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An increased weaning age and liquid feed enhances weight gain compared to piglets fed dry feed pre-weaning

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ABSTRACT

Increasing age and providing liquid creep feed could potentially increase the solid feed intake in pre-weaning piglets, which may in turn promote gut maturation and post-weaning feed intake, possibly lessening the severity of the growth-check associated with the suckling-to-weaning transition. Therefore, this study aimed to investigate if feeding dry- versus liquid creep feed (**DF** vs. **LF**) and weaning in week 4 or 5 (**4W** or **5W**) could accelerate maturational changes to the small intestines of pre-weaning piglets by increasing digestive and absorptive capacity. In a 2 × 2 factorial study the effect of weaning age (**WA**) and feeding strategy (**FS**) on weaning weight, pre-weaning accumulated gain (**AG**), and average daily gain was measured for 12 923 piglets. A subpopulation of 15 piglets from each treatment group (4WDF, 4WLF, 5WDF and 5WLF; n = 60) were sacrificed to assess the effects of WA and FS on weight of digestive organs, activity of maltase, lactase and sucrase, and gene expression level of sodium-glucose linked transporter 1 (*SGLT-1*), glucose transporter 2 (*GLUT2*) and peptide transporter 1 (*PepT1*) in the proximal part of the small intestine (**SI**). No interactions were found but average weaning weight was affected by WA ($P < 0.001$) and FS ($P < 0.001$), where 5W were heavier than 4W and LF were heavier than DF. Correspondingly, the average daily gain (**ADG**) was affected by both WA ($P = 0.003$) and FS ($P < 0.001$). Only WA affected the relative weight of the digestive organs, where stomach weight, weight of SI and colon weight were heavier in 5W piglets compared to 4W. Lactase activity tended to decrease with age ($P = 0.061$), but there was no difference in the activity of maltase or sucrase between any of the treatment groups. Similarly, there was no differences in gene expression level of *SGLT1*, *GLUT2* or *PepT1* between neither the two ages nor feeding strategies. In conclusion, both WA and FS affect weaning weight and weight gain of piglets in the pre-weaning period.

Implications

Pigs reared under conventional conditions are vulnerable at weaning, due to their relatively young age resulting in an immature gut with a low digestive capacity. Increasing the brush border enzyme activity and nutrient absorption prior to weaning through the provision of creep feed, or an additional week of suckling, could enhance intestinal maturation and digestive capacity prior to weaning. Liquid feeding and extended weaning age increased BW which could not be explained by changes in the gut. In practice, liquid feeding and an extended weaning period could result in heavier piglets, which might smoothen the suckling-to-weaning transition.

Introduction

Conventionally reared piglets are abruptly weaned at 3–4 weeks of age, which is considerably earlier than the gradual weaning of a 15–22 week suckling period observed under semi-natural conditions (Jensen & Stangel, 1992). Early weaning is incompatible with the ontogenetic maturation of the piglet gastrointestinal tract, as the development of the epithelial barrier, transport function, immune system and enteric nervous system does not finish until

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12–14 weeks of age (Moeser et al., 2017). Weaning has been shown to induce significant changes in the morphological structure and function of the small intestine (**SI**) during the early post-weaning period, including reduced activity of the brush border enzymes, shortened villous and increased crypt depth, overall reducing the digestive and absorptive capacity of the SI (Hampson, 1986; Hampson & Kidder, 1986; Pluske et al., 1997; Tsukahara et al., 2013). These changes and the reduced voluntary feed intake (**FI**), which is often observed in the immediate post-weaning period, cause a post-weaning growth check (Pluske et al., 1997). A commonly used preventive measure to accommodate the weaning transition is to provide dry creep feed to suckling piglets. This familiarises piglets with ingesting vegetable-based ingredients and teaches them to eat solid feed prior to weaning, potentially increasing the post-weaning FI. Indeed, a positive correlation between pre-weaning FI and the FI and growth in the immediate post-weaning period has been found (Pajor et al., 1991; Fraser et al., 1994; Bruininx et al., 2002; Berkeveld et al., 2007). Since piglets are to be reared without antimicrobial growth promoters and medicinal zinc oxide, utilising the correlation between pre- and post-weaning FI could be of even more importance, when preventing post-weaning enteric disorders and the post-weaning growth check. In fact, Berkeveld et al. (2007) argues that increased pre-weaning FI might prevent harmful changes to the intestinal structure, and thus the impaired gut function observed around weaning. Adding to this notion, increased pre-weaning ingestion of creep feed could potentially promote and accelerate the development and maturation of the digestive systems of piglets, such as increased activity of digestive enzymes, creating a smoother suckling-to-weaning transition (Muns & Magowan, 2018).

Age and duration of creep feed provision has proven to increase pre-weaning FI (Bruininx et al., 2002; Sulabo, Jacela, et al., 2010; Sulabo, Tokach, et al., 2010), therefore it seems reasonable to assume that an extended suckling period with provision of creep feed, could promote the pre-weaning intestinal development through both ageing and feed-related maturation. Similarly, the provision of liquid creep feed could increase the pre-weaning FI compared with the dry form (Blanchard et al., 2000; Martins et al., 2020), which in turn may further stimulate the maturation of the piglet's digestive system. Age alone has been shown to not give any significant benefits over the period from birth to slaughter (Edge et al., 2008) but the combination with liquid feed could possibly give a positive additive effect.

