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Gene expression and gastrointestinal function is altered in piglet small intestine by weaning and inclusion of *Cyberlindnera jadinii* yeast as a protein source

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ABSTRACT

This study investigated the effect of feeding *Cyberlindnera jadinii* yeast on the development of gastrointestinal function and health in piglets during the first two challenging weeks after weaning. Changes in gastrointestinal function were mainly attributed to weaning, and not to dietary treatment. The post-weaning (PW) transcriptome profiles differed between dietary treatments showing an overall higher number of differentially expressed genes (DEGs) in control piglets than in yeast-fed piglets. DEGs in jejunum and ileum were compared between sampling timepoints within each feeding group and divided into clusters with similar expression trends. Pathway enrichment analysis was run on each cluster to reveal PW physiological changes. Weaning induced downregulation of several immune functions in the control piglets, which was not as evident in the yeast fed piglets. The results indicate that feeding *C. jadinii* yeast can improve PW gut homeostasis and give more robust piglets.

1. Introduction

Weaning is a critical time for piglets because major changes occur, including abrupt transition in diet, maternal separation, change in environment, increased exposure to pathogens and dietary or environmental antigens (Campbell, Crenshaw, & Polo, 2013; Lallès, Bosi, Smidt, & Stokes, 2007), stress associated with litter mixing and establishment of a social hierarchy. High stress causes activation of physiological mechanisms to maintain body homeostasis (Jayaraman & Nyachoti, 2017), and when combined with reduced energy intake, may result in disruption of normal epithelial, immune and enteric nervous system development (Moeser, Pohl, & Rajput, 2017). Therefore, weaning is often associated with a post-weaning (PW) decline in growth performance and a higher frequency of diarrhea.

Several authors have described PW morphological changes such as villous atrophy and crypt hyperplasia (Cera, Mahan, Cross, Reinhart, & Whitmoyer, 1988; Degroote et al., 2020; Lallès et al., 2007), changes in enzyme activity (Hampson & Smith, 1986; Lindemann, Cornelius, El Kandelgy, Moser, & Pettigrew, 1986; Makkink, Negulescu, Guixin, & Verstegen, 1994) and increased intestinal permeability (Moeser et al., 2017; Spreeuwenberg, Verdonk, Gaskins, & Verstegen, 2001). The changes induced in the gastrointestinal tract (GIT) by weaning are temporary and can be divided into two periods: an initial acute period immediately after weaning, followed by a more progressive adaptative and maturational phase (Montagne et al., 2007). However, the severity is dependent by the piglets age at weaning (Moeser, Ryan, Nighot, & Blikslager, 2007). Epithelial atrophy implies a reduction of intestinal absorptive capacity. Wang et al. (2008), compared jejunal gene expression in weaned piglets and age-matched suckling piglets and found a PW reduction in gene expression associated with oxidative defense capacity, intestinal transport and utilization of dietary nutrients, immune response, synthesis of glycoproteins and proliferation and differentiation of intestinal epithelial cells, whereas water permeation across the intestinal wall was enhanced. The adverse intestinal morphological and functional change PW is attributed to low feed intake, which causes limited energy supply to the gut epithelium (Dong & Pluske, 2007; Jayaraman & Nyachoti, 2017). The withdrawal of milk from the sow does not only mean a change from liquid to solid feed, but also a change in the main energy source from milk fat and lactose to starch (Spreeuwenberg et al., 2001). The withdrawal of milk also stops

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the supply of maternal antibodies (IgA) and other bioactive compounds, thus increasing the risk of enteric diseases in piglets (Heo et al., 2013).

After the antimicrobial growth promoter ban in the EU in 2006, yeast or yeast extracts have been investigated as non-antibiotic functional products to improve gut function and health of piglets PW (Heo et al., 2013; Liu et al., 2018). The yeast cell wall consists of different polysaccharides, such as mannan oligosaccharides and β-glucans (Shurson, 2018; Øverland & Skrede, 2017). Mannan oligosaccharides are known to suppress the toxic activity of mycotoxins and reduce pathogenic intestinal colonization by blocking the binding of pathogenic bacteria to the intestinal wall (Spring, Wenk, Connolly, & Kiers, 2015). β-glucans are known to stimulate the gut immune system (Vetvicka & Oliveira, 2014). In the nursing period, piglets receive passive immunity through the sow's milk, which gradually declines as the piglets develop their own immune functions until natural weaning. Under commercial conditions, weaning occurs when the adaptive immune system of the piglets is immature (Moeser et al., 2017), and stimulation of the piglet's immune system is therefore thought beneficial. Yeast also contains about 6–10% nucleic acids in a dried inactivated state (Øverland & Skrede, 2017). In nutrient deficiency periods, dietary nucleotides may be important in tissues with a high turnover rate such as the intestinal mucosa (Sauer, Mosenthin, & Bauer, 2011). Supplementation of the diets of PW piglets with yeast or yeast extracts has been shown to increase villus height (VH) (Bontempo, Di Giancamillo, Savoini, Dell'Orto, & Domeneghini, 2006; Cruz et al., 2019; Van der Peet-Schwering, Jansman, Smidt, & Yoon, 2007), improve the main nutrient total tract digestibility (Shen et al., 2009) and reduce PW diarrhea. Cruz et al. (2019) investigated piglets after a four-week period PW and showed promising growth performance and health results of weanling piglets fed diets with *Cyberlindnera jaidinii* (previous name: *Candida utilis*) as a protein source.

The objective of this study was to investigate the effect of high dietary inclusion of *C. jadinii* yeast on GIT function and health development during the two first weeks PW, as assessed by fecal score, apparent ileal digestibility (AID) of nutrients, enzyme activity, gut morphology, and gene expression profiles.

2. Materials and methods

The experiment was performed between 30th of October and 13th of November 2017 at the Center for livestock production (SHF), NMBU, Ås, Norway, which is an animal experiment unit approved by the National Animal Research Authority (permit no. 174). All piglets were handled in accordance with laws and regulations controlling experiments with live animals in Norway (regulated by the "Animal Welfare Act" and "The Norwegian Regulation on Animal Experimentation" derived from the "Directive 2010/63/EU on the protection of animals used for scientific purposes").

2.1. Animals and housing

A total of 64 crossbred [(Norwegian Landrace × Yorkshire z-line) × (Duroc) and (Norwegian Landrace) × (Duroc)] weanling piglets, selected from eight litters, were included in the experiment. Average weaning age was 27.4 ± 1.15 days and the average weaning weight 10.24 ± 1.56 kg. Piglets were allocated to dietary treatment and day of dissection based on litter origin and weaning weight. There were six pens per diet and five or six piglets per pen. Piglets were housed in an environmentally controlled room, with temperature average of 19.9 ± 1.05 °C. Temperature was logged every morning. Pen size was 1 m × 1.58 m, with a 0.8 m^2 area of slatted concrete floor in the front of the pen. In the rear, low roofing, covering 1 m^2, constituted a sheltered resting area. A rubber mat was provided in the resting area.

Table 1
Dietary composition of experimental diets, calculated total crude protein (CP) content in diets and calculated crude protein content from yeast in diets.

	Dietary treatments	
Ingredient, g/kg as fed	Control	Yeast
Wheat	627.8	593.5
Barley	100.0	100.0
Oats	50.0	50.0
Soybean meal[1]	80.0	19.2
Potato protein conc.[2]	33.8	9.1
Fish meal[3]	20.0	4.8
Rapeseed meal[4]	20.0	4.9
Yeast - *Cyberlindnera jadinii*[5]	–	146.0
Rapeseed oil	19.7	23.4
Monocalcium phosphate	13.1	15.5
Limestone	9.2	9.4
Sodium chloride	7.2	5.5
Selenium premix	0.7	0.9
Iron(II) fumarate	0.4	0.4
Micro-mineral premix[6]	2.0	2.0
Vitamins[7]	2.2	2.2
L-Lysine	6.5	5.7
L-Methionine	2.1	2.9
L-Threonine	2.9	2.4
L-Valine	1.4	1.2
L-Tryptophan	0.9	0.9
Yttrium (III) oxide	0.1	0.1
Calculated CP content (%)	17.1	17.3
Ratio CP from yeast (% of total CP)	0.0	40.0

[1] Non-GMO soybean meal, Denofa AS, Fredrikstad, Norway
[2] Cargill, Denmark
[3] Nordsildmel AS, Egersund, Norway
[4] Expeller-pressed rapeseed cake, Mestilla, UAB, Klaipeda Lithuania
[5] Yeast meal (*C. jadinii*): 970 g DM/kg, 78 g ash/kg, 470 g crude protein/kg, 16 g ether extract/kg, 19.9 MJ/kg.
[6] "Mikro-Svin"; provided per kilogram of diet: 475 mg Ca; 3.4 mg Mg; 13.2 mg S; 120 mg Fe; 60 mg Mn; 120 mg Zn; 26 mg Cu; 0.6 mg I.
[7] Provided per kilogram of diet: 0.8 g Vitamin A; 0.3 g Vitamin E; 0.8 g Vitamin ADKB mix; 0.3 g Vitamin C (Stay C 35%).

2.2. Diets and feeding

C. jadinii yeast (LYCC-7549; Lallemand Yeast Culture Collection), previously classified as *Candida utilis* (torula yeast), was grown on sugars derived from lignocellulosic biomass from Norwegian spruce trees and beet molasses (1:1), as described in Øverland and Skrede (2017). The yeast was inactivated and drum dried.

The dietary treatments included: 1) control diet based on wheat, barley, oats, soybean meal (SBM), fishmeal (FM), potato protein concentrate and rapseed oil, and 2) experimental diet containing 14.6% inactivated *C. jadinii* yeast where the yeast replaced 40% of the protein ingredients in the control diet. Diets correspond to the Control and CU40 diets in Cruz et al. (2019). Dietary composition is shown in Table 1 and chemical composition in Table 2. Both diets contained an inert marker (0.01% Yttrium(III)oxide: Y_2O_3). Diets were formulated on net energy and standardized ileal digestibility (SID) values to be isoenergetic and isonitrogenous, and to meet or exceed the requirements for indispensable amino acids and all other nutrients for pigs of this age (NRC, 2012). The formulation of the diets was done in collaboration with Felleskjøpet Fôrutvikling AS, Trondheim, Norway, using their optimization least-cost program. Diets were produced by the Center for Feed Technology (ForTek, NMBU, Ås, Norway), and pelleted with a 3 mm diameter.

In the nursery period, piglets had access to the sow's diet as creep feed. In the experimental period, piglets had *ad-libitum* access to the experimental diets immediately after weaning from an automatic feeder (FRH-2 Domino A/S, Tørring, Denmark). Total feeding space was 43 cm (x 15 cm) with a metal bar dividing the feeding space in two. New feed

Table 2
Analyzed chemical composition of experimental diets.

