Body weight of newborn and suckling piglets affects their intestinal gene expression

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Abstract
Modern hyperprolific sows must deal with large litters (16–20 piglets) which reduce piglet birthweight with a concomitant increase in the proportion of small and intrauterine growth retarded piglets. However, larger litters do not only have a greater variation of piglet weights, but also a greater variation in colostrum and milk consumption within the litter. To further understand the impact that body weight has on piglets, the present study aimed to evaluate the degree of physiological weakness of the smallest piglets at birth and during the suckling period (20 d) compared to their middle-weight littermates through their jejunal gene expression. At birth, light piglets showed a downregulation of genes related to immune response (FAXDC2, HSPB1, PPARGC1a), antioxidant enzymes (SOD2m), digestive enzymes (ANPEP, IDO1, Sl), and nutrient transporter (SLC39A4) (P < 0.05) but also a tendency for a higher mRNA expression of GBP1 (inflammatory regulator) and HSD11β1 (stress hormone) genes compared to their heavier littermates (P < 0.10). Excluding HSD11β1 gene, all these intestinal gene expression differences initially observed at birth between light and middle-weight piglets were stabilized at the end of the suckling period, when others appeared. Genes involved in barrier function (CLDN1), pro-inflammatory response (CXCL2, IL6, IDO1), and stress hormone signaling (HSD11β1) over-expressed compared to their middle-weight littermates (P < 0.05). In conclusion, at birth and at the end of suckling period, light body weight piglets seem to have a compromised gene expression and therefore impaired nutrient absorption, immune and stress responses compared to their heavier littermates.

Lay Summary
Under hyperprolific situations, piglets must deal with a reduced birthweight and a severe sibling competition for nutrients. Therefore, body weight newborn and suckling piglets may also have physiological disadvantages compared to their middle-weight littermates. To further understand the impact that body weight has on piglets, the present study aimed to evaluate the degree of physiological weakness of the smallest piglets at birth and during the suckling period (20 d) compared to their middle-weight littermates through their jejunal gene expression. Newborn light piglets downregulated genes related to immunity, antioxidant, and digestive activities, but also a tendency to upregulate other genes related to inflammation and stress responses. At the end of the suckling period, those gene expression differences vanished while others appear. Light weight piglets showed lower expression of genes involved in barrier function, inflammation, and stress responses compared to their middle-weight littermates at birth. At birth and at the end of lactation, light piglets seem to have a compromised intestinal gene expression for nutrient absorption, immune and stress responses compared to their heavier littermates.

Key words: birthweight, gene expression, hyperprolific sows, neonatal pigs, sucking pigs

Abbreviations: ACTB, actin beta; ANPEP, alanyl aminopeptidase membrane; ALPI, alkaline phosphatase intestinal; B2M, beta-2-microglobulin; BW, body weight; CCL, chemokine ligand; CCK, cholecystokinin; CLDN, claudins; CRHR1, corticotropin releasing hormone receptor; DAO, d-amino acid oxidase; FDR, false discovery rate; FAXDC2, fatty acid hydroxylase domain containing; GCG, glucagon; GPX, glutathione peroxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GBP1, guanylate binding protein; HSP, heat shock protein; HNMT, histamine N-methyltransferase; HSD11β1, hydroxysteroid (11-beta) dehydrogenase 1; IDO1, indoleamine 2,3-dioxygenase; IFG, insulin-like growth factor; IFN, interferon gamma; IL, interleukin; IUGR, intrauterine growth restricted; MT, metallothionein; MUC, mucins; NR, nuclear receptor; OCLN, occludin; PYY, peptide YY; PPARGC1a, peroxisome proliferator activated receptor gamma; REG3G, regenerating family member 3 gamma; SLC, solute carrier; SI, sucrase-isomaltase; SOD, superoxide dismutase; TBP, TATA-box binding protein; TLR, toll-like receptor; TGF-β1, transforming growth factor beta 1; TFF, Trefoil factor; TNF, tumor necrosis factor; ZO, Zonula occludens

