Birth weight and nutrient restriction affect jejunal enzyme activity and gene markers for nutrient transport and intestinal function in piglets

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Research

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Abstract

Background

Significant variations in live birth weight of piglets have arisen due to increased sow prolificacy. Intestinal development and function may be affected by birth weight. Low birth weight (LBW) pigs may also have reduced feed intake, leading to further impairment of intestinal development. The objective of this study was to examine the intestinal development pattern of low and normal birth weight (NBW) piglets with normal (NN) or restricted (RN) neonatal feed intake.

Results

Jejunal intestinal samples were analyzed for target gene expression and enzyme activity at d 28 (weaning) 56 post-birth (post-weaning). At d 28, EAAC1 and B0AT1 were downregulated in LBW compared to NBW pigs ($P < 0.05$). On d 56, B0AT1 and ASCT2 were downregulated in RN compared to NN pigs ($P < 0.05$), regardless of birth weight. PepT1 expression was downregulated in LBW compared to NBW pigs at 28 d ($P < 0.05$), with no effects of treatments at 56 d. SGLT1 was upregulated in NBW-NN compared to LBW-NN pigs ($P < 0.05$) at 28 d. Alkaline phosphatase (ALP) was upregulated in LBW-RN at d 28. At d 56, CLDN-3 and ZO-1 were upregulated in NN compared to RN pigs ($P < 0.05$). There were no treatment effects on ALP, maltase, or sucrase activity at 28 d. However, at 56 d, ALP was upregulated in NBW-NN pigs while sucrase activity was upregulated in NN pigs.

Conclusion

The results demonstrate differences in jejunal gene expression associated with birth weight, with reduced expression of protein/amino acid transporters (PepT1, EAAC1, B0AT1) in LBW compared to NBW pigs. While neonatal nutrient restriction had minimal effects at 28 d, intestinal barrier function (Claudin-3 and ZO-1) and neutral amino acid transport (B0AT1 and ASCT2) were improved in pigs under normal compared to restricted feeding pre-weaning at d 56.

Introduction

There is significant variation in live birth weight of piglets within the same litter, which is primarily related to an increase in sow prolificacy [1, 2]. The survival and development of neonatal piglets is associated with live birth weight [3, 4] with piglets born at a lower birth weight at higher risk of poor growth and development, including inadequate intestinal development and function. In addition to their small size, low birth weight (LBW) piglets may have reduced access to nutrition in the pre-weaning period due to competition with larger litter mates, potentially exacerbating the negative effects of LBW on intestinal function. During the first few days after birth, intestinal development and function is crucial to the survival of the neonate, such that poorly developed intestine will lead to issues relating to poor nutrient
digestion and absorption and intestinal barrier function [5, 6]. The integrity of the intestinal barrier, increased enzyme activity, and improved nutrient transport function are not only important indicators of intestinal development but imply the potential ability of these pigs to absorb and metabolize nutrients efficiently for growth performance. It may be argued that the internal metabolism of the pigs will influence intestinal development and, as such, influencing factors which affect body metabolism, such as birth weight, may have an impact on intestinal development. Therefore, the objective of this study was to examine intestinal development and function of LBW and normal birth weight (NBW) piglets with either normal pre-weaning feed intake (NN) or restricted intake (RN).

Materials And Methods

The protocols used in this study were approved by the Animal Research Ethics Board of the University of Saskatchewan (Animal Use Protocol #20190042) and followed the Canadian Council of Animal Care guidelines for the care and use of farm animals in research.

Animals, housing, and experimental design

Details of the experimental procedure have been outlined previously [7]. Briefly, a total of 14 sows (Camborough Plus × C3378; PIC Canada Ltd.) were housed in farrowing crates at the Prairie Swine Centre (Saskatoon, SK) over 4 blocks and fed a standard commercial lactation diet. After farrowing, all piglets in the litter were individually weighed and identified as LBW or NBW based on previously established weight ranges in this population of pigs [8], with piglets < 1.5 kg initial body weight considered LBW. Postnatal nutrient restriction (RN) was induced in 4 target piglets per litter (2 LBW and 2 NBW) through isolation from the sow for 6 h/d from 0800–1400 h from d 3 post-farrowing to weaning (d 28), based on a modification of previously described methods [9, 10]. All other piglets were allowed unrestricted access to the sow [normal nutrition (NN)]. No additional feed was provided to the piglets before weaning. At the end of the suckling period, piglets were weaned onto a commercial nursery diet for 28 d (d 28–56 post-weaning) and housed in groups of 3–6/pen and fed standard commercial diets. At d 28 and 56 of the study, 8 pigs/treatment were euthanized with an overdose of isoflurane (oxygen flow at 1 L/min with 5% isoflurane) followed by exsanguination. After evisceration, sections of the small intestine (jejunum) were sampled and immediately snap-frozen in liquid nitrogen and then stored at -80°C until analysis.

