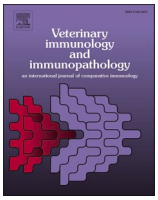
Veterinary Immunology and Immunopathology 255 (2023) 110533

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm



Weaning diet supplemented with health-promoting feed additives influences microbiota and immune response in piglets challenged with *Salmonella*

Martin Lessard [a,b,c,d], Guylaine Talbot [a,b,c,*], Nadia Bergeron [b], Luca Lo Verso [a,d], Bruno Morissette [c], Étienne Yergeau [b,e], Jacques J. Matte [a], Nathalie Bissonnette [a], Mylène Blais [a,b], Joshua Gong [f], Qi Wang [f], Sylvain Quessy [b], Frédéric Guay [b,d]

[a] *Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC J1M 0C8, Canada*
[b] *Swine and Poultry Infectious Diseases Research Center (CRIPA), Faculty of Veterinary Medicine (FMV), Université de Montréal, St-Hyacinthe, QC J2S 7C6, Canada*
[c] *Département de Biologie, Université de Sherbrooke, 2500 boulevard de l'Université, Sherbrooke, QC J1K 2R1, Canada*
[d] *Faculté des sciences de l'agriculture et de l'alimentation, Département de sciences animales, Université Laval, Québec, QC G1V 0A6, Canada*
[e] *Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique, Université du Québec, Laval, QC, Canada*
[f] *Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, ON N1G 5C9, Canada*

ARTICLE INFO

Keywords:
Immune response
Microbiota
Weaning feed
Gut health
Piglet

ABSTRACT

The aim of this study was to evaluate the potential of micronutrients and feed additives to modulate intestinal microbiota and systemic and mucosal immune responses in weaned pigs infected with *Salmonella*. At weaning, 32 litters of 12 piglets each were allocated to four dietary treatments: 1) control diet (CTRL), 2) CTRL supplemented with chlortetracycline (ATB), 3) CTRL supplemented with a cocktail of feed additives (CKTL); and 4) CKTL diet containing bovine colostrum in replacement of spray-dry animal plasma (CKTL+COL). The CKTL supplement included cranberry extract, encapsulated carvacrol and yeast-derived products and an enriched selenium and vitamin premix. Three weeks after weaning, four pigs per litter were orally inoculated with *Salmonella* Typhimurium DT104. Half of them were euthanized 3 days post-infection (dpi) and the other half, 7 dpi. The expression of *IL6, TNF, IL8,* monocyte chemoattractant protein 1 (*MCP1*), *IFNG,* cyclooxygenase 2 (*COX2*), glutathione peroxidase 2 (*GPX2*) and β-defensin 2 (*DEFB2*) showed a peaked response at 3 dpi ($P < 0.05$). Results also revealed that *DEFB2* expression was higher at 3 dpi in CTRL and CKTL groups than in ATB ($P = 0.01$ and 0.06, respectively) while *GPX2* gene was markedly increased at 3 and 7 dpi in pigs fed CKTL or CKTL+COL diet compared to CTRL pigs ($P < 0.05$). In piglets fed CKTL or CKTL+COL diet, intestinal changes in microbial communities were less pronounced after exposure to *Salmonella* compared to CTRL and progressed faster toward the status before *Salmonella* challenge (AMOVA $P < 0.01$). Furthermore, the relative abundance of several families was either up- or down-regulated in pigs fed CKTL or CKTL+COL diet after *Salmonella* challenge. In conclusion, weaning diet enriched with bovine colostrum, vitamins and mixture of feed additives mitigated the influence of *Salmonella* infection on intestinal microbial populations and modulate systemic and intestinal immune defences.

1. Introduction

In swine production, weaning is a stressful period during which growth performances are reduced (Heo et al., 2013) and important perturbation of the intestinal microbiota are induced (Castillo et al., 2007; Lallès et al., 2007b). Furthermore, weaning impairs intestinal barrier and immune functions and increases susceptibility to pathogenic bacteria such as enterotoxigenic *Escherichia coli* (Stokes et al., 2004; Lallès et al., 2007a; Schroyen et al., 2013). To improve growth performance and to prevent bacterial infection in livestock animals, antibiotics have been added to weaning feed as prophylactic treatment (Thacker, 2013). However, such use of antibiotics promotes the acquisition of antimicrobial resistance genes within bacterial populations of the intestinal microbiota which greatly increases the threat of facing more

* Corresponding author at: Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC J1M 0C8, Canada.
E-mail address: guylaine.talbot@agr.gc.ca (G. Talbot).

https://doi.org/10.1016/j.vetimm.2022.110533
Received 19 May 2022; Received in revised form 9 November 2022; Accepted 6 December 2022
Available online 7 December 2022

severe enteric infections in animals and humans, and consequently this practice has been restricted in many countries (van den Bogaard and Stobberingh, 1999). Worldwide the swine industries are under pressure to reduce the use of antibiotics while maintaining animal health and performance. Therefore, new feeding strategies aiming to modulate intestinal and systemic immune functions are required to improve the development and health of piglet gastrointestinal tract (GIT), limit antibiotic use and secure swine value chain.

So far, several feed additives have been assessed as alternatives to in-feed antibiotics because of their potential to improve gut health by modulating intestinal bacterial populations, epithelial barrier properties or immune functions (de Lange et al., 2010; Pluske, 2013). However, in most studies performed in pigs and other species, feed additives have been independently tested and have induced in most cases very specific and limited benefits on gut health parameters. Based on these observations, it appears that the combination of bioactive compounds with complimentary functional properties associated with gut health could be a more suitable approach than the use of single additive because of the potential synergy between additives to improve piglets intestinal microbiota and immune defences and to protect piglets from enteric infections.

In the present study, we proposed a feeding approach that included a combination of feed additives and nutrients that could improve gut health through their potential to modulate intestinal microbiota, epithelial integrity and immune defence. The selected feed additives included carvacrol, a yeast extract rich in glucans and mannans, cranberry extracts rich in phenolic compounds and bovine colostrum. They were chosen for their complementary prebiotic, antimicrobial, antioxidant and immunoregulatory functional properties. Bovine colostrum contains many bioactive molecules with immunoregulatory and antimicrobial properties (Cross and Gill, 2000; Stelwagen et al., 2009). The non-dialyzable material fraction of cranberry was reported to inhibit the production of proinflammatory cytokines and chemokines such as interleukin (IL) − 1β, IL-6, IL8, tumour necrosis factor-α (TNF-α) and RANTES by macrophages stimulated with lipopolysaccharide (LPS) from *E. coli* and other pathogens, including *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* (Bodet et al., 2006). Yeast, carvacrol and cranberry extracts, which provide together glucans, mannans and phenolic compounds, are also interesting to study in combination with bovine colostrum because of their complementary prebiotic, antimicrobial, antioxidant and immunoregulatory functional properties (Cross and Gill, 2000; Volman et al., 2008; Blais et al., 2014; Blais et al., 2015).

Dietary supplementation with organic selenium and vitamins A, D and B-complex was also considered for their potential to modulate immune functions (Stephensen, 2001; Wintergerst et al., 2007; Hoffmann and Berry, 2008; Bikle, 2011; Konowalchuk et al., 2013). Indeed, several studies demonstrated that vitamin A and vitamin D regulate tight junction molecule expression and intestinal barrier function (Kong et al., 2008; Cantorna et al., 2019). Furthermore, they are both involved in the regulation of innate and adaptive immunity and are required for the normal development of the immune system in the GIT (Veldhoen and Brucklacher-Waldert, 2012; Lauridsen et al., 2021). As previous results from our group showed that newborn piglets had very low body status for these vitamins, and colostrum or milk provision was insufficient to meet the corresponding requirements during lactation, these nutrients have been found to be more at risk of being deficient (Matte and Audet, 2020) and may compromise the development of natural and immune defences. With respect to vitamin E and selenium as constituent of the selenoenzyme glutathione peroxidase (GSH-Px), they both play a complementary critical function in protecting the internal cellular constituents from oxidative damage and in the regulation of the immune response (Finch and Turner, 1996) while B-vitamins are involved in various cellular metabolic pathways, and have thereby an indirect influence on functional properties of immune cells (Maggini et al., 2007; Lauridsen et al., 2021).

Therefore, the aim of this study was to investigate the influence of a dietary supplementation with cranberry extract, encapsulated carvacrol, yeast-derived products, fortified vitamin premix, organic Se, and bovine colostrum on the production of inflammatory cytokines, modulation of blood leukocyte populations and the gene expression profile of the ileal mucosa in weaned piglets infected with *Salmonella*. The effect of *Salmonella* infection and dietary treatments on intestinal microbial communities were also examined.

