Expression Patterns of Defensin Gene mRNA in Different Tissues of Wujin and Yuedawu Pigs and the Modulatory Effects of Antimicrobial Substances from the East Asian Migratory Locust on Their Expression in Immune-Stressed Cells (Postprint)

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Abstract

This study aimed to detect the differences in mRNA expression of β-defensin-1 (pBD-1), β-defensin-2 (pBD-2), and β-defensin-3 (pBD-3) genes among different tissues in Wujin pigs and Yedawu pigs with Wujin pig ancestry, and to investigate the regulatory effects of antimicrobial active substances from the Oriental migratory locust (LAAS) on immune stress parameters and defensin (pBDs) gene mRNA expression in porcine fetal skin fibroblasts (PFSF). Real-time fluorescent quantitative PCR was used to detect the differential mRNA expression of pBD-1, pBD-2, and pBD-3 genes in 12 tissues including heart, liver, lung, kidney, jejunum, ileum, duodenum, skin, muscle, pancreas, testis or ovary from 2-month-old Wujin pigs and Yedawu pigs; using PFSF cultured to the 4th-5th passage, an immune stress model was established by lipopolysaccharide (LPS) induction, and different concentrations (0, 75, 150, 300, 600, and 1,200 g/mL) of LAAS were added to DMEM/F12 culture medium to investigate the effects of LAAS on immune stress parameters and pBD-1, pBD-2, pBD-3 gene mRNA expression in PFSF. The results showed that: 1) Serum lysozyme (LSZ) activity and immunoglobulin G (IgG) content in Wujin pigs were significantly higher than those in Yedawu pigs ($P<0.05$), while serum nitric oxide (NO) content was highly significantly lower than that in Yedawu pigs ($P<0.01$). 2) The mRNA expression levels of pBD-1, pBD-2, and pBD-3 genes in most tissues were higher in Yedawu pigs than in Wujin pigs; the mRNA expression levels of pBD-1 and pBD-3 genes were relatively high in skin and ovary, while pBD-2 gene mRNA expression was relatively high in liver and kidney in both pig breeds. 3) 300

g/mL LAAS highly significantly decreased lactate dehydrogenase (LDH) activity in the culture medium (P<0.01); LAAS highly significantly increased NO content in the culture medium (except for normal cells in the 75 g/mL group) (P<0.01), with both normal and stressed cells reaching the maximum at 600 g/mL LAAS concentration; NOS activity in stressed cells was significantly or highly significantly higher than that in normal cells at LAAS concentrations of 0, 75, and 150 g/mL (P<0.05 or P<0.01); addition of 1,200 g/mL LAAS could highly significantly reduce LSZ activity in the culture medium (P<0.01), and the difference in LSZ activity between stressed and normal cells was significant or highly significant at LAAS concentrations of 150, 300, 600, and 1,200 g/mL (P<0.05 or P<0.01). 4) The mRNA expression levels of pBD-1, pBD-2, and pBD-3 genes in stressed cells were higher than those in normal cells; the expression levels of pBD-1, pBD-2, and pBD-3 genes mRNA reached the maximum at 150 g/mL LAAS concentration. The results suggest that there are obvious breed differences and tissue specificities in defensin gene expression between Wujin pigs and Yedawu pigs, and appropriate concentrations of LAAS can reduce LDH activity, increase NO content and NOS and LSZ activities, and upregulate defensin expression in both stressed and normal PFSF.

Full Text

Expression Patterns of β-Defensin Genes in Different Tissues of Wujin and Yuedawu Pigs and the Regulatory Effect of Locust Antibacterial Active Substances on Their Expression in Immune-Stressed Cells

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Abstract

This study aimed to investigate the differential mRNA expression of β-defensin-1 (pBD-1), β-defensin-2 (pBD-2), and β-defensin-3 (pBD-3) genes across different tissues of Wujin pigs and Yuedawu pigs (a crossbreed containing Wujin pig lineage), and to explore the regulatory effects of locust antibacterial active substances (LAAS) on immune stress parameters and pBDs gene mRNA expression in porcine fetal skin fibroblasts (PFSF). Using real-time quantitative PCR, we examined the expression differences of pBD-1, pBD-2, and pBD-3 genes in twelve tissues (heart, liver, lung, kidney, jejunum, ileum, duodenum, skin, muscle, pancreas, testes or ovary) of 2-month-old Wujin and Yuedawu pigs. PFSF cells at passages 4-5 were cultured and an immune stress model was established by lipopolysaccharide (LPS) induction. Different concentrations of LAAS (0, 75, 150, 300, 600, and 1,200 g/mL) were added to DMEM/F12 culture medium to investigate their effects on immune stress parameters and pBD-1, pBD-2, and

pBD-3 gene mRNA expression in PFSF cells.