Enzyme activity assay

The enzyme activities of intestinal digestive enzymes including intestinal alkaline phosphatase (ALP), maltase, and sucrase were determined. Briefly, approximately 500 mg of pulverized and frozen jejunal tissue samples were thawed in an ice-cold homogenizing buffer consisting of 50 mM D-mannitol and 0.1 mM phenylmethylsulphonyl fluoride (PMSF) at pH 7.4 (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) and homogenized on ice using a polytron homogenizer (Fisher Scientific, Ottawa, ON, Canada). After centrifugation (4000 rpm, 10 min, 4°C), the protein concentrations of the homogenate suspensions were determined using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). Alkaline phosphatase (EC. 3.4.11.2) activity was assayed according to the method of Engström [11]. Potassium fluoride
(Sigma-Aldrich Chemical Co. St. Louis, MO, USA) was added to inhibit the interference of intracellular acid phosphatase in the intestinal mucosa [12]. Incubations were conducted in a final volume of 0.50 mL containing homogenized tissue suspensions (0.100 to 0.200 mg protein), 2.0 mM potassium fluoride, 5.0 mM MgCl$_2$, and 40.0 mM p-nitrophenyl phosphate (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) at pH 10.5 for 15 min at 37°C. The enzyme reaction was stopped by adding 0.50 mL of 0.50 M NaOH (Sigma-Aldrich Chemical Co. St. Louis, MO, USA). The end-product of the enzyme reaction, p-nitrophenol, was measured using a Synergy™ H4 Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA) at 400 nm wavelength. Maltase (E.C. 3.2.1.20) and sucrase (E.C. 3.2.1.48) activities were assayed according to a previously established method [13]. Incubations were conducted in a final volume of 0.500 mL containing homogenized cell suspensions (0.200 to 0.300 mg protein), 100 mM maleate buffer (maleic acid dissolved in NaOH), and 60 mM maltose, 60 mM sucrose or 100 mM amylose, at pH 6.0 and 37°C for 20 min. Incubations were terminated by adding 70 mM ZnSO$_4$. The end-product of both enzyme reactions, D-glucose (Pointe Scientific, Inc.), was measured by spectrophotometric analysis at 500 nm. All enzyme assays were corrected for nonspecific blank readings.

**Real time polymerase chain reaction (RT-PCR)**

Total RNA of the jejunum tissue was extracted by the Trizol procedure (Invitrogen, Carlsbad, CA, U.S.) according to the manufacturer’s instructions. Total RNA was dissolved in 20 µL RNase-free water and concentration was determined using a NanoDrop 2000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE, U.S.) with purity ascertained (A260/A280) between 1.8 and 2.0. The total RNA (about 1 µg) from each sample was converted into cDNA using iScript cDNA Synthesis Kit (Bio-Rad, Mississauga, ON, Canada) according to the manufacturer’s instructions. The qPCR analysis was performed to quantify the target genes encoding for enzyme (ALP, SI), nutrient transporter (SGLT1, PepT1, EAAC1, ASCT2 and B$_0$AT1) and barrier function genes (ZO-1 and Claudin-3), as presented in Table 1. The cyclophilin-A (Cyc-A) gene was used as the housekeeping gene. The relative changes in gene expression levels of genes in the jejunum tissues normalized against Cyc-A were determined by using the $2^{-\Delta\Delta CT}$ method according to Livak and Schmitten [14].
Table 1
The primer sequences of the target genes and the internal reference gene

