Effects of Hydrolyzed Wheat Protein Supplementation in Late Gestation Diet on Reproductive Performance, Serum Biochemical Indices, Amino Acid Content, and Expression of Nutrient Transport-Related Genes in Placental Tissue of Sows: Postprint

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with hydrolyzed wheat protein (HWG) during late gestation on sow reproductive performance, serum biochemical parameters and amino acid concentrations, as well as the expression of nutrient transport-related genes in placental tissue. Thirty-three pregnant sows with 3-4 parities and similar expected farrowing dates were randomly allocated to 3 groups, with 11 replicates per group and 1 sow per replicate. Sows in the control group were fed a basal diet, while those in the experimental groups were supplemented with 1% and 2% HWG in the basal diet from days 90 to 114 of gestation, respectively; all diets were isoenergetic and isonitrogenous. The results showed: 1) Compared with the control group, dietary supplementation with 1% HWG significantly increased the average birth weight of live piglets ($P<0.05$), while supplementation with 2% HWG significantly increased both litter birth weight and litter weight of live-born piglets ($P<0.05$). 2) Compared with the control group, dietary supplementation with 1% and 2% HWG significantly increased the concentrations of asparagine, histidine, and ornithine in sow serum and histidine, isoleucine, leucine, phenylalanine, and tyrosine in umbilical cord serum on day 107 of gestation ($P<0.05$), and significantly decreased the concentrations of blood ammonia and glycine in sow serum and blood ammonia in umbilical cord serum ($P<0.05$); supplementation with 1% HWG significantly increased valine concentration in sow serum ($P<0.05$), while supplementation with 2% HWG significantly increased isoleucine and threonine concentrations in sow serum and the concentrations of arginine, glutamic acid, glutamine, glycine, methionine, serine, threonine, tryptophan, valine, albumin, glucose, and alkaline phosphatase activity in umbilical cord serum (P<0.05). 3) Compared with the control group, dietary supplementation with 1% HWG significantly increased the relative mRNA expression levels of neutral amino acid transporter 2 (SNAT2), glutamate transporter 3 (EAAT3), basic amino acid transporter 2 (LAT2), and glucose transporter 2 (GLUT2) in placental tissue (P<0.05), while supplementation with 2% HWG significantly increased the relative mRNA expression levels of neutral amino acid transporter 1 (SNAT1), SNAT2, EAAT3, LAT2, peptide transporter 1 (PepT1), peptide transporter 2 (PepT2), glucose transporter 1 (GLUT1), GLUT2, and glucose transporter 3 (GLUT3) in placental tissue (P<0.05). In conclusion, dietary supplementation with HWG during late gestation can enhance the expression of nutrient transport-related genes in placental tissue, promote the efficiency of maternal nutrient transport and fetal pig growth and development, and increase litter birth weight of piglets.

Full Text

Effects of Dietary Hydrolyzed Wheat Gluten in Late Pregnancy on Reproductive Performance, Serum Biochemical Parameters and Amino Acid Contents, and Placental Nutrient Transporter Gene Expression in Sows

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Abstract

This experiment investigated the effects of dietary hydrolyzed wheat gluten (HWG) supplementation during late gestation on sow reproductive performance, serum biochemical parameters and amino acid concentrations, and placental nutrient transporter gene expression. Thirty-three multiparous sows (3–4 parities) with similar expected farrowing dates were randomly allocated to three groups with 11 replicates per group and one sow per replicate. Sows in the control group received a basal diet, while those in the treatment groups received the basal diet supplemented with 1% or 2% HWG from days 90 to 114 of gestation. All diets were formulated to be isonitrogenous and isocaloric. The results showed that: (1) Compared with the control group, 1% HWG supplementation significantly increased the average birth weight of live piglets (P<0.05), while

