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Original Research Article

Aerial ammonia exposure induces the perturbation of the interorgan ammonia disposal and branched-chain amino acid catabolism in growing pigs

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ABSTRACT

Aerial ammonia exposure leads to tissue damage and metabolic dysfunction. However, it is unclear how different organs are coordinated to defend against aerial ammonia exposure. Twenty-four pigs were randomly divided into 4 groups, exposed to 0, 10, 25 or 35 mg/m^3 ammonia respectively for 25 d. After above 25 mg/m^3 ammonia exposure, decreased aspartate ($P = 0.016$), glutamate ($P = 0.030$) and increased ornithine ($P = 0.002$) were found in the ammonia-removing liver, and after high ammonia (35 mg/m^3) exposure, glutamine synthetase (*GS*) expression was increased ($P = 0.012$). An increased glutamate ($P = 0.004$) and decreased glutaminase (*GLS*) expression ($P = 0.083$) were observed in the lungs after high ammonia exposure. There was also an increasing trend of glutamine in the kidneys after high ammonia exposure ($P = 0.066$). For branched-chain amino acid (BCAA) catabolism, high ammonia exposure increased BCAA content in both the lungs and muscle ($P < 0.05$), whereas below 25 mg/m^3 ammonia exposure increased BCAA only in the lungs ($P < 0.05$). The expression of BCAA transaminase (*BCAT1/2*) and dehydrogenase complex (*BCKDHA/B* and *DBT*) were inhibited to a varying degree in the liver, lungs and muscle after above 25 mg/m^3 ammonia exposure, especially high ammonia exposure. The expression of *BCKDH* complex and glutamate-glutamine metabolism-related genes were highly expressed in the liver, followed by the lungs and muscle ($P < 0.01$), whereas the *BCAT2* expression was highest in the lungs ($P = 0.002$). Altogether, low ammonia exposure sufficed to evoke the urea cycle to detoxify ammonia in the liver. The process of ammonia removal in the liver and potential ability of the lungs to detoxify ammonia were enhanced with increasing ammonia. Furthermore, high ammonia exposure impaired the BCAA catabolism and decreased the transcripts of the BCAA catabolism-related enzymes, resulting in high BCAA content in extrahepatic tissues. Therefore, with aerial ammonia increasing, an increased urea cycle and glutamine synthesis were ammonia defensive strategies, and high ammonia exposure impaired the BCAA catabolism.

1. Introduction

Ammonia is the most abundant notorious gas emission from agricultural activities including animal husbandry and ammonia-based fertilizer applications (Behera et al., 2013; Ti et al., 2019; Zeng et al., 2018). Industrial processes and human activities, such as power plants, automobiles and airplane emissions, also give out ammonia (Boyle, 2017). Ammonia plays a vital role in the formation of $PM_{2.5}$ and haze, causing human and animal health problems over the past few decades (Bauer et al., 2016; Kim et al., 2020; Shen et al.,

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2019). Atmospheric ammonia exposure damages respiratory mucosa (Shi et al., 2019; Wang et al., 2020) and leads to inflammation or immunotoxicity in many other tissues of animals as well (An et al., 2019; Chen et al., 2019; Wang et al., 2019; Xu et al., 2020). Furthermore, aerial ammonia exposure disturbs lipid metabolism in farm animals (Sa et al., 2018; Zhang et al., 2019; Zhu et al., 2019). We found that exposure to 35 mg/m^3 aerial ammonia activated the mammalian target of rapamycin (mTOR) pathway, consequently up-regulating genes involved in lipogenesis and down-regulating lipolysis genes in the muscle of pigs (Tang et al., 2020). In contrast, aerial ammonia below 25 mg/m^3 has no such effect (Tang et al., 2019). Interestingly, serum and muscular branched-chain amino acids (BCAA, including valine, isoleucine and leucine) were increased by high ammonia exposure when the mTOR pathway was activated (Tang et al., 2020). Therefore, the perturbation of amino acids, particularly BCAA, is one of the critical routes via which aerial ammonia exposure disturbs lipid metabolism.

Ammonia is a normal metabolite of nitrogen-containing compounds in mammals. There is a stringent control mechanism to detoxify ammonia in the body. Ammonia can either lead to synthesis of urea or be incorporated into glutamine (Gln). The liver is the key organ for ammonia metabolism, where ammonia is irreversibly incorporated into urea in the periportal hepatocytes or is reversibly incorporated into Gln in the perivenous hepatocytes (Dimski, 1994; Matoori and Leroux, 2015). In mammals, ammonia is mainly excreted by the kidneys in the form of urea (Weiner et al., 2015). Compared with the urea route, Gln synthesis has a low capacity but a high-affinity to detoxify ammonia (Matoori and Leroux, 2015; Walker, 2014). Extrahepatic tissues, such as the muscle and brain, utilize the Gln synthesis route to detoxify ammonia, which is then transported to the liver to synthesize urea. Therefore, the presence of glutaminase and Gln synthetase, enzymes involved in the conversion of glutamate (Glu) and Gln, is a good indicator for the capability of ammonia detoxification in extrahepatic tissues. In the lungs, glutaminase and Gln synthetase were found (Svenneby and Torgner, 1987). Therefore, the lungs may serve an important role as they stand as the first line of dense when interacting with aerial ammonia. In the biological processes to detoxify ammonia, interorgan ammonia metabolism is coordinated, and Gln, Glu, and amino acids in the urea cycle all play central roles. Some amino acids, such as BCAA, can enhance ammonia detoxification by stimulating Glu synthesis (Holeček, 2017).

Due to the remarkable capacity of multiple organs to detoxify ammonia, a threshold exists before ammonia damages the tissues and interferes with metabolism. Our previous studies have demonstrated the different responses of serum amino acids in pigs exposed to various levels of aerial ammonia, where only high level of aerial ammonia exposure (35 mg/m^3) increased serum BCAA and activated the mTOR pathways. It is yet unclear what the threshold is, and where and how different ammonia-detoxifying organs coordinate at various levels of aerial ammonia exposure. Therefore, the current study aimed to investigate how the primary ammonia-removing tissues were coordinated upon various levels of aerial ammonia exposure, with a particular focus on amino acid metabolism.

2. Materials and methods

2.1. Ethics statement

All procedures used in this experiment have been approved by the Experimental Animal Welfare and Ethical Committee of Institute of Animal Science of Chinese Academy of Agricultural Sciences (IAS2017-2). Minimum numbers of pigs were used with an effort to minimize stress during handling.

2.2. Animals and design

Twenty-four 10-week-old Yorkshire × Landrace gilts (20.60 ± 0.28 kg) purchased from a commercial farm (Beijing Breeding Pig Co., LTD, Beijing, China) were randomly assigned into 4 groups (6 pigs in each group). Groups were firstly the control group (Con, < 2.5 mg/m^3 ammonia), secondly the low ammonia group (LA, 7.5 to 12.5 mg/m^3 ammonia), thirdly the medium ammonia group (MA, 22.5 to 27.5 mg/m^3 ammonia) and finally the high ammonia group (HA, 32.5 to 37.5 mg/m^3 ammonia). All animals were kept in an individual cage and each group was maintained in a separate controlled-environment chamber. The ToxiRAE Pro Ammonia (NH_3) Detector (RAE Systems, San Jose, CA, USA) was used to monitor the concentration of ammonia, and ammonia was sent into each chamber via a ventilation system before being mixed with air. During the 25-d experiment, the animals were given ad libitum access to clean drinking water and an amount of commercial feed (Appendix Table 1) equal to 4% to 5% of body weight per day. The average daily feed intake per pig (1.028 vs. 1.104 vs. 1.048 vs. 1.048 kg/d, $P = 0.353$) showed no difference among the 4 groups. After 25 d, all animals were humanely euthanized and the liver, lungs, kidneys, and longissimus dorsi muscle were removed, and tissue samples were collected and snap-frozen in liquid nitrogen for determination of free amino acids (FAA) and gene expression. The timeline is shown in Fig. 1A.

2.3. Quantification of free amino acid by targeted metabolomics

Sample preparation: About 80 to 100 mg tissues were homogenized in 600 μL of 0.1 mol/L hydrochloric acid using a high-through tissuelyser (SCIENTZ-48, SCIENTZ, Ningbo, China). The mixture was centrifuged at 25,000 × *g* for 20 min at 4 °C, and then the collected supernatant was diluted two times with 0.1 mol/L hydrochloric acid, then finally passed through a 0.45-μm Millex-LG filter (Millipore, Billerica, MA, USA) for LC-MS/MS analysis. The resultant residues of tissue were homogenized again in 1-mL RIPA lysis buffer (Solarbio, Beijing, China) containing a protease inhibitor cocktail (Roche, Basel, Switzerland) and phosphatase inhibitors (Biotopped, Beijing, China) to extract total protein, which was quantified with a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

Parameters of high-performance liquid chromatography/mass spectrometry: HPLC-MS/MS (Agilent, Santa Clara, CA, USA) equipped with an Agilent Poroshell 120 HILIC-Z column (3.0 × 100 mm, 2.7 μm, Agilent) was used to measure the quantity of FAA in the prepared samples. Briefly, 1 μL filtrate was injected into the HILIC-Z column at 25 °C to separate amino acids. The mobile phases consisted of 20 mmol/L ammonium formate in water solution (solvent A) and 20 mmol/L ammonium formate in acetonitrile-water (vol/vol: 9/1) solution (solvent B). The gradient for amino acid elution switched from 100% solvent B to 70% solvent B after 11.5 min, backed to 100% solvent B in 0.5 min and maintained for 3 min prior to next injection. The flow rate was 0.5 mL/min. Amino acids were determined by Agilent 6470 (Agilent) equipped with an ESI source in the positive ion mode. The gas and sheath gas temperature were set as 330 and 390 °C; gas and sheath gas flow were 13 and 12 L/min; the voltage of nebulizer, capillary and nozzle was 35 psi, 1.5 kV and 0 V, respectively.