| Gene Name          | Sequence (5′-3′)            | Accession Number | Product Size (bp) |
|--------------------|----------------------------|------------------|-------------------|
| **Enzyme genes**   |                            |                  |                   |
| ALP                | F: CCACTCCGCGCCACC         | XM_021097682.1   | 76                |
|                    | R: AAGAGCTCGTGGGTGAAGG     |                  |                   |
| SI                 | F: TGATAGGCCAGTGAGAGTGC    | XM_021069748.1   | 99                |
|                    | R: AGAGTTGAGTAAGGCTGCCA    |                  |                   |
| **Nutrient transporter genes** |            |                  |                   |
| SGLT1              | F: GGCTGGACGAAATGGGTG       | NM_001164021.1   | 153               |
|                    | R: ACAACCACCCAAATCAGAGC    |                  |                   |
| EAAC1              | F: GTTCCTGATTGCGGGAAGA      | NM_001164649.1   | 165               |
|                    | R: ATGGCGAATCGGAAAGGTT      |                  |                   |
| ASCT2              | F: GCCAGCAAGATTGTGGAGAT     | XM_003355984.4   | 206               |
|                    | R: GAGCTGGATGGAGTTCCAAA    |                  |                   |
| B0AT1              | F: AAGGCCCAGTACATGCTCAC    | XM_0033559855.4  | 102               |
|                    | R: CATAAATGCCCTCCACCAG     |                  |                   |
| PepT1              | F: CATCGCCATACCCCTTCTG     | NM_214347.1      | 143               |
|                    | R: TTCCCATCCATCGTGACATT    |                  |                   |
| **Barrier function genes** |            |                  |                   |
| ZO-1               | F: GATCCTGACCCCGGTGCTGA    | XM_021098896.1   | 200               |
|                    | R: TTGGTGGGGTTGGTG          |                  |                   |
| CLDN3              | F: CTACGACCAGCAAGGACTACG   | NM_001160075.1   | 123               |
|                    | R: TAGCATCTGGGTGGACTG       |                  |                   |
| **Internal reference gene** |            |                  |                   |
| CycA               | F: GCGTCTCCTTGACGCTGTT     | NM_214353.1      | 160               |
|                    | R: CCATTATGCGTGTGAAGTC      |                  |                   |

ALP; alkaline phosphatase, SI; sucrase-isomaltase, SGLT1; sodium/glucose cotransporter 1, EAAC1; excitatory amino acid transporter 1, ASCT2; glutamine transporter, B0AT1; neutral amino acid transporter, PepT1; peptide transporter 1, ZO-1; zonula occludens-1, CLDN3; claudin 3; CycA; Cyclophilin-A.
Statistical analysis

Data were tested for normality and outliers using the PROC UNIVARIATE model and the Shapiro–Wilk test in SAS version 9.4 (SAS Institute Inc.). Outliers were determined as a value ± 2 standard deviations away from the treatment mean using the studentized residual analysis. Different groups of piglets were sampled on d 28 and 56, and each time point was analyzed separately as a randomized complete block design with a 2 × 2 factorial arrangement. The fixed effects were 1) birth weight category (BWC) (LBW and NBW), 2) dietary treatment (RN and NN), and 3) their interaction. The block (sampling group) was included in the model as a random factor. Initially, gender effect was also included as a fixed effect model but was not significant hence removed from the model. Differences between treatment means were separated by the PDIFF option adjusted for Tukey-Kramer test. Significance differences were determined at $P \leq 0.05$; a trend toward significance was considered at $P < 0.10$.

Results

qPCR of target gene expression

Tables 2 and 3 show protein gene expression of jejunal tissue samples of piglets at 28 and 56 d, respectively.
Table 2
Nutrient transporter and tight junction protein gene expression of jejunal tissue sample 28-days post weaning

| Genes     | LBW    | NBW    | p-value |
|-----------|--------|--------|---------|
|           | RN     | NN     | RN      | NN     | SEM    | BWC    | DT     | BWC*DT |
| Claudin-3 | 0.578  | 1.000  | 0.471   | 0.546  | 0.17   | 0.086  | 0.126  | 0.279  |
| ZO1       | 0.836  | 1.001  | 0.734   | 0.997  | 0.26   | 0.835  | 0.405  | 0.847  |
| ALP       | 4.505a | 1.000b | 0.564b  | 2.384ab| 1.22   | 0.283  | 0.477  | 0.031  |
| SI        | 4.154  | 1.000  | 1.038   | 1.564  | 1.08   | 0.226  | 0.214  | 0.086  |
| EAAC1     | 1.968A | 0.998A | 3.198B  | 3.877B | 0.99   | 0.043  | 0.881  | 0.402  |
| B0AT1     | 1.228A | 1.001A | 1.867B  | 2.696B | 0.41   | 0.007  | 0.464  | 0.203  |
| PEPT1     | 1.545A | 1.000A | 2.519B  | 2.593B | 0.39   | 0.002  | 0.533  | 0.414  |
| SGLT1     | 1.627ab| 1.000b | 1.598ab | 2.127a | 0.27   | 0.048  | 0.853  | 0.038  |
| ASCT2     | 0.531  | 1.000  | 0.154   | 0.459  | 0.36   | 0.189  | 0.267  | 0.812  |

ALP; alkaline phosphatase, SI; sucrase-isomaltase, SGLT1; sodium/glucose cotransporter 1, EAAC1; excitatory amino acid transporter 1, ASCT2; glutamine transporter, B0AT1; neutral amino acid transporter, PepT1; peptide transporter 1, ZO-1; zonula occludens-1, CLDN3; claudin 3, CycA; Cyclophilin-A.

