are not yet clear. When a 250 mg FB_1/kg diet for 21 days failed to alter the PI-stearic acid proportion [23], 10 days of pure FB_1-intraperitoneal exposure (eq. 100 mg FB_1/kg diet) decreased its concentration, as well as the total SAT level [18]. We speculate that the depletion in lung-PI-stearic acid proportion was related to elevated activity of Δ9D and elongation enzymes (not analytically determined). Indeed, we noticed no change in the lungs' PI-stearic/oleic, but PI-stearic/behenic and PI-oleic/erucic were markedly lowered (data not shown). Notably, trials by [18,23] found high oleic acid and total MUFA concentrations, mostly attributed to the relatively high FBs' dose compared to ours.

When comparing PS and PI from lungs of highly FBs-intoxicated animals, strikingly similar FA patterns were noticed; the erucic acid and overall MUFA compensated the decrease in total SAT. The fact that erucic acid was the major contributor to high MUFA indicates its possible essential role in protecting against lung injury. Recently, it was reported [56] that PI displayed antagonistic properties to activated ligand by the Toll-like receptors (TOR)—a crucial event for the virulence of certain viruses of the lung. Despite the absence of a quantitative analysis of the entire PI pool, it has been shown that erucic acid inhibits the infection of influenza A and H7N9 viruses [57,58]. Its preventive mode of action consists of down-regulating pro-inflammatory mediators, pro-apoptotic signaling, and aggravating immunological inflammation, underpinning its potential role in lung protection and disease prognosis management [53].

In liver PI, the nervonic acid proportion decreased as a result of FBs' toxicity. A plausible mechanism behind decreased nervonic acid level is the translocation of acyl chains across bilayer lipid classes, which may alter hepato-signal transduction and membrane traffic. However, the present study did not investigate the related molecular signaling. Probably the most PI-lipid disintegration event was the proportional depletion of DHA (also occurred in lung-PI), which decreased the total sum of n-3 FAs and raised the n-6:n-3 ratio. [18] have reported a similar DHA's finding in rat liver-PI. In our case, the decrease in DHA appears independent of feed intake and lipid peroxidation (no marked change was noticed; see Table 8). This finding with n-3 FA patterns in PC supports the proposal of [23] that FBs hamper Δ5D and Δ6D activities in hepato-microsomal membranes.

3.7. Antioxidant Enzymes and Lipid Peroxidation

The lipid bilayers' peroxidation is a complex chain reaction encompassing enzymatic (catalyzed by the lipoxygenase family) and/or non-enzymatic reactions (generation and propagation of reactive oxygen species (ROS), uptake of oxygen, and disruption of the double bond in unsaturated FAs). Thus, lipid peroxidation ultimately leads to the destruction of cellular biomolecules, including membrane lipids [59,60]. According to numerous in vivo and in vitro reports (reviewed by [61]), oxidative stress is one of the FBs' mediated toxicity pathways. However, there is no full compliance whether oxidative stress is a direct or indirect route of the FBs-toxicity. According to [62], ROS generation is rather a consequence of FBs-toxicity than a direct toxicity event of FBs.

In this study, neither the low molecular weight antioxidant (GSH) nor the antioxidant enzyme (glutathione peroxidase (GPx)) of the glutathione redox system, or the end product of lipid peroxidation TBARS (expressed as malondialdehyde (MDA)) were altered in liver or lung tissues. It is important to highlight that the literature lacks data on the porcine pulmonary oxidative capacity under FBs exposure. However, pure doses of FB_1 (eq. 20, 50, and 100 mg FB_1/kg diet) were reported to induce lipid peroxidation in rat lungs, although not in a dose-dependent manner. Moreover, alterations in MDA levels varied among the time points—5 and 10 days of exposure [63]. Variation in oxidative findings may be attributable to the ceramide proportion within the tissue, in which the up-regulation of CerS has been associated with apoptosis and oxidative stress [64]. Anyhow, the compensatory re-modulation in the FA profile of investigated polar lipids likely assisted in the prevention of strong propagation of lipid peroxidation.

Our findings in the liver are similar to those obtained at 5 mg FBs/kg diet for 3 weeks [16], but inconsistent with those of [17], when 20 mg FBs/kg diet for 10 days elevated the lipid peroxidation biomarker and triggered the enzymatic defense system. We speculate that the variation in outcomes may be due to the high SAT and MUFA levels in hepatic membrane lipid fractions, such as PC and PI. [19] have reported that high SAT and MUFA levels refer to rigid membranes, a cellular defense mechanism against the attack of free radicals. In addition, the elevated proportion of oleic acid in PC and PE fractions possibly exhibited antioxidant properties that neutralized the end product of lipid peroxidation. Oleic acid has been reported to augment GSH biosynthesis in murine liver [65]. The novelty of our study is that it confirms the proposals that lipid profile alterations in bilayer lipids by FBs are rather a re-modulation mechanism than a lipid peroxidation-consequence [16], and that elevation of SAT and MUFA may express a protective mechanism against FBs toxicity [19].

3.8. Histopathology

Compared to studies performed on rats, there are fewer studies on porcine histological modifications induced by FBs exposure. Once, FBs target the swine lung and liver, and thus, histological modifications were expected as they have been reported in porcine tissues [8,17]. In the livers of piglets exposed to FBs, we observed a depletion in glycogen microvacuoles that was associated with a lower extent of cytoplasmic vacuolization. Furthermore, intoxicated animals exhibited scattered solitaire hepatocellular necrosis, swollen and scattered focal proliferation of MPS. These findings concur with the recently published findings of [8,17], demonstrating together the dose-dependent toxic effects of FBs on the liver. In contrast, our findings contradict those of [16] in piglets, where no liver lesions were identified. We attribute our detected hepatotoxicity to the FBs retention level and exposure period in the liver throughout the trial, since in the study of [16] a low FBs dose was administered. Notably, our hepato-histopathology was confirmed by alterations in serum biochemicals; 30 mg FBs/kg diet for 21 days elevated AST, ALP, and cholesterol concentrations (six-, five-, and two-folds, respectively; data not shown). The novelty of this study is that no marked lipid peroxidation was detected, strongly suggesting that the perpetuation of sphingolipids and hepatocellular lipids are the primary determinants of observed hepatotoxicity.

Among all treatments, the lungs of some animals showed mild focal interstitial lymphohistiocytic infiltrations (seen interstitially), as well as mild focal fibrosis of the visceral pleura. These histological alterations may relate to the PPE progression. However, a single piglet developed PPE upon exposure to 30 mg FBs/kg feed, revealing alveoli filled with finely granular, pale eosinophilic serous fluid. This finding appeared to be consistent with the studies [9,66,67] reporting that FBs, especially FB_1, are PPE inducers in swine. Events such as the blockage of L-type calcium channels and left ventricular hypertrophy in the heart have been established as mediators for PPE [9]. Anyhow, the majority of intoxicated piglets in our study did not develop PPE. According to [68], when weanling piglets fed FBs-contaminated diets in a dose response manner (175, 101, 39, 23, 5, and <1 mg FB_1 + FB_2/kg feed) for 14 days, only 175 mg FBs/kg feed induced PPE. Herein, we are rather aiming to present possible inconsistencies in results than establish that PPE development requires extremely high doses of FBs. Indeed, PPE has been observed in some weaned piglets upon exposure to 20 mg FB_1/kg diet for 10 days [17]. On the other hand, in a recent study by [8], a relatively very low dose of FBs (3.7 mg FBs/kg diet) for 28 days exerted pathomorphological changes in the weaned piglet heart without developing PPE. In the present study, hyperaemia and lesions found in the lungs may either be mediators for PPE development or represent subclinical evidence of a latent airway infection or immunosuppression of the respiratory system (none was tested) that implicated FBs exposure. FB_1 has been shown to exert an immunosuppressive effect in pigs exposed to relatively low level of 10 mg FB_1/kg feed [69], which was reported to aggravate the progression of respiratory pathogenicity induced by *Mycoplasma hyopneumoniae* [32] and

Pasteurella multocida [70]. However, further investigation in immunosuppression induction by FBs related to dose and exposure period are important.

4. Conclusions

The study investigated the effects of FBs in a dose-dependent manner on the FA profiles of membrane lipids from the liver and lungs of piglets. This is the first study to report alterations in the FAs profiles of porcine lung phospholipid classes in response to FBs-exposure. The relatively high dose of FB_1 had no marked effect on piglet production traits or oxidative stress markers. Only minor characteristic effects were detected in FA profiles of membrane lipid fractions. Alterations in linear dose-dependence were observed in liver PC, as well as lung PC and PS fractions. The obtained data revealed variations in tissue-specific membrane lipid responses. Furthermore, findings suggest alterations in enzymes involved in FA metabolism, such as elongase, Δ5D, Δ6D, and Δ9D. Disruption in these enzymes was characterized by the proportional depletion of polyunsaturated FAs and the augmentation of total monounsaturation. Alterations detected in membrane lipids are not assumed to be mainly responsible for liver and lung toxicities, rather than consequences/mediators of other events that might have led to tissues' injuries. The study lacks the quantitative determination of ceramide and polar lipids, which could provide additional explanation for the results. Therefore, further studies investigating the quantitative effects of FBs on lipidomes of the liver and lungs are highly important.

5. Materials and Methods

5.1. Experimental Design, Animals and Feeding

The study was a completely randomized design (CRD) that involved 18 weaned Danbred male piglets at the age of 35 days with semi-equal average body weights. Randomly, piglets were equally assigned to three treatments, with each group consisting of six animals. Each animal was kept individually in a metabolic cage with an area of 80 cm × 80 cm. In the beginning, all piglets underwent a 14-day adaptation period. After the adaptation period (at the exact age of 49 days), the initial body weight was determined for each animal individually. The duration of the feeding trial was 21 days; besides the control (FBs-free), one group was fed on a contaminated diet (15 mg FBs/kg; FB_1, FB_2, and FB_3 in a fungal culture), whereas the other group was fed a high FBs-contaminated diet (30 mg total FBs/kg).

The diet formulation and nutrient optimization were performed by Bonafarm-Bábolna Takarmány Kft (Bábolna, Hungary). The formulated diet was analyzed in accordance with [71], and its FA composition was determined (Table 9). Upon animals' arrival at the experimental unit, the portion of feed offered was estimated based on the initial body weight of piglets, and in correspondence to the actual consumption, the daily offered diets were adjusted. The diet was offered twice a day, in equal proportions (equivalent to 0.5 kg feed consumption/animal/day during the first week and gradually increased to reach 0.7 kg feed intake/animal/day at the last week). At the end of each day, the feed residual was measured back for the daily feed intake determination. During the whole study, water was offered *ad libitum* to the piglets via automatic drinkers. The house environmental conditions (such as temperature and humidity) were adjusted by the needs of weaned piglets.

At the end of the trial, the piglet bodyweight was determined individually. Later on, the piglets were euthanized by exsanguination after sedation (Euthanyl-Pentobarbital Sodium, 400 mg/mL, Dechra Veterinary Products, Shrewsbury, UK), and their liver and lung were sampled for analysis. From the jugular vein, the fresh blood was collected into heparinized (20 IU/mL whole blood) tubes and was centrifuged for 10 min at 1000× *g* (SIGMA 3-30KS refrigerated centrifuge, Osterode am Harz, Germany) for plasma separation. Both plasma and liver samples were immediately stored at −80 °C for further analysis.

5.2. Feed Mycotoxin Contamination

The fungal strain *Fusarium verticillioides* (MRC 826) was inoculated on pre-soaked, sterile maize kernels, in a form of spore suspension. The incubation was set to 25 °C for 5 weeks, and the final FBs concentration in dried culture material was harvested in different production batches. Details on fungal culture preparation were published earlier [72]. The final FB_1 concentrations were 2000–4000 mg/kg in the air-dried culture material harvested in different batches. The FB_2 concentration of the inoculum materials was ca. 30% of the FB_1 content and the FB_3 concentration was ca. 10–15% of the FB_1 content. The fungal culture was mixed into the ration of the experimental animals so as to provide a daily FBs (FB_1 + FB_2 + FB_3) feed concentration of 15 and 30 mg/kg. The diet fed to the control group did not contain detectable amounts of FBs, whereas the experimental diets were mixed with fungal culture to provide 15 and 30 mg FBs/kg diet. In the diets, the absence of FBs co-occurrence with deoxynivalenol (DON), zearalenone (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). The content of FBs was determined by the LC-MS-2020 mass spectrometer (Shimadzu, Kyoto, Japan) [73].

Table 9. The proximate and chemical composition of the experimental diet.

Component	Concentration
Crude protein (%)	17.50
Crude fat (%)	3.30
Crude fiber (%)	3.70
Crude ash (%)	5.00
Starch (%)	41.8
Lysine (g/kg)	1.11
Methionine (g/kg)	0.37
Ca (g/kg)	0.65
P (g/kg)	0.50
Na (g/kg)	0.18
DE (MJ/kg)	14.7
ME (MJ/kg)	14.1
Fatty acid (methyl ester)	%
C12:0	0.01
C14:0	0.19
C14:1	0.01
C15:0	0.02
C16:0	15.3
C16:1n7	0.33
C17:0	0.08
C18:0	5.61
C18:1n9	26.5
C18:1n7	1.13
C18:2n6	47.1
C18:3n3	2.60
C20:0	0.36
C20:1n9	0.39
C20:2n6	0.07
C20:4n6	0.08
C22:0	0.06
C24:0	0.06
C22:6n3	0.13

5.3. Fatty Acid Composition of Phospholipid Classes

The liver, lung (after frozen storage at −20 °C), and diet samples were homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in the 20-fold volume of chloro-

form:methanol (2:1), and the total lipid content (complex lipids) was extracted according to [74]. Solvents were ultrapure-grade (Merck Sigma-Aldrich, Schnelldorf, Germany) and 0.01% butylated hydroxytoluene was added to prevent FA oxidation.

In the case of the diet, the FAs were transmethylated by the base-catalyzed sodium-methoxide method of [75]. For the separation of phospholipid classes in liver and lungs, thin layer chromatography (TLC) was used. Extracted complex lipids were spotted onto pre-dried (110 °C, 2 h) 20 cm × 20 cm TLC plates (Sigma Cat. No.: 99570). The separation was performed in one dimension, using the eluent mixture of methyl acetate–isopropanol–chloroform–methanol–aqueous 0.025% KCl (25:25:25:10:9, *v*/*v*/*v*/*v*/*v*), developing the plate until the top, in an all-glass, covered TLC chamber [76]. Primuline spray (5 mg in 100 mL of acetone:water (80:20, Merck-Sigma Cat. No.: 206865, Schnelldorf, Germany)) was used to stain lipid spots, and detection was performed under ultraviolet light (365 nm). To identify PL classes, certified reference materials were used as follows: N-Acyl-D-sphingosine-1-phosphocholine (Merck-Sigma Cat. No. S0756), L-α-phosphatidylcholine (Merck-Sigma Cat. No.: P3556), L-α-phosphatidylethanolamine (Merck-Sigma Cat. No.: P7943), L-α-phosphatidylserine (Merck-Sigma Cat. No. 870336C), and L-α-phosphatidylinositol (from Glycine max, Merck-Sigma Cat. No. P6636). Identified fractions were scraped off from the plates and were extracted 3 times into the TLC eluent mixture. Subsequently, the solvent was evaporated entirely and lipids were trans-methylated with an acid-catalysed method, using 1% H_2SO_4 in methanol [77]. Fatty acid methyl esters were extracted into 150 µL ultrapure n-hexane for gas chromatography, which was performed on a GC-Shimadzu 2030 equipped with an AOC 20i automatic injector (Kyoto, Japan), a Phenomenex Zebron ZB-WAXplus capillary GC column (30 m × 0.25 mm ID, 0.25 µm film, Phenomenex Inc., Torrance, CA, USA) and a flame ionization detector (FID) detector. Characteristic operating conditions were: injector temperature: 220 °C; detector temperature: 250 °C; helium flow: 28 cm/sec. The oven temperature was graded: from 60 (2 min hold) to 150 °C, from 150 to 180 °C: 2 °C/min and 10 min at 180 °C, from 180 to 220 °C: 2 °C/min, and 16 min at 220 °C. The makeup gas was nitrogen.

