PROTEIN PROFILES OF SERUM, BRAIN REGIONS AND HYPOPHYSES OF PUBERTAL BOARS FED DIETS CONTAINING FUMONISIN B₁

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ABSTRACT

The effects of dietary fumonisin B₁ (FB₁), a toxin produced mainly by Fusarium verticillioides and F. proliferatum that grow on maize worldwide, on protein profiles of serum, brain regions and hypophyses were studied in 24 male Large White weanling pigs randomly divided into four groups (n = 6). In a completely randomized design, each group of the animals with six replicates, received one of the four diets containing 0.2, 5.0, 10.0 and 15.0 mg FB₁/kg constituting the Control, Diet 1, Diet 2 and Diet 3, respectively, in a 6-month feeding experiment. At the end of the feeding experiment, blood sample was collected from the ear vein of each animal for serum protein analysis. All the animals were slaughtered and the brains and the hypophyses were carefully dissected out to determine the total protein (TP) concentrations in the regional brain and hypophyses. Animals fed the Control diet and Diet 1 had significantly (P<0.05) higher serum protein profiles than those on Diets 2 and 3. It was observed that TP concentrations decreased significantly (P < 0.05) in the cerebellum, hypothalamus and the medulla oblongata as the dietary FB₁ concentration increased. The TP concentrations in these brain regions and hypophyses of the animals on Diets 1, 2 and 3 ranged from 42.1-105.6, 30.5-96.2 and 26.3-92.3 % of the Control, respectively. Chronic dietary exposure to FB₁ at concentrations above 5.0 mg/kg is a potential health risk that may interfere with protein metabolism and result in significantly reduced serum protein profiles. This may not be lethal to growing pigs but a potential health risk that may produce adverse physiological response in the animals.

Keywords: Brain; Fumonisin B₁; Hypophysis; Pigs; Protein; Serum.

INTRODUCTION

Fumonisins are mycotoxins produced by Fusarium moulds, most notably Fusarium verticillioides (=F. moniliforme) and F. proliferatum. Mycotoxins are natural contaminants of cereals and other food commodities throughout the world and they significantly impact human and animal health. The economic consequences of mycotoxin contamination are profound, and exposure of human and livestock to mycotoxin-contaminated foods is particularly a serious problem in the tropics (Reddy and Raghavender, 2008).

Fumonisins are contaminants of agricultural products, particularly maize worldwide (Nelson et al., 1991) and have been documented to cause various physiological responses in humans and animals. Although several naturally-occurring fumonisins are known, fumonisin B₁ (FB₁) has been reported to be the most abundant and most toxic which represents approximately 70% of the total concentration in naturally-contaminated foods and feeds. Toxicological studies on the fumonisins have consequently been concentrated on FB₁.

Fumonisins have been implicated as a causative agent in several animal and human diseases. The toxins have also been associated with different kinds of mycotoxicoses in domestic animals, such as leukoencephalomalacia in equines (Ross et al., 1993), pulmonary edema in pigs (Colvin and Harrison, 1992), hepatocellular carcinoma in rats (Gelderblom et al., 1994) and apoptosis in the livers of rats (Proctor, 2000). There are numerous reports that weanling piglets (Friend et al., 1982; Döll et al., 2003) and growing and finishing pigs (House et al., 2002; Dänicke et al., 2004) are particularly sensitive to feed-borne Fusarium mycotoxins. The major symptoms seen in pigs consuming Fusarium-contaminated feeds include reduced tissue protein synthesis (Dänicke et al., 2006) and immunomodulation (Swamy et al., 2003). Also, significantly altered concentrations of neurotransmitters in brain regions of starter pigs fed a blend of grains naturally contaminated with Fusarium mycotoxins have been reported (Swamy et al., 2002).

An estimation of the proteins in body fluids may be utilized as an assessment of the nutritive state
of the animal. The nutritive state may be dependent not only on the proper and adequate intake of protein building materials in the diet but may also be a reflection of the nutritive state existing within the animal body, reflecting alterations in metabolism (Gbore and Egbunike, 2009). Fumonisins inhibit sphingolipid metabolism in tissues, leading to an accumulation of sphingoid bases - sphinganine and sphingosine, which are intermediates in sphingolipid biosynthesis (Wang et al., 1991) and a variety of biological activities for sphingolipids have been reported (Wang et al., 1992). Alterations in the amounts of any of these by fumonisins could potentially result in a variety of biological and pathological effects. It was therefore hypothesized that fumonisin could alter protein synthesis in serum and the brain since the brain contains high levels of sphingolipids and proteins in the nervous system are known to occur in complexes with lipids. Based on the reviewed physiological effects of fumonisin on animals, this study was designed to determine the effects of chronic exposure to dietary FB on protein profiles in serum, brain regions and hypophyses of pubertal boars.