Introduction
Intensive pressure for a genetic selection of hyper prolificacy, based on sow reproductive performance (litter size; 16–20 total piglets born), has led to a reduction in the average piglet birthweight as well as an increase in the variability of birthweights within the litter (Peltoniemi et al., 2021). Thus, the proportion of small piglets in large litters as well as the frequency of intrauterine growth-restricted (IUGR) piglets has notably increased (Matheson et al., 2018; Ward et al., 2020). The initial birthweight variation within litter may reveal that in hyperprolific genetic lines, sows may not be able to ensure a satisfactory nutrition for the uneven litter, resulting in an...
intense pre- and post-natal sibling competition to acquire limited nutrients and a high mortality risk for weaker offspring (Ward et al., 2020). Additionally, most of these IUGR and light birthweight piglets may also show long-term negative effects on their organ structure, postnatal growth, and feed efficiency (Ji et al., 2017). Thus, modern commercial farms need to cull IUGR piglets because there is no effective nutritional or management support for their growth or survival during the suckling and post-weaning periods (Kraeling and Webel, 2015). Nonetheless, light birthweight piglets could show compensatory growth if given optimal management and dietary conditions (van Barneveld and Hewitt, 2016; Viott et al., 2018; Farmer and Edwards, 2021). Fetal or neonatal programming occurs during the gestation period leading to changes in gene transcription resulting in altered activities of metabolic pathways and homeostatic control processes (Burdge et al., 2007). Insufficient maternal nutrition during gestation can result in permanent fetal programming alterations (Kwon and Kim, 2017). In order to further understand the impact that the intrauterine fetal overcrowding has on piglets, the present study aimed to evaluate the changes in jejunal gene expression, as a key indicator of the intestinal function and development. Therefore, the gene expression of light-weight piglets at birth and at the end of the lactation period was compared with that of their middle-weight littermates, both born to hyperprolific sows under commercial conditions.

Materials and Methods

All animal experimentation procedures were approved by the Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH27888M2) in compliance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010).

Animals and housing

At 113 d post-mating (± 1.27), the litter characteristics of 80 litters (1,542 total born neonates) from 80 hyperprolific sows (DanBred hybrid line; Landrace × Yorkshire, parity 4.78 ± 1.69) were recorded. Sows and their litters were followed until weaning (21.6 ± 1.27 d). During the gestation and lactation periods, sows were kept under commercial feeding and management conditions. Gestation feed intake was individually controlled using a feeding electronic station (model Intec-Mac, Mannebeck, Schuttorf, Germany), whereas during lactation a 24 h recording was performed as described in Villagómez-Estrada et al., 2021. Briefly, a light piglet was defined as having a birth weight between 600 and 800 g (belonging to the lower quartile) at birth and a BW between 2,500 and 3,800 g on day 20, whereas a middle-weight littermate had a BW within the average of the litter at birth (1,200–1,300 g) and at the end of lactation (4,000–5,100 g). Piglets with no obvious characteristics of disease or injury were selected. Piglet sex was not considered as sampling indicator. Individual BW was examined and if it matched into the light or middle BW category, piglet was selected to obtain samples.

Selected piglets were removed from the sow, approximately 150 min (±15) after the start of farrowing, and euthanized by an overdose of sodium pentobarbital (Dolethal, Vetoquinol, S.A., Madrid, Spain). Samples for gene expression analysis were collected as described in Villagómez-Estrada et al., 2021. Briefly, at the mid-point of the jejunum, one sample of approximately 1.5 cm was collected, rinsed in PBS solution, snap frozen in 1 mL of RNA later (Deltalab, Barcelona, Spain), and stored at −80 °C until processing.