	Dietary treatments	
Nutrients, g/kg of DM	Control	Yeast
DM, g/kg	869.17	885.02
Crude protein	201.96	193.92
NDF	110.02	102.35
Starch	507.99	494.31
Crude fat	45.31	46.15
Ash	52.73	51.16
Phosphorus	8.01	9.08
Gross energy, MJ/kg	18.94	18.96
Indispensable AA, g/16 g N		
Arginine	5.28	5.04
Histidine	2.13	2.02
Isoleucine	3.82	3.90
Leucine	6.99	6.86
Lysine	7.15	7.01
Methionine	2.40	2.73
Phenylalanine	4.39	4.26
Threonine	4.97	5.23
Tryptophan	1.49	1.61
Valine	5.24	5.54
Dispensable AA, g/16 g N		
Alanine	3.62	4.18
Aspartic acid	7.83	7.30
Cysteine	1.45	1.36
Glutamic acid	20.14	21.35
Glycine	4.14	4.13
Proline	6.86	6.94
Serine	4.81	4.99
Tyrosine	2.63	2.64

was provided every morning, and average daily feed intake (ADFI) for the pen calculated. Piglets had *ad-libitum* access to clean drinking water from a drinking nipple located in the front of the pen next to the feeder.

Fecal consistency was assessed every day during the experimental period using a scoring system from 1 to 4 developed by Pedersen and Toft (2011), where fecal consistency scores one and two were considered normal while scoring three and four were considered as diarrhea. The score was given as a pen average with 0.25 intervals.

2.3. Sampling

Eight piglets (littermates of piglets included in the experiment) were sampled at the day of weaning (day zero) to provide a baseline time point to facilitate detection and interpretation of changes due to weaning and potential yeast-induced changes. At day two, four, seven and 14 PW eight piglets of each diet were sampled (one or two per pen). Live body weight was registered before sampling. All animals were euthanized using a captive bolt pistol, followed by exsanguination.

The abdominal cavity was opened immediately after exsanguination and the entire GIT removed. pH was measured in stomach and duodenal content. The small intestine was sampled at two sites: proximal jejunum (1 m aboral to the right lobe of pancreas) and ileum. Approximately 25 cm tissue of each segment were isolated, pH measured and digesta collected before it was washed with phosphate-buffered saline (PBS). Small pieces of tissue (5 × 5 mm) were collected from the two segments and mucosa was sampled from the jejunum segment by scraping with a glass slide. All samples were immediately frozen in liquid nitrogen, stored on dry ice during sampling, and then transferred to storage at −80 °C until analysis. For RNA sequencing, tissue samples of approximately 5x5 mm were stored in RNAlater (Merck, Germany) at −80 °C. Liver was weighted before samples were collected. Contents from the last two meters of the small intestine were collected for determination of AID. Samples were immediately frozen at −20 °C. Ileal content was later freeze dried, homogenized using a batch mill (A11 basic Analytical mill, IKA®, England), and chemically analyzed.

A pooled feed sample collected during the experiment was ground at 1 mm for chemical analysis of main nutrients and 0.5 mm for analysis of starch and yttrium, using a Fritsch Pulverisette 19 cutting mill (Fritsch, Idar-Oberstein, Germany).

2.4. Chemical analyses

Chemical analysis (Dry matter (DM), ash, crude protein (CP), starch, gross energy, neutral detergent fiber (NDF) and amino acids) of main ingredients and diets in triplicates, and singular analysis of ileal content were performed as described in Cruz et al. (2019) by the LabTek group at the Department of Animal and Aquacultural Science, NMBU, Ås, Norway. Organic matter was calculated by subtracting ash content. Crude fat was analyzed by Eurofins Food & Feed Testing Norway AS, Moss, Norway, using acid hydrolysis method with co-extraction with hydrochloric acid followed by extraction with petroleum ether. Marker (^{89}Y) and total phosphorus in feed and ileal samples were conducted at the Department of Environmental Sciences, NMBU. Samples were completely digested in concentrated nitric acid (HNO_3) in an UltraCLAVE III (Milestone, Sorisole, Italy) at 260 °C for 15 min, and diluted with deionized water before analysis of Y and P by inductively coupled plasma mass spectroscopy using an Agilent 8800 Triple Quadrupole ICP-MS/MS (Agilent Technologies Inc., Santa Clara, USA) in oxygen reaction mode.

2.5. Enzyme activities, IgA concentration and total protein

Amylase, trypsin and lipase activity were measured in intestinal content from the proximal jejunum, whereas alkaline phosphatase (ALP), leucine aminopeptidase (LAP) and maltase activities were measured in epithelium from the same intestinal segment. Samples were prepared as described by de Nanclares et al. (2017) and stored at −80 °C until further analyses.

The amylase, trypsin, ALP, LAP and lipase activity were determined using commercial kits (Abcam: ab102523, ab102531, ab83369, ab234627; Sigma-Aldrich: MAK048) according to the manufacturer's protocols. The maltase activity was determined following a modified protocol described by Dahlqvist (1968). In the final step of the protocol, glucose concentration was measured using a commercial kit (EIAGLUC, ThermoFisher). Total protein concentration was measured according to the Quick Start™ Bradford Protein Assay protocol (Bio-Rad Laboratories). Absorbance was measured using a SpectraMax M2e Microplate Reader (Molecular Devices).

Quantitative measurement of immunoglobulin A (IgA) in jejunal tissue were conducted using Abcam's IgA Pig enzyme-linked immunosorbent assay (ELISA) kit (Pig IgA, Colorimetric, Abcam, USA, ab190536). Approximately 70 mg of tissue were homogenized using a bead mill homogenizer with a lysis buffer. Supernatant was collected and stored at −80 °C after centrifugation. Total protein was determined as described above, and samples were normalized for protein concentration prior to IgA analysis, which followed the manufacture's protocol for the assay.

2.6. Morphology and morphometry

Intestinal tissues from jejunum and ileum were collected, processed and analyzed as described in detail in Cruz et al. [24]. Briefly, digital images were captured from tissue sections routinely stained with hematoxylin and eosin. VH was measured by from the tip of the villus to the villus-crypt-junction. Crypt depth (CD) was measured from the villus-crypt junction to the deepest portion of the crypt, adjacent to the *tunica muscularis mucosae*. Between four and seventeen villus and crypt complexes were measured in each intestinal segment from each piglet. For each villus-crypt-complex, a VH:CD ratio was calculated.

2.7. RNA extraction, library construction and RNA sequencing

Total RNA was extracted from proximal jejunal and ileal tissue from 56 piglets (day zero, two, four and seven PW) following the RNeasy Plus Universal Kit's protocol (Qiagen). Total RNA concentration and quality were determined using NanoDrop TM 8000 spectrophotometer (Thermo Fisher Scientific), and Agilent 2100 Bioanalyzer (Agilent Technologies). High quality samples (RIN ≥ 7) were sent for sequencing at the Norwegian Sequencing Centre (http://www.sequencing.uio.no). 84 samples (42 from ileum and 42 from jejunum: six from day zero, and six from each dietary treatment on day two, four and seven PW) were used for RNA-seq library preparation. TruSeq™ Stranded total RNA-seq (Illumina) library kit was used for library preparation following manufacturer's protocols targeting the mRNAs using their polyA tail. Libraries were pooled together and sequenced over 7 lanes of HiSeq 4000 (Illumina) employing 150 bp, paired end (Supplementary data 1).

2.8. Data analysis of RNA sequencing data

Raw reads was cleaned using BBDuk v34.56 (Bushnell, 2014) to trim/remove low quality reads, adapter sequences and PhiX (Illumina spike-in) using the following parameters: ktrim = r, k = 23, mink = 11, hdist = 1, tbo, tpe, qtrim = r, trimq = 15, maq = 15, minlen = 36, forcetrimright = 149. Cleaned reads were aligned to the *Sus scrofa* ENSEMBL genome Sscrofa 11.1 release 98 using HISAT v2.1.0 (Kim, Langmead, & Salzberg, 2015) using default parameters. Fragments mapping to the known genes from release 98 was counted using featureCounts v1.4.6-p1 (Liao, Smyth, & Shi, 2014) and differential expression was calculated using DESeq2 v1.22.1 R package (Love, Huber, & Anders, 2014). Differential expression analysis was carried out for ileum and jejunum independently.

To investigate the transcriptomic changes PW and the differences between the treatment groups, significantly differentially expressed genes (DEGs) between timepoints were included in separate heatmaps for each of the dietary treatment groups. Hierarchical clustering was used to group genes with similar expression trends. Genes from each cluster were then extracted and included in search for enriched KEGG pathways using the gprofiler2 version 0.1.5 package in R (Kolberg & Raudvere, 2019) with g:SCS multiple testing correction method applying significance threshold of 0.05. KEGG pathway enrichment analysis was also performed for DEGs between piglets fed the yeast and control diet on day 7 PW.

2.9. Calculations and statistical analyses

Liver index was calculated as: (*Liver weight, kg / Body weight kg*) * 100. AID coefficients were calculated as described by Maynard and Loosli (1969). For digestibility and enzyme activity results, an inter quartile range (IQR) test were run and values outside the range of three times IQR excluded. Two piglets, one from each treatment at day 4 PW, were excluded from the AID dataset due to low yttrium values, which affected all their AID values. Results are presented as mean values with error bars.

Figures were made using the ggplot (Wickham, 2016) and Hmisc package (Harrell Jr, Dupont, & others, 2018) in Rstudio Inc, version 1.1.456. Statistical analysis was performed using the mixed procedure in the SAS® software, V.9.4 (SAS Inst. Inc., Cary, NC). Day zero samples were not included in the statistics. The following model was used for all parameters:

$Y_{ijklmn} = \mu + diet_i + s\_day_j + (diet \times s\_day)_{ij} + breed_k + litter_l + pen_m(diet_i) + e_n$, where Y is one observation on piglet n; μ is the intercept; $diet_i$ is the fixed dietary treatment effect (i = 1,2); s_day_j is the fixed effect of sampling day PW (j = 1:4); $diet_i \times s\_day_j$ is the fixed interaction between dietary treatment and sampling day; $breed_k$ is the fixed effect of the breed of the piglet (k :1 = LLD and k : 2 = LZD); $litter_l$ is the random effect of the l-th litter $\sim N(I \times \sigma^2_{litter})$ here I is an identity matrix of dimensions and σ^2_{litter} is the variance component for litter; $pen_m(diet_i)$ is the random effect of pen within diet each $N(0, I \times \sigma^2_{pen(diet)})$; and e_n is the error of the n piglet $N(0, I \times \sigma^2_{es\_day})$, i.e. assumed heterogeneous per sampling day.

A Pearson correlation matrix between the digestibility coefficients, enzyme activities, morphology, and pH parameters was made using the rcorr function in the Hmsic package v.4.1–1 (Harrell Jr et al., 2018) and was visualized with the corrplot function in the corrplot package v 0.84 (Wei & Simko, 2017).

