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TESIS DOCTORAL

INTESTINAL IMMUNE RESPONSE IN POST-WEANING PIGLETS

RESPUESTA INMUNE INTESTINAL EN LECHONES POST-DESTETE

Dª. NIENKE DE GROOT

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Autor: D.ª Nienke de Groot

Director/es: D. Guillermo Ramis Vidal

D. Fernando Fariñas Guerrero

UNIVERSIDAD DE
MURCIA

DECLARACIÓN DE AUTORÍA Y ORIGINALIDAD
DE LA TESIS PRESENTADA EN MODALIDAD DE COMPENDIO O ARTÍCULOS PARA OBTENER EL TÍTULO DE DOCTOR

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INTESTINAL IMMUNE RESPONSE IN POST-WEANING PIGLETS

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PhD THESIS BY PUBLICATIONS

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Nienke de Groot, Fernando Fariñas, Carolina G Cabrera-Gómez, Francisco J Pallares, Guillermo Ramis. **Weaning causes a prolonged but transient change in immune gene expression in the intestine of piglets**. Journal of Animal Science. **2021** April; Volume 99, Issue 4. skab065, https://doi.org/10.1093/jas/skab065

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Nienke de Groot; Fernando Fariñas; Lluís Faba, Ellen Hambrecht, Carolina G. Cabrera-Gómez; Francisco J. Pallares; Guillermo Ramis. **Fermented rye with *Agaricus subrufescens* and mannan-rich hydrolysate based feed additive to modulate post-weaning piglet immune response**. Porcine Health Management. 2021; 7, 60. https://doi.org/10.1186/s40813-021-00241-y

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Nienke de Groot, Mariana Meneguzzi, Barbara de Souza, Matheus de O. Costa. **In Vitro Screening of Non-Antibiotic Components to Mitigate Intestinal Lesions Caused by *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and Salmonella enterica Serovar Typhimurium**. Animals 2022, 12, 2356. https://doi.org/10.3390/ani12182356

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This project started around 5 years ago, when executing product validation trials in swine gut health solutions we realized that we needed and wanted more in depth and profound results on inflammation response in the intestinal tract of weaned piglets. It turned out into a remarkably interesting project and finally resulted in the work that you have in your hands today.

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INTRODUCTION

The process of weaning is known to change the intestinal morphology, caused by the changes in feed intake pattern, for example changing from liquid to solid feed and a decrease in intake post-weaning, and/or physiological or environmental stressors (Pluske et al., 1997). Weaning can result in high morbidity and mortality resulting from post-weaning diarrhea (McCracken et al., 1999; Lallès et al., 2004; Fairbrother et al., 2005). Nowadays, the modern pork production industry requests increasingly high animal productivity and efficiency. In the past, these results were obtained using antimicrobial strategies such as the use of antibiotic growth promoters (AGPs), pharmaceutical levels of zinc oxide (ZnO) or a high use of preventive antibiotics. The injudicious use of antimicrobials selects for resistant bacterial strains, imposing a risk for human and animal health (Coculescu et al., 2014; Holyoake et al., 2009; Jia et al., 2006; Mirajkar et al., 2016). Restrictions imposed on antimicrobial drugs available for veterinary use demands improved on-farm management measures, biosecurity practices and the development of novel non-antimicrobial alternatives to treat and prevent infectious diseases (Diário Oficial da União, 2016; Hayes et al., 2001; J. L. Turner et al., 2001; U.S. Food & Drug Administration, 2016). To support animal growth and health without using antimicrobials, nutrition has an important role beyond solely supplying nutrients and energy, high-quality feed ingredients and feed additives therefore might be needed.

Another complicating factor in the modern pork production is the fact that nowadays sows are hyper prolific with an average litter size of >16. This means that more than half of all parturitions are longer than physiologically. This rapid increase is concerning and leads to a high

incidence of dystocia with subsequent negative consequences on piglet survival and sows' fertility and longevity (Edwards & Baxter, 2015; Oliviero et al., 2019). Furthermore increased heterogeneity of birth weight within large litters increases the competition for colostrum intake, with the smaller piglets being less competitive and vital, and therefore affecting negatively their immunity (Akdag & Arslan, 2009; Quesnel et al., 2008) resulting in lower vitality and reduced health and potentially increase the immune related problems after weaning.

The gastrointestinal tract (GIT) is essential in the maintenance of health in animals, and it contains a large mucosal immune system. The intestine forms a physical barrier that prevents toxic compounds and pathogens from entering the intestinal mucosa and systemic circulation, while the GIT simultaneously has a function for the uptake of nutrients. Feed interacts with the microbiota, and with the host mucosa, and mutual interaction exists between the three components (Niewold, 2015). The intestinal epithelium plays an active role in organ integrity and body defense locally (Eckmann, 1995; Pitman & Blumberg, 2000).

Results of several studies have demonstrated that weaning in pigs induces a breakdown in epithelial barrier function characterized by a significant decline in intestinal transepithelial electrical resistance and increased permeability (Hu et al., 2013; Moeser et al., 2007). At the same time that epithelial barrier function is disrupted, an upregulation of pro-inflammatory cytokines has been reported indicating a robust activation of the gastrointestinal immune system after weaning (McCracken et al., 1999; Pié et al., 2004; Hu et al., 2013). Previous research has shown that the process of weaning in piglets is associated with an increased inflammation response in the intestine (de Groot et al., 2021; Pié et al., 2004), and the potential negative effect of increased expression of inflammation markers on intestinal integrity (Moeser et al., 2017; Spreeuwenberg

et al., 2001), morphology of intestinal structures such as villous length and crypt depth (B. A. McCracken et al., 1995; Pié et al., 2004) and disruption of the microbiota (Gresse et al., 2017; Schachtschneider et al., 2013), favoring a delay in intestinal maturation and a predisposal to diseases (Jayaraman & Nyachoti, 2017; John R. Pluske et al., 2018).

One of the strategies that the host utilizes to avoid an inflammatory response against the microbiota is to implement an intestinal barrier, including the mucous layer and immunoglobulin (Ig) A, an antibody isotype specialized in mucosal protection (Cerutti & Rescigno, 2008; Gutzeit et al., 2014) and produced locally by plasma cells present in the mucosal wall. The naïve B-cell precursor of an IgA-secreting plasma cell are activated in the Peyer's patches and mesenteric lymph nodes. Production of IgA is controlled by cytokine-producing T-cells within the Gut-associated lymphoid tissue (GALT) and by cytokines released from the mucosa. Within the GALT, the Th1 cytokines, interferon γ (IFN-γ) and tumor necrosis factor-β (TNF-β), downregulate IgA production, whereas the Th2 cytokines, interleukin (IL)-4, IL-5, IL-6, and IL-10, upregulate IgA production (Kramer et al., 1995; Ramsay, 1995; Stokes, 2017). A balance between Th1 and Th2 response is necessary for maintaining normal IgA immune responses, with no response in favor of the other. Peyer's patches in the GALT are rich in cytokines with IgA-inducing functions, including TGF-β (Gonnella et al., 1998). IgA secreted into the gut lumen binds to the layer of mucus coating the epithelial surface, and it prevents the adherence of micro-organisms, as well as neutralizing their toxins or enzymes.

Recent research by Pluske et al. (2018) has shown the potential of different management measures such as reducing physical and physiological stressors, implementing nutritional strategies reducing intestinal microbial load, increasing digestion and preventing production and

activity of pro-inflammatory cytokines, all with the main goal to manipulate the immune system of pigs for improving performance, aiming to have an appropriate immune response for each specific circumstance, preventing to maximize the immune response (Pluske et al., 2018). Furthermore, nutritional strategies can modulate the complex interplay between the immune system and/or inflammatory responses and neuroendocrine mediators such as growth hormone and cortisol, thus having consequences on animal health and performances (Superchi et al., 2012).

Promising examples of nutritional strategies modulating the complex interplay between the immune system and/or inflammatory responses are organic acids (OA), pre- and probiotics and phytobiotics, which are being explored commercially as alternatives to antimicrobials (Bindels et al., 2015; Cook & Sellin, 1998; Dibner & Buttin, 2002; Gadde et al., 2017; Gibson et al., 2017; Keyser et al., 2008; Schönfeld & Wojtczak, 2016; Tugnoli et al., 2020). From a nutritional perspective, controlling early intestinal inflammation is certainly a challenge in managing post-weaning gut disorders and preventing intestinal inflammation due to pathogenic enteric organisms can be an important pillar to maintain the health of piglets. The objective of this study was to evaluate the gastrointestinal changes and immune modulation response, local in tissue and systemic, induced by the process of weaning and evaluate different nutritional strategies as feed additives to find a non-antimicrobial alternatives for supporting intestinal health and performance in piglets after weaning.

