ROLE OF PROBIOTICS IN COUNTERACTING THE EFFECT OF USUAL FEED CONTAMINANTS IN SWINE

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Abstract

The paper aims to investigate the potential of probiotics (a Lactobacillus polyculture-Lb) to counteract the effects of experimental co-contamination between E. coli, a common pathogenic bacterium in livestock farms responsible for post-weaning diarrhea in pigs and zearalenone, a Fusarium mycotoxin in order to achieve a nutritional strategy that reduces therapeutic interventions with antibiotics. For this purpose, growth performance, overall health status, immune response were assessed in 40 weaned piglets (10 piglets/group) distrusted in 4 groups: C- control, E1- E. coli+250ppb ZEA, E2- E. coli+250ppb ZEA+ Lb (product in development), E3- E. coli + 250ppb ZEA+ Lb (commercial product). Experimental groups received feed contaminated (E1, E2, E3) or not (C) with 250 ppb ZEA for the entire period of the trial (13 days). E coli was administered to E1, E2, E3 for 3 days at the beginning of the trial, followed by the administration of probiotic products for the rest of the period. Feeding piglets with co-contaminated feed had a tendency to decrease the body weight and feed consumption. No significant effect of the treatments was observed on the serum parameters. However, piglets from E2 group have a significant increase of IgA, IgM and IgG as compared with control, but no effect was observed on total antioxidant capacity or nitric oxide synthesis. In conclusion, the probiotic treatment can improve the immune status in piglets feed co-contaminated feed.

Key words: swine, zearalenone, *E. coli, Lactobacillus*

INTRODUCTION

The number of substances that can contaminate animal and human food varies greatly from heavy metals, pesticides, aromatic hydrocarbons to microrogans (bacteria or fungi) and their metabolites. Mycotoxins are the secondary products of fungi metabolism that can contaminate cereals and cereal products [1]. The most common and common mycotoxins are aflatoxins, fumonisins, deoxynivalenol, zearalenone, and Salmonella enterica serotypes, Escherichia coli spp, and Clostridium perfringens are among the pathogenic microorganisms most commonly found in livestock farms and leading to economic losses. After the EU has banned the use of antibiotics as growth factors, bacterial infections and co-contamination with other contaminants remain a serious problem. Bacterial (Escherichia coli, Salmonella etc) and contamination with mycotoxins produced by fungus (aflatoxins, fumonisins, deoxynivalenol, zearalenone, etc.). are very frequent [3]. Co-contamination produces significant adverse effects in local immune defense (intestinal mucosa) in addition to systemic side effects [2].

The pig is a highly sensitive species for both micotoxin and pathogen contamination that can affect performance, reproduction, and immune defenses. For example, post-weaning diarrhea, one of the causes of economic losses in this species, is caused by *Escherichia coli* (E. coli K88), *Salmonella*, *Rotavirus,* etc. after the interaction with intestinal epithelium and endotoxin secretion. Contamination with mycotoxins in this species can also have genotoxic, immunotoxic and carcinogenic effects. The concomitant presence of mycotoxins and pathogenic bacteria in feed for pigs may have additive, antagonistic or synergistic effects *as* observed *in the in* vitro experiments.

*L*actic *a*cid- bacteria (Lactobacillus sp.) are known for their immunostimulatory

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effect [3] *and they* are used as probiotics to counteract the negative effects of E. coli and other pathogens. The effect on the immune response varies depending on the species of lactobacilli; a large number of studies have been performed with individual acido-lactic strains and fewer have investigated the effect of acidolactic bacterial polycystics.

The paper aims to investigate the potential of probiotics (a Lactobacillus polyculture-Lb) to counteract the effects of experimental co-contamination between E. coli, a common pathogenic bacterium in livestock farms responsible for post-weaning diarrhea in pigs and zearalenone, a Fusarium mycotoxin in order to achieve a nutritional strategy that reduces therapeutic interventions with antibiotics.

MATERIAL AND METHODS

The experiment was performed on 40 weaned piglets (10 piglets/group) distributed in 4 groups: C- control, E1- E. coli+250ppb ZEA, E2- E. coli+250ppb ZEA+ Lb (product in development containing L. plantarum, L. paracasei, L. acidophilus), E3- E. coli + 250ppb ZEA+ Lb (commercial product). Experimental groups received feed contaminated (E1, E2, E3) or not (C) with 250 ppb ZEA for the entire period of the trial (13 days). E coli was administered to E1, E2, E3 for 3 days at the beginning of the trial, followed by the administration of probiotic products for the rest of the period.