3. Results

3.1. Growth performance

No health problems related to dietary treatments and no mortality were encountered during the experiment. Average daily gain (ADG) and ADFI did not differ between the treatments. ADG for the whole experimental period was 165 g/day and 163 g/day for control and yeast-fed piglets, respectively. ADFI was 287 g/day and 275 g/day for control and yeast-fed piglets, respectively. Liver index increased from day two to 14 W ($P < 0.001$) but did not differ between treatments ($P = 0.506$).

3.2. Fecal score and ileal DM

Fig. 1 shows the fecal score and ileal DM results. The fecal score increased (from approx. 1.2 to 2.5) for both treatments from day two to four PW. Average fecal scores from day four to seven for both treatments were above 2.5 (2.5 – 3), hence considered piglets being diarrheic. For the remainder of the experimental period, the fecal scores

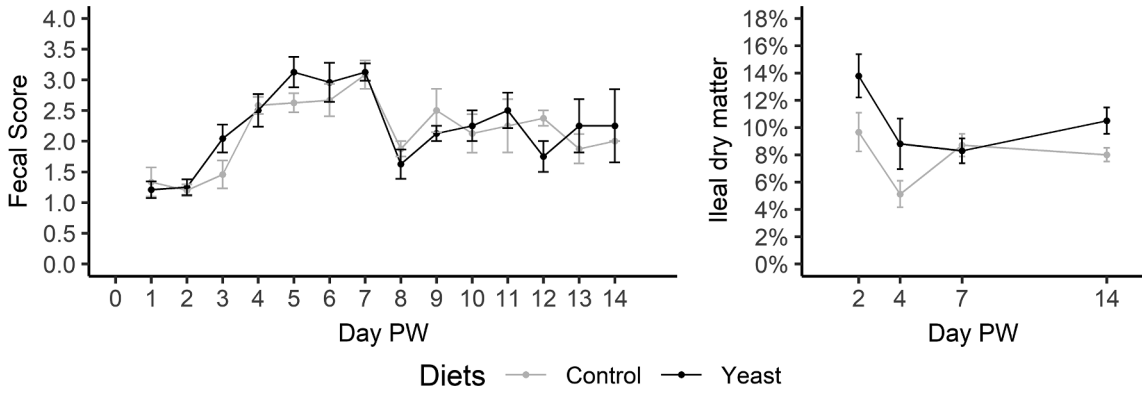

Fig. 1. Fecal score and dry matter content of ileal digesta of PW piglets fed the control diet or the yeast-based diet. Results are presented as mean values with error bars.

were < 2.5. Ileal DM showed changes consistent with fecal scoring. Ileal DM significantly decreased from day two to four PW and increased at day seven and 14 ($P = 0.019$). Piglets fed the yeast diet had significantly higher DM content in ileum compared with piglets fed the control diet ($P = 0.007$).

3.3. pH in intestinal segments

There was no significant effect of dietary treatment on pH measured in content from stomach ($P = 0.382$), duodenum ($P = 0.550$) or jejunum ($P = 0.857$). pH differed between sampling days ($P < 0.001$) in jejunum, where pH increased from 5.5 on day two to 6.2 on day four and 6.4 on day seven, before decreasing to 5.7 on day 14. There was a tendency for a sampling day effect in stomach pH ($P = 0.088$), but no sampling day effect was found in duodenum ($P = 0.468$).

3.4. Morphology

In jejunum, CD significantly differed between the PW sampling days ($P < 0.001$). The CD was lower on day two and four PW compared with day zero but increased from day four to day 14 PW (Fig. 2). No significant difference between sampling days was found for the jejunal VH ($P = 0.798$). The jejunal VH:CD ratio decreased from day two to 14 PW ($P < 0.001$). In ileum both VH ($P = 0.008$) and CD ($P < 0.001$) increased over time PW. No significant differences between dietary treatments and no significant interaction between diet and sampling day were found for any of these parameters.

3.5. Digestibility

The development of AID PW is shown in Fig. 3. Inclusion of yeast in the PW diet significantly improved AID of CP ($P = 0.033$) and tended to increase AID of phosphorus ($P = 0.089$). No significant effect of diet was found for AID of organic matter ($P = 0.239$), starch ($P = 0.652$) or fat ($P = 0.367$). The AID of organic matter tended to be affected by sampling day ($P = 0.063$). There was no significant interaction between diet and sampling day PW for the AID coefficients.

3.6. Enzyme activities

Development of lumen and brush border enzyme activities in the proximal jejunal mucosa is shown in Fig. 4. A clear increase in trypsin activity PW ($P = 0.001$) can be observed, whereas lipase activity decreased during the first days PW ($P = 0.012$). No differences were found for development of amylase activity PW ($P = 0.953$), and no effect of dietary treatment was found for any of the three enzyme activities measured in lumen of proximal jejunum (trypsin $P = 0.757$; lipase $P = 0.578$; amylase $P = 0.912$). Common for the development of maltase, ALP and LAP activities in the proximal jejunal mucosa, is an increase in activity during the first days PW, with a higher activity present in the yeast fed piglets, followed by a decrease in activity and similar activities between the dietary treatment groups. There was an effect ($P < 0.001$) of sampling day PW for all three brush border enzymes. Piglets fed the yeast diet had higher ($P = 0.014$) ALP activity than those fed the control diet, whereas maltase and LAP activity tended ($P < 0.1$) to be higher for the yeast diet. No interaction between diet and sampling day was found for any of the enzyme activity parameters.

3.7. IgA

IgA concentration showed similar pattern for both diets ($P = 0.920$) and differed between days PW ($P < 0.001$), with a decrease from day two to four PW followed by an increase in concentration over time (Fig. 5).

3.8. Correlations between parameters of GIT function

Fig. 6 shows a correlation plot of some of the individual measured parameters, including pH, enzyme activities, digestibility coefficients, ileal DM, VH and CD, and IgA. There were positive correlations between ileal DM and AID of organic matter ($r = 0.547$, $P < 0.001$), AID of CP ($r = 0.731$, $P < 0.001$), AID of phosphorus ($r = 0.534$, $P < 0.001$) and AID of crude fat ($r = 0.524$, $P < 0.001$). There were negative correlations between CD in jejunum and ileum and brush border enzymes maltase (jejunal CD: $r = -0.439$, $P < 0.001$; ileal CD: $r = -0.345$, $P = 0.003$), ALP (jejunal CD: $r = -0.404$, $P < 0.001$; ileal CD: $r = -0.412$, $P < 0.001$) and LAP (jejunal CD: $r = -0.545$, $P < 0.001$; ileal CD: $r = -0.565$, $P < 0.001$).

3.9. Gene expression

Using day zero as a baseline, Fig. 7 shows the number of DEGs in proximal jejunum at the different sampling days PW. The number of DEGs increased over time PW. In jejunum, the changes in DEGs were larger in the control than in the yeast piglets, at day four and seven. The number of DEGs at day four were 471 vs. 31 and on day seven 2855 vs. 83, for the control and yeast group, respectively.

Jejunal gene expression patterns over time for control piglets are shown in Fig. 8. The DEGs are divided in two cluster trends; a decreased relative expression PW (cluster 1) and an increased relative expression PW (cluster 2). The DEGs in cluster 1, which were downregulated with time PW, mainly enriched pathways related to environmental information processing and immune system processes. The DEGs in cluster 2 showed an increased expression pattern over time, and enriched pathways were mainly related to endocrine systems, metabolism and others such as genetic information processing (proteasome, protein processing in endoplasmic reticulum and ribosome).

Fewer DEGs were found between timepoints in the yeast group, in total 276 genes. The heatmap in Fig. 9 is also divided in two clusters, where metabolic pathways and protein digestion and absorption are enriched in the cluster with increasing expression trend PW, whereas DEGs in two enriched pathways related to the immune system; hematopoietic cell lineage and B cell receptor signaling pathway, have decreased expression patterns.

Few genes were significantly differentially expressed between dietary treatments on the specified sampling days. In jejunum on day seven, 476 DEGs were found between the control piglets and the piglets fed the yeast diet. Of these DEGs, 469 were upregulated in piglets fed yeast as compared with the control piglets. In total, 38 KEGG pathways were enriched ($P < 0.05$), i.e., upregulated in piglets fed yeast compared with control piglets on day seven (Fig. 10). Nine of these pathways were related to the immune system, and the most distinctly enriched pathway was intestinal immune network for IgA production, where 29% (12 out of 41) of the genes in this pathway were upregulated in the yeast group. Only seven genes were downregulated on day seven in jejunum of piglets fed yeast compared with control piglets (*COX20*, *DNAJC15*, *GDE1*, *MORC4*, *MRPS21*, *SYDE2* and *ZNF133*).

In ileum, fewer genes were differentially expressed (Table 3). In total, 2090 DEGs were found between any of the timepoints in the control group, with the largest difference being between day two and seven PW with 2038 DEGs. In the yeast fed piglets only 100 DEGs were found between timepoints.

In the ileum of control piglets two opposing cluster trends were identified; cluster 1 included DEGs with an overall increasing expression from day zero to two, followed by a decline below day zero level over time, and cluster 2 included genes with the opposite PW expression trend (Fig. 11). DEGs in cluster 1 enriched pathways involved in genetic information processing, and transport and catabolism (autophagy and mitophagy), along with the NOD-like and RIG-I-like receptor signaling pathways. In cluster 2, the DEGs were mainly enriched signaling pathways in environmental information processing and