Therefore, the aim of this study was to investigate if the activity of digestive enzymes in the brush border of the SI of pre-weaning piglets could be increased by promoting pre-weaning FI through provision of liquid versus dry feed during either a 4- or 5-week suckling period in a 2 × 2 factorial design. The hypotheses were (1) that liquid feed increases FI and thereby promotes increased level of digestive enzyme activity, (2) that an additional week of age will increase pre-weaning FI and thereby the activity of digestive enzymes through both natural ontogenetic development and an extended duration of creep feeding and (3) together both age and feed combined will increase level of digestive enzyme activity.

Material and methods

Animals and experimental design

A large-scale study was conducted in a Danish commercial sow herd producing (Landrace × Yorkshire) × Duroc crossbred piglets. The study was conducted from March 2020 to February 2021 and included 12 923 piglets at weaning in a 2 × 2 factorial design. Each treatment consisted of a combination of weaning age (**WA**) and feeding strategy (**FS**), where piglets were to be weaned in the 4th or 5th postnatal week (**4W** or **5W**) and received either dry or liquid creep feed (**DF** or **LF**). Thus, the four treatment groups were 4WDF, 5WDF, 4WLF and 5WLF. The choice to wean in week 4 or 5 was made based on Danish recommendations. A subpopulation of 60 piglets were euthanised for intestinal tissue sampling (n = 15). The sampled piglets were selected from five consecutive batches. Within each batch, piglets were born within three days of each other (d 0 ± 3). The piglets originated from 32 different litters and sows. Two piglets were randomly selected from each of 28 litters, and because of unforeseen circumstances the last four piglets had to be randomly selected from four separate litters. In the large-scale study, all piglets were weighed at day 3 and at weaning, and feed allowance was monitored daily. Euthanised piglets were weighed at day 3 and at euthanasia.

Housing and management

The herd had an average production size of 1 170 year-sows and weaned approximately 800 pigs per week. The farrowing unit consisted of 318 farrowing crates distributed in eight sections. Sows and litters were housed in traditional farrowing pens with crates and partially slatted floor. To stimulate the ingestion of creep feed, sows reared one additional piglet than their number of functional teats. Thus, litter size was determined by the sows' number of functional teats at day 3, where all litters were locked, and no more changes were allowed. Litter size at day 3 varied from 9 to 17 piglets per sow depending on functional teats. Shortly after birth, all pigs were treated with Vetrimoxin (Ceva Animal Health, Libourne, France). Two days after birth, all piglets were treated with Dozuril (Dopharma Research, Raamsdonksveer, The Netherlands) and male piglets were castrated.

Diet and feeding regime

To ensure adequate nutrient and energy intake, all litters had access to milk supplement (Danmilk™ Supreme 1.0, Agilia™, Videbæk, Denmark) the first eight postnatal days (**MS1**), and from day 9 to 17, piglets in LF litters had access to a second milk supplement (**MS2**; Danmilk™ Gain Agilia™, Videbæk, Denmark). Nutrient composition of MS1 and MS2 can be viewed in Table 1. The supplement was fed using computerised Mini Wet Feed Systems (BoPil A/S, Sønderborg, Denmark), which were installed in all farrowing pens housing experimental sows. Troughs were placed above the slatted floor in the separating wall between two pens, and thus two litters shared one feeder. Troughs were equipped with sensors, which registered if a trough was empty. All milk supplements and feed were allocated in the installed troughs. To ensure continuous access to feed, 20% of the daily ration would be allocated when a trough was empty. Both milk supplements and the liquid feed was allocated in this manner. Computer registrations of allocated amounts of milk supplement and liquid feed were used to estimate the average feed allowance. In dry fed litters, the feed was delivered through separate feeding tubes and registered separately. For DF piglets, the dry feed was provided 5–6 times per day to ensure constant availability. From day 9 until euthanasia or weaning, 4WDF- and 5WDF piglets had access to a dry zinc-free weaner diet as creep feed. The weaner diet was formulated in accordance with the current nutritional standards of SEGES Innovation P/S (Tybirk et al., 2021) (Table 2). Piglets in treatment group 4WLF and 5WLF received the same diet in liquid form from day 18 to euthanasia or weaning. Liquid feed was produced by mixing the feed with water, so that DM constituted 20%.

Table 1
Ingredients[1] and composition of piglet milk supplements.

	Milk Supplement	
Composition of milk supplement	MS1[2]	MS2[3]
Ingredients		
Dairy biproducts	X	X
Whey protein concentrate	X	X
Whey powder	X	X
Heat-treated wheat starch		X
Vegetable fat		X
Vegetable protein		X
Chemical analysis (DM basis)[4]		
Energy, MJ/kg DM	20.9	15.2
DM, %	97.0	94.7
CP, %	19.5	22.1
Crude fat, %	16.8	12.2
Ash, %	5.0	3.3
Lysine, %	1.81	1.70
Threonine, %	1.29	1.07
Methionine, %	0.41	0.50
Valine, %	1.19	1.21

[1] As the milk supplements are commercial products, the list of ingredients is known. However, the ratio between them is undisclosed. X indicates whether the ingredient was included in the diet.
[2] MS1 denotes Agilia™ Danmilk™ Supreme 1.0. Piglets in all treatment groups had access to MS1 from 24 hours postpartum until day 8.
[3] MS2 denotes Agilia™ Danmilk™ Gain. Piglets in liquid feed groups had access to MS2 from day 9 to 17 postpartum.
[4] Values from the chemical feed analysis performed by SEGES Innovation P/S.

