Animal Nutrition 12 (2023) 63–71

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Replacing $ZnSO_4$ with Zn-glycine in the diet of goat promotes the pancreatic function of the offspring

Jinhe Kang [a], Qiongxian Yan [a], Chuanshe Zhou [a], Xiaoling Zhou [b], Zhiliang Tan [a, *]

[a] CAS Key Laboratory for Agro-Ecological Processes in Subtropical Region, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Hunan Provincial Key Laboratory of Animal Nutrition & Physiology and Metabolism, South-Central Experimental Station of Animal Nutrition and Feed Science in Ministry of Agriculture, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha 410125, China
[b] College of Animal Science, Tarim University, Alaer 843300, China

ARTICLE INFO

Article history:
Received 17 June 2021
Received in revised form
20 June 2022
Accepted 12 August 2022
Available online 24 September 2022

Keywords:
Zinc
Pancreas
Enzyme
Proteomics
Kid

ABSTRACT

Zinc supplementation in the diet of goats affects pancreas development in offspring. However, the impact of maternal inorganic and organic zinc supplementation in offspring is poorly defined. In this study, 14 late-pregnant goats were assigned at random to the zinc sulfate group ($ZnSO_4$, $n = 7$) and the zinc-glycine chelate group (Zn-Gly, $n = 7$), respectively. Serum samples and pancreas tissue were collected from kids whose mothers were fed $ZnSO_4$ and Zn-Gly at the late pregnancy, respectively. Histologic examination showed no morphologic differences between the 2 groups. Pancreatic zinc content in kids tended to be increased when replacing $ZnSO_4$ with Zn-Gly. The serum insulin concentration was greater and glucagon less in the Zn-Gly group when compared to the $ZnSO_4$ group. The activities of lipase and chymotrypsin were enhanced when replacing $ZnSO_4$ with Zn-Gly. Proteomics results showed that 234 proteins were differentially expressed between the 2 groups, some of which were associated with the secretion of insulin, enzyme activity and signal transduction. The results suggested that supply of dietary Zn-Gly to goats during late pregnancy promoted pancreatic function in offspring compared with dietary $ZnSO_4$ supplementation. This provides new information about pancreatic function when supplementing different zinc sources in the diets of late pregnant goats.

1. Introduction

Maternal nutrition potentially affects the development of the offspring by modifying fetal programming through epigenetics. Fetal programming is an adaptive response to environmental conditions early in life which can alter gene expression, permanently affecting the structure and function of several organs and influencing susceptibility to metabolic disorders (Godfrey, 1998). The pancreas regulates pancreatic enzyme secretion on the one hand, and controls the secretion of insulin and glucagon on the other hand. Thus, the pancreas has both exocrine and endocrine function. Zinc (Zn) is an essential component for the function of the pancreas. It participates in the synthesis, crystallization, storage, secretion and signaling of insulin and glucagon, as well as the secretion of pancreatic enzymes (Egefjord et al., 2010; Kelleher et al., 2011; Slepchenko et al., 2015; Salim et al., 2008). Generally, the average Zn content in the tissues of fattening lambs and blood are 11.49 to 21.89 mg/kg and 0.25 to 0.60 mg/100 mL, respectively (Hou et al., 2021). The total zinc concentration in eukaryotic cells varies from 200 to 300 μmol/L. The content of Zn in pancreatic β-cells ranges from 10 to 20 mmol/L, and the majority of Zn is localized in the insulin secretory granules (Li, 2014; Duan et al., 2021; Zhao et al., 2019). Meanwhile, Zn^{2+} is transported across the membrane or into zymogen granules (ZGs) via Zn transporters in the pancreas (Kambe et al., 2017). Usually, there are 2 forms of Zn used in animal diets. One is inorganic Zn, including Zn chloride, Zn sulfate, Zn carbonate and Zn oxide. The other is organic Zn, including Zn gluconate and amino acid chelated Zn (Spears, 2003). Animal diets are often supplemented with inorganic Zn in the feed industry (Sobhanirad and Naserian, 2012). However, dietary supplementation of organic Zn has increased over the past 20 years (Huang et al., 2009). Studies on the bioavailability of these 2 kinds of Zn are not consistent (Huang et al., 2009). Some researchers have found that the

* Corresponding author.
E-mail address: zltan@isa.ac.cn (Z. Tan).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2022.08.014

bioavailability of amino acid chelated Zn was higher than that of inorganic Zn (Salim et al., 2010). On the contrary, other studies have shown that the bioavailability was similar between organic and inorganic Zn (Cao et al., 2000).

Previous studies have indicated that Zn source (Zn-chitosan vs $ZnSO_4$; Zn-Gly vs $ZnSO_4$) had a significant influence on the pancreatic Zn content in weaned piglets (Ma et al., 2021; Liu et al., 2021). We hypothesized that inorganic Zn and organic Zn had different effects on pancreatic function. The objective of the present study was to compare the effects of dietary inorganic and organic Zn addition in late pregnant goats on the pancreatic function in kids.

2. Materials and methods

The experiment was ratified by the Animal Care Committee (Permit No. ISACAS-02-2017-11), Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

2.1. Experimental design and sampling

Fourteen pregnant Xiangdong black goats (similar parity and the body weight was 36.0 ± 8.2 kg) were selected after oestrous synchronisation, and randomly divided into the Zn sulfate group ($ZnSO_4$, Tanke, China) and the glycine chelated Zn group (Zn-Gly, Tanke, China). The purity of both the $ZnSO_4$ and Zn-Gly was 98%. The Zn content of $ZnSO_4$ and Zn-Gly was 34.5% and 29%, respectively. Both groups had 7 goats. The preliminary experiment period was 7 d, and the formal experiment period was 45 d (from d 106 of gestation to parturition). The basal diet was formulated according to NRC (2007) to meet the nutritional requirements of goats in late pregnancy (goat weight, 30 kg; dry matter intake, 1.05 kg/d). $ZnSO_4$ and Zn-Gly were added to the basal diets respectively, and the total content of Zn in each diet was 82 mg/kg DM. The composition and ingredients of the experimental diets are given in Table 1. The contents of dry matter (DM), crude protein (CP), ether extract (EE) and ash in the diets were analyzed according to AOAC (1995). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured according to the methods of Van Soest et al. (1991) by a Fibretherm Fiber Analyzer (Gerhardt, Bonn, Germany). The Zn content in the diets was measured by ICP-OES as described by Solaiman and Min (2019). Goats were fed with the basal diet (Zn content, 22 mg/kg DM) without adding the Zn source after parturition. During lactation, all kids ($n = 9$ in each group) remained with the goats only during feeding. After weaning at d 60, all kids were fed with the same diet without adding the Zn source to eliminate the influence of an exogenous Zn source on the offspring. The composition and ingredients of diets for lactating goats and offspring are given in Table 2. Kids were fed at 08:00 and 18:00 each day. After fasting for 12 h, the kids were slaughtered 100 d after birth.