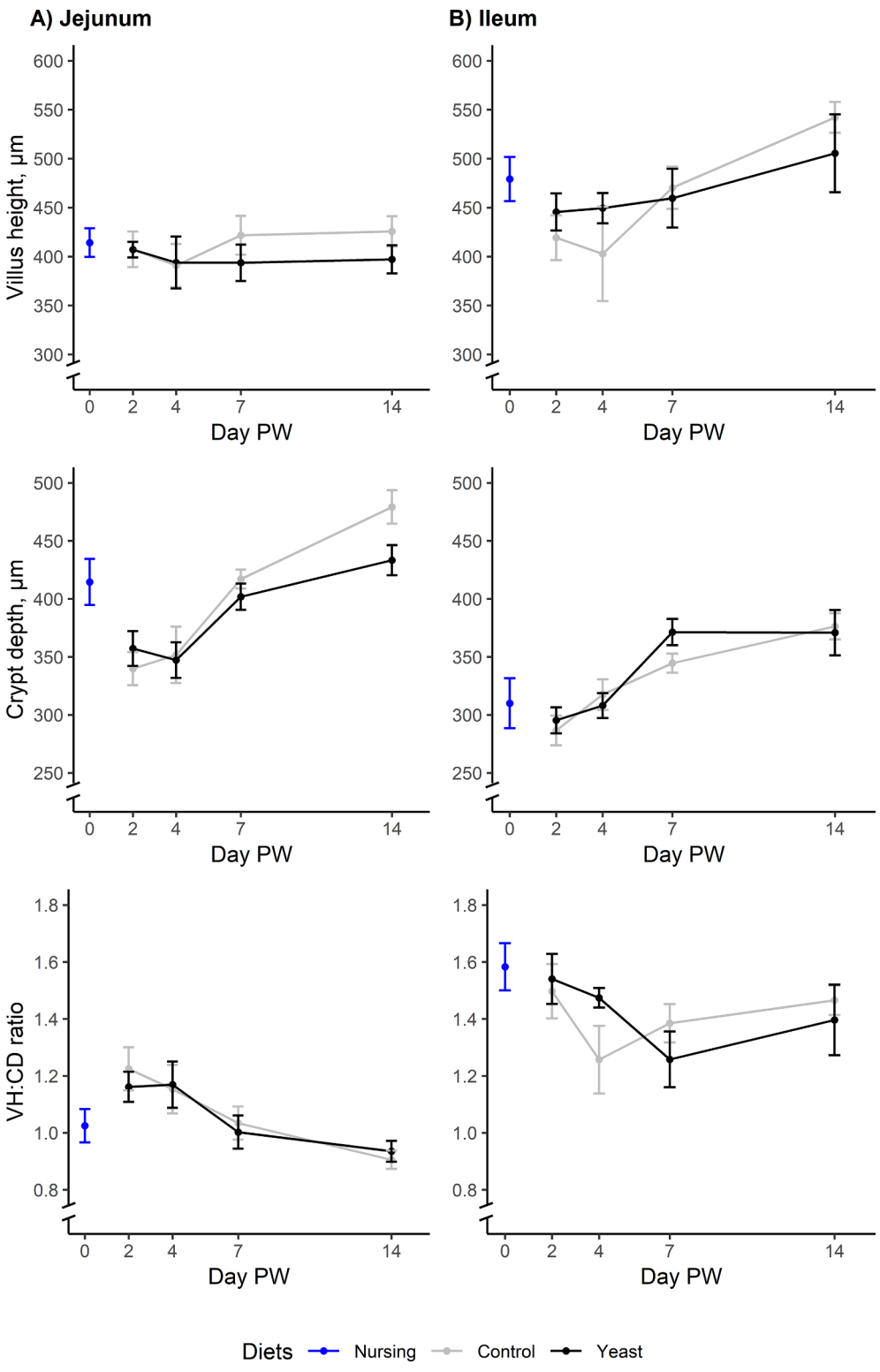

Fig. 2. Villus height (VH) and crypt depth (CD) in jejunum (A) and ileum (B) of PW piglets fed the control diet or the yeast-based diet. VH and CD at day zero (nursing) are indicated in blue. Results are presented as mean values with error bars.

pathways in different organismal systems.

In the ileum of yeast-fed piglets, three cluster trends were found, but in cluster 1 there were too few genes to determine pathway enrichment, and in cluster 3 only one human disease pathway was enriched. Cluster 2 included DEGs with an overall increasing PW relative expression trend. In cluster 2 four pathways were enriched; thyroid hormone synthesis, and three pathways related to protective host response and cytokine signaling networks (Fig. 12).

4. Discussion

In this study, we have investigated the development and maturation of gastrointestinal function and health in PW piglets fed two diets; a control diet or a diet with 14.6% inclusion of *C. jadinii* yeast. The focus

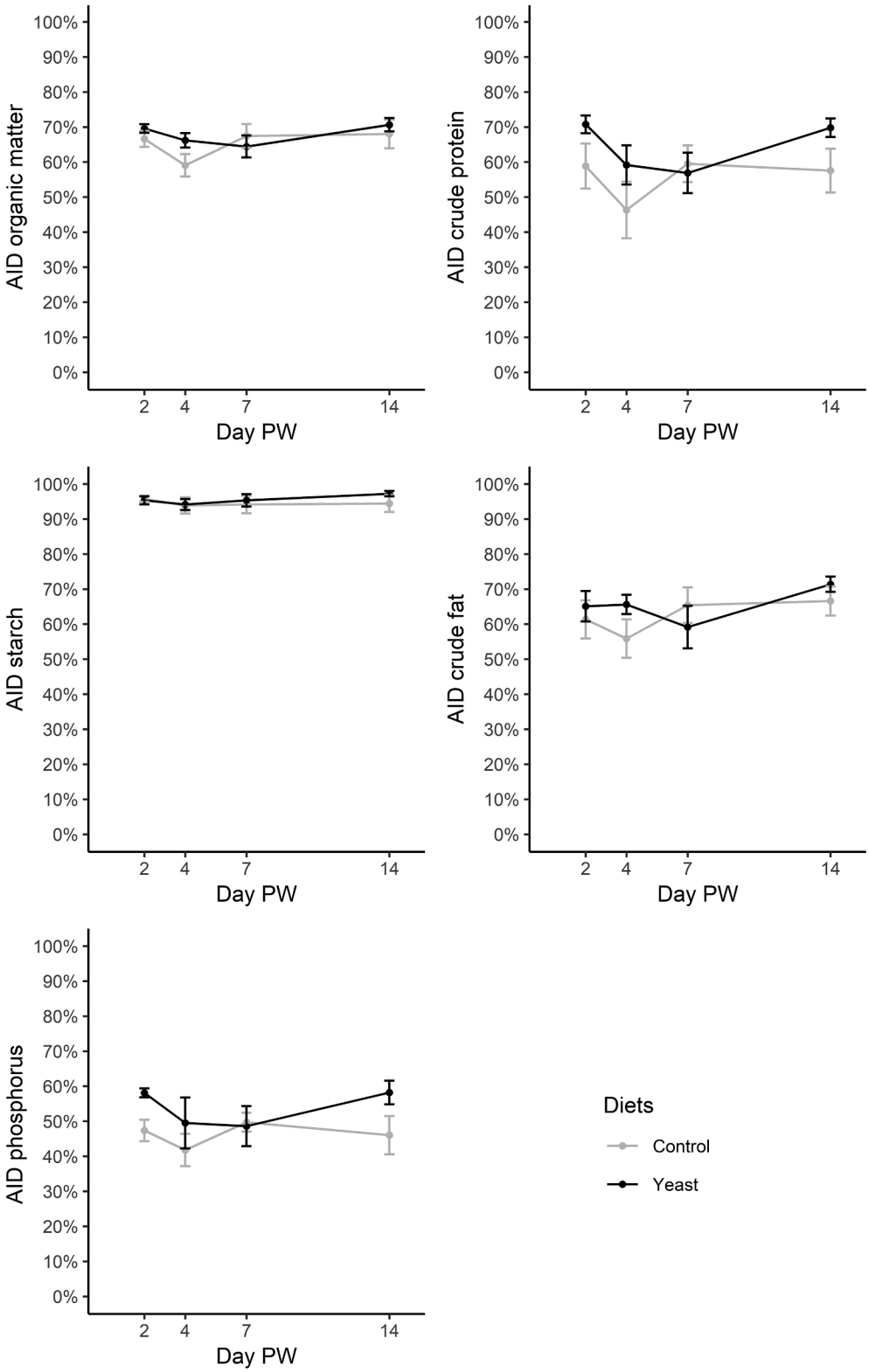

Fig. 3. Apparent ileal digestibility (AID) coefficients of organic matter, crude protein, starch, phosphorus and fat in PW piglets fed the control diet or the yeast-based diet. Results are presented as mean values with error bars.

of the study was on general intestinal function (i.e. pH, AID and enzyme activities), and the health-related parameters fecal score and intestinal morphometry. In addition, we have investigated the jejunal and ileal transcriptome at different time-points during the first week after weaning. In accordance with our previous experiment (Cruz et al., 2019), no differences in ADG or ADFI were observed between dietary treatments in the present experiment, confirming that yeast potentially can replace 40% of CP from the main protein sources traditionally used in piglet diets in Norway.

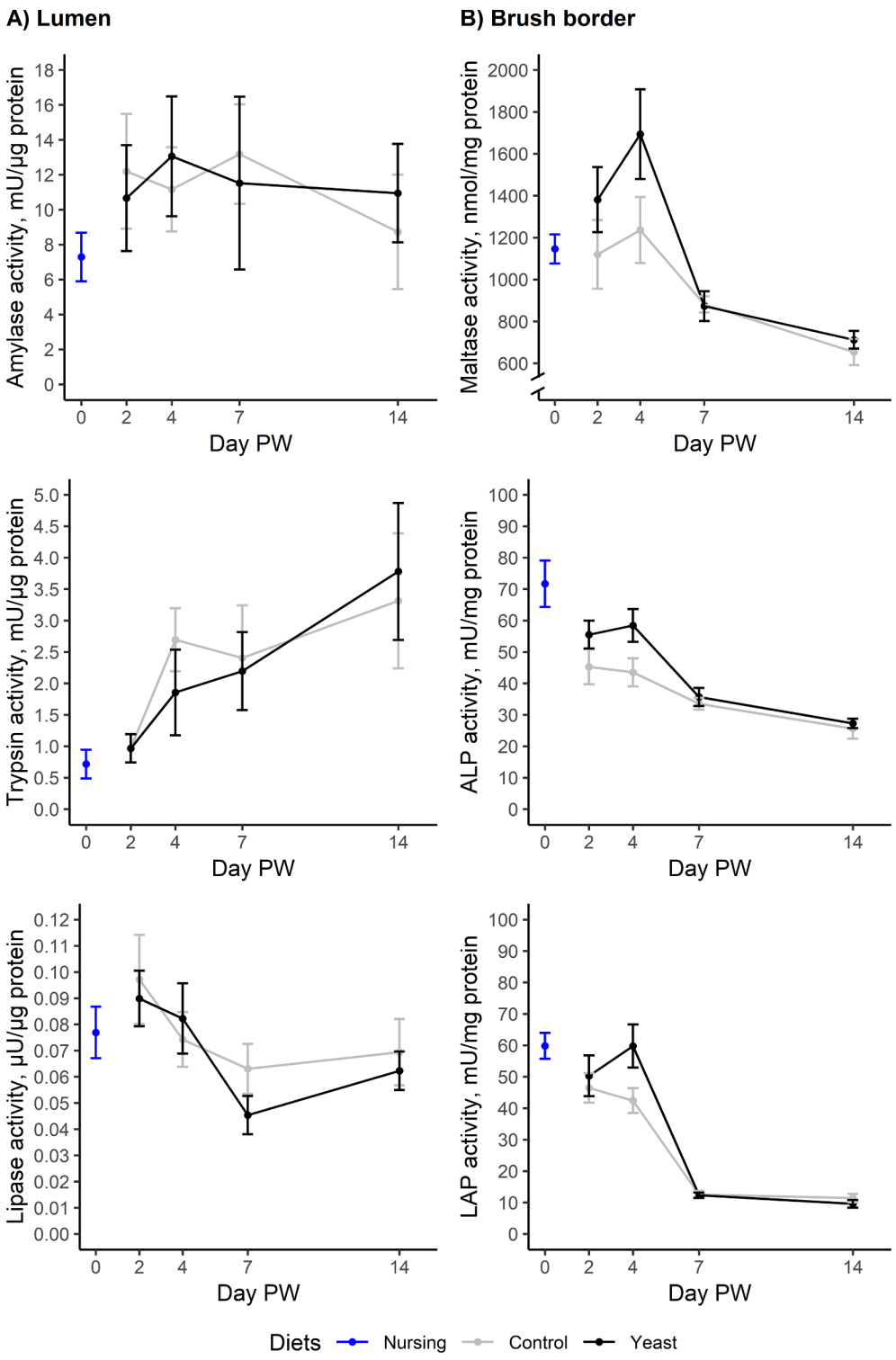

Fig. 4. Lumen (A) and brush border (B) enzyme activities in proximal jejunum of PW piglets fed the control diet or the yeast-based diet. Enzyme activities at day zero (nursing) is indicated in blue. All activities are expressed per µg or mg of protein. Results are presented as mean values with error bars.