Table 2
Ingredients and composition of pig weaner diet offered as creep feed.

	Feed[1]
Composition of feed	Weaner diet[2]
Ingredients, %	
Wheat	47
Barley	13
Rye	8
Fishmeal	9
Pig lard	3
Concentrate[3]	20
Chemical analysis (DM basis)[4]	
Energy, MJ/kg DM	15.4
DM, %	89.4
CP, %	18.8
Crude fat, %	6.9
Ash, %	4.7
Zinc, %	0
Lysine, %	1.48
Threonine, %	0.92
Methionine, %	0.48
Valine, %	0.92

[1] All piglets received the same feed. Dry feed piglets received the feed in its dry form from day 9 until euthanasia or weaning. Liquid feed piglets had access to the diet in liquid form from day 18 until euthanasia or weaning. Liquid feed was mixed with water so that DM constituted 20%.
[2] The diet was optimised after the current norms set by SEGES Innovation P/S (Tybirk et al., 2021).
[3] This is a commercial product with a known list of ingredients, but an undisclosed ratio between them. The ingredients are Soy protein concentrate, Lactose, Toasted soybeans, Wheat, Mono-dicalcium-phosphate, Shea- and palm fat, Saccharose, Natrium chloride, Soy oil, Barley, Wheat bran, Calcium carbonate, Wheat, Maize, Water-soluble onion residues, and Water-soluble grape seed residues.
[4] Values from the chemical feed analysis performed by SEGES Danish Pig Research Centre.

Tissue and organ sampling

All tissue and organ samples were collected 3 days prior to weaning. Piglets in the 4W group had a mean age of 21 ± 0.8 days at euthanasia, whereas 5W piglets were 28 ± 0.7 days of age. Before euthanasia, each pig was weighed, and the pig's sex was determined. The pigs were euthanised using a bolt pistol and exsanguinated, before removing the entire gastrointestinal tract. From each piglet, tissue samples from the proximal part of the SI were collected for later enzyme activity and gene expression analysis. The stomach, SI and colon were weighed both full and empty, and the length of the SI was measured. Samples intended for analysis of enzyme activity were immediately put in cryotubes and stored at −80 °C. The samples for gene expression analysis were put in cryotubes with RNAlater™ (Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania) and stored at −20 °C.

Analysis of enzyme activity

There were a few samples that did not generate data on disaccharidase activity. The results of maltase and lactase activity are based on 59 samples, where treatment group 5WDF consisted of 14 pigs (n_{5WDF} = 14). Sucrase activity was analysed for 56 samples, where the 4WLF group consisted of samples from 13 pigs (n_{4WLF} = 13), and both 4WDF and 5WDF consisted of 14 pigs (n_{4WDF} = 14, n_{5WDF} = 14), and 5WLF consisted of 15 pigs as planned (n_{5WLF} = 15). Mucosal maltase, sucrase and lactase activity was determined for the SI tissue samples homogenised in 1% Triton X-100. The ratio was 0.20 mg tissue per 1 mL Triton X-100 solution. The disaccharidase activity was determined using the method described by Dahlqvist (1968), where the concentration of generated glucose was measured via the absorbance change at 340 nm using an ELx 808 microplate reader (Bio-tek, Winooski, VT, USA). For all three disaccharidases, the generated quantity of glucose was determined by a nicotinamide adenine dinucleotide phosphate (NADP) chain reaction:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose 6-phosphate} + \text{ADP}.$$

$$\text{Glucose 6-phosphate} + \text{NADP} \xrightarrow{\text{G-6-P-DH}} \text{Gluconate-6-phosphate} + \text{NADPH} + \text{H}^{+}.$$

For each sample, enzyme activity measured in Units per liter (U/L) was calculated as:

$$\begin{aligned}\Delta c/t &= \frac{\Delta \boldsymbol{E}\times \boldsymbol{a}\ \ \text{mL}\times \boldsymbol{d}}{\left(\boldsymbol{t}\times \boldsymbol{b}(L\times \text{mol}^{-1}\times \text{cm}^{-1})\times \boldsymbol{c}\ \ \text{cm}\times \boldsymbol{e}\ \ \mu\text{L}\right)\times \boldsymbol{n}} \\ &= \frac{\Delta \boldsymbol{E}\times 3.22\ \ \text{mL}\times \boldsymbol{d}\times 10^{6}\ \ \mu\text{mol/mol}}{\left(60\ \ \text{min}\times 6300(L\times \text{mol}^{-1}\times \text{cm}^{-1})\times 0.93\ \ \text{cm}\times 0.1\ \ \mu\text{L}\right)\times \boldsymbol{n}} \\ &= \frac{\Delta \boldsymbol{E}\times 91.597\times \boldsymbol{d}}{\boldsymbol{n}}\end{aligned}$$

where **Δc/t** denoted the change in glucose concentration over the reaction time, **ΔE** was the change in absorption measured per minute, **a** was the total volume in a well and **d** was the dilution factor for the specific sample. The reaction times was denoted **t**, while **b** was the extinction coefficient for NADPH (6300 M^{-1} cm^{-1}). The well width was denoted as **c** and **e** was the sample volume. The number of glucose molecules generated per molecule hydrolysed disaccharide was denoted **n**, and for maltose, **n** = 2, while for sucrose and lactose, **n** = 1. Before performing any statistical analysis, U/L was converted to U/mg tissue by multiplying Δc/t with 0.2 mg tissue per mL triton.