2. Material and methods

2.1. Experimental design, housing, and dietary treatments

All animals were housed and slaughtered according to practices approved by the Animal Care Committee of Agriculture and Agri-Food Canada's Sherbrooke Research and Development Centre (Protocol CIPA No. 378) and of the Faculty of Veterinary Medicine of the University of Montreal (Protocol No. 11-Rech-1615). All procedures were in accordance with the code of practice of Canada (Canadian Council on Animal Care, 2009; National Farm Animal Care Council, 2015).

In this study, a total of 32 multiparous Yorkshire–Landrace sows and their litters housed at Agriculture and Agri-Food Canada's Sherbrooke Research and Development Centre (Sherbrooke, QC, Canada) were used in a randomized complete block design. Each block included eight sows and their litters, which were randomly distributed into four treatments (overall, n = 8 litters per treatment). Oestrus was synchronized before the sows were inseminated. Once oestrus was detected, two inseminations were performed with pooled semen of three Duroc boars provided by a local artificial insemination centre (CIPQ Inc., St-Lambert, QC, Canada).

Two weeks before parturition, the sows were housed in two farrowing rooms. Within the first 2 d after birth, the size of each litter was adjusted to 12 piglets. At weaning (20 ± 1 d of age), all litters were moved to the nursery section, where they were housed in a separate pen for each litter (1.9 m × 1.9 m) and assigned to one of the following four dietary treatments as described previously (Bissonnette et al., 2016): (i) the control weaning diet containing spray-dried plasma proteins at 35 g/kg (CTRL); (ii) the CTRL treatment plus the antibiotic chlortetracycline at 1.5 g/kg feed (ATB); (iii) the CTRL treatment plus a cocktail of dietary supplements (CKTL) composed of cranberry extract at 1 g/kg feed (kindly provided by Nutra Canada, Champlain, QC, Canada), encapsulated carvacrol at 0.1 g/kg feed as prepared in a previous study (Wang et al., 2009), selenized yeast and yeast-derived products (i.e. mannans and glucans) at 5 g/kg feed (kindly provided by Lallemand Inc., Montreal, QC, Canada), and extra vitamins A, D, E, and B complex at 3 g/kg feed; and (iv) the CKTL treatment with defatted bovine colostrum (CKTL+COL) (colostrum kindly provided by Sterling Technology, Brookings, SD, USA) at 50 g/kg instead of spray-dried plasma proteins. Supplementation of CKTL diets in different vitamins B ranged between 2 and 4 times and in vitamins A, D, and E between 3 and 5 times to compensate for reduced feed intake of piglets in the first few days after weaning and poor reserve in liposoluble vitamins. Indeed, sow prenatal transfer of vitamins A, D and E is limited and not fully compensated by the colostrum and milk postnatal transfer to neonatal piglets (Matte and Audet, 2020). All diets were manufactured by Shur-Gain (Regional East Office, Saint-Hyacinthe, QC, Canada) and were formulated to meet or exceed nutrient requirements recommended for weaned pigs by the National Research Council (National Research Council, 2012). The composition and calculated chemical analysis of each diet are presented in Supplementary Table S1. The piglets were weighed at 1, 7, 14, 21, and 35 d of age. They were fed ad libitum and had free access to water.

In the third week after weaning, six pigs per litter were transferred in level II biosafety facilities of the Veterinary School in St-Hyacinthe, University of Montreal, to perform a *Salmonella* challenge. All pigs were housed in the same room (one pen per dietary treatment) and had 7

days of acclimation before carrying out experimental infection as described below. At their arrival, they continued to have free access to water and experimental diets, which were tested negative for *Salmonella*. During the acclimation period, an individual clinical exam was done each day to evaluate general state (score 1–6), respiration, body temperature, and fecal consistency score (0−3). Fecal samples were collected 24 hrs after arrival to be sure that piglets were *Salmonella* free before the challenge. Two piglets were euthanized before challenge as negative control (Day 0) and samples of intestinal contents and ileal mucosa were taken as described below.

2.2. Salmonella challenge and necropsy procedures

Salmonella enterica serovar Typhimurium DT104 strain #4393 rifampicin resistant, originating from a clinical case of salmonellosis, was used for inoculation. The frozen stock culture was sub-cultured in nutrient broth overnight at 35 °C as described previously (Côté et al., 2004). A final concentration of approximately 1×10^8 colony-forming units per ml (cfu/ml) was used to orally infect the four animals one week after arrival. After challenge, individual clinical exam was done twice a day has described above. Rectal temperatures and rectal swabs were taken on days 1, 3 and 7 for qualitative bacteriology as previously described (Côté et al., 2004).

Blood samples were taken by venipuncture from the jugular vein before *Salmonella* infection (D0) and on days 2 and 6 post-infection (dpi) to determine concentration of inflammatory blood markers and to characterize lymphocyte populations as described below. At 3 and 7 dpi and before challenge as mentioned above before, two pigs per treatments were euthanized by intravenous overdose of sodium pentobarbital (Eutanyl Forte 540, Bimeda-MTC animal health, Cambridge, ON, Canada) after being anesthetised by intramuscular injections of 2 mg/kg xylazine (Xylamax, Bimeda-MTC animal health) and 20 mg/kg ketamine (Vetalar, Bioniche animal health, Belleville, ON, Canada). Body cavities were carefully opened to prevent contamination of internal organs by intestinal content. Mesenteric lymph nodes and the ileo-ceacal junction were collected to detect and quantify *Salmonella*. Samples of ileal mucosa as well as intestinal content from ileum and colon were also collected for analysis of gene expression and microbial populations, respectively, as described below.

2.3. Salmonella detection

The detection of *Salmonella* in feces, mesenteric lymph nodes and the ileo-ceacal junction was carried out using a modification of ISO6579 2002(E): Annex D, using brilliant green sulfa agar with rifampicin as the single isolation medium. Briefly, primary enrichment of intestinal contents was done in nutrient broth (1 g in 9 ml with incubation for 18–24 h at 35 °C) (Difco Laboratories, Detroit, MI, USA). One ml was transferred to 9 ml of tetrathionate brilliant green broth (BBL Microbiology Systems, Cockeysville, MD, USA) and incubated at 42 °C for 18–24 h. A loopful was inoculated on brilliant green sulfa agar plates (Difco Laboratories), and plates were incubated at 37 °C for 18–24 h. For confirmation, suspected colonies were streaked on triple sugar iron (Difco Laboratories) and Christensen's urea agars (Difco Laboratories).

2.4. Analysis of inflammatory markers in blood

Blood samples taken before *Salmonella* infection (D0) and 2 and 6 dpi were analysed to evaluate C-reactive protein (CRP), IL-8, TNF-α, interferon-γ (IFN-γ) and prostaglandin E_2 (PGE_2) in serum. Porcine DuoSet ELISA kits from R&D System (Minneapolis, MN, USA) were used to assay CRP, IL-8 and TNF-α, while IFN-γ was measured with a Swine IFN-γ CytoSet kit (ThermoFisher Scientific, MA, USA). As a reliable estimate of PGE_2, measurement of Prostaglandin E Metabolite (PGEM) was performed using a PGEM ELISA kit (Cayman chemical, Ann Arbor, MI, USA). Prior to each assay, all samples and controls were diluted to fit the standard curve and tested in duplicate according to the manufacturer's instructions. The inter-assay coefficients of variation for CRP, IL-8, TNF-α, IFN-γ and PGEM were 7.3%, 4.1%, 14.1%, 3.7% and 6.6%, respectively.

2.5. Analysis of lymphocyte populations by flow cytometry

To analyse lymphocyte populations, whole blood samples collected on D0 and at 2 and 6 dpi were diluted in Hanks balanced salt solution and then layered on Ficoll-Paque PLUS (GE Healthcare Life Science, Mississauga, ON, Canada) to isolate peripheral blood mononuclear cells (PBMC). Isolated PBMC were labelled with antibodies directed against different cell surface antigens (Table S2) to characterize the percentage of different lymphocyte populations including $CD3^-CD21^+$ B cells, $CD3^+CD4^+CD8\alpha^-$ T helper (Th) cells, $CD3^+CD4^-CD8\alpha^+$ cytotoxic T (Tc) cells, double positive $CD3^+CD4^+CD8\alpha^+$ T cells, $CD3^-CD4^-CD8\alpha^+CD16^+$ natural killer (NK) cells and $CD3^+\gamma\delta^+$ T cell subset as described previously (Lessard et al., 2018).