ABBREVIATIONS

ADG: average daily gain

BW: body weight

Ca^{2+}: Calcium

CCG: Compound control group

CDA: canonical discriminant analysis

cDNA: Complementary DNA

CFTR: Cystic fibrosis transmembrane conductance regulator

cGMP: Cyclic guanosine monophosphate

Cl^-: Chloride

CO_2: Carbon dioxide

COX-2: Cyclooxygenase-2

ELISA: Enzyme Linked Immuno Sorbent Assay

GALT: gut associated lymphoid tissue

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

GEE: Generalized estimating equations

GIT: gastrointestinal tract

H&E: Hematoxylin and eosin

HBSS: Hank's balanced salt solution

HCO^{3-}: Bicarbonate

IACUC: Institutional Animal Care and Use Committee

IFN: interferon

IL: interleukin

Ig: immunoglobulin

IV: intravenous

LB: Luria broth

LPS: Lipopolysaccharides

MCFA: Medium chain fatty acids

O_2: Oxygen

OA: Organic acids

PBMC: peripheral blood mononuclear cell

PBS: Phosphate buffered saline

PCA: Principal Component Analysis

PCG: Pathogen control group

PCR: polymerase chain reaction

PCV-2: porcine circovirus-2

PE: proliferative enteropathy

PIA: Porcine intestinal adenomatosis

PLS: Partial Least Square

SCFA: Short chain fatty acids

SD: Swine dysentery

TBS: tris buffered saline

TG: Treatment group

TGF-β: transforming growth factor-β

TJ: tight junction

TNBS: 2,4,6-trinitrobenzenesulfonic

TNF: tumor necrosis factor

ZO: Zonula occludens

OBJECTIVES

The main objective of the present thesis was to investigate the intestinal inflammation response in piglets after weaning, to find new strategies that can reduce this inflammation response, which damages intestinal integrity, cause gut disorders and cost energy. Due to the need to reduce antibiotic usage in production animals and minimize risk of bacterial resistance against antibiotics, relevant strategies may focus on reducing risk of pathogenic and zoonotic bacteria in the feed-to-food chain, mitigate post-weaning diarrhea, and achieve profitable systems. To achieve this goal, the thesis contemplates three different specific objectives:

1. Evaluate the inflammation response in piglets post-weaning systemically and locally in the different intestinal tissues, in commercial settings but with low infection pressure (as proved by low diarrhea response)
2. Evaluate the inflammation response in piglets post-weaning systemically and locally in the different intestinal tissues, caused by specific pathogen challenges using a previous described challenge method, using pathogens that are known for causing major economic losses due to poor growth performance, and increased production costs associated with treatments
3. To investigate non-antibiotic compounds with previously described antimicrobial effects that have been suggested to support gut health and reduce intestinal inflammation, as part of a nutritional strategy to modulate the complex interplay between the immune system and/or inflammatory responses and the consequences on animal health and performance

EXTENDED SUMMARY

Introduction

The process of weaning in piglets is known to change the intestinal morphology, caused by the changes in feed intake pattern, for example changing from liquid to solid feed and a decrease in intake post-weaning, and/or physiological or environmental stressors (Pluske et al., 1997). Weaning can result in high morbidity and mortality resulting from post-weaning diarrhea (McCracken et al., 1999; Lallès et al., 2004; Fairbrother et al., 2005). From a nutritional perspective, controlling early intestinal inflammation is certainly a challenge in managing post-weaning gut disorders. Preventing intestinal inflammation due to pathogenic enteric organisms can be an important pillar to maintain the health of piglets. The injudicious use of antimicrobials selects for resistant bacterial strains, imposing a risk for human and animal health (Coculescu et al., 2014; Holyoake et al., 2009; Jia et al., 2006; Mirajkar et al., 2016). Restrictions imposed on antimicrobial drugs available for veterinary use demands improved on-farm management measures, biosecurity practices, and the development of novel non-antimicrobial alternatives to treat and prevent infectious diseases (Diário Oficial da União, 2016; Hayes et al., 2001; J. R. Turner, 2009; U.S. Food & Drug Administration, 2016). Organic acids (OA), being short chain fatty acids (SCFA) and medium chain fatty acids (MCFA), prebiotics, phytobiotics, and enzyme inhibitors are currently being explored commercially as alternatives to antimicrobials (Bindels et al., 2015; Cook & Sellin, 1998; Dibner & Buttin, 2002; Gadde et al., 2017; Gibson et al., 2017; Keyser et al., 2008; Schönfeld & Wojtczak, 2016; Tugnoli et al., 2020). The main objective of the

present thesis was to investigate the intestinal inflammation response in piglets after weaning, to find new strategies to support piglet's health and performance.

Material and methods

The in vivo experiments performed in the present thesis involved the handling of animals. All the experimental *in vivo* procedures described in this research were reviewed and approved by the Bioethical Committee of the University of Murcia and applied under project license permit CEEA-OH 465/2018. The *in vitro* study is reported in accordance with ARRIVE guidelines for in vitro studies and were conducted following approval by the Institutional Animal Care and Use Committee (IACUC) from the University of Minnesota and was in accordance with the Canadian Council for Animal Care, being approved by the University of Saskatchewan Committee on Animal Care and Supply.

Farm location, Animals, Housing and Handling

The *in vivo* studies were performed at the Veterinary Teaching Farm of the University of Murcia and the farm fulfilled the guidelines of the European Union and local Spanish legislation in terms of production, health, biosecurity, and animal welfare. Pigs were housed in an environmentally controlled unit (25–27 °C) with natural light throughout the experiment. The pens (pen size 0.61 × 1.22 m) were full-slated and contained one nipple drinker ad libitum and one feeder with 4 spaces. Animals used were Large White, weaning was done at 22 ± 3 days of age and distribution between pens was done aiming for a 1:1 male:female ratio. Standard procedures entail on day 4 post-weaning a routine vaccination against PCV2 and no systematic medications in feed or water.

The *ex vivo* study studied healthy, commercial crossbred male pigs from high health herds of 6 weeks of age as tissue donors.

Sample collection and analysis

In the *in vivo* trials, animals were sacrificed at day 0, 15, 30 and 40 after weaning to obtain blood from the vena jugular and intestinal tissue samples. Tissue samples were obtained from the jejunum (middle section), ileum (5 cm adjacent to ileocecal valve), and colon (apex section of spiral). In the *in vitro* model, distal spiral colon was collected. In blood, total serum immunoglobulins IgA, IgE, IgG and IgM were quantified and flow cytometry was used to analyse subpopulations of different types of lymphocytes. In jejunum, ileum and colon tissue the amount of IgA secretory cells was counted in the lamina propria using the avidin biotine-peroxidase complex technique. Gene expression for cytokines IFN-α, IFN-γ, IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12p35 (IL-12α), IL-12p40 (IL-12β), TNF-α, and TGF-β was analysed in jejunum, ileum and colon tissue using relative quantification. Histopathology analyses were done to determine height of the villous (tip to villous-crypt junction) and depth of the crypt (from villous-crypt junction to the base of villous). In the *in vitro* model, the percentage of healthy epithelium and the mucus layer thickness covering explants was analysed. A digital image of each explant, covering its entire length, was analyzed in samples from colon segments.

Statistical analysis

Statistical analyses were performed using IBM SPSS 21 and the MIXED procedure of SAS. Significantly different means were separated using Tukey adjustment. Statistical significance and tendency were considered at $P \leq 0.05$ and $0.05 \leq P \leq 0.10$, respectively. Animal was the

experimental unit. Mucus layer thickness and percentage of healthy epithelium data were compared between challenge groups by generalized estimating equations (GEE) using an unstructured correlated working matrix while clustering by pig. The data followed a normal distribution.

Experimental Designs

Four experimental studies are included to achieve the objectives as mentioned before.

Objective 1: Evaluate the inflammation response in piglets post-weaning systemically and locally in the different intestinal tissues, in commercial settings but with low infection pressure (as proved by low diarrhea response)

Experimental design

A total of 108 piglets (Large White) were used, weaned at at 22 ± 3 d of age with an average body weight (BW) of 5.53 ± 1.19 kg and male:female 1:1. After weaning, all pigs were randomly allotted to 12 pens, with 9 animals per pen (pen size: 0.61 × 1.2 m) and offered unmedicated feed or water ad libitum. lndividual BW was recorded at 18 hour postweaning (day 0), days 15, 30, and 45, and average daily gain was calculated. Randomly selected piglets were similarly sacrificed per complete pen at day 0 (n = 10), day 15 (n = 26), day 30 (n = 34), and day 45 (n = 34) to obtain blood and tissue samples for immunohistochemical and histomorphometrical analyses.