To identify individual FAs, an authentic external FA standard mixture (Merck Sigma-Aldrich, Schnelldorf, Germany) was used. Fatty acid results were expressed as weight % of total FA methyl esters. The unsaturation index (UI) was calculated to express the number of double bonds in 100 FA chains. Calculation was performed with the LabSolutions 5.93 software, using the PostRun module (Shimadzu, Kyoto, Japan) with manual peak integration.

5.4. Antioxidant Status and Lipid Peroxidation

Samples of lung and liver were stored at −82 °C before analysis. Lipid peroxidation was assessed with the determination of thiobarbituric acid reactive substances and expressed as malondialdehyde, which was served as standard [78] in the 10-fold volume of tissue homogenate in physiological saline (0.65 *w*/*v*% NaCl). The amount of GSH and GPx activity was measured in the 10,000× *g* supernatant fraction of tissue homogenate. The quantification of the GSH was performed according to the method of [79], using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as a sulfhydryl reagent to form a yellow derivative, which is measurable at 412 nm. The activity of GPx was determined according to [80] applying an end-point direct assay with GSH and cumene hydroperoxide as co-substrates.

GSH concentration and GPx activity were calculated from the protein content of the 10,000× *g* supernatant fraction after centrifugation (10 min at 4 °C), which was measured by the Folin-phenol reagent [81]. In all instances, the color was measured with UV–Vis spectrophotometry in 10 mm pathway optical glass cuvettes.

5.5. Histological Preperation and Assessment

Tissue samples of the liver and lung were stored in 10% neutrally buffered formalin and embedded into paraffin. For light microscopic analysis, microtome slides of 5 micrometers were prepared and stained with hematoxyllin–eosin. Detected lesions in the liver were decreased glycogen content of liver cells, hepatocellular necrosis, and swelling and prolifer-

ation of MPS cells. In the lung, lesions included interstitial lymphohistiocytic infiltration and pleural fibrosis. The main pathological alterations have been described and scored according to their extent and severity as follows: 0 = no alteration, 1 = slight/small scale/few, 2 = medium degree/medium scale/medium number, 3 = pronounced/extensive/numerous. Images were taken on an Olympus BX43 microscope with a DP23 camera (Olympus, Tokyo, Japan).

Histopathological examination was performed in accordance with the Decree No. 9/2001 (03.30) of the Ministry of Health and the Ministry of Agriculture and Rural Development and the guidelines of the OECD Good Laboratory Practice for Chemicals [82].

5.6. Statistical Analysis

All data were tested for normality (Shapiro–Wilk test), whereas the extent of standard deviation was compared between groups with Levene's F test. After this, the univariate analysis of variance (ANOVA) was used on the control and total FB-fed group means, with the Least Significant Difference (LSD) "post hoc" test for detailed inter-group differences. For dose–response determination, the Pearson correlation was calculated between offered doses of FBs and further FA variables, always using individual data-pairs. Identified p-values > 0.05 were subjected to linear regression analysis, whereas only R^2 greater than 0.6 are reported. The software that performed data evaluation was IBM SPSS 20 [83]. For the significance level identification, the calculated probability of a p-value < 0.05 was set for all tests.

Principal Component Analysis (PCA) was performed on the FA profile of the different phospholipids from liver and lungs with the Unscrambler 9.7. software [84] to seek principal components describing the variance responsible for the "group formation" with the highest possible efficacy. The sole purpose of PCA was not to discriminate between certain groups of treatments based on their chemical composition, but rather to describe the basic orientation of the groups within the multidimensional space described by the variables investigated (e.g., FA profile elements). The orientation of the samples is described by the score plot, which shows the scores of each sample along with the first two principal components. The variable impact is presented with the loadings bar graph, which shows the contribution of the variance of each investigated variable to the full variance of the first principal component; that is, the values of the loadings graph are the weights for each original variable when calculating the principal component.

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4.4. A 65-day fumonisin B exposure at high dietary levels has negligible effects on the testicular and spermatological parameters of adult rabbit bucks

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A 65-Day Fumonisin B Exposure at High Dietary Levels Has Negligible Effects on the Testicular and Spermatological Parameters of Adult Rabbit Bucks

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Abstract: A 65-day study was undertaken to test the effects of two doses (10 and 20 mg/kg) of dietary fumonisin Bs (FB) on the rabbit male reproduction system. Body and testicular weight was not affected by the intoxication, neither the fatty acid composition of the testicular total phospholipids; the testis histological analysis failed to reveal any toxic effect. The FBs increased the testicular concentration and activity of reduced glutathione and glutathione peroxidase and decreased initial phase lipid peroxidation (conjugated dienes and trienes) in a dose dependent manner. Sperm morphology and chromatin condensation were monitored on Feulgen-stained smears. No significant differences were observed between the treatment groups and between sampling time points. The live cell ratio in the sperm (as assessed with flow cytometry) was not different among groups at any of the five sampling timepoints and was also identical within groups. Similarly, the spermatozoa membrane lipid profile was also identical in all three groups after the total intoxication period. In summary, it was demonstrated that FBs in an unrealistic and unjustified high dose still do not exert any drastic harmful effect on the leporine, male reproduction system, meanwhile slightly augmenting testicular antioxidant response.

Keywords: fumonisin B series; rabbit; testis; spermatozoa; membrane fatty acids; oxidative stress

Key Contribution: Adult rabbit bucks exposed to fumonisins at 10 and 20 mg/kg diet provided no marked testicular damage (negative histopathology; no weight change; unaltered membrane fatty acid profile; but minimally provoked antioxidant defense) and no detectable spermatological alteration (morphology; live cell ratio; membrane lipid profile) as a result of a 65-day exposure.

1. Introduction

Fumonisins are mycotoxins (fungal secondary metabolites) produced by *Fusarium verticillioides* and *proliferatum* filamentous fungi (*Liseola* section), mostly infecting cereal

commodities, the starch feed basis of monogastric farm animals. The 28 fumonisin analogues characterized since 1988 can be divided into four main groups: series A, B, C, and P [1], from which the B analogues are toxicologically the most hazardous, fumonisin B_1 (FB_1) being the most well-known and the most toxic [2]. Fumonisin occurrence is primarily frequent in corn; the prevalence was 78% in 2020 in the samples tested [3]. When considering rabbit feeds, the natural occurrence of fumonisins is relatively low, since corn is rarely exceeding 20% proportion in them; as we are currently not aware of any large scale screening dataset [3], the fumonisin contamination of rabbit feeds might be deduced from the relevant corn content [4].

The harmful effects of FB_1 are not only species specific in vertebrates (for reviews see: [5,6]), but its mode of action is also approached in detail [6] and provides organ specificity. In brief, FB_1 is a ceramide conformational analogue and thus a competitive inhibitor of CoA-dependent ceramide synthase [7]. Target organs of FB_1 are liver and kidney in most domestic animal species; the harmful effect on these organs is exerted via an altered (perturbed) sphingolipid metabolism, leading ultimately to apoptotic and oncotic necrosis, and carcinogenesis in rodents [6]. The consequences of fumonisin-mediated disruption of sphingolipid metabolism are most likely altered cell regulation, since the cellular concentrations of free sphingoid bases are increased and ceramide biosynthesis becomes inhibited. Both above compounds are capable in the induction of cell death, and, according to Riley et al. [6], tumorigenesis is basically initiated by the imbalance between ceramide (↓), sphingosine 1-phosphate (↑), and altered fatty acid (FA) profile [7].

Besides marked contribution to cell disruption, FB_1 has also been reported to initiate oxidative stress (induction of reactive oxygen species, ROS) in variable cell types, such as neural cell cultures [8] and iliac endothelial cells [9]. Indeed this specific point, the ROS-mediated initiation of the dis-regulation of the cellular membrane permeability [9,10] is a characteristic point of FB_1 toxicosis that also leads to cell necrosis or swelling. In addition, ROS production and the involvement of the antioxidant system is not limited to defined tissue or cell types, it seems to be a more general event [11], but this has never tested in the male reproduction system (only in one, in vitro approach by Minervini et al. [12] in an equine spermatozoa test).

Meanwhile the nephrotoxic, hepatotoxic, neurotoxic, and carcinogenic impact of FBs is generally well documented [2,7], concerning their in vivo, systematic effects on the male reproductive system and performance there is a relative lack of literature and is mostly limited to rabbits [13–15]. Though testicular and epididymal characteristics have been documented in detail, the possible oxidative stress and the accompanying lipid profile modifications in rabbit have not yet been addressed, especially not at high exposure levels. Moreover, in the field of spermatology, mostly quantitative data (volumetric proportions of testicular elements) have been published so far [15], and spermium abnormality types have not yet been tested.

Domestic rabbits have an economical importance within the animal production industry, moreover, rabbits are suggested to be an appropriate model species for reproductive toxicology studies [16].

As far as we are aware, neither oxidative stress, nor dose dependence has been tested in rabbits undergoing fumonisin B series feeding. This targeted study was thus aimed to test the leporine male response on a lower and higher FB dietary level in a (1.) dose dependent manner, in a relatively complex and long approach (to markedly exceed the 49 day-long spermatogenesis time), involving (2.) the testicular histopathology and lipid profile modifications, with (3.) the characterization of the spermatological traits (morphology and composition). The fumonisin dose used in this study was defined to be 2 and 4 times that of the least observed adverse effect level (LOAEL), as established by the European Food Safety Authority [17].

2. Results

2.1. Animal Performance

All biological parameters are shown sub-divided according to experimental groups. The initial and final bodyweight (BW) is shown in Table 1 and Figure 1. Since none of the recorded BW group-means differed (Figure 1 and Table 1), BW gain was not considered. As shown in Figure 1, the graded FB levels likewise lowered group mean BW, in a systematic manner, but without statistical significance. The feed intake was measured daily until day 25, then weekly, but provided no inter-group differences (data not shown).

Table 1. Internal organ (absolute and relative values), initial and final bodyweight values (means ± SD) of the control and intoxicated rabbit groups (n = 10/group; BW: bodyweight).

Group	Control				10 mg/kg				20 mg/kg			
Organ Weight (g)	**Mean**	±	**SD**		**Mean**	±	**SD**		**Mean**	±	**SD**	
liver	91.7	±	17.6	ab	96.9	±	13.2	b	80.9	±	11.6	a
kidney	19.8	±	1.92	ab	20.8	±	1.95	b	18.4	±	1.77	a
spleen	1.80	±	0.41		1.58	±	0.47		1.62	±	0.30	
testes	10.3	±	1.68		10.5	±	1.05		9.6	±	1.58	
rel. liver (%)	1.90	±	0.26	ab	2.07	±	0.29	b	1.75	±	0.17	a
rel. kidney (%)	0.41	±	0.04		0.44	±	0.04		0.40	±	0.04	
rel. spleen (%)	0.04	±	0.01		0.03	±	0.01		0.04	±	0.01	
rel. testes (%)	0.22	±	0.04		0.22	±	0.02		0.21	±	0.03	
initial BW (g)	4380	±	345		4387	±	335		4390	±	325	
final BW (g)	4819	±	350		4686	±	309		4619	±	486	

a,b: different superscripts indicate significant difference between group means at $p < 0.05$.

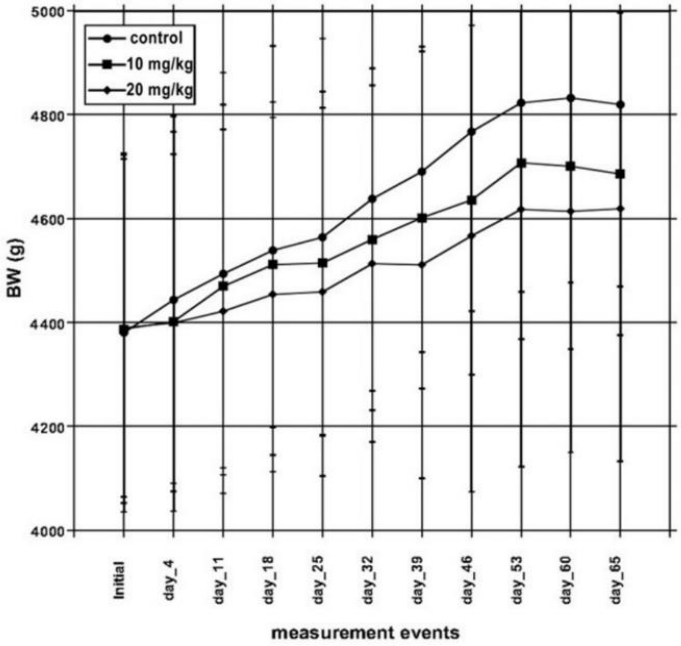

Figure 1. Bodyweight changes along the study period of the three rabbit groups (data points represent group means of each 10 individuals and error bars represent ± SD).

During dissection, the weight of internal organs was recorded. The liver and kidney absolute weights were different between the two intoxicated groups, but spleen and (paired) testis weights (Table 1) were not different among any of them. Relative liver weight showed

the same differences like the absolute weight, while other relative organ weights were identical in all three groups.

2.2. Spermium Morphology, Chromatin Integrity and Viability

The flow cytometric live and dead cellular distribution pattern is shown in Figure 2 in a representative sample, providing a very effective separation. Debris events were excluded from the analysis. When analyzing the live cell proportion within the total cell counts, no systematic difference could be established (Figure 3); differences were not significant even at $p < 0.05$ if analyzing the five consecutive samplings within the single groups and even if the three experimental groups were compared with each other at the five sampling events. Live/dead spermatozoa ratio at the ultimate sampling was 84.3 ± 2.95, 75.8 ± 7.36 and 74.4 ± 21.8, for the control, 10 and 20 mg/kg treatments, respectively.

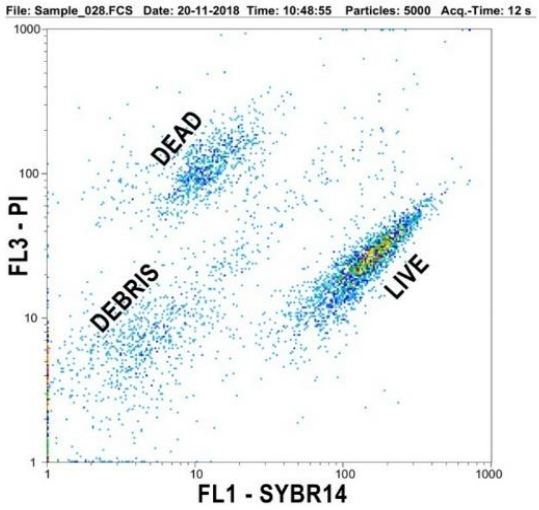

Figure 2. Flow cytometric dot-plot showing the green (SYBR14) and red (PI) fluorescence properties of live and dead spermatozoa; the lower left population was identified as debris and was excluded from data analysis.

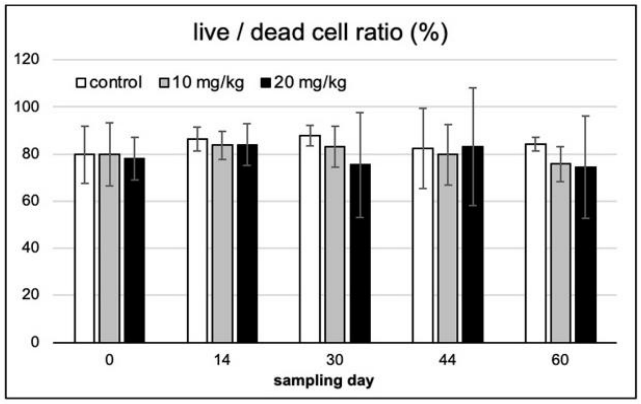

Figure 3. The live cell % data (as assessed from the flow cytometric measurements) of the sperm samples in the five consecutive sampling events, in the three experimental groups (means of 10 individuals ± SD).

2.3. Spermium Mophology and Distribution

On Feulgen-stained semen smears, abnormal spermatozoa and disturbances in chromatin condensation were recorded with light microscopic counting. Figure 4 provides the

typical demonstrations of some of the most frequent cellular defects observed. Morphological abnormalities were classified as head or tail defect; neither of these showed a significant difference over time and between treatments (Figures 5 and 6).