2.6. Analysis of gene expression in the ileum

Within 15 min after death, ileum slices were collected at 25 cm cranially from the cecum for RNA extraction and were immediately frozen in liquid nitrogen and stored at − 80 °C until assays were performed. Briefly, ileum slices were homogenized in RLT Plus (Qiagen, Toronto, ON, Canada) / β-mercaptoethanol / DX reagent (Qiagen) buffer using a Polytron homogenizer (Kinematica, New York, NY, USA). Total RNA was extracted with RNeasy Plus mini kit (Qiagen) following the manufacturer's recommendations and was resuspended in 50 μL. Total RNA was quantified using a NanoDrop spectrometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) at a wavelength of 260 nm. Purity was assessed by determining the ratio of absorbance at 260 and 280 nm (A260/A280). All samples had a ratio between 1.9 and 2.1. The ratio of absorbance at 260 and 230 nm (A260/A230) was also measured and all samples had a ratio between 1.7 and 2.1. A 1 μg aliquot of total RNA was reverse-transcribed with Superscript II reverse transcriptase (Invitrogen Canada Inc., Burlington, ON, Canada) using oligo (dT)12–18 primer (Invitrogen Canada Inc.) in a final volume of 20 μL, according to the supplier's instructions. The cDNA samples were diluted 1:15 in nuclease-free water and aliquots were stored at − 20 °C prior to real-time PCR analysis.

The mRNA quantification of the targeted genes listed in Table S3 was performed by real-time qPCR using Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as previously described (Lessard et al., 2015). Briefly, the mRNA expression levels were determined using a relative standard curve established by serial dilutions of a cDNA pool composed of all piglets included in the study. In order to confirm the specificity of the measured amplicons (i.e. the presence of only one amplicon), the melting curve was systematically analysed for all samples. Each run included a no-template control to detect DNA contamination of the reagents and each sample was tested in triplicate. All cDNA samples were analysed for the expression of three different reference genes: H3 histone family 3 A (H3F3A), ribosomal protein L32 (RPL32) and ribosomal protein S18 (RPS18).

2.7. Analysis of bacterial communities in the ileum and colon DNA using 16 S rRNA gene amplicon sequencing approach

Sampling of intestinal digesta samples and DNA extraction were performed as previously described (Morissette et al., 2018). General amplification and sequencing preparation were performed as described previously (Sanschagrin and Yergeau, 2014). Amplification of the 16 S rRNA gene was performed using the primer pair F343 (5′-TACGGRAGGCAGCAG-3′) and R533 (5′-ATTACCGCGGCTGCTGGC-3′) as previously described (Yergeau et al., 2014). All 16 S rRNA gene PCR products were run on 1.2% agarose gels and purified using the PureLink Quick Gel Extraction Kit (Invitrogen

Canada Inc.). PCR products were quantified using the Invitrogen™ Qubit™ dsDNA HS Assay Kit and Qubit Fluorometer (ThermoFisher Scientific) and combined in equimolar ratios before sequencing. We multiplexed 64 samples for each sequencing run, giving a total of two amplicon pools. Sequencing was performed on an Ion Torrent Personal Genome Machine (Thermo Fisher Scientific) using the 316 Chip and associated kit, following manufacturer protocols. The sequences were cleaned and analysed using MOTHUR ver. 1.39.5 following an adapted version of MOTHUR's 454 standard operation procedure, as previously described (Sanschagrin and Yergeau, 2014). The unique sequences were aligned using a MOTHUR adapted version of the SILVA database (silva reference files, release 102, https://mothur.org/wiki/silva_reference_files/). Sequences were attributed a taxonomic classification using the MOTHUR formatted Ribosome database project (RDP) trainset version 9 (https://mothur.org/wiki/rdp_reference_files/). Raw reads for each piglet faecal microbiota analysed in this study are available through the NCBI SRA database under BioProject accession PRJNA693822.

2.8. Statistical analysis

Blood inflammatory markers, flow cytometry cell populations and ileum gene expression data were analyzed using the MIXED procedure of SAS (SAS Statistical Analysis System 2013, SAS Institute Inc., Cary, NC, USA). The model included as fixed effects the factors dietary treatments (CTRL, ATB, CKTL and CKTL+COL), and day post *Salmonella* infection, with the litter as the experimental unit while the blocks was considered as a random effect. Dietary treatments were then compared using Tukey's test. When the interactions between dietary treatments and *Salmonella* infection reached significance and required further investigations, a separate ANOVA was performed. When necessary, percentage data of leukocyte populations as determined by flow cytometry were standardized by angular transformation prior to analysis. The results were considered significant at $P < 0.05$ or as a trend at $0.05 \leq P < 0.10$.

The Bray–Curtis distance matrices were used to produce ordination using nonmetric multidimensional scaling (NMDS). Sequenced 16 S rRNA amplicons were analysed to evaluate the influence of dietary treatments and *Salmonella* challenge on the beta-diversity of the bacterial communities in different groups using MOTHUR software and the GLIMMIX statistical procedure. Bacterial communities were analysed using Nonmetric multidimensional scaling (NMDS) with Yue & Clayton distance measure (thetayc) or Jaccard index (Jclass) to determine the impact of *Salmonella* infection on intestinal microbiota of supplemented pigs. Pairwise AMOVA analysis was used to determine if the microbiota was modified post-infection. The relative abundances of bacterial phyla and families were compared using ANOVA and GLIMMIX procedure using SAS.

3. Results

3.1. Clinical signs, fecal consistency scores and Salmonella excretion in feces

All piglets were *Salmonella* free before starting the experimental trial. For the clinical exam, no significant changes were observed between the groups. Most of the animals presented a normal respiration and a general fecal state score of one. At 3 and 4 dpi, pigs from ATB group showed less diarrhea ($P = 0.01$) compared to pigs from CTRL and CKTL groups (Figure S1). At 4 dpi, the number of pigs showing diarrhea was reduced in CKTL+COL group compared to pigs from CTRL and CKTL groups (results not shown). After 7 days, no difference was observed between groups.

After the infection, the presence of *Salmonella* was detected in the feces of most piglets at 1, 3 and 7 dpi. At 1 dpi, pigs from ATB group showed lower *Salmonella* fecal excretion level than CKTL group ($P = 0.05$). At 3 dpi, a lower *Salmonella* fecal excretion level was also measured in the ATB group compared to the CTRL group ($P = 0.02$) whereas there was no difference with pigs allocated to CKTL or CKTL+COL diet (results not shown). At 7 dpi, there was no difference between groups. *Salmonella* was also found in mesenteric lymph nodes and ileo-ceacal junction samples at 3 and 7 dpi in most piglets.

3.2. Blood inflammatory markers in pigs infected with Salmonella

Blood levels of CRP and TNF-α markedly increased in the first two days following *Salmonella* infection ($P < 0.001$), but only CRP induction was down regulated at 6 dpi compared to 2 dpi ($P = 0.005$; Table 1). A significant decrease in PGE_2 level was measured at 2 dpi and 6 dpi while lL-8 and IFN-γ were respectively up- and down-modulated ($P = 0.02$ and $P = 0.001$) at day 6 compared to day 2 (Table 1).

The increase of CRP levels between day 0 and 2 post-infection was less important in pigs fed ATB diet compared to pigs fed CKTL diet, while there was no difference in CRP concentration variation in pigs fed CTRL, CKTL or CKTL+COL (Table 2). Between day 2 and day 6 post-infection, CRP continued to increase only in CTRL group while pigs fed ATB, CKTL or CKTL+COL diet showed a negative variation during the same post-infection period resulting in significant difference between CTRL pigs and other groups (Diet × dpi interaction significant at $P = 0.02$, results not shown). No dietary effect was observed on IL-8 and IFN-γ levels after *Salmonella* infection (Table 2).

3.3. Blood lymphocyte populations in Salmonella challenged pigs

After challenge, Th and B lymphocyte percentages in blood significantly decreased with time (Table 3; $P_{dpi\ effect} = 0.01$ and <0.001, respectively). The effect was more important for the B cells as the reduction after 2 and 6 dpi was significant compared to day 0 and 2 dpi ($P < 0.001$ and 0.05, respectively). Regarding the $CD4^{-}CD8^{high}$ Tc and γδ T cell population, results indicated that both population percentages were significantly increased 2 dpi ($P < 0.001$). Percentage of NK cells tended to be also reduced 2 dpi compared to 6 dpi while the percentage of double positive $CD4^{+}CD8\alpha^{+}$ T cells was too low (less than 2%) to observe significant effect (data not shown). Only the γδ-T cell population was modulated by dietary treatments (Fig. 1). Results revealed that in pigs fed CKTL or CKTL+COL, the percentage of γδ T cells was increased compared to pigs fed ATB diet ($P < 0.05$ and $P < 0.01$, respectively).

