Fumonisin induced alteration of sphingolipid metabolism in piglets – Analytical and biological aspects

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Introduction

Fumonisin biomarkers

Sphingolipid metabolism

Feeding trials

Analysis

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Conclusion

Overview

- Aim of the work
- Fumonisin biomarkers
- Sphingolipid metabolism
- Analysis of sphingoid bases
- Feeding trials with piglets
- Effects of fumonisin B1 on sphingolipid metabolism in piglets
- Conclusion

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Aim of the work

Mycotoxins → Problem in feed production

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Development of feed additives working as mycotoxin deactivators

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Evaluation of efficiency: Need for mycotoxin biomarkers

Our aim: Evaluate biomarkers for monitoring fumonisin exposure of piglets

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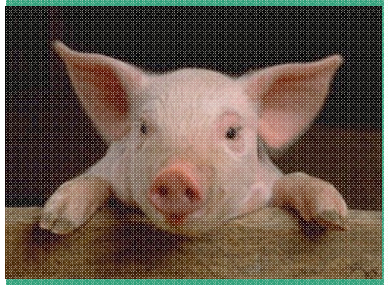
Requirements

- Biomarker in treatment group significantly different to biomarker in control group even at low dietary fumonisin concentrations (e.g. 5 ppm in feed)

- Uncomplicated sampling

- Simple and cheap, but nevertheless sensitive and rugged analysis



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Fumonisin biomarkers in pigs

Fumonisin B1 concentrations in

(faeces) | urine | plasma | tissues

Low absorption of fumonisins (< 5%)
→ excretion in urine only 1% of intake
→ Low plasma concentrations (≤ low ppb-range)

Partial conversion of FB1 to pHFB1 and HFB1 in the animal to different extent

Disruption of sphingolipid metabolism in

urine | plasma | tissues

One main way how fumonisins exert their toxicity

Promising literature data for urine, plasma and tissue analysis

Cheaper analysis (HPLC-FLD instead of HPLC-MS)

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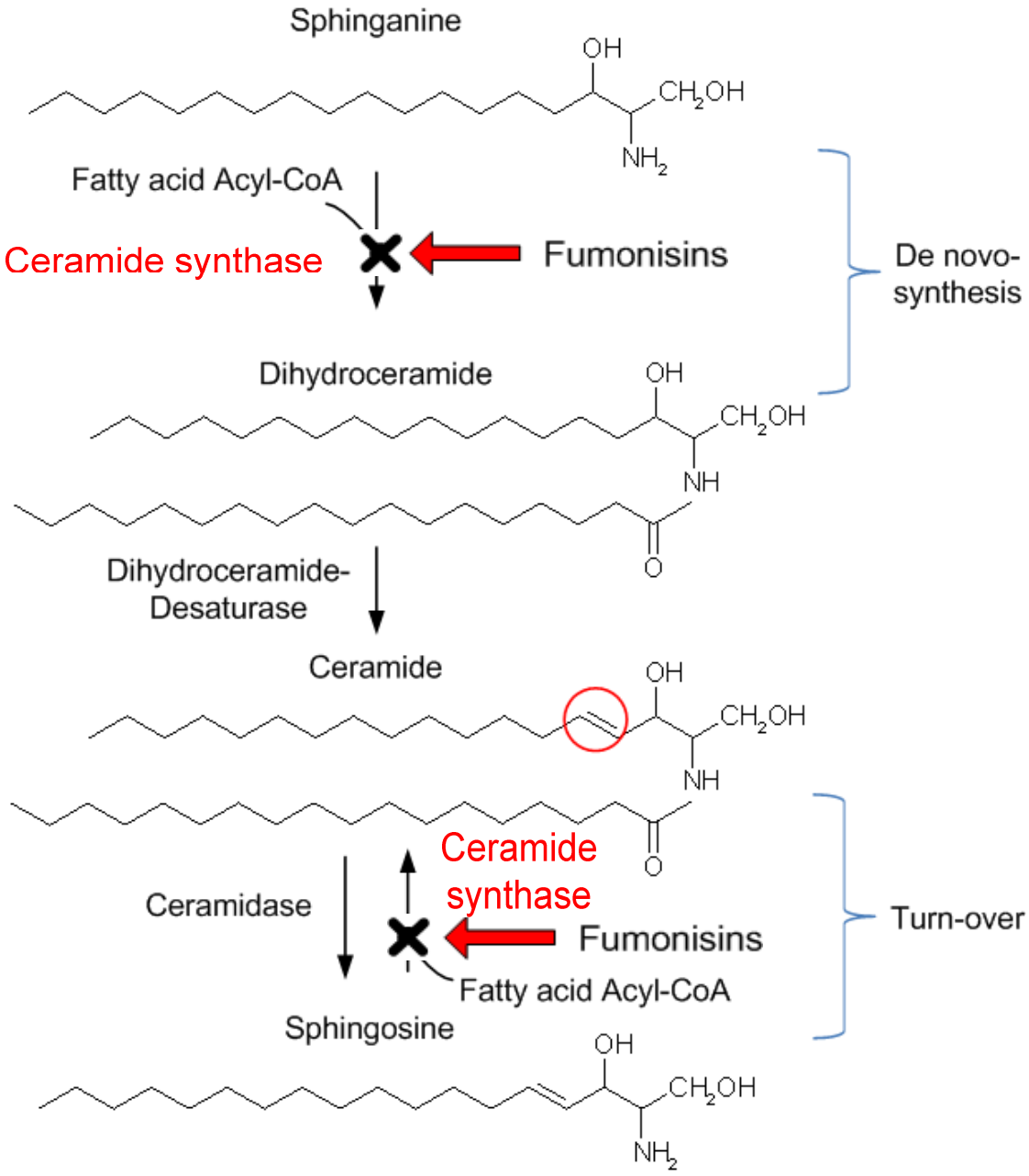
Sphingolipid metabolism