The results showed: (1) Serum lysozyme (LSZ) activity and immunoglobulin G (IgG) content were significantly higher in Wujin pigs than in Yuedawu pigs ($P < 0.05$), while serum nitric oxide (NO) content was extremely significantly lower ($P < 0.01$). (2) The mRNA expression levels of pBD-1, pBD-2, and pBD-3 genes were higher in most tissues of Yuedawu pigs compared to Wujin pigs. Both breeds exhibited high pBD-1 and pBD-3 mRNA expression in skin and ovary, whereas pBD-2 mRNA expression was predominantly high in liver and kidney. (3) LAAS at 300 g/mL extremely significantly decreased lactate dehydrogenase (LDH) activity in culture medium ($P < 0.01$). LAAS extremely significantly increased NO content in culture medium (except for the 75 g/mL group in normal cells) ($P < 0.01$), with both normal and stressed cells reaching peak NO levels at 600 g/mL LAAS. NOS activity in stressed cells was significantly or extremely significantly higher than in normal cells at LAAS concentrations of 0, 75, and 150 g/mL ($P < 0.05$ or $P < 0.01$). The addition of 1,200 g/mL LAAS extremely significantly reduced LSZ activity in culture medium ($P < 0.01$), with significant differences observed between stressed and normal cells at LAAS concentrations of 150, 300, 600, and 1,200 g/mL ($P < 0.05$ or $P < 0.01$). (4) The mRNA expression of pBD-1, pBD-2, and pBD-3 genes was higher in stressed cells than in normal cells, reaching maximum expression at 150 g/mL LAAS. These findings indicate that β-defensin gene expression exhibits significant breed-specific and tissue-specific patterns in Wujin and Yuedawu pigs. Appropriate LAAS concentrations can reduce LDH activity, increase NO content and NOS/LSZ activities, and upregulate defensin expression in both stressed and normal PFSF cells.

Keywords: Wujin pigs; Yuedawu pigs; β-defensins; immune stress; locust antibacterial active substances

Introduction

Porcine diseases are becoming increasingly complex, making the search for novel, safe, and effective antibiotic alternatives a critical research priority in swine nutrition and health management. Animal health represents a fundamental biological basis for survival, reproduction, and genetic potential expression. Nutrition and immunity are central themes in animal health research, as disease-resistant genes are closely associated with animal health, and nutritional interventions can improve immunity and modulate disease-resistant genes, thereby enhancing disease resistance and production performance. Antimicrobial peptides show promising potential as non-toxic, residue-free antimicrobial additives. Defensins (pBDs) constitute the largest family in the animal defense system, characterized as cysteine-rich cationic peptides that form three intramolecular disulfide bonds with molecular masses of 2-6 kDa. Based on disulfide bond positions and spatial structures, defensins are classified into α-defensins, β-defensins, and -defensins.

In pigs, only β-defensins have been identified, with β-defensin-1 (pBD-1), β-defensin-2 (pBD-2), and β-defensin-3 (pBD-3) being the most extensively studied [1]. Defensins exhibit broad-spectrum antimicrobial, antiviral activity, and non-specific cytotoxic effects, while also regulating adaptive immunity and other antimicrobial immune responses. Diamond et al. [2] first discovered defensins in bovine tracheal mucosal epithelial cells, consisting of 38-42 amino acid residues and primarily localized in mammalian skin and mucosal epithelial cells. Herrera et al. [3] reported that human pBD-2 and pBD-3 are closely associated with antiviral resistance mechanisms against HIV in adult oral epithelial cells. Kallóo et al. [4] demonstrated that selected reaction monitoring (SRM) enables rapid analysis of defensin correlations with biological samples.