Results

Results demonstrated an increase in IgA producing cells in the GIT on day 30 after weaning. Furthermore, the results confirmed the existence of different distribution patterns of immune

cells in the jejunum, ileum and colon. The study demonstrated that mRNA expression of pro- and anti-inflammatory cytokines varied according to type tissue analyzed and a difference in expression of cytokines was seen during the life of the piglet, with increasing levels of pro-inflammatory cytokines after weaning. IgA concentrations in plasma are increased over time, where the main increase occurred on days 30 and 45 after weaning. No correlation has been found between IgA producing cells in the different intestinal tissues and the cytokine gene expression in the tissue and/or in PBMCs. PCA indicated a separation between cytokine gene expression in different GIT tissues and in PBMC, with a clustering of cytokine gene expression in the small intestine (jejunum and ileum).

Conclusion

The changes in cytokine expression in time in both intestinal tissue as in blood found in this study indicate that the pigs seem to recover from intestinal inflammation, observed by an increase in pro-inflammatory cytokines as previously discussed, followed by an increase anti-inflammatory cytokine expression as a negative feedback. Results have shown that the process of weaning in piglets was associated with a prolonged and transient response in gene expression of intestinal inflammatory cytokines and an increased amount of IgA producing cells in the intestinal tissue. These increased responses were still present at days 15 and 30 post-weaning.

Objective 2: Evaluate the inflammation response in piglets post-weaning systemically and locally in the different intestinal tissues, caused by specific pathogen challenges using a previous described challenge method, using pathogens that are known for causing major economic losses due to poor growth performance, and increased production costs associated with treatments.

Experimental design

A total of 20 healthy, commercial crossbred male pigs from high health herds 6 weeks of age were used as tissue donors. For *Salmonella* Typhimurium and *Lawsonia intracellularis*, explants from five different tissue donors were evaluated and compounds F, S, and P were tested. Ten different pigs were used to challenge explants with *Brachyspira hyodysenteriae*: 5 were used for compound F, S, and P, and 5 additional pigs were used for compound D. For compound F, S and P; a total of 4 explants/pig for each combination group were used. For compound D alone a total of 12 explants/pig for each combination group were used.

Inocula contained on average 3.2 x 10^8 CFU/mL for S. Typhimurium, 7.9 x 10^7 genome copies/mL for *B. hyodysenteriae*, and 1 x 10^4 cells/mL for *L. intracellularis*. After the inoculum and the compound were prepared, both were mixed and then exposed to the explants. Explants in the pathogen control group (PCG) received 100 µL of inoculum of a given pathogen and explants in the combined compound control group (CCG) received 100 µL of compound only. The treatment group (TG; explant co-exposure to a given pathogen-compound combination), received 50 µL of 2x bacterial inocula and 50 µL of 2x compound dilution.

Results

Compound P treatment, a blend of MCFA and SCFA, improved explant epithelial coverage and decreased the accumulation of mucus and the expression of TNF-α mRNA following challenge with *B. hyodysenteriae*. A trend towards downregulation of IFN-γ mRNA expression following challenge was also observed. Compound S decreased the accumulation of mucus and reduced the expression of TNF-α and iNOS mRNA following challenge with *B. hyodysenteriae*. Explants

treated with compound F (prebiotic based on *Agaricus subrufescens* fermented rye) had higher epithelial coverage when challenged with *L. intracellularis* than those untreated. Explants infected with *B. hyodysenteriae* and treated with compound D (phytobiotic) had increased epithelial coverage and decreased levels of IL-1α, TNF-α, and IFN-γ when compared to infected untreated explants.

Conclusion

Findings suggest that the non-antimicrobial compounds studied may have beneficial effects for the host based on the explant model data shown. Compound P supported epithelial survival and reduced mucus thickness when explants were exposed to *B. hyodysenteriae.* Compound D has an immune-modulating effect in explants challenged with *B. hyodysenteriae*. Compound F prevented epithelial death following *L. intracellularis* exposure. The authors believe that further investigations are warranted to verify compound effectiveness in vivo.

Objective 3: To investigate non-antibiotic compounds with previously described antimicrobial effects that have been suggested to support gut health and reduce intestinal inflammation, as part of a nutritional strategy to modulate the complex interplay between the immune system and/or inflammatory responses and the consequences on animal health and performance.

Material and method

In this study 72 piglets (Large White) were used, weaned at 22 ± 3 days (d) of age. Pens were randomly allotted to two treatments with 8 pens per treatment and 9 animals per pen. Without receiving any feed before weaning, after weaning all pigs were allotted visually trying to avoid great difference in weight among the animals in the same pen. The dietary treatments consisted of a control diet and a treatment diet, being the control diet + 0.2% Fysal® Solute (FS), a feed additive consisting of a blend of mannan-rich hydrolyzed copra meal and fermented rye with A. subrufescens. Individual BW was recorded at 18 hour postweaning (day 0), days 15, 30, and 45, and average daily gain was calculated. Randomly selected piglets were similarly sacrificed per complete pen at day 0 (n = 10), day 15 (control n=11, FS n=10), day 30 (control n=6, FS n=12) and day 45 (control n=9, FS n=13) to obtain blood and tissue samples for immunohistochemical and histomorphometrical analyses.

Results

Average daily gain tended to be higher in FS treatment for day 0-15. The villous length in jejunal tissue was lower on day 15 for FS compared to control ($P < 0.05$). On day 30 a higher villous length in jejunal tissue and a tendency in ileal tissue were observed for FS compared to control. In ileal tissue the V/C ratio was higher in FS compared to control on day 30. The average count of IgA producing cells on day 15 was lower in piglets fed the FS diet in jejunum and ileum. On day 30, compared to control, the average count of IgA producing cells was lower in piglets fed with FS in jejunum, ileum and colon. The average count of IgA producing cells in colon was lower ($P < 0.05$) in piglets fed with FS on day 45 compared to control (Table 5, Figure 1).

On day 15, a higher IgA and IgM level in blood and on day 45, a higher IgG and IgM quantity was found in FS compared to control. Comparing gene expression of cytokines in jejunum, ileum, colon and PBMC over the three time points, it was observed that FS treatment reduced local tissue gene expressions of several cytokines, mainly on day 15 and 45, and increases gene expression in PBMC mainly on day 15.

Conclusion

In post-weaning piglets, feeding component FS, a blend of mannan-rich hydrolyzed copra meal and fermented rye with A. subrufescens, stimulates an immune modulation effect most evident at 15 days post-weaning. The effect includes a reduction of local intestinal inflammatory response, with emphasis in jejunum, accompanied with an increase in systemic (PBMCs) cytokine gene expression and a higher villous height in jejunum and ileum on day 30, while it was observed to be lower in jejunum on day 15.

Conclusion

Intestinal inflammation costs energy and can increase permeability and reduce digestion and growth performance in post-weaning piglets. This study shows that these effects can be prolonged, but the intestine can recover. Furthermore, non-antimicrobial additives can be supporting the intestinal health of these piglets, by reducing inflammation response and promoting intestinal integrity. This effect was also seen in disease challenges with pathogens known to cause enteropathogenic problems in swine production.

RESUMEN GENERAL

Introducción

Se sabe que el proceso de destete en lechones cambia la morfología intestinal, causado por los cambios en el patrón de ingesta de alimento, por ejemplo el cambio de alimento líquido a sólido y una disminución de la ingesta post-destete, y/o factores de estrés fisiológicos o ambientales (Pluske et al., 1997). El destete puede resultar en una alta morbilidad y mortalidad resultante de la diarrea post-destete (McCracken et al., 1999; Lallès et al., 2004; Fairbrother et al., 2005). Desde una perspectiva nutricional, el control de la inflamación intestinal temprana es sin duda un reto en el manejo de los trastornos intestinales post-destete. La prevención de la inflamación intestinal debida a organismos entéricos patógenos puede ser un pilar importante para mantener la salud de los lechones. El uso imprudente de antimicrobianos selecciona cepas bacterianas resistentes, lo que supone un riesgo para la salud humana y animal (Coculescu et al., 2014; Holyoake et al., 2009; Jia et al., 2006; Mirajkar et al., 2016). Las restricciones impuestas a los medicamentos antimicrobianos disponibles para uso veterinario exigen mejorar las medidas de gestión en las explotaciones, las prácticas de bioseguridad y el desarrollo de nuevas alternativas no antimicrobianas para tratar y prevenir las enfermedades infecciosas (Diário Oficial da União, 2016; Hayes et al., 2001; J. R. Turner, 2009; U.S. Food & Drug Administration, 2016). Los ácidos orgánicos (AO), siendo ácidos grasos de cadena corta (AGCC) y ácidos grasos de cadena media (AGCM), prebióticos, fitobióticos e inhibidores enzimáticos están siendo actualmente explorados comercialmente como alternativas a los antimicrobianos (Bindels et al., 2015; Cook & Sellin, 1998; Dibner & Buttin, 2002; Gadde et al., 2017; Gibson et al., 2017; Keyser et al., 2008; Schönfeld

& Wojtczak, 2016; Tugnoli et al., 2020). El objetivo principal de la presente tesis fue investigar la respuesta de inflamación intestinal en lechones después del destete, para encontrar nuevas estrategias para apoyar la salud y el rendimiento del lechón.

