Effects of 21-Day Weaning on Intestinal Morphology, Permeability, and Mucosal Barrier in Piglets: Postprint

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Abstract

This study was conducted to investigate the effects of weaning at 21 days of age on intestinal morphology, intestinal permeability, and intestinal mucosal barrier in piglets. A 2×3 two-factor completely randomized experimental design was employed, with group (suckling group, weaning group) and age (22, 24, and 28 days of age) as the two main effects. Six litters of healthy Large White piglets with similar body condition were selected, and 6 piglets with an average body weight of (6.1±0.2) kg were selected from each litter and randomly divided into 2 groups, namely the weaning group and the suckling group, with 18 piglets in each group. They were slaughtered at 22, 24, and 28 days of age, respectively, to determine their growth performance, intestinal morphology, intestinal permeability, and intestinal mucosal barrier. The results showed that: 1) At 28 days of age, the final body weight and average daily gain of piglets in the weaning group were extremely significantly lower than those in the suckling group ($P<0.01$). 2) Group and age had an extremely significant interactive effect on jejunal crypt depth and villus height/crypt depth, and ileal villus height of piglets ($P<0.01$); the crypt depth of jejunum and ileum in the weaning group was extremely significantly higher than that in the suckling group ($P<0.01$), while the villus height and villus height/crypt depth of jejunum and ileum were extremely significantly lower than those in the suckling group ($P<0.01$); the jejunal villus height/crypt depth of the suckling group at 28 days of age was extremely significantly higher than that at 22 and 24 days of age ($P<0.01$), and the ileal villus height at 24 days of age was extremely significantly higher than that at 22 days of age ($P<0.01$). 3) Group and age had a significant interactive effect on jejunal mucosal diamine oxidase (DAO) activity in piglets ($P<0.05$), and the DAO activity in jejunal and ileal mucosa of the weaning group was significantly lower than that of the suckling group ($P<0.05$). 4) Group and age had an extremely significant interactive effect on the mRNA expression level of occludin in jejunal

mucosa of piglets (P<0.01); the mRNA expression levels of zonula occludens-1 (ZO-1) in jejunal mucosa and occludin in jejunal mucosa of the suckling group first increased and then decreased with increasing age (P<0.05); compared with the suckling group, the mRNA expression levels of ZO-1 and occludin in ileal mucosa and occludin in jejunal mucosa at 24 days of age in the weaning group were significantly decreased (P<0.05); the mRNA expression level of ZO-1 in ileal mucosa at 24 days of age was significantly higher than that at 22 days of age (P<0.05). 5) Group and age had a significant interactive effect on the mRNA expression level of tumor necrosis factor- (TNF-) in jejunal mucosa of piglets (P<0.05); the mRNA expression level of TNF- in jejunal mucosa of the weaning group at 24 and 28 days of age was significantly higher than that of the suckling group (P<0.05), and the mRNA expression level of interleukin-10 (IL-10) in jejunal mucosa was significantly lower than that of the suckling group (P<0.05). The mRNA expression level of interleukin-1 (IL-1) in ileal mucosa decreased with increasing age (P<0.05). In conclusion, the intestinal development of suckling piglets tended to mature at 22-28 days of age, while weaning disrupted the structure of intestinal epithelial cells and the mRNA expression of tight junction proteins in piglets, causing intestinal villus shortening and shedding, increased intestinal permeability and inflammatory response, and reducing average daily gain.

Full Text

Effects of Weaning at 21 Days of Age on Intestinal Morphology, Permeability and Mucosal Barrier of Piglets

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Abstract

This experiment was conducted to investigate the effects of weaning at 21 days of age on intestinal morphology, permeability, and mucosal barrier function in piglets. A 2×3 factorial completely randomized design was employed, with group (nursing vs. weaning) and age (22, 24, and 28 days) as the two main factors. Six litters of healthy Yorkshire piglets with similar body condition were selected. From each litter, six piglets with an average body weight of (6.1±0.2) kg were randomly allocated to two groups at 21 days of age: a weaning group and a nursing group, with 18 piglets per group. Piglets were slaughtered at 22, 24, and 28 days of age to measure growth performance, intestinal morphology, intestinal permeability, and mucosal barrier function. The results showed: 1) At 28 days of age, the final body weight and average daily gain of weaned piglets were significantly lower than those of nursed piglets (P<0.01). 2) Significant interactions between group and age were observed for jejunal crypt depth,