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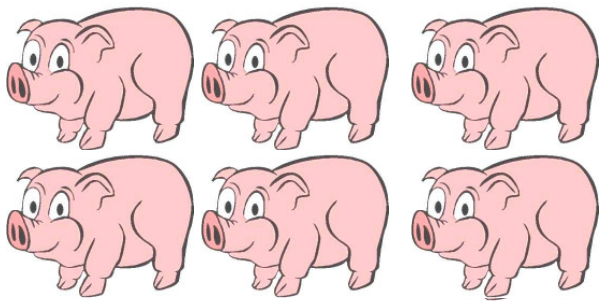
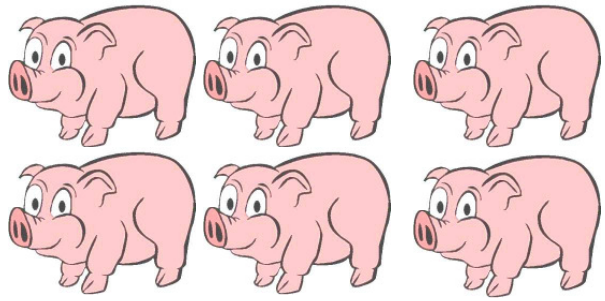
Feeding trial 1





Control group, basal diet

Fumonisin group, 2 mg FB1/kg BW by gavage in addition to basal diet

Day 0 → Plasma samples

Day 7 → Plasma samples

Day 14 → Plasma and tissue samples (lung, liver, kidney)

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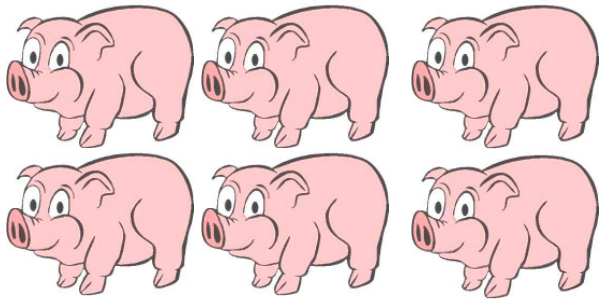
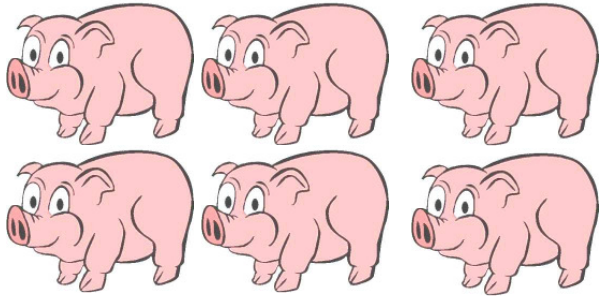
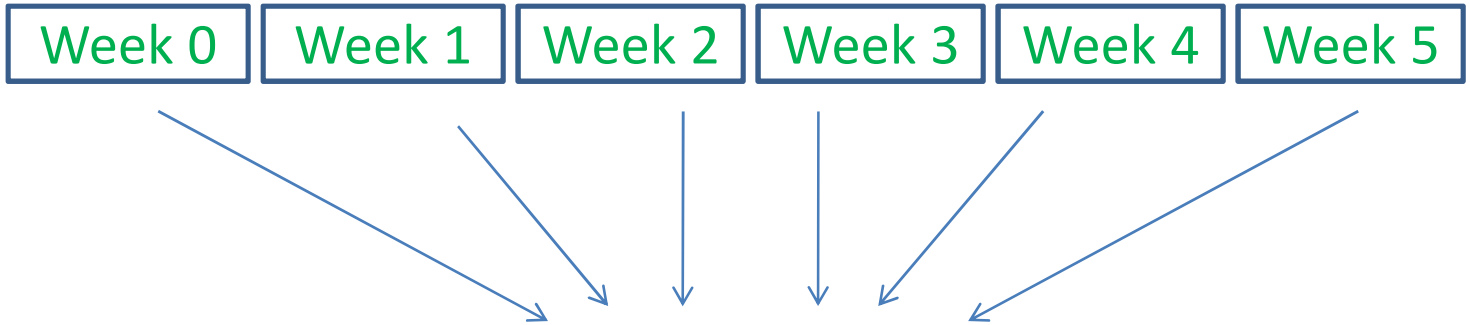
Feeding trial 2





Control group, basal diet

Fumonisin group, 4.6 mg/kg FB1 (6.4 mg/kg total fums) in feed provided ad libitum (~ 0.15 mg FB1/kg BW)