A strain of *E. coli* (K 88 ETEC) known for the enterotoxigenic effect and triggering of haemorrhagic diarrhea in piglets after weaning was used in this study.

The feed was manufactured in IBNA following a recipe for weaned piglets (NC-P2) that fulfilled the essential nutrients required for this category according to NRC 1988 based on: corn, wheat, sunflower and soybean meal. The chemical composition (crude protein, crude fat, cellulose and ash) of feed was determined after manufacturing by standardized international methods (ASRO, Standard Bulletin, SR ISO-2010) in the IBNA Chemistry Laboratory (Table 1). For experimental groups feed was artificially contaminated with ZEA (250ppb). Plasma markers were assessed using a biochemistry analyzer. Immunoglobulins and cytokines concentration was assessed using specific kits as already described.

Table 1 Chemical composition of experimental feed

Parameters (%)	***Proteins***	***Fat***	***Celulose***	***Ash***
Feed (NC-02)	18.17	1.98	4.88	4.12

RESULTS AND DISCUSSION

Piglets in the control group who received normal food did not show abnormal clinical signs throughout the experiment and no mortality was recorded. Contamination with *E. coli* caused diarrhea from day 2 of contamination (liquid faeces, score 2) in all three experimental lots. Piglets from experimental group 1 who consumed ZEA-contaminated feed and who were infected with *E. coli K88 ETEC* ($1x10^{10}$cfu / pig) and who did not receive probiotics showed the worst performances, their weight dropping by 15.91%, and daily average gain by 36% compared to controls. In E2 and E3 piglets group who received probiotics starting with day 4 of the experiment, their weight decreased by 13.07% in lot 2 (feed with ZEA + E. coli + probiotic 1) and 13.30% in lot 3 (fed with ZEA + E. coli + probiotic 2), and the daily average gain of 32.7% for group 2 and 33.7% for group 3. However, diarrhea persisted even after administering the probiotics for at least 1-2 piglets from the groups that received probiotics for 7 consecutive days, but at a lower score (score = 1). Co-contamination (ZEA-contaminated food and E. coli infection) did not influence feed consumption of piglets in the experimental lots, but an increase feed conversion was observed in piglets from E1 group due to the low bogy weight. (+ 53.2% lot 1) and also E2 and E3 (+ 27.7% E2 and respectively + 33% E3) as a consequence of the feed conversion efficiency (Table 2). It should be noted, however, that piglets that were treated with probiotic increased specific consumption was less than pigs without treatment.

Table 2 The effect of co-contamintion with ZEA , E. col and and treatement with probiotics on feed consumption

Groups	Feed consumption (kg)	Daily gain (kg)	Feed conversion (kg:kg)
Control	0.679 ± 0.03	0.481 ± 0.04	1.410 ± 0.10
E 1 (feed+ZEA+E.coli)	0.667 ± 0.04	0.308 ± 0.0	2.160 ± 0.05
E2 (feed+ZEA+E.coli+probiotic 1)	0.583 ± 0.03	0.324 ± 0.0	1.800 ± 0.07
E 3 (feed+ZEA +E.coli+probiotic 2)	0.600 ± 0.025	0.320 ± 0.0	1.875 ± 0.15

Values for glucose, cholesterol and triglyceride in the serum of pigs fed co-contaminated diet (ZEA + E. coli) were in the normal physiological limits for age of piglets used in the trial. Compared with plasma control, a glucose increase was recorded for all lots, but was significant ($p < 0.05$) for E3 group (Table 3). A slight decrease in cholesterol concentration was noted in pigs of E1 group (ZEA + E. coli co-contamination) and E2 group (co-contamination + Probiotic 1) and in contrast a slight increase in cholesterol and triglycerides was obtained in piglet plasma in experimental group 3 (co-contamination + probiotic 2). This suggests that ZEA and E. coli, but especially the commercial probiotic and the administered to group 3, are able to modulate glucose and lipid metabolism.