Gene expression analysis

Gene expression in jejunal tissue was performed on 56 genes using an Open Array Real-Time PRC Platform (Applied Biosystems, Waltham, MA). All genes were involved in multiple physiological functions closely related to intestinal health and were selected based on the literature and grouped according to their main function as follows: 1) barrier function genes such as the family members of Claudins (CLDN), mucins (MUC), zonula occludens (ZO), trefoil factor (TFF), and occludin (OCLN) (CLDN1, CLDN4, CLDN15, MUC2, MUC13, ZO1, TFF3, and OCLN); 2) genes involved in immune and inflammatory responses such as pattern recognition receptors, cytokines, chemokines, and stress proteins [toll-like receptor (TLR2, TLR4); interleukin (IL-1β, IL-6, IL-8, IL10, IL17A, IL22); interferon gamma (IFNG, IFNG1); tumor necrosis factor (TNF); transforming growth factor beta 1 (TGF-β1); chemokine ligand (CCL20, CXCL2); heat shock protein (HSPB1, HSPA4); regenerating family member 3 gamma (REG3G); peroxisome...
| Gene      | Name                     | Primer Forward (5'-3')                  | Primer Reverse (5'-3')                  | Probe (5'-3')                           |
|-----------|--------------------------|----------------------------------------|----------------------------------------|-----------------------------------------|
| CLDN1     | Claudin-1                | CTTCGACTCCTTGCTGAAATCTGA               | CTCCATGACCTTCATACACTCTCAT              | ACAGCACTTGGCAAGC                       |
| CLDN4     | Claudin-4                | CCTCCGTCGGTCTGGTCCTCAA                 | GAGGCAAGAGCCAGCAGA                    | CTTTGGTCGACCTTGG                      |
| CLDN15    | Claudin-15               | GCTATCTCCTGTAGCTCCTCAA                 | GGGACTCTCAACTCTGTTG                   | ACTTCTCAGCCCCCTTGA                    |
| MUC2      | Mucin 2                  | AAGGACGACACTCCTACCTACCTCAC             | GAGGCCAGGGAGAAATAGAC                  | CATGGTCAAGCCCC                        |
| MUC13     | Mucin 13                 | CAGTGGAGTTGGCTGAAAAAC                 | ATCAAGTCCGTCGGATCTATTTG              | TCCCTCATTAAAGACATCAC                  |
| ZO1       | Zonula occludens 1       | GCTATGTCGAGAATCCTGCGAAAAA             | TGGATCTTTCATTGCTCCATACAC              | TCCCACTTTTACACTTAC                    |
| TFF3      | Trefoil factor 3         | AGAACCTGCCGGTACCAT                   | CACACTGTTGGCGGAGAC                    | AGGCCAGAAGCTTCT                      |
| OCLN      | Occludin                 | CAGGATGCAAGCCCTCCAGATG                | CAGGCAATAGGAGTGAGCT                   | TGACATGACAGCATGC                      |
| TLR2      | Toll-like receptor 2     | CTCTCTTGGGCTGCTCTGTGA                 | AAAGAACGAGGTAGGCTTGG                  | AGACAAATACCTCCCA                      |
| TLR4      | Toll-like receptor 4     | CATCCCCACATCGTCAAGATCAT              | TCAATTGTCGATTTCCATCTCTTAGG            | ACAGAATACCCCTCCCA                      |
| IL1B      | Interleukin 1 beta       | GGTTGAAGTTGGCTGAAAAAC                 | ACAGGATCAGCTATC                      | AGATATACCCCTCCCA                      |
| IL6       | Interleukin 6             | CCAATCTGGGCTTCAATCAGGAG              | ACAGGCTGAGCTATC                      | AGATATACCCCTCCCA                      |
| IL8       | Interleukin 8             | GGAAATGAGTGCTTGGAGAGGT                | GAGATGGTGTGTGTGTGTG                   | TACGATATTTTTGAGAGAACACT               |
| IL10      | Interleukin 10            | TTGAGGCTGCGGGGCT                     | GAAGCTGTCATGTAAAGCAC                   | AACAAGGGAAGGGCGCT                     |
| IL17A     | Interleukin 17            | CACAGACTGCCCCTGACATTAT                | ATGTCCCAATATCCATGAGTTG                | AGCTAAGGCAAGTGGCGTC                    |
| IL22      | Interleukin 22            | TTGCTGGCAACACTCTGATAGATGCC            | GAGTGGTCTGATCCGTAAGTGTGATGGTGA        | CATGGCAGCATCCCTTCCCC                   |
| IFNγ      | Interferon gamma          | TGACCTTGTGGTTTTTCTGGGTTCCT            | CAGGGCTGATGAGATTTGGGTGA               | TGTTCAAGGACATTTTTATTTAT              |
| IFNγR1    | Interferon gamma receptor 1 | CATGTTGACACAAATCTTGTGGGTCA         | CAGTATGACGCGTGGAAATATGTC             | ATATATATACCCCCTCAACTCCCA              |
| TNF       | Tumor necrosis factor alpha | CACACGCTCCTTCCATGCTAC                  | CAGGGCTGATGAGATTTGGGTGA               | TGTTCAAGGCCATATGTA                    |
| TGFB1     | Transforming growth factor beta 1 | NGCGGACGCTCTCAGTCTG                    | CAGATGCAATGGAGACCTCAAG                | AGCAGCTCAGCATGTA                      |
| CCL20     | Chemokine (C-C motif) ligand 20 | CATGCTTCCTACCCACTGAGTATTTGGG        | CAGGGCTGATGAGATTTGGGTGA               | ATATATATACCCCCTCAACTCCCA              |