Analysis of nutrient transporter gene expression

The level of gene expression of sodium-glucose linked transporter 1 (*SGLT-1*), glucose transporter 2 (*GLUT2*) and peptide transporter 1 (*PepT1*) in the proximal SI was determined using relative quantification and Real-Time Polymerase Chain Reaction (RT qPCR). The RT qPCR analyses were carried out on all 60 samples. However, one sample did not generate a result of ΔCt for *SGLT1* despite of repeated attempts, thus the results of *SGLT1* gene

expression are based on 59 animals, with the 5WDF group consisting of 14 piglets (n_{5WDF} = 14). The same applies to the analysis of *PepT1* gene expression, though it was a 5WLF piglet, from which the defect sample originated. Thus, the 5WLF group consisted of 14 piglets (n_{5WLF} = 14) in the RT qPCR analysis of *PepT1* gene expression.

RNA was extracted from the SI tissue samples using a lysis reagent and RNeasy mini kits (Qiagen, Hilden, Germany) following the accompanying protocol. From each sample, 500 ng of RNA was used to synthesise cDNA by use of a Reverse Transcription kit (Qiagen, Hilden, Germany). The qPCR was performed on an Aria MX PCR system (Agilent Technologies, Santa Clara, California, USA) using the following cycling programme: 2-minute hot start at 95 °C, forty amplification cycles of 5 seconds at 95 °C and 20 seconds at 60 °C, and a melt cycle of 3 × 30 seconds at 95 °C, 65 °C and 95 °C respectively. The primer sequences can be seen in Table 3. The efficiency of all primers was evaluated by construction of linear standard curves for each primer pair. Porcine Beta-2-Microglobulin (*B2M*) served as the reference gene due to stable expression in the intestinal tissue samples, to which the normalised relative genetic expression of nutrient transporter genes was calculated. The ΔCt value for each of the separate genes was calculated for each of the original tissue samples and was chosen as a parameter because of its ability to be analysed using linear modelling and regression (Livak & Schmittgen, 2001).

Statistical analysis

Growth performance data was analysed for 12 923 piglets and a subsample of 60 piglets were sampled and analysed for organ, enzyme and gene expression.

Growth performance data were analysed and evaluated at SEGES Innovation P/S using a Proc Mixed model in SAS (SAS inst. Inc.). Data on feed allowance were estimates based on measurements per double pen averaged per pig. They are therefore presented as mean values of allocated DM per pig during the preweaning period without any analysis of statistical differences between treatment groups. The statistical analysis of piglet growth performance was based on individual measurements of BW at day three and at weaning, thus the experimental unit was piglet. The analysis was performed using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \rho_k + (\alpha\beta)_{ij} + \theta + \delta_\theta + \omega_{ijk} + \varepsilon_{ijk}$$

Y_{ijk} denotes the response variables of piglet BW at weaning, preweaning average daily gain and accumulated gain, and μ is the independent variable mean. The systematic effects of WA and FS are denoted α_i (i = 4W, 5W) and β_j (j = DF, LF), respectively. The systematic effect of sow parity is denoted ρ_k (k = 2, …, 8), and the model includes $(\alpha\beta)_{ij}$ as the interaction between WA and FS. θ is the random effect of sow and contains sow-dependant parameters, such as milk yield and genetics, and litter size is included in the model as δ_θ. Lastly, piglet BW at day three is denoted ω_{ijk} and is included as a covariate, and ε_{ijk} $N(0, \sigma^2)$ is the normally distributed random residual of a model. Data and model residuals were assessed and confirmed to be normally distributed. Least square means for piglet BW at weaning, average daily gain **(ADG)**, and accumulated gain were calculated for each treatment group.

Data from the sixty-piglet subpopulation were analysed using R 4.1.1 and R studio version 1.4.1717 (R Studio Team, 2020; The R Foundation, 2021), where each measurement or laboratory result was derived from a single piglet. The response variables of piglet BW at euthanasia, the weight and content of stomach, SI and colon, SI length, stomach, SI and colon weight relative to BW, disaccharidase activity, and lastly, ΔCt values for *SGLT1*, *GLUT2* and *PepT1* were analysed using linear mixed effect models, and models were reduced using AIC-stepwise reduction determining the significance level of the included systematic effects and their interactions. Using qqnorm, residuals of each model were evaluated to ensure they followed a normal distribution, thus confirming linear regression as a valid model choice for the statistical analysis. Using the Emmeans-function, least square means for each response variable were obtained, and are presented with the pooled SE of the mean (±SEM). Data analysis was based on the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + (\alpha\beta\delta)_{ijk} + \theta_l + \varepsilon_{ijkl}$$

