Acta Agraria Kaposváriensis (2006) Vol 10 No 2, 285-290
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# Biomarker in the detection of fumonisin toxicosis in pigs

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***ABSTRACT***

*The sphinganine to sphingosine ratio (Sa/So) after oral administration of 50 mg fumonisin $B_1$/animal/day for 10 days and after a 10-day elimination period was studied in weaned barrows. At the beginning of the experiment, then after a 10-day period of the toxin feeding and on the 10$^{th}$ day after the end of the toxin feeding blood samples were taken and from those the Sa/So ratio was determined. Directly after the 10-day period of the toxin feeding the Sa/So ratio was 6.2-fold higher, as compared to control group and it decreased only in a* slight *extent even after the 10-day elimination period. Based on the results, presumably the restitution in the Sa-So parameters is more slower than the elimination of fumonisin from the organism.*
(Keywords: sphinganine, sphingosine, fumonisin, biomarker, pig)

## INTRODUCTION

Fumonisins are a family of mycotoxins that were first isolated in South Africa in 1988 from cultures of *Fusarium verticillioides* (*Gelderblom et al*., 1988), followed soon thereafter by elucidation of the structures of the prevalent isoforms fumonisin $B_1$ ($FB_1$) and $B_2$ ($FB_2$) (*Bezuidenhout et al.* 1988). Leukoencephalomalacia in horses and pulmonary edema syndrome in pigs (Ross et al. 1990) were shown to be result of $FB_1$ administration (*Kellerman et al*., 1990; *Harrison et al.,* 1990), and field outbreaks were associated with fumonisin contamination (*Plattner et al.*, 1990) when analytical methods were developed (*Shephard et al*., 1990). Fumonisins were also implicated in esophageal cancer when they were found in home-grown maize in a high-incidence area of the Transkei region of South Africa (*Sydenham et al*., 1990). $FB_1$ has been demonstrated to cause liver and kidney cancer in rats and mice (*Gelderblom et al*., 1991), and the International Agency for Research on Cancer evaluated $FB_1$ as a Group 2B carcinogen, i.e., possibly carcinogenic to humans (*IARC*, 1993).

The structural similarity between sphinganine and fumonisin $B_1$ led to the hypothesis that this mycotoxin acts by disrupting the metabolism and/or function of sphingolipids (*Merrill et al.,* 1996). There is considerable support for the hypothesis that fumonisin-induced disruption of sphingolipid metabolism is an important event in the cascade of events leading to altered cell growth, differentiation, and cell injury observed both in vitro and in vivo (WHO, 2002). Complete inhibition of ceramide synthetase by fumonisins causes a rapid increase in the intracellular concentration of sphinganine and sometimes of sphingosine, both in vivo and in vitro (*Delongchamp* and *Young*, 2001; *Enongene et al*., 2000).

Numerous studies are available concerning the use of sphinganine to sphingosine ratio (Sa/So) in plasma during fumonisin exposure (*Riley et al*., 1993). In horses and

pigs, it appears predictive of fumonisin exposure. By contrast, in other studies conducted in pigs, even if Sa/So also increases during exposure to the toxin, hepatic biochemical parameters appears more sensitive (*Rotter et al.,* 1996). Similar results were also reported in certain studies conducted in rodents (*Castegnaro et al*., 1996). In avian populations, both Sa/So and bio-chemical parameters of hepatic damage increase during fumonisin exposure (*Bermudez et al*., 1995).

The aim of the study was to get data on Sa/So ratio after a high $FB_1$ exposure, to get information about the effect of $FB_1$ on the mentioned biomarker of fumonisin toxicosis. Moreover, to get information on the degree of its regression after the withdrawal of the toxin containing diet.

## MATERIALS AND METHODS

### Experimental animals, design of the experiment

Weaned castrated pigs of approximately 12–14 kg body weight were used. Animals (treated n=10; control n=6) were placed in metabolic cages during the trial. A *Fusarium verticillioides* fungal culture was mixed into the feed of the experimental animals, so as to provide a daily $FB_1$ intake of 50 mg/animal, i.e. 3.5–4 mg/kg body weight/day.

The experimentally produced fungal culture contained a high amount of $FB_1$; this was mixed in a pre-defined proportion to the feed reaching the aimed toxin concentration. This was performed according to the modified method of *Fazekas* (1998). At the beginning of the experiment (day 0), than after a 10-day period of the toxin feeding (day 10) and on the$10^{th}$ day after the end of the toxin feeding (day 20), blood samples were taken, and the Sa/So ratio was determined.

The experimental procedure, the determination of the Sa/So ratio were performed at the University of Kaposvár Faculty of Animal Science. The trial is approved by the Animal Experimentation Ethics Committee of the University of Kaposvár.

### Analysis

Analytical method for the determination of sphinganine sphingosine ratio was according to *Castegnaro et al*. (1998) after some modifications, as described below.

*Equipment*

An HPLC liquid chromatographic system (Shimadzu-Gynkotek) was coupled with manual injector port with 50 µl sample loop, a flourescence detector (Shimadzu RF551) and a data computing system (Chromeleon 4.30). Separation of sphingolipids was on a Supelco RP-Amyde C16 column maintained at room temperature.