| CXCL2     | Chemokine (C-X-C motif)   | GACATGCTCTCACTCCACTGAGTATTTGGG        | CAGGGCTGATGAGATTTGGGTGA               | ATATATATACCCCCTCAACTCCCA              |
| HSPB1     | Heat shock protein 27     | GGAGGATGACAGGTGACAGA                  | GAGATGGTGTGTGTGTGTG                   | TACGATATTTTTGAGAGAACACT               |
| HSPA4     | Heat shock protein 70     | TCAATATTGCTGCTGCTGCTG                | GAGATGGAGTTGGGTGGGTG                   | TACGATATTTTTGAGAGAACACT               |
| REG3G     | Regenerating-islet derived protein 3 | TGCTCTGAGTCTCTGCTTCAA                 | GAGATGGAGTTGGGTGGGTG                   | TACGATATTTTTGAGAGAACACT               |
| PPARG C1A | Peroxisome proliferative activated receptor gamma, coactivator 1 alpha | CTCTGGAGATCTGACGGGCTA                  | TGAGAAGGCCTAAAGAGTTAT                 | ACCCCCAACTTCTTC                      |
| FAXDC2    | Fatty acid hydrolyase domain containing 2 | CATGCTGCAACACTCACTCATAGTT            | GAGGATGCTGTTGTGTCCTTGTA               | TGTTCAAGGACAGCAAG                     |
| GBP1      | Guanylate binding protein 1 | AGAATCCCATACGAGAACTCAGGAGT    | CAGGATACGAGTGCAGGCGGTATTA            | TCAAGCTTAAAGAGAGTACAGC                |
| GPX2      | Glutathione peroxidase 2  | CAACTATTTGGGGAATCAGGAG               | GGGATAAGGTGGCGCAGATGG                | AGACTGGACAGACTCC                      |
| SOD2      | Superoxide dismutase      | CAGGGTCTGGCTGTTTCA                    | CAGGGCTGATGAGATTTGGGTGA               | CATGGCAGCATCCCTTCCCC                   |
| ALPL      | Intestinal alkaline phoshatase | ATGCTTCTCTTCTTTGTGGGCTACA             | GAGATGGAGTTGGGTGGGTG                   | TACGATATTTTTGAGAGAACACT               |
| S1        | Sucrase-isomaltase       | CGAACCCTTCTCTTCTGATGTTGTTGCA         | GAGATGGAGTTGGGTGGGTG                   | TACGATATTTTTGAGAGAACACT               |
| DAO1      | Diamine oxidase          | GGAACCAAGAGCCACTTCAACTACTCTC         | TGGGGAATCCAGGAGCCAG                    | CCGGACCGATCCACTGGAA                    |
| HNMT      | Histamine N-methyltransferase | TGTTGACACATGGCGGCTGAAACAT            | ACTTATATGCTGCTGCTGCTG                 | ACCAAAGTCAACAGACCT                   |
| ANPEP     | Aminopeptidase-N          | AGGGCAAGCTGCAAAAGAGTGG                | GTCGAAAGCTGAGAGGTGGGCAGT              | AGCAGCTCAGCATGTA                      |
| IDO1      | Indoleamine 2,3 dioxygenase | TTGGGAAATTTGGAGAAAGAGG               | CCGGAAATGAGAGAGATATCC                 | CAGCAGTGGCAGCCTAC                      |
| Gene     | Name                                           | Primer Forward (5'-3')                          | Primer Reverse (5'-3')                          | Probe (5'-3')                                      |
|---------|------------------------------------------------|-------------------------------------------------|-------------------------------------------------|--------------------------------------------------|
| GCG     | Glucagon                                       | AGCCGTGCCCAGGATTTT                              | CATCGTGACGTTTGCAATG                              | CACCAAGAGGAACAGAA                                  |
| CCK     | Cholecystokinin                                | CAGCAGGCTCAGCAACCAC                           | AATCCATCCAGCCTAATGTC                              | CAGCAGACAAATAAGTG                                 |
| IGF1R   | Insulin-like growth factor 1 receptor          | CCGACGCGGGAACAAC                              | TCAGGAAAGGACAAGAGACCAAA                           | CTACGTGAGATCCGCCA                                 |
| PYY     | Peptide tyrosine tyrosine                      | CAGAGTGATGGGAAACGTACA                          | CCTCTCGGACCAGCTGAC                               | CAAACTGCTCCTCCCTGAA                               |
| SLC5A1  | Solute carrier family 5 (sodium/glucose       | GCCCATCTTTCTCTTTACTGACA                        | TCCACCTTCCAGAAGAACAC                             | TTTATACGGATACCTTGCAGAC                           |
| SLC16A1 | Monocarboxylate transporter 1                  | CTTTGTTGACCTCAGAGATCTCT                     | CCAGTATGTGATATTAATGTACCTCCGCCG-                 | CCACACTTNTAGGTGTC                                |
| SLC7A8  | Solute carrier family 7 (amino acid transport| TGTCCCTTACGTGACTGCAATGT                        | GACAGGGCGACGGAAATG                               | CTGTAATTTTGAGAGAA                                 |
| SLC15A1 | Solute carrier family 15 (oligopeptide        | GTTTATCCCTTGAGCATTCTCTTCTMIN                  | AGTGCTCTTATCATTGACTAGTAAGAA                     | TCCAGAATGTTGAGTACAAAT                            |
| SLC13A1 | Solute carrier family 13 (sodium/sulfate      | GTGACCTTCACCAACATTGTACCTCT                     | ATCCAAAGTTGAGTACGAAT                            | ATTTCAAATATGCGCTACCC                              |