Insects possess unique immune mechanisms dominated by antimicrobial peptides in their defense system. Luo et al. [5] reported that locusts contain inducible antibacterial active substances. Wen et al. [6] demonstrated that silkworm antimicrobial peptides could replace traditional feed antibiotics in pig and poultry diets, suggesting that insect antimicrobial peptides may regulate animal defensin gene expression as immunomodulators. Indigenous Yunnan Wujin pigs, adapted to high-altitude ecological conditions, have developed biological characteristics including roughage tolerance, slow growth, and strong stress resistance (disease and antioxidant capacity) through long-term evolution. Yuedawu pigs, developed through modern genetic breeding techniques, contain 25% Wujin pig lineage, exhibiting improved lean meat percentage and growth rate, potentially accompanied by altered stress resistance. This study investigated the expression of antimicrobial pBD-1, pBD-2, and pBD-3 genes across different tissues of Wujin and Yuedawu pigs, and explored the regulatory effects of locust antibacterial active substances (LAAS) on pBD-1, pBD-2, and pBD-3 gene expression in porcine immune-stressed cells.

Materials and Methods

1.1 Experimental Animals and Serum Biochemical Index Determination

Twelve healthy 2-month-old Wujin pigs and twelve Yuedawu pigs with consistent origin, identical rearing conditions, and similar body weight (half male and half female) were randomly selected. Five milliliters of blood were collected from the anterior vena cava after fasting to prepare serum for determination of lysozyme (LSZ), immunoglobulin G (IgG), nitric oxide synthase (NOS), lactate dehydrogenase (LDH), and nitric oxide (NO) activities or concentrations. IgG content was measured using an automatic biochemical analyzer, while NO content and NOS, LDH, and LSZ activities were determined by colorimetric methods using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.2 Tissue Collection

Experimental piglets were slaughtered at 07:30 after fasting and weighing. Twelve tissues including heart, liver, lung, kidney, jejunum, ileum, duodenum, skin, muscle, pancreas, testes or ovary were collected. After removing adipose tissue, samples were rapidly minced, snap-frozen in liquid nitrogen, and stored at -80°C.

1.3 Cell Culture

Porcine fetal skin fibroblasts (PFSF) at passages 4-5 were cultured in DMEM/F12 (1:1) complete medium containing 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% glutamine at 37°C in a 5% CO_2 incubator.

1.4 Experimental Materials

Locust (*Locusta migratoria*) adult extracts were provided by the Monogastric Animal Nutrition Laboratory of Yunnan Agricultural University' s Key Laboratory of Animal Nutrition and Feed Science (extraction rate: 8.62%; antibacterial active substance concentration: 11.78 mg/mL). Antibacterial active substances were induced using a needle puncture method with *E. coli* immersion. Specifically, adult locusts were punctured in the abdomen and immersed in log-phase *E. coli* suspension for 10 minutes, then maintained for 24 hours before collection. Insect bodies were stored at -80°C overnight, homogenized, and centrifuged to collect the supernatant [5].

1.5 Establishment of LPS-Induced Porcine Cell Immune Stress Model

LPS was added to basal culture medium at concentrations of 0 (control), 0.1, 1.0, 10.0, and 100.0 g/mL to induce immune stress, with 24 replicates per group (one 24-well plate). Cell morphology was observed at 0, 9, 18, and 36 hours post-culture [Figure 1: see original paper]. Based on these observations, 10 g/mL LPS and 18 hours were selected as the experimental induction concentration and time point. In stressed cell cultures, medium was removed and cells were washed with phosphate-buffered saline (PBS) before adding 600 g/mL LAAS. The expression of pBD-1, pBD-2, and pBD-3 genes was detected at 0, 3, 6, 12, 24, and 48 hours, and the 24-hour time point (showing highest expression for all three genes) was selected for subsequent experiments. Subsequently, six LAAS concentrations (0, 75, 150, 300, 600, and 1,200 g/mL) were examined for their effects on pBD-1, pBD-2, and pBD-3 gene expression in PFSF cells.

Figure 1. PFSF in different states: A) untreated cells; B) immune-stressed cells induced by 10 g/mL LPS; C) cells cultured in LAAS-containing medium for 24 hours after stress; D) dead cells from excessive stress induced by 100 g/mL LPS.