Quantification of FAA: All amino acid standards were purchased from Sigma–Aldrich (Merck KGaA, Darmstadt, Germany). The typical residence time for the peak of each amino acid standard is shown in Fig. 1B and C. The relative quantification of each FAA was based upon the single-point calibration of available standards and the quantity of total protein was used to normalize the contents of FAA.

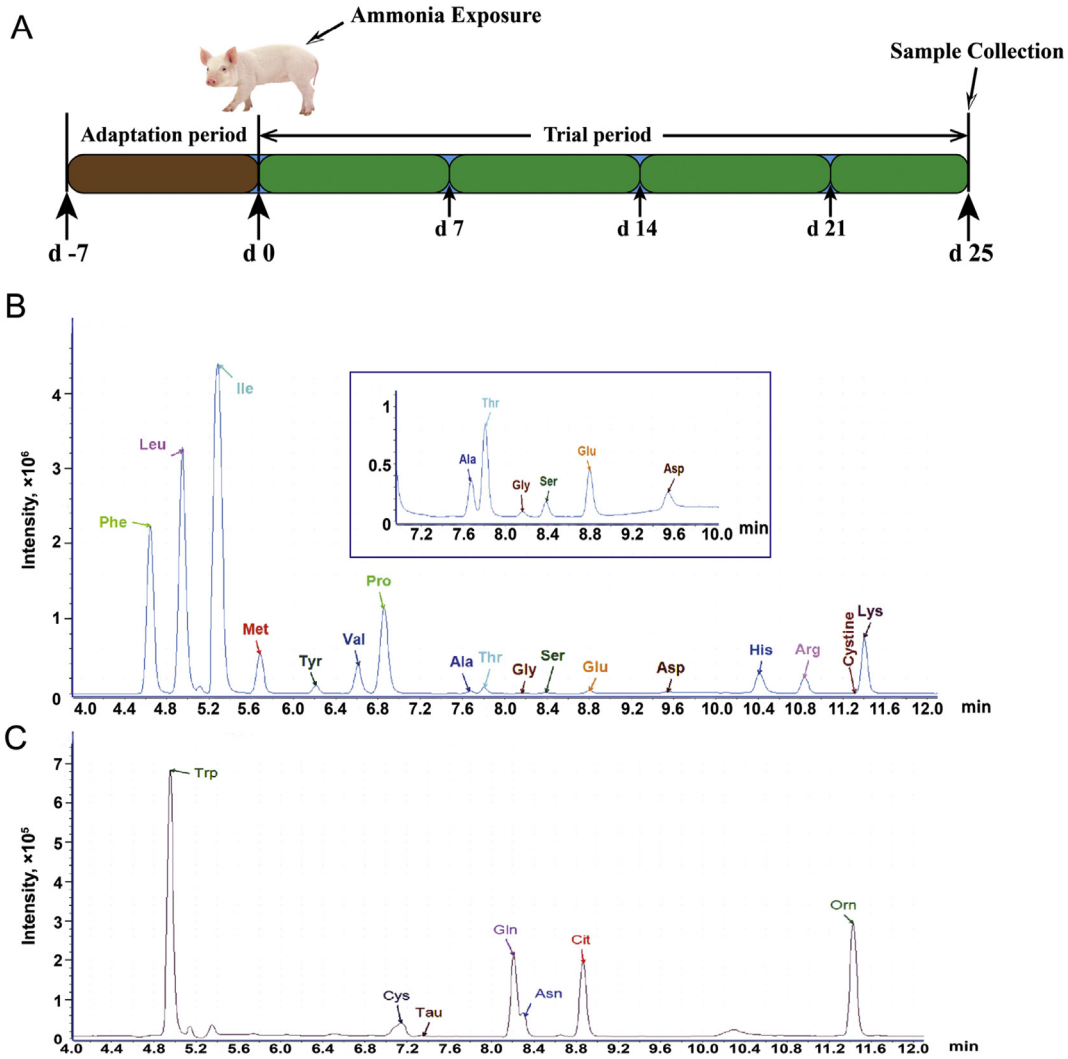


Fig. 1. Trial timeline and standard amino acid peak. Timeline of ammonia exposure trial (A) and the typical residence time for each 17-mix (B) or 7-mix (C) standard amino acid peak.

2.4. Detection of genes expression by qRT-PCR

Total RNA was isolated from muscle tissues with TRIzol reagent (Cat # 15596026, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. The concentration of each RNA sample was determined using the NanoDrop 2000 (Nanodrop Technologies, Wilmington, DE, USA). Genomic DNA contamination was removed by incubating with gDNA Eraser provided by the PrimeScript RT reagent kit (Cat # RR047A, Takara, Shige, Japan) before reverse transcription. qRT-PCR was conducted with a commercial kit (SYBR Premix Ex Taq, Cat # 420A, Takara). A single peak was checked to confirm the specificity of each primer (Appendix Table 2) set in the melting curves after 40 PCR amplification cycles. Relative expression of the target gene between different groups was calculated by $2^{-\Delta\Delta Ct}$ method using the reference genes, geometric mean of β-actin and *GAPDH*.