Table 1
Ingredients and composition of the experimental diets (DM basis, %).

Item	$ZnSO_4$	Zn-Gly
Ingredients		
Miscanthus sibiricus	40.00	40.00
Corn	34.66	34.66
Soybean meal	11.74	11.74
Fat powder	4.49	4.49
Soy protein concentrate	5.03	5.03
$Ca(HCO_3)_2$	0.50	0.50
$CaCO_3$	0.84	0.84
Premix[1]	2.34	2.34
NaCl	0.40	0.40
Nutrient levels[2]		
DM	91.79	92.17
CP	17.76	18.72
EE	2.50	3.14
ADF	29.30	31.24
NDF	45.42	47.64
OM	89.53	89.35
Zn, mg/kg	82.00	82.00

DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; OM = organic matter.
[1] One kilogram of premix contains vitamin A 100,000 IU, vitamin D_3 15,000 IU, vitamin E 300 IU, I (as potassium iodide) 0.1996 g, selenium 0.02 g, $CuSO_4 \cdot 5H_2O$ 0.04 g, $FeSO_4 \cdot H_2O$ 0.14 g, $MnSO_4 \cdot H_2O$ 0.38 g.
[2] Nutrient levels were measured values.

Table 2
Dietary composition of lactating goats and offspring (DM basis, %).

Item	Lactating goats	Item	Off springs
Ingredients		Ingredients	
Miscanthus sibiricus	40.00	*Miscanthus sibiricus*	40.00
Corn	34.66	Corn	10.02
Soybean meal	11.74	Soybean meal	8.00
Fat powder	4.49	Fat powder	10.93
Soy protein concentrate	5.03	Extruded soybean	19.27
$Ca(HCO_3)_2$	0.50	Whey powder	6.87
$CaCO_3$	0.84	$Ca(HCO_3)_2$	1.51
Premix[1]	2.34	$CaCO_3$	0.60
NaCl	0.40	Premix[3]	2.00
Nutrient levels[2]		NaCl	0.80
DM	89.31	Nutrient levels[2]	
CP	11.08	DM	90.05
EE	1.06	CP	14.10
ADF	25.91	EE	2.87
NDF	42.91	ADF	20.00
OM	88.59	NDF	37.60
Zn, mg/kg	22.00	OM	89.01
		Zn, mg/kg	21.46

[1] One kilogram of premix contained the following: vitamin A 100,000 IU, vitamin D_3 15,000 IU, vitamin E 300 IU, I (as potassium iodide) 0.1996 g, Se (as sodium selenite) 0.02 g, $CuSO_4 \cdot 5H_2O$ 0.04 g, $FeSO_4 \cdot H_2O$ 0.14 g, $MnSO_4 \cdot H_2O$ 0.38 g.
[2] Dry matter (DM), crude protein (CP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), organic matter (OM) and Zn were measured values.
[3] One kilogram of premix contained the following: VA 95,000 IU, VD 17,500 IU, KI 40 mg, $FeSO_4 \cdot 7H_2O$ 2.5 g, $CuSO_4 \cdot 5H_2O$ 0.8 g, $MnSO_4 \cdot H_2O$ 3 g, Na_2SeO_3 10 mg, $CoCl_2 \cdot 6H_2O$ 30 mg.

Blood samples of kids were collected from the jugular vein using vacutainer tubes before slaughtering, and then serum was obtained by centrifugation (3,000 × *g* for 15 min) and stored at −80 °C. The whole pancreas was collected, weighed and washed with PBS solution. Pancreas samples were frozen quickly in liquid nitrogen and stored at −80 °C. Each pancreas sample was used for metabolomics analysis. For proteomics analysis, every 3 pancreatic samples in the same group were pooled into 1 sample (Budzinski et al., 2019). Thus, biological replicates of metabolomics and proteomics were 9 and 3, respectively. Pancreas samples (1 cm × 1 cm) were then collected, washed with precooled saline solution, fixed with 4% paraformaldehyde for 1 d, paraffin-embedded and then stored at 4 °C for hematoxylin-eosin (HE) staining.

2.2. Mineral concentration determination

Pancreatic samples were collected and stored at −20 °C until analysis. The samples were digested with nitric acid-perchloric acid (4:1) and filtered to volume, and then sampled using an inductively coupled serum spectrometer (Agilent Technologies 5110 ICP-OES, USA). The total contents of Fe, P, Zn, Cu and K in the pancreatic samples were measured by ICP-OES as described by Solaiman and Min (2019).

2.3. Insulin and glucagon determination

The concentrations of insulin and glucagon in serum samples were measured by commercial goat insulin and glucagon ELISA kits

(Cusabio Biotech Co., Ltd., Hubei, China) and analyzed using a microplate reader (TECAN, Austria).

2.4. Pancreatic enzyme activities

The activities of lipase, α-amylase, chymotrypsin and trypsin in the pancreatic tissue were measured using assay kits (Cominbio Co., Suzhou, China) and analyzed using a microplate reader (TECAN, Austria).

2.5. HE staining

The paraffin-embedded pancreatic samples were cut to 5 μm thickness and stained with HE solution (Solarbio, Beijing Solarbio Science & Technology, Beijing, China) according to routine protocols (Slaoui et al., 2017). Specimens were observed under a BX53 microscope (Olympus, Tokyo, Japan) and assayed with Image proplus 6.0 Software (Media Cybernetics, Silver Spring, MD, USA). Three different sections from the pancreas of each kid were examined. Five different areas were selected in each slide.

2.6. Metabolomics analysis

This process was conducted according to the method described previously by Zhou et al. (2019). The pancreatic tissue (100 mg) was homogenized in cold 90% methanol twice and sonicated at low temperature (30 min, twice). The mixture was centrifuged at 14,000 × *g* for 20 min at 4 °C. Next, the supernatant was dried in a vacuum centrifuge, re-dissolved in acetonitrile/water (1: 1, vol/vol), and 2 μL supernatant was injected into an UHPLC-Q-TOF-MS system. A pooled quality control (QC) sample was obtained by mixing equal volumes of supernatant from each pancreatic sample to determine the stability of the analytical system.