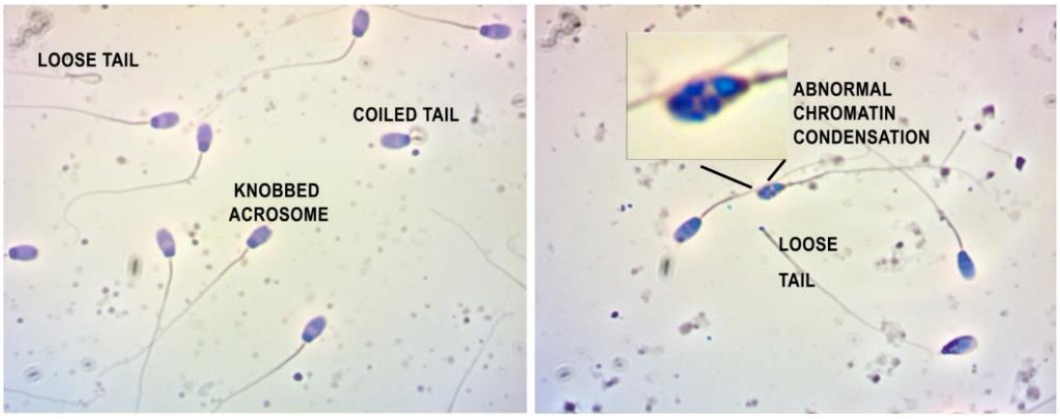

Figure 4. Abnormal morphology of the rabbit spermatozoa. (Feulgen staining, 400× magnification). Inner photo: abnormally condensed chromatin showing patchy staining pattern.

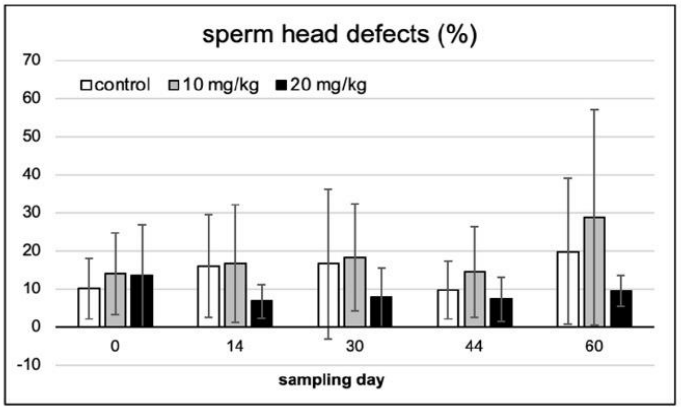

Figure 5. Sperm head defects in the five consecutive sampling events, in the three experimental groups (means of 10 individuals ± SD).

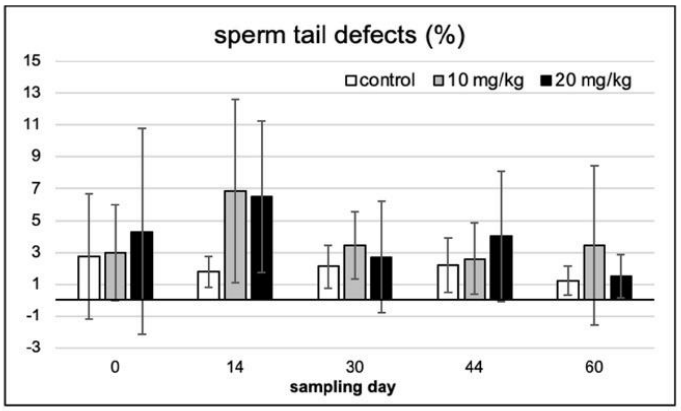

Figure 6. Sperm tail defects in the five consecutive sampling events, in the three experimental groups (means of 10 individuals ± SD).

Similarly, no significant effect among the treatment groups or over the exposure time (among sampling days within single treatments) was observed in sperm chromatin condensation (Figure 7).

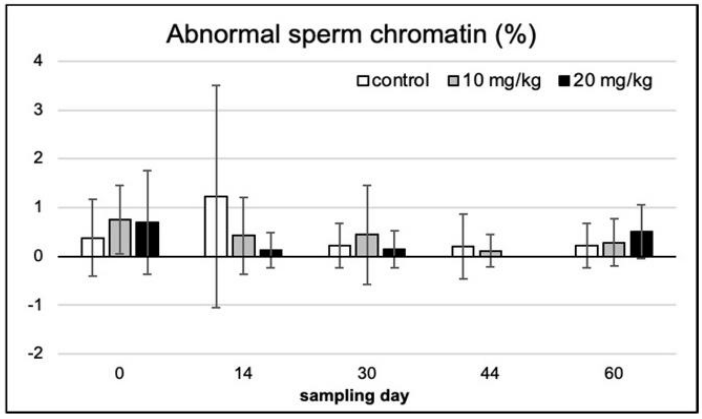

Figure 7. Abnormal sperm chromatin % (occurrence frequency) in the five consecutive sampling events, in the three experimental groups (means of 10 individuals ± SD).

2.4. Testicular Phospholipid Fatty Acid Composition

The testicular total phospholipid fatty acid profile of the three groups is given in Table 2. When comparing the group means, there was a detectable difference only in the proportion of C17:0, margaric acid (↑ in the 20 mg/kg group, $p = 0.002$ for the ANOVA model). Any other individual FAs or calculated variables failed to provide inter-group differences.

Table 2. The fatty acid (FA) composition (weight % of total FA methyl esters) of the testicular total phospholipids in the three experimental groups (means of 10 individuals ± SD).

Group	Control				10 mg/kg				20 mg/kg			
Fatty Acid	**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**	
C14:0	0.39	±	0.52		0.31	±	0.15		0.26	±	0.12	
C14:1n5	0.02	±	0.00		nd				nd			
C15:0	0.31	±	0.64		0.10	±	0.01		0.11	±	0.02	
C16:0	35.3	±	3.01		37.0	±	2.32		35.3	±	1.39	
C16:1n7	0.20	±	0.07		0.23	±	0.05		0.21	±	0.03	
C17:0	0.32	±	0.08	a	0.40	±	0.09	a	0.51	±	0.12	b
C18:0	29.0	±	8.17		25.0	±	4.82		24.6	±	3.16	
C18:1n9c	7.95	±	2.45		8.79	±	1.38		9.04	±	0.85	
C18:1n7	0.83	±	0.25		0.96	±	0.15		0.88	±	0.12	
C18:2n6	4.91	±	1.24		5.62	±	0.99		5.47	±	0.76	
C18:3n6	0.12	±	0.05		0.15	±	0.02		0.15	±	0.04	
C18:3n3	0.02	±	0.01		0.08	±	0.18		0.02	±	0.00	
C18:4n3	0.43	±	0.45		0.27	±	0.27		0.30	±	0.14	
C20:0	0.21	±	0.12		0.14	±	0.05		0.14	±	0.03	
C20:1n9	0.13	±	0.04		0.13	±	0.03		0.11	±	0.02	
C20:2n6	0.30	±	0.08		0.35	±	0.08		0.30	±	0.03	
C20:3n9 (Mead acid)	0.09	±	0.03		0.08	±	0.01		0.08	±	0.02	
C20:3n6	7.58	±	2.09		8.68	±	1.78		8.83	±	1.19	
C20:4n6	11.6	±	3.27		11.5	±	2.30		13.3	±	1.48	
C20:5n3	0.10	±	0.03		0.09	±	0.01		nd			
C22:0	0.06	±	0.06		0.03	±	0.01		0.03	±	0.01	
C22:5n3	0.18	±	0.07		0.15	±	0.06		0.18	±	0.04	
C24:0	0.13	±	0.10		nd				nd			
C22:6n3	0.12	±	0.05		0.11	±	0.03		0.13	±	0.06	

Table 2. *Cont.*

Group	Control			10 mg/kg			20 mg/kg		
Fatty Acid	**Mean**		**SD**	**Mean**		**SD**	**Mean**		**SD**
Saturated	65.6	±	9.27	62.9	±	6.04	61.0	±	3.73
Unsaturated	34.4	±	9.27	37.1	±	6.04	39.0	±	3.73
Monounsaturated	9.09	±	2.80	10.1	±	1.55	10.2	±	0.97
Polyunsaturated	25.3	±	6.60	26.9	±	4.59	28.8	±	2.83
n3	0.77	±	0.48	0.58	±	0.33	0.62	±	0.14
n6	24.5	±	6.36	26.3	±	4.47	28.1	±	2.79
n6/n3	39.5	±	18.5	64.5	±	43.0	46.7	±	9.80
Odd chain FA	0.61	±	0.59	0.49	±	0.10	0.61	±	0.13
Unsaturation index	93.6	±	24.9	98.1	±	16.7	106.1	±	10.5
Average FA chain length	17.68	±	0.13	17.67	±	0.11	17.74	±	0.07

a,b: different superscripts indicate significant difference between groups means at $p < 0.05$; nd: not detected.

Minor further alterations induced by mycotoxin treatment detected were that C14:1 n5, C20:5 n3 and C24:0 were present only in the control samples, while these were absent from the FBs fed animals' samples.

2.5. Testicular Lipid Peroxidation and Antioxidants

Results of the testicular antioxidant and lipid peroxidation parameters are shown in Table 3. FBs feeding significantly increased the concentration and activity of reduced glutathione (GSH) and glutathione peroxidase (GSHPx), in both intoxicated groups, as compared to the control, in a dose dependent manner (Figure 8). Initial phase lipid peroxidation decreased slightly (CD and CT, i.e. conjugated dienes and trienes) with the increasing FB level, while end-phase lipid peroxidation (MDA) was not proven.

Table 3. Testicular antioxidant and lipid peroxidation parameters in the three experimental rabbit groups (GSH: reduced glutathione; GSHPx: glutathione peroxidase; MDA: malondialdehyde; CD: conjugated dienes; CT: conjugated trienes, U: unit, A: absorbance).

Group	Control				10 mg/kg				20 mg/kg			
GSH (micromol/g prot.)	6.57	±	0.55	a	7.54	±	0.70	b	7.90	±	0.98	b
GSHPx (U/g prot.)	6.25	±	0.66	a	7.45	±	0.85	b	7.50	±	0.65	b
MDA (nmol/g)	44.7	±	9.11		39.0	±	7.17		38.5	±	7.22	
CD (A232 nm)	0.34	±	0.02	b	0.34	±	0.03	b	0.31	±	0.02	a
CT (A268 nm)	0.16	±	0.01	b	0.15	±	0.01	ab	0.14	±	0.01	a

a,b: different superscripts indicate significant difference between groups means at $p < 0.05$.

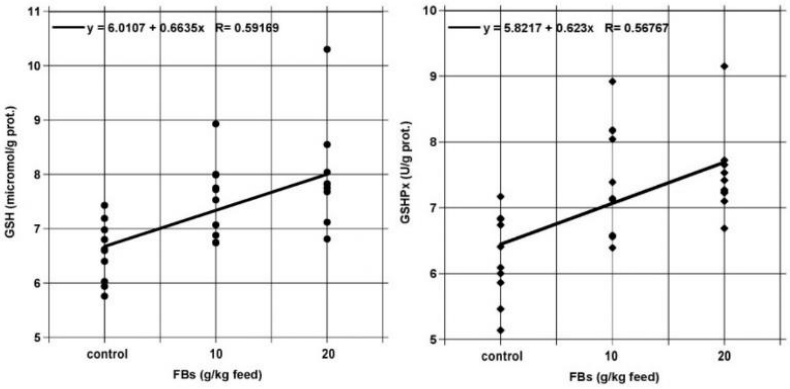

Figure 8. Linear dose-dependence for GSH (●) and GSHPx (♦) in the rabbit testis.

2.6. Testis Histology

The testicular tissue sections were evaluated in all animals and provided no group dependent differences. A typical section pair is shown in Figure 9a,b.

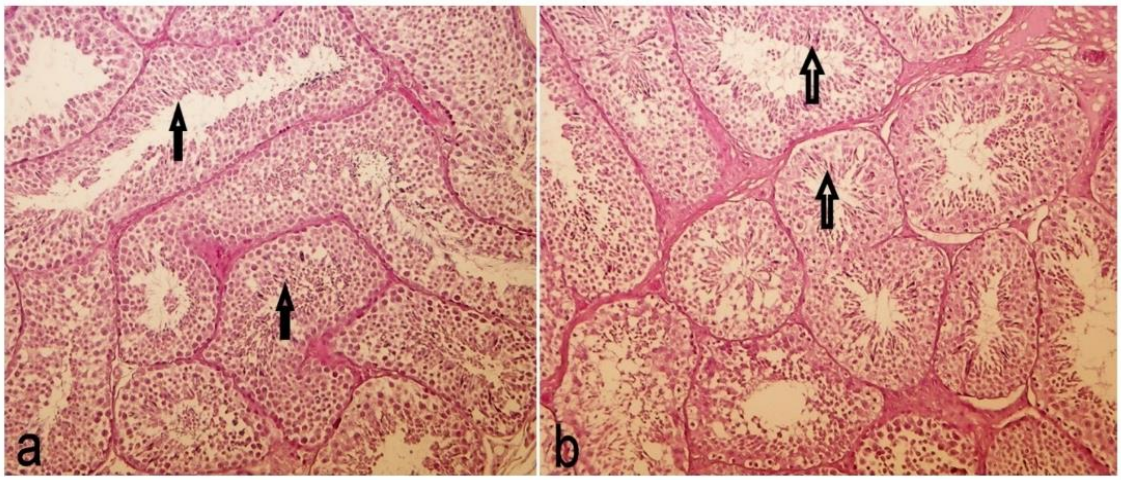

Figure 9. Testis histology of a control (**a**) and an intoxicated (**b**; 20 mg/kg fumonisin Bs, 65 days exposure) rabbit buck (Hematoxilin Eosin staining, 200× magnification).

Both the cell layers of spermiogenesis and mature spermiocytes (⇑) are well visible in the ductuli, without any detectable toxic effect.

2.7. Spermium Membrane Fatty Acid Profile

We only determined the control vs. the two intoxicated cases at the last sampling event, and since there was absolutely no systematic inter-group difference detectable, further, retrospective analysis was avoided. Data of the spermium phospholipid fatty acid profile are presented in Table 4.

Table 4. Phospholipid fatty acid (FA) composition (weight % of total FA methyl esters) of the washed spermia after 65 days of FBs exposure of rabbit bucks to 0, 10, and 20 mg/kg diet FBs (n.d.: not detected).