Development of general GIT function and health PW

The increased fecal score and diarrhea incidence between day four and seven PW in both treatments are consistent with the cohort study by Madec, Bridoux, Bounaix, and Jestin (1998), that reported PW diarrhea from around day four to nine PW for piglets at approximately the same age. The ileum DM results also corresponded with the observed fecal score pattern. High gastric pH is commonly reported PW and a failure of acidification in the stomach might increase the risk for PW diarrhea (Heo et al., 2013). In all gut segments measured, pH was numerically highest in the first week PW, which correlated positively with increased fecal score and decreased ileal DM. Because pH in the GIT varies with time postprandial, and because the time from feeding to sampling varied among piglets in the present experiment, pH in the different GIT segments showed large variation. Infection of *Escherichia coli* is a major factor causing PW diarrhea, which weakens mucosal and

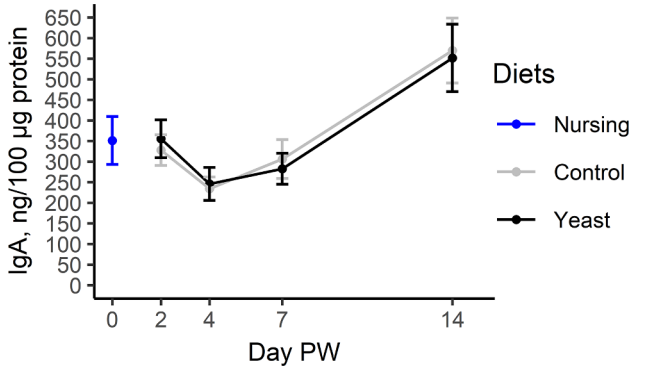

Fig. 5. IgA concentration in proximal jejunal tissue in PW piglets fed the control diet or the yeast-based diet. IgA concentration at day zero (nursing) is indicated in blue. Results are presented as mean values with error bars.

cellular barrier function and increases secretion of water and electrolytes into the small intestinal lumen (Heo et al., 2013). Although, even when not infected, Nabuurs, Hoogendoorn, and Van Zijderveld (1994) reported decreased net absorption of fluids on day four and seven PW compared with suckling piglets, which corresponds to our ileal DM results. No difference in diarrhea was seen between the yeast and the control treatment in our experiment. In our previous experiment (Cruz et al., 2019), we also reported no difference in fecal score between the diets in the second week, but we found improved fecal score with the similar yeast diet in the fourth week PW, and higher DM in feces on day seven and 21 PW.

The piglets digestive capacity increases as it grows older (Manners, 1976). However, abrupt weaning temporarily disturbs GIT maturation. These temporary PW changes were divided into two periods by Montagne et al. (2007). First five days PW constitutes an acute period with deterioration of the GIT structure and function. This initial phase is followed by a more progressive adaptative and maturational phase. The piglets in the study by Montagne et al. (2007) was weaned at 21 days of age, fasted for 48 h and tube fed, controlling the feed intake and simulating a critical weaning situation with no energy intake the first few days. Although our piglets were one week older at weaning and not restricted from feed intake PW, we observed a reduction in some of the functional parameters at the first sampling time-points PW, with an initial recovery on day 14 PW. Even though not significant, AID coefficients for all nutrients showed a numerical decrease in the early PW period at day four and/or day seven. All digestibility coefficients were positively correlated with the DM of ileal digesta, except for starch digestibility. Trypsin activity in jejunal content increased with day PW, which was also reported by Makkink et al. (1994) for piglets weaned at the same age. The reduction in lipase activity until day seven followed by an increased activity has also been reported by several authors (Cera, Mahan, & Reinhart, 1990; Marion et al., 2003) The decrease in lipase activity PW can explain the PW decrease in AID of fat.

Low feed intake during the first days PW is considered the main cause for the functional and structural changes occurring in the acute PW phase (Jayaraman & Nyachoti, 2017; Montagne et al., 2007). In the nursing period, lactose is an important energy source for the intestinal epithelial cells (Spreeuwenberg et al., 2001), and deprivation of luminal substrates for mucosal epithelial cell growth is a consequence of weaning (Montagne et al., 2007). A reduction in villus length equates to a reduction in the mature enterocyte population and affects functional and absorptive capacity. In jejunum, VH remained relatively stable PW, whereas an increase in VH from day two to seven PW was observed in ileum. These results are not consistent with Montagne et al. (2007). These investigators reported that the acute effect of weaning was primarily seen in the proximal part of the small intestine, while changes in ileum occurred five days PW in piglets weaned at 21 days of age and fasted for two days PW. In our study, ileal DM was positively correlated with ileal VH:CD ratio, where piglets with higher villi or lower crypts

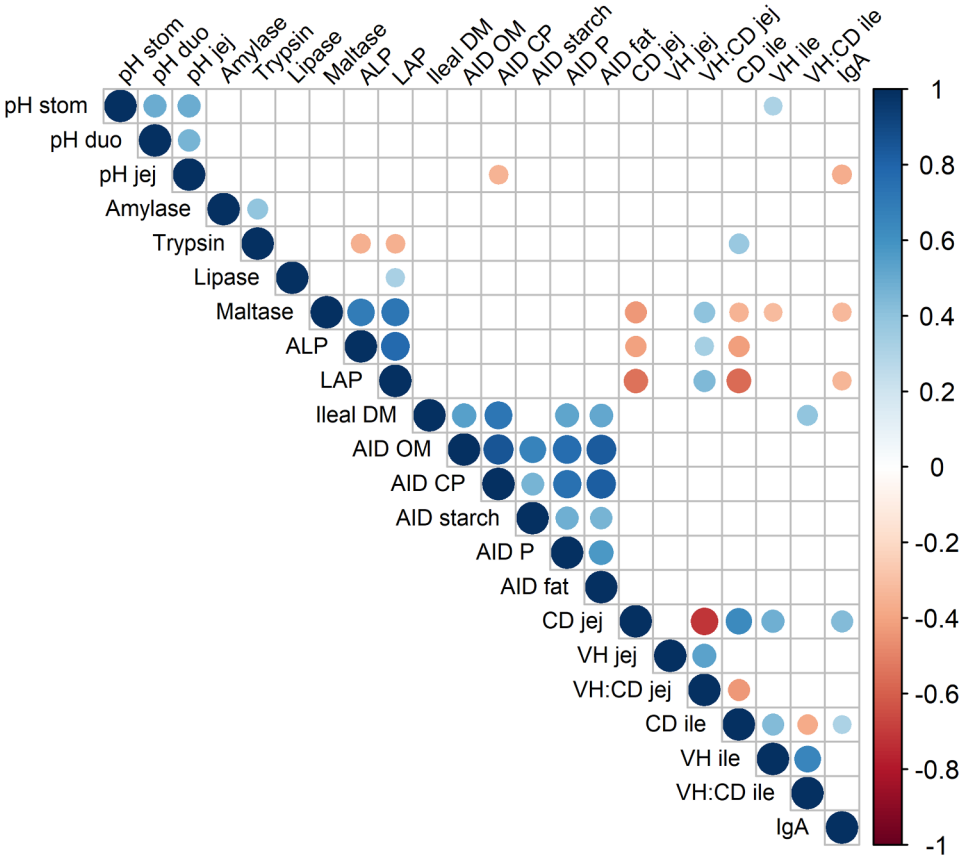

Fig. 6. Correlogram including digestibility coefficients, enzyme activities, pH and morphology parameters. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. Only significant correlations ($P < 0.01$) are visualized.

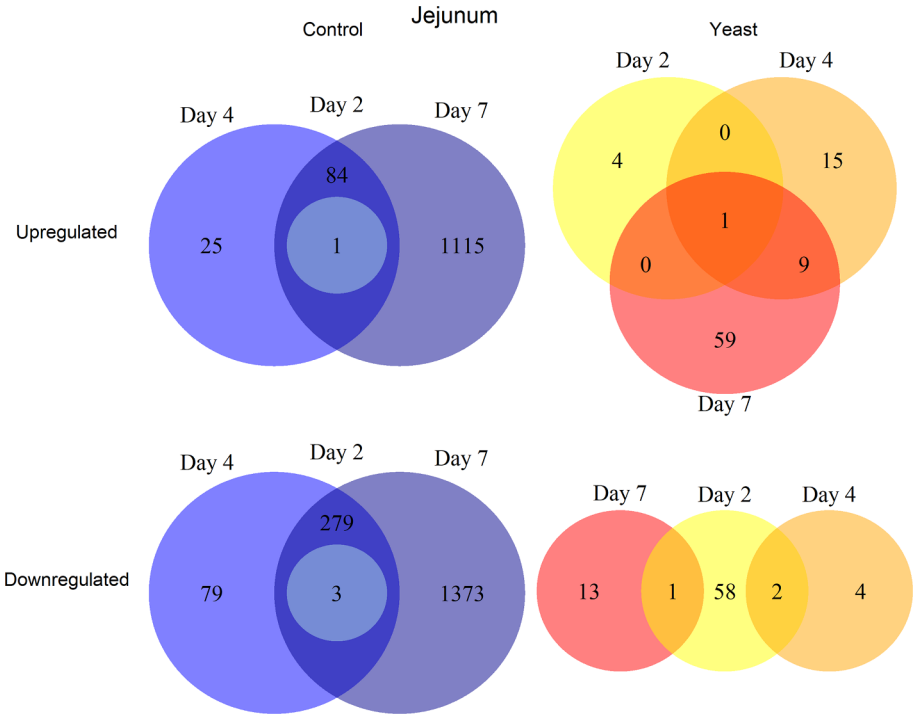

Fig. 7. Venn diagram showing number of DEGs at the different sampling days PW compared with day zero in jejunum.

had less watery content in ileum. These findings support the overall results that functional parameters deteriorated early PW. Shorter villus and deeper crypts have fewer absorptive and more secretory cells, and is associated with PW diarrhea (Nabuurs, Hoogendoorn, Van der Molen, & Van Osta, 1993). CD in both jejunum and ileum increased from day two to seven PW, and correlates negatively with the decreased jejunal activity of the brush border enzymes maltase, ALP and LAP. Increase in CD implies an increased rate of enterocyte production. The continual renewal of the intestinal epithelial cells takes place by differentiation along the crypt-villus axis (CVA) expanding from stem cells located near the base of the crypts (Yang, Xiong, & Yin, 2013). It is reported that enzyme activity, including ALP, aminopeptidase N and sucrase, increases during maturation along the CVA (Fan, Stoll, Jiang, & Burrin, 2001). An increased rate of enterocyte production and migration, indicated by an elongation of the crypts, might lead to more immature cells with lower digestive enzyme activities and nutrient uptake capacity. Yang, Wang, Xiong, and Yin (2016), reported a global change in protein expression during maturation along the CVA. They found an increased mucosal energy metabolism, which is crucial for digestion and absorption processes.