Material y métodos

Los experimentos in vivo realizados en la presente tesis implicaron la manipulación de animales. Todos los procedimientos experimentales in vivo descritos en esta investigación fueron revisados y aprobados por el Comité de Bioética de la Universidad de Murcia y aplicados bajo el permiso de licencia de proyecto CEEA-OH 465/2018. El estudio in vitro se informa de acuerdo con las directrices ARRIVE para estudios in vitro y se llevaron a cabo tras la aprobación del Comité Institucional de Cuidado y Uso de Animales (IACUC) de la Universidad de Minnesota y fue de acuerdo con el Consejo Canadiense para el Cuidado de Animales, siendo aprobado por el Comité de Cuidado y Suministro de Animales de la Universidad de Saskatchewan.

Ubicación de la granja, animales, alojamiento y manejo

Los estudios in vivo se realizaron en la Granja Docente Veterinaria de la Universidad de Murcia y la granja cumplía las directrices de la Unión Europea y la legislación local española en materia de producción, sanidad, bioseguridad y bienestar animal. Los cerdos se alojaron en una unidad de ambiente controlado (25-27 °C) con luz natural durante todo el experimento. Los corrales (tamaño del corral 0,61 × 1,22 m) eran de rejilla completa y contenían un bebedero de tetina ad libitum y un comedero con 4 espacios. Los animales utilizados eran Large White, el destete se realizó a los 22 ± 3 días de edad y la distribución entre corrales se hizo procurando que la

proporción macho:hembra fuera de 1:1. Los procedimientos estándar incluyen una vacunación rutinaria contra PCV2 el día 4 post-destete y ninguna medicación sistemática en el pienso o el agua. En el estudio ex vivo se estudiaron cerdos machos comerciales sanos procedentes de piaras de alta sanidad de 6 semanas de edad como donantes de tejidos.

Recogida y análisis de muestras

En los ensayos in vivo, los animales fueron sacrificados los días 0, 15, 30 y 40 después del destete para obtener sangre de la vena yugular y muestras de tejido intestinal. Se obtuvieron muestras de tejido del yeyuno (sección media), el íleon (5 cm adyacentes a la válvula ileocecal) y el colon (sección del ápice de la espiral). En el modelo in vitro, se recogió colon espiral distal. En la sangre, se cuantificaron las inmunoglobulinas séricas totales IgA, IgE, IgG e IgM y se utilizó la citometría de flujo para analizar las subpoblaciones de distintos tipos de linfocitos. En el tejido del yeyuno, el íleon y el colon se contó la cantidad de células secretoras de IgA en la lámina propia mediante la técnica del complejo avidina biotina-peroxidasa. La expresión génica de las citocinas IFN-α, IFN-γ, IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12p35 (IL-12α), IL-12p40 (IL-12β), TNF-α y TGF-β se analizó en tejido de yeyuno, íleon y colon mediante cuantificación relativa. Se realizaron análisis histopatológicos para determinar la altura de la vellosidad (desde la punta hasta la unión vellosidad-cripta) y la profundidad de la cripta (desde la unión vellosidad-cripta hasta la base de la vellosidad). En el modelo in vitro, se analizó el porcentaje de epitelio sano y el grosor de la capa de moco que recubría los explantes. En las muestras procedentes de segmentos de colon se analizó una imagen digital de cada explante, que abarcaba toda su longitud.

Análisis estadístico

Los análisis estadísticos se realizaron utilizando IBM SPSS 21 y el procedimiento MIXED de SAS. Las medias significativamente diferentes se separaron mediante el ajuste de Tukey. La significación estadística y la tendencia se consideraron a $P \leq 0,05$ y $0,05 \leq P \leq 0,10$, respectivamente. El animal fue la unidad experimental. Los datos del grosor de la capa de moco y el porcentaje de epitelio sano se compararon entre los grupos de desafío mediante ecuaciones de estimación generalizada (GEE) utilizando una matriz de trabajo correlacionada no estructurada mientras se agrupaban por cerdo. Los datos siguieron una distribución normal.

Diseños experimentales

Se incluyen cuatro estudios experimentales para alcanzar los objetivos antes mencionados.

Objetivo 1: Evaluar la respuesta inflamatoria en lechones post-destete de forma sistémica y local en los diferentes tejidos intestinales, en entornos comerciales pero con baja presión de infección (como demuestra la baja respuesta diarreica).

Diseño experimental

Se utilizó un total de 108 lechones (Large White), destetados a los 22 ± 3 d de edad con un peso corporal (PC) medio de 5,53 ± 1,19 kg y machos:hembras 1:1. Tras el destete, todos los cerdos se distribuyeron aleatoriamente en 12 corrales, con 9 animales por corral (tamaño del corral: 0,61 × 1,2 m), y se les ofreció pienso no medicado o agua ad libitum. Se registró el PC individual a las 18 horas del destete (día 0), los días 15, 30 y 45, y se calculó la ganancia media diaria. De forma similar, se sacrificaron lechones seleccionados al azar por corral completo el día 0 (n = 10), el día

15 (n = 26), el día 30 (n = 34) y el día 45 (n = 34) para obtener muestras de sangre y tejidos para análisis inmunohistoquímicos e histomorfométricos.

Resultados

Los resultados demostraron un aumento de las células productoras de IgA en el intestino grueso el día 30 después del destete. Además, los resultados confirmaron la existencia de diferentes patrones de distribución de las células inmunitarias en el yeyuno, el íleon y el colon. El estudio demostró que la expresión de ARNm de citocinas pro y antiinflamatorias variaba según el tipo de tejido analizado y se observó una diferencia en la expresión de citocinas durante la vida del lechón, con un aumento de los niveles de citocinas proinflamatorias después del destete. Las concentraciones de IgA en plasma aumentan con el tiempo, produciéndose el principal incremento en los días 30 y 45 tras el destete. No se ha encontrado correlación entre las células productoras de IgA en los diferentes tejidos intestinales y la expresión génica de citoquinas en el tejido y/o en PBMCs. El ACP indicó una separación entre la expresión génica de citocinas en los diferentes tejidos del TGI y en las PBMC, con una agrupación de la expresión génica de citocinas en el intestino delgado (yeyuno e íleon).

Conclusiones

Los cambios en la expresión de citoquinas en el tiempo tanto en tejido intestinal como en sangre encontrados en este estudio indican que los cerdos parecen recuperarse de la inflamación intestinal, observada por un aumento de citoquinas pro-inflamatorias como se ha comentado anteriormente, seguido de un aumento de la expresión de citoquinas anti-inflamatorias como

retroalimentación negativa. Los resultados han demostrado que el proceso de destete en lechones se asoció con una respuesta prolongada y transitoria en la expresión génica de citoquinas inflamatorias intestinales y una mayor cantidad de células productoras de IgA en el tejido intestinal. Estas respuestas aumentadas seguían presentes en los días 15 y 30 post-destete.

Objetivo 2: Evaluar la respuesta inflamatoria en lechones post-destete a nivel sistémico y local en los diferentes tejidos intestinales, causada por desafíos patógenos específicos utilizando un método de desafío previamente descrito, utilizando patógenos que son conocidos por causar importantes pérdidas económicas debido a un pobre rendimiento en el crecimiento, y un aumento de los costes de producción asociados a los tratamientos.

Diseño experimental

Se utilizaron como donantes de tejidos 20 cerdos machos comerciales sanos, procedentes de piaras de alta sanidad de 6 semanas de edad. Para Salmonella Typhimurium y Lawsonia intracellularis, se evaluaron explantes de cinco donantes de tejido diferentes y se probaron los compuestos F, S y P. Se utilizaron diez cerdos diferentes para desafiar a los explantes con Brachyspira hyodysenteriae: 5 se utilizaron para el compuesto F, S y P, y 5 cerdos adicionales se utilizaron para el compuesto D. Para el compuesto F, S y P, se utilizó un total de 4 explantes/cerdo para cada grupo de combinación. Sólo para el compuesto D se utilizó un total de 12 explantes/cerdo por cada grupo de combinación.