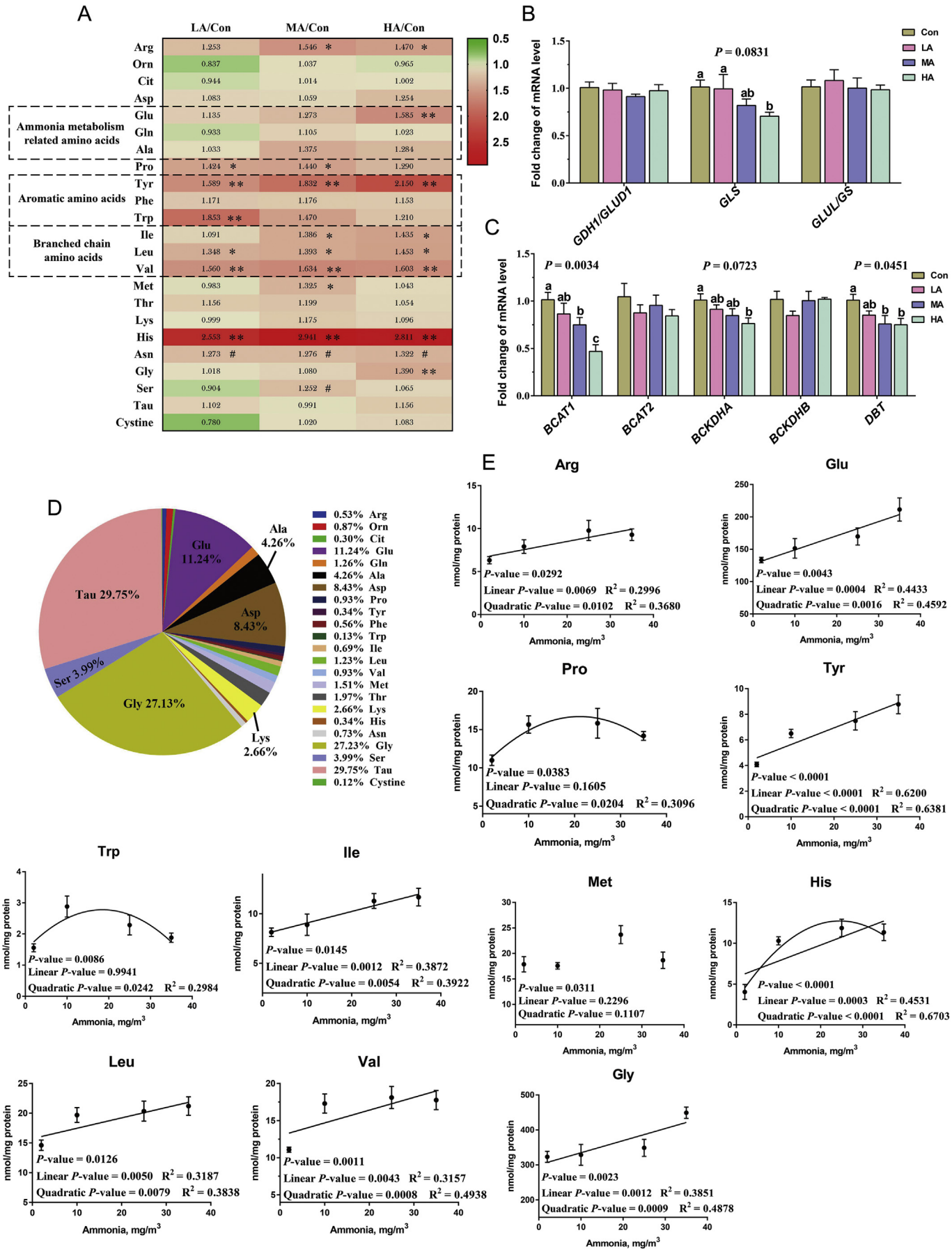
2.5. Statistical analysis

Statistical analysis was performed using JMP software (JMP version 10.0.0, SAS Institute, Cary, NC, USA) for Windows. One-way ANOVA was used to test for differences among 4 groups in FAA contents and gene expression related to ammonia detoxification or BCAA catabolism. In contrast, the Kruskal–Wallis signed-rank test was applied to test whether the expression of genes related to Gln metabolism and BCAA catabolism differed among tissues. Student *t*-test and Wilcoxon test were considered as post-hoc multiple comparison methods for the parameter test and nonparametric test, respectively. Linear and quadratic regression models were performed on related sensitive indicators to fit the effect of ammonia on affected amino acids in different tissues. All data were presented as mean ± SE and $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Free amino acids and expression level of genes related to ammonia metabolism in the lung

In the lungs, the most abundant FAA were taurine (Tau, 29.75%) and glycine (Gly, 27.33%), which consisted of approximately 90% of total FAA with Glu (11.24%), asparagine (Asp, 8.43%), alanine (Ala, 4.26%), serine (Ser, 3.99%), lysine (Lys, 2.66%) and threonine (Thr, 1.97%) (Fig. 2D). Among amino acids related to ammonia


A
LA/Con
MA/Con
HA/Con
Arg 1.253 1.546 * 1.470 *
Orn 0.837 1.037 0.965
Cit 0.944 1.014 1.002
Asp 1.083 1.059 1.254
Glu 1.135 1.273 1.585 **
Gln 0.933 1.105 1.023
Ala 1.033 1.375 1.284
Pro 1.424 * 1.440 * 1.290
Tyr 1.589 ** 1.832 ** 2.150 **
Phe 1.171 1.176 1.153
Trp 1.853 ** 1.470 1.210
Ile 1.091 1.386 * 1.435 *
Leu 1.348 * 1.393 * 1.453 *
Val 1.560 ** 1.634 ** 1.603 **
Met 0.983 1.325 * 1.043
Thr 1.156 1.199 1.054
Lys 0.999 1.175 1.096
His 2.553 ** 2.941 ** 2.811 **
Asn 1.273 # 1.276 # 1.322 #
Gly 1.018 1.080 1.390 **
Ser 0.904 1.252 # 1.065
Tau 1.102 0.991 1.156
Cystine 0.780 1.020 1.083
Ammonia metabolism related amino acids
Aromatic amino acids
Branched chain amino acids
0.5
1.0
1.5
2.0
2.5
B
Fold change of mRNA level
P = 0.0831
Con
LA
MA
HA
GDH1/GLUD1
GLS
GLUL/GS
C
Fold change of mRNA level
P = 0.0034
P = 0.0723
P = 0.0451
BCAT1
BCAT2
BCKDHA
BCKDHB
DBT
D
Glu 11.24%
Ala 4.26%
Asp 8.43%
Tau 29.75%
Ser 3.99%
Gly 27.13%
Lys 2.66%
0.53% Arg
0.87% Orn
0.30% Cit
11.24% Glu
1.26% Gln
4.26% Ala
8.43% Asp
0.93% Pro
0.34% Tyr
0.56% Phe
0.13% Trp
0.69% Ile
1.23% Leu
0.93% Val
1.51% Met
1.97% Thr
2.66% Lys
0.34% His
0.73% Asn
27.23% Gly
3.99% Ser
29.75% Tau
0.12% Cystine
E
Arg
P-value = 0.0292
Linear P-value = 0.0069 R² = 0.2996
Quadratic P-value = 0.0102 R² = 0.3680
Glu
P-value = 0.0043
Linear P-value = 0.0004 R² = 0.4433
Quadratic P-value = 0.0016 R² = 0.4592
Pro
P-value = 0.0383
Linear P-value = 0.1605
Quadratic P-value = 0.0204 R² = 0.3096
Tyr
P-value < 0.0001
Linear P-value < 0.0001 R² = 0.6200
Quadratic P-value < 0.0001 R² = 0.6381
Trp
P-value = 0.0086
Linear P-value = 0.9941
Quadratic P-value = 0.0242 R² = 0.2984
Ile
P-value = 0.0145
Linear P-value = 0.0012 R² = 0.3872
Quadratic P-value = 0.0054 R² = 0.3922
Met
P-value = 0.0311
Linear P-value = 0.2296
Quadratic P-value = 0.1107
His
P-value < 0.0001
Linear P-value = 0.0003 R² = 0.4531
Quadratic P-value < 0.0001 R² = 0.6703
Leu
P-value = 0.0126
Linear P-value = 0.0050 R² = 0.3187
Quadratic P-value = 0.0079 R² = 0.3838
Val
P-value = 0.0011
Linear P-value = 0.0043 R² = 0.3157
Quadratic P-value = 0.0008 R² = 0.4938
Gly
P-value = 0.0023
Linear P-value = 0.0012 R² = 0.3851
Quadratic P-value = 0.0009 R² = 0.4878
nmol/mg protein
Ammonia, mg/m³

metabolism (Fig. 2A and Appendix Table 3), Glu increased when ammonia reached 35 mg/m^3 ($P = 0.004$). For BCAA, leucine (Leu, $P = 0.013$) and valine (Val, $P = 0.001$) were greater in pigs exposed to varying concentrations of aerial ammonia (LA, MA and HA) than the controls, and isoleucine (Ile) was greater in MA and HA group pigs ($P = 0.015$) (Fig. 2A and Appendix Table 3). In addition, arginine (Arg) was greatly increased when aerial ammonia reached 25 mg/m^3 ($P = 0.029$), and both histidine (His, $P < 0.001$) and tyrosine (Tyr, $P < 0.001$) were increased as well when the pigs were exposed to aerial ammonia as shown in Fig. 2A and Appendix Table 3. Regression analyses demonstrated a linear relation between aerial ammonia exposure with Arg, Glu, Tyr, Ile, Leu, Val and Gly, while a quadratic relation was revealed between aerial ammonia exposure and proline (Pro), tryptophan (Trp) and His ($P < 0.05$, Fig. 2E).

Further investigation focusing on the glutamate-glutamine metabolism demonstrated a decreased trend in mRNA abundance of glutaminase (*GLS*) in the HA group compared with the Con group ($P = 0.083$, Fig. 2B). But no difference was found in the expression of Glu dehydrogenase 1 (*GDH1*) or Gln synthetase (*GS*) between the Con group and ammonia exposure groups ($P > 0.05$, Fig. 2B). Since BCAA in the lung were increased, gene expression related to BCAA catabolism was also examined. No significant difference was found in the BCAA transaminase 2 (*BCAT2*) and branched-chain α-keto dehydrogenase β subunit (*BCKDHB*) ($P > 0.05$, Fig. 2C). The expression of *BCAT1* ($P = 0.003$) and dihydrolipoamide branched-chain transacylase (*DBT*, *BCKDH E2* subunit; $P = 0.045$) was decreased in a dose-dependent way in the ammonia-exposed groups (MA and HA, Fig. 2C). A linear downward trend was found in branched-chain α-keto dehydrogenase α subunit (*BCKDHA*) ($P = 0.072$, Fig. 2C).

3.2. Free amino acids and expression level of genes related to ammonia metabolism in the liver

In the liver, Glu (27.81%), Gln (18.83%) and Gly (13.94%) are the 3 most abundant FAA (Fig. 3E). As shown in Fig. 3A and Appendix Table 4, exposure to aerial ammonia significantly enhanced ornithine (Orn) in the liver of pigs by approximately 2-fold ($P = 0.002$). Liver Asp and Glu were reduced when aerial ammonia reached 25 mg/m^3 ($P < 0.05$). Ala content was higher in HA group pigs ($P = 0.017$) while Gln had a decreased trend in MA group pigs ($P = 0.059$). Few changes were found in other AA when aerial ammonia was increased. Regression analyses demonstrated a linear relationship between aerial ammonia exposure with Orn, Glu, Ala, Asp and His ($P < 0.05$), while a quadratic relationship was observed between aerial ammonia exposures and Gln ($P = 0.062$) (Fig. 2F).