*Sphingolipid extraction*

KCl (1.5 ml, 0.8%) and KOH (50 µl, 1 M) were added to 500 µl of serum. The mixture was extracted with 5 ml of ethyl acetate by gentle agitation with vertical shaker for 20 min. The phases were separated by centrifugation 600 g, 4 min. 4 ml of organic phase was evaporated to complete dryness at 50 $^{o}$C under $N_2$.

*O-phtalaldehyde (OPA) derivatisation*

The derivatisation mixture consisted of 5 mg OPA in 1 ml of MeOH containing 8 µl of 2-mercaptoethanol and 5 ml of 3% boric acid solution (pH 10.5 with 1 M KOH). Following the ethyl acetate extraction the dried samples were dissolved in 100 µl of methanol-water mixture 9:1 by vortex shaking for 30 sec and derivatisated for at least 30 min of the above 50 µl OPA mixture.

*HPLC analysis of the derivates*

The derivates were analysed by HPLC with fluorescence detection (ex.: 340 nm em.: 455 nm). The column (Supelco RP Amide-C16 150x4mm, 5 µm) was kept at room temperature and the isocratic eluent (MeOH-water 84:16) flowrate maintained at 1 ml/min. The injection volume was 50 µl of derivated samples or standards.

*Statistical analysis*

Statistical analysis of the data obtained was carried out by the SPSS statistical software package using the version 7.5. All basic data (means, standard deviations, extreme values) were evaluated. Data were analyzed with one-way analysis of variance (ANOVA) and the least significant difference (LSD) test was used to compare means. Probability levels less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

It can be generally established that the fumonisin exposure in pigs caused a marked elevation in the concentration of free sphinganine, and to a lesser extent in sphingosine, with a consequent increase in the sphinganine to sphingosine ratio (*Table 1*). These findings show good agreement with the results obtained in the experiment of *Gumprecht et al*. (1998) on swine.

The sphinganine to sphingosine ratio was significantly increased on the last day of the toxin feeding (day 10), as a result of the toxin dose applied. The sphinganine to sphingosine ratio of fumonisin-treated pigs' plasma was significantly greater than those of control pigs' plasma, and than of those samples taken on day 0 (*Table 1*).

**Table 1**

**Influence of oral administration of 50 mg fumonisin $B_1$/animal/day on the ratio of sphinganine to sphingosine (Sa/So) in plasma**

| Date | Sa/So (mean ± S.D.) | |
|---|---|---|
| | Control group (n=6) | Treated group (n=10) |
| Day 0 | $0.43^{a} \pm 0.04$ | $0.40^{a} \pm 0.10$ |
| Day 10 | $0.46^{a} \pm 0.03$ | $2.85^{b} \pm 0.48$ |
| Day 20 | $0.42^{a} \pm 0.06$ | $2.33^{c} \pm 0.21$ |

$^{abc}$Means with different superscripts are different at the 1% probability level.

Directly after the 10-day period of the toxin feeding the Sa/So ratio was 6.2-fold higher, as compared to control group. This parameter decreased only in a slight extent, i.e. even after the 10-day elimination period it was 5.5-fold higher than at the beginning of the experiment.

Fumonisin residues accumulated in liver and kidney when pigs were fed [$^{14}$C] $FB_1$ at dietary concentrations of 2–3 mg $kg^{-1}$ for 24 days (*Prelusky et al*., 1996). When this was followed by clean food, residue levels dropped to about 35% of peak values after 3 days, and were only marginally above the detectable limits after 9 days. Sphingoid bases increased in serum earlier (within 24 h) (*Gumprecht et al*., 1998) at even much lower doses of fumonisin (≤5 mg $kg^{-1}$) (*Riley et al.,* 1993) than liver enzymes and before morphologic tissue alterations, suggesting that the serum concentration of sphingoid

bases could be used as a sensitive biomarker of fumonisin exposure. However, the role of altered sphingolipid biosynthesis in the pathogenesis of fumonisin toxicity is still under investigation. There are no data available about the total restitution concerning the parameters of sphinganine to sphingosine ratio. Based on the results of the present study and the earlier published data about the elimination of $FB_1$ (*Prelusky et al*., 1996), presumably the restitution in the Sa-So parameters is slower than the elimination of fumonisin from the organism. Therefore, to clarify entirely the elimination of $FB_1$ from the organism, including following up of the Sa/So ratio, further animal studies are needed with long-term elimination period.

## CONCLUSION

The ratio of sphinganine to sphingosine increases after exposure to fumonisins; these increases occurred long before any indication of cytotoxicity (*Riley et al*., 1994). The results of the in vitro studies suggested that changes in the relative amounts (i.e. in the ratio) of free sphinganine and free sphingosine might be useful as a biomarker for fumonisin consumption in animals (*Riley et al.*, 1994). Based on the results of the present study and the earlier published data about the elimination of $FB_1$ (*Prelusky et al*., 1996), the restitution of the Sa-So parameters to the physiological values would presumably be slower than the elimination of fumonisin from the organism.

The determination of Sa/So alteration kinetics would give us the possibility to verify the consumption of fumonisin contaminated diet even when the toxin is already not detectable in it. Therefore, the objective of our future studies will be to determine the Sa/So ratio as a presumptive test for identifying animals that consumed a fumonisin-contaminated feed.

## ACKNOWLEDGEMENTS

The research was supported by the Hungarian Academy of Sciences (project no. B04074) and the Ministry of Education (NKFP 4/024/2004).

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