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Effects of Dietary Inclusion of Prebiotic, Probiotic and Synbiotic on the Intestinal Glucose Absorption of Broiler Chickens

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Abstract: Due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters, there is an increasing interest in finding alternatives to antibiotics in poultry production. The effects of prebiotics and probiotics or direct fed microbials (DFM) on gut health and performance in poultry as well as other species are studied. The interactions between intestinal microbiota, the gut epithelium and the immune system are important in the competitive exclusion process. Such feed additives have already been shown to affect relevant functions of the intestinal mucosa such as lowering the secretory response to theophylline or stimulating sodium/glucose cotransport in rat, but knowledge of the plausible interactions between food contaminants and natural components has not yet been studied. In this study we examined the effects of prefeeding of a microbial feed additive (Lactobacillus sp.), prebiotic (chicory rich in inulin) and synbiotic feed additive (combination of probiotic strain Enterococcus faecium, prebiotic chicory rich in inulin and immune stimulating substances derived from sea algae) on glucose transport of isolated jejunal mucosa of broiler chicks in the presence or absence of deoxynivalenol by the Ussing chamber technique. The addition of glucose on the mucosal side in Ussing chamber produced a significant increase in shortcircuit current (Isc) (P < 0.001) in all treated groups relative to the basal values. This increase in Isc for prebiotic and probiotic feed additives is equivalent to an increase of about 2 times that for the basal values and 3 times for the synbiotic group, while in the control group is about half fold that for the basal value. Further addition of DON to the mucosal solution decreased the D-glucose-stimulated current and returned to the basal value. In the second experiments, the addition of D-glucose to the mucosal side after preincubation of the control tissues with DON had no effect on the Isc (P > 0.05). While, the glucose addition after preincubation of the tissue with DON produced a higher increase in the lsc from the basal values in the prebiotic group (70%), probiotic group (20%) and the synbiotic group (26%) compared with the control group (13%), suggesting that the dietary prebiotic, probiotic and synbiotic supplementation of the broilers increased the glucose transport in the presence of DON which could be promising to reduce the alterations caused by DON on gut physiology. This may offer the host protection against the negative effects of DON on intestinal glucose absorption. Thus, this study supports the concept that probiotics, prebiotic and synbiotic may exert beneficial effects in the gastrointestinal tract.

Keywords: Broiler, prebiotic, probiotic, synbiotic, intestinal absorption, glucose

Introduction

The Ussing chamber technique is one of the most powerful methods to measure transepithelial ion transport across a variety of epithelial membranes including intestinal mucosa. 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, two 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 mycotoxins. Another advantage to use

Ussing chambers is to incubate the intestinal tissue with certain substances (toxin or inhibitors) added directly into the chambers and consequently monitoring their effects on glucose and amino acids transport. Some substances can be detrimental to the intestine, inducing electrolyte secretion or decreasing barrier function. However, some substances show a beneficial effect. This approach constitutes a rapid way to evaluate the effect of substances on intestinal physiology and can also elucidate the mechanisms of action of those additives. 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. It was shown that DON induces both acute and chronic alteration of the epithelial barrier function and nutrient transport in chickens (Awad et al., 2004; 2005a, b; 2007a). We also reported that the addition of 5 mmol/L of glucose to the mucosal side increased the Isc in the control tissues (Awad et al., 2004). While, DON decreased the D-glucose stimulated lsc, suggested that DON decreased the absorption of glucose. Furthermore, the morphological alterations caused by DON in the intestine and the decreased feed conversion are associated with the impaired absorption of nutrients. Direct-fed microbials (probiotics) have been utilized to improve animal performance by maintaining the normal microflora of host animals. The main action of probiotics is a reinforcement of the intestinal mucosal barrier against deleterious agents (Fioramonti et al., 2003). But the prebiotic has been defined as "a non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the intestine" (Gibson and Roberfroid, 1995; Young et al., 1998). As such, they are completely available for fermentation by intestinal flora. The unique properties of chicory inulin and oligofructose are that they selectively stimulate the growth of bifidobacteria, lactobacilli, and certain butyrate-producing bacteria (Hold et al., 2003). Since the gastrointestinal mucosa is the surface of contact with pre- or probiotics, it seems evident that the first effects of those additives relate to digestive function.