The untargeted metabolic analysis was conducted using a UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex TripleTOF 6600) with a dual electrospray ionization source (ESI, Shanghai Applied Protein Technology Co., Ltd). In both ESI positive and negative modes, the mobile phase contained 25 mmol/L ammonium acetate and 25 mmol/L ammonium hydroxide in water (A) and acetonitrile (B). The gradient was 95% B for 0.5 min and linearly reduced to 65% in 7 min, and then decreased to 40% in 1 min and kept for 1 min, and then increased to 95% in 0.1 min, with a 3 min re-equilibration period employed. The mass spectrometric (MS) experiment was performed with the ESI source under the following conditions: Ion Source Gas1 (Gas1) and Ion Source Gas2 (Gas2) were both set to 60 psi, curtain gas (CUR) pressure was 30 psi, source temperature was 600 °C, Ion Spray Voltage Floating (ISVF) was ±5,500 V. In MS only acquisition, the mass range of the instrument was from *m*/*z* 60 to 1,000 Da, and the accumulation time for TOF MS scan was set at 0.20 s/spectra. The mass range was set in *m*/*z* 25.1000 Da with 0.05 s/spectra of the accumulation time for production scan in auto MS/MS acquisition. The product ion scan was acquired using information dependent acquisition (IDA) with high sensitivity mode selected. The parameters were set as follows: the collision energy (CE) was fixed at 35 V with ±15 eV; declustering potential (DP) were 60 V (+) and - 60 V (−); exclude isotopes within 4 Da, candidate ions to monitor per cycle: 10.

The raw MS spectra were converted to MzXML files using ProteoWizard MSConvert, and then imported into XCMS software which was used for nonlinear alignment, automatic integration and extraction of the peak intensities. The obtained data sets were introduced into SIMCA-P (version 16.1, Umetrics, Umea, Sweden) to perform partial least squares discriminant analysis (PLS-DA). The fitness and predictive capability of the PLS-DA models were evaluated by the cumulative R^2 and Q^2. Student's *t*-test with false discovery rate (FDR) was employed for univariate analysis between the 2 groups. The metabolites expressed differentially were recognized with variable importance for projection (VIP) values > 1.0 and adjusted *P*-values < 0.05. The metabolomics data have been deposited in the MetaboLights with the dataset identifier MTBLS4664.

2.7. Proteomics analysis

This process was conducted according to the methods described previously by Yan et al. (2018). The pancreatic tissue was incubated in a lysis buffer (7 mol/L urea, 2 mol/L thiourea, 100 mmol/L DTT, 5% SDS and protease inhibitor cocktail) at 4 °C for 1 h, and centrifuged at 40,000 × *g* for 1 h at 4 °C. The supernatant was collected, and the protein concentration was determined by the RC DC Protein Assay (Bio-Rad Laboratories, Inc.). The total protein of the pancreatic sample was reduced with 5 mmol/L TRIS-(2-carboxyethyl) phosphine at 60 °C and then alkylated with 10 mmol/L methyl methanethiosulfonate for 30 min at room temperature. The protein was digested with sequencing-grade modified porcine trypsin (Promega) overnight at 37 °C. The peptide mixture was labeled with eight different iTRAQ labelling reagents according to the manufacturer's instructions (Applied Biosystems). The labeled peptide was fractionated by strong cation exchange chromatography on a 20AD HPLC system (Shimadzu) using a polysulfoethyl column (2.1 mm × 100 mm, 5 μm, the Nest Group Inc.). The peptide mixture was reconstituted and acidified with buffer A (10 mmol/L KH_2PO_4 in 25% of acetonitrile, pH 2.8) and eluted with a gradient of 0 to 80% buffer B (350 mmol/L KCl, 10 mmol/L KH_2PO_4 in 25% of acetonitrile, pH 2.8) in buffer A at a flow rate of 200 μL/min for 60 min. The elution was monitored by absorbance at 214 nm, and fractions were collected, desalted and concentrated by centrifugation.

After being dried down by the rotary vacuum concentrator, each fraction was dissolved in solvent A containing 5% acetonitrile and 0.1% formic acid and analysed with TripleTOF 5600 systems (AB SCIEX) in an information-dependent mode. MS/MS spectra were searched using MASCOT engine (Matrix Science, London, UK; version 2.2) embedded into Proteome Discoverer 1.4. The parameter sets were as follows: enzyme: trypsin; max missed cleavages: 2; peptide mass tolerance: ± 20 ppm; fragment mass tolerance: 0.1 Da; peptide FDR: ≤0.01; fixed modifications: carbamidomethyl. The *P*-value was calculated from the *t*-test for differentially expressed proteins (DAPs). The DAPs were recognized with a *P*-value < 0.05 and the fold changes > 1.2 or < 0.8 (Ma et al., 2020). Functional analysis of the DAPs was conducted using the gene ontology (GO) annotation (http://www.geneontology.org/). GO enrichment analysis was performed to identify GO terms. Analyses of GO enrichment were applied based on Fisher's exact test. Functional categories and pathways with *P*-values < 0.05 were considered significant. The proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD03315.

2.8. Statistical analysis

An independent sample *t*-test was utilized to evaluate the statistical significance of values (mean ± standard deviation of the mean) by using SPSS 21.0 software (IBM; Armonk, NY, USA). Significant differences between the 2 groups were defined as $P < 0.05$, and a tendency was defined as $0.05 < P < 0.1$.