To further investigate urea cycle-related genes in the liver, ornithine transcarbamoylase (*OTC*), the enzyme catalyzing the synthesis of citrulline (Cit), was less expressed in the MA and HA groups ($P < 0.05$), whereas no significant difference was found for the carbamoyl phosphate synthase 1 (*CPS1*), argininosuccinate synthase (*ASS*), argininosuccinate lyase (*ASL*) and arginase 1 (*ARG1*) (Fig. 3B). When we tested the expression of glutamate-glutamine metabolism-related genes, only *GS* was found to be increased in the HA group ($P = 0.012$, Fig. 3C). Regarding BCAA catabolism, the ammonia exposure groups decreased the expression of *BCAT1/2* and *BCKDHA/B* in a dose-dependent manner. The expression level of *BCAT1* tended to be lower ($P = 0.087$) and *BCAT2* was significantly lower in the HA group ($P < 0.05$) when compared with the Con group (Fig. 3D). The liver *BCKDHA/B* significantly decreased in the MA and HA groups ($P < 0.05$, Fig. 3D).

3.3. Free amino acids and expression level of genes related to ammonia metabolism in the longissimus dorsi muscle

The top 4 abundant FAA in the muscle (Fig. 4D) were Ala (33.66%), Tau (16.48%), Gly (15.14%) and Cystine (12.19%). The specific FAA content in muscle was shown in Fig. 4A and Appendix Table 5. Except for increased Asp in the HA group ($P = 0.039$), no differences in AA associated with glutamate-glutamine metabolism were found in pigs exposed to ammonia. Compared with the Con group of pigs, Leu ($P = 0.002$) and Ile ($P = 0.001$) were significantly increased, and Val ($P = 0.084$) tended to increase in the HA group. Other AA, Tyr ($P < 0.001$) and Thr ($P < 0.001$) were increased when aerial ammonia reached 25 mg/m^3 and phenylalanine (Phe) was increased when aerial ammonia reached 35 mg/m^3 ($P = 0.014$). Regression analyses demonstrated that the altered AA (including Thr, Asp, Pro, Tyr, Phe, Ile, Leu and Val) and aerial ammonia exposure presented a linear relationship ($P < 0.05$, Fig. 4E).

For genes related to the glutamate-glutamine metabolism, no significant differences were found in the expression level of *GDH1, GLS* and *GS* between the Con group and the groups exposed to ammonia ($P > 0.05$, Fig. 4B). For genes related to the BCAA catabolism, *BCAT2* ($P = 0.032$) and *BCKDHA* ($P = 0.004$) were lower in the HA group than in the Con group (Fig. 4C). *BCAT1* in the HA group was lower than in the LA group ($P = 0.065$), and no differences were found in *BCKDHB* and *DBT* among treatments (Fig. 4C).

3.4. Free amino acids in the kidney

In the kidneys, Glu (43.97%), Gly (20.97%), Tau (8.67%), Ala (6.6%), Gln (4.16%), Asp (3.33%) and Ser (2.62%) consisted of 90% of total FAA (Fig. 5B). Only a significant elevation in Val was observed in pigs exposed to 25 and 35 mg/m^3 ammonia ($P = 0.002$, Fig. 5A and Appendix Table 6). A trend was observed for higher Leu ($P = 0.082$) and Gln ($P = 0.066$) in the HA group pigs compared to the Con group pigs, which, however, was not statistically significant (Fig. 5A and Appendix Table 6). No change in other FAA was observed ($P > 0.05$) between the Con group and ammonia exposure group pigs. The changed AA (including Val, Leu and Gln) and aerial ammonia exposure also presented a linear relationship ($P < 0.05$, Fig. 5C).

3.5. Spatial distributions of enzymes involved in glutamate-glutamine and BCAA catabolism

For enzymes involved in the glutamate-glutamine metabolism, the greatest expression of *GDH1*, the enzyme catalyzing the synthesis of Glu from α-ketoglutarate, was found in the liver, which was 14 times or 87.5 times greater than that in lung or muscle, respectively ($P = 0.005$, Fig. 6A). The most abundant *GS*, the enzyme catalyzing the conversion of Gln from Glu, was found in the liver, which was 3.7 times greater in the lung and 8.3 times greater in the muscle ($P = 0.001$, Fig. 6A). No obvious alteration of *GLS* expression, the enzyme to convert Gln to Glu, was found between in liver and

Fig. 2. Effects of various ammonia exposure on the alterations of free amino acids and related genes expression in lungs of pigs. Fold change of free amino acids (A) and the expression of glutamine synthesis related genes (B) or branched-chain amino acid catabolism related genes (C) in the lungs of pigs after different ammonia exposure. Compositions of free amino acids (D) and linear or quadratic regression models fit the effect of ammonia on the content of sensitive amino acids in pig lung (E) ($n = 5$ to 6 pigs/group). Gene expression data are expressed as mean ± SE. One-way ANOVA was applied to test for differences among groups. $^{*}P < 0.05$,$^{**}P < 0.01$,$^{\#}P < 0.10$. $^{a, b}$ Different letters indicate significant difference among groups at $P < 0.05$. Con is control group, LA, MA and HA are low ammonia group (10 mg/m^3), medium ammonia group (25 mg/m^3) and high ammonia group (35 mg/m^3), respectively.

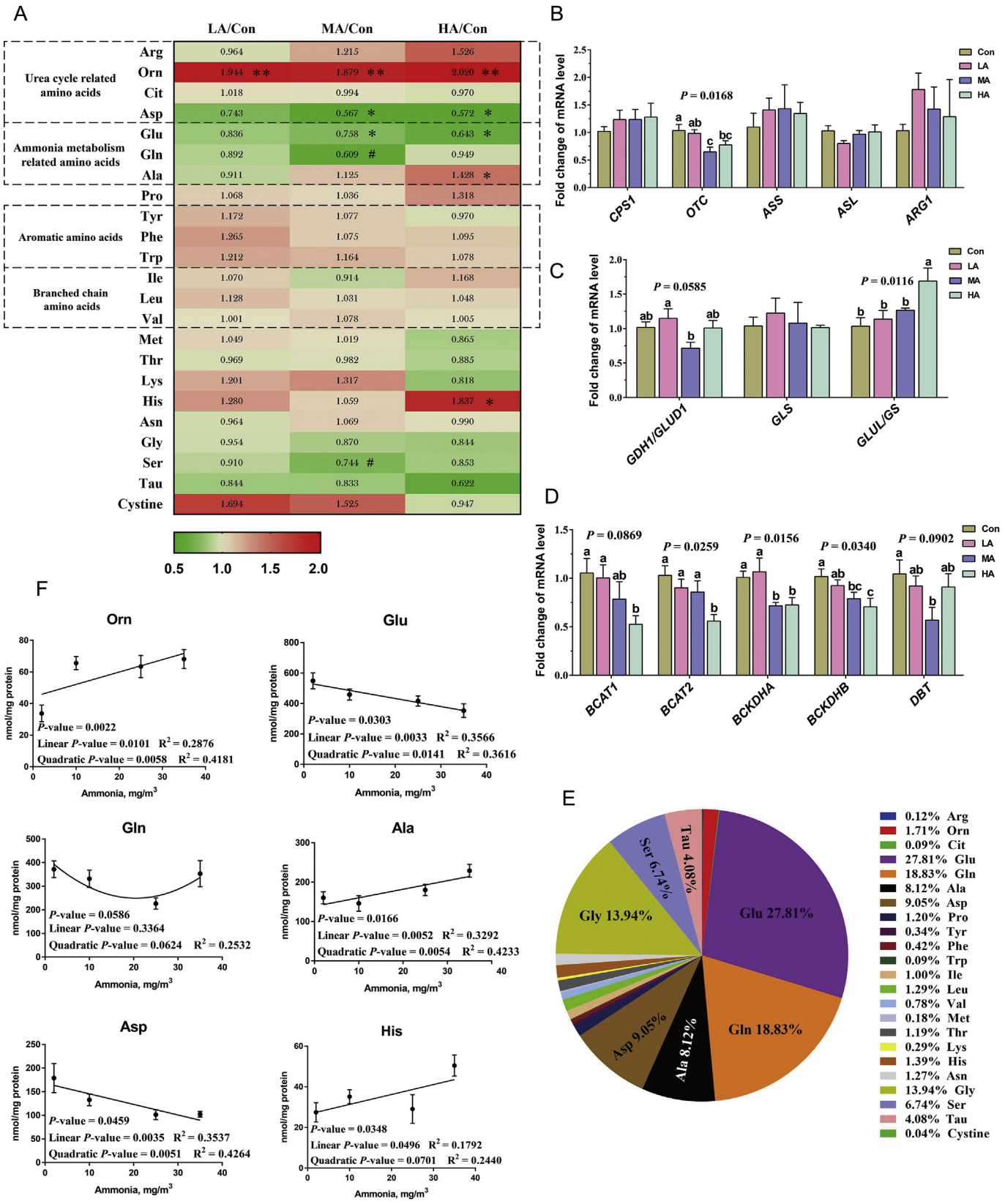


Fig. 3. Effects of various ammonia exposure on the alterations of free amino acids and related genes expression in liver of pigs. Fold change of free amino acids (A) and the expression of urea cycle related genes (B), glutamine synthesis related genes (C) or branched-chain amino acid catabolism related genes (D) in the liver of pigs after different ammonia exposure. Compositions of free amino acids (E) and linear or quadratic regression models fit the effect of ammonia on the content of sensitive amino acids in pig liver (F) (n = 5 to 6 pigs/group). Gene expression data are expressed as mean ± SE. One-way ANOVA was applied to test for differences among groups. $^{*}P < 0.05$,$^{**}P < 0.01$,$^{\#}P < 0.10$. $^{a, b}$ Different letters indicate significant difference among groups at $P < 0.05$. Con is control group, LA, MA and HA are low ammonia group (10 mg/m^3), medium ammonia group (25 mg/m^3) and high ammonia group (35 mg/m^3), respectively.