Table 3 The effect of co-contamination with ZEA , E. col and treatement with probiotics on plasmatic markers of lipid and energetic metabolism

Parameters	Control	Experimental 1 (ZEA+ E.coli)	Experimental 2 (ZEA+E.coli+ probiotic 1)	Experimental 3 (ZEA+E.coli+ probiotic 2)
Glucose (mg/dL)	84.47 ± 11.8	101.52 ± 9.8	112.60 ± 6.2	117.20* ± 7.9
Cholesterol (mg/dL)	68.57 ± 4.4	61.33 ± 4.8	63.56 ± 3.8	74.28 ± 5.9
Triglicerides (mg/dL)	84.78 ± 4.9	70.00 ± 5.7	84.53 ± 4.0	101.54*≠ ± 4.0

**=significant versus control; ≠ significant versus E1 si E2*

Plasma protein markers (total protein, bilirubin, albumin, creatinine) synthesized in the liver, reflecting the metabolism of internal organs, liver, kidneys, were not influenced by experimental treatments (Table 4). A slight decrease in urea concentration was recorded in the plasma of piglets exposed to co-contamination (E1) compared to control and an increase in its concentration was observed in E3 group (ZEA + E.coli + probiotic 2) which was significantly different ($p < 0.05$) compared to the control group, E1 (co-contamination) and E2 (co-contamination plus probiotic 2). No significant effect was observed on the parameters that reflects the liver metabolism.

The innate immune response is the first line of defense of the animal body involving cell populations such as phagocytes, macrophages and neutrophils responsible for recognizing and counteracting microbial invasion [7]. It is known that both pathogenic bacteria such as *E. coli* and mycotoxins produce in these cells an increase in the synthesis of nitrogen monoxide (NO), an important microbicidal molecule [9]. NO is also an important regulatory molecule that influences many aspects of the inflammatory cascade, being an important mediator in both acute and chronic inflammation, as well as in countering oxidative stress acting directly as an antioxidant in the removal of reactive oxygen species (ROS) such as anion superoxide ($O2^-$) or indirectly as the signaling molecule by altering the gene expression. In the present experiment, contamination of feed with zearalenone and infection with E. coli did not produce any effect on the level of NO in plasma (Table 5). In contrast, a significantly increased NO concentration was recorded in the pigs receiving the commercial probiotic treatment (E3). It is known that lactic acid bacteria and *Bifidobacterium* have a stimulating effect on macrophage activity, leading to an increase in their ability to produce NO [6].

Table 4 The effect of co-contamination with ZEA, E. coli and treatement with probiotics on concentration of markers of proteic metabolism

Parameters	Control	Experimental 1 (ZEA+E.coli)	Experimental 2 (ZEA+ E.coli+probiotic 1)	Experimental 3 (ZEA+ E.coli+probiotic 2)
Total proteina (mg/dL)	5.12 ± 0.2	4.87 ± 0.2	5.43 ± 0.1	5.18 ± 0.4
Albumine (g/dL)	3.22 ± 0.1	3.07 ± 0.0	3.22 ± 0.0	3.39 ± 0.3
Total bilirubine (mg/dL)	0.12 ± 0.0	0.11 ± 0.0	0.10 ± 0.0	0.11 ± 0.0
Creatinine (mg/dL)	0.87 ± 0.0	0.77 ± 0.0	0.85 ± 0.0	0.80 ± 0.0
Urea (mg/dL)	23.60 ± 2.4	18.34 ± 1.3	21.30 ± 3.0	30.12*≠§ ± 1.8

**-significant versus control; ≠ - significant versus E1 si E2, §- significant versus E2.*

Table 5 The effect of co-contamination with ZEA , E. coli and treatement with probiotics on the synthese of nitric oxyde in plasma

Groups	NO (µM/L)	P-value*
Control	5.72 ± 0.50	NS
Experimental 1 (ZEA+E.coli)	5.54 ± 0.41	NS
Experimental 2 (ZEA+E.coli+probiotic 1)	6.89 ± 0.98	NS
Experimental 3 (ZEA+E.coli+probiotic 2)	7.83* ± 1.01	0.03

**-significant versus control*

The immune response humoral mediated contributes to the neutralization of toxins, viral and bacterial particles by antibody synthesis (immunoglobulin-Ig) as effector molecules. [5] The three classes of immunoglobulins M, G and A are differently involved in the immune response. IgM is the first antibody produced after first exposure to various antigens and it is found to have the highest concentration in circulating fluids [4]. In the present experiment, co-contamination or probiotic treatment did not produce significant effects on IgM concentration; but a slight decrease in IgM was recorded (-13.15%) in experimental group 3, which was not significant when compared to control IgM. IgG, the major component of the humoral immune response is the antibody synthesized after the second exposure to bacterial, viral, fungal, antigens, and provides long-lasting immunity. In this study, co-contamination and treatment with probiotic 1 led to an increase in plasma IgG (+ 5.11% for E1 and + 33.20% for E2); an increase in IgG was also obtained with probiotic 2, but less than with probiotic 1.