Group	Control			10 mg/kg			20 mg/kg		
	Mean		SD	Mean		SD	Mean		SD
C14:0	2.61	±	0.50	2.32	±	0.57	2.57	±	0.92
C15:0	0.19	±	0.07	0.49	±	0.34	0.46	±	0.35
C16:0	25.0	±	0.28	26.3	±	1.05	26.4	±	0.98
C16:1	0.68	±	0.37	0.80	±	0.12	0.78	±	0.61
C17:0	0.74	±	0.49	1.13	±	0.61	1.04	±	0.12
C17:1n7	0.18	±	0.03	0.24	±	0.12	0.28	±	0.14
C18:0	47.5	±	3.76	39.0	±	9.26	41.1	±	6.85
C18:1n9	9.62	±	3.41	14.9	±	6.85	13.3	±	6.96
C18:1n7	0.61	±	0.06	0.82	±	0.33	0.97	±	0.44
C18:2n6	5.01	±	1.98	8.54	±	3.90	7.16	±	2.45
C18:3n6	0.02	±	0.01	0.09	±	0.06	0.06	±	0.03
C18:3n3	0.10	±	0.01	0.08	±	0.07	0.05	±	0.02
C20:0	n.d.			0.15	±	0.03	0.17	±	0.15
C20:1n9	0.08	±	0.02	0.08	±	0.05	0.02	±	0.01
C20:3n9 (Mead acid)	0.31	±	0.05	0.26	±	0.15	0.28	±	0.19
C20:3n6	3.18	±	0.80	2.21	±	1.14	2.64	±	1.28
C20:4n6	3.11	±	0.93	1.80	±	0.91	1.93	±	1.05

Table 4. *Cont.*

Group	Control			10 mg/kg			20 mg/kg		
	Mean		SD	Mean		SD	Mean		SD
C20:5n3	0.28	±	0.01	0.21	±	0.07	0.17	±	0.05
C22:0	0.11	±	0.01	n.d.			0.05	±	0.04
C22:1n9	0.08	±	0.01	n.d.			0.07	±	0.01
C24:0	0.41	±	0.01	0.11	±	0.06	n.d.		
C22:6n3	0.37	±	0.02	0.29	±	0.18	0.34	±	0.11
C24:1n9	0.19	±	0.16	0.19	±	0.07	0.14	±	0.13
Saturated	76.4	±	4.17	69.6	±	8.99	71.9	±	8.23
Unsaturated	23.6	±	4.17	30.4	±	8.99	28.1	±	8.23
Monounsaturated	11.4	±	4.00	17.0	±	7.34	15.5	±	8.30
Polyunsaturated	12.2	±	0.17	13.4	±	1.88	12.6	±	0.07
n3	0.56	±	0.14	0.51	±	0.24	0.56	±	0.04
n6	11.3	±	0.26	12.6	±	2.18	11.8	±	0.15
n6/n3	20.8	±	5.59	37.4	±	36.4	21.1	±	1.62
Odd chain FA	0.93	±	0.56	1.62	±	0.93	1.50	±	0.23
Unsaturation index	47.4	±	1.15	51.7	±	7.28	49.5	±	4.37
Average FA chain length	17.54	±	0.01	17.45	±	0.04	17.45	±	0.01

3. Discussion

3.1. Animal Performance

The rabbit production performance has also been tested during FB_1 intoxication in our earlier study, when 10 mg/kg FB_1 for 4 weeks did not compromise rabbit buck growth [18]. Ewuola (2009) [13] fed rabbit bucks FB_1 (0.13–5–7.5–10 mg/kg diet) for 196 days and found no throwback in BW. At the same FB_1 concentration range for 175 days, the onset of puberty was delayed by the two higher doses (i.e., 7.5 and 10 mg/kg), but bodyweight at puberty was not significantly influenced by the toxin. Most probably the exposure time length is the explanation of the unaltered growth, since growth inhibition has already been proven for FB_1 at 35 mg/kg diet (rats) [19]. The slight, non-significant, but likewise mycotoxin dose associated BW curves in Figure 1 might refer to some growth depression, but without statistical difference, thus it is void to discuss it.

From the splanchnic organs the higher fumonisin dose decreased liver (absolute and relative) and kidney weight; hepatotoxic and nephrotoxic effect of FB_1 in rabbit is well known [20], but it seems to depend as well on exposure time. In a shorter setting (4 weeks at 10 mg/kg) we found hepatic mitochondrial modifications, but without organ mass difference [18]. In contrast to earlier rabbit testicular results [13,14] with a likewise dose-associated organ weight increase, here we failed to detect any alteration in the testicular weights. We suppose that the reason of this may be that in relevant studies [13,14] growing rabbits were enrolled, meanwhile we started to feed adult, producing males in this study.

3.2. Testicular Phospholipid Fatty Acid Composition

The phospholipids (PL) of biological systems are parts of an adaptive, responsive, sensitive domain [21]. We are not aware for any reference data for rabbits in the literature, only Morin (1967) [22] published results for rabbit testicular PLFA. The analysis of whole testicular phospholipids is beyond doubt an approach focusing on multiple cell types; however, this is the lipid fraction that possesses the highest proportion of the polyunsaturated FAs. Testes and spermia show characteristic lipid composition that is rich in longchain polyunsaturated FA (PUFA), primarily DPA (C22:5n3, docosapentaenoic acid) in rats and other rodents [23]. When the channeling of dietary PUFA was checked, it was reported that the primary site of PUFA incorporation is the Sertoli cell population. When exposed to toxic stimulus by FB_1 (at 7.5 and 10 mg/kg diet for 196 days), Sertoli cell lesion was reported to occur at a moderate level in rabbit testis [12]. Anyway, fumonisin B series induced effect has not yet been tested on the testicular total PLFA profile, especially not at

that high exposure level used in this study. Thus, we challenged the animals with a more drastic intoxication, but what we found was negligible.

The only FA that provided a significant proportional alteration was margaric acid (C17:0). This acid is absorbed after coecotrophy into the tissue lipids and is not a product of de novo synthesis. Since the diet did not contain C17:0 in a marked proportion (Table 5), neither feed intake was different among the groups (data analyzed, but not shown), we assume that a change of C17:0 is related to the amount of ingested coecotroph by the host animal, as shown in growing rabbits with the increasing importance of coecotrophy [23,24]. Though we are not aware of the real biological reason, in multiple studies in the past our team detected the significant modification of odd chain FAs (mostly C15:0 and C17:0) in the tissue PL FA profile (rabbit liver ↑, rat liver: C17:0 ↓) [18,25].

Table 5. Chemical and fatty acid composition of the basal diet of the experimental rabbits.

Chemical Composition		Fatty Acid (Diet)	Weight % of Total FAME
Dry material (%)	89.0	C12:0	0.05
Crude protein (%)	14.5	C14:0	0.18
Ether extract (%)	2.4	C15:0	0.14
Crude fibre (%)	17.1	C16:0	14.3
Ash (%)	7.5	C16:1n7	0.20
Lysine (%)	0.90	C17:0	0.12
Methionine (%)	0.41	C18:0	2.73
Calcium (%)	0.88	C18:1n9	36.8
Phosphorus (%)	0.52	C18:1n7	0.84
Sodium (%)	0.19	C18:2n6	38.6
Vitamin A (IU/kg)	14000	C18:3n3	3.83
Vitamin D3 (IU/kg)	1300	C20:0	0.42
Vitamin E (mg/kg)	107	C20:1n9	0.50
Digestible energy (MJ/kg)	9.7	C20:2n6	0.04
		C21:0	0.04
		C20:4n6	0.07
		C22:0	0.61
		C24:0	0.45
		C22:6n3	0.08
		Saturated	19.0
		Unsaturated	80.9
		Monounsaturated	38.3
		Polyunsaturated	42.6
		n3	3.91
		n6	38.7
		n6/n3	9.89
		Odd chain FA	0.26
		Unsaturation index	127.8
		Average FA chain length	17.75

The fatty acid profile of testicular phospholipids in germinal and Sertoli cells is a function driven property and is a sensitive indicator of essential FA (EFA) supply or its disturbance [26]. EFA deprivation is reflected in the testicular lipids after 9–14 days [26] and is as well detectable on the cellular accretion of Mead acid (C20:3 n9). We recorded Mead acid proportion (Table 2), but were unable to report dose or group dependent variations, meanwhile present in the tissue PLs in detectable amounts.

In summary, feed intake was identical in the three groups, and feed fatty acid profile (Table 5) was relatively rich in EFA, deficiency was not supposed (Mead acid proportion), meanwhile the overall level of PL unsaturation was relatively low. When EFA deficiency is discussed, not merely the dietary FA supply, but the intensification of lipid peroxidation must as well be considered.

3.3. Testicular Lipid Peroxidation and antioxidants

The fumonisin intoxication increased the testicular GSH level, as compared to the control; the increase was not only significant, but provided linear dose dependence (Figure 8). Such a direct oxidative effect of fumonisins has not yet been reported, but heat stress in rabbits has been found to act similarly [27]. According to Aydilek et al. [28], the overall improvement of rabbit antioxidant capacity (e.g., by vitamin E feeding) is accompanied by increased testicular GSH level, and in a more wide context, this effect was shown in case of fumonisin intoxication in porcine liver (20 mg FB_1/kg diet for 10 days) [29], and at a lower fumonisin level (10 mg/kg dietary FB_1 for 4 weeks) the opposite was found in the liver of rabbits [18]. Directly relevant mammalian comparison for fumonisin intoxication is unavailable, but at a high and even low dietary FB_1 dose (600 and 10 mg/kg diet) chicks and broilers provide hepatic oxidative stress [30]. The FBs induced direct oxidative stress is a less studied topic. In contrast, oxidative stress (post-ischemic reperfusion) itself in the leporine testis is a known phenomenon and is accompanied by the increased activity of the enzymatic antioxidant system (GSHPx) [31]. Since testis is a relatively hypoxic microenvironment enriched with a high unsaturated fatty acid proportion [31], its oxidative damage indicators are relatively sensitive.

Currently, there is no full compliance whether fumonisins are direct oxidative stress inducers or this plausible effect is playing a carcinogenic role [32]. Anyway, the molecular mechanisms behind fumonisin toxicity in liver and kidney are supposed to be linked with early events of oxidative stress [33], but the role of them in the male reproductive organs is less studied. As well, a direct link between fumonisins and the glutathione redox system is not fully elucidated; in glutathione peroxidase-1/catalase knocked out (KO) mice FB_1 toxicity was not influenced by the existence or lack of the induced mutation (KO), referring to a likewise indirect relationship between FB_1 and oxidative stress [34]. If fumonisin is really not a direct induction factor of the slight oxidative stress detected, then there shall be a plausible alternative process responsible for the dose dependent increase in the tissue GSH level and the associated reaction of GSHPx. Recent and emerging evidence at the molecular level suggests the disruption of mitochondria and excessive generation of toxic, reactive oxygen species as additional mechanisms of toxicity [32]. We assume that quickly proliferating cells (like hepatic and germinal cells) of rabbits may be prone for FBs induced oxidative stress at a subclinical intoxication level, without markedly compromised function. A more general view might be the supposal of slight cellular apoptosis and necrosis, which has only been shown in the renal and hepatic cases [34], but this has not been proven or tested in this study. Ultimately, it shall be added that FBs acted like a slight pro-oxidants in the rabbit testis, augmenting non-enzymatic and enzymatic adaptation (GSH and GSHPx), leading to the lowered concentration of conjugated fatty acid derivatives (dienes and trienes), as early phase lipid peroxidation indicators. This plausible adaptation (exposure: 65 days) was effective, since PLFA profile was unaltered and MDA (as late peroxidation indicator) as well.

3.4. Testis Histology

The testicular tissue sections were evaluated in all animals and provided no group dependent differences at all. Intact germinal epithelium, spermiogenesis and mature spermiocytes are well visible in the ductuli, without any detectable toxic effect.

In a prolonged setting [13], the paired testes weight, the seminiferous tubule diameter, and the volumetric proportion of the testicular elements with the exception of the secondary spermatocytes and the Leydig cells provided FB_1 associated alterations. Though we did not analyze all cell types in such detail as did Ewuola and Egbunike [13,14], we did not find any difference, neither in the paired testicular weight (Table 1), nor in the seminiferous tubule diameter. However, as compared to the data of Ewuola and Egbunike [13,14], the present study enrolled markedly larger bucks and the paired testicular weight was more than two-times higher, as compared to the cited source data. We were unable to detect any

patho-morphological signs, while related works [13] refer to depletion of sperm reserves as a mycotoxin effect.

In summary, adult, producing rabbits seem to tolerate relatively high (10 and 20 mg/kg) fumonisin intoxication without detectable testicular patho-morphological alterations.

3.5. Spermium Phospholipid Fatty Acid Profile

Fatty acids in male reproduction system are associated with cellular membrane fluidity, acrosome reaction, sperm motility, and viability [5]. Though we did not detect any inter-group differences as possibly evoked by the toxic effect, additionally we found a relatively low level of (poly)unsaturation in the FA profile [35]. Comparing data to those of similarly prepared rabbit spermatozoa PL FAs, our rabbit samples had a higher level of saturated fatty acids, lower mono-unsaturation (11.4 vs. 15.9 %), markedly lower n3 proportion, but higher n6 FA ratio (5.7 vs. 11.3%). Comparing our dataset to a very early report [36], we found more similar data, but still the present n3 FA proportion was lower. The basic difference observed between the two datasets may be based on the nutritional provision of the n3 fatty acids. The dietary FA profile seems to provide basic support that our animals were taking up a low n3 FA amount (Table 5), but their essential fatty acid demand was fulfilled, as shown by the Mead acids constant proportion (Tables 2 and 4).

When seeking FB_1-induced spermium compositional or functional modifications, Ewuola and Egbunike [13] reported decreasing sperm mass activity, motility, and live proportion of spermatozoa of the rabbits in parallel with an increase in the dietary FB_1 concentration. Mice, rats, and rabbits undergo the disruption of sphingolipid metabolism as a result of FB_1 feeding, but at sub-clinical levels morphologic evidence is lacking [5]. Fumonisin exposure is not limited to the perturbation of the sphingolipid metabolism; it induces imbalances in phosphoglycerolipid and fatty acid metabolism, though never shown for spermia in vivo; additionally, there is only one equine report on an in vitro test [11]. Neither their nor our test results are robust; these suggest that spermia are not sensitive targets of FBs. Though we directly targeted phosphatides, these were found to be absolutely unresponsive on the treatment applied.

The ultimate reason for this may be (1) the low sensitivity against oxidative stimuli, as shaped by the fatty acid profile that has been a result partly of the (2) less unsaturated diet. In addition, we must admit that not only the membrane composition, but (3) other parameters (live cell proportion, sperm morphology, and chromatin status were as well practically unaltered).

4. Conclusions

When feeding producing, adult, male rabbit bucks with high fumonisin levels for over a whole testicular cycle, no marked alteration was detected at five sampling timepoints in the spermatozoa endpoints (live cell proportion, morphological distribution, membrane lipid profile) and only minimally increased antioxidant defense was provoked in the testes (without lipid profile or histological modifications). Results refer to minimal pro-oxidant effect of fumonisins on the male reproductive system without marked harmful effect on the tested spermatological traits.

5. Materials and Methods

5.1. Animals and keeping

Altogether, 3 × 10 Pannon White rabbit bucks were enrolled in the study at the starting age of 24 weeks. The animals were already in production and underwent sperm collection weekly once before the study. Bodyweight (BW) and feed intake (FI) was recorded throughout the experimental period (BW: Table 1 and Figure 1; FI was recorded, but not shown). Feed was offered ad libitum, as well as drinking water from nipple drinkers. Feed (a commercial rabbit buck feed without medication) chemical composition and fatty acid profile is given in Table 5. The somatic data of the three groups before the experiment and at slaughter are given in Table 1. The rabbits were caged individually in a

rabbit stable of controlled environment. The photoperiod was natural in the stable (2018 October–November). The study lasted for a total of 65 days and on day 67 animals were euthanized by exsanguination after sedation (Euthanyl-Pentobarbital Sodium, 400 mg/mL, Dechra Veterinary Products, Shrewsbury, UK) and splanchnic organs, testes, and blood were sampled. During the study period, altogether five times, sperm samples were taken for cell integrity and viability analysis. Ejaculate collection was performed with water (37 °C) filled artificial vaginas having a collection tube as an attachment, performed by the caretaker, using a rabbit fur as a phantom. The collection tubes were immediately incubated to 37 °C.

5.2. Feed Mycotoxin Contamination

The basic feed was of commercial origin (Table 5). A *Fusarium verticillioides* fungal culture of high FB_1 concentration (for production details see: [37] culture name: RL 596) was mixed into the ration of the experimental animals, so as to provide a daily FBs (FB_1+FB_2+FB_3) feed concentration of 10 and 20 mg/kg. The mycotoxin concentration of the control and the experimental feed was determined with LC-MS) [38]. The limit of detection (LOD) for FB_1 was 3 µg/kg. The diet fed to the control group did not contain detectable amounts of FBs (the full absence of deoxinivalenol, zearalenone and T-2 toxin was as well controlled and confirmed).

5.3. Evaluation of Sperm Morphology, Chromatin Integrity, and Viability

Fresh semen samples were immediately transferred to the laboratory at 37 °C. The samples were used for the preparation of smears and for differential staining for flow cytometry.