1.6 Determination of LDH, NOS, LSZ Activities and NO Content in Cell Culture Medium

LDH activity was measured by colorimetry, NO content by nitrate reductase method, NOS activity by colorimetry, and LSZ activity by turbidimetry. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.7 Total RNA Extraction

Total RNA from tissues and cells was extracted using the RNAsimple Total RNA Kit (Tiangen, Beijing) according to the manufacturer' s instructions (1 mL lysis buffer per 50-100 mg tissue). After grinding and homogenization, chloroform was added for extraction. Purified total RNA was dissolved in ultrapure water, and purity/concentration were assessed spectrophotometrically at 260 nm and 280 nm. Samples with OD_{260}/OD_{280} ratios between 1.8-2.0 were accepted.

1.8 Reverse Transcription

Equal concentrations of total RNA samples were used to prepare reverse transcription reaction mixtures (10 µL total volume) following the kit protocol (TaKaRa, Japan). Complementary DNA (cDNA) products for real-time PCR were stored at -20°C.

1.9 Conventional PCR Results

Conventional PCR was performed using reverse-transcribed RNA as template. PCR products were analyzed by 2% agarose gel electrophoresis [Figure 2: see original paper]. Clear, bright bands without non-specific products indicated specific amplification of target genes.

Figure 2. 2% agarose gel electrophoresis of PCR products. Marker: 100 bp DNA ladder; 1: pBD-1 gene; 2: pBD-2 gene; 3: pBD-3 gene; 4: β-actin; 5: glyceraldehyde-3-phosphate dehydrogenase gene; 6: 18S rRNA.

1.10 Real-Time Fluorescence Quantitative PCR

The cDNA was used for real-time quantitative PCR of reference genes (glyceraldehyde-3-phosphate dehydrogenase [GAPDH], 18S rRNA, β-actin [ACTB]) and target genes (pBD-1, pBD-2, pBD-3). Primers were designed using Primer Express Software (PE Applied Biosystems, CA) with parameters listed in Table 1. Eva Green I dye-based reactions were performed on a Bio-Rad CFX96TM Real-Time PCR System (USA) in 20 µL volumes containing: 10 µL SsoFastTM EvaGREEN® Supermix (Bio-Rad, USA), 1.0 µL each of forward and reverse primers (100 µmol/L), 2.0 µL cDNA template, and nuclease-free water to 20 µL. Samples were loaded in 96-well plates (Bio-Rad, USA) with optical caps. Cycling conditions: 95°C for 10 min; 40 cycles of 95°C for 5 s, 60°C for 20 s, and 72°C for 15 s.

Table 1. Parameters of primers for RT-qPCR

Gene	Primer Sequences (5'-3')	Amplification length/bp	GenBank accession No.
β-defensin-1 (pBD-1)	5'-ACCGCCTCCTCCTTGTAT-3' 5'-GGTGCCGATCTGTTTCAT-3'	-	NM_{213838}.1
β-defensin-2 (pBD-2)	5'-CCTGCTTACGGGTCTTG-3' 5'-TCTGCTGTGGCTTCTGG-3'	-	NM_{214442}.1
β-defensin-3 (pBD-3)	5'-GAAGTCTACAGAAGCCAAAT-3' 5'-GGTAACAAATAGCACCATAA-3'	-	NM_{214444}.1
β-actin (ACTB)	5'-TCTGGCACCACACCTTCT-3' 5'-TGATCTGGGTCATCTTCTCAC-3'	-	DQ178122
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	5'-ACATCAAGAAGGTGGTGAAG-3' 5'-ATTGTCGTACCAGGAAATGAG-3'	-	AF017079.1
18S rRNA	5'-CTGCCTTCCTTGGATGTG-3' 5'-GCGGCTTTGGTGACTCTA-3'	-	HQ127318.1

1.11 Data Analysis

Defensin expression levels in all tissues were normalized to GAPDH, 18S rRNA, and ACTB as reference genes, with final results expressed as relative mRNA expression levels calculated using the Pfaffl method [7]. Data were processed in Excel and analyzed using SPSS 19.0 software, with results presented as mean ± standard deviation. Inter-tissue gene expression differences were analyzed by Duncan' s multiple comparison test. $P < 0.05$ was considered significant and $P < 0.01$ extremely significant.