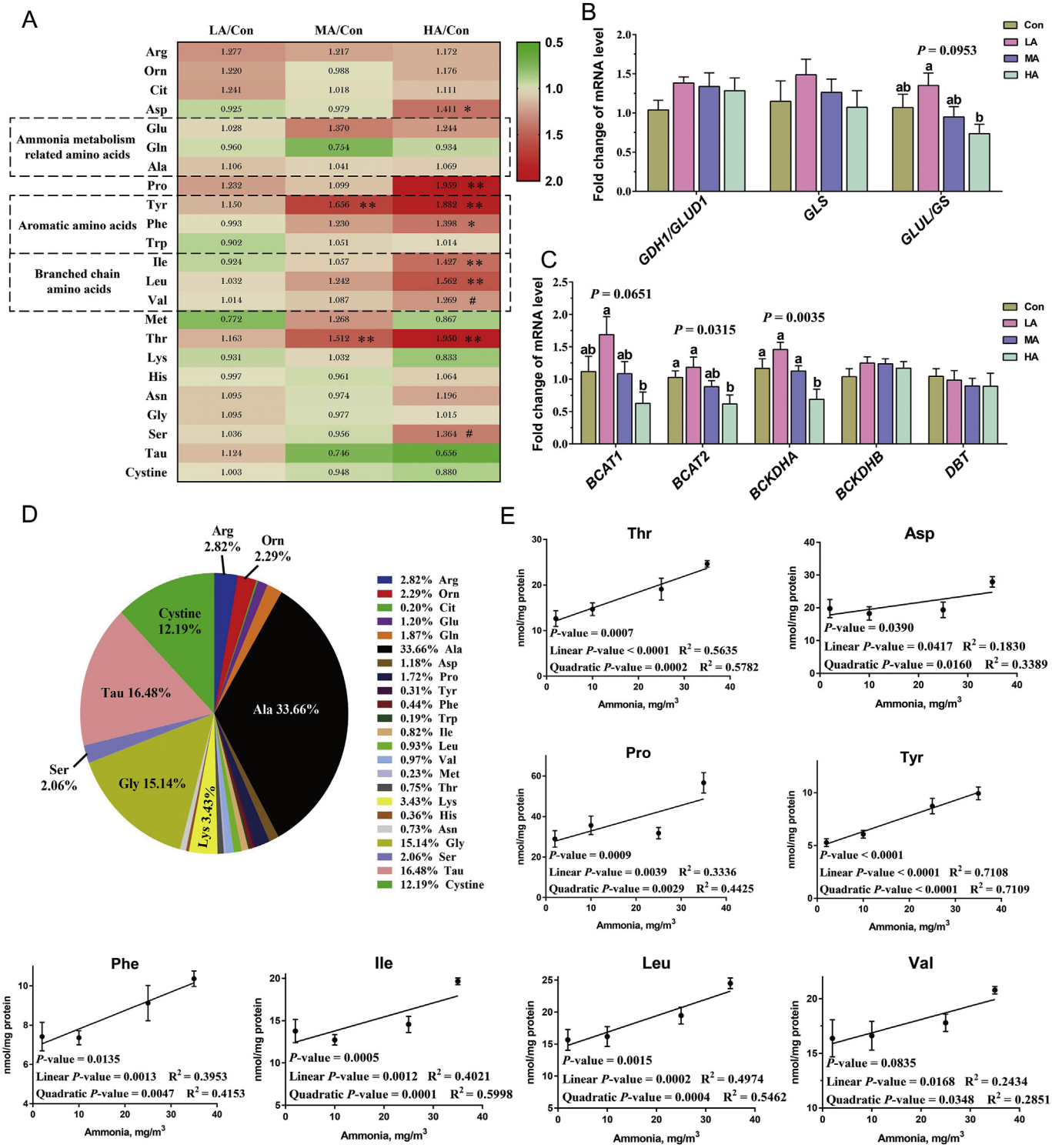


Fig. 4. Effects of various ammonia exposure on the alterations of free amino acids and related genes expression in muscle of pigs. Fold change of free amino acids (A) and the expression of glutamine synthesis related genes (B) or branched-chain amino acid catabolism related genes (C) in the muscle of pigs after different ammonia exposure. Compositions of free amino acids (D) and linear or quadratic regression models fit the effect of ammonia on the content of sensitive amino acids in pig muscles (E) ($n = 5$ to 6 pigs/group). Gene expression data are expressed as mean ± SE. One-way ANOVA was applied to test for differences among groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\#}P < 0.10$. $^{a, b}$ Different letters indicate significant difference among groups at $P < 0.05$. Con is control group, LA, MA and HA are low ammonia group (10 mg/m^3), medium ammonia group (25 mg/m^3) and high ammonia group (35 mg/m^3), respectively.

lungs, which is more than 25 times higher than that in the muscle ($P = 0.003$, Fig. 6A).

For enzymes involved in BCAA catabolism, *BCAT1* and BCKD complex subunits were highly expressed in the liver, where liver *BCAT1* was 1.7 times and 22 times greater, liver *BCKDHA* was 15.4 and 67.7 times greater, and liver *BCKDHB* was 10.2 and 50.1 times greater than that in the lungs and muscle ($P < 0.01$, Fig. 6B). The highest expression of *BCAT2* was found in the lungs ($P < 0.01$,

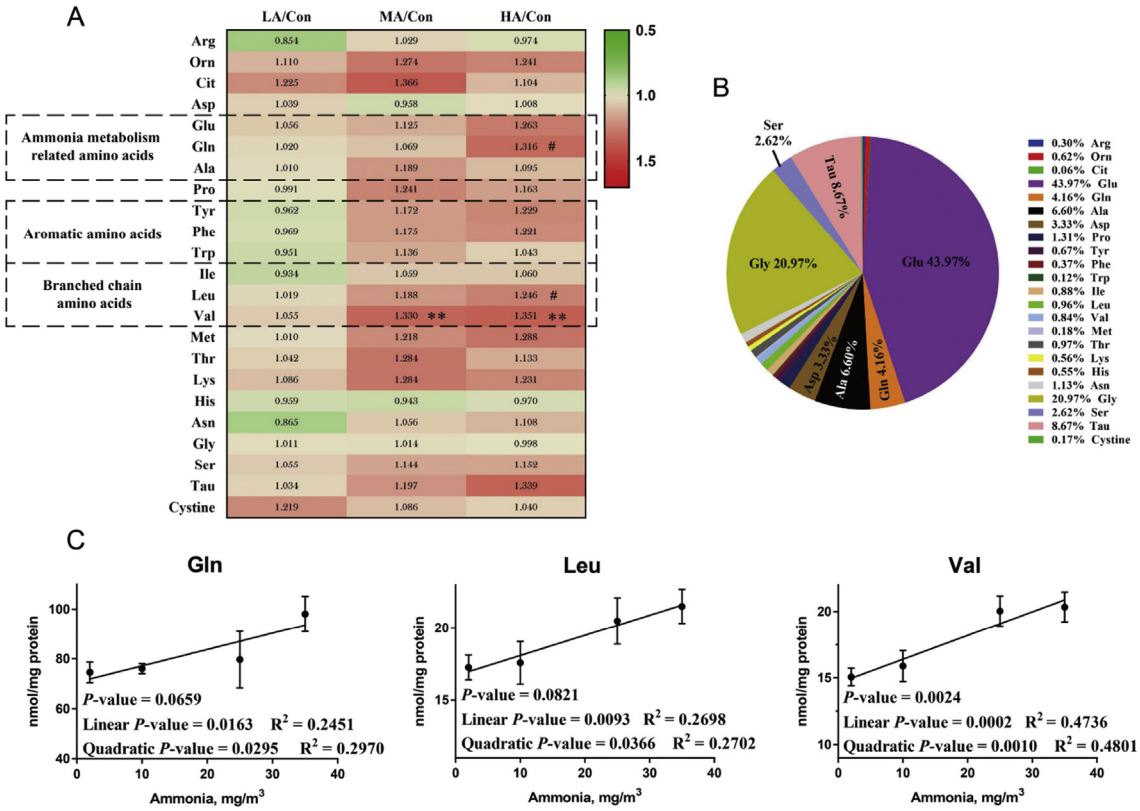


Fig. 5. Effects of various ammonia exposure on the alterations of free amino acids in kidney of pigs. (A) Fold change of FAA in the kidney of pigs exposed to different ammonia. (B) The compositions of free amino acids. (C) Linear or quadratic regression models fit the effect of ammonia on the content of sensitive amino acids in pig kidney. $n = 5$ to 6 pigs/group. One-way ANOVA was applied to test for differences among groups. #$P < 0.10$, $^{**}P < 0.01$. Con is control group, LA, MA and HA are low ammonia group (10 mg/m^3), medium ammonia group (25 mg/m^3) and high ammonia group (35 mg/m^3), respectively.