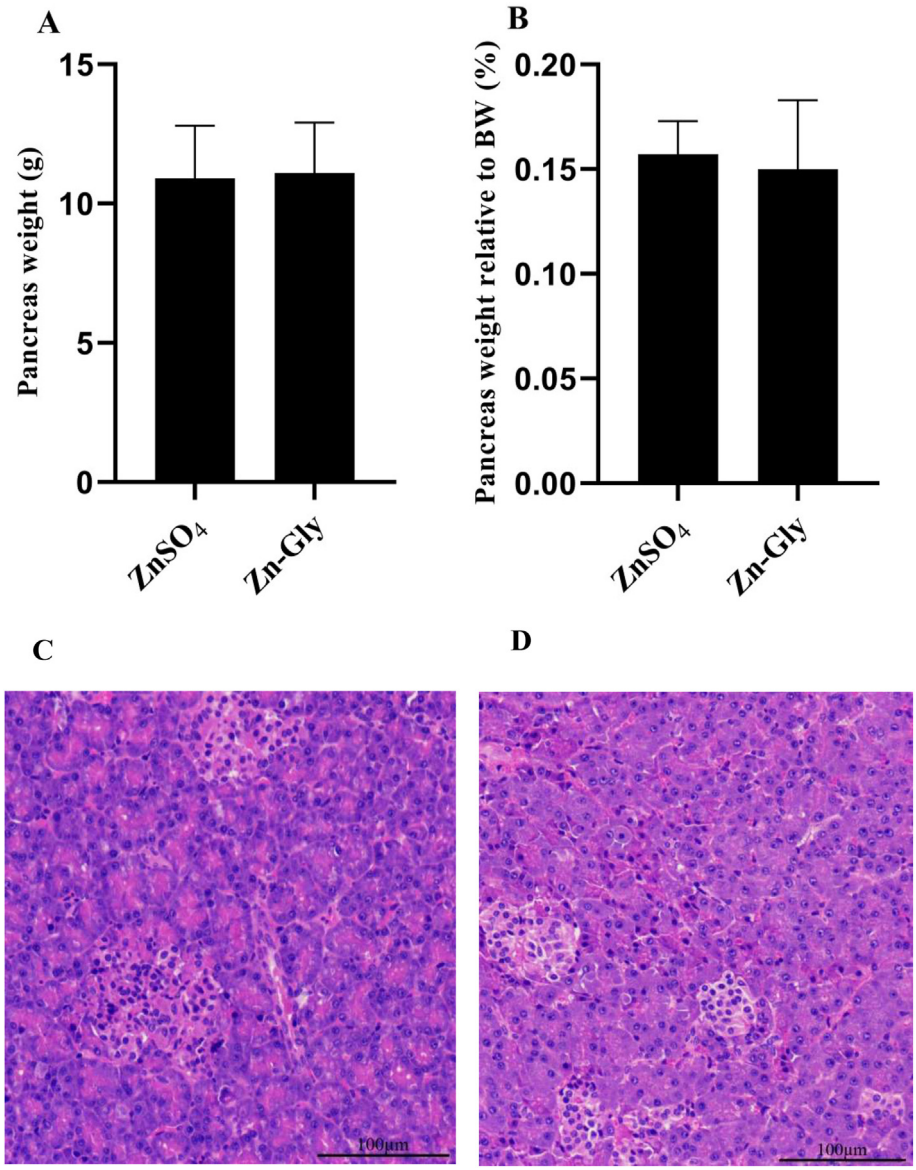

Fig. 1. Pancreas weight (A) and the percentage of pancreas weight to body weight (B) of kids; Morphology of pancreatic tissue sections stained with hematoxylin-eosin stain (HE stain) in kids whose mothers were fed with $ZnSO_4$ (C) or Zn-Gly (D) diets (200× magnification).

3. Results

3.1. Pancreas weight

The weight of pancreas and the percentage of pancreas weight to body weight were comparable ($P > 0.05$) between 2 groups (Fig. 1A and B).

3.2. Hematoxylin-eosin staining

There were no significant differences in the structure of the pancreas between the 2 groups (Fig. 1C and D). The islet and acinar cells in the kids of both groups were structured clearly, arrayed and distributed equally. Interstitial oedema and the infiltration of inflammatory cells were not observed in the kids of both groups.

3.3. The concentrations of mineral elements

Zn-Gly supplementation significantly decreased ($P < 0.05$) the Cu content and tended to increase the contents of Ca ($P = 0.096$) and Zn ($P = 0.085$) compared with the $ZnSO_4$-treated group. However, the contents of P, Fe, Mg and Mn were comparable ($P > 0.05$) between the 2 groups (Table 3).

3.4. Insulin and glucagon contents

The Zn-Gly addition significantly increased ($P < 0.01$) the plasma insulin concentration and significantly decreased ($P < 0.01$) the glucagon content compared with the $ZnSO_4$ group (Fig. 2A and B).

Table 3
Mineral elements in pancreatic tissue of kids (mg/kg, $n = 9$).

Item	$ZnSO_4$	Zn-Gly	*P*-value
Ca	345.55 ± 53.22	409.00 ± 65.88	0.096
Cu	6.33 ± 1.95	4.22 ± 0.80	0.034
P	3947 ± 340.36	3781 ± 332.29	0.414
Zn	22.74 ± 3.54	26.91 ± 4.02	0.085
Fe	18.83 ± 2.67	21.36 ± 3.50	0.189
Mg	198.18 ± 16.56	190.03 ± 23.18	0.499
Mn	1.64 ± 0.21	1.43 ± 0.26	0.147