3.4. Intestinal gene expression in pigs infected with Salmonella

The expression of genes involved in the regulation of inflammatory response (*TNF-α, IL-6, IL-8,* monocyte chemoattractant protein 1 (*MCP1*), *IFN-γ* and *IL-10*), oxidative stress (inducible nitric oxide synthase (*iNOS*), prostaglandin-endoperoxide synthase 2 (*PTGS2*) and glutathione Peroxidase *2* (*GPX2*)) and barrier function (β-defensin-2 (*βDEF2*)), were significantly upregulated at 3 dpi in pig ileal tissue

Table 1
Effect of Salmonella challenge on serum C-Reactive Protein (CRP), prostaglandin E2 (PGE2), IL-8 and IFN-γ levels.

Variables	Days post-infection (dpi)			SEM	*P* value[a]		
	D0	2 dpi	6 dpi		dpi	D0 vs 2 dpi	2 dpi vs 6 dpi
CRP (ug/ml)	11.0	46.0	35.2	3.1	< 0.001	< 0.001	0.005
PGE_2 (pg/ml)	151.0	107.1	85.9	10.5	< 0.001	< 0.001	< 0.001
IL-8 (pg/ml)	246.2	210.2	317.8	41.0	0.10	n.s.	0.02
IFN-γ (pg/ml)	11.9	10.4	5.4	2.4	n.s.	n.s.	0.001

[a] n.s.: not significant.

Table 2
Effect of dietary treatments on serum C-Reactive Protein (CRP), IL-8 and IFN-γ levels in *Salmonella* infected piglets at different days post infection (dpi).

Variables	Delta dpi	Dietary treatments				SEM	*P* value[a]				
		CTRL	ATB	CKTL	CKTL+COL		Diet	CTRL vs ATB	ATB vs CKTL	CKTL vs CKTL+COL	CTRL vs CKTL+COL
CRP (ug/ml)	Δ0–3	34.18	23.38	44.73	36.00	5.43	0.05	n.s.	0.005	n.s.	n.s.
	Δ3–7	15.47	-4.76	-9.00	-21.34	7.77	0.01	0.06	n.s.	n.s.	0.01
IL-8 (pg/ml)	Δ0–3	-25.09	25.80	-49.92	-94.94	55.3	n.s.	n.s.	n.s.	n.s.	n.s.
	Δ3–7	132.35	44.03	138.28	75.72	92.7	n.s.	n.s.	n.s.	n.s.	n.s.
IFN-γ (pg/ml)	Δ0–3	6.70	-4.84	-1.07	-6.59	6.35	n.s.	n.s.	n.s.	n.s.	n.s.
	Δ3–7	-2.71	10.92	1.96	-16.91	12.02	n.s.	n.s.	n.s.	n.s.	n.s.

[a] n.s.: not significant

Table 3
Effect of *Salmonella* challenge on the percentage of blood mononuclear immune cell subsets.

Variable	Days post-infection (dpi)			SEM	*P* value[a]		
	D0	2 dpi	6 dpi		Dpi	D0 vs 2 dpi	2 dpi vs 6 dpi
T lymphocytes ($CD3^+$):							
- Helper ($CD4^+CD8^-$)	22.1	20.7	19.3	1.0	0.01	n.s.	n.s.
- Cytotoxic ($CD4^-CD8^{high}$)	6.0	10.0	10.4	0.7	<0.001	<0.001	n.s.
- γδ ($γδ^+$)	17.8	23.5	23.7	1.4	<0.001	<0.001	n.s.
B lymphocytes ($CD3^-CD21^+$)	21.5	15.9	12.8	0.9	<0.001	<0.001	0.05
NK cells ($CD3^-CD8α^{low}CD16^+$)	15.2	13.5	16.1	1.5	0.10	n.s.	0.10

[a] n.s.: not significant

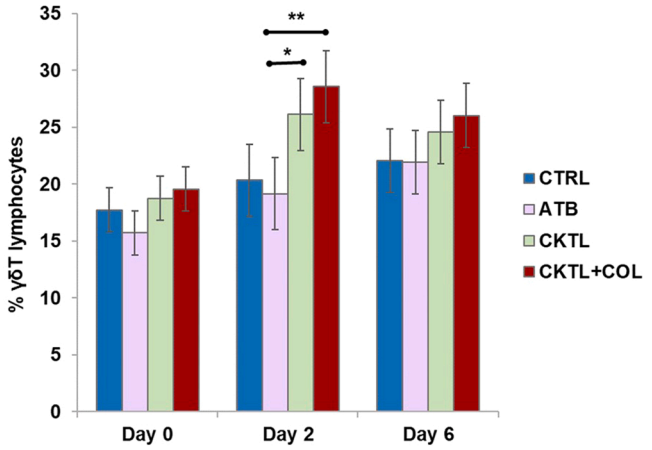


Fig. 1. Dietary effect on gamma-delta T cells in *Salmonella* infected pigs. *' * *Two days after infection, the percentage of γδ T cells was significantly increased in pigs fed CKTL or CKTL+COL compared to pigs fed ATB diet at $P < 0.05$ and $P < 0.01$, respectively.

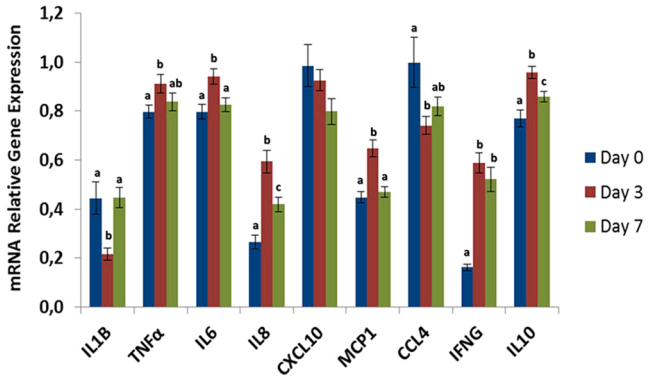


Fig. 2. *Salmonella* infection influenced expression of intestinal inflammatory cytokines and chemokines. [a, b, c] Bars accompanied with different letters indicate a significant day effect after *Salmonella* infection at $P < 0.05$.

($P < 0.05$) while *IL-1* expression was decreased (Figs. 2 and 3). However, *claudin 3–4* and *occludin* were not modulated by *Salmonella* infection. At 7 dpi, the expression of most genes had returned to their basal level.

Among the genes induced by *Salmonella* infection, only the expression of two genes, β-defensin-1 (*DEFB1*) *and GPX2,* was modulated by dietary treatments (Fig. 4). Results indicated that the *DEFB1* gene was less expressed in challenged pigs fed ATB diet than in pigs fed CTRL and CKTL diet ($P = 0.01$ and 0.06, respectively). Results also showed no difference between CTRL, CKTL and CKTL+COL groups. On the other hand, the expression of *GPX2* gene was upregulated in pigs fed CKTL and CKTL+COL compared to the CTRL and ATB groups ($P < 0.05$ and $P < 0.01$, respectively, Fig. 4). Moreover, the combination of bovine colostrum and CKTL diet further increased the *GPX2* expression ($P < 0.01$) suggesting an additive effect between feed additives and bovine colostrum molecules. Seven days post-infection, the expression of *GPX2* almost returned to the basal levels in all groups except for pigs fed CKTL+COL in which the expression was still slightly upregulated compared to other groups ($P < 0.05$).

3.5. Modulation of intestinal microbial populations by diet in Salmonella challenged pigs

The effects of the *Salmonella* infection on the composition of the ileal and colonic microbiota of piglets fed the experimental diets were also evaluated. First, NMDS ordination results showed that colonic microbiota profile was not affected by dietary treatment at 0 dpi (AMOVA at $P = 0.1$; Fig. 5A). However, the microbiota was affected by dietary treatments at 3 and 7 dpi (AMOVA at $P = 0.006$ and $P < 0.001$, respectively; Fig. 5B-C). This was ascribed to a clear separation of the microbial communities of piglets fed the ATB diet compared to piglets fed all the other dietary treatments ($P < 0.04$). Furthermore, the colonic microbiota of CKTL+COL piglets tended to differ from that of CKTL