2% HWG supplementation significantly increased total litter birth weight and total live litter birth weight (P<0.05). (2) Dietary supplementation with 1% and 2% HWG significantly elevated serum concentrations of asparagine, histidine, and ornithine in sows, as well as histidine, isoleucine, leucine, phenylalanine, and tyrosine in umbilical cord serum on day 107 of gestation (P<0.05), while significantly decreasing ammonia and glycine concentrations in sow serum and ammonia in umbilical cord serum (P<0.05). The 1% HWG group also showed increased serum valine in sows (P<0.05), whereas the 2% HWG group exhibited significantly higher serum isoleucine and threonine in sows, and increased arginine, glutamate, glutamine, glycine, methionine, serine, threonine, tryptophan, valine, albumin, glucose, and alkaline phosphatase activity in umbilical cord serum (P<0.05). (3) Compared with the control, 1% HWG supplementation significantly upregulated mRNA expression of sodium-coupled neutral amino acid transporter 2 (SNAT2), excitatory amino acid transporter 3 (EAAT3), L-type amino acid transporter 2 (LAT2), and glucose transporter 2 (GLUT2) in placental tissue (P<0.05). The 2% HWG supplementation significantly increased mRNA expression of SNAT1, SNAT2, EAAT3, LAT2, peptide transporters 1 and 2 (PepT1 and PepT2), and glucose transporters 1, 2, and 3 (GLUT1, GLUT2, and GLUT3) (P<0.05). These findings indicate that HWG supplementation during late gestation enhances placental nutrient transporter gene expression, improves maternal-fetal nutrient transfer efficiency, promotes fetal growth and development, and consequently increases litter birth weight.

Keywords: hydrolyzed wheat gluten; gestation sows; reproductive performance; serum biochemical parameters; amino acids; placenta

Introduction

Wheat is one of China's most important cereal crops, rich in protein, amino acids, and minerals. Beyond human consumption, wheat serves as a major energy feed ingredient. With rising domestic corn prices, wheat and its byproducts have gained increasing application in the feed industry due to their nutritional value comparable to corn and relatively stable pricing. Wheat gluten (also known as vital wheat gluten or active gluten flour) is the primary byproduct of wheat starch production, composed of gliadin, glutenin, albumin, and globulin, with the first two proteins accounting for approximately 90% of wheat protein content. However, wheat gluten' s unique structure results in low water solubility and high viscosity, limiting its application in feed formulations. Recent advances in enzymatic hydrolysis technology have enabled the production of hydrolyzed wheat gluten (HWG), also known as hydrolyzed wheat active peptides, which exhibits improved solubility and digestibility while containing various bioactive peptides, thereby expanding its utility in feed manufacturing.

HWG contains approximately 80% crude protein and is rich in amino acids and small peptides, particularly glutamine, glutamate, and leucine, with glutamine comprising about 30% of total amino acids. Glutamine and glutamate serve as crucial energy substrates and signaling molecules for intestinal development in pigs. Approximately 67% of dietary glutamine and 97% of glutamate are metabolized in the small intestine, where they maintain intestinal epithelial cell proliferation and renewal while preserving intestinal barrier function and tight junction protein expression. Previous research demonstrated that 1% dietary glutamine supplementation increased plasma glutamine levels, improved intestinal villus architecture, enhanced tight junction protein expression, and reduced intestinal permeability in weaned piglets. This effect on tight junction proteins is mediated primarily through calmodulin-dependent protein kinase pathways. Additionally, glutamine and glutamate can alleviate oxidative stress-induced apoptosis in porcine intestinal epithelial cells, thereby maintaining intestinal barrier integrity. Leucine, an essential amino acid constituting about 7% of total body amino acids, promotes intestinal villus development, stimulates protein synthesis and amino acid transporter expression, and improves growth performance when supplemented in piglet diets.

As a high-quality plant-derived protein source, HWG is widely used in Europe in creep feeds for piglets, aquafeeds, calf milk replacers, and pet foods. Supplementation with HWG in weaned piglet diets significantly increases average daily gain, reduces feed conversion ratio and diarrhea incidence, and improves intestinal morphology by increasing villus height and decreasing crypt depth, thereby mitigating the adverse effects of weaning stress on intestinal mucosa and growth. HWG can partially replace fish meal, reducing feed costs. During late gestation, rapid fetal and mammary gland development occurs concurrently with dramatic changes in maternal hormones and nutrient metabolism, making maternal nutritional status critical for fetal growth and reproductive performance. However, research on HWG supplementation in sow diets remains scarce. Therefore, this study investigated the effects of HWG supplementation during late gestation on sow reproductive performance, serum biochemical parameters and amino acid concentrations, and placental nutrient transporter gene expression to provide a scientific basis for HWG application in sow production.

Materials and Methods

Experimental Material

The HWG used in this experiment was provided by Zhengzhou Newwill Nutrition Technology Co., Ltd., and its amino acid composition is presented in Table 1 .