villus height/crypt depth ratio, and ileal villus height (P<0.01). The weaning group exhibited significantly greater crypt depth in both jejunum and ileum (P<0.01) and significantly lower villus height and villus height/crypt depth ratio in both segments (P<0.01) compared to the nursing group. The jejunal villus height/crypt depth ratio in the nursing group at 28 days was significantly higher than at 22 and 24 days (P<0.01), while ileal villus height at 24 days was significantly higher than at 22 days (P<0.01). 3) A significant group × age interaction was found for jejunal mucosal diamine oxidase (DAO) activity (P<0.05), with weaned piglets showing significantly lower DAO activity in both jejunal and ileal mucosa (P<0.05). 4) A significant group × age interaction was observed for occludin mRNA expression in jejunal mucosa (P<0.01). The mRNA expression of occludin in jejunal mucosa of nursed piglets and ZO-1 in jejunal mucosa increased initially and then decreased with age (P<0.05). Compared to the nursing group, weaned piglets showed significantly reduced mRNA expression of ZO-1 and occludin in ileal mucosa and occludin in jejunal mucosa at 24 days (P<0.05). Ileal ZO-1 mRNA expression at 24 days was significantly higher than at 22 days (P<0.05). 5) A significant group × age interaction was detected for tumor necrosis factor- (TNF-) mRNA expression in jejunal mucosa (P<0.05). The TNF- mRNA expression in jejunal mucosa of weaned piglets at 24 and 28 days was significantly higher than in nursed piglets (P<0.05), while interleukin-10 (IL-10) mRNA expression was significantly lower (P<0.05). Ileal IL-1 mRNA expression decreased with increasing age (P<0.05). In conclusion, the intestinal development of nursing piglets tends to mature between 22 and 28 days of age, whereas weaning disrupts intestinal epithelial cell structure and tight junction protein mRNA expression, causing villus shortening and shedding, increased intestinal permeability, inflammatory responses, and reduced average daily gain.

Keywords: weaning piglets; intestinal morphology; intestinal permeability; intestinal mucosal barrier

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Introduction

Traditional piglet weaning age in China is approximately 56 days, but modern swine production systems have adopted early weaning (typically 21–35 days) to maximize production efficiency. However, the digestive tract of piglets younger than 35 days is not fully developed, and the transition from liquid milk to less palatable solid feed severely impacts the intestinal environment. Weaning is further complicated by relocation, adaptation to new housing, and mixing of piglets, all of which induce weaning stress. This stress leads to reduced feed intake, digestive disorders, diarrhea, and slow growth—collectively known as "early weaning syndrome." Therefore, selecting an appropriate weaning age that balances sow turnover efficiency and piglet health is crucial for optimizing overall farm productivity.

The intestine serves as both the primary site for nutrient absorption and a critical immune organ in animals. Weaning stress severely damages intestinal morphology and function, impairing the mucosal barrier and causing morphological changes and increased intestinal permeability, which ultimately compromises future growth performance and immune function. Understanding intestinal developmental patterns is fundamental for selecting and optimizing weaning age. While previous studies have focused on the effects of different weaning ages (14–35 days) on growth performance and digestive enzyme activities, few have examined post-weaning intestinal morphological and functional development. This study investigates developmental changes in intestinal structure, tight junction proteins, and cytokines in piglets aged 21–28 days and the damage caused by weaning at 21 days, providing a basis for rational early weaning timing and strategies to prevent weaning stress injury.

Materials and Methods

1.1 Experimental Design and Diets A 2×3 factorial completely randomized design was employed, with group (nursing vs. weaning) and age (22, 24, and 28 days) as the two main factors. Six litters of healthy Yorkshire piglets with similar body condition (10–12 piglets per litter) were selected. At 21 days of age, six piglets with an average body weight of (6.1±0.2) kg were chosen from each litter and randomly divided into two groups: a weaning group (transferred to nursery pens and fed a basal diet) and a nursing group (remaining with sows to continue nursing). Weaned piglets were housed individually in pens with temperature and lighting conditions identical to nursed piglets, maintained at approximately 30°C. All piglets had ad libitum access to water. Weaned piglets were fed a corn-soybean meal basal diet twice daily at 08:00 and 16:00 in wet mash form. The basal diet was formulated as powder according to NRC (2012) nutrient standards to meet the requirements of 5–10 kg piglets. Diet composition and nutrient levels are shown in Table 1 .