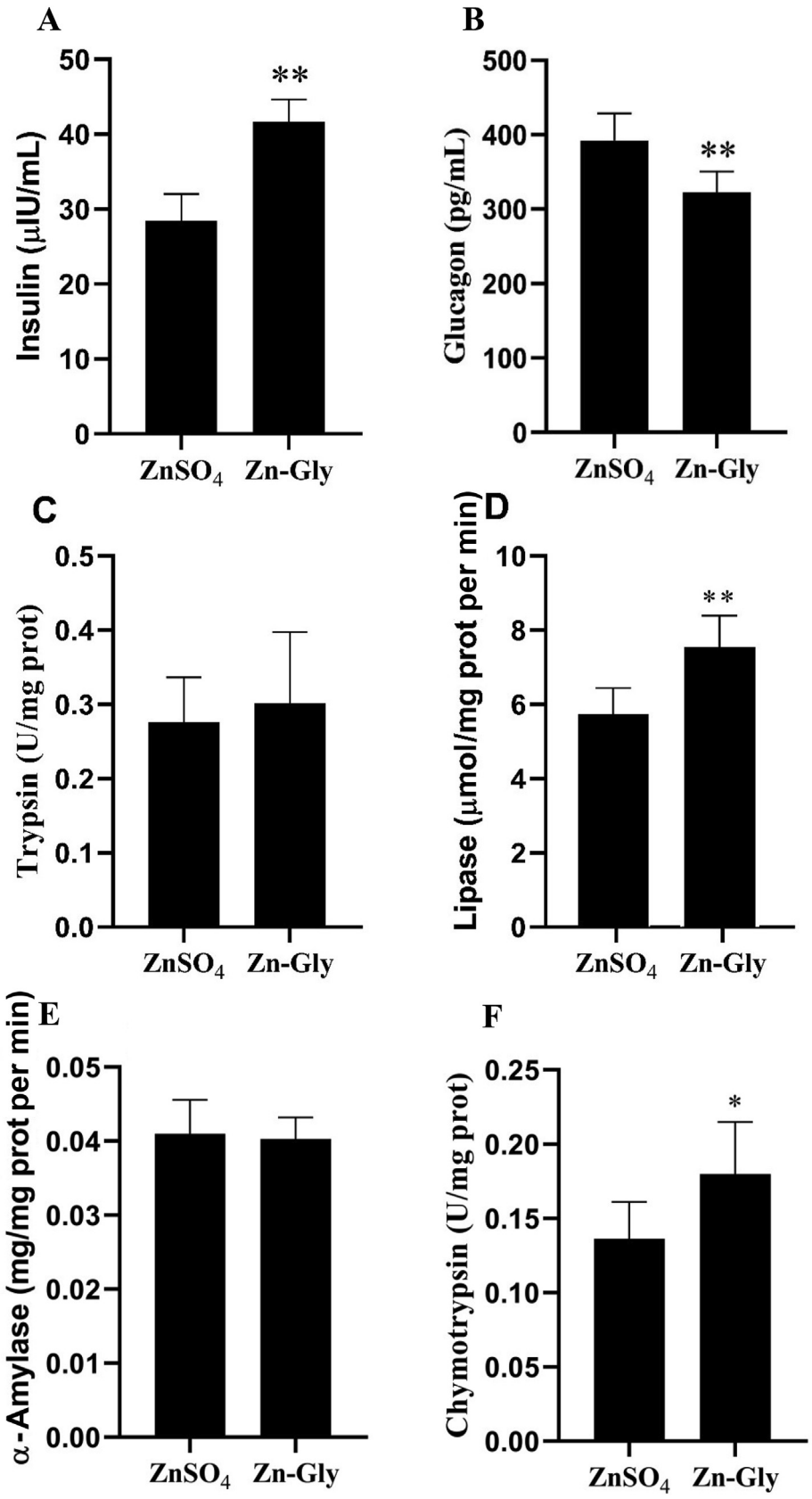

Fig. 2. Plasma insulin (A) and glucagon (B) contents of kids; (C–F) activities of digestive enzymes in pancreatic tissues of kids. *$P < 0.05$, **$P < 0.01$.

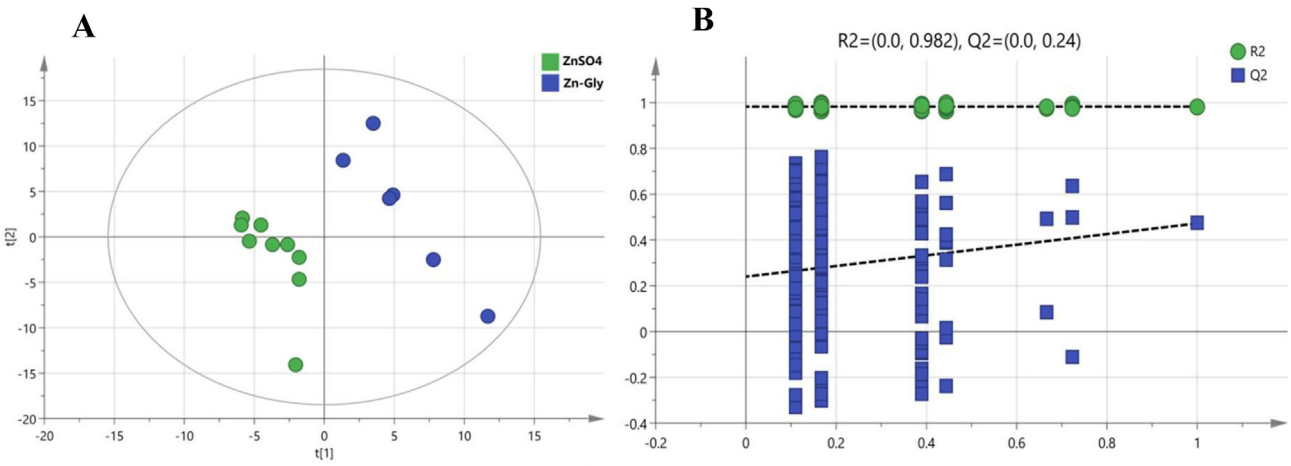

Fig. 3. Metabolite profiling analysis of pancreas of kids. (A) PLS-DA score plot derived from liquid chromatography-mass spectrometry-based metabolomics analysis of kids fed with $ZnSO_4$ (green circles) and kids with Zn-Gly (blue circles). (B) Statistical validation of the PLS-DA model by permutation testing.

3.5. Activities of digestive enzymes

The Zn-Gly supplementation significantly ($P < 0.05$) increased the activities of chymotrypsin and lipase compared with the $ZnSO_4$ group. There were no differences ($P > 0.05$) in the activities of a-amylase and trypsin between the 2 groups (Fig. 2C–F).

3.6. Metabolomics analysis

A supervised PLS-DA was performed using 2 components ($R^2 = 0.982$, $Q^2 = 0.24$), and the PLS-DA analysis showed a clear separation in metabolite profiles between **2** groups (Fig. 3A and B). A total of 444 metabolites were identified by matching the secondary mass spectrogram of the metabolites with the database of the company, in which 249 metabolites were detected in the positive model and 195 metabolites were detected in the negative model in this study. However, there were no differentially expressed metabolites between the 2 groups (Appendix Table 1).

3.7. Proteomics analysis

A total of 234 proteins expressed differentially were found between the 2 groups (Appendix Table 2), therein 103 proteins were up-regulated and 131 proteins were down-regulated in the Zn-Gly group compared to the $ZnSO_4$ group. Clustering analysis based on GO nomenclature revealed that the highest proportion of changed proteins was located in the cell (18.18%) and cell parts (18.18%). Proteins located in the organelle (17.10%) and organelle parts (10.47%) were the 2 next largest groups that were differentially regulated in the pancreas. Categorical analysis based on molecular function revealed that the majority of changed proteins in the pancreas between the 2 groups were associated with binding (50.37%) and catalytic activity (33.21%). Clustering analysis based on biological process revealed that proteins involved in metabolic processes and biological regulation constituted the largest functional groups, comprising about 14.50% and 11.97%, respectively (Fig. 4A). The GO enrichment analysis is shown in Fig. 4B. The biological processes were involved in the regulation of kidney development and epithelial cell differentiation, activation of cysteine-type endopeptidase activity and chaperone cofactor-dependent protein refolding. The molecular functions were related to acetyltransferase activity, neurotransmitter receptor regulator activity and acetylcholine receptor regulator activity. The cellular components were related to intracellular, mRNA cleavage factor complex, NADH dehydrogenase complex, mitochondrial respiratory chain complex 1 and membrane microdomain.