Plasma samples

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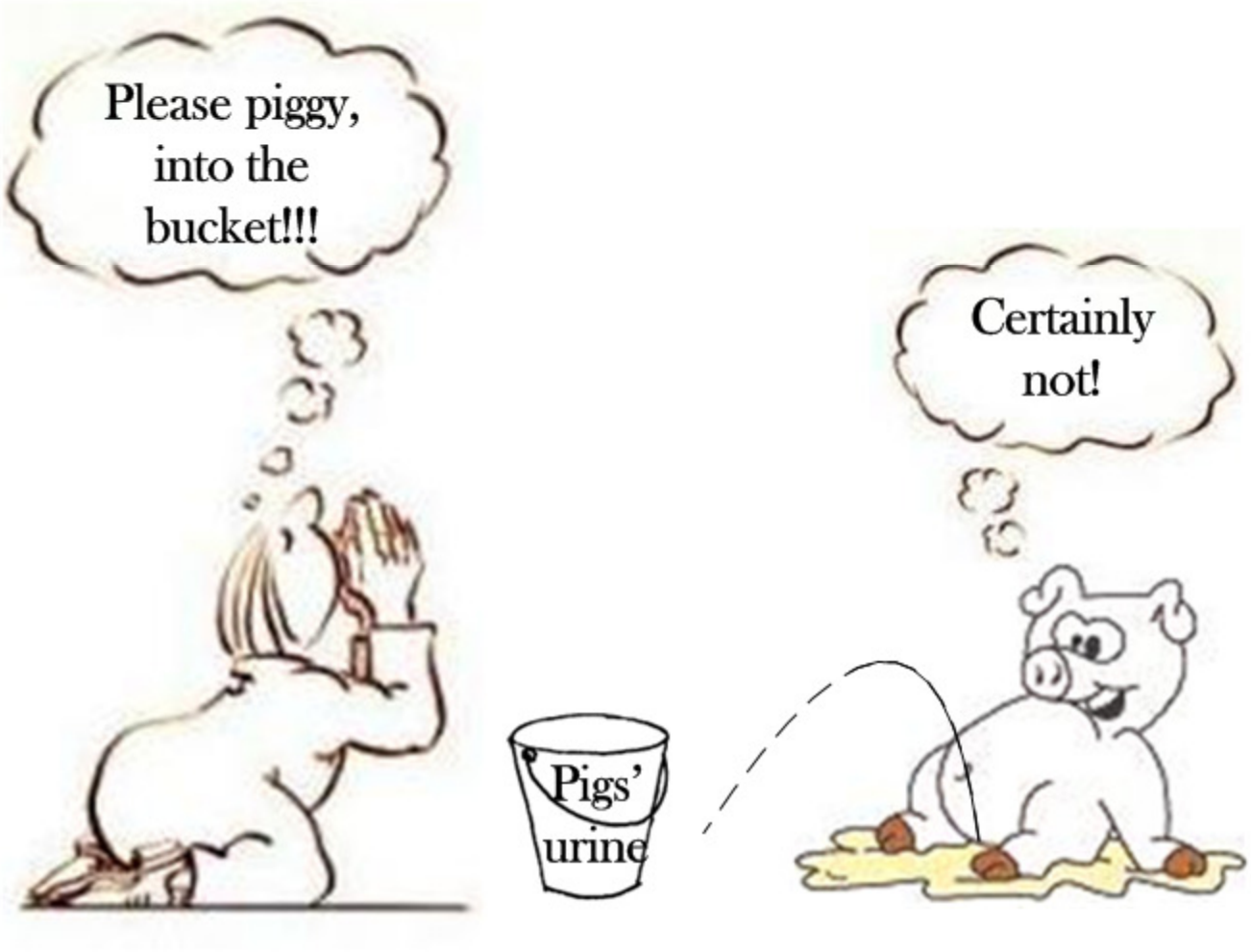
Collection of plasma and tissue samples, but no urine collection because of

- Dependence of sphingoid base concentrations on the amount of cells in urine
- Tricky sampling



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Sphingoid base analysis (1)

TISSUES (stored at -80°C)

Homogenization of ≥ 1 g of tissue in the 4-fold volume of cold K_2HPO_4 buffer

50-100 µl of homogenate + internal standards (C17-So, C20-Sa)

PLASMA (stored at -20°C)

200 µl plasma + internal standards (C17-So, C20-Sa)

0.4 ml of $CHCl_3$

1.3 ml of KOH in MeOH (0.154 M)

Shaking for 1 h at 37°C

1.2 ml of $CHCl_3$

3 x washing with alkaline water, evaporation of organic phase

Derivatization with OPA

HPLC-FLD analysis

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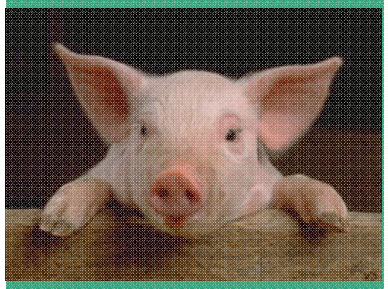
Sphingoid base analysis (2): Challenges

- Homogeneity of tissue samples
- Coelution of internal standards C17-So and C20-Sa with matrix compounds in liver and lung samples
- Baseline increase in chromatograms requiring regular column washing
- Limited stability of opa-derivatized samples – FMOC-derivatization is no suitable alternative
- Acidic mobile phase required for HPLC-separation