1.2 Sample Collection At 22, 24, and 28 days of age, piglets were fasted for 12 hours before blood collection from the anterior vena cava using heparinized tubes. Blood was left to stand for 1 hour and centrifuged at 4,000 r/min to prepare plasma, which was stored at -80°C. Following litter-matching principles, two piglets from each litter (one from each group, totaling six per group) were selected, euthanized by exsanguination, and the abdominal cavity was opened to isolate the jejunum and ileum. The anterior jejunum and terminal ileum were collected, gently rinsed with cold physiological saline, and the mucosa was scraped with a scalpel, aliquoted into 2 mL cryovials, snap-frozen in liquid nitrogen, and transferred to -80°C storage. Approximately 1 cm intestinal rings from jejunum and ileum were immersed in 4% paraformaldehyde for fixation.

1.3 Analytical Methods

1.3.1 Growth Performance Piglets were weighed at 22 and 28 days of age to calculate average daily gain post-weaning (based on 12 piglets grown to 28 days). Health status and diarrhea incidence were observed daily to calculate diarrhea rate:

Diarrhea rate (%) = (Number of piglets with diarrhea during trial period) / (Trial days × Number of piglets in group) × 100.

1.3.2 Intestinal Morphology Fixed jejunal and ileal rings were paraffin-embedded, sectioned (3-4 m), stained with hematoxylin-eosin (HE), and mounted with neutral resin. Morphology was observed under an optical microscope. Leica LAX software was used for photography and quantitative analysis of villus height and crypt depth in jejunum and ileum.

1.3.3 DAO Activity in Plasma and Intestinal Mucosa Intestinal mucosal tissue was homogenized to prepare 10% tissue homogenates. DAO activity in plasma and intestinal mucosal tissue fluid was measured using commercial kits (Nanjing Jiancheng Bioengineering Institute).

1.3.4 Gene Expression in Intestinal Mucosa Total RNA was extracted from jejunal and ileal mucosa using QIAGEN kits, and RNA purity and concentration were determined using a micro-spectrophotometer (Bio-drop). cDNA was synthesized following the PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa) protocol. Reaction systems were prepared according to the SYBR® Premix Ex Taq™ kit instructions. Real-time PCR conditions were: 95°C predenaturation for 30 s; 40 cycles of 95°C denaturation for 10 s and 60°C annealing/extension for 30 s; melting curve analysis was performed according to the Bio-Rad CFX 96 instrument manual. mRNA expression levels were calculated using the 2-ΔΔCt method with -actin as the reference gene. Primer sequences are shown in Table 2 and were synthesized by Invitrogen.

1.4 Statistical Analysis Data were analyzed using JMP 10.0 software. Growth performance was analyzed by t-test, while other indices were analyzed by 2×3 factorial ANOVA with Tukey' s multiple comparison test. $P<0.05$ was considered significant and $P<0.01$ highly significant.

Results

Effects of Weaning at 21 Days on Growth Performance As shown in Table 3 , initial body weight (at weaning) did not differ significantly between groups ($P>0.05$). At 28 days (7 days post-weaning), final body weight and average daily gain were significantly lower in the weaning group compared to the nursing group ($P<0.01$). Diarrhea incidence during days 1-3 post-weaning was higher in the weaning group but not statistically significant ($P>0.05$).

Effects of Weaning at 21 Days on Intestinal Morphology As shown in Figure 1 [Figure 1: see original paper], weaned piglets exhibited jejunal villus shedding and crypt indentation. Table 4 reveals that jejunal villus height was significantly lower in the weaning group ($P<0.01$). Significant group × age interactions were observed for jejunal crypt depth and villus height/crypt depth ratio ($P<0.01$). Jejunal crypt depth was significantly greater in the weaning group ($P<0.01$), decreasing gradually with age in nursed piglets but increasing in weaned piglets. No significant difference in jejunal crypt depth was observed at 22 days ($P>0.05$), but it was significantly higher in weaned piglets at 24 and 28 days ($P<0.01$). The villus height/crypt depth ratio was significantly lower in the weaning group ($P<0.01$), with the nursing group showing significantly higher values at 28 days compared to 22 and 24 days ($P<0.01$), while no age-related differences were observed in the weaning group ($P>0.05$).

