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Animal Feed Additive and the Effect of the *Fusarium* Toxin Deoxynivalenol on the Electrophysiological Measurement of Transepithelial Ion Transport of Young Chickens with Ussing Chamber Technique

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Abstract: The presence of mycotoxins in poultry feeds is a significant factor for financial losses to animal industries. Ingestion of mycotoxin-contaminated feed by chickens causes injury to the gastrointestinal tract. DON has negative effects on the active transport of some nutrients in the small intestine of chickens. We tested the hypothesis that prefeeding with probiotic (*Eubacterium* sp.) or inulin, as a prebiotic, would attenuate these effects. Whereas, there is evidence in chicken that dietary supplementation with probiotic and prebiotic affect the intestinal microflora, increased the paracellular permeability and increased the villus length and villus area of the small intestine. The question of whether these changes affect the toxic effects of DON on the electrogenic glucose transport in the chicken intestine or not needs to be clarified. Therefore, an experiment was conducted to study the effects of DON in the presence or absence of dietary (*Eubacterium* sp.) or inulin on the electrophysiological response of the gut to glucose. The results indicated that in the absence of clinical signs and without impaired performance, DON appeared to alter the gut function of broilers. The addition of *Eubacterium* sp. may be useful in counteracting the toxic effects of DON on intestinal glucose transport. But, the dietary inulin supplementation of the broilers improved the glucose transport in the presence of DON and kept it at normal levels.

Key words: Mycotoxins, poultry feeds, animal industries, gastrointestinal tract

INTRODUCTION

The trend for a more natural nutrition has raised the interest in natural plant based ingredients for both human and animal nutrition. Herbal feed additives comprise a wide variety of herbs, spices and essential oils. Apart from enhancing the taste of food and its flavour, such feed additives are believed to exert positive effects on digestion and intestinal health. Important effects associated with herbal additives are the prevention of digestive disturbance, an improved feed utilization and animal performance. The EU decision to ban the use of antibiotic feed additives accelerated probiotic application in animal nutrition. Nevertheless, a sound scientific basis for the evaluation of conditions under which probiotics might be beneficial is largely missing.

The ussing chamber is an *in vitro* technique in which intestinal tissue is collected and immediately mounted as a flat sheet between two half-chambers, establishing a luminal and a serosal side. This technique allows the measurement of actively transported ions as well as the permeability of the tissues, 2 parameters relevant for the evaluation of gut health. Ussing chambers have been used to describe the changes of intestinal physiology occurring at acute or chronic feeding of different mycotoxins.

Deoxynivalenol (DON) is the most common trichothecene mycotoxin detected globally in feedstuffs. As a mycotoxin, DON causes losses in livestock production and poses a health problem to livestock and humans consuming contaminated cereal products. All animal species tested have been shown to be susceptible to DON. The mode of toxic action of DON is the inhibition of protein synthesis, thus affecting rapidly dividing cells, such as those of the gastrointestinal tract and immune system. This induces susceptibility to diseases.

We observed that DON induces an acute and chronic alteration of the epithelial barrier function and nutrient transport in chickens (Awad *et al.*, 2004, 2005a, b, 2007). It seems likely that the morphological changes in the intestine and the decreased feed conversion are linked to an impaired absorption of nutrients. The detoxification of DON is of major practical interest and the use of feed additives with *Fusarium* toxin degrading properties might be one method for accomplishing this (Binder *et al.*, 1998) with regard to costs and practicability of a potential detoxification procedure. Direct-fed microbials (probiotics) have been utilized to improve animal performance by maintaining the normal microflora of host animals. The mode of action of probiotics still remains unclear.

A beneficial effect of pro- and pre-biotics on the rat intestine, human biopsies or epithelial cell monolayers has been demonstrated using Ussing chambers. In chickens, the influence of the source of dietary ingredients or the physical form of the diet seems limited. Another way to use Ussing chambers is to incubate the intestinal tissue with different substances added directly into the chambers.

Some substances can be detrimental to the intestine, inducing electrolyte secretion or decreasing barrier function. However, some substances show a beneficial effect. Although this approach has limits and should be combined with *in vivo* measurements, it constitutes a rapid way to evaluate the effect of substances on intestinal physiology and can also, at least partly, elucidate the mechanisms of action of those substances. Therefore, the objective of the present studies were to investigate the influence of 10 mg/kg DON in the presence or absence of a microbial feed additive on the electrical properties of isolated jejunum mucosa of broiler chicks and the effect of prefeeding prebiotic (inulin) on the electrophysiological parameters in the presence of DON.

MATERIALS AND METHODS

In the first experiment, 277, 1-d-old broiler chicks were distributed randomly into three groups, either in the presence or absence of a microbial feed additive (*Eubacterium sp.*). Feed consumption, body weight gain and feed: gain ratio were measured weekly during the 6-week experiment. Intestinal segments were immediately taken from proximal jejunum; the tissue was mounted in modified Ussing chambers. Transmural Potential Difference (PD), short-circuit current (Isc) and electrical Resistance (Rt) were measured. Thereafter, 5 mmol/L glucose was added to the mucosal side.