4. Discussion

Pregnant goats digest and absorb nutrients, which are transferred to the fetus through the placenta. Therefore, supplementation of Zn in pregnant goats can be transferred to their fetuses to maintain Zn status. However, organic Zn is absorbed more effectively and excreted less than inorganic Zn in ruminants, which can affect the Zn status of the offspring (Spears, 2003). This might explain why Zn content in the pancreas of Zn-Gly group kids tended to increase compared with $ZnSO_4$ group kids in this study. A similar result was reported by Kwiecień et al., who found that the Zn concentration in broiler liver was increased when replacing zinc oxide supplementation with Zn-Gly addition (Kwiecien et al., 2017). Additionally, despite increasing Zn content in the pancreas with Zn-Gly addition, no histological changes were observed in the present study, indicating that the Zn level was not toxic to the pancreas, but might affect the metabolism of pancreatic cells.

It is well known that Zn is profoundly involved in pancreatic endocrine function, mainly because insulin secretion from pancreatic β-cells is regulated by Zn (Cooper-Capetini et al., 2017; Morais et al., 2019). In the present study, the higher serum insulin level in the Zn-Gly group indicated that Zn-Gly addition enhanced insulin secretion from pancreatic β-cells. This result was inconsistent with previous reports that the serum insulin concentrations were comparable in Holstein dairy cows when supplemented with $ZnSO_4$ and Zn methionine (Sobhanirad and Naserian, 2012). This discrepancy might be due to the difference in the variety and physiological stage of the animals, the dosage of zinc supplementation, and chelated amino acid. Furthermore, experiments in isolated islets have also shown that the addition of exogenous insulin inhibited the secretion of glucagon (Ravier and Rutter, 2005). Therefore, the glucagon level in the Zn-Gly group was lower than that in the $ZnSO_4$ group in this study, which might be the result of increased Zn^{2+} and insulin content in kids. Similar reports have shown that glucagon secretion of pancreatic α cells and isolated islets was inhibited by Zn^{2+} (Gyulkhandanyan et al., 2008).

Digestive enzymes, produced by pancreatic acinar cells, play an essential role in digesting nutrients (Williams, 2019). The increase in chymotrypsin and lipase activity in the Zn-Gly group in this study was consistent with previous reports that Zn addition enhanced the activity of chymotrypsin and lipase in the pancreas of piglets (Pieper

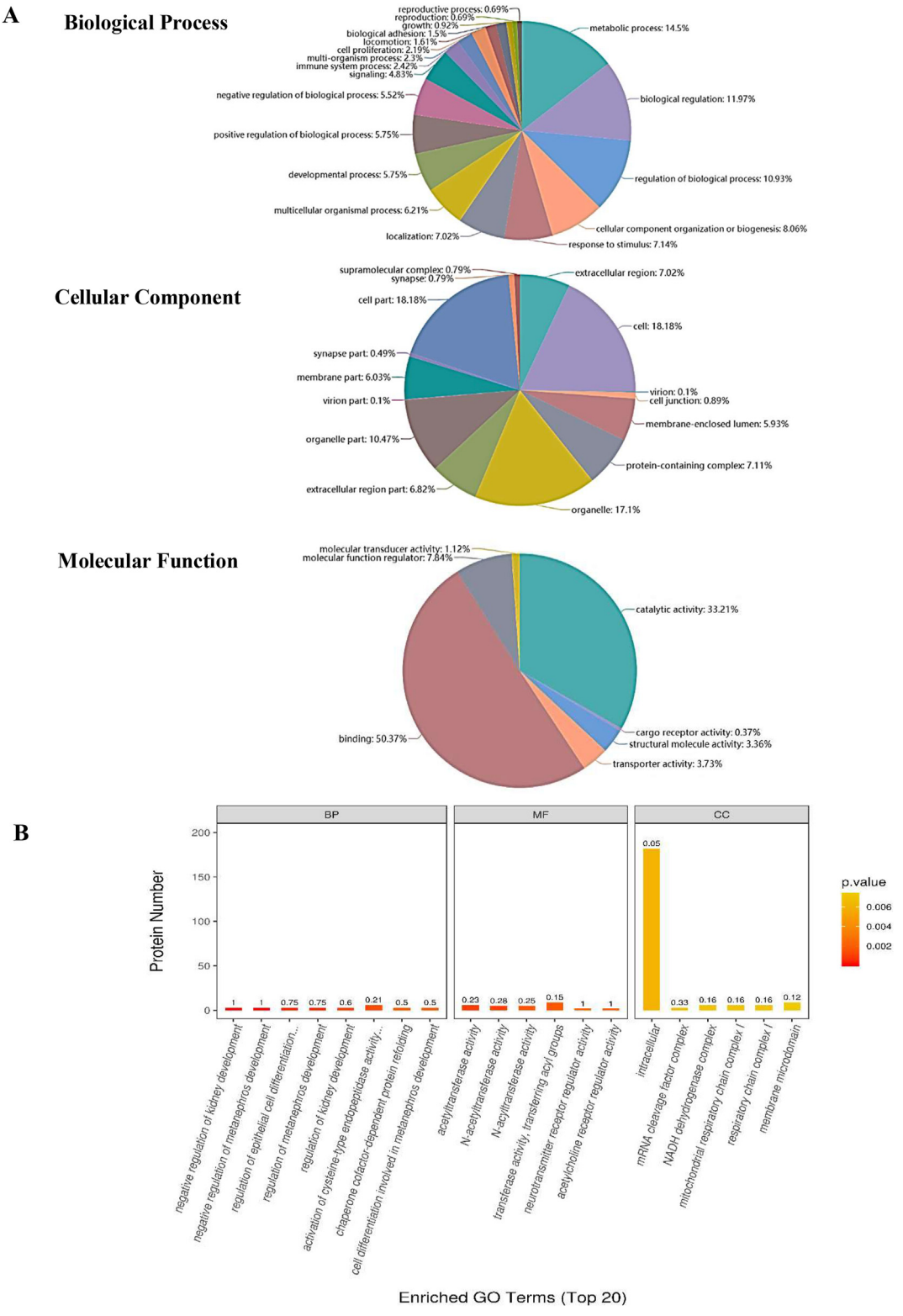

Fig. 4. (A) Classification of the differentially expressed proteins in the pancreas of kids whose mothers were fed with $ZnSO_4$ and Zn-Gly. (B) The top 20 most enriched GO terms based on proteomics analysis between kids fed with $ZnSO_4$ and Zn-Gly. BP = biological process; MF = molecular function; CC = cellular component; GO = gene ontology.