LBW; Low birth weight, NBW; Normal birth weight, RN; restrictive nutrition; NN; normal nutrition, BWC; birth weight category, DT; dietary treatment.

A,B Uppercase letters within a row represent main effect significant difference (P< 0.05)

a,b Lowercase letters within a row represent significant treatment interaction (P< 0.05)
Table 3
Nutrient transporter and tight junction protein gene expression of jejunal tissue 56 d post weaning

| Genes    | LBW  | NBW  | p-value         |
|----------|------|------|-----------------|
|          | RN   | NN   | RN            | NN            | SEM   | BWC | DT    | BWC*DT |
| Claudin 3| 0.952B | 1.510A | 0.250B | 2.171A         | 0.37  | 0.955 | 0.002 | 0.075  |
| ZO1      | 0.596B | 2.098A | 0.420B | 3.293A         | 0.52  | 0.338 | 0.0003 | 0.200  |
| ALP      | 0.688b  | 7.321b  | 0.656b  | 20.934a        | 3.11  | 0.041 | 0.0002 | 0.041  |
| SI       | 2.594b  | 4.977b  | 1.097b  | 15.718a        | 2.28  | 0.052 | 0.0009 | 0.012  |
| EAAC1    | 0.574  | 1.371  | 1.593  | 1.931          | 0.53  | 0.148 | 0.294 | 0.669  |
| B0AT1    | 1.264b  | 2.776A  | 0.685b  | 4.455A         | 0.69  | 0.435 | 0.0007 | 0.116  |
| PEPT1    | 1.114  | 1.235  | 1.820  | 1.150          | 0.49  | 0.532 | 0.581 | 0.427  |
| SGLT1    | 0.679  | 0.552  | 0.595  | 0.971          | 0.20  | 0.413 | 0.539 | 0.222  |
| ASCT2    | 0.556b  | 1.520A  | 0.130B  | 3.018A         | 0.61  | 0.388 | 0.004 | 0.127  |

ALP; alkaline phosphatase, SI; sucrase-isomaltase, SGLT1; sodium/glucose cotransporter 1, EAAC1; excitatory amino acid transporter 1, ASCT2; glutamine transporter, B0AT1; neutral amino acid transporter, PepT1; peptide transporter 1, ZO-1; zonula occludens-1, CLDN3; claudin 3, CycA; Cyclophilin-A.

LBW; Low birth weight, NBW; Normal birth weight, RN; restrictive nutrition; NN; normal nutrition, BWC; birth weight category, DT; dietary treatment.

A, B Uppercase letters within a row represent main effect significant difference (P< 0.05)

a, b Lowercase letters within a row represent significant treatment interaction (P< 0.05)

At weaning (d28), piglets within the NBW category had a significantly higher expression of EAAC1, B0AT1 and PepT1 compared to piglets with LBW (P< 0.05), with no effect of nutrient restriction at either time point (P> 0.05). Further, ZO1, Claudin-3, SI and ASCT2 were neither affected by birth weight or nutrition level (P> 0.05). Expression of SGLT1 and ALP was significantly affected by the interaction of birth weight and nutrition level. Specifically, ALP expression appeared to be higher in LBW-RN pigs compared to LBW-NN and NBW-RN pigs but similar to NBW-NN pigs (P< 0.05). Expression of SGLT1 was reduced in the LBW-NN compared to NBW-NN pigs and intermediate with the other treatments (P< 0.05).

At d56, there were no significant treatment effects for SGLT1, PepT1 or EAAC1 (P> 0.05). Generally, there was a significant effect on nutrient restriction group (RN or NN) on Claudin-3, ZO1, ASCT1 and B0AT1. Specifically, NN piglets, regardless of BWC, had greater expression of Claudin-3, ZO1, ASCT1, and B0AT1 compared to RN piglets (P< 0.01). There was a significant interaction between BWC and nutrition on ALP.
and SI expression. Specifically, there was an increased expression of ALP and SI in NBW-NN piglets compared to all other treatments ($P<0.05$).