Figure 2 [Figure 2: see original paper] shows changes in ileal morphology. Table 5 indicates that, unlike the jejunum, a significant group × age interaction existed for ileal villus height ($P<0.01$). Ileal villus height was significantly higher in the nursing group ($P<0.01$), with significantly greater values at 24 days compared to 22 days ($P<0.01$), whereas no age-related differences were observed in the weaning group ($P>0.05$). Ileal crypt depth was significantly greater in the weaning group ($P<0.01$) and increased gradually with age ($P<0.05$). The ileal villus height/crypt depth ratio was significantly lower in the weaning group ($P<0.01$), with no significant age-related differences in either group ($P>0.05$).

Effects of Weaning at 21 Days on DAO Activity Table 6 shows a significant group × age interaction for jejunal mucosal DAO activity ($P<0.05$), with significantly lower activity in the weaning group ($P<0.01$). Plasma DAO activity did not differ significantly between groups at any age ($P>0.05$). Ileal mucosal DAO activity was significantly lower in the weaning group ($P<0.05$), though no significant age-related differences were observed in either group ($P>0.05$).

Effects of Weaning at 21 Days on Tight Junction Protein mRNA Expression Figure 3 [Figure 3: see original paper] demonstrates a significant group × age interaction for occludin mRNA expression in jejunal mucosa ($P<0.01$). In nursed piglets, jejunal occludin mRNA expression increased initially then decreased with age ($P<0.05$), while no significant age-related changes were observed in weaned piglets ($P>0.05$). Compared to the nursing group, weaned piglets showed a trend toward lower jejunal occludin mRNA expression at 22 days ($P=0.05$) and significantly lower expression at 24 days ($P<0.05$). ZO-1 mRNA expression in jejunal mucosa followed a similar pattern of initial increase then decrease with age ($P<0.05$). Weaned piglets exhibited significantly lower mRNA expression of ZO-1 and occludin in ileal mucosa ($P<0.05$), while ileal ZO-1 mRNA expression at 24 days was significantly higher than at 22 days ($P<0.05$).

Effects of Weaning at 21 Days on Cytokine mRNA Expression Figure 4 [Figure 4: see original paper] shows a significant group × age interaction for TNF- mRNA expression in jejunal mucosa ($P<0.05$), with significantly higher expression in weaned piglets at 24 and 28 days ($P<0.05$). IL-10 mRNA expression in jejunal mucosa was significantly lower in the weaning group ($P<0.05$). Age had no significant effect on jejunal cytokine mRNA expression ($P>0.05$).

Figure 5 [Figure 5: see original paper] reveals that, unlike jejunal mucosa, no significant group × age interactions were observed for cytokine mRNA expression in ileal mucosa ($P>0.05$). Ileal IL-1 mRNA expression decreased with increasing age ($P<0.05$). No significant differences in ileal cytokine mRNA expression were observed between groups ($P>0.05$).

Discussion

The digestive tract and immune function of piglets are not fully developed at weaning, and the microbial community is unstable. Weaning exposes piglets to environmental, nutritional, and psychological challenges that cause physiological dysfunction. Studies have shown that intestinal diseases often accompany weaning stress, indicating its critical role in disease susceptibility. In this study, weaning at 21 days induced significant stress, manifested by decreased body weight, an 86.46% reduction in average daily gain, and 16.67% diarrhea incidence on day 1 post-weaning, severely compromising growth performance.

The intestine is a primary target of weaning stress. The jejunum and ileum are critical segments for nutrient absorption, and their villus morphology and functional development directly affect nutrient uptake. Weaning caused villus shortening and shedding with crypt indentation in both segments, demonstrating that 21-day weaning severely impairs intestinal morphology and development. Reduced villus height decreases absorptive surface area, representing a major factor contributing to the significant decline in average daily gain.

Intestinal villi are the primary sites for nutrient absorption, where epithelial cells transport amino acids, glucose, and minerals into the bloodstream. Damage to this region impairs nutrient absorption. DAO, primarily located in intestinal mucosa or villus epithelial cells, is a key regulatory enzyme in polyamine metabolism and plays an important role in cell proliferation. DAO activity is closely correlated with villus height, epithelial integrity, and maturity. In this study, jejunal DAO activity decreased gradually with age in nursed piglets but showed no significant changes in weaned piglets, suggesting continuous intestinal development during lactation with ongoing epithelial cell proliferation and differentiation. Reduced DAO activity at 28 days indicates maturing intestinal epithelium. Significantly lower ileal DAO activity in weaned piglets demonstrates that 21-day weaning severely damages epithelial integrity and maturity, causing villus shortening.