Experimental Animals, Grouping, and Management

Thirty-three Large White sows at day 90 of gestation (3–4 parities) with similar expected farrowing dates were randomly allocated to three groups with 11 replicates per group and one sow per replicate. A basal diet for gestating sows was formulated according to NRC (2012) standards. The control group received the basal diet, while treatment groups received the basal diet supplemented with 1% or 2% HWG from days 90 to 114 of gestation. All diets were made isonitrogenous and isocaloric using alanine and corn starch, with essential amino acids balanced. Diet composition and nutrient levels are shown in Table 2 . Sows were fed twice daily (06:00 and 14:00) at 3 kg/day. One week before the expected farrowing date, sows were transferred to thoroughly disinfected farrowing rooms, and routine management procedures were followed throughout the trial.

Sample Collection and Analysis

Reproductive Performance Measurement: Within 4 hours after farrowing, total piglets born, live-born piglets, stillbirths, total litter birth weight, and live litter birth weight were recorded for each sow. Average birth weight and average live birth weight were calculated.

Serum Biochemical Parameters: On day 107 of gestation, six sows per group were randomly selected for jugular vein blood collection. During farrowing, umbilical cord blood was collected from six randomly selected sows per group. Blood samples were allowed to clot at room temperature for 1 hour, then centrifuged at 3,000×g for 10 minutes. Serum was harvested and stored at -20°C for subsequent biochemical analysis.

Serum Free Amino Acid Analysis: Serum free amino acid concentrations were determined by high-performance liquid chromatography (HPLC). Sample preparation involved thawing serum samples on ice, mixing 20 L serum with 80 L of 1.5 mol/L HClO to precipitate proteins, vortexing, and incubating at 4°C for 30 minutes. After adding 40 L of 2 mol/L K CO to neutralize pH and vortexing, samples were centrifuged at 14,800 r/min for 10 minutes. The supernatant (50 L) was diluted threefold with HPLC-grade water, then mixed with 50 L of 1.2% benzoic acid and 700 L HPLC water in an autosampler vial for analysis using a Thermo Fisher Scientific HPLC system.

Placental Nutrient Transporter Gene Expression: When placentas were expelled during natural farrowing, chorionic villus samples were collected and stored at -20°C. Total RNA was extracted using TRIpure reagent kit (Aidlab Biotechnologies, Beijing), and RNA concentration was determined using a nucleic acid/protein analyzer (IMPLEN, Germany). Complementary DNA was synthesized (Tiangen Biotech, Beijing) and analyzed by real-time quantitative PCR (7500 Real-Time PCR, Applied Biosystems, USA) using primers listed in Table 3 . The 10 L reaction mixture contained 1 L cDNA template, 0.3 L each of forward and reverse primers (10 mol/L), 5 L 2×SuperReal Premix, 0.2 L Rox dye, and nuclease-free water. Cycling conditions were: 95°C for 15 minutes, followed by 40 cycles of 95°C for 10 seconds and 60°C for 32 seconds. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal reference gene, and relative mRNA expression was calculated using the $2^{(-\Delta\Delta Ct)}$ method.

Statistical Analysis

Data were analyzed using one-way ANOVA in SAS 9.1.3 software and graphed using GraphPad Prism 6.0. Results are expressed as means ± standard error. Differences were considered significant at P<0.05.

Results

Effects of HWG on Sow Reproductive Performance

As shown in Table 4 , compared with the control group, 1% HWG supplementation significantly increased the average birth weight of live piglets (P<0.05), while 2% HWG supplementation significantly increased both total litter birth weight and total live litter birth weight (P<0.05). Dietary HWG supplementation had no significant effects on total piglets born per litter, live-born piglets per litter, stillbirths, or average birth weight of all piglets (P>0.05).

Effects of HWG on Serum Biochemical Parameters

Table 5 presents the effects of HWG on serum biochemical parameters. Dietary supplementation with 1% HWG significantly decreased blood ammonia concentrations in both sow and umbilical cord serum on day 107 of gestation (P<0.05). The 2% HWG supplementation significantly increased albumin, glucose, and alkaline phosphatase activity in umbilical cord serum while reducing blood ammonia in both sow and cord serum (P<0.05).