A beneficial effect of pro- and prebiotics on the rat intestine, human biopsies or epithelial cell monolayers has been demonstrated Ussing chambers (Michail and Abernathy, 2002; Mangell et al., 2002). In chickens, the influence of dietary ingredients or the microorganism has not yet been studied. So far, little information are available pertaining to the effect of pre- and probiotics dietary supplementation on the intestinal glucose transport of broilers in the presence or absence of DON. As we found the dietary inulin supplementation of the broilers improved the glucose transport in the presence of DON and kept it as normal (Awad et al., 2007b). Therefore, the objective of the present studies was to investigate the influence of prefeeding of a microbial feed additive (Lactobacillus sp.), prebiotic (chicory rich in inulin) and synbiotic feed additive (combination of probiotic strain Enterococcus faecium, prebiotic chicory rich in inulin and immune stimulating substances derived from sea algae) on the electrical properties of isolated jejunum mucosa of broiler chicks in the presence or absence of DON.

Materials and Methods

Eight hundred 1 day-old broiler chicks (Ross 308) were obtained from a commercial hatchery. The birds were randomly divided into four groups (200 birds/group) and housed in pens of identical size in a deep litter system with wood shaving floor. Each group has 8 replicates (25 birds/pen). The control group was fed a basal diet. The prebiotic group (derived from chicory rich in inulin), synbiotic group [Biomin® IMBO; combination of probiotic strain Enterococcus faecium (DSM 3530), added to the starter diet with 5 x 108 cfu/kg and 2.5 × 108 cfu/kg to the grower diet, prebiotic derived from chicory rich in inulin, and immune-modulating substances derived from sea algae] and probiotic group (supplemented with 1x108 cfu/kg of a homofermentative and a heterofermentative Lactobacillus sp.) were fed a basal diet supplemented with 1kg/ton of feed of the respective additive. The chicks were fed with the starter diets from days 1 to 13 and grower feed from day 14-35. The feed additives were delivered by Biomin® GmbH, Herzogenburg, Austria. The birds had free access to water and feed. The climatic conditions and lighting programme were computerfollowed commercial operated and the recommendations. Environmental temperature in the first week of life was 35°C, and decreased to 25°C till the end of experiment. During the first week 22 hours of light were provided with a reduction to 20 hours afterwards. At the end of the feeding period, 5 birds (6 replicates /bird) from each group were killed and the basal and glucose stimulated transmural potential difference (PD), shortcircuit current (Isc) and electrical tissue conductivity (Gt) were measured in the isolated gut mucosa to characterize the electrical properties of the gut. After preparation of stripped intestinal sheets (removal of serosal layer), the tissue was mounted in modified Ussing chambers with an active area of 1 cm². The serosal and mucosal surfaces of the tissues were bathed in 5 mL of Ringer solution with the following composition (mmol/L): CaCl₂, 1.2; MgCl₂, 1.2; Na₂HPO₄, 2.4; NaH₂PO₄, 0.4; NaHCO₃, 25; KCL, 5; NaCl, 115; mannitol, 20 for the serosal side. Ringer solution was added to mucosal side and 5 mmol D-glucose was added instead of mannitol. All chemicals were dissolved in distilled water and mixed thoroughly in a 1-L flask. The pH of the solution was adjusted to 7.4 using a pH meter. The incubation medium was continuously gassed with a mixture of 95% O₂ and 5% CO₂, and the temperature of the mixture was kept at 38°C. Continuous oxygenation provided recirculation of the incubation solutions by means of a gas lift. The tissues were first incubated under open circuit conditions for 20 min and then voltage clamped by fixing the voltage at 0 mV. All three parameters were recorded. Thereafter, the D-glucose was added at the mucosal side (final concentration:5 mmol/L). The electrical response to glucose was measured as the peak response obtained

approximately 1 min after addition of the solution. Moreover, 50 µg of DON/5 mL was added to the luminal side after addition of D-glucose to investigate the effect of DON on the glucose transport. Further experiments were performed to investigate the mechanisms of action of DON. The tissues were incubated with 5 mL of Ringer solution on the luminal side containing DON (50 µg of DON/5 mL of Ringer solution) for 1 hr. The electrical response was measured after addition of DON into the chamber. Thereafter, D-glucose (5 mmol/ L) was added to the luminal side after preincubation of the tissues with DON. The basal lsc and Gt are expressed as actual values, whereas the effect of D-glucose to the mucosal side on the electrical variables is shown as the amount of change.

Statistical analysis: Statistic SPSS program version 15.0 was used for data analysis. Kolmogorov Smirnov test was used to test the normal distribution of the data. Basal values of Isc and Gt (6 replicates per bird) were compared between groups by ANOVA and subsequently, Duncan's multiple range test was used for group comparison. Paired samples t-test was used for the comparison of the different measurements within the same group. Significance was denoted by P < 0.05.