Here Y_{ijkl} denotes piglet BW at euthanasia, weight and content of digestive organs and SI length, relative organ weight, disaccharidase activity or gene expression level, and μ is the independent mean of the variable. α_i is the systematic effect of WA (i = 4W, 5W), β_j is the systematic effect of FS (j = DF, LF) and δ_k is the systematic effect of sex (k = Female, Male). Second-order interactions between main factors are denoted $(\alpha\beta)_{ij}$, $(\alpha\delta)_{ik}$ and $(\beta\delta)_{jk}$. The second-order interactions were included in the analysis, as the AIC-stepwise reduction revealed that for 12 out of 20 models the interactions were explanatory. The third-order interaction, $(\alpha\beta\delta)_{ijk}$, was found to be non-significant for all response variables (p > 0.10). θ_l is the random effect of Sow (l = 1, …, 32). ε_{ijkl} $N(0, \sigma^2)$ is the normally distributed random residual of a model. Therefore, the final model is as follows:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + \theta_l + \varepsilon_{ijkl}$$

To allow comparison of the response variables between groups, Tukey Kramer adjusted LS means were calculated when relevant.

Table 3
Nucleotide sequences of forward and reverse primers for select porcine nutrient transporter genes and reference gene.

	Nucleotide sequence	
Item	Forward primer (from 5′ to 3′)	Reverse primer (from 5′ to 3′)
Transporter		
SGLT1	AATGCGGCTGACATCTCTGT	CCAACGGTCCCACGATTAGT
GLUT2	AGTTGGCGCTATCAACACGA	CACAAGTCCCACCGACATGA
PepT1	CAGACTTCGACCACAACGGA	TTATCCCGCCAGTACCCAGA
B2M	CAAGATAGTTAAGTGGGATCG	TGGTAACATCAATACGATTTC

Abbreviations: *SGLT1* = Sodium-Glucose-cotransporter-1, *GLUT2* = Glucose Transporter 2, *PepT1* = Peptide Transporter-1 and *B2M* = Beta-2-Microglobulin. Additionally, A = adenine, C = Cytosine, G = guanine and T = Thymine.

Results

Feed allowance and growth performance

The registered feed allowance was used to estimate and compare the average amount of feed available per piglet for each treatment group. To make the results comparable, feed allowance was calculated on DM basis and can be seen in Table 4. Results of growth performance can be seen in Table 5. There were no interaction effects only main effects, where piglets in 5W litters were heavier at weaning compared with 4W piglets (P < 0.001), and similarly, LF piglets were heavier than DF piglets (P < 0.001). Piglets in 5W litters showed greater accumulated gain compared with 4W piglets (P < 0.001), and the accumulated gain was greater in LF compared to DF piglets (P < 0.001). The ADG of 5W piglets was higher than that of 4W piglets, and LF piglets had a higher ADG than DF piglets (P_{WA} = 0.003, P_{FS} < 0.001).

BW at euthanasia and organ weights

Results of BW at euthanasia and organ parameters can be seen in Table 6. At euthanasia, the 5W-piglets were heavier compared to

Table 4
Mean estimates[1] of DM allowance per pig for the four treatment groups.

WA	4W		5W	
FS	DF	LF	DF	LF
Milk supplement, g DM/piglet				
MS1	99	96	87	88
MS2	0	325	0	278
Weaner diet, g DM/piglet				
Dry	694	0	1 387	0
Liquid	0	329	0	843
Total feed allowance, g DM/piglet	790	750	1 480	1 210

Abbreviations: WA = weaning age, FS = feeding strategy, 4W = weaning in week 4, 5W = weaning in week 5, DF = dry feed, LF = liquid feed, MS1 = Agilia™ Danmilk™ Supreme 1.0 (piglets in all treatment groups had access to MS1 from 24 hours postpartum until day 8, MS2 = Agilia™ Danmilk™ Gain (piglets in liquid feed groups had access to MS2 from day 9 to 17 postpartum.
[1] Simple mean values of estimated feed allowance. The values are estimates of total allocated feed quantity for each of the respective diets. No statistical analysis was performed on feed-related data.

Table 5
Results[1] of pre-weaning growth performance of piglets provided with either dry- or liquid creep feed and weaned in their 4th or 5th week of life.

	Treatment group						
WA	4W		5W			*P*-value[2]	
FS	DF	LF	DF	LF	SEM	WA	FS
No. of pigs	3 216	3 318	3 113	3 276			
Mean age at weaning	23.9	23.9	31.0	30.9			
BW at weaning[3], kg	6.15	6.37	7.87	8.10	0.05	<0.0001	<0.0001
Accumulated gain[4], kg	4.50	4.73	6.23	6.46	0.05	<0.0001	<0.0001
ADG, g/day	215	225	222	232	1.95	0.003	<0.0001

Abbreviations: WA = weaning age, FS = feeding strategy, 4W = weaning in week 4, 5W = weaning in week 5, DF = dry feed, LF = liquid feed, ADG = average daily gain.
[1] For each treatment group, results are presented as least squares means with pooled SEM values.
[2] Probability values for the main effects of weaning age and feeding strategy.
[3] These pigs were reared past weaning and entered the weaner unit. Therefore, a weaning weight was recorded.
[4] The accumulated gain during the lactation period calculated as the average difference in weight at day 3 and at weaning.