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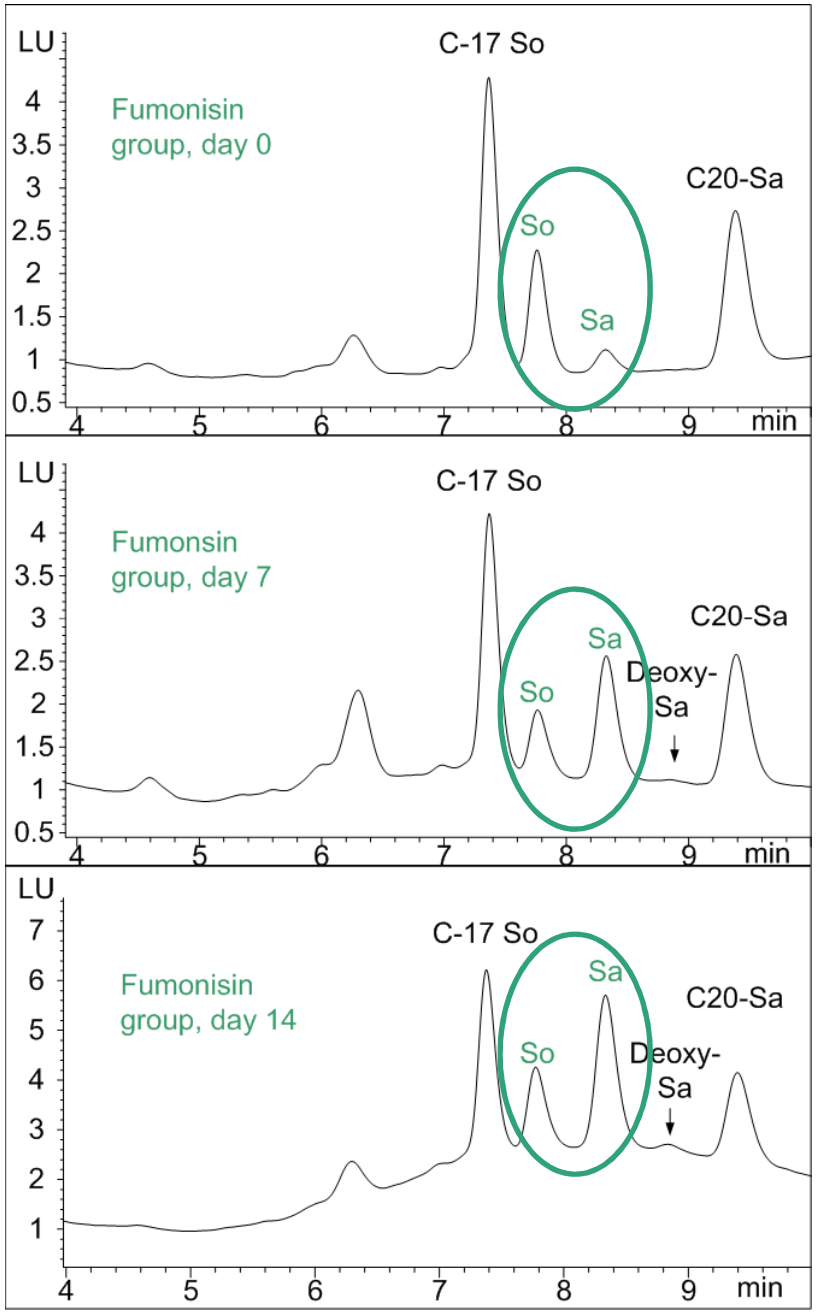
Feeding trial 1: Chromatograms of plasma samples



Column: C6 Phenyl (50 x 2 mm, 5 µm, Phenomenex) equipped with a pre-column of the same material

Flow rate: 0.5 ml/min

Temperature: 25 ℃

Injection volume: 25 µl

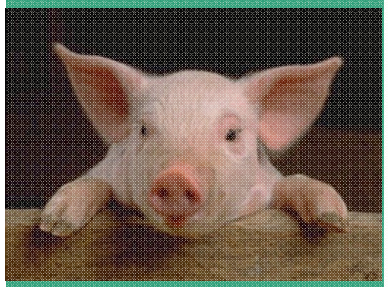
Mobile phase A: 60% aqu. MeOH containing 0.1% glacial acetic acid

Mobile phase B: 90% aqu. MeOH containing 0.1% glacial acetic acid

Gradient elution:

Time (min)	%B
0	0
4.5	84
6.8	90
9	90
9.5	100
13.9	100
14	0
16	0

Fluorescence detection: λex: 335 nm, λem: 440 nm



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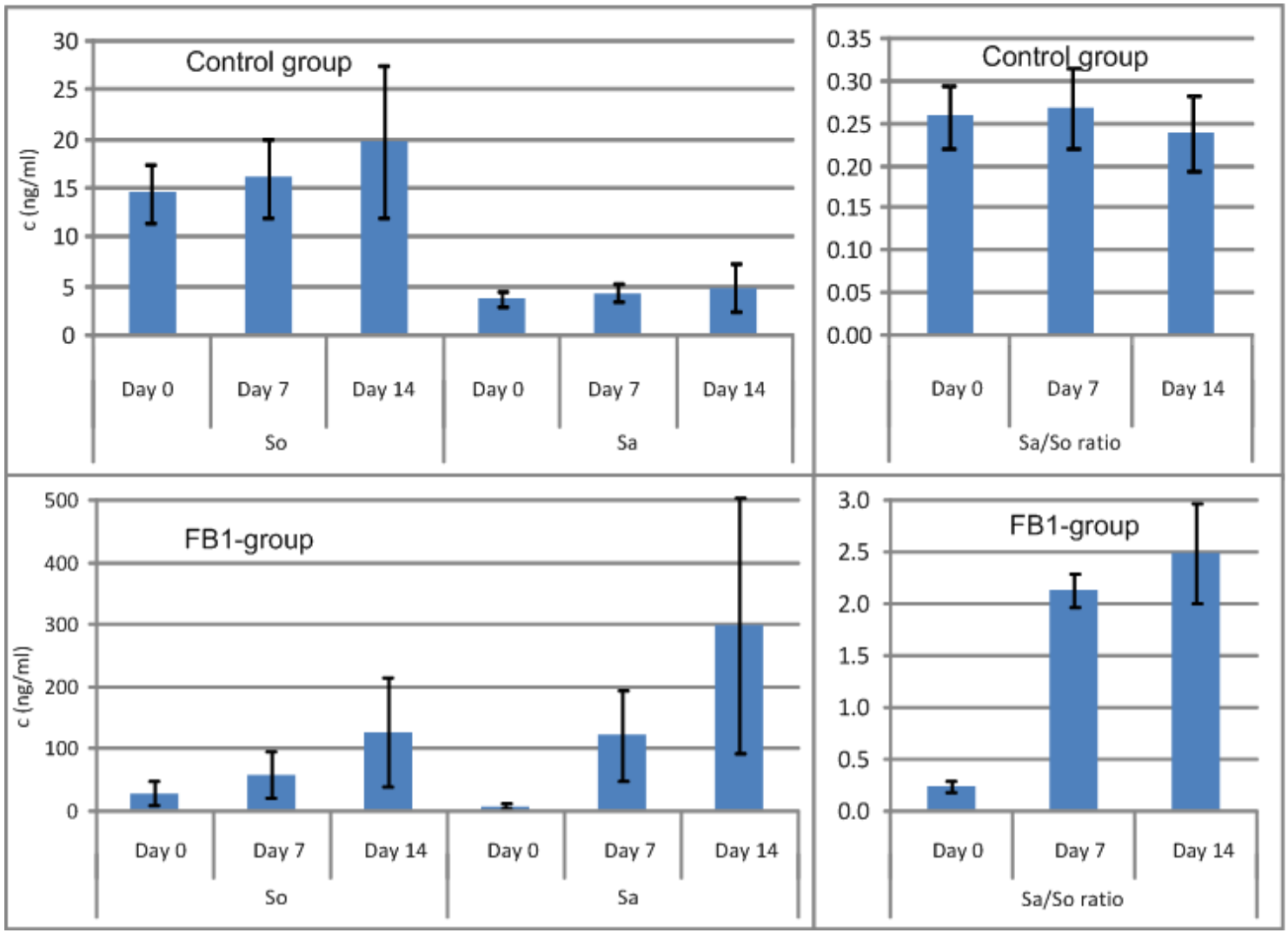
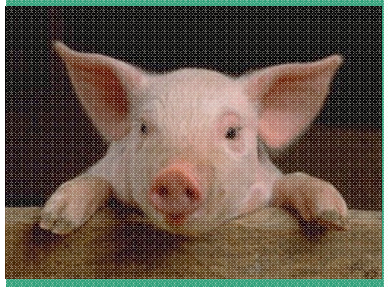
Feeding trial 1 (2 mg FB1/kg BW/day): Concentrations and ratios in plasma samples (N = 6)