Los inóculos contenían una media de 3,2 x 108 UFC/mL para S. Typhimurium, 7,9 x 107 copias del genoma/mL para B. hyodysenteriae y 1 x 104 células/mL para L. intracellularis. Una vez preparados el inóculo y el compuesto, ambos se mezclaron y se expusieron a los explantes. Los

explantes del grupo de control de patógenos (PCG) recibieron 100 µL de inóculo de un patógeno determinado y los explantes del grupo de control de compuesto combinado (CCG) recibieron 100 µL de compuesto solamente. El grupo de tratamiento (TG; coexposición de los explantes a una determinada combinación de patógeno y compuesto), recibió 50 µL de inóculo bacteriano 2x y 50 µL de dilución de compuesto 2x.

Resultados

El tratamiento con el compuesto P, una mezcla de MCFA y SCFA, mejoró la cobertura epitelial del explante y disminuyó la acumulación de moco y la expresión de TNF-α mRNA tras la exposición a B. hyodysenteriae. También se observó una tendencia a la disminución de la expresión de ARNm de IFN-γ tras la exposición. El compuesto S disminuyó la acumulación de moco y redujo la expresión de TNF-α y ARNm de iNOS tras la exposición a B. hyodysenteriae. Los explantes tratados con el compuesto F (prebiótico a base de centeno fermentado de Agaricus subrufescens) presentaron una mayor cobertura epitelial cuando fueron desafiados con L. intracellularis que los no tratados. Los explantes infectados con B. hyodysenteriae y tratados con el compuesto D (fitobiótico) presentaron una mayor cobertura epitelial y menores niveles de IL-1α, TNF-α e IFN-γ en comparación con los explantes infectados no tratados.

Conclusiones

Los hallazgos sugieren que los compuestos no antimicrobianos estudiados pueden tener efectos beneficiosos para el hospedador basándose en los datos del modelo de explante mostrados. El compuesto P favoreció la supervivencia epitelial y redujo el grosor del moco cuando los explantes se expusieron a B. hyodysenteriae. El compuesto D tiene un efecto inmunomodulador en los

explantes expuestos a B. hyodysenteriae. El compuesto F previno la muerte epitelial tras la exposición a L. intracellularis. Los autores creen que se necesitan más investigaciones para verificar la eficacia del compuesto in vivo.

Objetivo 3: Investigar compuestos no antibióticos con efectos antimicrobianos previamente descritos que se ha sugerido que favorecen la salud intestinal y reducen la inflamación intestinal, como parte de una estrategia nutricional para modular la compleja interacción entre el sistema inmunitario y/o las respuestas inflamatorias y las consecuencias sobre la salud y el rendimiento de los animales.

Material y método

En este estudio se utilizaron 72 lechones (Large White), destetados a los 22 ± 3 días (d) de edad. Los corrales se asignaron aleatoriamente a dos tratamientos con 8 corrales por tratamiento y 9 animales por corral. Sin recibir ningún alimento antes del destete, tras el destete todos los cerdos se asignaron visualmente intentando evitar grandes diferencias de peso entre los animales del mismo corral. Los tratamientos dietéticos consistieron en una dieta control y una dieta de tratamiento, siendo la dieta control + 0,2% de Fysal® Solute (FS), un aditivo alimentario consistente en una mezcla de harina de copra hidrolizada rica en manano y centeno fermentado con A. subrufescens. Se registró el peso corporal individual a las 18 horas posdestete (día 0) y a los días 15, 30 y 45, y se calculó la ganancia media diaria. De forma similar, se sacrificaron lechones seleccionados al azar por corral completo el día 0 (n = 10), el día 15 (control n=11, FS

n=10), el día 30 (control n=6, FS n=12) y el día 45 (control n=9, FS n=13) para obtener muestras de sangre y tejidos para análisis inmunohistoquímicos e histomorfométricos.

Resultados

La ganancia media diaria tendió a ser mayor en el tratamiento con SL durante los días 0-15. La longitud de las vellosidades en el tejido yeyunal fue inferior en el día 15 para el SL en comparación con el control ($P < 0,05$). En el día 30 se observó una mayor longitud de las vellosidades en el tejido yeyunal y una tendencia en el tejido ileal para el SL en comparación con el control. En el tejido ileal, la relación V/C fue superior en el SL en comparación con el control el día 30. El recuento medio de células productoras de IgA en el día 15 fue inferior en los lechones alimentados con la dieta FS en yeyuno e íleon. En el día 30, en comparación con el control, el recuento medio de células productoras de IgA fue inferior en los lechones alimentados con FS en yeyuno, íleon y colon. El recuento medio de células productoras de IgA en el colon fue inferior ($P < 0,05$) en los lechones alimentados con SL el día 45 en comparación con el control (Tabla 5, Figura 1).

En el día 15, se observó un mayor nivel de IgA e IgM en sangre y en el día 45, una mayor cantidad de IgG e IgM en el SL en comparación con el control. Al comparar la expresión génica de las citocinas en el yeyuno, el íleon, el colon y las PBMC en los tres puntos temporales, se observó que el tratamiento con SL redujo las expresiones génicas en los tejidos locales de varias citocinas, principalmente en los días 15 y 45, y aumentó la expresión génica en las PBMC principalmente en el día 15.

Conclusiones

En lechones post-destete, la alimentación con el componente FS, una mezcla de harina de copra hidrolizada rica en manano y centeno fermentado con A. subrufescens, estimula un efecto de modulación inmunitaria más evidente a los 15 días post-destete. El efecto incluye una reducción de la respuesta inflamatoria intestinal local, con énfasis en el yeyuno, acompañada de un aumento de la expresión génica de citoquinas sistémicas (PBMCs) y una mayor altura de las vellosidades en el yeyuno y el íleon en el día 30, mientras que se observó que era menor en el yeyuno en el día 15.

Conclusiones generales

La inflamación intestinal cuesta energía y puede aumentar la permeabilidad y reducir la digestión y el rendimiento del crecimiento en lechones post-destete. Este estudio demuestra que estos efectos pueden prolongarse, pero que el intestino puede recuperarse. Además, los aditivos no antimicrobianos pueden favorecer la salud intestinal de estos lechones, reduciendo la respuesta inflamatoria y promoviendo la integridad intestinal. Este efecto también se observó en desafíos de enfermedades con patógenos conocidos por causar problemas enteropatógenos en la producción porcina.

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PUBLICATIONS

Article 1:

https://doi.org/10.1093/jas/skab065

Journal of Animal Science. 2021. **Weaning causes a prolonged but transient change in immune gene expression in the intestine of piglets**

Controlling gut inflammation is important in managing gut disorders in the piglet after weaning. Establishing patterns of inflammation markers in the time subsequent to weaning is important for future research to determine if interventions are effective in controlling gut inflammation. The objective of this study was to evaluate the intestinal inflammatory response during the post-weaning period in piglets. A 45-day study included 108 piglets (weaned at 22 days (d), body weight 5.53 ± 1.19 kg), distributed in 12 pens with nine pigs per pen. Histomorphometry, gene expression of pro- and anti-inflammatory cytokines and the quantity of Immunoglobulin (Ig) A producing cells were measured in jejunum, ileum and colon on days 0, 15, 30 and 45 post-weaning. Cytokine gene expression in peripheral blood mononuclear cells (PBMC) and Ig quantities were analysed in blood from piglets on day 0, 15, 30 and 45 post-weaning. Histomorphometrical results showed a lower villus length directly after weaning. Results demonstrated a post-weaning intestinal inflammation response for at least 15 days post-weaning by upregulation of IgA producing cells and IFN-γ, IL-1α, IL-8, IL-10, IL-12α and TGF-β in jejunum, ileum and colon. IgM and IgA was upregulated at day 30 post-weaning. IgG was downregulated at day 15 post-weaning. The results indicate that weaning in piglets is associated with a prolonged and transient response in gene expression of pro- and anti-inflammatory cytokines and IgA producing cells in the intestine.