4W-piglets ($P < 0.001$), and the extra week generated a 1.55 ± 0.2 4 kg difference in BW. Feeding strategy did not affect BW at euthanasia. Sex was insignificant ($P = 0.54$), however, there was a significant interaction effect of WA and Sex ($P = 0.03$). Sex had an additive effect on BW when combined with WA, because the difference in BW in 4W and 5W males (1.95 kg ± 0.37, $P < 0.001$) was higher than the difference between 4W- and 5W females (1.16 k g ± 0.37, $P = 0.02$). The significant effect of the interaction across both WA and Sex, generated a difference of 1.67 ± 0.37 kg between 4W females and 5W males ($P < 0.001$), and 1.45 ± 0.37 kg between 4W males and 5W females ($P < 0.01$), with 5W pigs being the heaviest. Relative organ weights were calculated as gram empty organ weight per kg BW at euthanasia. As shown in Table 6, the relative organ weights were only affected by WA, where relative organ weights were higher in older piglets, ($P_{stomach} < 0.01$, $P_{SI} < 0.01$, $P_{colon} = 0.02$).

Maltase, lactase and sucrase activity

Maltase, lactase and sucrase activity was not affected by any of the main explanatory factors, WA, FS or Sex, or their interactions, as shown in Table 7. There was, however, a tendency for WA to affect lactase ($P = 0.061$), and as expected the extra week of age decreased the activity. The difference in lactase activity between 4W- and 5W piglets was 726 U/mg tissue. Sex tended to affect sucrase activity ($P = 0.051$), where female pigs had the highest activity averaging at 1 290 U/mg SI tissue. Male pigs showed a sucrase activity of 1 055 U/mg SI tissue.

Gene expression of nutrient transporters

As shown in Table 7, the gene expression level of nutrient transporters *SGLT1* and *PepT1* was not affected by any of the main factors or their interactions. The level of *GLUT2* gene expression was not affected by any of the main factors on their own, nor the interactions of WA × FS or FS × Sex. However, the WA × Sex interaction proved to be significant ($P = 0.002$) and influenced the piglet SI resulting in 5W male piglets having a lower expression of *GLUT2* genes compared to 5W female piglets. 5W male piglets had an average ΔCt value of 8.40 ± 0.49, while 5W female piglets had an average ΔCt value of 7.40 ± 0.49.

Discussion

For this study, it was hypothesised, that an additional week of suckling together with the allocation of LF would stimulate maturational changes to the SI measured as BW gain, organ weight gain, increased brush border disaccharidase activity and expression of selected nutrient transporter genes. The activities of maltase, lactase and sucrase were chosen as parameters, as they attest to the digestive capacity of the SI. The digestion of carbohydrates was prioritised above other nutrients, as carbohydrates were the main nutritional component in the provided supplementary feed.

Growth performance and organ measurements

BW at euthanasia and weaning were analysed, as weaning weight is widely accepted as an indicator for the robustness of piglets at weaning, because weaning weight is positively correlated with the post-weaning ADG (Cabrera et al., 2010; He et al., 2016). Nevertheless, the present study confirms that age is a determining factor for BW, as both BW at euthanasia and weaning were significantly affected by age. Both WA and FS improved ADG, suggesting an increased utilisation of the ingested creep feed at 5 weeks of age and when piglets received LF. The inconsistent effect of FS on organ parameters, and the fact that FS was primarily significant in its interaction with WA, could suggest that the pre-weaning maturation and development of the digestive organs is

Table 6
Results[1] of body and organ measurements of piglets provided with either dry- or liquid creep feed and weaned in their 4th or 5th week of life.

	Treatment group							
WA	4W		5W			*P*-value		
FS	DF	LF	DF	LF	SEM	WA	FS	WA × FS
n	15	15	15	15				
Mean age, days	21.8 ± 0.8	22.1 ± 0.9	28.4 ± 0.5	29.0 ± 0.8				
BW at euthanasia, kg	6.97	6.58	8.38	8.29	0.333	<0.0001	0.403	0.659
Weight of organs, g								
Stomach								
Full weight	138.5	98.6	208.4	208.8	17.8	<0.0001	0.253	0.269
Empty weight	36.0	33.2	46.1	49.1	1.56	<0.0001	0.890	0.075
Content	102.5	65.5	162.4	159.8	17.1	0.0001	0.238	0.323
Small intestine								
Full weight	329[a]	279[a]	450[b]	533[b]	30.8	<0.0001	0.662	0.038
Empty weight	261[a]	239[a]	334[b]	378[b]	15.9	<0.0001	0.564	0.049
Content	68.0	39.1	112.9	154.8	17.3	0.0001	0.765	0.051
Length, m	8.88[a,b]	8.36[a]	9.61[b,c]	10.51[c]	0.306	0.0001	0.564	0.028
Colon								
Full weight	136	124	192	209	9.47	<0.0001	0.847	0.126
Empty weight	71.5	66.7	95.3	96.9	3.68	<0.0001	0.585	0.388
Content	64.5[x]	56.8[x]	95.7[y]	112.2[y]	6.64	<0.0001	0.529	0.078
Relative organ weight, g/kg BW								
Relative stomach weight	5.21	5.07	5.49	6.00	0.227	0.014	0.460	0.167
Relative weight of SI	37.9	36.6	40.0	46.4	2.32	0.016	0.295	0.106
Relative colon weight	10.4	10.2	11.4	11.9	0.553	0.020	0.801	0.587

Abbreviations: 4W = weaning in week 4, 5W = weaning in week 5, DF = dry feed, LF = liquid feed, SI = small intestine.
[1] Results are presented as least squares means with pooled SEM values for each treatment group. Each treatment consisted of a combination of weaning age (WA) and feeding strategy (FS).
[a–c] Least squares means without a common superscript differ significantly ($P < 0.05$).
[x,y] Least squares means without a common superscript tend to differ ($P < 0.10$).