Fig. 6B), and no difference in *BCAT2* expression was found between the liver and muscle.

4. Discussion

Ammonia is toxic to cells and must be either converted into urea in the liver or be incorporated into Gln. During the process of ammonia detoxification, many AA are involved. In the present study FAA were profiled across the tissues of the lungs, liver, muscle and kidneys, which are considered as major ammonia-removing tissues in pigs. Our data demonstrated that FAA profile and enzymes catalyzing the glutamate-glutamine conversion greatly differ at the transcriptional level among these ammonia-removing tissues. These tissues displayed various sensitivities to aerial ammonia, as indicated by changes in FAA composition (Table 1). At the lowest level of ammonia exposure (10 mg/m^3), BCAA, Trp, His and Pro in the lungs, and Orn in the liver, were increased. When aerial ammonia was increased to 25 mg/m^3, Arg in the lungs, as well as Tyr and Thr in the muscle, were increased and Glu, Gln and Asp in the liver were decreased. When the level of aerial ammonia reached the highest level (35 mg/m^3), it led to the most abundant changes in the amino acid profile in all examined tissues. These observations suggested that the lungs and liver have superior sensitivity to inhaled ammonia, especially the lungs.

The liver, as the major ammonia metabolising organ, adopted both the urea cycle and the Gln route to detoxify ammonia (Dimski, 1994; Matoori and Leroux, 2015). These two routes are present in functional groups of hepatocytes and have different capacity and affinity. The urea cycle utilized a free ammonia and the other ammonia from Asp to synthesize urea. As urea leaves the cycle, Arg is converted to Orn to restart the urea cycle. Following its function in ammonia metabolism, the ammonia-induced changes in FAA profile mainly occur in AA integrally involved in the two routes. As shown, Asp was decreased, and Orn was increased when aerial ammonia started at 10 mg/m^3 and linear relationships were found between Asp or Orn and the aerial ammonia levels. Thus, the urea cycle is quickly enhanced at the low level of aerial ammonia (Fig. 7E). With the accumulation of urea produced, greater serum urea was observed when aerial ammonia reached 35 mg/m^3 (Tang et al., 2020). Despite higher differences from the trend point of view, no significant difference was found in the gene expression of *CPS1*, *ASS*, *ASL* and *ARG1*, suggesting the enhanced urea cycle may be independent of gene transcription of enzymes involved in the urea cycle. However, the high level of ammonia exposure may also down-regulate enzymes (such as *OTC*) of the urea cycle via the p53 (Li et al., 2019) and limit the process of conversion of Arg to Orn. The highest expression of *GDH1*, *GLS* and *GS* indicates the importance of glutamate-glutamine metabolism in the liver. Free Glu in liver dropped gradually after exposure to 25 or 35 mg/m^3 ammonia and the level of *GS* sharply increased after exposure to 35 mg/m^3 (Fig. 7F). Our previous studies found that serum Gln increased until aerial ammonia reached 35 mg/m^3 (Tang et al., 2019, 2020). These findings suggest the biosynthesis of Gln is favored at a high level of ammonia exposure. There is a discrepancy in liver Gln, which was reduced by 25 mg/m^3 aerial ammonia exposure but did not change after 10 or 35 mg/m^3 ammonia exposure. There are several sources of liver Gln. Extrahepatic tissue deposits ammonia in the form of Glu or Ala, which are transported to the liver to metabolize into urea. Therefore, the level of liver Gln is not only affected by the biosynthesis from Glu but also by the uptake of Gln.

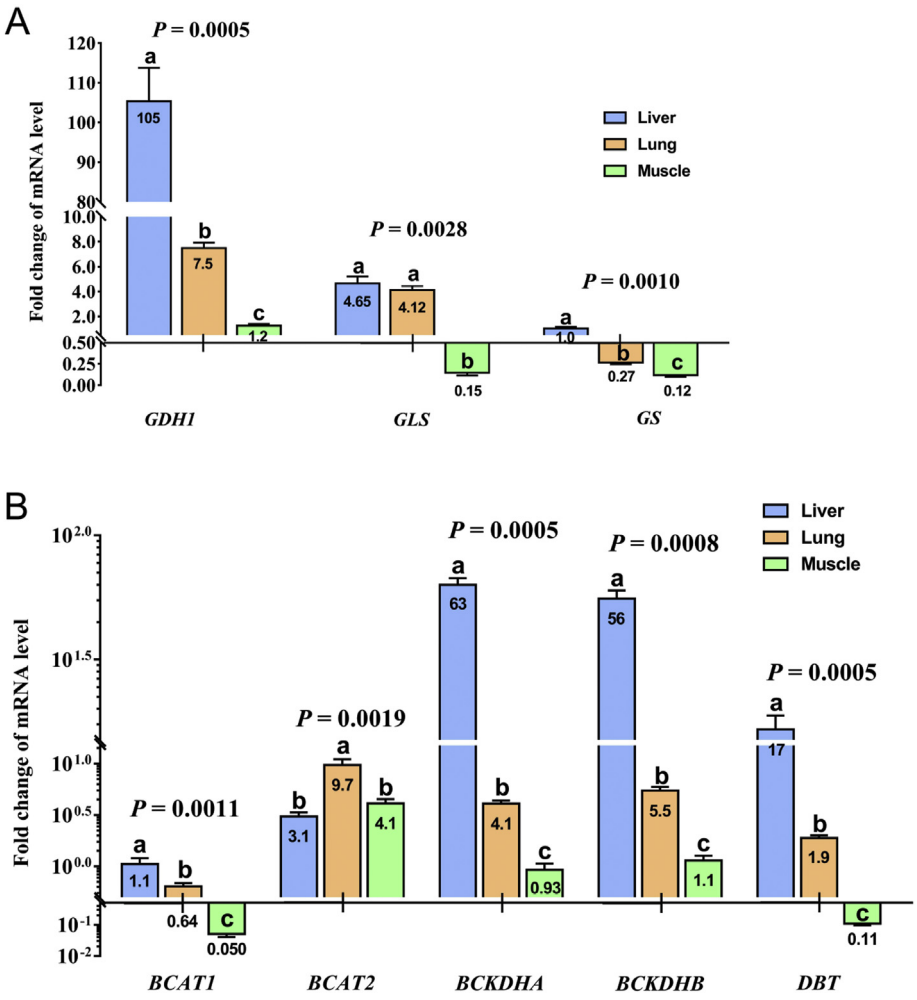


Fig. 6. Spatial distributions of amino acid metabolism-related genes. The expression of glutamine synthesis related genes (A) and branched-chain amino acid catabolism related genes (B) in different tissues. All data are expressed as mean ± SE. Kruskal–Wallis signed-rank test (nonparametric test) was applied to test for differences among groups. Different letters indicate significant differences in each gene abundance among different tissues. *GDH1* = GLu dehydrogenase 1; *GLS* = glutaminase; *GS* = glutamine synthetase; *BCAT1/2* = branched-chain amino acid aminotransferase 1/2; *BCKDHA/B* = branched-chain α-keto dehydrogenase α/β subunit; *DBT* = dihydrolipoamide branched-chain transacylase.

As shown by the spatial distribution of enzymes associated with glutamate-glutamine metabolism, the lungs have fewer transcripts of enzymes than the liver, but more transcripts than the muscle. Therefore, the lungs can play an important role in removing ammonia, especially when the respiratory tract directly interacts with aerial ammonia. Most of the inhaled ammonia gets dissolved in the mucus of the upper respiratory tract and approximately 75% gets excreted in exhaled air, so long-term ammonia exposure causes some absorption into the systemic circulation (Padappayil and Borger, 2020; Silverman et al., 1949). The potential ammonia-removing ability of the lungs was increased by the high level of ammonia exposure, which was indicated by decreased *GLS* expression and increased Glu content in the lungs (Fig. 7G). Lung Arg was increased after exposure to 25 mg/m^3. Unlike in the liver, Arg is catabolized to synthesize NO by nitric oxide synthase (NOS) or to synthesize Pro and polyamines by arginase in the lungs (King et al., 2004). It is reported that high concentrations of NO may be detrimental by promoting inflammation/cytokines and by causing tissue injury (Earnest et al., 2004). Several studies have reported that oxidative damage and inflammation in the respiratory tract have occurred after aerial ammonia exposure (Shi et al., 2019; Wang et al., 2020; Xiong et al., 2016), which is closely related to the dysregulation in Arg metabolism in lung tissue. Therefore, during higher aerial ammonia, conversion to Pro is favored, while Arg metabolism is disturbed, as shown by increased Pro and Arg in the lung. Meanwhile, histamine, a sign of inflammation (Holecek, 2020) and Tyr, mediators of oxidative stress (Ipson and Fisher, 2016), were greatly increased by ammonia exposure. These findings suggest a low level of ammonia might induce inflammatory responses in the lungs.