Results

2.1 Serum Biochemical Indices of Wujin and Yuedawu Pigs

As shown in Table 2, serum LSZ activity and IgG content were significantly higher in Wujin pigs than in Yuedawu pigs ($P < 0.05$), while serum NO content was extremely significantly lower ($P < 0.01$). Serum LDH and NOS activities were lower in Wujin pigs but without significant differences ($P > 0.05$).

Table 2. Serum biochemical indices of Wujin and Yuedawu pigs

Items	Yuedawu pigs	Wujin pigs
Lysozyme (LSZ) (U/mL)	214.18$±7.63$^b|294.67±9.86$^a\|\|$ImmunoglobulinG(IgG)(mg/mL)$\|65.86±2.46$^b|81.27±2.08$^a\|\|$I

In the same row, values with different small letter superscripts differ significantly ($P < 0.05$), and with different capital letter superscripts differ extremely significantly ($P < 0.01$). Values with no letter or the same letter superscripts show no significant difference ($P > 0.05$).

2.2 Tissue-Specific Expression Patterns of pBD Genes

2.2.1 pBD-1 Gene mRNA Expression Across Tissues As shown in Figure 3-a, pBD-1 gene mRNA was detected in all tissues of both Wujin and Yuedawu pigs by real-time quantitative PCR. In Wujin pigs, pBD-1 expression was highest in skin and ovary, and lowest in heart and kidney. In Yuedawu pigs, expression was similarly high in skin and ovary, but lowest in heart and muscle.

2.2.2 pBD-2 Gene mRNA Expression Across Tissues As shown in Figure 3-b, pBD-2 gene mRNA was detected in all tissues of both breeds. In Wujin pigs, expression was highest in liver and kidney, and lowest in heart and muscle. In Yuedawu pigs, expression was also highest in liver and kidney, but lowest in muscle and pancreas.

2.2.3 pBD-3 Gene mRNA Expression Across Tissues As shown in Figure 3-c, pBD-3 gene mRNA was detected in all tissues of both breeds. In Wujin pigs, expression was highest in skin and testes, and lowest in heart and duodenum. In Yuedawu pigs, expression was highest in skin and ovary, and lowest in muscle and jejunum.

For the same breed, columns with different small letters differ significantly ($P < 0.05$) and with different capital letters differ extremely significantly ($P < 0.01$). For the same tissue, columns marked with differ significantly ($P < 0.05$) and ** differ extremely significantly ($P < 0.01$). The same applies to Figure 4.*

Figure 3. mRNA expression differences of pBD-1, pBD-2, and pBD-3 genes in different tissues of Wujin and Yuedawu pigs.

2.3 Effects of LAAS on Stress Parameters in PFSF Culture Medium

As shown in Table 3, in normal cells, 300 g/mL LAAS extremely significantly decreased LDH activity compared to the control group ($P < 0.01$), with activity increasing again at higher concentrations. In stressed cells, 300 g/mL LAAS also extremely significantly reduced LDH activity ($P < 0.01$), followed by increased activity at higher concentrations.

Regardless of cell type, NO content in culture medium was extremely significantly higher in all LAAS groups compared to the control (except the 75 g/mL group in normal cells) ($P < 0.01$), peaking at 600 g/mL LAAS in both normal $(16.50\$\pm3.76\mu g/mL)and stressed cells(20.39\pm\$2.81$ g/mL).

In normal cells, NOS activity increased with LAAS concentration. In stressed cells, NOS activity decreased with increasing LAAS concentration. NOS activity in stressed cells was significantly or extremely significantly higher than in normal cells at LAAS concentrations of 0, 75, and 150 g/mL ($P < 0.05$ or $P < 0.01$).

In normal cells, LSZ activity was extremely significantly lower than control at 600 and 1,200 g/mL LAAS ($P < 0.01$). In stressed cells, LSZ activity was extremely significantly lower than control at 300 and 1,200 g/mL LAAS ($P < 0.01$). Significant differences between stressed and normal cells were observed at 150, 300, 600, and 1,200 g/mL LAAS ($P < 0.05$ or $P < 0.01$).