et al., 2015). However, replacing $ZnSO_4$ with Zn-Gly had no effect on activity of trypsin and α-amylase in the current study. The reason for this phenomenon might be that although both trypsin and chymotrypsin are serine proteases, their substrate specificities are different. Trypsin favors basic residues like lysine and arginine; chymotrypsin favors aromatic residues like phenylalanine, tyrosine, and tryptophan (Ma et al., 2005). Studies in pigs have shown that pancreatic enzyme synthesis was affected by dietary Zn supplementation. However, enzyme secretion was not influenced by Zn addition (Hedemann et al., 2006). Furthermore, 80% of the whole

body glycine is used for protein synthesis and the flexibility of active sites in enzymes is provided by glycine (Razak et al., 2017). Although the Cu content was decreased in the pancreas compared with the $ZnSO_4$-treated group, the activities of pancreatic enzymes were not influenced by the Cu content (Hedemann et al., 2006). As Cu may not be a co-factor to pancreatic enzymes, Cu content would have the least influence on enzyme activity. Therefore, the reason for the different enzyme activity between the 2 groups might be due to zinc accumulation and glycine supplementation. The mechanisms were not entirely clear and need further investigation.

In pancreatic acinar cells, digestive enzymes are synthesized and stored in ZGs. Ras-related protein Rab-8A is localized to ZGs and involved in ZG formation and apical trafficking of pancreatic enzymes (Williams et al., 2009). The ZGs number was decreased and granule marker proteins were accumulated when Rab8 was silenced. In this study, the proteomic analysis showed that the protein level of Rab-8A was upregulated when replacing $ZnSO_4$ with Zn-Gly, suggesting that Zn-Gly might promote the number of ZGs, which was one of the reasons for the different enzyme activity between the 2 groups.

Generally, the elevation of proteins related to translation has a beneficial effect on protein synthesis (Haque and Spremulli, 2008). In our work, several components of protein synthesis, including eukaryotic initiation factors 3 (EIF3), transcriptional activator protein Pur-beta, elongator complex protein 3, transcription factor BTF3 homolog 4, mediator of RNA polymerase II transcription subunit 20 and ribosomal protein L35, were significantly upregulated in kids in the Zn-Gly group compared with kids in the $ZnSO_4$ group. This result might indicate that these proteins are directly related to the synthesis of proteins, which directly responded to the supplementation of different Zn sources.

The increased β-catenin, F-box-like/WD repeat-containing protein TBL1XR1 and the reduced S-phase kinase-associated protein 1 (Skp1), casein kinase I isoform X9 in the Zn-Gly group compared with $ZnSO_4$ group in this study indicated that the Wnt signaling pathway was modulated by replacing $ZnSO_4$ with Zn-Gly. The Wnt signaling pathway is closely involved in insulin resistance and pancreatic function. Furthermore, β-catenin can regulate the transcription of downstream genes. Thus, the synthesis and secretion of insulin and the growth and regeneration of pancreatic β-cells were affected (Chen et al., 2021). These results suggested that the Wnt signaling pathway might be related to the insulin secretion enhanced by Zn-Gly addition.

The pterin-4-alpha-carbinolamine dehydratase (PCD) functions both as a metabolic enzyme and as a transcriptional coactivator of transcription factor hepatocyte nuclear factor 1 (Wang et al., 2015). Meanwhile, gephyrin can regulate the release of insulin. Inhibition of gephyrin resulted in lower insulin secretion in pancreatic β-cells (Tattikota et al., 2013). Moreover, PCD and gephyrin were found to be involved in folate biosynthesis. Apoptosis of pancreatic β-cells was induced and synthesis of insulin was inhibited when folate was deficient (Hsu et al., 2013). In this study, the expression levels of PCD and gephyrin were upregulated in the Zn-Gly group compared with the $ZnSO_4$ group, which was consistent with the higher plasma insulin level in the Zn-Gly group in the current study. It was likely that Zn-Gly enhanced the secretion of insulin by upregulating the expression levels of PCD and gephyrin.

5. Conclusions

This study revealed that replacing dietary $ZnSO_4$ with Zn-Gly in the diets of goats inlate pregnancy promoted pancreatic function in offspring. This provides new information about pancreatic function when supplementing with organic and inorganic zinc sources in late pregnancy. In addition, differentially expressed proteins need to be validated by using western blotting and ELISA methods.

Author contributions

Conceptualization: **Jinhe Kang**, **Qiongxian Yan**, and **Chuanshe Zhou;** Investigation: **Jinhe Kang, Qiongxian Yan**; Software: **Xiaoling Zhou**; Methodology: **Jinhe Kang**; Formal Analysis: **Xiaoling Zhou, Jinhe Kang**; Data curation: **Jinhe Kang**, **Qiongxian Yan**; Resources, Supervision, Funding Acquisition and Writing – Review & Editing: **Zhiliang Tan**; Writing - Original Draft Preparation: **Jinhe Kang**; Project Administration, **Qiongxian Yan**, **Chuanshe Zhou**.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, 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.

Acknowledgements

The authors wish to acknowledge the financial support received from the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant NO. XDA 26040304), Natural Science Foundation of Hunan Province (2020JJ5634), National Natural Science Foundation of China (31760678).

Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.08.014.

References

AOAC. Official methods of analysis. 16th ed. Arlington: Association of Official Analytical Chemists; 1995.
Budzinski IGF, de Moraes FE, Cataldi TR, Franceschini LM, Labate CA. Network analyses and data integration of proteomics and metabolomics from leaves of two contrasting varieties of sugarcane in response to drought. Front Plant Sci 2019;10:1524.
Cao J, Henry PR, Guo R, Holwerda RK, Toth JP, Littell RC, et al. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J Anim Sci 2000;78:2039–54.
Chen J, Ning C, Mu JJ, Li DN, Ma Y, Meng XJ. Role of Wnt signaling pathways in type 2 diabetes mellitus. Mol Cell Biochem 2021;476:2219–32.
Cooper-Capetini V, de Vasconcelos DAA, Martins AR, Hirabara SM, Donato J, Carpinelli AR, et al. Zinc supplementation improves glucose homeostasis in high fat-fed mice by enhancing pancreatic beta-cell function. Nutrients 2017;9:1150.
Duan MP, Li T, Liu B, Yin SH, Zang JC, Lv CY, et al. Zinc nutrition and dietary zinc supplements. Crit Rev Food Sci 2021. https://doi.org/10.1080/10408398.2021.1963664.
Egefjord L, Petersen AB, Rungby J. Zinc, alpha cells and glucagon secretion. Curr Diabetes Rev 2010;6:52–7.
Godfrey KM. Maternal regulation of fetal development and health in adult life. Eur J Obstet Gynecol Reprod Biol 1998;78:141–50.
Gyulkhandanyan AV, Lu HF, Lee SC, Bhattacharjee A, Wijesekara N, Fox JEM, et al. Investigation of transport mechanisms and regulation of intracellular Zn^{2+} in pancreatic alpha-cells. J Biol Chem 2008;283:10184–97.
Haque ME, Spremulli LL. Roles of the N- and C-terminal domains of mammalian mitochondrial initiation factor 3 in protein biosynthesis. J Mol Biol 2008;384:929–40.
Hedemann MS, Jensen BB, Poulsen HD. Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. J Anim Sci 2006;84:3310–20.
Hou PX, Liu YH, Wang JD, Ma JF, Yu Y, Zhang SR, et al. Effects of adding amino acid in diets on slaughter performance, meat quality and trace element contents in blood and tissue of fatttening sheep. Chiese J Anim Sci 2021;33:563–71.
Hsu HC, Chiou JF, Wang YH, Chen CH, Mau SY, Ho CT, et al. Folate deficiency triggers an oxidative-nitrosative stress-mediated apoptotic cell death and impedes