Few differences were found in PW development of GIT function and structure between the control and the yeast fed piglets. However, an overall higher CP digestibility was recorded for the yeast group, but in accordance with our previous study (Cruz et al., 2019), yeast did not affect trypsin activity. Moreover, in our previous study (Cruz et al., 2019), we reported increased ATTD of CP, crude fat and phosphorus in piglets fed a similar yeast-based diet. The increased ALP activity PW in the yeast fed piglets, suggest an improved PW intestinal health. Intestinal ALP is essential for gut homeostasis, and loss of intestinal ALP expression is associated with increased intestinal inflammation and dysbiosis (Fawley & Gourlay, 2016). Enhanced intestinal ALP activity in ileum has been reported by Xiong et al. (2015) when feeding a PW diet containing a yeast product with fermentation products and hydrolyzed yeast cell walls from *Saccharomyces cerevisiae*.

Development of small intestinal gene expression PW

In this study, we have focused on patterns of pathway enrichment PW and on differences between the two feeding groups, rather than on differential expression patterns of singular genes. We investigated the PW gene expression pathway patterns separately for the two feeding groups, followed by a discussion about the differences between diets. It is clear that weaning causes activation of stress responses, alteration in immune functions and metabolism, but the differences vary with time PW (Tao & Xu, 2013). Several studies indicate that weaning before three weeks of age induces more transcriptome alterations than weaning at a later age (Inoue et al., 2015; Li, Rajput, Fernandez, & Moeser, 2018). In our study, piglets were weaned at four weeks of age, making them more developed and robust than many of the piglet studies reported in the literature. However, we found clear changes in gene expression patterns PW.

The DEGs detected in jejunum of the control group PW were mainly classified into three important KEGG categories; nutrient metabolism, environmental information processing and immunoregulation. DEGs in enriched pathways related to metabolism, endocrine systems such as insulin signaling, and some of the cellular processes such as endocytosis and lysosome, showed increased expression in jejunum over time PW. These results indicate an overall increased functionality of the jejunum over time PW, which is contrary to findings reported by Wang et al. (2008) and Bomba et al. (2014). These investigators reported a decline in gene expression related to nutrient metabolism in the first week PW. However, in the study by Wang et al. (2008), piglets were younger, weaned at 21 days of age, and in both studies the piglets were fed a corn- and soybean meal based diet, contrary to our grain based diet. While metabolism related pathways improved in jejunum PW in the present study, a declining expression trend was found for DEGs involved in pathways connected to environmental information processing, immune system and other cellular processes such as cellular senescence, phagosome and regulation of actin cytoskeleton.

Whereas most of the enriched pathways in the immune system were downregulated in jejunum of control piglets over time PW, three enriched pathways (Cytosolic DNA-sensing pathway, NOD-like receptor

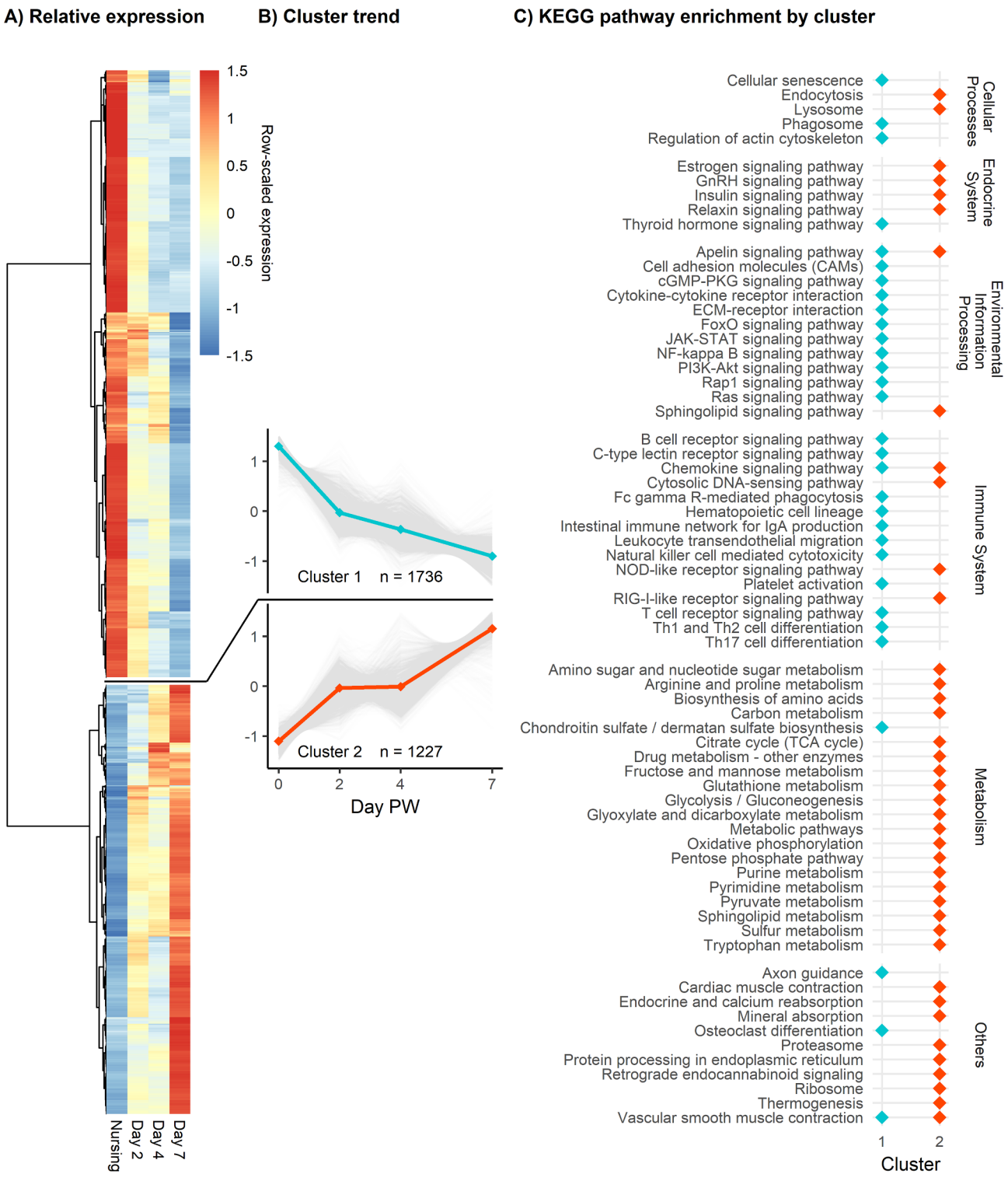

Fig. 8. Jejunal gene expression pattern over time for control piglets. A) Heatmap showing transcriptional changes in jejunum over time for piglets fed control diet. Heatmap includes 2 963 genes differentially expressed (adjusted P < 0.05) between any of the timepoints. Transcript abundance was row-scaled to highlight changes in the expression of individual genes, B) Genes with similar expression patterns across the timepoints were separated into two main clusters using Ward's method. Trend lines are based on mean scaled values in each cluster. C) KEGG pathway enrichment analysis was run on each cluster. Each point represents a significantly enriched pathway (P < 0.05). Pathway parents which includes less than five pathways are gathered and assigned as "Others". Pathways related to human disease were excluded in the figure but can be found in the supplementary data together with an overview of DEGs in pathways (Supplementary data 2).

signaling pathway and RIG-I-like receptor signaling pathway) responsible for pathogen detection and generating innate immune responses were upregulated. The diet change at weaning causes ingestion of new bacteria and feed antigens, which challenges and trains the intestinal tissue. An increased expression of genes in the endocytosis pathway was found in jejunum of the control group PW. By contrast, Wijtten, van der Meulen, and Verstegen (2011), suggest that a PW reduction in endocytosis rate is a strategy to prevent antigen overload excessively activating the immune system. A complex regulation of the GIT immune system is important to prevent excessive activation and

