Effects of Dietary Epidermal Growth Factor Supplementation on Serum Biochemical Parameters, Serum Free Amino Acids, and Small Intestinal Mucosal Hydrolyzed Amino Acid Content in Weaned Piglets: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary epidermal growth factor (EGF) supplementation on serum biochemical indices, serum free amino acids, and intestinal mucosal hydrolyzed amino acid contents in weaned piglets. Forty-two healthy 21-day-old weaned piglets with similar body weight were randomly assigned to three groups, with 14 replicates per group and one piglet per replicate. Group I served as the control group fed a basal diet; Groups II and III were fed experimental diets supplemented with 200 and 400 μg/kg EGF in the basal diet, respectively. The trial period lasted for 14 days. The results showed: 1) Dietary EGF supplementation had no significant effect on average daily gain, average daily feed intake, or feed-to-gain ratio in weaned piglets ($P>0.05$). 2) On day 7 post-weaning, serum uric acid content in Group II was significantly higher than that in Group I ($P<0.05$), while serum immunoglobulin A contents in Groups II and III were significantly lower than that in Group I ($P<0.05$). On day 14 post-weaning, serum creatinine content in Group III was significantly lower than that in Group I ($P<0.05$), and the serum aspartate aminotransferase/alanine aminotransferase ratio in Group III was significantly lower than those in Groups I and II ($P<0.05$). Dietary EGF supplementation had no significant effect on serum urea nitrogen, glucose, total protein, albumin, globulin, immunoglobulin M, immunoglobulin G, insulin-like growth factor-1, growth hormone, albumin/globulin ratio, or activities of aspartate aminotransferase and alanine aminotransferase in weaned piglets ($P>0.05$). 3) On day 7 post-weaning, serum taurine, alanine, citrulline, and histidine contents in Group II were significantly lower than those in Group III ($P<0.05$), and serum arginine, glutamic acid, and serine contents in Group II were significantly

lower than those in Groups I and III (P<0.05); there were no significant differences in other serum amino acid contents among groups (P>0.05). 4) On day 14 post-weaning, compared with Groups I and III, jejunal mucosal valine and ileal mucosal cysteine contents in Group II were significantly increased (P<0.05), and jejunal mucosal serine, glutamic acid, alanine, cysteine, isoleucine, phenylalanine, and histidine contents in Group II also showed an increasing trend (P<0.10). In conclusion, dietary supplementation with high-dose EGF can reduce the contents of serum immunoglobulin A and other biochemical indices, while dietary supplementation with low-dose EGF can reduce some amino acid contents in serum and intestinal mucosa.

Full Text

Effects of Dietary Epidermal Growth Factor on Serum Biochemical Indices, Serum Free Amino Acids, and Intestinal Mucosal Hydrolytic Amino Acids in Weaned Piglets

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Abstract

This experiment investigated the effects of dietary epidermal growth factor (EGF) supplementation on serum biochemical indices, serum free amino acids, and intestinal mucosal hydrolytic amino acids in weaned piglets. Forty-two healthy 21-day-old weaned piglets with similar body weight were randomly allocated into three groups, each containing 14 replicates with one piglet per replicate. Group I served as the control and received a basal diet, while groups II and III received the basal diet supplemented with 200 and 400 μg/kg EGF, respectively. The experimental period lasted 14 days. The results showed: (1) Dietary EGF supplementation had no significant effects on average daily gain, average daily feed intake, or feed-to-gain ratio (P > 0.05). (2) On day 7 post-weaning, serum uric acid content in group II was significantly higher than in group I (P < 0.05), while serum immunoglobulin A (IgA) content in groups II and III was significantly lower than in group I (P < 0.05). On day 14 post-weaning, serum creatinine content in group III was significantly lower than in group I (P < 0.05), and the serum aspartate aminotransferase/alanine aminotransferase ratio in group III was significantly lower than in groups I and II (P < 0.05). Dietary EGF supplementation had no significant effects on serum urea nitrogen,