insulin biosynthesis in rinm5f pancreatic islet beta-cells: relevant to the Pathogenesis of Diabetes. PLoS One 2013;8:e77931.
Huang YL, Lu L, Li SF, Luo XG, Liu B. Relative bioavailabilities of organic zinc sources with different chelation strengths for broilers fed a conventional corn-soybean meal diet. J Anim Sci 2009;87:2038–46.
Kambe T, Matsunaga M, Takeda TA. Understanding the contribution of zinc transporters in the function of the early secretory pathway. Int J Mol Sci 2017;18:2179.
Kelleher SL, McCormick NH, Velasquez V, Lopez V. Zinc in specialized secretory tissues: roles in the pancreas, prostate, and mammary gland. Adv Nutr 2011;2:101–11.
Kwiecien M, Winiarska-Mieczan A, Milczarek A, Klebaniuk R. Biological response of broiler chickens to decreasing dietary inclusion levels of zinc glycine chelate. Biol Trace Elem Res 2017;175:204–13.
Li YV. Zinc and insulin in pancreatic beta-cells. Endocrine 2014;45:178–89.
Liu X, Ma YF, Chen LJ, Yu XN, Feng J. Effects of different zinc sources on growth performance, antioxidant capacity and zinc storage of weaned piglets. Livest Sci 2021;241:104181.
Ma N, Yang YJ, Liu XW, Li SH, Qin Z, Li JY. Plasma metabonomics and proteomics studies on the anti-thrombosis mechanism of aspirin eugenol ester in rat tail thrombosis model. J Proteonomics 2020;215:103631.
Ma WZ, Tang C, Lai LH. Specificity of trypsin and chymotrypsin: loop-motion-controlled dynamic correlation as a determinant. Biophys J 2005;89:1183–93.
Ma X, Qian MQ, Yang Zh, Xu T, Han XY. Effects of zinc sources and levels on growth performance, zinc status, expressions of zinc transporters, and zinc bioavailability in weaned piglets. Animals 2021;11:2515.
Morais JBS, Severo JS, Beserra JB, Soares De Oiveira AR, Cruz KJC, Melo SRD, et al. Association between cortisol, insulin resistance and zinc in obesity: a mini-review. Biol Trace Elem Res 2019;191:323–30.
Pieper R, Martin L, Schunter N, Tudela CV, Weise C, Klopfleisch R, et al. Impact of high dietary zinc on zinc accumulation, enzyme activity and proteomic profiles in the pancreas of piglets. J Trace Elem Med Biol 2015;30:30–6.
Ravier MA, Rutter GA. Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic alpha-cells. Diabetes 2005;54:1789–97.
Razak MA, Begum PS, Viswanath B, Rajagopal S. Multifarious beneficial effect of nonessential amino acid, glycine: a review. Oxid Med Cell Longev 2017;2017:1716701.
Salim HM, Jo C, Lee BD. Zinc in broiler feeding and nutrition. Avian Biol Res 2008;1:5–18.
Salim HM, Lee HR, Jo C, Lee SK, Lee BD. Effect of sources and levels of zinc on the tissue mineral concentration and carcass quality of broilers. Avian Biol Res 2010;3:23–9.
Slaoui M, Bauchet AL, Fiette L. Tissue sampling and processing for histopathology evaluation. Methods Mol Biol 2017;1641:101–14.
Slepchenko KG, Daniels NA, Guo A, Li YV. Autocrine effect of Zn^{2+} on the glucose-stimulated insulin secretion. Endocrine 2015;50:110–22.
Sobhanirad S, Naserian AA. Effects of high dietary zinc concentration and zinc sources on hematology and biochemistry of blood serum in Holstein dairy cows. Anim Feed Sci Technol 2012;177:242–6.
Solaiman SG, Min BR. The effect of high levels of dietary zinc on growth performance, carcass. characteristics, blood parameters, immune response and tissue minerals in growing Boer-cross goat kids. Small Rumin Res 2019;177:167–74.
Spears JW. Trace mineral bioavailability in ruminants. J Nutr 2003;133:1506s–9s.
Tattikota SG, Sury MD, Rathjen T, Wessels HH, Pandey AK, You XT, et al. Argonaute2 regulates the pancreatic beta-cell secretome. Mol Cell Proteomics 2013;12:1214–25.
Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.
Wang DL, Coco MW, Rose RB. Interactions with the bifunctional interface of the transcriptional coactivator dcoh1 are kinetically regulated. J Biol Chem 2015;290:4319–29.
Williams JA. Cholecystokinin (CCK) regulation of pancreatic acinar cells: physiological actions and signal transduction mechanisms. Compr Physiol 2019;9:535–64.
Williams JA, Chen XQ, Sabbatini ME. Small G proteins as key regulators of pancreatic digestive enzyme secretion. Am J Physiol-Endoc M 2009;296:405–14.
Yan QX, Xu JZ, Wu XS, Su DD, Tan ZL. Stage-specific feed intake restriction differentially regulates placental traits and proteome of goats. Br J Nutr 2018;119:1119–32.
Zhao TJ, Huang QF, Su YN, Sun WY, Huang Q, Wei W. Zinc and its regulators in pancreas. Infammopharmacology 2019;27:453–64.
Zhou XL, Yang H, Yan QX, Ren A, Kong ZW, Tang SX, et al. Evidence for liver energy metabolism programming in offspring subjected to intrauterine undernutrition during midgestation. Nutr Metab 2019;16:20.