So: Sphingosine
Sa: Sphinganine



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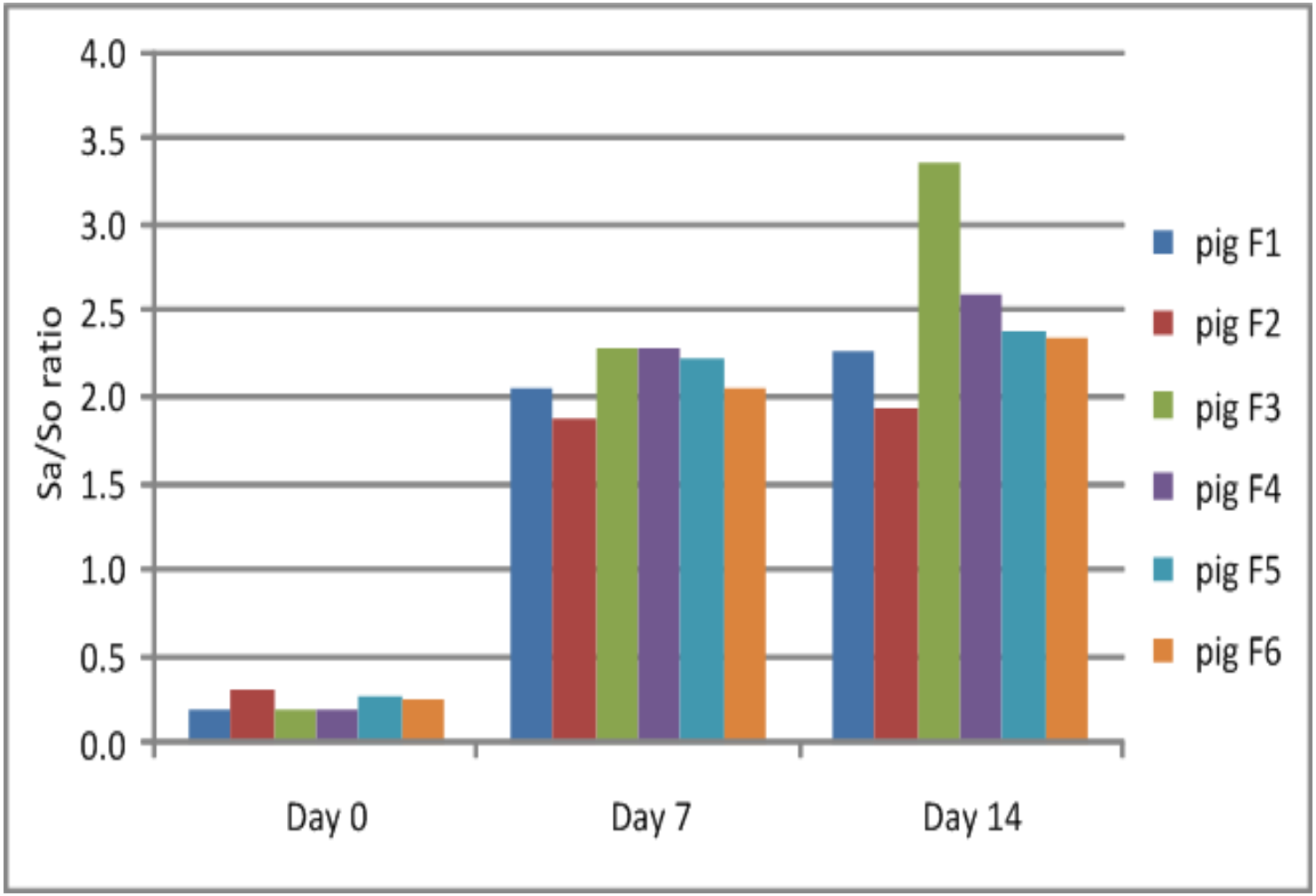
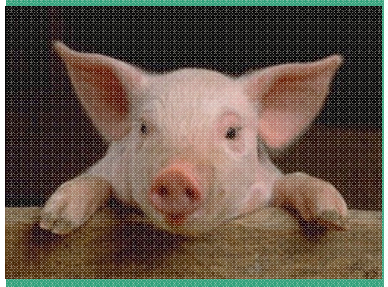
Feeding trial 1:

Sa/So ratios in plasma samples of individual pigs of the fumonisin group





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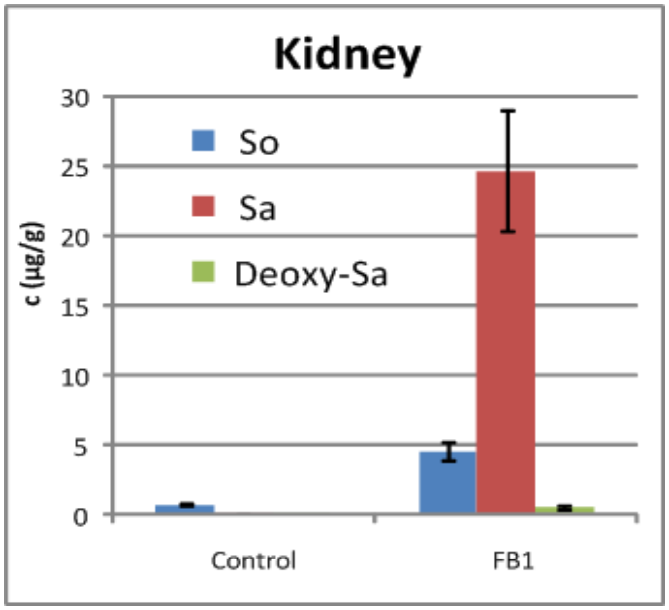
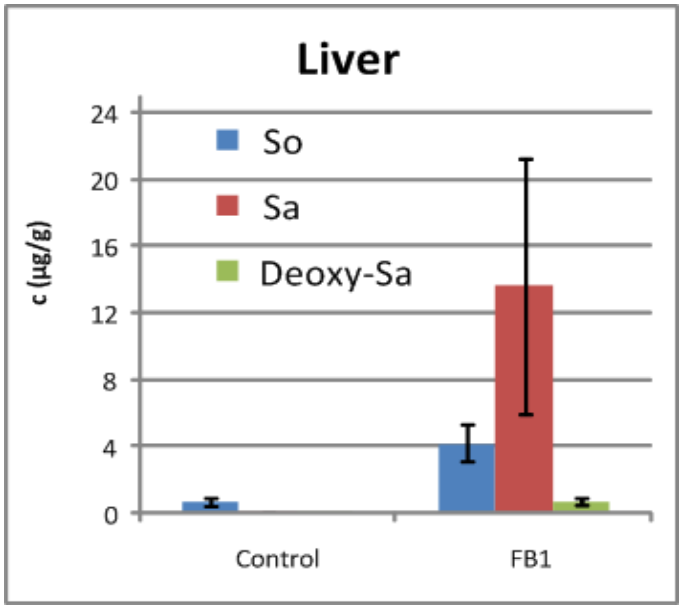
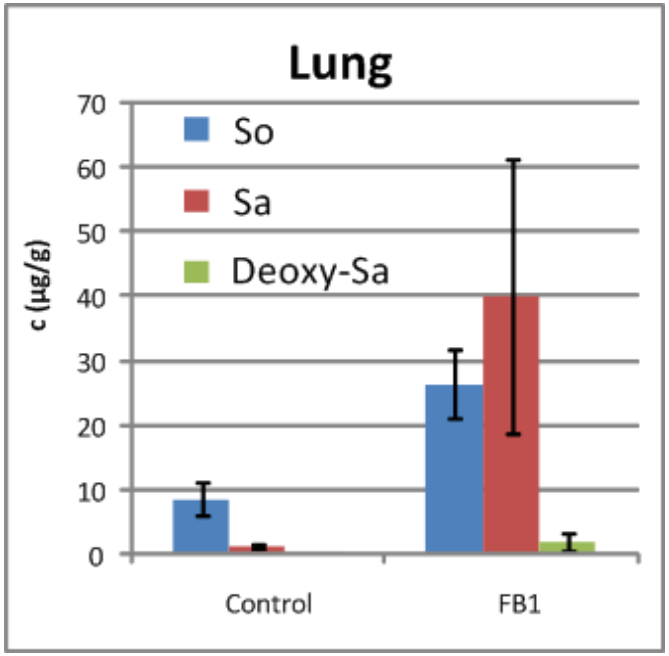
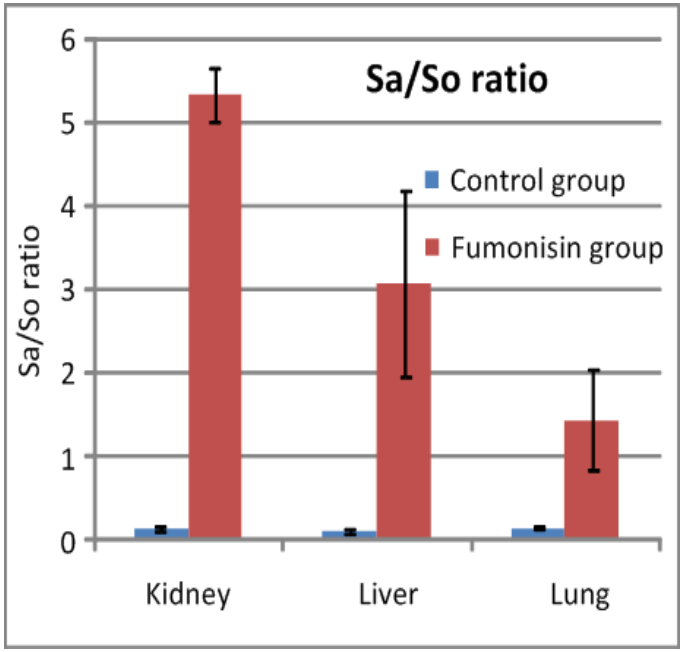
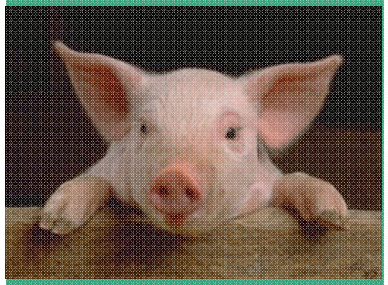
Feeding trial 1:
Concentrations and ratios in tissue samples (N = 6)