In the second experiment, 40 1-d-old broilers were randomly divided into 2 groups, each comprising 20 birds. Chicks in group A were fed a basal diet and group B was fed the basal diet with 1.0% inulin. The diets were provided *ad libitum* for a period of 5 weeks. At the end of the feeding period, 5 birds from each group were killed by stunning and bleeding. Intestinal segments were immediately taken from the mid-jejunum. The intestine was rinsed with ice-cold buffer and transported in ice-cold oxygenated incubation buffer to the laboratory. The intestinal segments were opened along the mesenteric border and washed free of any remaining intestinal contents with buffer solution at 4°C. The epithelial sheets were mounted in modified Ussing chambers. Isolated epithelia were incubated in D-glucose-free buffer mucosally and serosally in Ussing chambers for at least 30 min. Thereafter, 5 mmol/L glucose was added to the mucosal side. After 1-min incubation with glucose, DON was added to the buffer solution. In further experiments, D-glucose was added to the luminal side after prior incubation of the tissues with 10 µg DON/mL.

Data were compared by a paired t-test to evaluate the effects of both substrates before and after their addition on Isc and Rt.

RESULTS AND DISCUSSION

The general performance of the birds was not influenced ($p > 0.05$) by feeding DON. The addition of the probiotic to DON contaminated diet did not improve the general performance of broilers. The addition of 5 mmol/L D-glucose on the luminal side of the jejunum increased ($p < 0.001$) the Isc in the control and the DON-probiotic fed groups compared to basal conditions, while this parameter decreased ($p = 0.004$) for the DON fed group (Table 1).

Glucose like other nutrients is transported by carrier systems and they are usually co-transported with Na^+ . Thus, if those nutrients are added to the mucosal side of intestinal tissues, the carrier mediated transport is stimulated, with a concomitant rise in the uptake of Na^+ . In the present studies, the addition of glucose to the mucosal side increased the Isc. This finding supports the results of Amat *et al.* (1999) and Aschenbach *et al.* (2002) who found that the increase in Isc after glucose addition to luminal side was due to a stimulation of transepithelial Na^+ transport.

DON seems to have specific effects by reducing nutrient absorption (Rotter *et al.*, 1996). In the present studies, DON decreased ($p < 0.05$) the Isc after addition of D-glucose. The results confirm that DON decreases the absorption of glucose. This could be attributed to the effect of DON on D-glucose/sodium-dependent transporter (Maresca *et al.*, 2002).

According to results of experiments to date it appears that microorganisms are the main living organisms applicable for mycotoxin deactivation. In the present study, the addition of the eubacteria to the DON contaminated feed of the broilers effectively alleviated the alterations caused by DON, indicating that *Eubacterium sp.* as a probiotic feed additive can influence electrophysiological parameters of the gut in the presence of DON. This may be due to improve nutrient absorption because of a reduction of toxin resulting from the bio-transformation of DON to de-epoxy-DON resulting in a loss of toxicity (Fuchs, 1999). In the second experiments, the Isc and Rt in the control and inulin birds was similar, as no significant difference

Table 1: The short-circuit current (Isc) in isolated mucosa of broilers after addition of D-glucose

Treatments	Basal Isc ($\mu\text{A}/\text{cm}^2$)	Isc after glucose addition ($\mu\text{A}/\text{cm}^2$)	p-value
Control	16±7.56 ^b	50±7.65 ^a	0.001
DON	33±7.75 ^a	26±7.49 ^b	0.004
DON-Probiotic	18±7.15 ^b	50±7.65 ^a	0.001

Within the same row, means with no common superscript differ statistically ($p < 0.001$, Paired t - test). Results are reported as means ± S.E.M

was noted between control and the dietary inulin-supplemented groups. However, the R_t was higher ($p < 0.05$) in the jejunum exposed to DON in control group ($423 \pm 16 \Omega \text{cm}^2$) compared with the basal values ($342 \pm 13 \Omega \text{cm}^2$) and the values after addition of glucose ($360 \pm 22 \Omega \text{cm}^2$). In addition, the I_{sc} was not affected in jejunum in the control group ($p > 0.05$) by the addition of glucose after preincubation of tissues with DON, suggesting that DON inhibited the Na^+ D-glucose co-transport.

The results of this study are in agreement with our previous findings that DON has a specific inhibitory effect on glucose absorption. However, in the dietary inulin supplemented group in both jejunum and colon, the addition of glucose after preincubation of tissues with DON increased the I_{sc} . The present study indicated that inulin supplementation improved the glucose absorption in the presence of the acute toxicity of DON. This can be explained by that many of the physiological effects associated with inulin consumption are directly linked to its selective promotion of specific strains of gut microorganisms, particularly bifidobacteria and lactobacilli (Gibson *et al.*, 1995; Jackson *et al.*, 1999; Kaur and Gupta, 2002). The bifidobacteria and lactobacilli produce Short Chain Fatty Acids (SCFA) and the increase in SCFA contributed to elevated serum GLUT2 (a glucose transporter) and serum glucagon-like peptide- (GLP-2) and proglucagon mRNA (Trappenden and McBurney 1998), which subsequently regulated and improved the glucose transport in the presence of DON and kept it at normal levels.

Conclusion: Diets contaminated with DON below levels that induce negative impact on health and performance could affect small intestinal morphology and alters gut function in broilers. The alterations caused by DON were reduced by supplementing the DON containing diets with a probiotic feed additive. Although, inulin did not modify the electrogenic transport of glucose, it improved the glucose absorption in the presence of the acute toxicity of DON, suggesting that the dietary inulin supplementation may be useful in reducing the toxic effects of DON on intestinal glucose transport. This indicates that in case of DON contamination of feedstuffs the addition of feed additive would be a proper way to counteract the possible impacts due to this mycotoxin. Hence, the probiotics and prebiotic may be potent and safe means to reduce absorption and increase excretion of deoxynivalenol from the body.

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