Article 2 doi.org/10.1186/s40813-021-00241-y

Porcine Health Management. 2021. **Fermented rye with *Agaricus subrufescens* and mannan-rich hydrolysate based feed additive to modulate post-weaning piglet immune response**

The process of weaning in piglets is often associated with an increased inflammation response in the intestine and compromised intestinal integrity and morphology favoring a delay in intestinal maturation and a predisposal to diseases. Research has shown the potential of different nutritional strategies to reduce the production of pro-inflammatory cytokines, with the main goal to manipulate health and performance of pigs. Promising examples of nutritional strategies are fungal fermented products and their derivatives which are described to contain several compounds that may play a role in gastrointestinal health and pathogenic bacteria control. Products from *Agaricus subrufescens* mushroom are reported to contain prophylactic and therapeutic properties including antimicrobial and immunomodulatory. This study analysed the post-weaning immune status in intestinal tissue and blood of piglets, with the objective to evaluate the gastrointestinal health and immune modulation response induced by a blend of mannan-rich hydrolyzed copra meal and fermented rye with *A. subrufescens*. Intestinal histomorphology demonstrated a villus height reduction in jejunum and increase in ileum on day 15, while increased villous height in jejunum and ileum on day 30. The results showed that in post-weaning piglets, the feed additive stimulates an immunomodulation effect most evident at 15 days post-weaning, with significant lower expression of cytokines Interferon (IFN) γ, Interleukin (IL) 1α, IL-1β, IL-6, IL-8, IL-10 and Transforming Growth Factor (TGF) β in jejunum, accompanied with an increase in peripheral blood mononuclear cells (PBMC) cytokine gene expression of IL-1β, IL-6, IL-8, IL-10, IL-12p35 (IL-12α), IL-12p40 (IL-12β), Tumor Necrosis Factor (TNF) α, IFN-α, and TGF-β. In piglets fed the feed additive, the quantity of Immunoglobulin (Ig) A producing cells in jejunum, ileum was reduced on day 15 and 30 post-weaning, and on day 30 and 45 post-weaning in colon tissue. Natural Killer (NK) cells count in blood were increased at day 15 post-weaning in the piglets fed the feed additive. This study implies the potential of the blend including mannan-rich hydrolyzed copra meal and fermented rye with *A. subrufescens* on immune modulation in the intestine of post-weaning piglets.

Article 3 https://doi.org/10.3390/ani12182356

Animals. 2022. ***In Vitro*** **Screening of Non-Antibiotic Components to Mitigate Intestinal Lesions Caused by *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Salmonella enterica* Serovar Typhimurium**

Swine dysentery, ileitis, and porcine salmonellosis are production-limiting diseases of global importance for swine production. Currently, the prevention, treatment, and control of these diseases still relies on antimicrobials. The goal of this study was to evaluate the effectiveness of four commercially available non-antimicrobial compounds in preventing lesions caused by the bacteria cited above using an in vitro intestinal culture model. A total of five pigs per pathogen were used and multiple compounds were evaluated. For compound F (a fungal fermented rye), S (a blend of short and medium chain fatty acids), and P (a synergistic blend of short and medium chain fatty acids, including coated butyrates), a total of four explants/pig for each treatment were used, while for compound D (an extract of carob and thyme) only 12 explants/pig for each treatment were used. Explants were exposed to a combination of pathogen only (n = 4/compound/pig), compound only (n = 4/compound/pig), or pathogen and compound (n = 4/compound/pig) and sampled at two time-points. Histopathology and gene expression levels were evaluated to investigate the treatment effect on explants. Short and medium-chain fatty acids, and an extract of carob and thyme, was found to mitigate lesions due to B. hyodysenteriae exposure. A fungal fermented prebiotic increased healthy epithelial coverage when explants were exposed to L. intracellularis or S. Typhimurium. These findings represent a step towards finding alternatives to antimicrobials usage and control of swine dysentery, ileitis, and salmonellosis in pork production.

APPENDIX

Table 1: Composition of the experimental diets (as fed-basis) in the *in vivo* studies

Item	Phase 1 day 0-14	Phase 2 Day 15-45
Ingredients, %		
Barley	29.98	25.00
Wheat	24.00	26.89
Corn	17.17	19.50
Soybean Meal 47 crude protein	6.00	16.83
Ca carbonate	0.45	0.61
Monocalcium phosphate	0.75	0.78
Soybean oil	3.50	3.67
Intestinal swine mucosal hydrolyzate[1]	2.50	0.00
Milkpowder	5.00	0.00
Fysal MP[2]	0.30	0.30
Salt	0.30	0.44
L-Valine (96.5%)	0.050	0.025
DL-Methionine (99%)	0.175	0.175
L-Lysine HCl (98%)	0.542	0.525
L-Threonine (98%)	0.258	0.250
L-Tryptophan (98%)	0.033	0.008
Protein concentrate[3]	6.00	2.00
Trouwmix 30 premix[4]	3.00	3.00

[1]protein source as hydrolysed peptides from porcine intestinal mucosa

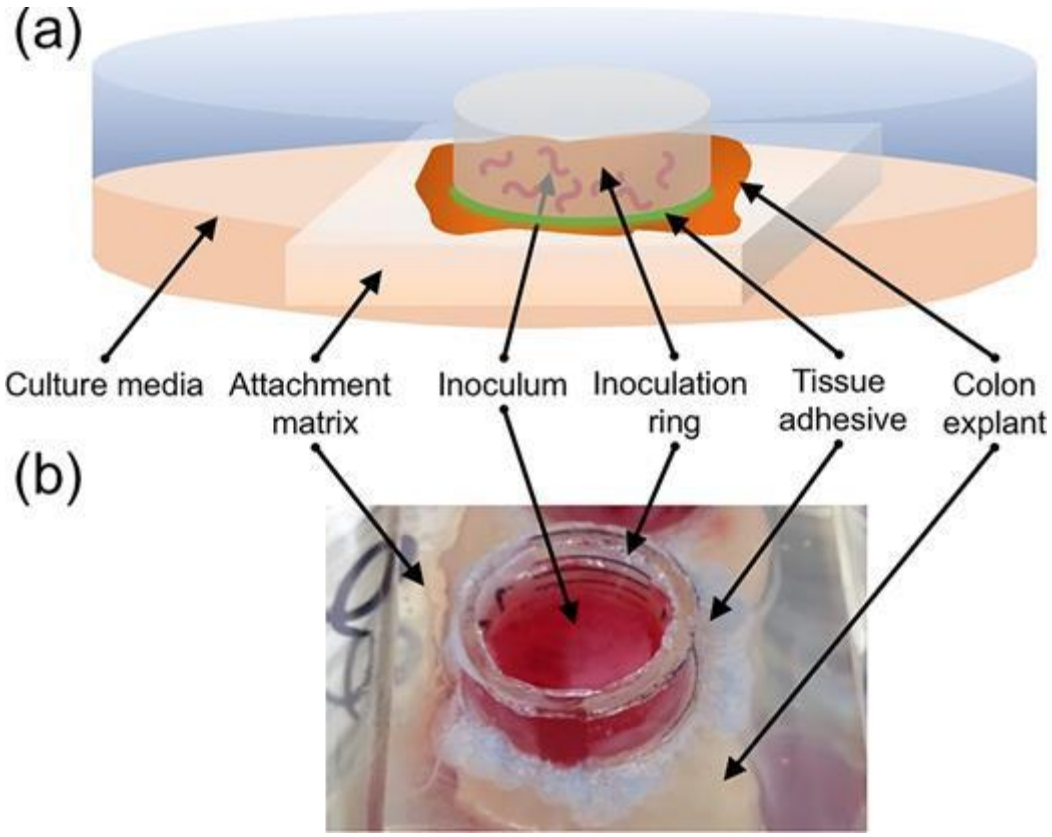
[2]blend of free and buffered organic acids (Formic, acetic, propionic acid)

[3]gluten meal, extruded soybean meal, potato protein

[4]Vitamin and mineral premix provided the following per kilogram of diet: vitamin A, 15,000 IU; vitamin D, 2000 IU; vitamin E, 100 IU; 30 µg of vitamin B12; vitamin K, 2 mg; D-pantothenicacid 15 mg as calcium pantothenate; 30 mg of nicotinicacid; choline, 150 mg as betaine hydrchloride; Mn, 50 mg as manganese oxide; Zn, 105 mg as zinc oxide; Fe, 100 mg as iron sulphate; Cu, 120 mg as copper sulphate; I, 1.5 mg as potassium iodide; Se, 0.42 mg as sodium selenite; 6-phytase 1500 Phytase Unit (FTU).

Figure 1: In vitro challenge model

Diagram of explant setup (**a**) and example of setup used in this study (**b**). Individual explants were cultured on top of an agar attachment matrix that isolated it from the media. Tissue adhesive was dispensed on the outer aspect of the inoculation ring, preventing leakage of inoculum to the basolateral sides of the explants.