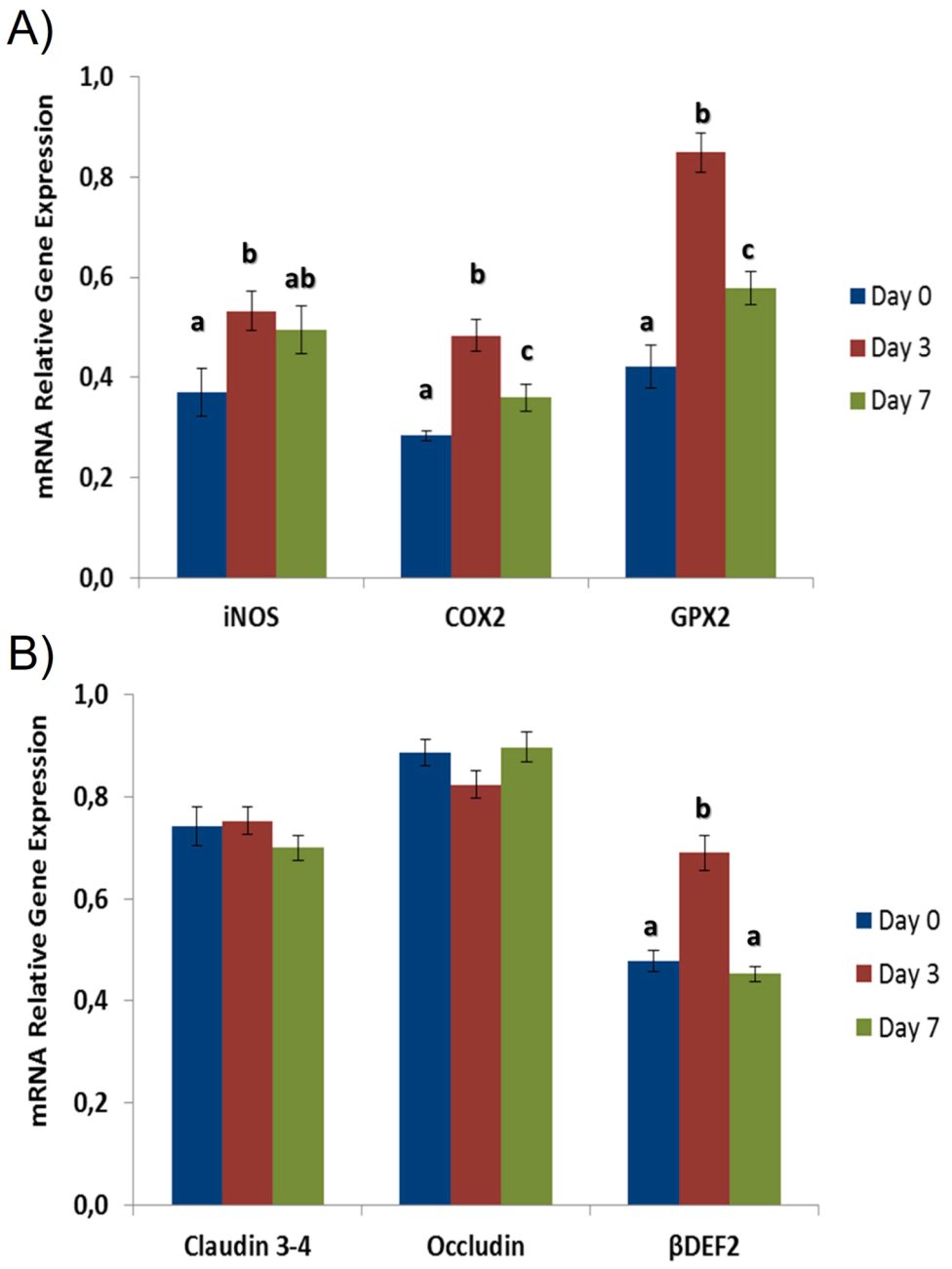


Fig. 3. Influence of *Salmonella* infection on expression of genes involved in oxidative stress (A) and intestinal defence functions (B). [a, b, c] Bars accompanied with different letters indicate a significant day effect after *Salmonella* infection at $P < 0.05$.

animals at 3 and 7 dpi ($P = 0.06$ and $P = 0.09$, respectively). In the ileum, no significant difference between the dietary treatments was observed on the microbiota composition (results not shown).

Further data analysis revealed that the composition of the intestinal microbiota was differently modulated after *Salmonella* challenge in CTRL piglets compared to pigs allocated to the other dietary treatments. In fact, both ileal and colonic bacterial communities were significantly modulated in CTRL piglets at 3 and 7 dpi compared to day 0 ($P < 0.05$; Fig. 5-D and E), whereas for the ATB group, no significant change in the ileal microbiota was measured, but a tendency was evidenced at the colonic level ($P = 0.08$; Fig. 5-F). In this group, the multiple comparisons analysis revealed that colonic microbiota at day 0 differed from that at 7 dpi ($P = 0.007$). In contrast, piglets fed the CKTL+COL diet also exhibited a rapid change in ileal microbiota at 3 dpi compared to day 0 ($P = 0.004$; Fig. 5-G), but at 7 dpi results indicated that ileal microbiota had progressed toward the status before *Salmonella* challenge. In the colon, no marked effect of this dietary treatment was observed on the microbiota (data not shown). Conversely, neither the ileal nor the colonic microbiota of piglets fed CKTL diet were significantly affected by the infection (data not shown).

Furthermore, to compare the effects of the *Salmonella* challenge on the intestinal bacterial communities of weanling piglets fed different dietary treatments, bacterial 16 S rRNA gene sequences were clustered in operational taxonomic units (OTUs), which were then classified in the RDP taxonomy. Relative abundances at the family level are graphically represented in Fig. 6. In the ileum, where the Firmicutes were the most dominant phylum for all the dietary treatments (data not shown), the abundances of some families were significantly affected by the *Salmonella* challenge (Fig. 6A). First, the *Peptostreptococcaceae* family was significantly reduced at 3 dpi in the CTRL, ATB and CKTL+COL groups

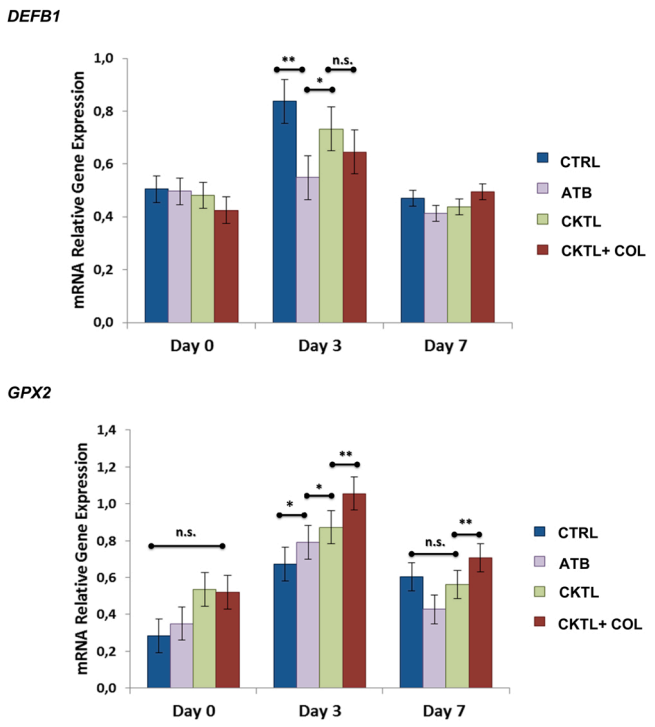


Fig. 4. Influence of *Salmonella* infection on expression of genes involved in oxidative stress (*GPX2*) and intestinal defense functions (*DEFB1*). Bars accompanied with * or ** show significant dietary effects at $P < 0.05$ and $P < 0.01$, respectively. Bars accompanied with n.s. indicate that no significant effect between treatments were detected.

($P < 0.05$; Fig. 6A). However, the relative abundance of this family slightly increased between 3 and 7 dpi and no significant differences were detected between days 0 and 7 (Fig. 6A). In addition, in the ATB group the values relative to the *Clostridiaceae* family significantly decreased at 3 dpi when compared to day 0 ($P = 0.04$) but returned to pre-infection levels at 7 dpi (Fig. 6A). Furthermore, feeding weanling piglets with the CKTL+COL diet was associated with a reduction of *Erysipelotrichaceae* family both at 3 and 7 dpi, as well as a marked increase at 3 dpi of the *Lactobacillaceae* family ($P < 0.05$; Fig. 6A), which returned to pre-infection levels four days later.

In the colon, Firmicutes and Bacteroidetes were the most dominant phyla for all diets (data not shown). In the CTRL group, the relative abundance of *Lachnospiraceae* were reduced at 3 dpi compared to day 0 (20–11% at $P < 0.02$), whereas that of the *Lactobacillaceae* increased (3–13% at $P < 0.05$; Fig. 6B). However, the relative abundance of both families returned close to pre-infection levels four days later and no significant differences were detected between days 0 and 7. Conversely, compared to day 0 the *Veillonellaceae* family gradually increased in CTRL animals at 3 and 7 dpi (0.6–3% at $P = 0.06$ and $P = 0.03$, respectively; Fig. 6B). In addition, in piglets receiving the CKTL diet the relative abundances of *Clostridiaceae* and *Peptostreptococcaceae* were reduced at 3 and 7 dpi compared to day 0, whereas the *Veillonellaceae* increased ($P < 0.05$; Fig. 6B). Similarly, the family *Streptococcaceae* was reduced in this group at 3 dpi ($P = 0.04$), but its relative abundance slightly increased between 3 and 7 dpi and no significant differences were detected between days 0 and 7 (Fig. 6B). Furthermore, feeding piglets with the CKTL+COL diet was associated with an increase of the *Streptococcaceae*, *Veillonellaceae* and *Ruminococcaceae* families from 3 to 7 dpi ($P < 0.05$; Fig. 6B), whereas no difference between day 0 and 3 dpi was detected.