**Enzyme activity**

Enzyme activity results are reported in Fig. 1, with the top panel showing ALP, maltase, and sucrase at d 28 and lower panel shows results for d 56. On d 28, there were no significant effects of treatment on any of the measured parameters ($P>0.05$). On d 56, there was an interaction between birth weight and nutrient restriction, with the NBW-NN group having greater ALP activity compared to the other treatments ($P<0.05$). Further, we observed a nutrition level effect on sucrase activity where piglets fed the NN diets regardless of the BWC had a significantly higher sucrase activity ($P<0.05$). There were no other treatment effects on any other measured enzyme activity.

**Discussion**

We previously developed and validated a model of birth weight and neonatal undernutrition, which demonstrated intermittent suckling as an effective strategy to induce nutrient restriction in pigs [7]. Since our model identified major differences in performance and organ development at 56 d, as influenced by BWC but not neonatal nutrient restriction, it is important to understand specific effects of BWC and nutrient restriction on intestinal development and function. Therefore, the objective of the present study was to determine if jejunal enzyme activity and nutrient transporter and tight junction protein gene expression measured at 28 or 56 d of age are affected by BWC (LBW vs. NBW) and neonatal nutrient intake (RN vs. NN).

**Birth weight and nutrition effect on enzyme activity**

Enzyme activity in the gut is important in the digestion and absorption process of nutrients, therefore, when enzyme activity is downregulated, there is potential for a negative impact on nutrient absorption. In the present study, we observed an upregulation of ALP in LBW-RN compared to LBW-NN and NBW-RN pigs at 28 d. Alkaline phosphatase is directly involved in intestinal immune response and, when expression is reduced, may predispose the gut to changes in the gut microbiome and intestinal inflammation and permeability [15]. Intestinal inflammation has been reported in LBW [16] as well as neonatally nutrient restricted pigs [17]. The higher expression of ALP at weaning suggests a adaptive mechanism in the gut to potential stressors, as ALP is known as a potent regulator of inflammation [15]. On the other hand, ALP expression and enzyme activity were upregulated in NBW-NN compared to the other groups at 56 d. This may be explained by intestinal protective roles of ALP recently reviewed by Lallès [18], which may indicate improved gastrointestinal health as a result of higher birth weight and neonatal nutrient intake. Huygelen et al. [19] reported no effect of birth weight (low vs. normal) on ALP expression in the small intestine of pigs at weaning, which confirms the findings in the present work showing that neonatal nutrient allowance may improve intestinal ALP activity, particularly long-term (e.g., 56 d post weaning). Maltase is a brush border enzyme involved in effectively hydrolyzing $\alpha$-1,4 and $\alpha$-1,6 linkages for the digestion of several carbohydrates [20]. Sucrase-isomaltase is also a highly prevalent
and enzyme for digestion of different dietary carbohydrates, which supports intestinal physiology and immune response [21]. In the present study, we observed no effect of the experimental treatments on maltase or sucrase activities at 28 d, which was consistent with the lack of effect of treatments on SI expression. Interestingly, at 56 d, SI was upregulated in NBW-NN compared to the other groups and sucrase activity was upregulated in NN compared to RN pigs. Indeed, a recent study reported expression of SI at 14 times higher in NBW compared to LBW piglets and an increased expression of SI with age in NBW but not in LBW [22]. Moreover, it is well known that increased access to milk intake early-in-life augments gastrointestinal development later-in-life [23], which may contribute to improved digestion and absorption of carbohydrates by the offspring. Since the adult pattern of distribution of brush-border carbohydrases is achieved around 8 weeks (Kidder and Manners, 1980), our findings indicate a better adaptation of NBW-NN pigs to the weaning transition from milk- to cereal-based diet and highlight that neonatal nutrient restriction may play an important role in this establishment.

**Birth weight and nutrition effect on target gene expression**

At 28 d, Claudin-3 tended to be upregulated in NBW compared to LBW pigs, with no effects of treatments on ZO-1 and ASCT2. Conversely, at 56 d, Claudin-3 and ZO-1 were upregulated in NN compared to RN pigs. Our findings suggest an improved intestinal physical barrier of NBW pigs around weaning. Interestingly, intestinal barrier function (Claudin-3 and ZO-1 expression) was influenced by neonatal nutrient allowance and not body weight at 56 d. Tissue stability and barrier function are influenced by tight junction protein function, with higher expression being positively correlated with enhanced barrier function [24]. A previous report indicated that milk/colostrum consumption may further enhance tight junction proteins expression [25], which is in line with our results, which demonstrated increased expression of tight junction proteins in NN pigs. This may relate to increased gut barrier protection and improve the ability of pigs to cope with intestinal disturbances post-weaning [26].