From: Costa et al., 2017. https://doi.org/10.1093/femspd/ftx032

Figure 2: Histopathology images

Figure 2.1: Immunohistochemical expression of IgA in the jejunum of animals from *in vitro* studies. The same sections were used, at different magnifications for histomorphometry and count of IgA producing cells. 1A and 1B day 15, 2A and 2B day 30, 3A and 3B day 45

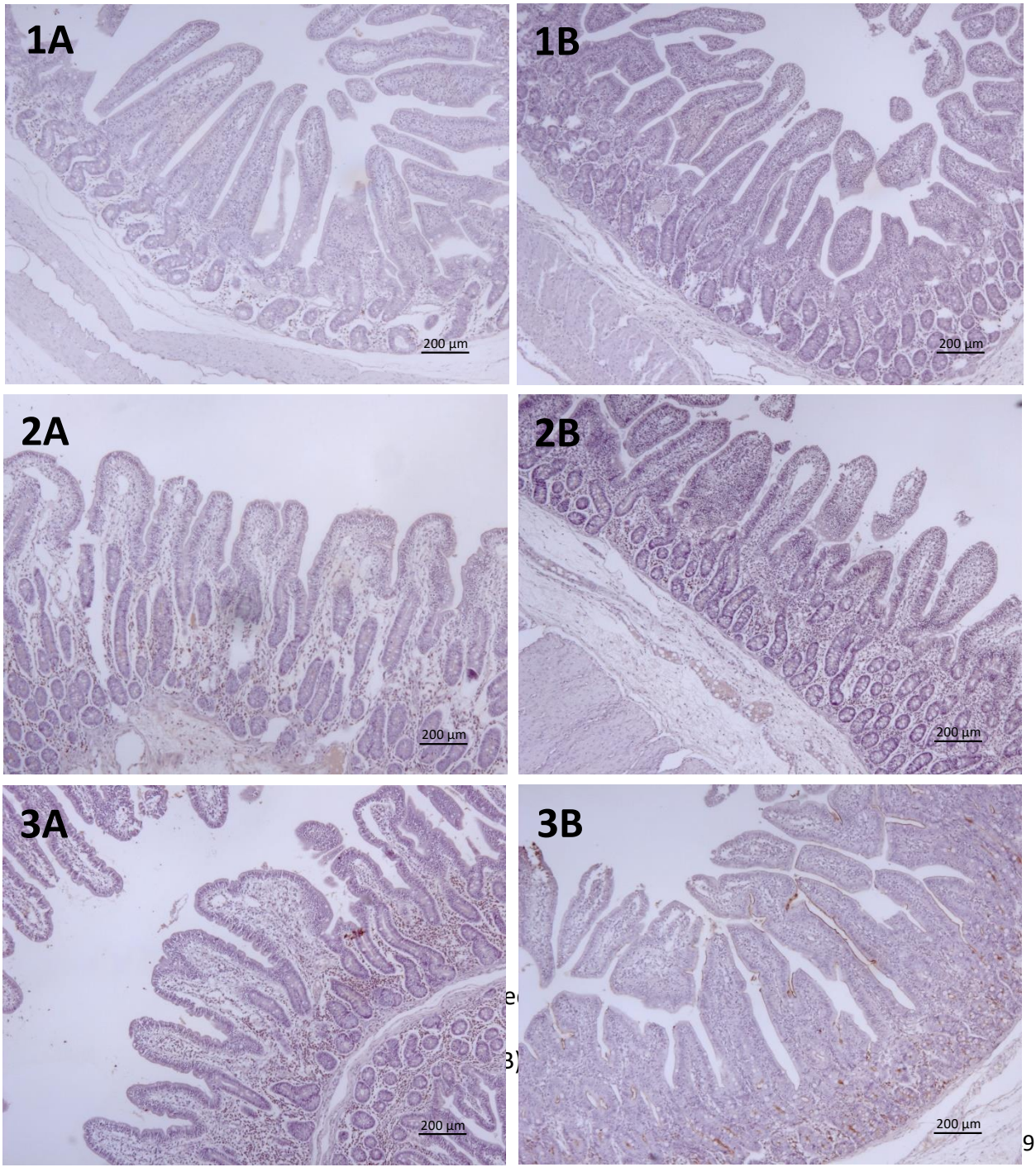
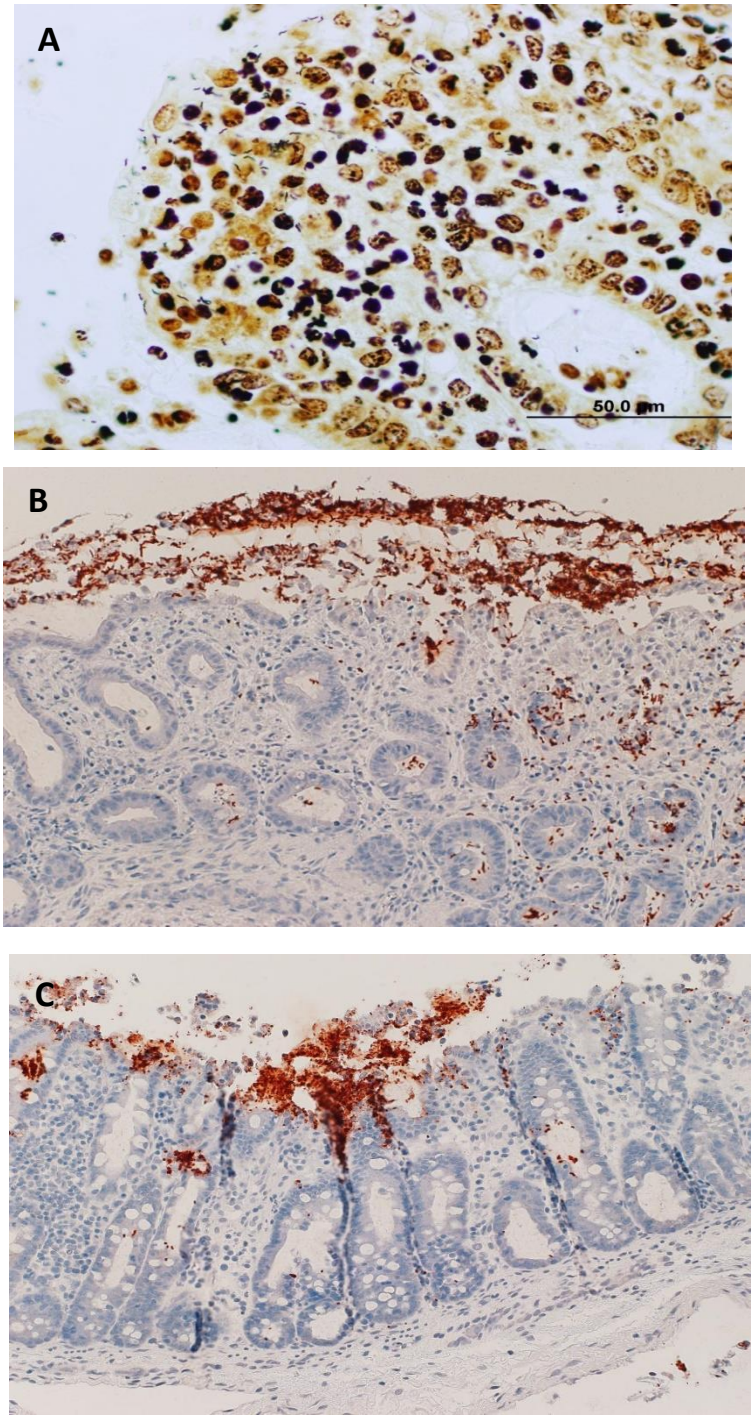


Figure 3: Examples of Warthin-faulkner-stained explant inoculated with *L. intracellularis* (A), immunohistochemistry for *B. hyodysenteriae* (B) and *S. typhimurium* (C)



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AGRICULTURE, DAIRY & ANIMAL SCIENCE

10/62

JCR YEAR	JIF RANK	JIF QUARTILE	JIF PERCENTILE
2021	10/62	Q1	84.68
2020	11/63	Q1	83.33
2019	12/63	Q1	81.75
2018	16/61	Q2	74.59
2017	12/60	Q1	80.83

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VETERINARY SCIENCES

17/169

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2021	17/169	Q1	90.24
2020	19/167	Q1	88.92
2019	18/167	Q1	89.52
2018	32/161	Q1	80.43
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VETERINARY SCIENCES - SCIE

LANGUAGES	REGION	1ST ELECTRONIC JCR YEAR
English	ENGLAND	2019

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BMC	CAMPUS, 4 CRINAN ST, LONDON N1 9XW, ENGLAND	1 issue/year