Results

The addition of 5 mmol of D-glucose/L on the mucosal side produced a significant increase in Isc (P < 0.001) in all treated groups relative to the basal values. This finding indicated that the D-glucose-stimulated current across the mucosa had to be attributed to an increase in glucose transport. This increase in the Isc from the basal values after glucose addition was higher in the prebiote group (19 µA/cm²) and probiotic group (15 μA/cm²) than the control group (10 μA/cm²). This increase in Isc for prebiotic and probiotic feed additives is equivalent to an increase of about 2 times that for the basal values and 3 times for the synbiotic group, while in the control group is about half fold that for the basal value. This result suggests that the addition of prebiotics or probiotics to broiler feed increased the sodium glucose transport and the addition of a synbiotic product to broiler diets produced more glucose transport than addition of prebiotic or probiotic. Further addition of 50 µg of DON/5 mL to the mucosal solution decreased the D-glucose-stimulated current and returned to the basal value, suggesting that DON entirely inhibited Na+-Dglucose cotransport. Furthermore, the decrease in the Isc from the stimulated glucose values was lower in the group supplemented with synbiotic (11 µA/cm²), prebiotic group (18 µA/cm²), and probiotic group (19 μA/cm²) compared with the control group (29 μA/cm²) (Table 1). However, the conductivity of jejunal tissues remained unaffected by the dietary supplementations and there was no significant difference (P > 0.05)

Table 1: Effect of feed additives supplementation on short-circuit current (Isc) $(\mu A/cm^2)$ after addition of glucose and DON

Isc

lecrease after
Naddition from
ose response ²
29
18
11
19
6

¹The difference between the peak Isc after glucose addition and the basal value. ²The difference between the peak Isc after glucose addition and the value after DON addition)

between the groups (Table 2). In the second experiments, the addition of D-glucose to the mucosal side after preincubation of the control tissues with DON had no effect on the Isc (P > 0.05). While, the glucose addition after preincubation of the tissue with DON produced a higher increase in the lsc from the basal values in the prebiotic group (38 µA/cm² with a percentage of 70%), probiotic group (9 µA/cm² with a percentage of 20%) and the synbiotic group (5 µA/cm² with a percentage of 26%) compared with the control group (4 µA/cm² with a percentage of 13%), suggesting that the dietary prebiotic, probiotic and synbiotic supplementation of the broilers increased the glucose transport in the presence of DON In vitro which could be promising to reduce the alterations caused by DON on gut physiology (Table 3).

Discussion

The intestinal microbiota plays a vital role in the normal nutritional, physiological, immunological and protective functions of the host animals (Vispo and Karasov, 1997). The composition and metabolic activity of the intestinal microbiota can be influenced by the diet (Netherwood et al., 1999). There is a growing interest in the use of a variety of oligosaccharides as prebiotics and microbials as probiotics to promote animal health by altering the intestinal microbial community. A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota and that is not digested by the host digestive enzymes (Gibson et al., 2004). The major functions of enterocytes are to act as a protective barrier shielding the body from organisms and substances that do not serve as nutrients (Metcalfe et al., 1991). Probiotics have been reported to enhance the maintenance and function of the epithelial barrier. The results reported here indicated that the conductivity of jejunal tissues remained unaffected by the dietary supplementations supports the concept that those additives enhance the maintenance and function of the epithelial barrier. The efficacy of probiotics may be

Table 2: Tissue conductance (Gt) across the isolated jejunum of

	Gt			
Dietary treatment	Basal	Gt after glucose addition	Gt after DON addition	
Control	3	3	2	
Prebiotic	4	5	4	
Synbiotic	2	2	2	
Probiotic	3	3	2	
SEM	0.33	0.39	0.28	