The IgA level, the most produced mucosal antibody, plays a key role in the mucosal immunity of placenta in the piglets treated with both probiotic 1 (+ 79.56%) and probiotic 2 mix of Lactobacillus and Bifidobacterium, + 12.90%) (Table 6). The effect of co-contamination and probiotics on the immune defense response at the cellular level was investigated by the assessment of cytokines as mediators synthesized by immune cells that provide intracellular "communication" by transmitting different signals inside cells and key factors in physiological processes such as inflammation. Cytokines and released chemokines activate and recruit other cells at the site of infection, ultimately triggering the adaptive immune response.

It is well known that mycotoxins and pathogenic bacteria have the ability to interact with the inflammatory response, but very few data are known about the effect of pathogen-mycotoxin co-contamination (ZEA + E. coli). In the spleen, the biggest lymphoid organ, *E. coli* + ZEA induced the synthesis of proinflammatory cytokines IL-1β, IL-6, IL-8

and TNF-α (Figure 1). The commercial Probiotic 2 (polyculture of *Lactobacilli* and *Bifidobacter*) decreased the level of IL-1β, IL-6, IL-8 and TNFα while the probiotic 1 diminished the concentration of IL-6, TNF-α and IFN gamma (Figure 1). Also, both probiotic significantly increase the IL-4 synthesis, an anti-inflammatory cytokine.

Table 6 The effect of co-contamination with ZEA , E. coli and treatement with probiotics on the concentration of immunoglobulins in plasma

Groups	IgA (mg/mL)	IgM (mg/mL)	IgG (mg/mL)
Control	1.86 ± 0.22	4.26 ± 0.46	9.97 ± 1.20
Experimental 1 (furaj+ZEA+E.coli)	1.71 ± 0.35	4.03 ± 0.34	10.48 ± 1.06
Experimental 2 (furaj+ZEA+E.coli +probiotic 1)	3.34 ± 0.55*	4.43 ± 0.57	13.28 ± 2.63
Experimental 3 (furaj+ZEA+E.coli+ probiotic 2)	2.10 ± 0.32	4.70 ± 0.66	12.81 ± 1.31

**-significant versus control*

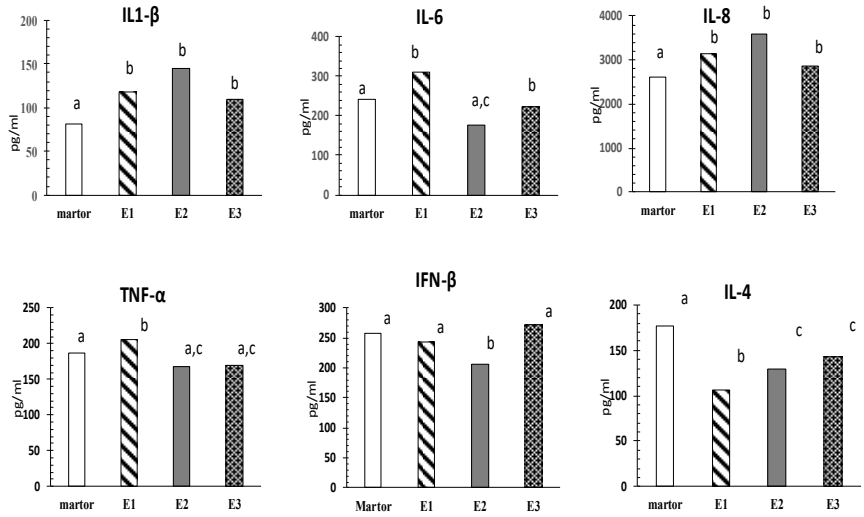

Figure 1. The effect of co-contamination with ZEA, E. coli and treatement with probiotics on the concentration of cytokines in spleen. [a,b,c,] indicates significant differences between treatments (P<0.05)

CONCLUSION

In conclusion, both products based on a mixture of Lactobacillus was able to reduce the inflammation induced by the feed co-contamination with *E.coli* and zearalenone, and increased the humoral immune response (IgA and IgG synthesis). Our results represent an argument for the use of probiotics in counteracting the toxic effect of both E.coli and zearalenone, as frequent feed contaminants.

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