Finally, in piglets fed the CKTL+COL diet the relative abundance at day 0 of the *Clostridiaceae* family was lower when compared to the CKTL group ($P = 0.049$; Fig. 6B). Furthermore, in piglets receiving the CKTL+COL diet at 7 dpi the *Streptococcaceae* family was higher when compared to piglets of the ATB and CKTL groups, as well as the relative abundance of the *Ruminococcaceae* family when compared to the ATB group ($P < 0.05$; Fig. 6B).

4. Discussion

The aim of this work was to describe the influence of health-promoting feed additives given in weaning diet on immune response and intestinal microbiota in weaned piglets exposed to *S. enterica* serovar Typhimurium.

As reported previously, *Salmonella* infection modulated the concentration of blood markers involved in regulation of inflammatory reaction such as TNF-α and CRP, a protein of the acute phase reaction, which was increased (Loughmiller et al., 2007), while others such as PGE_2 and IFN-γ were decreased with time of infection or transiently decreased such as IL-8 at 3 dpi compared to D0 and 7 dpi (Balaji et al., 2000; Collado-Romero et al., 2010; Knetter et al., 2015). However in these previous studies, it has been reported that IL-8 and IFN-γ are increased after *Salmonella* challenge while TNF-α and PGE_2 are not altered (Balaji et al., 2000). Discrepancy between the results may be due to experimental conditions, times of sampling and *Salmonella* challenge doses and strains used in different experiments.

Interestingly, in pigs fed ATB diet, the increase of CRP was less marked in the first two dpi than in pigs fed CKTL diet while there was no difference between pigs fed CTRL, CKTL and CKTL+COL. It was between days 2 and 6 post challenge that CRP decreased markedly in pigs fed CKTL or CKTL+COL diet. These results indicated that ATB treatment attenuated the activation of the acute phase response while the CKTL diet containing bovine colostrum was the most efficient to downregulate its production in the days following the inflammatory peak response compared to CTRL group. Therefore, the presence of both bovine colostrum and ingredients added through the CKTL of feed additives contributed to dampen the acute phase response. While precise mechanisms of action have not been specifically investigated in this project, results on intestinal gene expression and microbiota discussed in the following sections provide some potential explanations. Unfortunately, such dietary effect was not observed for IL-8 and IFN-γ. Possible explications for these results could be that chosen times to evaluate the dietary effect on the expression of different cytokines were not optimal as these genes are transiently expressed and therefore vary with time and in magnitude after induction of inflammatory response in *Salmonella* infected pigs. Moreover, the variation in the expression of these genes between pigs was very important suggesting that the kinetics and magnitude of the expression of these genes may differ between pigs in response to *Salmonella* challenge.

In the present study, we also reported that Th and B lymphocyte populations in blood decreased after *Salmonella* challenge while γδ-T cell percentage was enhanced. Results also revealed that γδ-T cell population was also increased in pigs fed CKTL or CKTL+COL compared to pigs fed ATB diet. As reported in previous studies, circulating leukocyte populations are modulated in *Salmonella* infected animals. It was observed that sick animals displayed lower levels of circulating $CD8^+$ or $CD4^+$ T cells and higher levels of γδ T cells (Berndt et al., 2006; Scharek-Tedin et al., 2013; Kreuzer et al., 2014). Such increase in γδ T cells may be partly related to the relative reduction of other leukocyte populations. However, it cannot be excluded that the intestinal inflammatory response induced by *Salmonella* infection was not responsible for the relative changes in different cell populations. Indeed, in *Salmonella*-infected mice, an increase in intestinal γδ T cells and natural killer cell cytotoxic activity has been reported, while the proportions of B and $CD4^+$ T lymphocytes in the spleen decreased (Li et al., 2012; Rosche et al., 2015). Therefore, changes in circulating lymphocytes could be due to inflammatory response induced by *Salmonella* infection and migration of lymphocytes to the sites of infection such as the intestine and other peripheral lymphoid tissues.

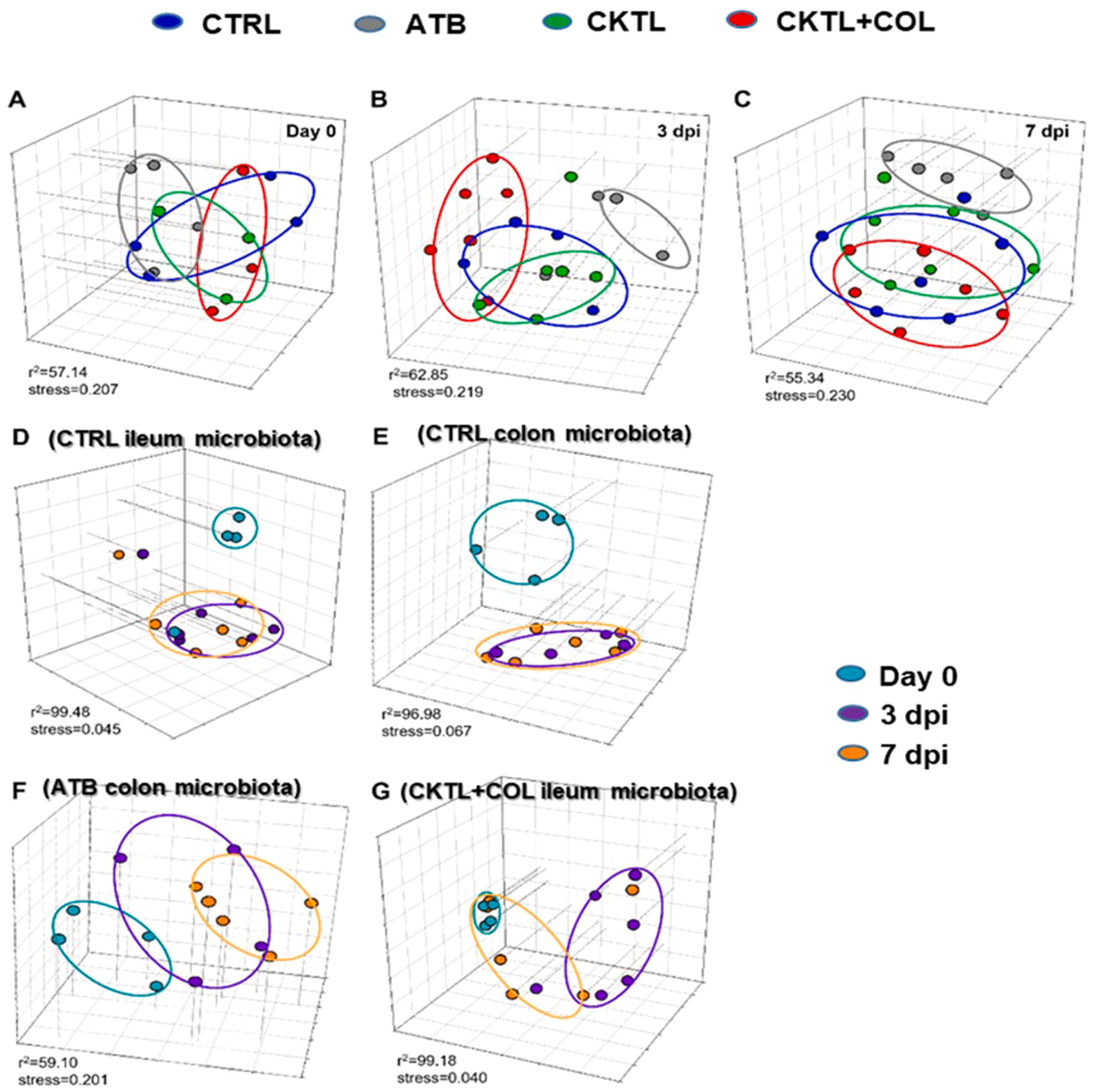


Fig. 5. NMDS graphic representation of the colonic microbiota in *Salmonella* infected piglets fed different diets at day 0 (A), 3 dpi (B) and 7 dpi (C) and NMDS graphic representation of the influence of *Salmonella* infection on the ileum (D) and colon (E) microbiota in piglets fed the CTRL diet, and on colon (F) and ileum (G) microbiota in piglets fed the ATB and CKTL+COL diets, respectively.