At 28 d, EAAC1 (glutamate transporter; [27]) and B0AT1 (neutral amino acids transporter; [28]) were downregulated in LBW compared to NBW pigs, which is in agreement with previous studies and suggests that intestinal normal absorptive function and mucosal growth may be decreased in LBW pigs due to decreased amino acid availability for protein synthesis [29, 30]. Conversely, at 56 d, there was no effect of treatment on EAAC1 expression; but both B0AT1 and ASCT2 (glutamine transporter) were downregulated in RN pigs compared to NN pigs, with no influence of birth weight. These findings confirm the lack of effect of birth weight on small intestine development as we have previously reported [7]. Moreover, neutral amino acids, including proline [31] and glycine [32] are nutritionally essential for milk-fed pigs and neonatal nutrient restriction may have decreased expression of their transporters later-in-life. Peptide transporter 1 (PepT1, di- and tripeptides transporter; [33]) has been reported that PepT1 is highly expressed in LBW pigs [34]. However, there is great variability in expression across the different segments of the intestine [35, 36], which may explain discrepancies among studies. In the present study, expression of PepT1 was downregulated in LBW compared to NBW pigs at 28 d and showed no effects at 56. This observation suggest that birth weight may influence the absorption of nutrients (e.g., peptides amino acids) in neonatal pigs, likely due to reduced capacity of the gut to feed intake and enzyme secretion.
Again, the sodium-dependent glucose transporter 1 (SGLT1, sodium-dependent glucose uptake in the small intestine; \[37\]) was upregulated in NBW-NN compared to LBW-NN pigs at 28 d, with no effects observed at 56 d. The results indicate that, when piglets fed a normal level of nutrients, their birth weight will have an influence on glucose transport capacity; in this case the results show that pigs with NBW have higher glucose transport capacity than LBW pigs. These results are aligned with another study which reported, that SGLT1 expression is influenced by feeding but this may be restricted by birth weight \[38\].

**Conclusions**

In summary, our results showed that the main differences in jejunal gene expression at 28 d were associated with differences in birth weight, with reduced expression of protein/amino acid transporters (PepT1, EAAC1, B\(^0\)AT1) in low birth weight compared to normal birth weight pigs. These findings may indicate insufficient intestinal protein synthesis. Neonatal nutrient restriction had minimal effects at 28 d, however, at 56 d, intestinal barrier function (Claudin-3 and ZO-1) and neutral amino acid transport (B\(^0\)AT1 and ASCT2) were improved in normal nutrition compared to restricted nutrition pre-weaning. Moreover, normal birth weight and normal nutrition pigs had improved gastrointestinal development and functionality compared to the other groups. Further research is needed to improve intestinal amino acid absorption in low birth weight piglets and to attenuate weaning transition in milk-fed piglets.

**Abbreviations**

- ALP: alkaline phosphate
- ASCT-2: glutamine transporter
- B\(^0\)AT1: neutral amino acid transporter
- CLDN-3: claudin-3
- Cyc-A: Cyclophilin-A
- EAAC-1: Glutamate transporter
- LBW: low birth weight
- NBW: normal birth weight
- NN: normal nutrition
Declarations

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Author contributions

DAC, MOW, LAR, and CY designed the study, MOW, LAR conducted the study, QL and BD conducted the lab analysis, MOW, LAR and JCP analyzed the data and wrote the manuscript. All authors reviewed and approved the manuscript for publication.

Ethics approval and consent to participate

The experimental protocols were approved by the University of Saskatchewan's Animal Research Ethics Board under protocols 20190042 and followed Canadian Council on Animal Care guideline

Availability of data and materials

Data are available from the corresponding author upon reasonable request.

Competing interests

All authors declare no conflicts of interest, financial or otherwise.

Consent for publication
Not applicable.

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Figures
Figure 1

Enzyme activity in jejunal tissue for piglets d 28 and d 56 post weaning. LBW; Low birth weight, NBW; Normal birth weight, RN; restrictive nutrition; NN; normal nutrition, BWC; birth weight category, DT; dietary treatment.