| SLC11A2 | Solute carrier family 11 (proton-coupled      | GTCTTTGCGCAGAGCTTTTTT                         | ACCACGCCCTCCTTGTAGA                              | CCAACCAGACGGTGT                                  |
| SLC30A1 | Solute carrier family 30 (zinc transporter)   | AATTGGGACGCGAGATCCA                           | TCTCTGATAAGATCCTACACTTG                          | AAAAGTCACAGAATGTC                                 |
| SLC39A4 | Solute carrier family 39 (zinc transporter)   | ATCTTTGGCCTTTCTCTCTT                         | GCCACCCGACACCTTTAG                               | CTGTCATACCGACTAGTCA                                |
| MT1A    | Metallothionein 1A                             | TGAATCCGCTGTCTCTCTCT                          | CAGAGGACGCAGCTCTCTT                              | ACGGTCAAAACCTGAGA                                 |
| CRHR1   | Corticotropin releasing hormone receptor 1     | CAGGGCCCATGATTGGG                             | CCGAGTTGCTGTGACGATG                              | CTGATCAATTTACTTCC                                 |
| NR3C1   | Glucocorticoid receptor                        | GGAATAACCCAGATTTCAGGAAC                      | CCAAGAAACACTCATTGAAATCTG                        | TGCAAATAGACCTCCT                                 |
| HSD11B1 | Hydroxysteroid (11-beta) dehydrogenase 1       | GTCACTAGAAAGACACTCCTCAGAGAGTTG                | GCGAAGGTCTATCGCTACATC                           | TCTCTGACACACTAGTGAAT                            |
| B2M     | Beta-2-microglobulin                           | TCACCTCTAAACGCTTGGGATCA                       | CGGTTATGCTGTGCTCAGTCC                          | ACGCAGACATCTGATGATA                               |
| GAPDH   | Glyceraldehyde-phosphate-dehydrogenase         | TCGTGTCAAGCTATTTCTCTGGTA                      | TCCCTGCTGTGCTCTTGCT                            | AATTGGTCTACAGCAACAG                              |
| TBP     | TATA-box binding protein                       | CAGAATGATCAAACGGGAATTGT                      | CTGCTGTGACTTTAGCACCCTGTTA                       | TTTTGTGCTGTGAAAAGTTGTA                            |
proliferator activated receptor gamma (PPARGC1a); fatty acid hydroxylase domain containing (FAXDC2) and guanylate binding protein (GBP1); 3) antioxidant enzymes genes (glutathione peroxidase, GPX2; superoxide dismutase, SOD2); 4) digestive enzymes and hormones genes involved in the digestion and metabolism processes [alkaline phosphatase intestinal (ALPI); sucrase-isomaltase (SI); d-amino-acid oxidase (DAO1); histamine N-methyltransferase (HNMT); alanyl aminopeptidase membrane (ANPEP); indoleamine 2,3-dioxigenase (IDO1); glucagon (GCG); cholecystokinin (CCK); insulin-like growth factor (IGF1R); and peptide YY (PYY)]; 5) nutrient transport coding genes ([solute carrier family (SLC5A1, SLC16A1, SLC7A8, SLC15A1, SLC13A1, SLC11A2, SLC30A1, SLC39A4) and Metallothionein (MT1A)]; and 6) stress response genes [corticotropin releasing hormone receptor (CRHR1); nuclear receptor (NR3C1); and hydroxysteroid (11-beta) dehydrogenase (HSD11B1)]. Four housekeeping genes were used to calculate the relative values for gene data [actin beta (ACTB); beta-2-microglobulin (B2M); glyceraldehyde-3-phosphate dehydrogenase (GAPDH); and TATA-box binding protein (TBP)]. The RNA gene expression analysis was performed according to the methodology described in Villagómez-Estrada et al., (2021). Briefly, from 50 mg of frozen jejunum tissue, the RNA was obtained using the RiboPure kit (Ambion, Austin, TX) and following the manufacturer’s instruction. The quality and quantity of RNA was assessed with a NanoDropND-1000 spectrophotometer (NanoDrop products, Wilmington, DE), whereas the RNA integrity was checked with Agilent Bioanalyzer-2100 equipment (Agilent Technologies, Santa Clara, CA). Reverse transcription of approximately 1 μg of total RNA to single-stranded cDNA in a final volume of 20 μL was performed using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) and random hexamer primers. The thermal cycler conditions applied were as follows: 25 °C 10 min; 37 °C 120 min; 85 °C 5 min; and 4 °C hold. Description of primers used are shown in Table 1. Gene expression analysis was performed in duplicates for each sample. Data were collected and analyzed using the ThermoFisher Cloud software 1.0 (Applied Biosystems) applying the 2-ΔΔCT method for relative quantification and using the sample with the lowest expression as a calibrator. The maximum cycle relative threshold was adjusted at 26, amplification score < 1.240, quantification cycle confidence > 0.8, and the maximum standard deviation between duplicates was set at < 0.38.