Interestingly, pigs fed CKTL-enriched diet displayed higher levels of circulating γδ T cells. An increase of γδ T cells has been shown to be associated with the development of a protective immune response to *Salmonella* organisms (Berndt and Methner, 2001; Li et al., 2012). As CKTL-enriched diets seem to modulate intestinal immune response of *Salmonella* infected animals, it is more likely that observed γδ T cell changes in pigs fed CKTL-enriched diets at two dpi were associated with immunomodulation effects rather than direct dietary immunostimulatory effects. Moreover, prior enrolling pigs in the challenge study, all the pigs have been fed their respective experimental diet for three weeks and no dietary effect has been observed on leukocyte populations at D0. Therefore, these results suggest that dietary supplementation with feed additives and nutrients that have the potential to improve gut health by modulating intestinal microbiota and immune defences may influence intestinal immune response to *Salmonella* infections and modulate activation and migration of different circulating populations of lymphocytes.

In the gut, *Salmonella* infection induced inflammatory reaction and activation of the immune response as shown by the pronounced changes at 3 dpi in the ileum expression of several genes involved in the regulation of inflammatory response (*TNF-α, IL6, IL8, MCP1, IFN-γ* and *IL10*), oxidative stress (*iNOS, PTGS2* and *GPX2*) and barrier function (*βDEF2*). By 7 dpi, most effects on gene expression were mitigated indicating that the inflammatory response of infected animals was in the resolution phase. These results go along with previous studies reporting that a massive transcriptional dysregulation occurs in the ileum as shown by

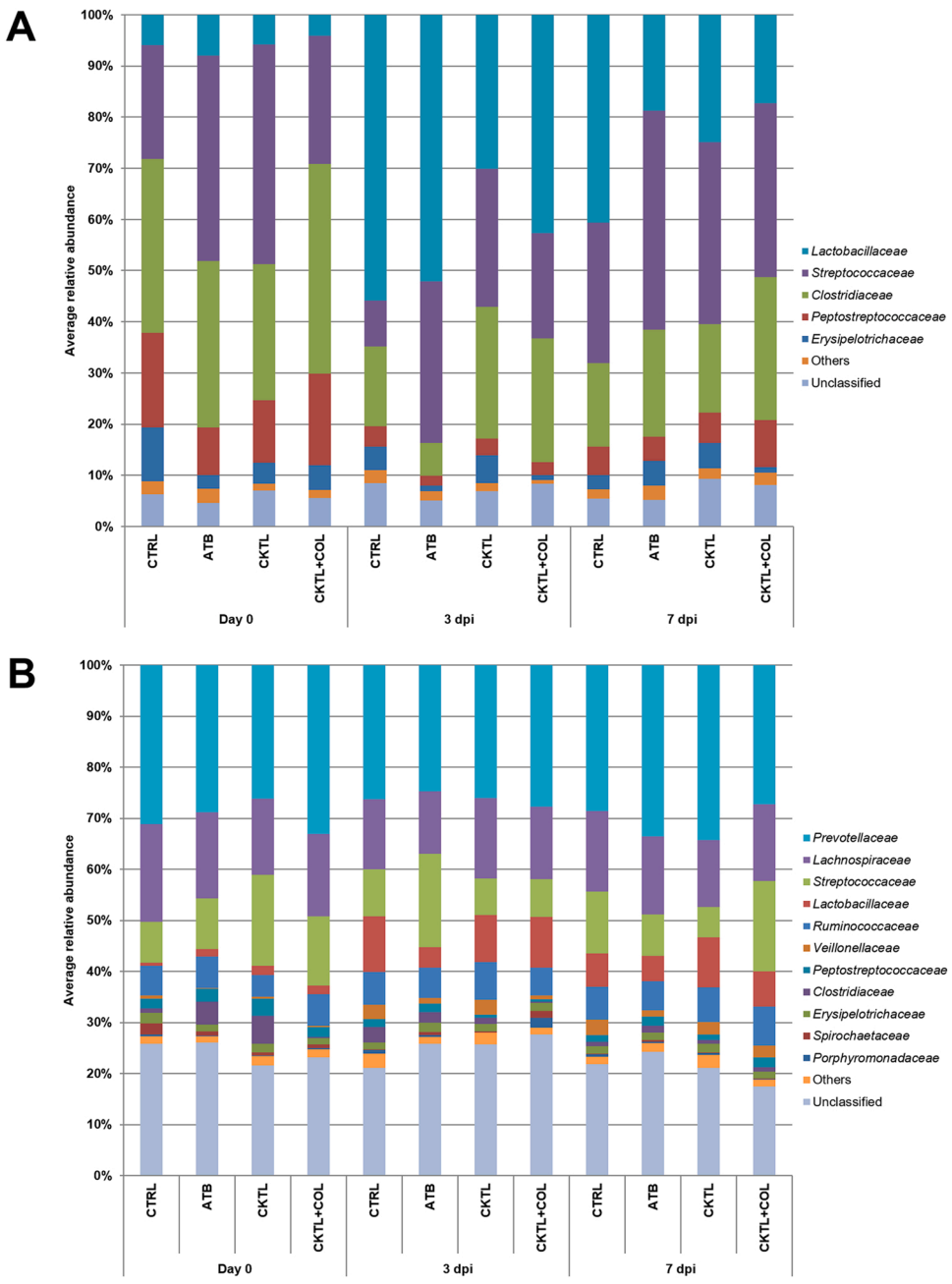


Fig. 6. Effects of the *Salmonella* challenge on the ileum (A) and colon (B) bacterial communities of weanling piglets fed different dietary treatments.

the overexpression of several ileum inflammatory genes in the first 2 dpi (Collado-Romero et al., 2010; Uribe et al., 2016).

In this study, we also observed that in piglets fed diet supplemented with a mixture of feed additives and vitamin premix, and specially in presence of colostrum, the expression of genes involved in defense response to the bacterium such as *βDEF1* and metabolic processes induced by inflammatory reaction such as *GPX2* are respectively down- and up-regulated compared to pigs fed control diet while the expression of key inflammatory cytokines was not affected. As inflammatory cytokines are transiently produced and varied during infection, time of sampling at a given day after challenge provide a limited picture of metabolic processes occurring during an inflammatory response. Therefore, the observed differences in the expression of *GPX2* and *βDEF1* suggest that inflammatory cytokines may not always be the best markers to identify subtle effects of dietary treatments on the regulation of the immune response that may have significant influence on defense mechanisms throughout the infection. Other inflammatory and immune modulators such as leukotrienes, prostaglandins, chemokines, toll-like receptors, cytokines (TGFβ1, IL-10, IL-12, etc.) and transcription factors (NFkB, TRP53) that are induced in *Salmonella* challenged pigs (Blais et al., 2015; Knetter et al., 2015; Uribe et al., 2016; Huang et al., 2018) could be considered to better understand the influence of dietary treatments on regulation of innate and adaptive immunity. For instance, in a previous study we have reported that bovine colostrum whey treatment decreases the expression of early and late inflammatory genes as well as the transcriptional activation of NF-kB induced by heat-killed *Salmonella* in the porcine intestinal epithelial cell IPEC-J2 and modulates the expression of a significant number of genes involved in cell migration, adhesion and proliferation in colostrum whey-treated cells (Blais et al., 2014; Blais et al., 2015).

Compared to the plasma protein-enriched diet containing the CKTL supplement, the results indicate that bovine colostrum had stronger

effects on priming the immune response and modulating the intestinal recovery following *Salmonella* infection. Although plasma proteins contain similar bioactive compounds as bovine colostrum such as immunoglobulins, cytokines and growth factors, the difference between both feed additives may be due to the presence of bioactive compounds in colostrum that are more relevant to support intestinal maturation and gut health (Stoy et al., 2014). Indeed, colostrum contains several types of proteins, hormones, lipids and other molecules that are involved in the maturation and development of the immune system and the establishment of healthy intestinal microbiome (Gopal and Gill, 2000; van Hooijdonk et al., 2000; Newburg et al., 2005; McGrath et al., 2015). However, previous studies also reveal that both products improve voluntary feed intake and growth rate during the immediate post-weaning period in pigs (Van Dijk et al., 2002; Huguet et al., 2012; Lo Verso et al., 2020) and can attenuate intestinal inflammation by modulating the production of pro-inflammatory cytokines in host response against pathogenic micro-organisms (Moller et al., 2011; Blais et al., 2015). However, up to now, there is no precise results describing the differences in their specific mode of actions, as previously reported (King et al., 2008).

