PROTEIN PROFILES OF SERUM, BRAIN REGIONS AND HYPOPHYSES OF PUBERTAL BOARS FED DIETS CONTAINING FUMONISIN B_1

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ABSTRACT

The effects of dietary fumonisin B_1 (FB_1), 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 FB_1/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 FB_1 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 FB_1 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 B_1; Hypophysis; Pigs; Protein; Serum.

INTRODUCTION

Fumonisins are mycotoxins produced by *Fusarium* moulds, most notably *Fusarium* v*erticillioides* (=*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 occur as 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 B_1 (FB_1) 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 FB_1.

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 sphingolipids 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_1 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 ^{o}C and 120 kPa. The autoclaved maize grits were then cultured with a toxigenic strain of *F. verticillioides* (MRC 286) obtained from the Plant Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria to produce FB_1 as previously described (Nelson *et al.,* 1994). The uncultured maize grits were used to formulate the Control diet, while three other diets were formulated with the cultured maize grits substituted for the uncultured maize grits at various proportions. Samples of homogenously mixed diets were quantified in replicates for FB_1 and other common Fusarium mycotoxins including zearalenone, deoxynivalenol (DON, vomitoxin) and T-2 toxin, using mycotoxin quantitative CD-ELISA test kits (Neogen, Lansing, MI, USA). The concentration of FB_1 in the Control diet was 0.2 mg/kg while the concentrations of FB_1 in the other three diets were adjusted to 5.0, 10.0, and 15.0 mg/kg, constituting Diets 1, 2, and 3, respectively. All other Fusarium mycotoxins screened were below the detection limit of 0.2 mg/kg diet.

Animals and Feeding

The feeding experiment was carried out at the Animal Physiology Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria (7^{o}20'N, 3^{o}50'E, 200 m above sea level with an average daytime temperature of 24-25 ^{o}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 $\pm$ 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 diaminetetraacetic 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 FB_1 for 24 weeks are shown in Table 2. The table shows that the serum proteins were dietary FB_1 dose-dependent. The serum proteins decline with an increase in the dietary FB_1 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 FB_1/kg, which were significantly ($P < 0.05$) higher than the serum proteins of those fed Diet 3 containing 15 mg FB_1/kg.

The effect of varied dietary FB_1 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 FB_1. The total protein concentrations in the pons, hippocampus, midbrain and the hypophyses tended to decline with an increase in the dietary FB_1 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

	Physiological phase		
Ingredient	Weanling	Pre-pubertal	Pubertal
Maize*	40.00	30.00	20.00
Soybean meal	20.00	15.00	8.50
Palm kernel cake	20.00	25.00	45.00
Wheat offal	14.00	14.30	5.00
Rice husk	-	11.00	17.80
Fish meal	3.00	2.00	1.00
Fixed ingredients**	2.70	2.70	2.70
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 FB_1 levels for each treatment.

**Contained 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.

Table 2: Serum Proteins of Pubertal Boars Fed Different Concentrations of Dietary FB_1

Component	Control 0.2 mg FB_1	Diet 1 5 mg FB_1	Diet 2 10 mg FB_1	Diet 3 15 mg FB_1	SEM
Total protein (g/L)	119.70[a]	119.75[a]	88.30[b]	81.00[c]	3.30
Albumin (g/L)	46.20[a]	47.50[a]	40.30[b]	35.20[c]	2.70
Globulin (g/L)	73.50[a]	72.25[a]	48.00[b]	45.80[c]	1.10
Albumin/Globulin	0.63[b]	0.66[b]	0.84[a]	0.77[a]	0.11

[abc] Means on the same row with different superscripts differ significantly ($P<0.05$).
SEM = Standard error of means

Table 3: Total Protein (g/dl) in the Brain Regions and Hypophyses of Pubertal Boars Fed Dietary FB_1

Brain Region	Control 0.2 mg FB_1	Diet 1 5 mg FB_1	Diet 2 10 mg FB_1	Diet 3 15 mg FB_1	SEM
Pons	0.52	0.45	0.37	0.25	0.031
Cerebellum	0.60[a]	0.45[ab]	0.25[b]	0.24[b]	0.010
Amygdala	0.30	0.42	0.30	0.29	0.015
Hippocampus	0.55	0.35	0.37	0.35	0.014
Hypothalamus	0.95[a]	0.40[b]	0.29[b]	0.25[b]	0.021
Cerebral cortex	0.21[b]	0.45[a]	0.25[ab]	0.30[ab]	0.007
Mid Brain	0.38	0.35	0.31	0.35	0.006
Medulla oblongata	0.90[a]	0.95[a]	0.40[b]	0.55[b]	0.006
Adenohypophysis	0.47	0.45	0.31	0.30	0.005
Neurohypophysis	0.26	0.25	0.25	0.24	0.004

[ab] Means on the same row with different superscripts differ significantly ($P<0.05$).
SEM = Standard error of means

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_1 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 (Iyayi 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_1, the results probably indicate interference of serum protein metabolism by FB_1 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_1. 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_1 could perturb protein biosynthesis in pigs as well.

The overall reduction in circulating albumin with an increase in dietary FB_1 concentrations suggests that the vascular endothelial cells might have been compromised by FB_1 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,

2000). Carlson *et al.* (2001) observed significantly elevated free sphinganine in the livers of rainbow trout fed diets containing 23 mg or more FB_1/kg. The accumulation of sphinganine appears to be responsible for most of the deleterious effects of fumonisins, although depletion of complex sphingolipids impairs the function of some membrane proteins, such as the folate transporter (Stevens and Tang, 1997). The reduction in the total serum protein and albumin values may have a significant implication on the physiological state such as immunosuppression in the boars. 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_1/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_1 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_1. 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_1 in this regard.

The significant reduction in some brain regional protein concentrations with an increase in the dietary FB_1 concentrations in this study also lend credence to the hypothesis of adverse influence of dietary *Fusarium* mycotoxins on protein metabolism. The significantly lower concentrations of total protein in the cerebellum of the animals with an increase in the dietary FB_1 may be an indication of the interference of FB_1 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_1. 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_1 might be secondary effects of the altered total protein in these brain regions of the animals.

CONCLUSION

This study suggests that dietary FB_1 has an adverse influence on protein metabolism in animals chronically exposed to feeds containing >5.0 mg FB_1/kg. Feed contaminated with *F. verticillioides* that will yield above 5.0 mg FB_1/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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