Effects of HWG on Serum Amino Acid Concentrations

Dietary HWG supplementation significantly altered serum amino acid profiles in both sows and umbilical cord blood. As shown in Table 6 , 1% and 2% HWG supplementation significantly increased asparagine, histidine, and ornithine concentrations in sow serum while decreasing glycine levels (P<0.05). The 1% HWG group also showed elevated valine in sow serum (P<0.05), whereas the 2% HWG group exhibited increased isoleucine and threonine in sow serum (P<0.05).

In umbilical cord serum (Table 7), 1% HWG supplementation significantly increased histidine, isoleucine, leucine, phenylalanine, and tyrosine concentrations (P<0.05). The 2% HWG group showed significant increases in arginine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine (P<0.05).

Effects of HWG on Placental Nutrient Transporter Gene Expression

Figure 1 [Figure 1: see original paper] illustrates the effects of HWG on placental nutrient transporter mRNA expression. Compared with the control, 1% HWG supplementation significantly increased mRNA expression of SNAT2, EAAT3, LAT2, and GLUT2 (P<0.05). The 2% HWG supplementation significantly upregulated SNAT1, SNAT2, EAAT3, LAT2, PepT1, PepT2, GLUT1, GLUT2, and GLUT3 mRNA expression ($P<0.05$).

Discussion

Fetal nutrient supply during gestation depends on both maternal nutritional status and placental transport efficiency. In commercial swine production, feed restriction is commonly practiced during gestation to prevent excessive maternal fat deposition, but this may also limit fetal nutrient availability and growth. Approximately 60% of fetal weight gain occurs during late gestation, particularly between days 90 and 114, making the potential negative impacts of feed restriction most pronounced during this critical period. Protein nutrition plays a vital role in regulating fetal growth, development, and survival, with protein deficiency or imbalance potentially reducing fetal weight gain, decreasing uniformity, and increasing the incidence of intrauterine growth restriction (IUGR).

HWG is a natural plant-derived protein rich in glutamine, glutamate, leucine, and bioactive peptides, though its effects on fetal development have not been previously reported. In this study, 2% HWG supplementation during late gestation significantly increased total litter birth weight and live litter birth weight, consistent with previous findings that dietary glutamine supplementation improves live litter weight and reduces within-litter variation. Analysis of serum amino acids revealed that HWG supplementation enhanced amino acid concentrations in both sow and umbilical cord blood, suggesting improved maternal-fetal amino acid transfer. Specifically, 1% and 2% HWG increased asparagine, histidine, and ornithine in sow serum while reducing glycine, and elevated multiple essential amino acids in umbilical cord serum, indicating enhanced fetal amino acid availability.

Serum biochemical analysis showed that HWG supplementation significantly reduced blood ammonia concentrations in both sows and umbilical cord blood, while 2% HWG increased albumin, glucose, and alkaline phosphatase activity in cord serum. These findings suggest that HWG improves fetal nutrient supply and protein utilization efficiency, which may contribute to the observed increase in piglet birth weight. The placenta serves as the primary interface for maternal-fetal nutrient transfer, with amino acid and glucose transporters playing crucial regulatory roles. This study demonstrated that 2% HWG supplementation significantly upregulated mRNA expression of multiple amino acid transporters (SNAT1, SNAT2, EAAT3, LAT2), peptide transporters (PepT1, PepT2), and glucose transporters (GLUT1, GLUT2, GLUT3) in placental tissue. Since glucose is the primary energy substrate for fetal and placental tissues, and amino acids can stimulate protein synthesis via the mTOR pathway to regulate fetal growth, these results suggest that HWG enhances fetal development by modulating placental nutrient transport capacity.

Overall, the results indicate that HWG supplementation during late gestation improves sow serum amino acid status, modulates placental amino acid and glucose transporter expression, enhances maternal-fetal nutrient transfer and protein utilization efficiency, and promotes fetal growth, ultimately increasing litter birth weight. Given China' s abundant wheat gluten resources, optimizing enzymatic hydrolysis processes to produce high-quality HWG could facilitate more efficient utilization of existing protein resources, reduce dependence on imported soybean meal, and promote sustainable development of the swine industry.

Conclusion

Dietary supplementation with HWG during late gestation enhances placental nutrient transporter gene expression, improves maternal-fetal nutrient transfer efficiency, promotes fetal growth and development, and increases total litter birth weight and live litter birth weight in sows.

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