In previous studies carried out in our laboratory, results revealed that bovine colostrum whey decreased inflammatory response of intestinal epithelial cells incubated in presence of enterotoxigenic *E. coli* and *Salmonella* components. The results also indicate that colostrum can control inflammatory processes by decreasing pathogenic bacteria binding to epithelial cells, by moderating inflammatory signalling pathways and/or by promoting the integrity of the epithelium (Blais et al., 2015). Moreover, results reveal that the expression of a significant number of genes involved in wound healing, adhesion and proliferation is affected in colostrum whey-treated cells (Blais et al., 2014). *In vivo* studies carried out by different research groups also provided clear evidence that bovine colostrum products stimulated digestive function and protected against intestinal inflammation (Boudry et al., 2007; Jørgensen et al., 2010; Moller et al., 2011; Huguet et al., 2012; Stoy et al., 2014).

The current results also suggest that the increased metabolic response to reactive oxygen species, as shown by increased *GPX2* gene expression, could be also associated to dietary enrichment in vitamins (E, A and D) and organic Se. As a matter of fact, these nutrients play complementary roles in the regulation of the innate and adaptive immune responses, and numerous studies have characterized their role in the modulation of the immune system, as reported by many articles and reviews (Stephensen, 2001; Maggini et al., 2007; Mora et al., 2008; Spinas et al., 2015; Cantorna et al., 2019).

Although these results clearly indicated that the CKTL+COL diet contributed to improve gut health through its action on the regulation of defense response to combat *Salmonella* infection and on microbiota (discussed in the following section), further studies are required to better understand the synergistic effect between the dietary components on piglet's intestinal immune defenses to pathogens.

As previously reported, *S. enterica* serovar Typhimurium is able to successfully infect pigs in a short timeframe (Argüello et al., 2018). The following local inflammation, due to the activation of the immune response, triggers some modifications in the gut environment which favour the survival of *Salmonella* and prompt changes in the gut microbiota (Drumo et al., 2015; Argüello et al., 2018). Similarly, in our study *Salmonella* infection rapidly caused important modifications in the intestinal bacterial communities as evidenced by the NMDS ordination results 3 dpi. Results also indicated that microbiota changes were more evident in the CTRL group, where a large and persistent shift of both ileal and colonic microbiota was recorded. Conversely, in piglets fed diet supplemented with CKTL or CKTL+COL, intestinal microbiota changes seemed to be attenuated after exposure to *Salmonella*. As a matter of fact, the NMDS ordination results showed no variation in CKTL piglets due to *Salmonella* infection, whereas in CKTL+COL piglets a rapid change in ileal microbiota at 3 dpi was evidenced. This variation, however, was temporary and minor compared to the shift seen in the CTRL group. Intestinal dysbiosis recovery was also faster in CKTL+COL piglets as shown by the ileal microbiota shift progression toward the status before *Salmonella* challenge, at 7 dpi. These results highlight the potential of these dietary supplementations to attenuate microbial dysbiosis and to improve gut health recovery of weaned piglets after infections.

These observations are supported by different studies reporting that several components included in CKTL and colostrum may help preventing gut infections and promote the gastrointestinal health. As a matter of fact, bovine colostrum possesses antimicrobial and prebiotic properties that may support the presence of beneficial microbial populations and reduce the abundance of potential pathogenic species (Menchetti et al., 2016; Poulsen et al., 2017). Furthermore, previous studies have already proved the ability of some molecules normally present in bovine colostrum, such as lactoferrin, to bind to *Salmonella* surface and block its adhesion in vitro, as well as to reduce the severity, mortality and the degree of inflammation induced by *Salmonella* infection in mice (Bessler et al., 2006; Mosquito et al., 2010). Concerning the CKTL ingredients, yeast-derived mannans and β-glucans have shown to protect pigs against enteropathogenic bacteria such as *E. coli* and *Salmonella* spp., preventing the adhesion to the intestinal villi and subsequent colonization and dissemination of bacterial pathogens (Kogan and Kocher, 2007). Furthermore, cranberry extracts can display inhibitory activity against pathogens such as *E. coli* and *Salmonella* because of their high content in phenolic acids (Wu et al., 2008). Carvacrol, as one of the main components of oregano oil, has also gained interest for its antimicrobial potential against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, molds and yeasts, and even at sub-lethal concentrations it may inhibit *Salmonella* motility in vitro (Nostro and Papalia, 2012). Encapsulation had been shown to increase the amount of carvacrol release in the intestine in our previous study, therefore, improving its antimicrobial activity (Zhang et al., 2016).

As previously reported, the severity of the damage caused by *Salmonella* infection in the intestinal epithelium may correlate with an increase in synergistic (with respect to *Salmonella* infection) or opportunistically pathogenic bacteria and a depletion of beneficial (such as *Lactobacillus* spp.) or competing bacteria (e.g. *Clostridium* spp. and *Ruminococcus*) (Argüello et al., 2018). Such changes could contribute to the pathogen's ability to colonize the gut successfully (Argüello et al., 2018). In our study, the further analysis by OTUs of the bacterial 16 S rRNA gene sequences also showed that some specific families were differently modulated after *Salmonella* challenge. The *Peptostreptococcaceae* were among the most affected bacterial families, and their relative abundance was somehow reduced in all the dietary treatments. However, such reduction was temporary in the ileum of the CTRL, ATB and CKTL+COL group, whereas it persisted until 7 dpi in the colon of CKTL piglets. *Peptostreptococcus* species are commensal bacteria well known for their capacity to produce the metabolite indoleacrylic acid, which promotes intestinal epithelial barrier function and mitigates inflammatory responses (Wlodarska et al., 2017). These bacteria have already shown to be markedly affected by *S. enterica* serovar Enteriditis in chickens, their relative abundance being inversely correlated to that of the *Enterobacteriaceae* family at different stages of the infection (Mon et al., 2015).

Interestingly, in piglets fed the CKTL+COL diet the relative abundance at 7 dpi of the *Streptococcaceae* family was higher when compared to piglets of the ATB and CKTL groups, as well as the relative abundance of the *Ruminococcaceae* family when compared to the ATB group. *Streptococcus* populations are considered as primary fermenters of diet-derived simple sugars in the intestine and the presence of lactose may enhance their fermentative activity (Thomas et al., 2011). Concerning the *Ruminococcaceae,* this family seems to be related to the *Salmonella*-shedding status of the host, with its relative abundance being higher in low *Salmonella* shedder, when compared to high *Salmonella* shedder, pigs (Bearson et al., 2013). Consequently, they are thought to help limiting the colonization and the shedding exacerbation after

Salmonella infection (Bearson et al., 2013). Furthermore, the *Ruminococcaceae*, together with the *Prevotellaceae*, *Lachnospiraceae* and *Veillonellaceae* families, assemble a functional group in the intestine of healthy piglets, because of their metabolic capacities that are indispensable for host survival (Zhang et al., 2018). In fact, they are positively correlated with the metabolism of amino acid, energy, cofactors and vitamins in the porcine large intestine (Zhang et al., 2018).

5. Conclusion

In conclusion, results showed that blood inflammatory markers and leukocytes, and intestinal expression of several genes involved in regulation of immune response were markedly modulated in *Salmonella* infected weaned piglets. Data also indicated that after *Salmonella* challenge, beta-diversity of bacterial communities is also markedly affected. In piglets fed with CKTL or CKTL+COL diet, intestinal changes in microbial communities were less pronounced after exposure to *Salmonella* and progressed faster toward the status before *Salmonella* challenge. Weaning diet enriched with bovine colostrum, vitamins and mixture of feed additives also modulated the intestinal expression of genes involved in regulation of immune defence as well circulating lymphocyte populations and inflammatory factors. Mechanisms of action are still not completely resolved and further studies are required to better understand the influence of different dietary components on piglet's intestinal immune defenses and microbial communities in response to *Salmonella* infection and on interactions between the host and the microbiota.

Funding

This work was funded by Agriculture and Agri-Food Canada and Swine Innovation Porc, in partnership with Shur-Gain-Nutreco and Lallemand Animal Nutrition.

Declaration of Competing interest

The authors declare that they have no competing interests.

Acknowledgements

On behalf of all authors, very special thanks go to Dre Ann Letellier (FMV, Université de Montréal) for her contribution in the conception and realisation of the project. The authors also want to sincerely thank Frédéric Beaudoin, Karoline Lauzon and Nathalie Gagnon for their help in laboratory analysis and data compilation, Steve Méthot for his support in statistical analysis of data, and the staff of the Swine Complex, under the supervision of Mélanie Turcotte, who took care of the pigs during the animal phase.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetimm.2022.110533.

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