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TITLE	CITATION COUNT

14

CATEGORY

AGRICULTURE, DAIRY & ANIMAL SCIENCE

9/79

JCR YEAR	JCI RANK	JCI QUARTILE	JCI PERCENTILE
2021	9/79	Q1	89.24
2020	10/80	Q1	88.13
2019	10/79	Q1	87.97
2018	11/78	Q1	86.54
2017	12/75	Q1	84.67

CATEGORY

VETERINARY SCIENCES

15/169

JCR YEAR	JCI RANK	JCI QUARTILE	JCI PERCENTILE
2021	15/169	Q1	91.42
2020	18/167	Q1	89.52
2019	16/167	Q1	90.72
2018	25/161	Q1	84.78
2017	25/157	Q1	84.39

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CATEGORY

AGRICULTURE, DAIRY & ANIMAL SCIENCE - SCIE

VETERINARY SCIENCES - SCIE

LANGUAGES	REGION	1ST ELECTRONIC JCR YEAR
English	SWITZERLAND	2018

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MDPI	ST ALBAN-ANLAGE 66, CH-4052 BASEL, SWITZERLAND	24 issues/year

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TITLE	CITATION COUNT
	14

Journal Impact Factor Trend 2021 Export

ARTICLE 1

https://doi.org/10.1093/jas/skab065

Weaning causes a prolonged but transient change in immune gene expression in the intestine of piglets

Nienke de Groot, †,‡,1 Fernando Fariñas, || Carolina G. Cabrera-Gómez, ||
Francisco J. Pallares, $ and Guillermo Ramis||

†Trouw Nutrition Innovation, Amersfoort 3811 MH, The Netherlands, ‡
Instituto de Inmunología Clínica y Enfermedades Infecciosas, Málaga, Spain, ||Dpto. Producción Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain, $ Dpto. Anatomía y Anatomía Patológica Comparadas, Universidad de Murcia, Murcia, Spain

Abstract

Controlling gut inflammation is important in managing gut disorders in the piglet after weaning. Establishing patterns of inflammation markers in the time subsequent to weaning is important for future research to determine whether interventions are effective in controlling gut inflammation. The objective of this study was to evaluate the intestinal inflammatory response during the postweaning period in piglets. A 45-d study included 108 piglets (weaned at 22 d, body weight 5.53 ± 1.19 kg), distributed in 12 pens with nine pigs per pen. Histomorphometry, gene expression of pro- and antiinflammatory cytokines, and the quantity of immunoglobulin (Ig) A producing cells were measured in jejunum, ileum, and colon on days 0, 15, 30, and 45 postweaning. Cytokine gene expression in peripheral blood mononuclear cells and Ig quantities were analyzed in blood from piglets on days 0, 15, 30, and 45 postweaning. Histomorphometrical results showed a lower villus length directly after weaning. Results demonstrated a postweaning intestinal inflammation response for at least 15 d postweaning by upregulation of IgA producing cells and IFN-γ, IL-1α, IL-8, IL-10, IL-12α, and TGF-β in jejunum, ileum, and colon. IgM and IgA were upregulated at day 30 postweaning. IgG was downregulated at day 15 postweaning. The results indicate that weaning in piglets is associated with a prolonged and transient response in gene expression of proand anti-inflammatory cytokines and IgA producing cells in the intestine.

Key words: cytokines, immunoglobulin, inflammation, intestine, piglets, weaning

ARTICLE 2

https://doi.org/10.1186/s40813-021-00241-y

Fermented rye with Agaricus subrufescens and mannan-rich hydrolysate based feed additive to modulate post-weaning piglet immune response

Nienke de Groot1,3* , Fernando Fariñas2, Lluís Fabà1, Ellen Hambrecht1, Carolina G. Cabrera-Gómez3, Francisco J. Pallares4 and Guillermo Ramis3

[1]Trouw Nutrition Innovation, Amersfoort 3811 MH, The Netherlands, [2] Instituto de Inmunología Clínica y Enfermedades Infecciosas, Málaga, Spain, [3]Dpto. Producción Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain, [4]Dpto. Anatomía y Anatomía Patológica Comparadas, Universidad de Murcia, Murcia, Spain

Abstract

Background: The process of weaning in piglets is often associated with an increased infammation response in the intestine and compromised intestinal integrity and morphology, favoring a delay in intestinal maturation and a pre- disposal to diseases. Research has shown the potential of diferent nutritional strategies to reduce the production of pro-infammatory cytokines, with the main goal to manipulate health and performance of pigs. Promising examples of nutritional strategies are fungal fermented products and their derivatives which are described to contain several compounds that may play a role in gastrointestinal health and pathogenic bacteria control. Products from Agaricus subrufescens mushroom are reported to contain prophylactic and therapeutic properties including antimicrobial and immunomodulatory properties.

Results: This study analysed the post-weaning immune status in intestinal tissue and blood of piglets, with the objective to evaluate the gastrointestinal health and immune modulation response induced by a blend of mannanrich hydrolyzed copra meal and fermented rye with A. subrufescens. Intestinal histomorphology demonstrated a villus height reduction in jejunum and increase in ileum on day 15, while increased villous height in jejunum and ileum on day 30. The results showed that in post-weaning piglets, the feed additive stimulates an immunomodulation efect most evident at 15 days post-weaning, with signifcant lower expression of cytokines Interferon (IFN) γ, Interleukin (IL) 1α, IL-1β, IL-6, IL-8, IL-10 and Transforming Growth Factor (TGF) β in jejunum, accompanied with an increase in peripheral blood mononuclear cells (PBMC) cytokine gene expression of IL-1β, IL-6, IL-8, IL-10, IL-12p35 (IL-12α), IL-12p40 (IL-12β), Tumor Necrosis Factor (TNF) α, IFN-α, and TGF-β. In piglets fed the feed additive, the quantity of Immunoglobulin (Ig) A producing cells in jejunum, ileum was reduced on day 15 and 30 post-weaning, and on day 30 and 45 post-weaning in colon tissue. Natural Killer (NK) cells count in blood were increased on day 15 post-weaning in the piglets fed the feed additive.

Conclusion: This study implies the potential of the blend including mannan-rich hydrolyzed copra meal and fermented rye with A. subrufescens on immune modulation in the intestine of post-weaning piglets.

Keywords: Infammation, Cytokines, Additive, Intestine, Piglets, Weaning

ARTICLE 3

https://doi.org/10.3390/ani12182356

In Vitro Screening of Non-Antibiotic Components to Mitigate Intestinal Lesions Caused by *Brachyspira hyodysenteriae, Lawsonia intracellularis* and *Salmonella enterica* Serovar *Typhimurium*

Nienke de Groot [1], Mariana Meneguzzi [2], Barbara de Souza [3] and Matheus de O. Costa [4,5,*]

[1] Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Murcia, 30100 Murcia, Spain [2] Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55455, USA, [3] Departamento de Clínica and Cirurgia Veterinárias, Escola de Veterinária, Federal University of Minas Gerais, Belo Horizonte 31270-901, Brazil. [4] Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada. [5] Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, 3584 CS Utrecht, The Netherlands

Abstract: Swine dysentery, ileitis, and porcine salmonellosis are production-limiting diseases of global importance for swine production. They are caused by infection with *Brachyspira hyodysenteriae*, *Lawsonia intracellularis*, and *Salmonella enterica* serovar *Typhimurium*, respectively. Currently, the prevention, treatment, and control of these diseases still relies on antimicrobials. The goal of this study was to evaluate the effectiveness of four commercially available non-antimicrobial compounds in preventing lesions caused by the bacteria cited above using an in vitro intestinal culture model. A total of five pigs per pathogen were used and multiple compounds were evaluated. For compound F (a fungal fermented rye), S (a blend of short and medium chain fatty acids), and P (a synergistic blend of short and medium chain fatty acids, including coated butyrates), a total of four explants/pig for each treatment were used, while for compound D (an extract of carob and thyme) only 12 explants/pig for each treatment were used. Explants were exposed to a combination of pathogen only (n = 4/compound/pig), compound only (n = 4/compound/pig), or pathogen and compound (n = 4/compound/pig) and sampled at two time-points. Histopathology and gene expression levels were evaluated to investigate the treatment effect on explants. Short and medium-chain fatty acids, and an extract of carob and thyme, was found to mitigate lesions due to *B. hyodysenteriae* exposure. A fungal fermented prebiotic increased healthy epithelial coverage when explants were exposed to *L. intracellularis* or *S. Typhimurium.* These findings represent a step towards finding alternatives to antimicrobials usage and control of swine dysentery, ileitis, and salmonellosis in pork production.

Keywords: swine; in vitro organ culture (IVOC); intestinal health; pathogen; feed additive