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Influence of a blend of essential oils and an enzyme combination on growth performance, microbial counts, ileum microscopic anatomy and the expression of inflammatory mediators in weaned piglets following an *Escherichia coli* infection

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ABSTRACT

This study evaluated the effects of nutritional supplementation with a blend of essential oils (EO, thymol and cinnamaldehyde) and an enzyme combination (XB, xylanase and β-glucanase), given alone or together, on growth performance, microbial counts, ileum microscopic anatomy and the expression of inflammatory mediators in weaned piglets challenged with *Escherichia coli* K88. One hundred and ninety-two weaned piglets were allocated to 8 treatments in a 35-day experiment with a 2 × 4 factorial design that compared 2 levels of oral *E. coli* challenge [sham (−) or infected (+)] under different dietary treatments [fed the basal diet (CTR) either with or without EO or XB individually or in combination (EOXB)]. Half of the piglets were orally challenged with *E. coli* O149:F4 (K88) on day 8 and 48 piglets (1 piglet/pen) were slaughtered on day 35. The *E. coli* challenge was found to decrease the average daily gain (ADG) and the gain to feed (*G*:*F*) ratio from days 7 to 14 ($P < 0.01$) and to increase the fecal score from 1 to 5 days post-inoculation ($P < 0.01$). EOXB supplementation decreased the fecal score compared to the challenged CTR animals during the first week post-challenge ($P = 0.02$). The *E. coli* challenge increased the populations of fecal *Clostridia*, *E. coli* and coliforms on day 9 ($P < 0.01$) and increased *E. coli* and coliforms counts on day 14 ($P < 0.01$ and $P = 0.01$, respectively). Dietary EO and EOXB reduced the fecal coliforms count compared to the CTR group on day 14 ($P = 0.02$ and $P < 0.01$, respectively). Furthermore, EOXB supplementation reduced the coliforms count ($P < 0.01$) and tended to decrease the *E. coli* count ($P = 0.051$) compared to the CTR group in the cecum digesta. Dietary EOXB also decreased the crypt depth and increased the villus-to-crypt ratio in piglets compared to the CTR group ($P < 0.01$). The *E. coli* challenge up-regulated the expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1α, toll-like receptor (TLR)2 and TLR4 ($P < 0.01$, $P = 0.01$,

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; *C*, crypt depth; EO, essential oils; EOXB, essential oils + xylanase and β-glucanase; *G*:*F*, gain to feed ratio; HE, hematoxylin and eosin; IL, interleukin; RAU, relative arbitrary units; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; *V*, villus height; *V*:*C*, the villus height to crypt depth ratio; XB, xylanase and β-glucanase.

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$P = 0.03$, $P = 0.01$, respectively), and EOXB supplementation down-regulated the expression of TNF-α ($P < 0.01$) and IL-6 ($P = 0.046$) compared to the EO group. The results suggest that the combination of EO and XB supplementation may have beneficial effects on the modulation of fecal consistency, microbial counts and ileum microscopic anatomy in weaned piglets following *E. coli* infection.

1. Introduction

Weaning is associated with marked changes in the histology and biochemistry of the gastrointestinal tract (GIT), which decrease digestive and absorptive capacity and contribute to post-weaning diarrhea (Pluske et al., 1997). Increased susceptibility to infections and post-weaning diarrhea are major causes of mortality and morbidity in weaned pigs worldwide and are estimated to account for as much as 50% of the economic losses incurred during production (Cutler et al., 2007). Enterotoxigenic *Escherichia coli* (ETEC) K88 infection is one of the most important causes of post-weaning diarrhea in pigs (Fairbrother et al., 2005), and oral challenge with ETEC K88 has often been used to evaluate the ability of feed additives to reduce infection or modulate the gastrointestinal microbial response in weaning pigs (Bosi et al., 2004; Kiarie et al., 2011).