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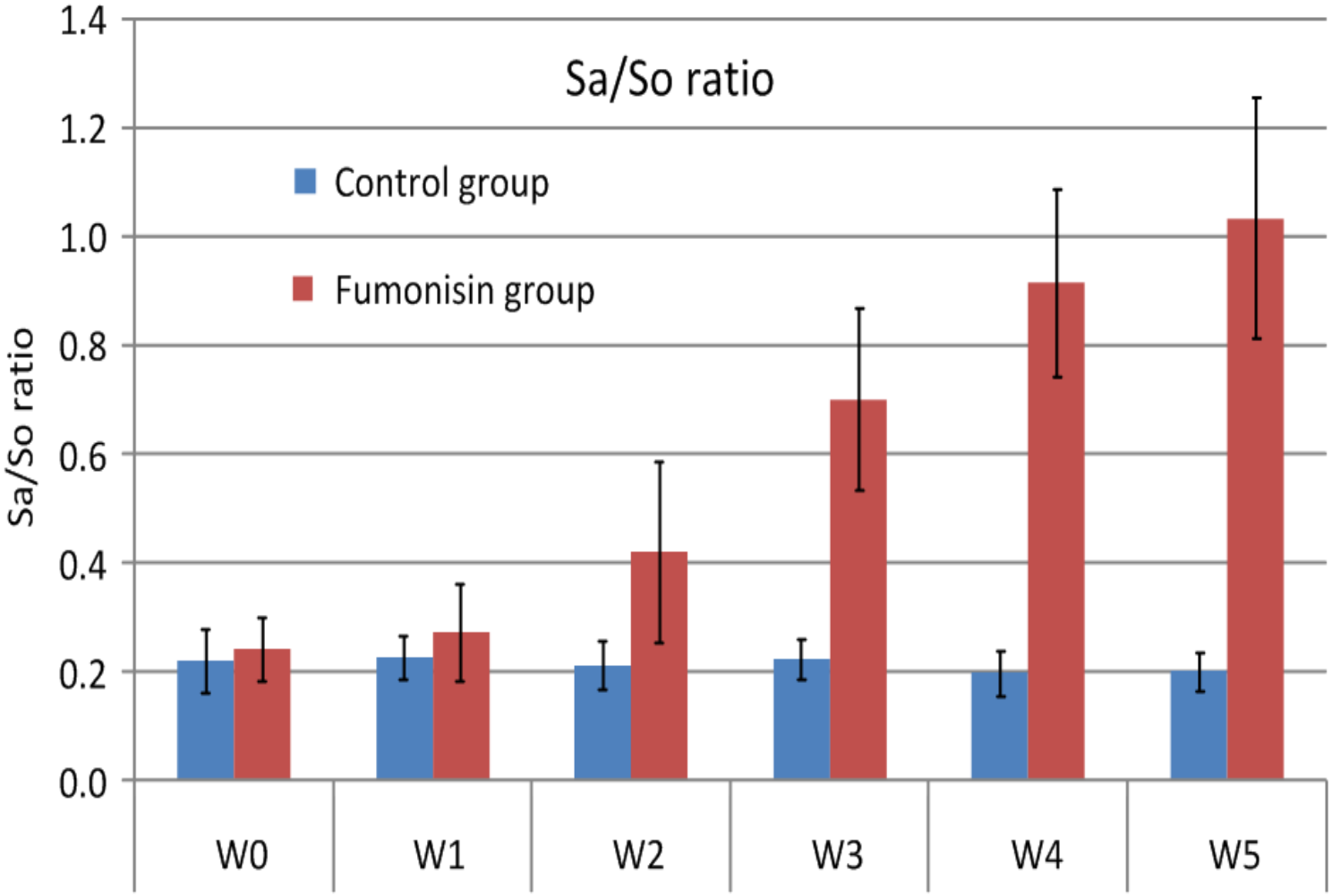
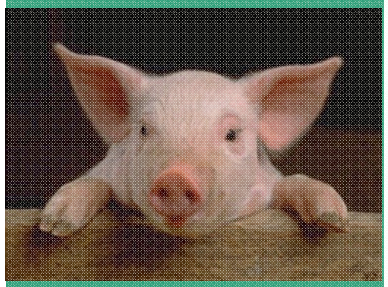
Feeding trial 2:
Sa/So ratios in plasma samples (N = 6)