potentiated by the several methods: the selection of more efficient strains; gene manipulation; the combination of several strains and the combination of probiotics and synergistically acting components. Synbiotics, a combination of prebiotics and probiotics may also stimulate the gut function. Therefore, the objective of this study was to evaluate the effects of pre-, pro- and synbiotics on the intestinal nutrient absorption. Although a marked proportion of the beneficial effects of probiotics so far discussed seem to be attributable to certain epithelial function, there are relatively few experimental data to support this hypothesis. In In vitro Ussing chamber experiments acute stimulation of chloride net absorption was found in jejunum and descending colon of rats when large amounts of Saccharomyces boulardii were added to the mucosal side (Krammer and Karbach, 1993). In descending colon prostaglandin induced Cl net secretion was reduced by this treatment, indicating specific effects on second messenger-mediated secretory processes. Ussing chamber experiments with epithelial tissues from mid-jejunum obtained from pigs treated with probiotic (Saccharomyces boulardii) revealed a significant decline of the mucosal secretory response after stimulation with theophylline (Winckler et al., 1998). In the same animal model a marked increase in Na⁺dependent glucose transport in jejunum was observed (Breves et al., 2000). Eberl (2005) found that after oral treatment of rats with S. boulardii, there was a marked stimulation of sodium dependent D-glucose uptake into brush border membrane vesicles with a corresponding increase of the sodium D-glucose cotransporter-1, (SGLT-1). In a previous study (Awad et al., 2004) we found that DON decreased the glucose transport and tissue conductivity. It was hypothesized that the prefeeding with a prebiotic, probiotic and synbiotic would attenuate the toxic effect of DON at the gut level. To our knowledge, no data are available pertaining to the effect of prebiotic, probiotic and synbiotic on the intestinal nutrient transport of broilers in the presence DON. Therefore, the current study tested the hypothesis that the dietary supplementations can enhance and improve the intestinal glucose transport following the acute DON exposure of broilers. The results reported here indicate that dietary prebiotic, probiotic and synbiotic can

influence electrophysiological parameters of the gut, indicating that the addition of prebiotics or probiotics to broiler feed increased the sodium glucose transport and the addition of a synbiotic product to broiler diets produced more glucose transport than addition of prebiotic or probiotic. Many of the physiological effects associated with prebiotic consumption are directly linked to its selective promotion of specific strains of gut bifidobacteria microorganisms, particularly lactobacilli. The bifidobacteria and lactobacilli produce short chain fatty acids (SCFA) and lactic acid and a range of other antimicrobial compounds. Kruh et al. (1995) showed that butyrate is a cofactor involved in growth and differentiation of mucosal cells. Tappenden and McBurney (1998) found that an increase in SCFA contributed to elevated serum GLUT2 (a glucose transporter) and serum glucagon-like peptide- (GLP-2) and proglucagon mRNA of rat intestine. Additionally, Rehman et al. (2007) found that the supplementation of inulin resulted in an increase in the villus height of jejunal mucosa of broilers and it is speculated that an increase in villus height is paralleled by an increase digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems (Pluske et al., 1996). Moreover, Fioramonti et al. (2003) indicated that a number of probiotics are able to modulate some characteristics of digestive physiology, such as intestinal permeability, mucosal immunity and mucus layer. They indicated that the probiotics reduce the alteration in gut permeability to molecules or bacteria and reduce mucus degradation. Our results indicated that the synbiotic, probiotic and prebiotic increased the active glucose transport. In the present study, DON decreased (P < 0.05) Isc after addition of glucose to the luminal side of jejunum, which could indicate that the glucose induced Isc was altered by DON. Furthermore, the supplementation of probiotic and synbiotic can be useful in reducing toxic effects of DON on intestinal glucose absorption. Additionally, the dietary prebiotic increased the lsc after the addition of glucose after preincubation of tissues with DON. In the present study, the prebiotic appeared to improve the glucose transport in the presence of DON in commercial broilers and offers a promising approach to restore microbial communities and to support barrier function of the epithelia by their prebiotic action. This may offer the host protection against the negative effects of DON on intestinal glucose absorption. Increased absorption of glucose after feeding of probiotic may also be linked to increase passive absorption of glucose and facilitate its transport in the gastrointestinal tract (Chichlowski et al., 2006). Therefore, we can conclude that the prebiotic, probiotic and synbiotic produced an increase in glucose absorption which could reduce the adverse effect of DON on glucose absorption in the small intestine of

Table 3: Effect of feed additives supplementation on glucose transport across the isolated intestinal mucosa after preincubation of the tissues with DON in broilers

	Isc			
Dietary treatment	Basal Isc with DON	Isc increase after glucose addition ¹		
Control	30	4		
Prebiotic	54	38		
Synbiotic	19	5		
Probiotic	45	9		
SEM	8	12		

¹The difference between the peak lsc after glucose addition and the basal value

broiler chickens. Therefore, this study supports the concept that probiotics, prebiotic and synbiotic can exert beneficial effects in the gastrointestinal tract.

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