MATERIALS AND METHODS

Fumonisin Production and Diets
Maize grits in 500 g quantities soaked with 200 ml of distilled water were placed into autoclavable polypropylene bags and autoclaved for 1h at 121 °C and 120 kPa. The autoclaved maize grits were then cultured with a toxigenic strain of F. verticilloides (MRC 286) obtained from the Plant Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (7 20'N, 3 50'E, 200 m above sea level with an average daytime temperature of 24-25 °C and relative humidity 80-85 %). The study was approved by the local Institutional Animal Ethics Committee and performed in accordance with Guide for the Care and Use of Laboratory Animals (NRC, 1996).

After a 2-week physiological adjustment period, 24 clinically normal male Large White weanling pigs of about 8 - 9 weeks of age averaging 6.94 ± 0.26 kg housed in individual concrete floor indoor pens were randomly divided into four groups, with six animals housed individually per group. Each group received one of the four diets. The feeding experiment, which lasted 6 months, was divided into 3 physiological phases [weanling (starter), pre-pubertal (grower) and pubertal (finisher)]. The animals were fed their respective diets ad libitum daily. The gross compositions of the experimental diets, fed during weanling, pre-pubertal and pubertal's phases for 6, 10 and 8 weeks, respectively, are shown in Table 1.

Serum and Brain Tissue Protein Determination
At the end of the experiment, blood sample was collected from the ear vein of each animal into Monoject® vacutainer without Ethylene diaminotetraacetic acid (EDTA). Thereafter, all the animals were stunned mechanically prior to bleeding, quickly decapitated and the brains and hypophyses immediately removed, freed of all adhering meninges and blood vessels. The brains and the hypophyses obtained were dissected on ice-cold porcelain tile into the pons, cerebellum, amygdala, hippocampus, hypothalamus, cerebral cortex, mid-brain, medulla oblongata, adenohypophysis and neurohypophysis as previously described (Egbunike, 1981). The blood samples obtained were centrifuged at 4000 rpm
for 10 minutes and separated sera analysed for the evaluation of serum proteins at the Chemical Pathology Unit of the University College Hospital, Ibadan, Nigeria. The serum total protein was determined by the Biuret method of Reinhold (1953) using a commercial kit (Randox Laboratories Ltd, U.K.). The albumin value was obtained by bromocresol green method of Doumas et al. (1971), while the globulin and albumin-globulin ratio were obtained according to the method of Coles (1986).

The brain and hypophyseal total protein concentrations were determined by Biuret method according to Reinhold (1953) immediately after the brain and hypophyseal samples from each animal was homogenized (1%, w/v) with a Potter-Elvehjem homogenizer in 0.1 M ice-cold phosphate buffer containing 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA).

**Statistical Analysis**

The experimental design used was complete randomized. All data obtained were subjected to one-way statistical analysis of variance (ANOVA) procedure of SAS (2001). The significant treatment means were compared at 5% probability level using the Duncan multiple range test option of the same software.

**RESULTS AND DISCUSSION**

The serum protein profiles of pubertal boars fed different concentrations of dietary FB₁ for 24 weeks are shown in Table 2. The table shows that the serum proteins were dietary FB₁ dose-dependent. The serum proteins decline with an increase in the dietary FB₁ concentrations. The serum proteins of animals fed the Control diet and Diet 1 were significantly (P < 0.05) higher than the serum protein values of those fed Diet 2 containing 10 mg FB₁/kg, which were significantly (P < 0.05) higher than the serum proteins of those fed Diet 3 containing 15 mg FB₁/kg.

The effect of varied dietary FB₁ on total protein in the brain regions and hypophyses of pubertal boars are shown in Table 3. Results showed significant (P < 0.05) decline in the total protein concentrations in the cerebellum, hypothalamus and the medulla oblongata with an increase in the dietary FB₁. The total protein concentrations in the pons, hippocampus, midbrain and the hypophyses tended to decline with an increase in the dietary FB₁ concentrations while the concentrations in the amygdala did not follow any particular trend. The total protein concentrations in the brain regions and hypophyses of the animals on Diet 3 ranged from 26.32-92.31 % of those on the Control diet.

**Table 1: Compositions (%) of the Test Diets for the Various Physiological Phases**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Physiological phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weanling</td>
<td>Pre-pubertal</td>
</tr>
<tr>
<td>Maize*</td>
<td>40.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>20.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>20.00</td>
<td>25.00</td>
</tr>
<tr>
<td>Wheat offal</td>
<td>14.00</td>
<td>14.30</td>
</tr>
<tr>
<td>Rice husk</td>
<td>-</td>
<td>11.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Fixed ingredients**</td>
<td>2.70</td>
<td>2.70</td>
</tr>
</tbody>
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**Nutritional levels**

| Crude fibre (%)         | 5.35 | 9.82 | 10.83 |
| Crude protein (%)       | 20.38 | 17.97 | 15.30 |
| DE*** (kcal/kg)         | 2700.00 | 2270.00 | 2240.00 |

*Mixture of *Fusarium*-cultured and non-cultured maize in various proportions to achieve desired dietary FB₁ levels for each treatment.

**Supported by**

- Dicalcium phosphate (1.50), Oyster shell (0.50), Salt (0.45)
- Minerals-vitamins premix (0.20), Methionine (0.01) and Lysine (0.04).