Skeletal muscle, with a much lower expression of *GS*, is considered as a major ammonia-removing organ due to its large mass, especially during the dysfunction of the liver (Adeva et al., 2012; Hakvoort et al., 2017; He et al., 2010; Olde Damink et al., 2002). Here, no significant alterations were observed in the level of Gln or Glu content and the mRNA level of *GDH*, *GLS* or *GS* with increasing concentration of ammonia exposure. Thus, the skeletal muscle contributed little to ammonia detoxification during ammonia exposure with a functional liver (Fig. 7A). The kidneys play a fundamental role in ammonium homeostasis and urea excretion (Adeva et al., 2012; Weiner et al., 2015). The elevated Gln content in kidneys and increased urea in serum after high ammonia exposure indicated the enhanced process of ammonia exclusion (Fig. 7I).

Table 1
The summary of the altered amino acids and genes expression across 4 different tissues after various concentrations of ammonia exposure.

Tissues	Ammonia concentration		
	LA (10 mg/m^3)	MA (25 mg/m^3)	HA (35 mg/m^3)
Lung	BCAA: Leu↑, Val↑ Aromatic AA: Tyr↑, Trp↑ Pro↑, His↑	BCAA: Leu↑, Val↑, Ile↑ Aromatic AA: Tyr↑ Arg↑, Pro↑, Met↑, His↑ *BCAT1* expression↓, *DBT* expression↓	Glu↑, *GLS* expression↓ BCAA: Leu↑, Val↑, Ile↑ Aromatic AA: Tyr↑ Arg↑, His↑, Gly↑ *BCAT1* expression↓, *BCKDHA* expression↓, *DBT* expression↓
Liver	Urea cycle: Orn↑	Urea cycle: Orn↑, Asp↓, *OTC* expression↓ Glu↓, Gln↓ *BCKDHA/B* expression↓, *DBT* expression↓	Urea cycle: Orn↑, Asp↓, *OTC* expression↓ Glu↓, *GS* expression↑ Ala↑, His↑ *BCKDHA/B* expression↓
Muscle	___	Aromatic AA: Tyr↑ Thr↑	BCAA: Leu↑, Val↑, Ile↑ Aromatic AA: Tyr↑, Phe↑ Asp↑, Pro↑, Thr↑, Ser↑ *BCAT2* expression↓, *BCKDHA* expression↓
Kidney	___	BCAA: Val↑	BCAA: Leu↑, Val↑ Gln↑
Serum[1]	Val↓	Aromatic AA: Tyr↑	Orn↑, Gln↑ Aromatic AA: Tyr↑, Phe↑, Trp↑ BCAA: Leu↑, Ile↑, Val↑ His↑, Asn↑

BCAA = branched-chain amino acid; *BCKDHA/B* = branched-chain α-keto dehydrogenase α/β subunit; *DBT* = dihydrolipoamide branched-chain transacylase; *OTC* = ornithine transcarbamoylase; *BCAT* = branched-chain amino acid aminotransferase; *GLS* = glutaminase; *GS* = glutamine synthetase.
[1] By Tang et al. (2020, 2019).

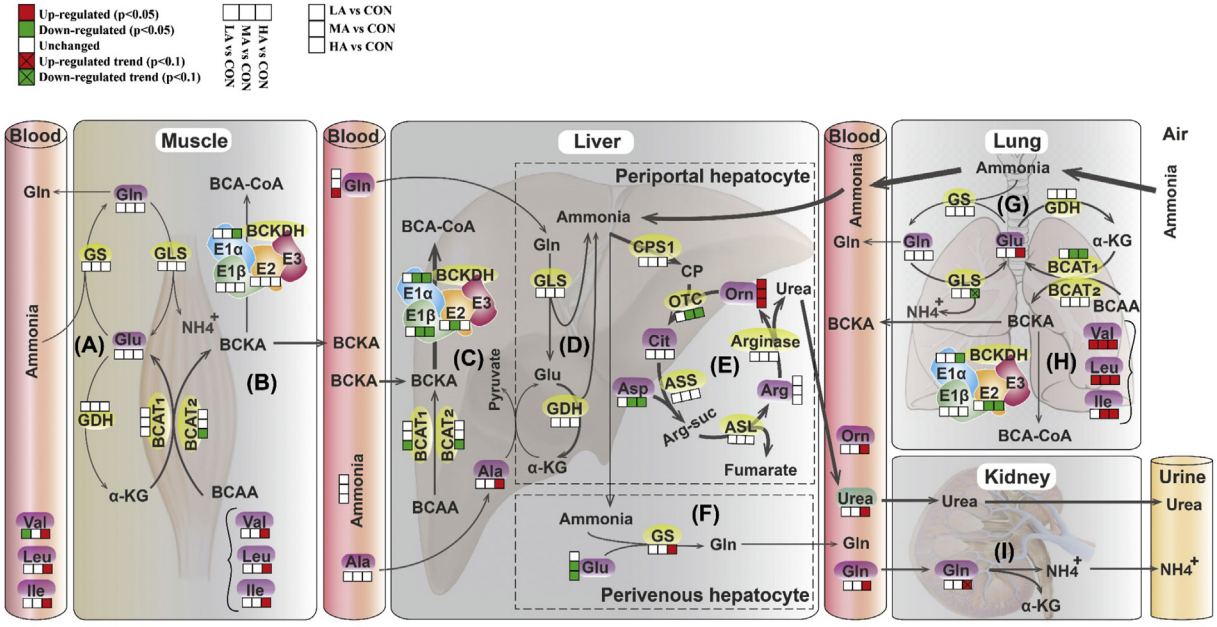


Fig. 7. The increased ammonia detoxifying and impaired branched-chain amino acid (BCAA) catabolism on the condition of various ammonia exposure. Little effect of ammonia exposure on glutamate-glutamine metabolism in muscle (A). Relative high ammonia exposure inhibited the BCAA catabolism in muscle (B), lungs (H) and liver (C). Unchanged glutaminolysis (D), increased urea cycle (E) and glutamine synthesis (F) were found in liver. Ammonia exposure improved the potential ability of ammonia-removing in lungs (G). Glutaminolysis yields ammonia in kidney (I). GS = glutamine synthetase; GLS = glutaminase; GDH = glutamate dehydrogenase; α-KG = α-ketoglutarate; BCAT = branched-chain amino acid aminotransferase; BCKDH = branched-chain α-keto dehydrogenase complex; E1 = BCKDH E1 subunit (including BCKDHA and B); E2 = BCKDH E2 subunit (also called DBT, dihydrolipoamide branched-chain transacylase); E3 = BCKDH E3 subunit; BCA-CoA = branched-chain acyl-coenzyme A; BCKA = branched-chain keto acid; BCAA = branched-chain amino acid; CPS1 = carbamoyl phosphate synthase 1; ASS = argininosuccinate synthase; ASL = argininosuccinate lyase; OTC = ornithine transcarbamoylase; Arg-suc = argininosuccinate.

Previously, we found serum BCAA were increased by 35 mg/m^3 ammonia exposure (Tang et al., 2020) and high ammonia exposure also increased the BCAA in the muscle. In the lungs, the level of BCAA (Leu, Ile and Val) were increased when pigs were exposed to all 3 levels of ammonia exposure. As shown by the gene expression data of BCAA transaminase and dehydrogenase complex in the liver, the breakdown of BCAA was inhibited by high-level aerial ammonia. Some researchers have found that decreased hepatic *BCKDH* might contribute to the increased plasma BCAA (Shin et al., 2014). Our further study found that the expression of *BCAT2*/*E1α*

subunit in muscle and *E1α/E2* subunits in the lungs showed a substantial decline after high ammonia exposure. Although BCAA were not changed in the liver, gene expression of BCAA catabolic enzymes were inhibited by 25 or 35 mg/m^3. Therefore, the elevated serum BCAA was very likely due to the decreased BCAA catabolism during high ammonia exposure (Fig. 7B, C and 7H). This result is a supplement to fill the gap in knowing how the ambient ammonia perturbs lipid metabolism via the mTOR pathway in our previous study (Tang et al., 2020). Many studies have shown BCAA can stimulate ammonia detoxification by increasing Gln synthesis in the muscle (Dam et al., 2011; Holecek, 2013; Holeček, 2017). However, the breakdown of BCAA was inhibited and could not provide Glu for ammonia removal in our study. At this point, how aerial ammonia could interfere with BCAA catabolic enzymes needs further investigation.

5. Conclusion

In summary (Fig. 7), low inhaled ammonia entered the circulation to evoke the urea cycle to detoxify ammonia in the liver. When the liver was functional, the muscle appeared to contribute little in ammonia disposal via the Gln route. The ammonia-removing process (including urea cycle and Gln synthesis) in the liver and potential ability of ammonia detoxification in the lungs were enhanced with increasing ammonia. High ammonia exposure inhibited the catabolism of BCAA by suppressing the expression of BCAA catabolism-related enzymes, consequently increasing BCAA in serum or extrahepatic tissues. Overall, the process of ammonia removal was raised to defend against the increasing aerial ammonia and high ammonia exposure disrupted the BCAA catabolism. Our study delineated the process of increased ammonia detoxification and impaired BCAA catabolism after various levels of ammonia exposure by monitoring alterations in FAA profiles across several ammonia-removing tissues. These findings may also be applicable to evaluate the adverse impacts of aerial ammonia on humans.