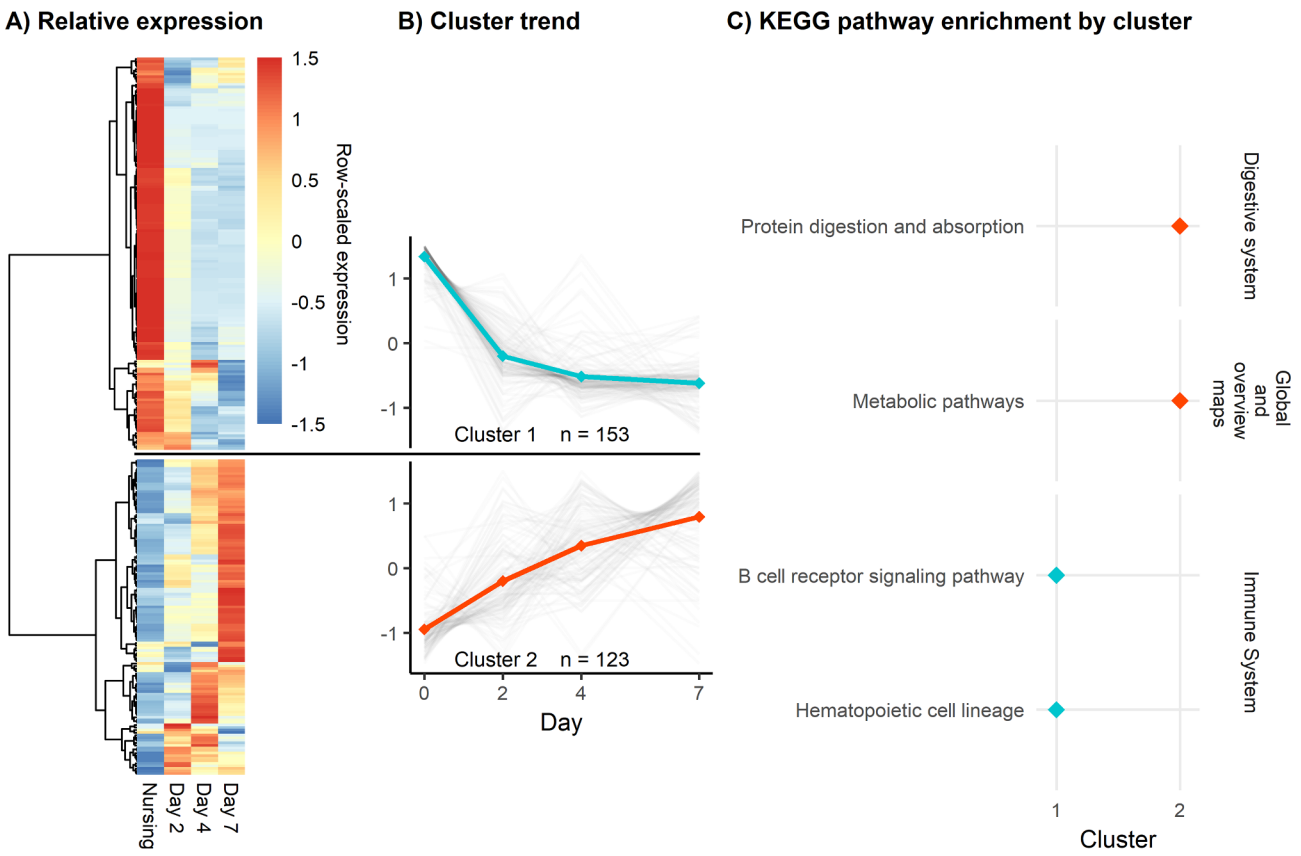

Fig. 9. Jejunal gene expression patterns over time for the yeast-fed piglets. A) Heatmap showing transcriptional changes PW in jejunum for piglets fed yeast diet. Heatmap includes 276 genes differentially expressed (adjusted P < 0.05) between any of the timepoints. Transcript abundance was row-scaled to highlight changes in the expression of individual genes, B) Genes with similar expression patterns across the timepoints were separated into two clusters using Ward's method. Three clusters were selected as a minimum number to represent data, and to ensure large enough groups to run pathway enrichment analysis. Trend lines are based on mean scaled values in each cluster. C) KEGG pathway enrichment analysis was run on each cluster. Each point represents a significantly enriched pathway (P < 0.05). Pathways related to human disease were excluded in the figure but can be found in the supplementary data together with an overview of DEGs in pathways (Supplementary data 2).

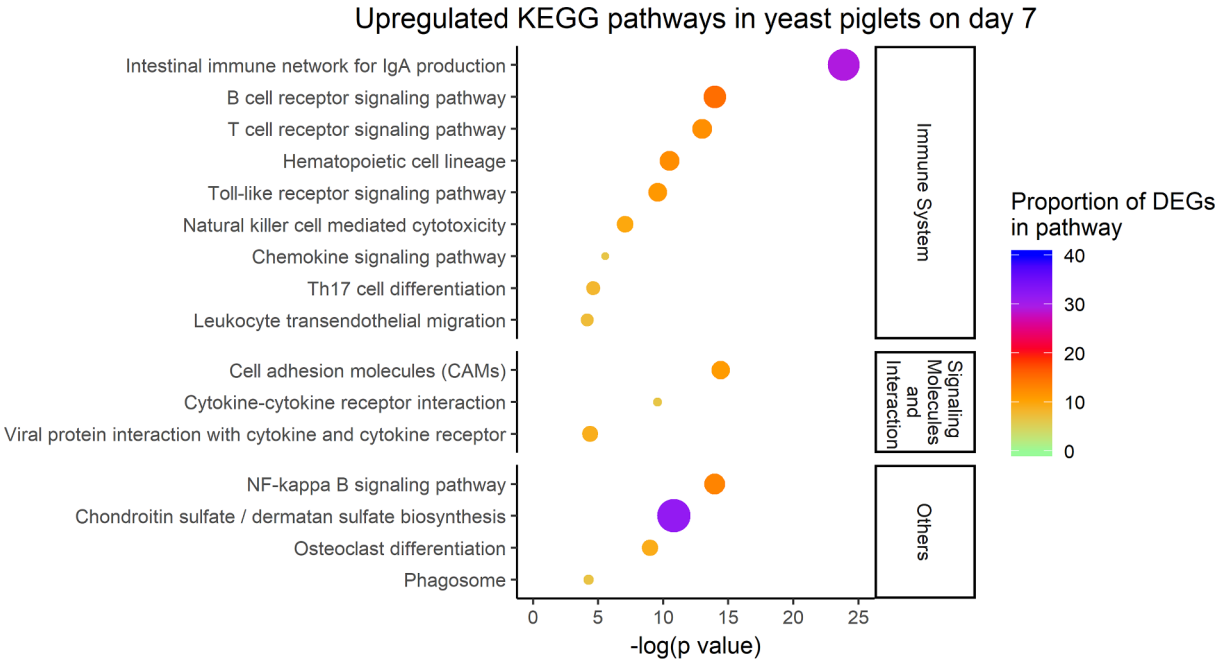

Fig. 10. KEGG pathways upregulated in jejunum on day seven for yeast group compared with control. Pathways are sorted by -log(p value) within pathway parent. High -log(p value) equals low p value. Point color and size represent proportion of DEGs found in pathway. Pathways related to human disease were excluded in the figure but can be found in the supplementary data together with an overview of DEGs in pathways (Supplementary data 3).

Table 3
Number of DEGs in ileum.

	Treatment vs D0		
	Control	Yeast	Yeast vs Control
Upregulated			
Day 2	21	0	0
Day 4	0	1	27
Day 7	28	41	0
Downregulated			
Day 2	1	0	3
Day 4	0	1	0
Day 7	2	2	0

inflammation in response to antigenic substances. Mechanisms to suppress immune activation when challenged with new microorganisms and antigens such as at birth and weaning, are important for an optimal and long-term maturation of the immune system (Moeser et al., 2017). On the other hand, in case of a breach in the epithelial barrier, the GIT immune system must be able to respond rapidly, and this balance of control and reactiveness is critical for optimal GIT health (Moeser et al., 2017).

In ileal tissue from control piglets, DEGs in the NOD-like receptor signaling pathway, RIG-I-like receptor signaling pathway, autophagy pathway and mitophagy pathway were upregulated on day two PW compared with pre-weaning levels followed by decreased expression to below pre-weaning level on day seven. Autophagy is activated in response to stress and starvation, which is relevant in the acute PW period, and is the cell's way of removing dysfunctional components. A reduction in VH in ileum was seen between day zero and day two, which corresponds to the upregulation of autophagy in this tissue, while the expression of the pathway is decreased in the following regeneration period. The activation of ubiquitin mediated proteolysis on day 2 with following decline also confirms the increased turnover of damaged tissue in the acute PW phase. An activation of NOD-and RIG-I-like receptor signaling pathway along with apoptosis was also found by Bomba et al. (2014), five days PW in ileum of piglets weaned at day 28. Correspondingly, an opposite trend was seen for ileal genes in the enriched pathways focal adhesion, ECM-receptor interaction and PI3K-Akt signaling, which all play essential roles in cell maintenance, motility and proliferation. Weaning induced enhancement of apoptosis and inhibition of epithelial cell proliferation have been previously reported in jejunal tissue (Zhu et al., 2014).

Comparing PW gene expression between diets

Changes in gene expression PW corresponds with the known functional and structural changes occurring in the intestinal tissue.

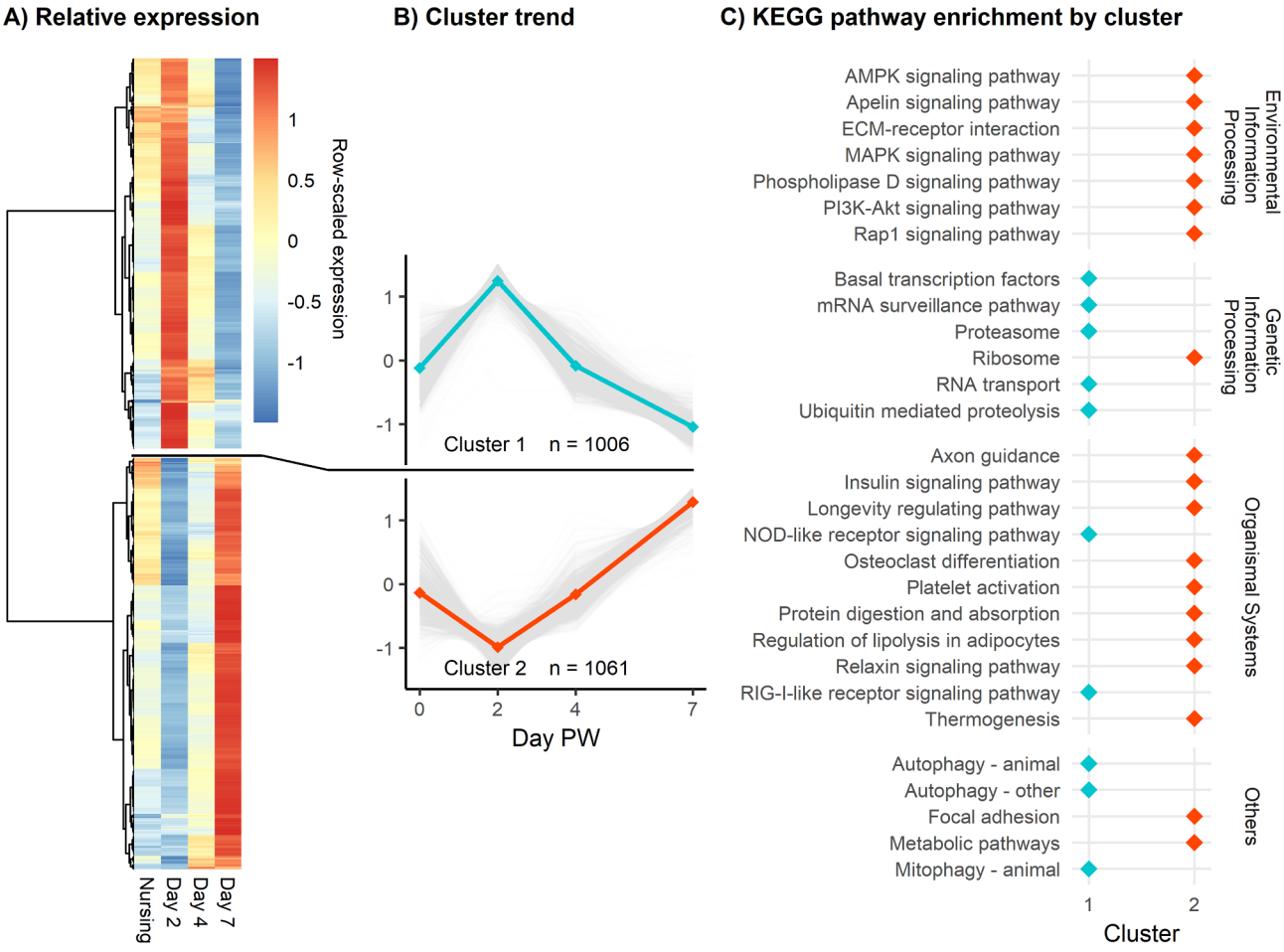

Fig. 11. Ileal gene expression patterns over time in ileum for control piglets. A) Heatmap showing transcriptional changes in ileum over time for piglets fed control diet. Heatmap includes 2067 genes differentially expressed (adjusted $P < 0.05$) between any of the timepoints. Transcript was row-scaled to highlight changes in the expression of individual genes, B) Genes with similar expression patterns across the timepoints were separated into two main clusters using Ward's method. Trend lines are based on mean scaled values in each cluster. C) KEGG pathway enrichment analysis was run on each cluster. Each point represents a significantly enriched pathway ($P < 0.05$). Pathways related to human disease were excluded in the figure but can be found in the supplementary data together with an overview of DEGs in pathways (Supplementary data 2).