Smears were dried at room temperature and were stained after Feulgen with a staining kit (Merck-Sigma, Schnelldorf, Germany, Cat. No. 1079070001) according to Barth and Oko [39]. The smears were protected with cover plates using Entellan mounting medium (Merck–Sigma Cat. No. 1079600500) and the cell evaluation was based on visual counting (200 cells/smear) on digital images taken at 400× magnification with an Olympus CX-41 (Olympus, Tokyo, Japan) phase contrast microscope equipped with a digital camera.

Flow cytometry was performed with a Molecular Probes Inc. (Eugene, OR, USA) LIVE/DEAD sperm Viability Kit (L-7011) containing SYBR14 and propidium iodide (PI). The staining protocol followed the description of Nagy et al. [40]. In brief, 100 nM SYBR 14 working solution (Component A of the LIVE/DEAD Sperm Viability Kit, diluted 10-fold with dimethyl sulfoxide 10 µl), and 2.4 mM PI stock solution (undiluted Component B of LIVE/DEAD Sperm Viability Kit, 2 µl) were added to 1 mL sperm (extended to approximately 1×10^6/mL in pre-warmed phosphate buffered saline). Samples were incubated at 37 °C for 10 min in darkness.

The samples were transferred immediately after incubation for flow cytometric analysis. A Partec CyFlow Space equipment (Sysmex Partec GmbH, Görlitz, Germany) was operated with the FloMax software (ver. 2.9.), with a two-laser design (20 mW at 488 nm blue solid state laser and a 40 mW at 635 nm red diode laser). The flow speed was 25 µl/sec and acquisitions were stopped after recording 5000 total events. SYBR14 fluorescence (FL) intensity was recorded on detector FL1 (green) while PI fluorescence intensity on FL3 (red), on log scale. Data files were stored in standard FCS file format.

Flow cytometric results were evaluated with the FloMax software (ver. 2.9., Partec GmbH, Görlitz, Germany), and the live/dead cell ratio expressed as % was handled as end result.

5.4. Determination of the Testicular and Spermium Phospholipid Fatty Acid Composition

Samples of circa 300 mg raw testicular tissue (after frozen storage at −70 °C) and the feed were homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in 20-fold volume of chloroform:methanol (2:1 v:v) and total lipid content (complex lipids) was extracted [41]. Sperm samples of the last sampling underwent 3× washing in 10-fold

volume of phosphate buffered saline, and the washed cells were extracted (to gain complex lipids) as above. Solvents were ultrapure-grade and 0.01% w:v butylated hydroxytoluene was added to prevent fatty acid oxidation. For the separation of lipid fractions, extracted complex lipids were transferred to glass chromatographic columns, containing 300 mg silica gel (230–400 mesh) for 10 mg of total lipids [42]. Neutral lipids were eluted with 10 mL chloroform for the above fat amount, then 15 mL acetone:methanol (9:1 v:v) was added, while 10 mL pure methanol eluted the total phospholipids. This latter fraction was evaporated under a nitrogen stream and was transmethylated with a base-catalyzed $NaOCH_3$ method [43]. Fatty acid methyl esters were extracted into 250 µL ultrapure n-hexane for gas chromatography. After a separation on a Phenomenex Zebron ZB-Wax capillary column (30 m × 0.25 mm × 0.25 micrometer film, Phenomenex Inc., Torrance, CA, USA). The chromatographic evaluation was performed with the LabSolutions 5.93 software, using the PostRun module (Shimadzu, Kyoto, Japan) with manual peak integration. Fatty acid composition was expressed as weight % of total FA methyl esters (g FAME/100 g of total FAME). The identification of the FAs was performed based on the retention time of a certified reference material external standard FA mix (Supelco 37 Component FAME Mix, Merck–Sigma Aldrich, CRM47885).

5.5. Testicular Antioxidant Status and Lipid Peroxidation

For the determination of lipid peroxidation and antioxidant status, whole tissue samples were stored at −70 °C until analysis. Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) levels with 2-thiobarbituric acid method [44], and the determination of conjugated dienes (CD) and trienes (CT) according to the photometric method of AOAC (1984) [45]. The concentration of reduced glutathione (GSH) was measured by the method of Sedlak and Lindsay [46] and the activity of glutathione peroxidase (GSPHx) according to Lawrence and Burk [47].

5.6. Histopathology

Tissue specimens were stored in 10% neutrally buffered formalin and were embedded into paraffin. For light microscopic analysis microtome slides of five micrometer were prepared and stained with hematoxylin-eosin. The main pathological alterations have been described and scored according their extent and severity as follows: 0 = no alteration, 1 = slight/small scale/few, 2 = medium degree/medium scale/medium number, 3 = pronounced/extensive/numerous. The histopathological analysis was performed according to the Act #2011 (03.30) of the Hungarian Ministry of Agriculture and Rural Development and was in accordance with the ethical guidelines of the OECD Good Laboratory Practice for Chemicals [48].

5.7. Statistical Analysis

For the comparison of the group means (enzyme activity, initial and final bodyweight, fatty acid profile data within single rows) univariate (FBs concentration as grouping variable) analysis of variance (ANOVA) was used, with the LSD (least significant difference) "post hoc" test for detailed the inter-group differences. The distribution of the different morphologic spermium groups was compared with the chi^2 probe. Spermatological variables gained from the five consecutive samplings, at each sampling time were compared with ANOVA; time dependent alteration of the different three groups was tested with repeated measures analysis. For all tests significance level was set to $p \leq 0.05$. IBM SPSS 20 for Windows (2010) [49] was used for the evaluation.

5.8. Ethical Issues

The experiments were carried out according to the regulations of the Hungarian Animal Protection Act. The allowance number for the studies was SOI/31/00308-1/2017 (KA2114) (date of approval: 27 March 2017).

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request due to restrictions e.g., privacy or ethical (The data presented in this study are available on request from the corresponding author. The data are not publicly available due to [large dataset and data are not self explanatory].).

Conflicts of Interest: The authors declare no conflict of interest.

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5. GENERAL DISCUSSION

In the first study, the fatty acid compositions of PC, PE, and PI in the renal and hepatic tissues of rats were investigated after intraperitoneal exposure to FB_1 at dietary doses equivalent to 0, 20, 50, and 100 mg/kg^{-1} for durations of 5 and 10 days. It is important to highlight that a part of this study was published in an earlier paper, where FB_1 doses above 20 mg/kg^{-1} were capable of compromising rat performance and feed intake, as well as inducing lipid peroxidation and histological lesions in the liver and kidneys (Szabó *et al.*, 2018). It is established that the kidney and liver are the primary target organs for FB_1 toxicosis in rodents, in a species-specific manner. The current study revealed that FB_1 causes more alterations in the kidney, particularly in a dose- and time-dependent manner, suggesting the roles of sphingolipid disruption and oxidative stress. These findings align with those of Voss *et al.* (2001), who observed more pronounced nephrotoxicity than hepatotoxicity in rats exposed to FB_1. Concerning the kidney, there is a lack of data on the fatty acid composition of various phospholipid fractions in response to FB_1, and no study has reported dose- and time-dependent fatty acid responses to FB_1. Among the inspected fractions, renal PC exhibited the most dose- and time-dependent fatty acid responses (characterized by a negative dose-response between FB_1 and PUFAs (both n6 and n3) and a positive dose-response with MUFAs), followed by renal PI (negative dose-response in ΣPUFA). Notably, liver PE was the only investigated fraction providing an R^2 value above 0.6, revealing a positive dose-response in Σn3 fatty acids.

The observed fatty acid changes (C20:2 n6 (DGLA), AA, EPA, docosapentaenoic acid (C22:5 n3, DPA-n3), and DHA) in renal PC were consistent with Zhao *et al.* (2015), confirming their potential role in nephropathy. DHA, notable for its reductive capacity, is sensitive to oxidative stress. Zhao *et al.* (2015) reported that renal PCs containing DHA and palmitic acid were targeted by the carcinogen aristolochic acid, a finding corroborated by the present study. A further marked reduction in the PC fraction was the proportion of AA, a common eicosanoid precursor. Its cleavage from phospholipids, catalyzed by PLA2, results in free AA and LysoPC. Although PLA2 activity was not analytically determined in this study, the decrease in AA and other n3 fatty acids suggested PLAS2 increased activity.

In the case of renal PI, there are limited toxicological reports, specifically regarding alterations in AA levels (Hiraide *et al.*, 2016). AA levels remained unchanged under all experimental settings (dose and time), but a decrease in DHA and an increase in both palmitic and stearic acid were observed. DHA, a precursor of D-resolvins, may undergo selective cleavage (Weylandt *et al.*, 2012). The findings also

highlight the role of lipid peroxidation (increased MDA), as decreases in ΣPUFA and its respective fatty acids were observed.

Renal PE displayed marked modifications in Σn6 and its respective LA, indicating proportional decreases. LA, an essential fatty acid, has been proposed as a biomarker of chronic renal failure in rats (Zhao *et al.*, 2013). The decrease in LA and its metabolites could be attributed to reduced feed intake due to FB_1 intoxication, which would also indicate a decrease in fatty acids derived from LA metabolism. Notably, C20:3 n6, AA, and Σn6 were decreased in renal PI. LA metabolites (PE18:4/22:4, PE20:3/18:1, PE20:0/18:0, PE22:5/20:3, PE16:0/20:4, PE18:2/20:4, and PE16:0/18:2) have been implicated in aristolochic acid-induced nephropathy and ochratoxin A exposure (Yang *et al.*, 2019; Zhao *et al.*, 2015). These authors also observed elevated levels of ΣSFA during nephropathy, a finding corroborated by this study. The decrease in ΣPUFA and its respective fatty acids might be a result of oxidation associated with previously published histological modifications, including tubular degeneration, necrosis, the detachment of tubular epithelial cells, atrophic or fully absent internal epithelium, and undeveloped hyalin cones at 50 and 100 mg kg^{-1} dietary dose equivalents (Szabó *et al.*, 2018). Renal failure, reported during nephrotoxicity (Yang *et al.*, 2019; Zhao *et al.*, 2015), has been proposed to emerge from renal mitochondrial dysfunction and the oxidation of fatty acids (Yang *et al.*, 2019).

Alterations in the fatty acid compositions of rat liver phospholipid fractions were observed, albeit not as pronounced (neither dose- nor time-dependent) as they were in the kidney. In the liver PCs, fatty acid modifications (ΣMUFA, oleic acid, DGLA, and AA), to some extent, were somewhat similar to those in the kidney PCs. Burger *et al.* (2007) reported similar findings in microsomal membranes at a relatively drastic FB_1 dose (250 mg/kg feed), suggesting a shift towards more rigid membranes. An increase in the oleic acid proportion is thought to counteract the impact of other PUFAs on membrane homeostasis and fluidity (Gelderblom *et al.*, 2002). Thus, the increase in MUFAs possibly compensates for the decrease in PUFAs; however, it still indicates potential changes in hepatocellular membrane rigidity, although membrane rigidity was not determined. Though PUFAs are prone to oxidation, this scenario seems plausible in the 5-day setting but not in the 10-day setting. Oxidative stress (elevated MDA) was observed only in the 5-day setting, confirming that PUFAs are susceptible to oxidation. However, by day 10, the liver mitigated ROS and increased Σn3 in the PC since no marked difference was detected between the control and intoxicated animals. This finding suggests that the marked depletion of DGLA and AA was caused by the production of their bioactive lipid

mediators, which are involved in inflammation. (Abel *et al.*, 2001) proposed a role for the decrease in AA in primary hepatocyte nodule formation, while Gelderblom *et al.* (1999) endorsed the inhibitory role of DGLA bioactive lipid mediators.

Gelderblom *et al.* (2002) previously reported fatty acid alterations in rat liver PI fractions due to FB_1 exposure. Proportional increases in ΣMUFA, oleic acid, and n6 and n3 fatty acids were observed after exposure to 250 mg FB_1/kg feed for 3 weeks. Similar findings were noted on day 10 of this study, but not on day 5. Depletions in ΣPUFA were observed after 5 days, suggesting a possible role of oxidation in these findings. The results from days 5 and 10 suggest that the potential effects may vary with exposure duration. It is plausible that during longer exposure (10 or 21 days), enzymatic antioxidant adaptation compensates for lipid peroxidation progression, as indicated by glutathione peroxidase (Szabó *et al.*, 2018).

Investigations into the rat PE fraction revealed minimal and largely nonsystematic fatty acid modifications. Notable alterations included DGLA depletion in intoxicated rats and elevations in the proportion of DHA and Σn3 in rats exposed to 100 mg FB_1. In addition, Σn3 was the only parameter showing a dose-response on day 10. Earlier studies by Burger *et al.* (2007) and Gelderblom *et al.* (2002) reported increased MUFAs and PUFAs in the rat microsomal membrane PE fraction following exposure to 100 or 250 FB_1/kg diet for 3 weeks, suggesting potential changes in D6D and D5D enzyme activities. However, the current study revealed a decrease in Σn6, which corresponded to an increase in Σn3. This finding suggests that fatty acid modulation did not affect ΣPUFA. Moreover, Burger *et al.* (2007) reported similar findings, but a comprehensive explanation for the increased Σn3 proportion in the PE fraction is still lacking. According to Gelderblom *et al.* (2002), this may necessitate a targeted study on phospholipid-metabolizing enzymes. The fatty acid indices used to estimate D6D and D5D activity decreased in the FB_1-treated groups, specifically the ratio of C18:2n6/C18:3n6 (LA/GLA), indicating D5D inhibition. This suggests an increase in GLA proportions. However, the data illustrate no effect of FB_1 on GLA but a decrease in LA, possibly due to lower dietary intake in intoxicated rats compared to controls.

The second study investigated the effects of oral FBs (FB_{1+2+3} at 15 and 30 mg/kg feed for 3 weeks) on the enzymatic regulation of cation flux in porcine erythrocytes by measuring the Na^+/K^+ ATPase activity and fatty acid composition of erythrocytes. This study also examined the potential relationship (correlation) between erythrocyte fatty acids and Na^+/K^+ ATPase activity. In regard to cation active transport, the observed increases in Na^+/K^+ ATPase activities were consistent with earlier findings in

rabbits exposed to FB_1 (Szabó *et al.*, 2014); however, this study additionally revealed a positive dose-response with an R^2 value of 0.58. These data suggest that ceramide, a typical FB target of action (via CerS inhibition), plays a role in altering intracellular ceramide accumulation rates. Ceramides are known to directly modulate Na^+/K^+ ATPase activity in basolateral membrane of renal cells (Cabral *et al.*, 2010), a finding also reported in an *in vitro* HepG2 cell model (Kreydiyyeh and Dakroub, 2014). Ceramides can also inhibit the ouabain-sensitive sodium pump in renal cells, through protein kinase A and C modulation (Cabral *et al.*, 2010). However, the ouabain-sensitive sodium pump is ubiquitous and influenced by multiple factors, including hormonal changes, substrate concentration, and the physicochemical properties of the embedding membranes (Wu *et al.*, 2004; Zhang *et al.*, 2008). Disturbances in K^+ homeostasis may be attributed to FB_1's modulation of the apoptotic process, which typically involves changes in cellular ion exchange dynamics, with K^+ as a triggering factor (Hughes *et al.*, 1997; Kreydiyyeh and Dakroub, 2014). It is important to highlight that apoptosis and proliferation were not analytically assessed in this study; therefore, further investigations are necessary to support this proposal.

In an earlier study by Szabó *et al.* (2014), minimal modifications in the fatty acid composition of rabbit erythrocytes were observed after 14 and 28-days of exposure to a 10 mg FB_1/kg diet. In the current study, both lower and higher FBs' doses resulted in divergent effects, which are difficult to interpret. Focusing specifically on the divergent alterations provoked by the two doses (lower vs. higher), increased oleic acid and AA, decreased ΣSFA (decreased palmitic acid), and elevated ΣMUFA were observed. These modifications, associated with the higher dose of FBs, unequivocally indicate a more rigid physicochemical property of the cell membrane. Furthermore, these alterations were unrelated to the peroxidative effect, as neither the antioxidant enzymatic activity nor the end product of lipid peroxidation in erythrocytes was altered. Despite the high proportion of PUFAs in erythrocytes, they lack a nucleus and, notably, mitochondria. Thus, an increase in lipid peroxidation was not anticipated in this study.