Author contributions

Shanlong Tang: Conceptualization, Software, Formal analysis, Writing - Original Draft, Visualization. **Chang Yin:** Methodology. **Jingjing Xie:** Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Jinglin Jiao:** Formal analysis. **Liang Chen:** Methodology. **Lei Liu:** Resources. **Sheng Zhang:** Writing - Review & Editing. **Hongfu Zhang:** Conceptualization, Supervision, Project administration, Funding acquisition.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix

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References

Adeva MM, Souto G, Blanco N, Donapetry C. Ammonium metabolism in humans. Metabolism 2012;61:1495–511.
An Y, Xing H, Zhang Y, Jia P, Gu X, Teng X. The evaluation of potential immunotoxicity induced by environmental pollutant ammonia in broilers. Poultry Sci 2019;98:3165–75.
Bauer SE, Tsigaridis K, Miller R. Significant atmospheric aerosol pollution caused by world food cultivation. Geophys Res Lett 2016;43:5394–400.
Behera SN, Sharma M, Aneja VP, Balasubramanian R. Ammonia in the atmosphere: a review on emission sources, atmospheric chemistry and deposition on terrestrial bodies. Environ Sci Pollut Res Int 2013;20:8092–131.
Boyle E. Nitrogen pollution knows no bounds. Science 2017;356:700–1.
Chen D, Miao Z, Peng M, Xing H, Zhang H, Teng X. The co-expression of circRNA and mRNA in the thymuses of chickens exposed to ammonia. Ecotoxicol Environ Saf 2019;176:146–52.
Dam G, Keiding S, Munk OL, Ott P, Buhl M, Vilstrup H, Bak LK, Waagepetersen HS, Schousboe A, N M. Branched-chain amino acids increase arterial blood ammonia in spite of enhanced intrinsic muscle ammonia metabolism in patients with cirrhosis and healthy subjects. Am J Physiol Gastrointest Liver Physiol 2011;301:G269.
Dimski DS. Ammonia metabolism and the urea cycle: function and clinical implications. J Vet Intern Med 1994;8:73–8.
Earnest CP, Morss GM, Wyatt F, Jordan AN, Colson S, Church TS, Fitzgerald Y, Autrey L, Jurca R, Lucia A. Effects of a commercial herbal-based formula on exercise performance in cyclists. Med Sci Sports Exerc 2004;36:504–9.
Hakvoort TB, He Y, Kulik W, Vermeulen JL, Duijst S, Ruijter JM, Runge JH, Deutz NE, Koehler SE, Lamers WH. Pivotal role of glutamine synthetase in ammonia detoxification. Hepatology 2017;65:281–93.
He Y, Hakvoort TB, Kohler SE, Vermeulen JL, de Waart DR, de Theije C, ten Have GA, van Eijk HM, Kunne C, Labruyere WT, Houten SM, Sokolovic M, Ruijter JM, Deutz NE, Lamers WH. Glutamine synthetase in muscle is required for glutamine production during fasting and extrahepatic ammonia detoxification. J Biol Chem 2010;285:9516–24.
Holecek M. Branched-chain amino acids and ammonia metabolism in liver disease: therapeutic implications. Nutrition 2013;29:1186–91.
Holecek M. Histidine in health and disease: metabolism, physiological importance, and use as a supplement. Nutrients 2020;12:848.
Holeček M. Branched-chain amino acid supplementation in treatment of liver cirrhosis: updated views on how to attenuate their harmful effects on cataplerosis and ammonia formation. Nutrition 2017;41:80–5.
Ipson BR, Fisher AL. Roles of the tyrosine isomers meta-tyrosine and ortho-tyrosine in oxidative stress. Ageing Res Rev 2016;27:93–107.
Kim E, Kim BU, Kim HC, Kim S. Direct and cross impacts of upwind emission control on downwind $PM_{2.5}$ under various NH_3 conditions in Northeast Asia. Environ Pollut 2020;268:115794.
King NE, Rothenberg ME, Zimmermann N. Arginine in asthma and lung inflammation. J Nutr 2004;134:2830S–6S.
Li L, Mao Y, Zhao L, Li L, Wu J, Zhao M, Du W, Yu L, Jiang P. p53 regulation of ammonia metabolism through urea cycle controls polyamine biosynthesis. Nature 2019;567:253–6.
Matoori S, Leroux JC. Recent advances in the treatment of hyperammonemia. Adv Drug Deliv Rev 2015;90:55–68.
Olde Damink SWM, Deutz NEP, Dejong CHC, Soeters PB, Jalan R. Interorgan ammonia metabolism in liver failure. Neurochem Int 2002;41:177–88.
Padappayil RP, Borger J. Ammonia toxicity. In: StatPearls. StatPearls Publishing Copyright © 2020; 2020. StatPearls Publishing LLC., Treasure Island (FL).
Sa RN, Xing H, Luan SJ, Sun YB, Sun CY, Zhang HF. Atmospheric ammonia alters lipid metabolism-related genes in the livers of broilers (Gallus gallus). J Anim Physiol Anim Nutr 2018;102:e941–7.
Shen D, Wu S, Li Z, Tang Q, Dai P, Li Y, Li C. Distribution and physicochemical properties of particulate matter in swine confinement barns. Environ Pollut 2019;250:746–53.
Shi Q, Wang W, Chen M, Zhang H, Xu S. Ammonia induces Treg/Th1 imbalance with triggered NF-kappaB pathway leading to chicken respiratory inflammation response. Sci Total Environ 2019;659:354–62.
Shin AC, Fasshauer M, Filatova N, Grundell LA, Zielinski E, Zhou JY, Scherer T, Lindtner C, White PJ, Lapworth AL, Ilkayeva O, Knippschild U, Wolf AM, Scheja L, Grove KL, Smith RD, Qian WJ, Lynch CJ, Newgard CB, Buettner C. Brain insulin lowers circulating BCAA levels by inducing hepatic BCAA catabolism. Cell Metabol 2014;20:898–909.
Silverman L, Whittenberger JL, Muller J. Physiological response of man to ammonia in low concentrations. J Ind Hyg Toxicol 1949;31:74–8.
Svenneby G, Torgner IA. Localization and function of glutamine synthetase and glutaminase. Biochem Soc Trans 1987;15:213–5.
Tang S, Xie J, Wu W, Yi B, Liu L, Zhang H. High ammonia exposure regulates lipid metabolism in the pig skeletal muscle via mTOR pathway. Sci Total Environ 2020;740:139917.

Tang S, Xie J, Zhang S, Wu W, Yi B, Zhang H. Atmospheric ammonia affects myofiber development and lipid metabolism in growing pig muscle. Animals (Basel) 2019;10:2.
Ti C, Xia L, Chang SX, Yan X. Potential for mitigating global agricultural ammonia emission: a meta-analysis. Environ Pollut 2019;245:141–8.
Walker V. Ammonia metabolism and hyperammonemic disorders. Adv Clin Chem 2014;67:73–150.
Wang S, Li X, Wang W, Zhang H, Xu S. Application of transcriptome analysis: oxidative stress, inflammation and microtubule activity disorder caused by ammonia exposure may be the primary factors of intestinal microvilli deficiency in chicken. Sci Total Environ 2019;696:134035.
Wang W, Shi Q, Wang S, Zhang H, Xu S. Ammonia regulates chicken tracheal cell necroptosis via the LncRNA-107053293/MiR-148a-3p/FAF1 axis. J Hazard Mater 2020;386:121626.
Weiner ID, Mitch WE, Sands JM. Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephrol 2015;10:1444–58.
Xiong Y, Tang X, Meng Q, Zhang H. Differential expression analysis of the broiler tracheal proteins responsible for the immune response and muscle contraction induced by high concentration of ammonia using iTRAQ-coupled 2D LC-MS/MS. Sci China Life Sci 2016;59:1166–76.
Xu Y, Li Z, Zhang S, Zhang H, Teng X. miR-187-5p/apaf-1 axis was involved in oxidative stress-mediated apoptosis caused by ammonia via mitochondrial pathway in chicken livers. Toxicol Appl Pharmacol 2020;388:114869.
Zeng Y, Tian S, Pan Y. Revealing the sources of atmospheric ammonia: a review. Current Pollution Reports 2018;4:189–97.
Zhang M, Chengdong H, Ming L, Yunxia Q, Wenbin X, Fanxing M, Rixin W. Modulation of lipid metabolism in juvenile yellow catfish (Pelteobagrus fulvidraco) as affected by feeding frequency and environmental ammonia. Fish Physiol Biochem 2019;45:115–22.
Zhu ZX, Jiang DL, Li BJ, Qin H, Meng ZN, Lin HR, Xia JH. Differential transcriptomic and metabolomic responses in the liver of Nile Tilapia (Oreochromis niloticus) exposed to acute ammonia. Mar Biotechnol 2019;21:488–502.