Previous studies have indicated that dietary supplementation with certain essential oils (EO) or enzymes might have beneficial effects on animal health and performance due to properties other than their functional characteristics related to antimicrobial action or improved nutrient digestibility, respectively (Windisch et al., 2008; Zijlstra et al., 2010). *In vitro* studies have shown that thymol and carvacrol exhibit antibacterial (Sivropoulou et al., 1996; Dorman and Deans, 2000; Lambert et al., 2001) and antifungal properties (Adam et al., 1998; Manohar et al., 2001). Cinnamaldehyde from cinnamon has also shown antimicrobial characteristics (Mancini-Filho et al., 1998). Improvements in nutrient digestibility, intestinal microscopic anatomy and growth performance have also been observed when the diets of weaned piglets or broiler chicks have been supplemented with xylanase or β-glucanase (Mathlouthi et al., 2002; Diebold et al., 2004; Fan et al., 2009).

Our previous study suggested that the combination of EO and XB had the potential to modulate the GIT environment and the inflammatory mediators of weaned piglets under the standard condition of a commercial farm without enterotoxigenic bacterial challenges (Jiang et al., 2015). However, these compounds might be more beneficial to the GIT environment of weaned piglets during a stressful event, such as an *E. coli* challenge, that could impact the health status of piglets. Thus, this work was conducted to determine whether the addition of an essential oil blend (thymol and cinnamaldehyde), an enzyme mixture (xylanase and β-glucanase) or a combination of both to the diets of weaned piglets would protect against a pathogenic *E. coli* infection by improving the microscopic anatomy and microbiology of the GIT environment or by down-regulating the gene expression of inflammatory mediators. Also, this study investigated whether there would be a synergistic effect between the essential oils and the enzymes.

2. Materials and methods

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the University of Milan (Protocol No. Dan.Piglet.EOSW0511).

2.1. Experimental animals and housing

The experiment was carried out at the Animal Production Research and Teaching Centre of the Polo Veterinario, Università degli Studi di Milano (Lodi, Italy). A total of 192 crossbred (Stambo HBI × Dalland) piglets (8.64 ± 1.54 kg) were assessed at weaning (24 ± 2 days) for initial BW and litter of origin and randomly allotted to eight treatments in a completely randomized design. There were 2 experimental rooms with 24 pens each, and each room was used for either *E. coli* challenged or unchallenged pigs. The environmental conditions were electronically controlled in both of the experimental rooms; each room was equipped with 24 pens with slatted floors (4 piglets/1.20 m × 1.00 m-pen), and each pen was equipped with two standard nursery pig bite-style nipple drinkers or stainless steel nursery push-lever bowl drinkers and a self-feeder. A combination of daylight and artificial light was used, and ventilation was achieved using variable-speed fans. A starting temperature of 28 °C was adjusted weekly to reach a final temperature of 24 °C.

Piglets were raised for 35 days in 8 different groups in a 2 × 4 factorial design comparing 2 oral *E. coli* challenges [sham (−) or infected (+)] and 4 different dietary treatments: the basal weaning diet supplemented with (1) no additive (CTR), (2) a 0.05 g/kg blend of essential oils (EO), (3) a 0.1 g/kg enzyme combination (XB), and (4) a 0.05 g/kg EO + 0.1 g/kg XB (EOXB). The supplemental doses were applied based on our previous study (Jiang et al., 2015). Each treatment consisted of 6 replicates, and each pen represented one replicate. Together, the different treatments were designated as follows: (1) CTR−, (2) EO−, (3) XB−, (4) EOXB−, (5) CTR+, (6) EO+, (7) XB+, and (8) EOXB+. On day 8 of the trial, all of the piglets in the *E. coli* challenge group received an oral dose of a 4 mL solution containing 10^9 CFU of *E. coli* O149:F4 K88-positive strain (Lombardy and Emilia Romagna Experimental Zootechnic Institute, Brescia, Italy). The K88-positive strain, which was isolated from pigs with colibacillosis, also expressed heat-labile (LT) and heat-stable B (STb) toxins and was further prepared as described