Table 7
Results[1] of disaccharidase activity and gene expression level of *SGLT1*, *GLUT2* and *PepT1* in the proximal SI of piglets provided with either dry- or liquid creep feed and weaned in their 4th or 5th week of life and their interactions (n = 15).

	Treatment group							
WA	4W		5W			*P*-value		
FS	DF	LF	DF	LF	SEM	WA	FS	WA × FS
Disaccharidase activity, U/mg								
Maltase	2 808	2 941	2 924	2 510	389	0.711	0.692	0.493
Lactase	2 119[x]	2 483[x]	1 632[y]	1 517[y]	371	0.061	0.717	0.531
Sucrase	1 284	1 318	1 151	938	232	0.281	0.635	0.618
Gene expression, ΔCt								
SGLT1	3.28	4.06	3.48	3.15	0.422	0.404	0.635	0.184
GLUT2	7.96	8.63	7.94	7.86	0.443	0.386	0.627	0.403
PepT1	5.47	5.48	5.46	4.99	0.625	0.642	0.618	0.677

Abbreviations: 4W = weaning in week 4, 5W = weaning in week 5, DF = dry feed, LF = liquid feed, SI = small intestine, *SGLT1* = Sodium-Glucose-cotransporter-1, *GLUT2* = Glucose transporter 2, *PepT1* = Peptide Transporter-1.
[1] Results are presented as least squares means for each treatment group. Each treatment consisted of a combination of weaning age (WA) and feeding strategy (FS). Results are presented with pooled SEM values.
[x,y] Least squares means without a common superscript tend to differ ($P < 0.10$).

driven primarily by age. However, it may be that the effect of ingested feed becomes evident after weaning, when piglets no longer rely on sows milk (Pluske et al., 2003; Bruininx et al., 2004; Wijtten et al., 2011; Everaert et al., 2017).

The present study showed that LF only had a pre-weaning effect when interacting with WA and only affected the SI response variables. Comparing empty SI weight in the 4WDF group, which represents the current Danish standard of weaning and creep feeding, with the SI weight of 5WDF and 5WLF piglets, it seems there is potential for an additive gain in SI growth when combining increased weaning age with providing liquid feed. This was evident when comparing the SI length across treatment groups. The small intestines of 5WLF piglets were longer than those of 4WDF and 4WLF piglets, showing that a combination of 5W and LF generated a FS dependent effect. These findings are in accordance with results by Callesen et al. (2006), where feed composition only affected growth performance when piglets were weaned at five weeks of age. Additionally, Pajor et al. (1991) found that larger piglets ingested most creep feed, which suggests that older and thereby larger piglets may show a stronger effect of FS.

That FS only affected a few of the parameters investigated could indicate that the selected piglets did not ingest sufficient amounts of feed to elicit an effect, or that the proportion of piglets ingesting creep feed was low. This may be caused by piglets feeding primarily om sow milk, and though all sows reared one additional piglet than their number of functional teats, it may not have induced enough hunger and motivation for piglets to increase their creep feed intake. Bruininx et al. (2004) found that 56% of piglets could be identified as 'moderate' or 'good eaters' of creep feed, thus nearly half of the piglets were either 'non-eaters' or impossible to categorise. Similarly, a study by Kuller et al. (2007) found that 76% of piglets were 'non-eaters' in litters having unlimited access to the sow and creep feed from day 7 until weaning at day 25. Additionally, there is a large variation in creep feed intake between individual piglets (Pajor et al., 1991). Considering sheer numbers, it could be, that the size of the subpopulation (n = 60) was inade-

quate to convey the same pre-weaning effect of FS, which was found in the larger population of nearly 13 000 piglets, because not enough 'eaters' were included.

Evaluating the average accumulated DM feed allowance, the results could indicate a high intake of supplementary milk and feed compared with earlier findings of cumulative feed disappearance reported by Bruininx et al. (2002). However, using feed allowance did not account for residual feed or waste and was measured at litter level, and is therefore an overestimation of the actual FI per piglet. Byrgesen et al. (2021) found that the average daily DM disappearance per piglet in litters fed liquid creep feed was continuously increased from day 10 to day 18 compared with litters fed dry creep feed. However, there was no difference in DM disappearance from day 18 to 24. Byrgesen et al. (2021) also found that there was no difference in the proportion of piglets categorised as 'eaters' between DF and LF piglets, as the treatment groups had 68% and 53% 'eaters' respectively. On the contrary, there was a tendency for a higher proportion of 'eaters' in dry fed litters at d 21 and a higher proportion at day 24 (Byrgesen et al., 2021). These findings could indicate that dry fed piglets might have a sudden increase in FI around day 18. Other studies have observed a similar pattern, where dry creep feed disappearance increased steadily with age and then more suddenly at day 16–25 of age (Bruininx et al., 2002; Pluske et al., 2007; Sulabo, Jacela, et al., 2010; Collins et al., 2013). In the present study, this sudden increase in feed intake could coincide with the time LF piglets started receiving a weaner diet and might therefore explain why there is no clear difference in the estimated DM allowance between DF and LF piglets, and by extension why there is no effect of FS. Additionally, the different creep feeding durations may have contributed to the lack of FS effect in the subpopulation, as duration has been found positively correlated to the proportion of 'eaters' within a litter (Sulabo et al., 2007), presumably because longer durations allow for increased familiarisation with the specific feed type (Devillers & Farmer, 2009).