Statistical analysis

Data of gene expression were analyzed with one-way ANOVA considering BW piglet category as a main factor. The effect of sow was not considered within the statistical model. Two separate analyses were performed, at birth and at the end of the lactation period. Gene expression data were subjected to a logarithmic transformation to get closer to the Gaussian distribution. The Benjamini and Hochberg false discovery rate (FDR) multiple testing correction was also performed using the p.adjust function of R software v.3.4.3 (Benjamini and Hochberg, 1995). All statistical analyses were performed using R software and Bioconductor software (Gentleman et al., 2004). Significance was declared at a probability P ≤ 0.05, and tendencies were considered when P-value was between >0.05 and <0.10 using Tukey adjust.

Table 2. Reproductive performance and growth of sow and piglets during the studied period

| Item                      | Mean | SD  |
|---------------------------|------|-----|
| Sows, n                   | 80   | –   |
| Sow BW, kg                | 7.45 | 1.22|
| Gestation (d 35)          | 248.3| 36.97|
| Gestation (d 110)         | 270.9| 28.80|
| Weaning (d 24)            | 259.7| 33.32|
| Average daily feed intake gestation, kg | 2.33 | 0.09 |
| Average daily feed intake lactation, kg  | 7.45 | 1.22 |
| Reproductive parameters   |      |     |
| At birth                  |      |     |
| Total born pigs¹, n       | 19.3 | 4.02|
| Pigs born alive, n        | 16.1 | 3.44|
| Born alive litter birth weight, kg | 21.0 | 3.90 |
| Born alive pig weight, kg | 1.3  | 0.20|
| At weaning                |      |     |
| Total weaned pigs, n      | 14.2 | 1.69|
| Litter weaning weight, kg | 74.7 | 14.63|
| Weaned pig mean BW, kg    | 5.3  | 0.82|
| BW Q1                     | 3.6  | 0.52|
| BW Q2                     | 4.8  | 0.27|
| BW Q3                     | 5.7  | 0.23|
| BW Q4                     | 7.0  | 0.73|
| At sampling²              | 10   | –   |
| Litter, n                 |      |     |
| Birthweight light, kg     | 0.7  | 0.10|
| Birthweight middle, kg    | 1.3  | 0.17|
| Weaning light, kg         | 3.1  | 0.48|
| Weaning middle, kg        | 4.7  | 0.34|

SD, standard deviation.
¹Total born includes born alive and stillborn piglets.
²Data are arithmetic means of 10 piglets for each category and period.

Results

Description of the sow and litter performance is shown in Table 2. The average BW of the sampled light and middle-weight piglets at birth was 741 and 1,367 g, whereas at the end of the suckling period was 3,125 and 4,733 g, respectively. All samples showed an adequate amplification. The effect of piglet BW on the intestinal expression of some genes involved in multiple physiological functions is shown in Table 3. At birth, light piglets showed a down-regulation of genes from immune response (FAXDC2, HSPB1, PPARGC1a), antioxidant enzymes (SOD2m), digestive enzymes (ANPEP, IDO1, SI), and nutrient transport (SLC39A4) (P < 0.05), but also a tendency for a higher mRNA expression of GBP1 (immune/inflammation response) and HSD11B1 (stress enzyme) compared to their heavier littersmates (P < 0.10). In fact, the tendency for HSD11B1 higher gene expression in light piglets was also observed at the end of the sucking period. At that age, the light piglets of the litter had a higher mRNA expression of five genes involved in barrier function (CLDN1), pro-inflammatory response (CXCL2, IL6), digestive enzyme (IDO1), and stress hormone signaling (HSD11B1) compared to the middle-weight piglets (P < 0.05). Likewise,
Table 3. Relative gene expression in light and middle weight piglets at birth and at weaning (20 d)