A Pearson correlation analysis between altered enzyme activities and fatty acid composition revealed a negative correlation with PUFAs, particularly n6 fatty acids. Free fatty acids (FFAs), or those released from the membrane by PLA2, typically inhibit the Na^+/K^+ ATPase (Therien and Blostein, 2000), but this study revealed only minor modifications, with no marked depletion of these fatty acids within erythrocyte membranes. Else *et al.* (2003) reported similar associations between sodium-potassium pump activity and n6 fatty acids, as well as additional positive correlations with long-chain

and Σn3 fatty acids. Despite the high incorporation of palmitic acid, oleic acid, and LA into erythrocytes, where they largely remain as FFAs, they did not alter the molecular activities of ion pump enzymes. It is important to understand that the dataset presented is based on fatty acid methyl esters released from ester bonds with base catalyzed methyl ester formation (Christie, 1982), thus excluding all FFAs. Despite ongoing debates about the roles of fatty acids in the sodium-potassium pump, these results highlight its sensitivity to the fatty acid composition of membranes.

In the third study, the performance of piglets and their fatty acid compositions in liver and lung membrane lipid fractions (SM, PC, PE, PS, and PI) were investigated. Notably, animal efficiency (growth and feed intake) remained unaltered with either a 15 or 30 mg FBs/kg diet for 28 days, contradicting reports by Fodor *et al.* (2005) and Gbore (2009). This discrepancy may be due to variations in experimental settings such as dose, exposure period, genotype, age, and mycotoxin source form and purity. Notably, the lung weight (absolute or relative) was unaffected, and PPE was detected only in a single intoxicated piglet, suggesting the absence of severe toxicity.

Upon assessing the fatty acid composition of SM in both the liver and lung, ΣSFA exceeded 70%, which is consistent with prior reports (Dobrzyń and Górski, 2002). In the liver SM, the highest dose of FBs increased lauric (C12:0) and behenic acids (two-fold and 1.5-fold, accordingly), while decreasing arachidic acid without altering ΣSFA. This finding supports Loiseau *et al.*'s (2015) findings in piglet liver, where 1.5 mg FB_1/kg BW for 9 days increased the level of SM-d18:1/22:0. These patterns suggest high ketoacyl-CoA synthase activity (markedly increased SM-C22:0/C20:0, *data not shown*) and/or inhibition of CerS due to FBs exposure. In the lung SM, the effects of FBs were more pronounced, with decreased ΣSFA, and increased erucic acid (C22:1 n9) and ΣMUFA. The depletion in ΣSFA, possibly indicating decreased CerS-3 activity, was due to myristic acid (C14:0) depletion. Erucic acid and MUFA generally have a protective effect against cytotoxicity, especially in cancer cell lines, but oleic acid is associated with metabolic and inflammatory lung diseases (Lopez *et al.*, 2014). Hence, the high proportions of erucic and oleic acids in the lungs may be the result of diverse stimuli, a protective mechanism and an injury trigger, respectively.

In both tissue types, the PC fractions showed increased levels of myristic and oleic acids, while stearic acid levels decreased markedly. These findings agree with previous *in vivo* and *in vitro* studies (Ali *et al.*, 2021; Burger *et al.*, 2007; Riedel *et al.*, 2015), including the first study on rats (Szabó *et al.*, 2019). The depletion of stearic acid is likely due to elevated SCD activity, as indicated by the low PC-C18:0/C18:1n9 and PC-C16:0/C16:1n7 ratios in the tissues studied. Alterations in MUFAs (notably,

dose-dependent increases in oleic and erucic acids) could substantially affect the health of the lungs. These findings are likely similar to those reported by Ali *et al.* (2021) for total phospholipids in piglet lungs. The elevation in ΣMUFA may compensate for the depletion in ΣSFA level, which is crucial for membrane rigidity. This is supported by the decreased ΣSFA-to-ΣMUFA ratio (data not shown). Rather than merely ensuring membrane rigidity maintenance, the increase in ΣMUFA levels may alter transmembrane signaling and the cell cycle (Cao *et al.*, 2008). There were also notable decreases in n6 (LA and AA) and n3 (DPA and DHA) fatty acids in PCs, partially corroborating the findings of Burger *et al.* (2007) and Szabó *et al.* (2019). However, the findings for AA, Σn6, and its ratio to Σn3 were inconsistent with those of Ali *et al.* (2019), likely due to differences in the fractions analyzed and exposure duration. Notably, the depletion of AA in PC suggests increased PLA2 activity, which preferentially cleaves AA and DHA from phospholipids (Ghosh *et al.*, 2006; Strokin *et al.*, 2003). The concurrent depletion of AA, DPA, and DHA in the intoxicated groups may also indicate inhibited D5D and D6D activities. This is further supported by the findings in the porcine lung-PC fraction, which showed remarkable modifications in PC-C20:4n6/C20:3n6 (ΔD5, low in the highest FBs setting, data not shown), PC-C18:3n6/C18:2n6 (Δ6D, low in all FBs treated animals, data not shown), and PC-DPA and DHA (both depleted in lungs).

Among all animal species, only the rat liver PE fraction has been reported to be susceptible to FB exposure, shown in the first study in this dissertation (Burger *et al.*, 2007; Riedel *et al.*, 2015; Szabó *et al.*, 2019). In the present study, the liver PE showed resistance to modifications of its SFAs, unlike the lung PE, which exhibited a decrease in behenic acid. Indeed, a diet of 20 mg FB_1/kg for 9 days did not significantly alter total phospholipids in the piglet liver (Ali *et al.*, 2019). This negligible change could be due to the species-specific effects of FBs and the lower doses used compared to those used for the rats. Notably, in both tissues, the levels of MUFAs (oleic and erucic acids) tended to increase in response to FBs, consistent with data from PC (Szabó *et al.*, 2019) and PE fractions (Riedel *et al.*, 2016). In rat models, these fatty acid elevations have been attributed to high SCD enzyme activity (Burger *et al.*, 2007; Riedel *et al.*, 2015). However, Ali *et al.* (2019) reported no modification in the MUFAs of intoxicated piglets, suggesting that these alterations are likely period-dependent rather than dose-dependent (Ali *et al.*, 2021). MUFAs appeared to compromise the depletion of n3 fatty acids, resulting in an elevated Σn6: Σn3 ratio. Similar findings for n3 fatty acids have been reported in piglets (Ali *et al.*, 2021) and rats (Burger *et al.*, 2007; Riedel *et al.*, 2015). Notably, in this study, these modifications were independent of oxidative stress, as neither the enzymatic antioxidant

system nor MDA levels were affected by the dose applied. This finding implies that these fatty acid alterations are likely caused by changes in enzymatic activities involved in membrane lipid fraction remodulation. When comparing Σn3 levels between PC and PE (Σn3-PC/PE), tissues showed organ-specific responses; liver levels increased while lung levels decreased.

A single study on rats explored the potential modulation of the PS fatty acid composition in the liver by FBs (Gelderblom *et al.*, 2002). To some extent, the undertaken study revealed patterns of nonresponsive ΣSFA, but not ΣMUFA. Meanwhile, rat liver showed proportional increases in oleic and ΣMUFA levels; the piglet liver showed no modification in oleic acid. However, nervonic acid increased threefold in the hepatocellular PS from piglets, and a 1.5-fold increase in erucic acid was observed in the lung PS. The protective properties of these fatty acids have been greatly acknowledged (Li *et al.*, 2019; Liang *et al.*, 2020). Despite marked decreases in SFAs in lung PS, modifications in behenic and lignoceric acids were more pronounced, indicating that FB_1 decreases in dose-dependent. These reductions remarkably contributed to the decrease in ΣSFA, which was compromised by ΣPUFA, particularly through elevated levels of n6 fatty acids (LA, DGLA, and AA). In mammals, the *de novo* production of n6 fatty acids is independent of SFAs. Furthermore, the study indicated identical feed intake, suggesting a possible role for membrane fatty acid remodeling in these alterations. These n6 fatty acids play a crucial role in signaling and inflammation; thus, their modifications may have a profound impact on PS-signaling. The lung also showed a reduction in PS-DHA, which is likely to decrease in the PC, PE, and PI fractions. The PS concentration has been found to be influenced by intracellular DHA levels (Hamilton *et al.*, 2000), which may underpin the potential change in the PS accumulation rate in pulmonary total phospholipids. However, our data are not quantitative; thus, further studies are needed to support this proposal.

Neither liver nor lung PI fractions showed a significant dose-dependent response to FBs in terms of fatty acid composition. However, a marked decrease in stearic acid was observed in the lung PI, differing from findings in the rat liver PI (Szabó *et al.*, 2019). This depletion likely resulted from increased SCD and/or elongase activities, as indicated by decreased ratios of PI-C18:0/C20:1n9 and PI-C18:0/C20:0 (data not shown). Notably, the high concentrations of oleic acid and ΣMUFA, found in trials by Gelderblom *et al.* (2002) and Szabó *et al.* (2019) were mostly attributed to higher doses of FBs used in comparison to this study. When comparing PS and PI from the lungs of highly FBs-intoxicated animals, similar fatty acid patterns were observed; increases in erucic acid and ΣMUFA levels compensated for the decrease in ΣSFA. Erucic acid, which is increased in lung PI, has been

reported to exhibit protective properties by suppressing ligands activated by Toll-like receptors (TORs), thereby protecting against respiratory infections (Voelker and Numata, 2019). Moreover, the nervonic acid proportion decreased in the liver PI. This depletion could be attributed to membrane fatty acid remodeling events that modify membrane trafficking, although this phenomenon was not investigated in this study. The most marked PI-lipid disintegration event was the proportional depletion of DHA in PIs from both tissues, which decreased Σn3 and increased the Σn6: Σn3 ratio. This finding, also reported in the rat PI fraction by Szabó *et al.* (2019), does not implicate the role of lipid peroxidation (as MDA was not altered), but instead suggests possible inhibition of D5D and D6D due to intoxication by FBs, as demonstrated in the rat liver (Gelderblom *et al.*, 2002).

Intoxicated piglets had notably more histological lesions in the liver, corroborating the dose-dependent toxic effects of FBs on the liver (Ali *et al.*, 2019; Terciolo *et al.*, 2019). This hepato-histopathology was confirmed by serum biochemical alterations; a 21-day diet of 30 mg FBs/kg resulted in elevated aspartate transaminase, alkaline phosphatase, and cholesterol concentrations (six, five-, and two-folds, respectively; data not shown). Notably, no substantial lipid peroxidation was detected, suggesting that the perpetuation of sphingolipids and hepatocellular lipids primarily determines the observed hepatotoxicity. When the lung was assessed, only one animal exposed to the highest dose of FBs developed PPE, a typical toxicological feature of FBs in swine. It was not anticipated that PPE would be absent in most of the intoxicated animals. When weanling piglets were fed FBs-contaminated diets in a dose-dependent manner (175, 101, 39, 23, 5, and <1 mg FB_{1+2}/kg feed) for 14 days, only 175 mg FBs/kg feed induced PPE (Motelin *et al.*, 1994). This study aimed to highlight potential inconsistencies in the results rather than establish that PPE development requires extremely high FBs' doses. The detrimental impacts of fumonisins on animals usually result from a multifaceted sequence of events that are influenced by a variety of factors. Under the presented design, the observed variability in PPE can be attributed to animal counter-regulation processes and individual variability since other influencing factors were under control. Indeed, the animal's response to high and low doses of mycotoxin can vary depending on the threshold and its ability to neutralize and/or eliminate toxins and their derivatives. In addition, despite the animal homogeneity was highly considered during experimental planning and execution, individual genetic variability could also be present and not fully controlled. However, identifying the exact factors/events is likely challenging in *in vivo* models due to the immense number of biological events and their potential interactions at the same time.

The fourth study investigated the potential effects of FBs (10 and 20 mg/kg diet for 65 days) on the male reproductive system, including the fatty acid composition of total phospholipids from the testis and sperm, antioxidant defense enzymes, lipid peroxidation, potential histological lesions, and sperm viability, morphology, and integrity in rabbits. Animal performance was also assessed in this study. The rabbits exhibited resistance to FBs exposure, with no fallback in body weight gain or feed intake, consistent with the findings of Ewuola (2009) and Szabó *et al.* (2016b). Notably, the testicle weight remained unchanged, contradicting findings of Ewuola (2009) and Ewuola and Egbunike (2010a). This discrepancy may be due to the use of adult male bucks in this study as opposed to growing rabbits in other studies.

Regardless of the dose, the phospholipid fatty acid composition of spermia revealed no marked modifications. This was accompanied by no substantial alterations in spermium viability, morphology, chromatin integrity, or livability, indicating a negligible effect of FBs on the spermium. In contrast, Ewuola (2009) reported a decrease in sperm mass activity, motility, and live proportion in rabbit spermatozoa with an increase in dietary FB_1 concentration. Notably, their study involved a longer exposure period (196 days), suggesting that the absence of FBs toxicity to sperm might be time-dependent. Another possible factor could be the age of the animals, as this study involved adult rabbit bucks, not growing rabbits like in other studies.

The testicular tissue also provided a more likely negligible response, as demonstrated by the activation of the antioxidant defense system (GSH and GPx increased in a dose-dependent manner) and a proportional increase in margaric acid (C17:0) in total phospholipids. There are no reports yet available on testicular membrane fatty acids, except for Morin (1967), who reported the fatty composition of testicular phospholipids. This tissue is characterized by an elevated level of ΣPUFA, with PUFAs primarily incorporated in Sertoli cells (Retterstøl *et al.*, 2001). This study examined whole testicular tissue, which is novel, especially at the highest doses compared to other studies. Notably, the fatty acid that responded (increased) was margaric acid, which is not produced via the mammalian *de novo* pathway but is likely absorbed after caecotrophy. This proposal seems plausible, as feed intake was identical across all animal groups.

The testis, a relatively hypoxic microenvironment with a high proportion of UFAs (Zhang *et al.*, 2016), is sensitive to oxidative damage. FBs intoxication increased testicular GSH and GPx levels compared to those in the control group, with these increases showing a linear dose dependency. Moreover, a direct oxidative effect of FBs has not been reported; similar effects have been observed

with heat stress in rabbits (Hosny *et al.*, 2020). It remains unclear whether FBs are direct oxidative stress inducers or whether this effect contributes to carcinogenesis (Sheik Abdul and Marnewick, 2020). The molecular mechanisms underlying fumonisin toxicity in the liver and kidney are thought to be linked to early oxidative stress events (Wang *et al.*, 2016), but their role in male reproductive organs has been less studied. If fumonisin is not a direct inducer of the detected slight oxidative stress, other factors, such as mitochondrial disruption, could plausibly be involved. However, the adaptation (exposure: 65 days) was effective, as PUFAs remained unaltered, and MDA was also unaffected. Thus, FBs appear to have a negligible effect after 65 days of intoxication, as confirmed by the absence of marked histological lesions across the groups.

6. CONCLUSIONS AND RECOMMENDATIONS

The initial study revealed that the rat kidney was highly reactive, showing modifications in the membrane fatty acid compositions of different phospholipid fractions due to low dosage and short-term exposure to FB_1. The most responsive phospholipid fraction in the rat renal cortex was PC, which displayed fatty acid proportional shifts in Σn6, Σn3, and consequently, ΣPUFA (dose-dependent decreases), while ΣMUFA showed a dose-response increase. This dose-dependent pattern was somewhat partial and present in PIs, particularly in ΣPUFA. The rat liver demonstrated less sensitivity to FB_1, with the PE fraction revealing a dose-response relationship. These results align with previously published histopathological and peroxidation endpoint findings (Szabó *et al.*, 2018). In respect to this, alterations in membrane fatty acids could potentially be used to determine the extent of histological sensitivity. Although the role of lipid peroxidation in depleting PUFAs has been acknowledged, indices relating to enzymes involved in lipid metabolism suggest possible alterations in enzyme activity. Therefore, further investigation of phospholipid-metabolizing enzymes is important for understanding the potential impact of enzyme perturbation on cellular membrane remodeling.