Table 3. Effect of LAAS on stress parameters in PFSF culture medium

Items	Cell type	LAAS concentration (g/mL)	0	75	150	300	600	1,200
LDH (U/L)	Normal	5,468.71$±96.97^{Aa}\|5,094.46±109.23^{Aab}\|5,468.71±96.97^{ABbc}\|5,094.46±109.23^{Bc}\|5,468.71± \|23.79±1.05^{ABbc} * *|31.61±1.33^{ABab} * |23.79±1.05^{ABab}|31.61±1.33^{ABabc}|23.79±1.05^{Ba}|||$Stressed$|31.61±1.33^{Aa}* |23.79±1.05^{Ab} * *|31.61±1.33^{ABab} * |23.79±1.05^{Bb}|31.61±1.33^{ABb}|23.79±1.05^{b}||$LSZ(U/mL)$|$Normal$|294.67±9.86^{Aa}|214.18±7 |214.18±7.63^{ABa} * *|294.67±9.86^{Bb} * *|214.18±7.63^{Bb} * |||$Stressed$|294.67±9.86^{cc}|214.18±7.63^{cc}|294.67±9.86^{Bb}* |214.18±7.63^{Dd} * *|294.67±9.86^{Aa} * *|214.18±$7.63 *						

In the same row, values with different small letter superscripts differ significantly ($P < 0.05$) and with different capital letters differ extremely significantly ($P < 0.01$). In the same column, values of the same index with differ significantly ($P < 0.05$) and ** differ extremely significantly ($P < 0.01$).*

2.4 Effects of LAAS on pBD-1, pBD-2, and pBD-3 Gene mRNA Expression in PFSF

As shown in Figure 4-a, pBD-1 mRNA expression was higher in stressed cells than in normal cells, with extremely significant differences at 0, 75, and 300 g/mL LAAS ($P < 0.01$) and a significant difference at 150 g/mL ($P < 0.05$). Expression increased with LAAS concentration in both cell types, peaking at 150 g/mL before declining.

As shown in Figure 4-b, pBD-2 mRNA expression was higher in stressed cells than in normal cells, with extremely significant differences at 0, 75, 150, and 300 g/mL LAAS ($P < 0.01$) and a significant difference at 600 g/mL ($P < 0.05$). Expression increased with LAAS concentration, reaching maximum at 150 g/mL before decreasing.

As shown in Figure 4-c, pBD-3 mRNA expression was higher in stressed cells than in normal cells, with extremely significant differences at 0 and 75 g/mL LAAS ($P < 0.01$) and significant differences at 150 and 1,200 g/mL ($P < 0.05$). Expression increased with LAAS concentration, peaking at 150 g/mL before declining.

Figure 4. Effect of LAAS on mRNA expression of pBD-1, pBD-2, and pBD-3 genes in PFSF.

Discussion

3.1 Changes in Serum Biochemical Indices

Blood immunoglobulin concentration is a crucial indicator of piglet immune function and health status. IgG provides both immunological replacement and immunomodulatory therapeutic effects [8]. Our results showed that Wujin pigs had significantly higher serum IgG content than Yuedawu pigs, while exhibiting lower serum NO content and LDH and NOS activities. NO plays an important role in immune responses by non-specifically killing bacteria, fungi, parasites, and tumor cells, thereby enhancing non-specific immunity. Appropriate NO levels help boost host immune capacity. Wujin pigs also showed significantly higher serum LSZ activity, which has been shown to improve piglet performance, reduce diarrhea rates, and enhance immunity in livestock production [9]. These results suggest that the immune function of the bred Yuedawu pig has undergone adaptive changes.

3.2 Breed Differences and Tissue Specificity

This study found that both Wujin and Yuedawu pigs exhibited high pBD-1 expression in skin and ovary, high pBD-2 expression in liver and kidney, similar to results reported by Veldhuizen et al. [10] in crossbred pigs (Yorkshire × Dutch Landrace). The pBD-3 expression pattern resembled pBD-1, with high expression in skin, testes, and ovary, which partially differs from Sang et al. [11] who reported higher pBD-3 expression in duodenum, liver, skin, testes, and epididymis of distantly crossbred pigs. These discrepancies may be attributed to breed and age differences, as previous studies have demonstrated that defensin gene expression patterns vary with age and health status [12-13]. To date, no studies have reported defensin gene expression differences across tissues in indigenous Yunnan highland Wujin pigs and Yuedawu pigs.