| Function                      | Gene               | Birth weight | Weaning weight |
|-------------------------------|--------------------|--------------|----------------|
|                               | Light | Middle | SEM | P    | FDR | Light | Middle | SEM | P    | FDR |
| Barrier function              |        |        |     |      |     |        |        |     |      |     |
| Claudin-1                     | CLDN1  | 1.10   | 0.94 | 0.071 | 0.322 | 0.650 | 2.41   | 1.02 | 0.091 | 0.046 | 0.415 |
| Immune response               |        |        |     |      |     |        |        |     |      |     |
| Fatty acid hydrolase domain   | FAXDC2 | 1.70   | 3.12 | 0.063 | 0.010 | 0.138 | 107.57 | 129.83 | 0.152 | 0.702 | 0.887 |
| Guanylate binding protein 1   | GBP1   | 0.31   | 0.06 | 0.141 | 0.062 | 0.319 | 1.53   | 1.19 | 0.093 | 0.806 | 0.907 |
| Heat shock protein 1          | HSPB1  | 1.27   | 1.81 | 0.029 | 0.002 | 0.083 | 3.47   | 3.33 | 0.045 | 0.970 | 0.992 |
| Peroxisome proliferative      | PPARGC1α | 2.87  | 4.79 | 0.062 | 0.012 | 0.138 | 2.73   | 2.67 | 0.102 | 0.391 | 0.815 |
| antioxidant receptor gamma,   |        |        |     |      |     |        |        |     |      |     |
| coactivator 1 alpha           |        |        |     |      |     |        |        |     |      |     |
| Interleukin 6                 | IL6    | 1.18   | 1.44 | 0.119 | 0.517 | 0.820 | 6.38   | 1.90 | 0.097 | 0.007 | 0.165 |
| Chemokine ligand 2            | CXCL2  | 1.20   | 0.90 | 0.078 | 0.263 | 0.650 | 10.99  | 4.08 | 0.101 | 0.025 | 0.283 |
| Antioxidant enzyme            |        |        |     |      |     |        |        |     |      |     |
| Superoxide dismutase          | SOD2m  | 1.17   | 1.37 | 0.027 | 0.100 | 0.462 | 1.40   | 1.20 | 0.059 | 0.673 | 0.887 |
| Digestive enzyme              |        |        |     |      |     |        |        |     |      |     |
| Aminopeptidase-N              | ANPEP  | 0.61   | 0.72 | 0.027 | 0.047 | 0.311 | 1.68   | 1.44 | 0.092 | 0.615 | 0.887 |
| Indoleamine 2,3-dioxygenase   | IDO1   | 1.40   | 4.72 | 0.120 | 0.040 | 0.310 | 12.90  | 3.30 | 0.115 | 0.024 | 0.283 |
| Sucrase-isomaltase            | SI     | 7.27   | 55.70 | 0.171 | 0.006 | 0.131 | 130.06 | 84.69 | 0.573 | 0.206 | 0.815 |
| Nutrient transporter          |        |        |     |      |     |        |        |     |      |     |
| Solute carrier family 39 member 4 (Zn transporter) | SLC39A4 | 1.31 | 2.19 | 0.074 | 0.028 | 0.258 | 1.88   | 1.64 | 0.095 | 0.752 | 0.887 |
| Stress enzyme                 |        |        |     |      |     |        |        |     |      |     |
| Hydroxysteroid (11-beta) dehydrogenase 1 | HS-D11β1 | 2.88 | 1.92 | 0.029 | 0.062 | 0.319 | 3.60   | 1.73 | 0.056 | 0.002 | 0.079 |
| Corticotropin releasing hormone receptor 1 | CRHR1 | 2.33 | 2.39 | 0.094 | 0.982 | 0.985 | 7.15   | 3.63 | 0.120 | 0.060 | 0.451 |

1Data are means of 10 piglets for each category and correspond to back-transformed values. Gene expression values are indicated as ratios of cycle relative threshold value for each gene normalized to that of the reference sample. Only significant (P-value < 0.05) and tendency (P-value < 0.10) differences are presented. SEM, standard error of the mean; FDR, false discovery rate.