glucose, total protein, albumin, globulin, immunoglobulin M, immunoglobulin G, insulin-like growth factor-1, growth hormone, albumin/globulin ratio, or aspartate aminotransferase and alanine aminotransferase activities ($P > 0.05$). (3) On day 7 post-weaning, serum taurine, alanine, citrulline, and histidine contents in group II were significantly lower than in group III ($P < 0.05$), while serum arginine, glutamate, and serine contents in group II were significantly lower than in groups I and III ($P < 0.05$). No significant differences were observed among groups for other serum amino acids ($P > 0.05$). (4) On day 14 post-weaning, compared with groups I and III, group II showed significantly increased valine content in jejunal mucosa and cysteine content in ileal mucosa ($P < 0.05$), with a tendency for increased serine, glutamate, alanine, cysteine, isoleucine, phenylalanine, and histidine contents in jejunal mucosa ($P < 0.10$). In conclusion, high-dose EGF supplementation reduced serum IgA and other biochemical indices, whereas low-dose EGF supplementation decreased the content of certain amino acids in both serum and intestinal mucosa.

Keywords: EGF; weaned piglets; serum biochemical indices; amino acids; intestine

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Early segregated weaning is widely employed in modern swine production because it shortens the reproductive cycle of sows, increases annual farrowing frequency and facility utilization, and reduces vertical transmission of maternal diseases, thereby improving piglet health and growth performance. However, the digestive systems of early-weaned piglets are immature, and weaning stress often causes digestive dysfunction, weakened disease resistance, growth retardation, and even mortality, resulting in significant economic losses. Epidermal growth factor (EGF) is a 53-amino-acid single-chain polypeptide secreted primarily by the submandibular glands, Brunner' s glands of the duodenum, and pancreas, and released into duodenal fluid, saliva, pancreatic juice, blood, and milk. EGF is the most abundant peptide in sow' s milk and plays a crucial regulatory role in piglet intestinal development. Studies have shown that EGF binding to intestinal mucosal receptors improves intestinal morphology, nutrient digestion and absorption, and growth performance in early-weaned piglets. Dietary EGF supplementation reduces diarrhea incidence, enhances immunity, improves growth performance, and promotes intestinal development.

However, few reports exist on the effects of EGF on intestinal amino acid composition, serum free amino acid content, and serum biochemical indices in weaned piglets. This study aimed to investigate these effects to provide a theoretical foundation for the broader application of EGF in pig production.

1.1 Experimental Animals and Group Design

Forty-two 21-day-old "Duroc × Landrace × Yorkshire" weaned piglets weighing (6.40 ± 0.44) kg were randomly divided into three groups with 14 replicates each and one piglet per replicate. Group I (control) received a corn-soybean

meal basal diet, while groups II and III received the basal diet supplemented with 200 and 400 µg/kg EGF, respectively. The basal diet composition and nutrient levels are shown in Table 1. The EGF premix, using spray-dried egg yolk antibodies as a carrier at a concentration of 4,000 mg/kg, was provided by Safet (Changsha) Biotechnology Co., Ltd.

1.2 Management

The experiment was conducted at the animal facility of the Sub-Tropical Agricultural Ecology Research Institute, Chinese Academy of Sciences. Before the trial, the facility was disinfected sequentially with NaOH solution and disinfectant, then rinsed with clean water and thoroughly dried. Piglets were housed individually in pens with slatted floors, allowed ad libitum access to feed and water via nipple drinkers. Daily feed intake was measured as the weight difference between the first feeding of the day and the remaining feed in buckets and troughs. Body weight was measured every 7 days to calculate average daily feed intake, average daily gain, and feed-to-gain ratio. Piglet appetite and behavior were monitored, and natural ventilation was maintained. The experimental period lasted 14 days.