***Calculated Digestible Energy values.
Reduced tissue protein synthesis has been reported to be one of the major symptoms seen in pigs consuming *Fusarium*-contaminated feed (Dänicke et al., 2006). The significantly lower serum protein values in pubertal boars fed Diets 2 and 3 in this study indicated that the dietary FB might have induced some alterations in protein metabolism in the animals, since serum protein synthesis is reported to be related to the amount of available protein in the diet (Iyaiyi and Tewe, 1998). The dietary mycotoxin, fed over long period, might have elicited some pathological and physiological changes in the animals, leading to poor digestion, poor absorption or poor utilization of dietary protein. The results suggest that the toxin might have inhibited protein metabolism as reported for sphingolipid synthesis (Riley et al., 1996) or protein digestibility and absorption as observed in the animals (Gbore and Egbunike, 2007). Since all the pubertal boars were fed isonitrogenous diets which contain only varied levels of FB, the results probably indicate interference of serum protein metabolism by FB as previously reported in rabbits (Ewuola and Egbunike, 2008; Gbore and Akele, 2010) and fingerlings (Gbore et al., 2010) fed different concentrations of dietary fumonisin or FB. Patulin, a secondary metabolite of a number of fungal species was reported to interfere with protein biosynthesis (Arafat and Musa, 1995). Also, the disruption of cellular membrane structure by cytotoxic mycotoxins, which interferes with vital cellular processes, including protein synthesis, has been reported (Guerre et al., 2000). Results from this present study suggest that FB could perturb protein biosynthesis in pigs as well.

The overall reduction in circulating albumin with an increase in dietary FB concentrations suggests that the vascular endothelial cells might have been compromised by FB as a result of leakage of albumin out of the vascular space and into the surrounding tissues. Also, the results of this study suggest that the pubertal boars fed Diets 2 and 3 might have suffered liver impairment since the liver is the sole site of formation of albumin (Coles, 1986). Liver has been reported to be the primary target organ for toxicity caused by fumonisins in all species tested thus far (EHC, 1986).
The mean serum total protein values for pubertal boars fed Diets 2 and 3, which were significantly lower than those fed the Control diet and Diet 1, were also lower than 89.0 ± 4.80 g/L (= 8.90 ± 0.48 g/dl) reported by Mitruka and Rawnsley (1981) as the mean serum total protein value for the normal male pigs. This probably implies that there was significant alteration in protein metabolism in the animals fed diets containing > 5 mg FB/kg resulting in significant alterations in serum protein profiles from chronic hepatic and/or gastrointestinal disorders.

The brain plays a modulating role on all body functions and is able to keep the metabolism of the other glands and body systems in check. Age, nutrition, enzymes and hormones have direct effects on brain functions (Adejumo and Egbunike, 2004). The generally high protein concentrations in the hypothalamus, cerebellum and the medulla oblongata, and the moderate concentrations observed in the pons, hippocampus and midbrain could be directly linked to their functional roles. The hypothalamus, for instance, secretes hormones, which are largely protein in nature. Also, the more secretory nature of the adenohypophysis over the neurohypophysis may be responsible for the higher protein concentrations observed in the adenohypophysis, especially since it has direct anatomical link with the hypothalamus (Adejumo and Egbunike, 2002). It is noteworthy however; that the protein concentrations in the medulla oblongata, cerebellum and hypothalamus significantly decline with an increase in the dietary FB concentrations. It is expected that the hypothalamus and hypophyses would normally have higher protein contents on account of their functions in protein hormone production but the significant decline in the protein concentrations observed in the hypothalamus in this study may indicate influence of FB. Since the hypothalamus functions as a coordinator of the brain's other processes, including metabolism and neurosecretions, more work is required to elucidate the mechanism of action of FB, in this regard.

The significant reduction in some brain regional protein concentrations with an increase in the dietary FB concentrations in this study also lend credence to the hypothesis of adverse influence of dietary F. verticillioides mycotoxins on protein metabolism. The significantly lower concentrations of total protein in the cerebellum of the animals with an increase in the dietary FB may be an indication of the interference of FB with neural mechanisms involved with protein synthesis and the rate of turnover in the cerebellum. The protein concentrations in the hypothalamus of the animals fed Diets 1, 2 and 3, which were about 28-42 % of the controls, may suggest hypofunction of this brain region in hormonal control in animals exposed to >5.0 mg/kg dietary FB. Sharma and Bahadur (1982) reported that neurosecretory cell number and total protein concentration in the brain are related to reproductive phenomenon. The reported reduced sperm production (Gbore and Egbunike, 2008), delayed sexual maturity (Gbore, 2009a), and inferior semen qualities (Gbore, 2009b) by the growing pigs fed increased concentrations of dietary FB might be secondary effects of the altered total protein in these brain regions of the animals.

CONCLUSION
This study suggests that dietary FB has an adverse influence on protein metabolism in animals chronically exposed to feeds containing >5.0 mg FB/kg. Feed contaminated with F. verticillioides that will yield above 5.0 mg FB/kg for a 6-month period may not be lethal to growing pigs, but present a potential health risk that may produce adverse physiological response in growing pigs.

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