W: Week



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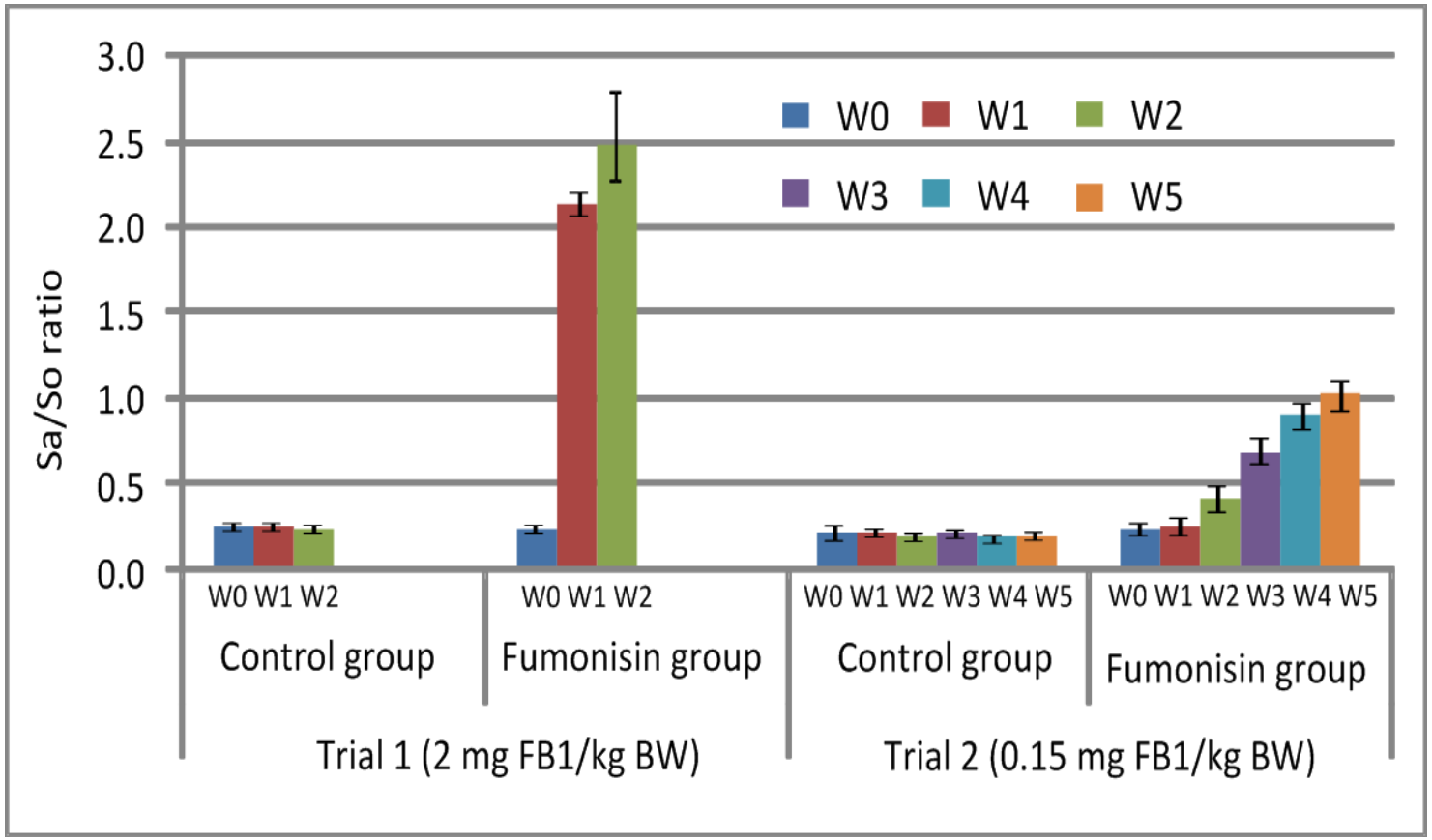
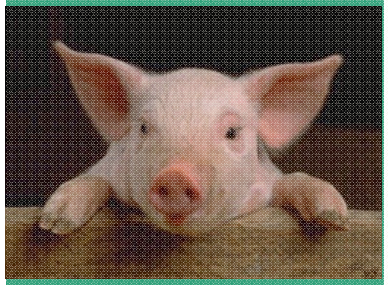
Comparison of Sa/So ratios in plasma in feeding trials 1 and 2



Consumption of 2 mg FB1/kg BW for 1 week results in significantly greater Sa/So ratios than consumption of 0.15 mg FB1 and 0.06 mg FB2/kg BW for 6 weeks



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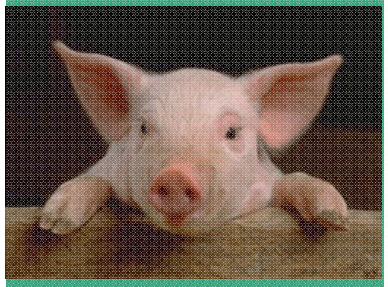
Conclusion (1)

- Big differences in concentrations of sphingoid bases and in the Sa/So ratios of pigs of one feeding group
- Despite that, the Sa/So ratios in all samples of piglets fed 2 mg/kg BW FB1 were significantly greater than Sa/So ratios in the control group
- Sa/So ratios in plasma of all piglets fed 0.15 mg/kg BW FB1 were significantly greater than Sa/So ratios in plasma of control piglets after ≥ 2 weeks of FB1-treatment
- Greatest Sa/So ratios were observed in kidney
- Greatest absolute concentrations of sphingoid bases were obtained in lung samples



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Conclusion (2)

- A concentration of 5 mg FB1/kg feed (EFSA-suggestion) is sufficient for significant changes in sphingolipid metabolism *in plasma* of piglets after ≥ 2 weeks

- The Sa/So ratio in plasma of piglets is a suitable biomarker for investigating the efficiency of fumonisin deactivating feed additives provided a fumonisin group and a control group are used for ≥ 2 weeks, preferably ≥ 4 weeks

- However, for confirmation of expected exposure to fumonisins a combination of several biomarkers is advisable (e.g. Sa/So ratio in plasma and presence of FB1 and its hydrolyzed forms in faeces of living animals)

Acknowledgements

Special thanks to

Dieter Moll (Biomin)
Bertrand Grenier (INRA Toulouse)
Isabelle Oswald (INRA Toulouse)
Irene Schöner (IFA Tulln)
Anita Seeböck (IFA Tulln)

Thank you for your attention!