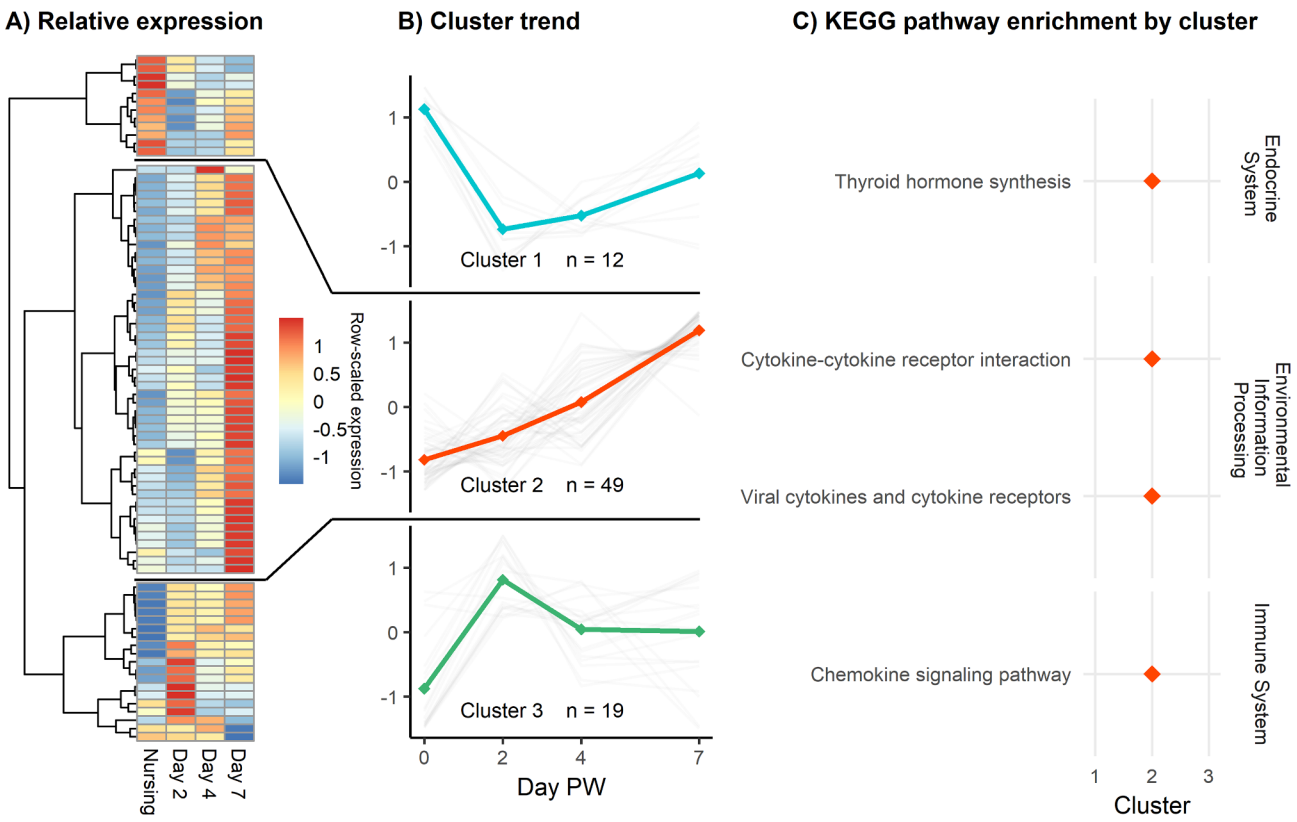

Fig. 12. Ileal gene expression patterns over time in ileum for yeast-fed piglets. A) Heatmap showing transcriptional changes in ileum over time for piglets fed yeast diet. Heatmap includes 80 genes differentially expressed (adjusted P < 0.05) between any of the timepoints. Transcript abundance was row-scaled to highlight changes in the expression of individual genes, B) Genes with similar expression patterns across the timepoints were separated into three main clusters using Ward's method. Cluster 1 contains too few DEGs to determine pathway enrichment. Trend lines are based on mean scaled values in each cluster. C) KEGG pathway enrichment analysis was run on each cluster. Each point represents a significantly enriched pathway (P < 0.05). Pathways related to human disease were excluded in the figure but can be found in the supplementary data together with an overview of DEGs in pathways (Supplementary data 2).

Interestingly, these PW changes in gene expression observed in the control group were not seen to the same extent in the yeast group, suggesting that piglets fed the yeast diet were more tolerant of weaning stress. When plotting the PW expression trend in the yeast-fed piglets for the DEGs in the control group, the overall trend for cluster 1 differs (Figure Supplementary figure 1). For the piglets fed yeast, the overall expression of these immune-related genes declined to a larger extent than for the piglets fed the control from day zero to day two PW, but the immunosuppression seemed to recover faster. This suggests an earlier maturation of the GIT immune system in the yeast fed piglets. On the other hand, there were few DEGs between the two treatments on day two and four PW, whereas significant differences were found on day seven. The genes upregulated in the yeast group on day seven, as compared to the control group, were mostly associated with immune system pathways. Several of the immune system associated enriched pathways in the yeast group on day seven were the same pathways that declined with time PW in the control group. Of the 469 upregulated DEGs in the yeast group compared with control on day seven, 414 were the same genes that were significantly downregulated in the control group compared with day zero. This finding provides evidence that there are different gene expression patterns PW between the two feeding groups. It also suggests that the DEGs on day seven are not caused by a PW upregulation in the yeast group as such, but a reduced expression PW in the control group, as these genes were not found to be significantly enriched with time PW in the yeast group.

The NF-κB signaling pathway, which was enriched in the jejunum of yeast-fed piglets compared with control on day seven, consists of dimers involved in the regulation of immunity, inflammation and cell survival. NF-κB activation has also been shown to regulate intestinal homeostasis through controlling apoptosis (Siggers & Hackam, 2011). Another enriched pathway was the Toll-like receptor signaling pathway, which is responsible for detecting pathogens by responding to the membrane component of Gram-positive or Gram-negative bacteria and for activating innate immune responses. Key genes in this pathway, the transmembrane receptors *TLR1*, *TLR2* and *TLR4* were upregulated in the jejunum of yeast piglets on day seven compared with control. *TLR4* is involved in both defense against pathogens and maintaining tolerance to commensal bacteria (Frosali et al., 2015). In the epithelium, *TLR4* activation is important in recruiting defense against pathogens in case of epithelial injury, and absence might result in severe mucosal damage (Frosali et al., 2015). Similar to *TLR4*, *TLR2* also mediates pro-inflammatory signaling, and activation might suppress mucosal inflammation and enhance barrier integrity (Siggers & Hackam, 2011).

The upregulation of the intestinal immune network for IgA production pathway on day seven in the yeast-fed piglets was not confirmed by quantification of IgA in the jejunal tissue. IgA immunoglobulins are constantly secreted from the tissue and the tissue concentration might therefore not reflect the production of the protein. Improved IgA secretion in jejunum mucosa PW when feeding live or superfine powder of *S. cerevisiae* was reported by Zhy et al. (2017). However, these piglets were weaned at 14 days of age. Moreover, Zhy and coworkers found no effect of heat-killed whole yeast, which is more similar to our yeast product. Although there was no difference in jejunal IgA concentration between the dietary treatments when measured by ELISA, the PW development of IgA concentration matches the PW expression trend in the control group for genes in the intestinal immune network for IgA production pathway. IgA is an important antibody, working as a first-line barrier by limiting antigen transition from the

intestinal lumen to blood and controlling the intestinal microbiota (Pabst, 2012).

Fatty acid-binding protein 6 (*FABP6*), was not involved in any of our significant pathways, but is important in the transportation and metabolism of long-chain fatty acids in the intestine and has been proposed as a potential biomarker for intestinal barrier dysfunction (Celi, Verlhac, Calvo, Schmeisser, & Kluenter, 2019). Intestinal FABP is highly present in mature enterocytes and high concentrations in plasma are correlated with intestinal mucosa damage (Niewold, Meinen, & Van der Meulen, 2004). It is therefore relevant to mention the expression trend of this gene. The jejunal *FABP6* expression decreased for both diets from day zero to day two (Figure Supplementary figure 2B). In the control piglets, the expression remained low, whereas it increased to above day 0 level for the yeast-fed piglets with time PW. The increased expression might be a result of rebuilding after intestinal damage, suggesting that higher expression levels indicate, as previously discussed, an enhanced rebuilding phase in the yeast-fed piglets.

To summarize and conclude, the results showed that yeast is a suitable protein ingredient for PW piglets. For the gut functional parameters, the differences were more significant for sampling day than for dietary treatment. However, inclusion of 14.6% of *C. jadinii* yeast improved PW AID of protein and brush border enzyme activities early PW. Reduction of brush border enzyme activity correlated with an increase in CD indicating a rebuilding process of the intestinal villi, which was also supported by the transcriptomics results where pathways involved in these processes showed the same trends. Although less evident in the yeast-fed group, the PW jejunal gene expression pattern in both dietary treatments showed a decreased PW relative expression of genes in pathways related to immune system processes. There was also an increased PW relative expression of DEGs in metabolic pathways, especially in the control group. The largest differences between the two dietary treatments were seen on day seven PW, where several pathways related to the immune system were upregulated in the yeast-fed piglets compared with control. Thus, there seems to be an PW immunosuppression in jejunum of the control piglets. In ileum, the PW transcriptional changes were also less evident in the yeast-fed piglets compared with control piglets, and the transcriptomic trends match the functional results well, with an early PW critical phase followed by a rebuilding phase. These results indicate that feeding *C. jadinii* yeast can improve PW gut homeostasis and give more robust piglets.

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CRediT authorship contribution statement

Ingrid Marie Håkenåsen: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration. **Margareth Øverland:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Ragnhild Ånestad:** Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Caroline Piercey Åkesson:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Arvind Y.M. Sundaram:** Formal analysis, Writing - original draft, Writing - review & editing. **Charles McLean Press:** Conceptualization, Investigation, Writing - review & editing. **Liv Torunn Mydland:** Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104118.

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