The intestinal mucosal barrier comprises epithelial cells, mucins, gut-associated lymphoid tissue, secreted cytokines, antimicrobial peptides, and microbial flora, forming a natural defense system. Epithelial cells create a mechanical barrier through tight junctions. Plasma DAO activity serves as an important marker of mechanical barrier damage, remaining low under normal conditions but increasing when intestinal mucosa is compromised. In this study, plasma DAO activity increased by 10.67% post-weaning but did not reach significance due to individual variation. Significantly reduced mRNA expression of ZO-1 and occludin in ileal mucosa and higher ZO-1 expression at 24 days indicate that 21-day weaning suppresses tight junction protein expression at the transcriptional level, disrupting the mucosal barrier and increasing permeability. These findings align with previous studies showing reduced ZO-1 and occludin mRNA expression post-weaning that gradually recovers by 28 days, and decreased expression under stress conditions.

The mechanical barrier formed by epithelial cells selectively permits water and ions while blocking toxins and pathogens. Weaning-induced barrier damage allows harmful substances to enter, potentially activating gut-associated lymphoid tissue including lamina propria lymphocytes, Peyer' s patches, and mesenteric lymph nodes, triggering immune responses and cytokine secretion. Weaned piglets showed significantly higher TNF- and lower IL-10 mRNA expression in jejunal mucosa. Previous studies have reported rapid upregulation of pro-inflammatory cytokines (IL-1 , IL-6, TNF-) post-weaning, indicating intestinal inflammation. Dendritic cells in the mucosa continuously capture antigens transported by goblet cells, secrete IL-10 and TGF- , and migrate to mesenteric lymph nodes to activate naïve T cells into Tregs, playing a crucial role in immune tolerance. Knockout of IL-10 or its receptor in humans and mice causes severe inflammation and increased pro-inflammatory cytokine secretion. Unlike the jejunum, weaning did not significantly affect cytokine mRNA expression in ileal mucosa, suggesting superior immune stability in the ileum under weaning stress, possibly due to abundant mucosa-associated lymphoid tissue. Peyer' s patches, concentrated in the ileum, are functional immune sites containing CD4+ T cells, CD8+ T cells, B cells, and macrophages that execute adaptive immunity. When antigens are delivered to these sites, B cells are activated for humoral immunity, while dendritic cells can capture antigens to activate T cells for stronger immune responses. The robust immune tolerance observed in ileal mucosa may be attributed to this mechanism.

Weaning timing must consider both sow turnover and piglet survival. Current production systems typically wean at 21 or 28 days. The 21–28 day period represents a critical developmental window that should be determined based on physiological status. This study demonstrates that nursed piglets show continuous villus development from 22–28 days, with peak barrier function at 24 days (evidenced by highest tight junction protein mRNA expression and reduced pro-inflammatory TNF- expression). Collectively, these indicators identify 24 days as a critical point in intestinal development. At 24 days post-21-day weaning, villus damage was most severe, with shortest villus height, most severe shed-

ding, highest plasma DAO activity, dramatically reduced tight junction protein expression, and elevated pro-inflammatory cytokines. Thus, 24 days represents the most severe stress injury timepoint, attributable to both the progression of stress responses and significant developmental changes around this age. Although intestinal morphology and barrier function showed some recovery by 28 days, weaning after completion of digestive tract development at 24 days is recommended. This study also identified key mucosal cytokines affecting barrier function. Numerous studies have reported feed additives that improve growth performance and maintain intestinal health. Therefore, combining optimal weaning timing with novel feed additives that promote anti-inflammatory cytokine expression and strengthen barrier function will help piglets better cope with weaning stress.

Conclusion

1. Intestinal development in nursing piglets matures between 22 and 28 days of age.
2. Weaning at 21 days disrupts intestinal epithelial cell structure, causing villus shortening and shedding, impairing nutrient absorption, inducing diarrhea, and reducing average daily gain.
3. Weaning at 21 days suppresses tight junction protein mRNA expression, damages barrier function, increases intestinal permeability, and triggers inflammatory responses.
4. Twenty-four days of age is a critical timepoint for intestinal development, and weaning after this age is recommended.

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Note: Figure translations are in progress. See original paper for figures.