The present study did confirm that the development of the pre-weaning piglet digestive system is highly dependent on age (Nielsen, 1973; Everaert et al., 2017), as body and organ parameters were consistently affected by WA. The effect of WA on the digestive organ weight found in the present study is in agreement with earlier findings by Pluske et al. (2003). Additionally, the present study observed a 70% SI weight gain in 5W piglets compared to 4W piglets, which is the same percental SI weight gain from day 29 to day 36 found by Pluske et al. (2003).

Digestive and absorptive capacity of the small intestine

To evaluate the digestive capacity of the SI, the brush border maltase-, lactase-, and sucrase activity, in addition to the level of *SGLT1*, *GLUT2,* and *PepT1* gene expression as a marker for absorptive capacity, were analysed. In general, these parameters were unaffected by both WA and FS, except lactase activity, which tended to decrease with age. The unaffected enzyme activity findings are supported by those of (Pluske et al., 2003), who did not find any effect of age, sex or weaning weight on the brush border enzyme activity before weaning. However, a study by Amdi et al. (2021) found that providing milk replacement with a DM content consisting of up to 40% wheat starch increased the activity of maltase and sucrase in the proximal SI, suggesting that vegetable-based carbohydrates can stimulate maturational changes in the brush border and accelerate the activity of digestive enzymes. However, it is worth noting that the piglets in the study were artificially reared, and thus their SI would not have adapted to ingesting sow milk (Amdi et al., 2021). The piglets in the present study had continuous access to their respective sow, and therefore, it is assumed that they were primarily nourished through suckling sow's milk. Though Pluske et al. (2003) did not find a pre-weaning effect on enzyme activity, there was an effect of weaning age in the post-weaning period, where the activity of maltase and sucrase was increased in pigs weaned at day 28 compared with day 14. Pluske et al. (2003) contribute this to the removal of sow milk and the sudden ingestion of feed.

Contrasting the present study, Byrgesen et al. (2021) did find an effect of LF on the pre-weaning level of maltase-, lactase-, and sucrase activity in the proximal SI in pigs euthanised on day 25 postpartum, where the activity of all three enzymes was lower in LF fed piglets. The authors argued that the substrate itself may be an influencing factor, as the enzyme activity in LF fed pigs was lower in spite of the greater DM disappearance compared with DF fed pigs (Byrgesen et al., 2021). This suggests that the unequal duration of creep feeding in this study may have influenced the potential FS effect on enzyme activity, as the shorter duration and the reduced DM content in LF could make it difficult for LF fed piglets to ingest enough substrate to affect the SI brush border enzyme activity. However, no actual quantification of how much DM a piglet needs to ingest to generate a response in the enzyme activity has previously been established.

It seems reasonable to apply similar arguments as to why no pre-weaning effect of WA or FS was found on *SGLT1, GLUT2* and *PepT1* gene expression level. Moran et al. (2010) found that the expression of *SGLT1* in weaned 28-day-old piglets remained constant when fed diets containing up to 40% carbohydrates, and that surpassing 50–60% carbohydrate upregulates the expression of *SGLT1* by increasing the luminal concentration of glucose. Therefore, the present results of *SGLT1* expression, and the lack of difference between treatment groups, suggest that none of the piglets ingested enough feed to increase the luminal glucose concentration and upregulate *SGLT1*. If piglets of the subpopulation were in fact primarily nourished by sow milk, then the carbohydrate ingestion would not come close to surpassing 40%, as lactose only constitutes approximately 6% of sow milk (Klobasa et al., 1987; Aguinaga et al., 2011).

Conclusion

Weaning age and feeding strategy affects weaning weight and weight gain of piglets in the pre-weaning period. This could potentially be explained by accelerated maturational changes in the SI, more specifically altered brush border disaccharidase activity. However, the present study found that the growth of the SI is primarily driven by age, with the effect of feeding strategy being additive when piglets are weaned in week 5. Age and feeding strategy did not affect the pre-weaning activity of maltase, sucrase or lactase, nor the expression of *SGLT1*, *GLUT2* and *PepT1* genes in the proximal part of the SI. Future studies would benefit from including intermittent suckling, artificially reared piglets or post-weaning measurements to elucidate if FS effects become evident, when piglets cannot ingest sow milk. Furthermore, future studies should include the categorisation of 'eaters' and 'non-eaters' and include a more representative measurement of feed intake than feed allowance.

Ethics approval

All procedures involving animals were conducted in accordance with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study (The Danish Ministry of Justice, 1995) and were approved under the AEIRB approval number: 2021-10-PNH-018A.

Data and model availability statement

The data/models were not deposited in an official repository. The data/models of the subpopulation that support the study findings are available from the authors upon request.

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Declaration of interest

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