1.3 Sample Collection

On days 7 and 14 of the experiment, seven piglets were randomly selected from each group. Blood (10 mL) was collected from the anterior vena cava, followed by intravenous injection of 4% pentobarbital sodium solution for anesthesia before slaughter. After euthanasia, the abdominal cavity was opened to remove the gastrointestinal tract. The small intestine was anatomically divided into duodenum, jejunum, and ileum. Mucosal samples from the middle jejunum and ileum were rapidly collected, snap-frozen in liquid nitrogen, and stored at -80°C. Blood samples were allowed to clot at room temperature for 2 hours, then centrifuged at 3,000 rpm for 10 minutes at 4°C to separate serum, which was immediately stored at -80°C for amino acid and biochemical analysis.

1.4 Analytical Methods

Serum Biochemical Indices: Serum samples were centrifuged at 3,000 rpm for 10 minutes at 4°C, and the supernatant was used for biochemical analysis. Assay kits were purchased from Hunan Yonghe Sunshine Biotechnology Co., Ltd., and measurements were performed using a Toshiba TBA-120FR automatic biochemical analyzer.

Serum Free Amino Acids: A 600 µL serum sample was mixed with an equal volume of 8% sulfosalicylic acid in a 1.5 mL centrifuge tube, vortexed thoroughly, and precipitated overnight at 4°C. The mixture was then centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was transferred to a new tube, filtered through a 0.22 µm membrane, and analyzed using a Hitachi L-8900 amino acid analyzer.

Mucosal Hydrolytic Amino Acids: Mucosal samples were ground into powder under liquid nitrogen. A 0.1 g sample was placed in an ampoule with 10 mL of 6 mol/L hydrochloric acid. The ampoule was sealed with an alcohol lamp and hydrolyzed at 110°C for 24 hours. The hydrolysate was transferred to a 100 mL volumetric flask, rinsed several times with double-distilled water, and brought to volume. A 1 mL aliquot was filtered through a 0.45 µm membrane and analyzed using a Hitachi L-8900 amino acid analyzer.

1.5 Data Processing and Statistical Analysis

All data were analyzed using SPSS 22.0 software. Results are expressed as "mean ± standard deviation." One-way ANOVA was performed, and Duncan' s multiple comparison test was used when significant differences were detected ($P < 0.05$). A trend was considered when 0.05 $P < 0.10$.

2.1 Effects of Dietary EGF on Growth Performance and Serum Biochemical Indices

As shown in Table 2, dietary EGF supplementation had no significant effects on average daily gain, average daily feed intake, or feed-to-gain ratio of weaned piglets ($P > 0.05$).

Table 3 shows that on day 7 post-weaning, dietary EGF significantly affected serum uric acid and immunoglobulin A (IgA) contents ($P < 0.05$). Serum uric acid in group II was significantly higher than in group I ($P < 0.05$), while serum IgA in groups II and III was significantly lower than in group I ($P < 0.05$). On day 14 post-weaning, dietary EGF significantly affected serum creatinine and aspartate aminotransferase/alanine aminotransferase ratio ($P < 0.05$). Serum creatinine in group III was significantly lower than in group I ($P < 0.05$), and the aspartate aminotransferase/alanine aminotransferase ratio in group III was significantly lower than in groups I and II ($P < 0.05$). No significant differences were observed among groups for other indices ($P > 0.05$).

2.2 Effects of Dietary EGF on Serum Free Amino Acid Content

Table 4 shows that on day 7 post-weaning, serum taurine, alanine, citrulline, and histidine contents in group II were significantly lower than in group III ($P < 0.05$), though no significant differences existed between groups III and I or between groups II and I. Serum serine, glutamate, and arginine contents in group II were significantly lower than in groups I and III ($P < 0.05$), with a tendency for lower aspartate and proline ($P < 0.10$). No significant differences were observed for other amino acids ($P > 0.05$). On day 14 post-weaning, serum citrulline content in group II tended to be higher than in groups I and III ($P < 0.10$), with no significant differences for other amino acids ($P > 0.05$).