In species other than rabbits, specifically swine (weaned piglets), the activity of the sodium-potassium pump was assessed during the second study. The diet contained FBs at levels of 15 and 30 mg/kg, which notably altered the Na^+/K^+ ATPase activity of erythrocytes after a period of three weeks, showing a dose-dependent increase. This observation suggests that erythrocyte damage or functional changes are a feature of FBs and are not limited to rabbits. The substantial increase in sodium pump activity is most likely triggered by FB_1's inhibitory effect on CerS. This particular mycotoxin induces a state associated with nonoxidative stress, characterized by a marked increase in sodium pump activity and a complex fatty acid profile of membrane lipids, offering pertinent correlations for all n-6 fatty acids. It is postulated that additional research is required to elucidate the role of cellular lipids in the proven regulatory function of Na^+/K^+ ATPase. It is highly likely that these studies will need to segregate lipid classes into more relevant subclasses prior to conducting fatty acid analysis.

In a relatively partially similar approach to the first study, the third investigation assessed the impact of FBs on the fatty acid profiles of membrane lipids in piglets' livers and lungs, with the effects observed to be dose-dependent. This is the first study to document modifications in the fatty acid profiles of phospholipid classes in the livers and lungs of piglets following exposure to FBs. The relatively high dosage of FBs did not have a marked effect on piglet production traits or oxidative stress markers. Only subtle, characteristic effects were identified in the fatty acid profiles of membrane

lipid fractions. Dose-dependent linear alterations were noted in liver PC as well as in lung PC and PS fractions. The data collected revealed variations in tissue-specific responses in membrane lipids. Additionally, the findings indicate a distortion in the activities of enzymes related to fatty acid metabolism, such as elongase, D5D, D6D, and Δ9D. These enzyme alterations were characterized by a proportional decrease in PUFAs and an increase in ΣMUFA. The changes observed in membrane lipids are not primarily considered to be responsible for liver and lung toxicity but rather are consequences or mediators of other events that may have led to tissue damage. This study did not include the quantitative determination of ceramide and polar lipids, which could offer further insight into the findings. Therefore, additional studies investigating the quantitative effects of FBs on the lipidomes of the liver and lungs are highly important.

Since the toxicity potential of FBs to the reproductive systems of animals, such as rabbits and swine, has been reported, the third study assessed the effects of FBs on adult male rabbit bucks. Exposure to FBs over an entire testicular cycle did not result in substantial modifications at five sampling points in the spermatozoa endpoints (proportion of live cells, morphological distribution, membrane lipid profile). However, a slight increase in antioxidant defense was observed in the testes without any striking alterations in the lipid profile or histological modifications. However, a minimal pro-oxidant effect of FBs on the male genital system was observed, with no marked detrimental impact on the examined spermatological traits. Thus, the reproductive system of adult male bucks appears to be resistant to the applied mycotoxins over a period of 65 days. Apparently, additional studies involving a longer exposure period and the inclusion of growing rabbits would provide further understanding of potential response variations.

7. NEW SCIENTIFIC RESULTS

The scientific outcomes presented herein are derived from four distinct studies conducted during the course of this doctoral research.

1. Observations from the rat study indicate that alterations in the fatty acid composition of hepatocellular membranes may likely occur at lower fumonisin B1 (FB_1) exposure levels (< 50 mg FB_1/kg^{-1} dietary dose equivalent for 5 days) compared to the high doses (150 and 250 mg/kg diet for a period over 21 days) reported in earlier studies.
2. Fatty acid alterations induced by FB_1 in rat kidneys were the most prevalent (especially at 100 mg FB_1/kg^{-1} dietary dose equivalent for 10 days), providing a negative dose-response for polyunsaturated fatty acids, and a positive dose-response for saturated fatty acids and monounsaturated fatty acids (MUFAs) of phosphatidylcholines (PC) and phosphatidylinositols (PI). In contrast, liver phosphatidylethanolamine was the only phospholipid fraction that exhibited a positive dose-response for the sum of omega-3 fatty acids (Σn3).
3. The modulation of ion exchange pump, Na^+/K^+ ATPase activity, by FB_{1+2+3} (FBs) in porcine erythrocytes was proven, a phenomenon previously not deliberately investigated in any species other than rabbits.
4. An increase in erythrocyte Na^+/K^+ ATPase activity was observed in a dose-dependent manner following the administration of FBs (15 and 30 mg/kg feed for 21 days) for the first time, at least in the studied animal model (pig) and its age. Furthermore, the findings indicated a negative correlation ($r > -0.6$) between the activities of Na^+/K^+ ATPase and omega-6 (n6) fatty acids, including linoleic, eicosadienoic, dihomo-γ-linolenic and arachidonic acids.
5. Similar to rats, piglets exposed to FBs (15 and 30 mg/kg diet for 21 days) exhibited altered fatty acid compositions of various phospholipid fractions, especially PC, phosphatidylethanolamine, and phosphatidylserine (PS), in the lungs and livers.
6. In piglets, dose-dependent responses were observed between the administered doses of FBs (15 and 30 mg/kg feed) and fatty acids (C16:1 n7, C18:1 n9, and sum of MUFAs (ΣMUFA)) in liver PCs, as well as in pulmonary PCs (C22:6 n3, Σn3, and Σn6:Σn3) and PSs (C20:0, and C24:0).
7. Based on the findings in piglets, oxidative stress (as assessed by malondialdehyde) does not appear to be directly involved in FBs toxicity, suggesting that fatty acid modifications and

histological lesions may occur independently of oxidative stress and involve other events, such as disruption of lipid metabolism and membrane remodeling enzymes.

8. Adult male rabbits appear to be resistant to doses (10 and 20 mg/kg diet) of FBs above the EU-permitted levels for over 65 days, as these mycotoxins did not compromise body weight or feed efficiency, or alter the membrane lipids of their reproductive system.

8. SUMMARY

First study

Graded exposure to fumonisin B1 (FB_1) in rats has been demonstrated to modify hepatic membrane lipids, a fact corroborated both *in vivo* and *in vitro*. Within this study, male Wistar rats (n = 4 × 6) were intraperitoneally administered pure FB_1 (0, 20, 50, or 100 mg kg^{-1} dietary dose equivalent) for 5 or 10 days to evaluate the dose- and time-dependent impacts on the fatty acid profiles of renal and hepatic phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE). In the kidney, the PC fatty acid profile displayed an increase in saturation (ΣSFA) within 5 days, and after 10 days, a marked decrease in polyunsaturation (ΣPUFA) was observed (total n3 (Σn3), total n6 (Σ n6), unsaturation index (UI), and average FA chain length (ACL)), majorly with a linear dose-response. A further marked dose-dependent increase in overall monounsaturation (ΣMUFA) was observed in renal PC. Similar patterns, to a lesser extent, were observed in the renal PI fatty acids, displaying a decrease in PUFA (provided a dose-response), UI, and ACL over both 5 and 10 days, while the renal PE fraction revealed a decrease in Σn6 and an increase in ΣSFA, but this was observed only after 5 days. In the liver, PC displayed an increase in saturation (C16:0) and a decrease in polyunsaturation (C20:3 n6, and C20:3 n3). Similar findings were observed in the PI fatty acid profile after 5 days. However, the fatty acid compositions of PC and PI did not respond to FB_1 in a dose-dependent manner. In the PE fatty acid profile, there was a decrease in C20:3 n6 and Σn6, but a dose-dependent increase in Σn3. The results revealed pronounced renal sensitivity (providing the most dose-dependent responses), corroborating our earlier published findings regarding oxidative stress and histopathological modifications. Notably, membrane fatty acid modifications in rat tissues occurred at a relatively low exposure to FB_1 (less than 50 mg/kg diet), in contrast to the higher doses used in other studies.

Second study

The impact of fumonisins on cellular membrane compositional modification and Na^+/K^+ ATPase activity in rabbit erythrocytes is established, however, such effects have not been investigated in other animal species. Within the undertaken study, weaned piglets (n = 6 animals/group) aged 35 days were fed a diet containing FBs (FB_1, FB_2, and FB_3) at concentrations of 0, 15, and 30 mg/kg for 21 days to assess the potential dose-dependent modifications in erythrocyte membranes induced by the mycotoxins. The activity of Na^+/K^+ ATPase, which is ouabain-sensitive, was measured in the membranes of lysed erythrocytes. The fatty acid profile of the membrane was analyzed, along with

antioxidant and lipid peroxidation endpoints. After 3 weeks of exposure, a significant difference (*p*-value = 0.036; R^2 = 0.58) in the activity of the sodium pump in the erythrocyte membrane was observed between the control group and the 30 mg/kg group, which was dose-dependent. The fatty acid profile of the membrane was highly saturated, with nonsystematic differences between groups. Upon pooling the data, a negative association was observed between the activity of the sodium pump and all individual membrane n6 fatty acids. Regardless of the applied dose of FBs, intracellular antioxidants (such as reduced glutathione (GSH) and glutathione peroxidase (GPx)) and lipid peroxidation indicators (conjugated dienes, trienes, and malondialdehyde (MDA)) showed no response. It is hypothesized that the effect of a CerS inhibitor (FBs) is exerted on the cell membrane. This effect was dose-dependent, resulting in an increase in sodium pump activity that was negatively associated with n6 fatty acids, and no lipid peroxidation was observed.

Third study

The rat model is predominantly investigated in terms of FB_1-induced lipid membrane alterations, while investigations involving other species are infrequent. In the case of swine, the liver and lungs are the organs most affected by FBs. In this study, male piglets that had been weaned were divided into groups of six and fed a diet containing FBs at 0, 15, or 30 mg/kg, and the feeding period lasted for three weeks. The purpose of this study was to assess the fatty acid composition of various classes of membrane lipids, the level of lipid peroxidation, and any histomorphological changes in the liver and lungs. The growth performance of the piglets and the level of lipid peroxidation remained unaltered; however, there was an increase in the overall liver lesion score. A linear dose-response relationship was observed in the liver PC for C16:1 n7, C18:1 n9, and ΣMUFA, and in the lung PC for C22:6 n3, Σn3, and the ratio of Σn6 to Σn3. C20:0 and C24:0 had similar patterns in the pulmonary PS. Modifications associated with the highest dosage of FBs were observed in various lipid classes. In the lung SM, there was a decrease in ΣSFA and an increase in ΣMUFA. In PCs, Σn6 decreased and Σn6:Σn3 increased in the liver, whereas in the lungs, ΣMUFA and ΣPUFA increased. In PSs, the decrease in Σn3 detected in the liver, while increases in ΣMUFA and Σn6:Σn3 were observed in the lungs. The hepatic PS showed an increase in Σn6:Σn3, whereas the lung PS exhibited a decrease in ΣSFA, an increase in ΣPUFA, and an increase in Σn6 and its ratio to Σn3. In the PI, Σn6:Σn3 decreased in the liver, and in the lungs, there was an increase in C22:1 n9, a decrease in C22:6 n3, a decrease in ΣSFA, and an increase in ΣMUFA. In summary, exposure to FBs did not impair growth or induce marked lipid peroxidation. However, hepatotoxicity was evident from the histopathological lesions

observed over the exposure period and at the administered doses. Fatty acid modifications were independent of lipid peroxidation, suggesting a disruption in fatty acid metabolism, which corroborated previous findings in rats. This perturbation in membrane lipids was most observed in PCs.

Fourth study

Rabbits are proposed as suitable model organisms for studies in reproductive toxicology. In this trial, the effects of FBs on the reproductive system of male rabbits (n = 10 animals/group) were assessed after 65 days of oral administration of two doses (10 and 20 mg/kg diet). Neither intoxication affected the weights of the body and testicles, nor did intoxication markedly affect the fatty acid composition of the total phospholipids in the testes. These findings were consistent with the absence of a marked effect on histological traits. However, in the testicles, the FBs increased the concentration and activity of GSH and GPx, respectively, and decreased the initial phase of lipid peroxidation (conjugated dienes and trienes) in a dose-dependent manner. Using Feulgen-stained smears, the sperm morphology and chromatin condensation were monitored. No substantial differences were detected between the treatment groups or between the sampling time points. The ratio of live cells in the sperm, as assessed with flow cytometry, was not distinct among the groups at any of the five sampling time points and was also identical within the groups. Similarly, the lipid profile of the spermatozoa membrane was identical in all three groups after the total intoxication period. In summary, even at unrealistically and unjustifiably high doses, FBs do not exert any severe harmful effects on the leporine, the male reproductive system of rabbits. However, FBs slightly induced antioxidant responses in rabbit testes.

9. ÖSSZEFOGLALÁS

Első tanulmány

Ebben a tanulmányban hím Wistar patkányokat (n = 6 állat/csoport) kezeltünk tiszta fumonizin B_1-gyel (FB_1: 0, 20, 50 és 100 mg kg^{-1} takarmány dózis ekvivalens) intraperitoneálisan, 5, illetve 10 napig, a dózis- és expozíciós időfüggő hatások vizsgálata céljából, a vese és a máj foszfatidilkolin (PC), foszfatidilinozitol (PI) és foszfatidiletanolamin (PE) frakciók esetében. A vesében a PC frakció zsírsavai közül növekedést igazoltunk a telített zsírsavakban (ΣSFA) 5 napot követően, majd pedig 10 nap után jelentős csökkenést figyeltünk meg a többszörösen telítetlen zsírsavak arányában (ΣPUFA) (összes n3 (Σn3), összes n6 (Σ n6), telítetlenségi index (UI) és átlagos szénlánchossz (ACL)), a legtöbb esetben lineáris dózisválasz formájában. A vese PC frakcióban további jelentős dózisfüggő növekedést mutatott az összes egyszeresen telítetlen zsírsav (ΣMUFA) részaránya. Hasonló mintázatokat, de enyhébb formában figyeltünk meg a vese PI zsírsavakban, amelyek csökkenést mutattak a PUFA, (dózisfüggő formában), UI és ACL esetében, mind 5, mind pedig 10 nap alatt, míg a vese PE frakció csökkenést mutatott a Σn6 és növekedést a ΣSFA esetekben, de ez csak 5 nap után volt megfigyelhető. A májban a PC frakció zsírsavprofilja növekedést mutatott a telítettségben (C16:0) és csökkenést a többszörösen telítetlen C20 zsírsavak arányában (C20:3 n6 és C20:3 n3). Hasonló eredményeket figyeltünk meg a PI zsírsavprofilban 5 nap után, azonban a PC és PI zsírsavai nem reagáltak dózisfüggő módon az FB_1-re. A PE zsírsavprofilban aránycsökkenést mutatott a C20:3 n6, és a Σn6, míg a dózisfüggő növekedést mutatott a Σn3 zsírsavcsoport. Az eredmények kifejezett érzékenységet jeleznek a vese esetében, megerősítve korábban publikált eredményeinket az oxidatív stresszel és a kórszövettani változásokkal kapcsolatosan.

Második tanulmány

Ebben a tanulmányban 35 napos korú választott malacokat (n = 6 állat/csoport), 0, 15 és 30 mg/kg FB sorozatú mikotoxinokat (FB_1, FB_2 és FB_3) tartalmazó kísérleti takarmánnyal kezeltünk 21 napig, elsősorban toxindózis-függő módosulások jellemzésére vörösvértest membránokban. A ouabain-szenzitív Na^+/K^+ ATPáz aktivitását mértük lizált sejtek membránjaiban, valamint elemeztük a membrán zsírsavprofilt, illetve az antioxidáns és a lipid-peroxidációs paramétereket. 3 hét expozíció után szignifikáns különbség ($p = 0,036$; $R^2 = 0,58$) volt megfigyelhető a nátrium pumpa aktivitásában az eritrocita membránban a kontroll csoport és a 30 mg/kg takarmány-dózist kapott csoport között, a változás pedig dózisfüggő volt. A sejtmembrán zsírsavprofilja határozottan telített volt, a csoportok közötti eltérések nem voltak szisztematikusak, de az enzimaktivitás és zsírsavprofil adatok együttes

értékelésekor negatív összefüggést figyeltünk meg a nátrium pumpa aktivitása és az összes egyedi membrán n6 zsírsav aránya között. Függetlenül az alkalmazott FB toxinok dózisától, az intracelluláris antioxidánsok (például a redukált glutation (GSH) és a glutation-peroxidáz (GPx)) és a lipid-peroxidációs mutatók (konjugált diének, triének és malondialdehid (MDA)) nem mutattak értékelhető választ. Feltételezhető, hogy a FB mikotoxinok ceramid szintézis gátló hatása a sejtmembrán összetételére hatott. Ez a hatás dózisfüggő volt, ami növekedést eredményezett a nátrium pumpa aktivitásában, amely negatívan korrelált az n6 zsírsavakkal, viszont nem járt együtt lipid-peroxidációval.