Discussion

The intestinal mucosa possesses a complex function; it does not only play an important role in epithelial barrier and nutrient digestion but is also part of a well-organized immune system (Okumura and Takeda, 2017). Like BW, the development and functionality of the gastrointestinal tract can be impaired due to prenatal events (Dong et al., 2014; Farmer and Edwards, 2021). This becomes especially important at weaning when stress and pro-inflammatory events occurred. In the present study, intestinal gene expression at birth showed the immaturity of the light piglets intestine due to a reduced activation of digestive (ANPEP, IDO1, SI), nutrient transport (SLC39A4), immunity/inflammation (FAXDC2, GBP1, HSPB1, PPARGC1α), and antioxidant (SOD2m) genes accompanied also by an overexpression of the stress enzyme gene HSD11β1. For instance, the down-regulated digestive genes such as ANPEP, IDO1, and SI are mainly involved in the final digestive process of proteins, amino acids, and carbohydrates but may also act in an anti-inflammatory way (Trevisi et al., 2012; Xu et al., 2015). Moreover, the down-regulation of the nutrient transport gene (SLC39A4) of Zn, considered as the major intracellular Zn transporter (Martin et al., 2013), may be involved in the changes observed in other physiological genes that need Zn for their proper expression (Suttle, 2010). Considering that the small intestine plays an essential role not only in diet digestion and nutrient absorption but also in immune response, this reduced gene expression observed in piglets of light birthweight may seriously compromise immunity functionality, as previously noted (Michiels et al., 2013; Qi et al., 2019; Li et al., 2021). Indeed, a decreased development of gastrointestinal tract (e.g., length, weight, and secretory capacity), even after 18 to 28 d post-weaning, has been observed in piglets with light birthweight compared to their average birth weight littersmates (912 g vs. 1,287 g BW; Michiels et al., 2013). Noteworthy, an over-expression of HSD11β1 gene was found in piglets with lower BW both at birth and at weaning. The HSD11β1 gene is encoding for the enzyme that converts inactive cortisone to the active cortisol, therefore regulating tissue glucocorticoid levels and playing a critical role in metabolism and inflammatory response (Nixon et al., 2012; Huang et al., 2020). The fact that it remains elevated until the end of lactation shows that the fetal distress and the neonatal inflammatory condition are not reverted with age,
hence suggesting that this negative impact may last until later in life. Since newborn piglets were not allowed to consume colostrum, further studies are needed to assess the influence of colostrum, the primary source of nutrients and immunoglobulins to piglets, in improving these physiological functions of piglets impaired by uterine overcrowding.

Excluding HSD11β1 gene, all these intestinal gene expression differences initially observed at birth between light and middle-weight piglets disappeared at the end of the suckling period, indicating the normal acquisition of these functions along the suckling phase.

Interestingly, at the end of the suckling period, a positive up-regulation of CLDN1 gene (barrier function) was observed in light piglets, together with an overexpression of some important pro-inflammatory genes (IL6 and CXCL2, IDO1) and stress hormone receptor (CRHR1, HSD11β1) compared to heavier piglets. On the one hand, the upregulation of CLDN1, an essential structural and functional component of tight junctions (Günzel and Yu, 2013), may suggest an enhancement in the regulation of intestinal permeability. However, the concomitant upregulation of stress hormone signaling (CRHR1, HSD11β1) and pro-inflammatory genes (IL6) and their cofactors (IDO1) might be also highlighting the stressful status that light piglets are experiencing. Particularly, HSD11β1 and CRHR1 genes have been suggested as stress-induced mediators of an impaired intestinal barrier and hypersecretion on early weaned piglets (Meddings and Swain, 2016). The IL6, CRHR1, HSD11β1 and IDO1 genes are also negatively affects several physiological functions by increasing pro-inflammatory and stress responses. Among the pathophysiological changes associated with proinflammatory cytokines and stress molecules is the redistribution of nutrients, such as energy and amino acids, that were initially destined for growth (Huntley et al., 2018). It could be speculated that in young pigs, nutrient and immune provisions during the lactation period are not enough to satisfy the needs of the poorly developed piglets that must compete with their littermates for milk provision. In fact, light piglets have shown a deteriorated post-natal and post-weaning development (Bérard et al., 2008; Ji et al., 2017; Rodrigues et al., 2020), with impaired characteristics of organs and carcasses (Rehfeldt and Kuhn, 2006; Bérard et al., 2008) compared to those of piglets with heavier weights. The impaired physiological functions of light piglets observed in the present study support the concept that the unsatisfactory nutrient supply to the fetus/neonate is an important factor influencing their postnatal physiological performance (Larriestra et al., 2006; Farmer and Edwards, 2021).

In conclusion, light BW piglets at birth and at the end of suckling period seem to have an impaired gut development and nutrient absorption, as well as higher pro-inflammatory responses compared to their average weight littermates. Further studies are needed to investigate the short- and long-term consequences of the gut immaturity of light BW piglets, coming from large litters, on their feed efficiency as well as their abilities to cope with post-weaning challenges.

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Conflict of Interest Statement
The authors declare no real or perceived conflicts of interest.

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