2.3 Effects of Dietary EGF on Jejunal and Ileal Mucosal Hydrolytic Amino Acid Content

As shown in Table 5, dietary EGF had no significant effects on jejunal mucosal hydrolytic amino acid content on day 7 post-weaning ($P > 0.05$). On day 14 post-weaning, valine content in jejunal mucosa of group II was significantly higher than in group I ($P < 0.05$). Compared with groups I and III, group II showed a tendency for increased serine, glutamate, alanine, histidine, cysteine, isoleucine, and phenylalanine contents in jejunal mucosa ($P < 0.10$). No significant differences were observed for other amino acids ($P > 0.05$).

Table 6 shows that dietary EGF had no significant effects on ileal mucosal hydrolytic amino acid content on day 7 post-weaning ($P > 0.05$). On day 14 post-weaning, cysteine content in ileal mucosa of group II was significantly higher than in groups I and III ($P < 0.05$), with a tendency for higher histidine content ($P < 0.10$). No significant differences were observed for other amino acids ($P > 0.05$).

3.1 Effects of Dietary EGF on Growth Performance and Serum Biochemical Indices

Blood is a crucial component of the internal environment and serves as a medium for substance exchange between the body and external environment, as well as among various tissues. Due to the focus on physiological metabolism and the limited number of animals, differences in growth performance among groups were not statistically significant despite numerical variations. Uric acid is the final product of purine metabolism, formed through the hydrolysis of purine nucleotides to guanine and adenine, followed by deamination to xanthine and subsequent oxidation. Uricase in pigs oxidizes uric acid to allantoin for urinary excretion, maintaining stable blood levels. In this study, serum uric acid transiently increased in the 200 μg/kg EGF group on day 7 post-weaning, but not in the 400 μg/kg group, suggesting a dose-dependent effect. Since EGF, as a small peptide, is not absorbed but acts primarily on intestinal mucosa, dietary EGF may modulate purine metabolism through regulation of intestinal digestion, absorption, or metabolic functions. No previous studies have reported EGF effects on uric acid production, warranting further investigation into the underlying mechanisms. Serum creatinine, a common indicator of renal function, reflects glomerular filtration capacity. The significant reduction in serum creatinine on day 14 post-weaning with 400 μg/kg EGF suggests improved glomerular filtration. The aspartate aminotransferase/alanine aminotransferase ratio indicates liver function; elevation occurs with hepatocellular damage, and its significant reduction with 400 μg/kg EGF suggests a hepatoprotective effect. Serum IgA content reflects immune capacity, and its significant reduction on day 7 post-weaning with both EGF doses suggests EGF may suppress immune responses. Therefore, dietary EGF modulates metabolism, hepatic and renal function, and immunity, though the mechanisms require further elucidation.

3.2 Effects of Dietary EGF on Serum Free Amino Acids and Mucosal Hydrolytic Amino Acids

Blood amino acid content is influenced by multiple factors including environment, nutrition, and disease. Serum free amino acids directly participate in amino acid metabolism and protein deposition, reflecting nutritional status. The intestine is a metabolically demanding tissue with energy and amino acid requirements far exceeding average body levels relative to tissue weight. Rapid intestinal epithelial cell turnover requires substantial nutrients for cellular components, while nutrient absorption consumes considerable energy. Dietary EGF modulates intestinal epithelial cell turnover, increasing villus height and crypt depth. This study showed that on day 14 post-weaning, the 200 μg/kg EGF group had higher hydrolytic amino acid contents in jejunal and ileal mucosa compared with control and 400 μg/kg groups, while most serum amino acids were lower. Thus, low-dose EGF may enhance mucosal amino acid deposition by improving intestinal morphology, consequently reducing blood amino acid levels. Further research should investigate the relationship between intestinal amino acid metabolism and epithelial cell turnover, as well as the effects of dietary EGF on amino acid requirements of weaned piglets.

4. Conclusion

High-dose dietary EGF supplementation reduces serum IgA and other biochemical indices, while low-dose supplementation decreases the content of certain amino acids in serum and intestinal mucosa.

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