As the first barrier between the organism and environment, skin damage facilitates direct pathogen invasion, leading to inflammation and disease. Our findings of high pBD-1 and pBD-3 expression in skin of both breeds suggest that abundant cutaneous defensins enhance disease resistance.

The small intestine serves as both the primary site for digestion/absorption and an important immune organ. An et al. [14] reported that pBD-1 and pBD-3 expression in porcine intestine increases from birth to 60 days of age. Yuedawu pigs showed higher pBD-1 and pBD-3 expression in small intestine than Wujin pigs, indicating adaptive changes in intestinal immune function in this crossbreed containing Wujin lineage.

Defensin gene expression was detected in heart, kidney, and muscle tissues. Consistent expression patterns were observed in liver and kidney, with Yuedawu pigs showing higher expression than Wujin pigs. Notably, pBD-2 expression in liver and kidney was extremely significantly higher than in other tissues, representing the highest expression among all 12 examined tissues, consistent with

Veldhuizen et al. [10] in crossbred pigs.

In pancreas, pBD-1 expression was lower in Yuedawu than Wujin pigs, while pBD-2 and pBD-3 expression were higher. The relationship between these expression differences and pancreatic function requires further investigation. Overall, defensin gene expression was higher in most tissues of Yuedawu pigs compared to Wujin pigs.

3.3 Changes in Cellular Immune Stress Parameters

This study utilized locusts as experimental material, employing the needle puncture with *E. coli* immersion method to induce antibacterial active substances. Zou [15] screened various induction methods for housefly larvae antimicrobial peptides and identified pig manure rearing as optimal. Suo et al. [16] found that needle puncture with *E. coli* produced the strongest antimicrobial activity in yellow mealworm and beet armyworm larvae. LDH, present in all porcine tissues and organs, is an internationally recognized indicator for assessing cell viability, growth status, and damage, as damaged cells release LDH through cytolysis. Luo et al. [17] reported that L-arginine reduces LDH activity while increasing NO content and NOS/LSZ activities in PFSF culture medium. Our results showed that LPS stimulation elevated immune stress parameters including NO content and LDH, NOS, and LSZ activities, while appropriate LAAS concentrations helped maintain normal cell morphology and growth. Studies have shown that promoting inducible NOS production through leukocyte reduction can protect intestines from inflammatory damage [18].

3.4 LAAS Induction of Defensin Gene Expression in PFSF

Cell culture results demonstrated that LAAS induced defensin gene expression, with LPS-stressed cells showing significantly higher expression than normal cells. Although the molecular mechanisms of defensin production remain unclear, bacterial components such as LPS and flagellin are recognized by pattern recognition receptors to initiate immune defense responses [19], leading to abundant defensin production. Studies have shown that LPS can induce defensin production in epithelial cells [20], consistent with our findings.

Within our tested LAAS concentration range, induction of defensin gene expression was observed, with 150 g/mL LAAS significantly enhancing pBD-1, pBD-2, and pBD-3 mRNA expression. Research indicates that insect antimicrobial peptides exhibit broad inhibitory effects against bacteria, fungi, viruses, tumor cells, and pathogens [21], supporting our results. The optimal dose-response relationship between LAAS concentration and β-defensin expression in porcine tissues requires further investigation. Our findings on the regulatory effects of different tissues and LAAS concentrations on defensin gene expression in immune-stressed cells and corresponding immune stress parameters suggest that the mechanisms by which LAAS enhances porcine immunity and disease resistance warrant deeper investigation.

Conclusions

1. Wujin pigs exhibited significantly higher serum LSZ activity and IgG content, and extremely significantly lower serum NO activity compared to Yuedawu pigs.
2. Defensin gene expression showed significant breed-specific and tissue-specific patterns in Wujin and Yuedawu pigs. Both breeds displayed high pBD-1 and pBD-3 expression in skin and ovary, and high pBD-2 expression in liver and kidney.
3. Appropriate LAAS concentrations reduced cellular LDH activity while increasing NO content and NOS/LSZ activities.
4. Appropriate LAAS concentrations upregulated defensin expression in both stressed and normal cells, with maximal pBD-1, pBD-2, and pBD-3 mRNA expression observed at 150 g/mL LAAS.

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