Harmadik tanulmány

Ebben a tanulmányban választott hímivarú malacokat hatos csoportokra osztottunk, és 0, 15 vagy 30 mg/kg FB-t tartalmazó kísérleti takarmányt kaptak, háromhetes expozíció keretében. A cél az volt, hogy megvizsgáljuk a különböző membránlipid osztályok/frakciók zsírsavösszetételét, a lipid peroxidáció mértékét és a májban és a tüdőben esetlegesen bekövetkező szövettani változásokat. A malacok termelési tulajdonságai és a lipid peroxidáció szintje nem változott, azonban fokozott mértékű szövettani elváltozásokat igazoltuk a májban. Lineáris mikotoxin dózis-választ igazoltunk a máj PC frakciójában a C16:1 n7, C18:1 n9 és ΣMUFA arányok esetében, valamint a tüdő PC frakcióban a C22:6 n3, Σn3 és a Σn6 és Σn3 zsírsavak arányában. Hasonló megfigyeléseket tettünk a tüdő PS frakciójában a C20:0 és C24:0 esetében. A tüdő SM frakcióban csökkenést tapasztaltunk a ΣSFA esetében és növekedést a ΣMUFA arányban. A PC frakcióban a Σn6 csökkent, és a Σn6:Σn3 arány növekedett a máj esetében, míg a tüdőben a ΣMUFA és a ΣPUFA arányok emelkedtek. A PS frakcióban a Σn3 csökkenését igazoltuk a májban, míg a tüdőben a ΣMUFA és a Σn6:Σn3 arány emelkedését figyeltük meg. A máj PS növekedést mutatott a Σn6:Σn3 arány vonatkozásban, míg a tüdő PS csökkenést mutatott a ΣSFA-ban, növekedést a ΣPUFA-ban, és növekedést a Σn6-ban és annak arányában a Σn3-hoz képest. A PI frakcióban a Σn6:Σn3 csökkent a májban, és a tüdőben növekedést tapasztaltunk a C22:1 n9-ben, csökkenést a C22:6 n3-ban, csökkenést a ΣSFA-ban, és növekedést a ΣMUFA-ban. Összefoglalva, az FB expozíció nem hátráltatta növekedést és nem indukált jelentős lipid-peroxidációt. Azonban a hepatotoxicitás nyilvánvaló volt az alkalmazott expozíciós időszak és alkalmazott dózisok esetében, elsősorban az igazolt megfigyelt szövettani elváltozások alapján. A zsírsav aránymódosulások függetlenek voltak a lipid peroxidációtól, ami a zsírsav metabolizmus zavarát sugallta, megerősítve a patkányokban korábban publikált eredményeket.

Negyedik tanulmány

Ebben a vizsgálatban takarmányeredetű FB mikotoxinok baknyulak szaporodási szervrendszerére (n = 10 állat/csoport) gyakorolt hatásait elemeztük 65 napos *per os* adagolás és két dózis esetében (10 és 20 mg/kg takarmány dózis). Az intoxikáció nem befolyásolta a test és a here súlyát, sem a herék teljes foszfolipidjeinek zsírsavösszetételét és szövettani képet igazolható szinten, azonban a herékben az FB mikotoxinok növelték a GSH és a GPx koncentrációját és aktivitását, illetve csökkentették a lipid peroxidáció kezdeti fázisát jelző konjugált diének és triének szintjeit, dózisfüggő módon. Feulgen-festett kenetek segítségével követtük nyomon a spermiumok morfológiáját és a kromatin kondenzációját. Nem találtunk jelentős különbségeket a kezelt csoportok és az egyes a mintavételi időpontok adatai között. A sperma élő sejtjeinek aránya, melyet áramlási citometriával értékeltünk, nem volt különböző a csoportok között az öt mintavételi időpont egyikén sem, és azonos volt a csoportokon belül is. Hasonlóképpen, a spermatozoa membránjának lipidprofilja azonos volt mindhárom csoportban a teljes expozíció után. Összefoglalva, azt tapasztaltuk, hogy még viszonylag magas (nem realisztikus) dózisokban sem gyakorolnak az FB-k súlyos káros hatást a hímivarú nyulak szaporodási szervrendszerére, de kis mértékben fokozzák az antioxidáns védelem mértékét a herékben.

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12. PUBLICATION AND PRESENTATIONS

12.1. Peer-reviewed papers relevant to the dissertation

12.1.1. Ali O, Szabó A. Fumonisin Distorts the Cellular Membrane Lipid Profile: A Mechanistic Insight. *Toxicology*. 2024. Doi: 10.1016/j.tox.2024.153860. **Q1, IF: 4.922.**

12.1.2. Ali O, Szabó A. Review of Eukaryote Cellular Membrane Lipid Composition, with Special Attention to the Fatty Acids. *Int J Mol Sci.* 2023;24(21):15693. Doi: 10.3390/ijms242115693. **D1, IF: 6.266**.

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12.1.4. Szabó A, Nagy S, **Ali O**, Gerencsér Z, Mézes M, Balogh KM, Bartók T, Horváth L, Mouhanna A, Kovács M. A 65-Day Fumonisin B Exposure at High Dietary Levels Has Negligible Effects on the Testicular and Spermatological Parameters of Adult Rabbit Bucks. *Toxins (Basel).* 2021;13(4):237. Doi: 10.3390/toxins13040237. **Q1, IF: 4.796**.

12.1.5. Szabó A, **Ali O,** Lóki K, Balogh K, Mézes M, Bartók T, Horváth L, Kovács M. Orally Administered Fumonisins Affect Porcine Red Cell Membrane Sodium Pump Activity and Lipid Profile Without Apparent Oxidative Damage. *Toxins (Basel).* 2020;12(5):318. Doi: 10.3390/toxins12050318. **Q1, IF: 4.796**.

12.1.6. Szabó A, Fébel H, **Ali O,** Kovács M. Fumonisin B1 induced compositional modifications of the renal and hepatic membrane lipids in rats – dose and exposure time dependence. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2019;36(11):1722-1739. Doi: 10.1080/19440049.2019.1652772. **Q2, IF: 3.530**.

12.2. Peer-reviewed papers on the subject of the dissertation but not incorporated

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12.2.3. Szabó A, Szabó-Fodor J, Kachlek M, Mézes M, Balogh K, Glávits R, **Ali O**, Zeebone YY, Kovács M. Dose and Exposure Time-Dependent Renal and Hepatic Effects of Intraperitoneally Administered Fumonisin B_1 in Rats. *Toxins (Basel).* 2018; 10(11):465. Doi: 10.3390/toxins10110465. **Q1, IF: 4.796**.

12.2.4. Szabó A, Szabó-Fodor J, Fébel H, Mézes M, Balogh K, Bázár G, Kocsó D, **Ali O**, Kovács M. Individual and Combined Effects of Fumonisin B_1, Deoxynivalenol and Zearalenone on the Hepatic and Renal Membrane Lipid Integrity of Rats. *Toxins (Basel).* 2017; 10(1):4. Doi: 10.3390/toxins10010004. **Q1, IF: 4.796**.

12.3. Peer-reviewed papers not relevant to the topic of the dissertation

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12.3.2. Kulcsár S, Turbók J, Kövér G, Balogh K, Zándoki E, Gömbös P, **Ali O,** Szabó A, Mézes M. The Effect of Combined Exposure of Fusarium Mycotoxins on Lipid Peroxidation, Antioxidant Defense, Fatty Acid Profile, and Histopathology in Laying Hens' Liver. *Toxins.* 2024; 16(4):179. DOI: 10.3390/toxins16040179. **Q1, IF: 4.796**.

12.3.3. Gebremichael A, Szabó A, Sándor ZJ, Nagy Z, **Ali O**, Kucska B. Chemical and Physical Properties of African Catfish (Clarias gariepinus) Fillet Following Prolonged Feeding with Insect Meal-Based Diets. *Aquac Nutr.* 2023; 2023:6080387. Doi: 10.1155/2023/6080387. **Q1, IF: 4.218**.

12.3.4. Yakubu HG, **Ali O,** Szabó A, Tóth T, Bazar G. Feeding Mixed Silages of Winter Cereals and Italian Ryegrass Can Modify the Fatty Acid and Odor Profile of Bovine Milk. *Agriculture*. 2023; 13(2):381. DOI: 10.3390/agriculture13020381. **Q2, IF: 3.916**.

12.3.5. Zeebone YY, Bóta B, Halas V, Libisch B, Olasz F, Papp P, Keresztény T, Gerőcs A, **Ali O,** Kovács M, Szabó A. Gut-Faecal Microbial and Health-Marker Response to Dietary Fumonisins in Weaned Pigs. *Toxins (Basel).* 2023;15(5):328. Doi: 10.3390/toxins15050328. **Q1, IF: 4.796**.

12.3.6. Varga-Visi É, Jócsák I, Kozma V, Lóki K, **Ali O**, Szabó A. Effects of Surface Treatment with Thymol on the Lipid Oxidation Processes, Fatty Acid Profile and Color of Sliced Salami

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12.3.7. Yakubu HG, **Ali O**, Ilyés I, Vigyázó D, Bóta B, Bazar G, Tóth T, Szabó A. Micro-Encapsulated Microalgae Oil Supplementation Has No Systematic Effect on the Odor of Vanilla Shake-Test of an Electronic Nose. *Foods*. 2022;11(21):3452. Doi: 10.3390/foods11213452. **Q1, IF: 5.738**.

12.3.8. Ali O, Petrási Z, Donkó T, Fébel H, Mézes M, Szabó A. Muscle fibre membrane lipid composition in musculus *biceps femoris* of pigs reared in indoor or outdoor systems. *Journal of Animal and Feed Sciences*. 2021;30(3):238-247. Doi:10.22358/jafs/139275/2021. **Q2, IF: 1.876**.

12.3.9. Kócsó DJ, **Ali O**, Kovács M, Mézes M, Balogh K, Kachlek ML, Bóta B, Zeebone YY, Szabó A. A preliminary study on changes in heat shock protein 70 levels induced by Fusarium mycotoxins in rats: *in vivo* study. *Mycotoxin Res*. 2021;37(2):141-148. Doi: 10.1007/s12550-021-00425-z. **Q2, IF: 3.612**.

12.4. Peer-reviewed papers published in Hungarian scientific journal

12.4.1. Ali O. A Brief Review of the European Directive on 3Rs and Facilitating Animal Experimentation. *ACTA AGRARIA KAPOSVÁRIENSIS*. 2024; 27 (1).

12.5. Submitted papers in peer-reviewed journals and not relevant to the topic of the dissertation

12.5.1. Szabó A, Emri M, Tóth Z, Fajtai D, Donkó T, Petneházy Ö, Kőrösi D, Repa I, Takács A, Kisiván T, Gerencsér Z, **Ali O**, Turbók J, Bóta B, Gömbös P, Romvári R, Kovács M. Measurement of hepatic glucose (18F-fluorodeoxyglucose) uptake with positron emission tomography-magnetic resonance imaging in Fumonisin B intoxicated rabbit bucks. Scientific Reports. 2024. **Q1, IF: 4.393**.

12.6. Abstracts

12.6.1. Oral presentations

12.6.1.1. **Ali O**, Mézes M, Balogh KM, Kovács M, Mouhanna A, Szabó A. Dose-dependent effects of the dietary fumonisin b series on the liver membrane lipids of weaned piglets. 30th International Symposium Animal Science Days, Zadar, Croatia, 21-23 September, 2022.

12.6.1.2. Szabó A, Nagy S, **Ali O**, Gerencsér Z, Mézes M, Kovács M, Effects of Fumonisins with Other *Fusarium* Toxins on Weaned Piglets: Membrane Lipids and Clinical Chemistry Approach. GINOP Conference 2021, Kaposvár, Hungary, 15 December, 2021.

12.6.1.3. **Ali O,** Mézes M, Balogh K, Kovács M, Szabó A. Effects of Fumonisins with Other *Fusarium* Toxins on Weaned Piglets: Membrane Lipids and Clinical Chemistry Approach. GINOP Conference 2021, Kaposvár, Hungary, 15 December, 2021.

12.6.1.4. Szabó A, Nagy S, **Ali O**, Gerencsér Z, Mézes M, Balogh KM, Bartók T, Horváth L; Mouhanna A, Kovács M. Effects of Fumonisin B Series on the Male Rabbit Reproductive System: Testicular and Spermatological Investigations. 29th Animal Science Day International Symposium, Gödöllő, Hungary, 13-17 September, 2021.

12.6.1.5. **Ali O.** Subchronic exposure to fumonisin exerts negligible effects on the reproductive system of adult male buck. Elektronikus PhD Hallgatói és Oktatói Workshop az állattenyésztési kutatások területén, Kaposvár, Hungary, 4 May, 2021.

12.6.1.6. **Ali O,** Szabó A, Kovács M. The effects of oral fumonisin B1 exposure on the renal and hepatic lipid metabolism. 17th Global Toxicology and Risk Assessment Conference, Budapest, Hungary; 22-24 October, 2018.

12.6.2. Posters

12.6.2.1. **Ali O**, Mézes M, Balogh KM, Kovács M, Szabó A. Fumonisin B Series' Effects on the Lung Membrane Lipids of Piglets. TOX'2022 Tudományos Konferencia, Zalakaros, Hungary, 12-14 October, 2022.

12.6.2.2. Szabó A, **Ali O,** Lóki K, Balogh K, Mézes M, Bartók T, Horváth L, Kovács M. Sensitivity of the porcine hepatic cell membrane to fumonisin B1 during short exposure period. MycoKey-MycoTWIN Conference 2021, Bari, Italy, 9-12 November, 2021.

13. CURRICULUM VITAE

Mr. Omeralfaroug Abdalla Ibrahim Ali was born on March 8, 1992, in Omdurman, Sudan. His educational journey began in his local area at Abukadook Primary School (**1997–2005**) and continued at Model Musa Aldaw High School for boys (**2005–2008**), where he successfully passed the National High School Exam. In **September 2008**, he was admitted to the College of Veterinary Medicine and Animal Production at the Sudan University of Science and Technology (SUST) in Khartoum, Sudan, under the Public Sudanese Educational Support System. He graduated in **September 2013** with a First-Class Honours BSc degree in Animal Production Science and Technology.

Following his graduation, he was appointed as a teacher assistant at the College of Veterinary Medicine, SUST, in **January 2014**, where he worked for 1 year and 8 months. In September 2015, he was awarded the FAO-Hungarian Government Scholarship to pursue an MSc in Animal Nutrition and Feed Safety at Kaposvár University (now known as the Hungarian University of Agriculture and Life Science, MATE), Hungary. He successfully completed this program, graduating with an **excellent CGPA** in **June 2017**.

In **September 2018**, he joined the Doctoral School of Animal Science at MATE-Kaposvár Campus to pursue his doctoral studies under the supervision of Prof. Dr. András Szabó. His doctoral dissertation topic was "Fumonisin B Series' Implications on Biomembranes' Fatty Acid Composition of Specific Tissues from Rats, Swine and Rabbits". The research activities undertaken on this topic were financed by Mycotoxins in the Food Chain Research Group (MTA-KE-SZIE, now known as HUN-REN-MATE).

Immediately upon his graduation from the MSc program in **June 2017**, he joined Kaposvár University as a junior researcher through HUN-REN-MATE, where he began working at the Laboratory of Lipid Analysis. He continues to work in the same laboratory, which has recently been incorporated into the Institute of Animal Physiology and Nutrition, MATE. His job position involves various activities, primarily conducting experiments, providing lectures and practice to students, and strengthening collaboration with other research teams.