Severe Pantothenic Acid Deficiency Induces Alterations in The Intestinal Mucosal Proteome of Starter Pekin Ducks

Jing Tang

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Yulong Feng

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Bo Zhang

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Yongbao Wu

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Zhanbao Guo

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Suyun Liang

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Zhengkui Zhou

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Ming Xie

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Shuisheng Hou (✉ hous@263.net)

chinese academy of agricultural sciences, institute of animal science

Research article

Keywords: pantothenic acid deficiency, intestinal hypofunction, mucosal proteomics, hypoglycemia, actin cytoskeleton

Posted Date: August 25th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-46187/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Pantothenic acid deficiency (PAD) results in growth depression and intestinal hypofunction of animals. However, the underlying mechanisms remain to be established. We investigated intestinal mucosal proteome changes induced by PAD in ducks to explain its effects on growth and intestine.

Results: A total of 128 one-day-old Pekin ducks were divided into two groups, with 8 replicates and 8 birds per replicate. All the ducks were fed either a PAD or a pantothenic acid adequate (control, CON) diet for 16 days. High mortality, growth retardation, fasting hypoglycemia, reduced plasma insulin, and oxidative stress were observed in the PAD group compared to the CON group. Furthermore, PAD induced morphological alterations of the small intestine indicated by reduced villus height and villus surface area of duodenum, jejunum, and ileum. The duodenum mucosal proteome of ducks showed that 198 proteins were upregulated and 223 proteins were downregulated (> 1.5 -fold change) in the PAD group compared to those in the CON group. Pathway analysis of these proteins exhibited the suppression of glycolysis and gluconeogenesis, fatty acid beta oxidation, tricarboxylic acid cycle, oxidative phosphorylation, oxidative stress, and intestinal absorption in the PAD group, indicating impaired energy generation and abnormal intestinal absorption. We also show that nine out of eleven proteins involved in regulation of actin cytoskeleton were upregulated by PAD, probably indicates reduced intestinal integrity.

Conclusion: PAD leads to growth depression and intestinal hypofunction of ducks, which are associated with impaired in energy generation, abnormal intestinal absorption, and regulation of actin cytoskeleton processes. These findings have important implications for understanding the effect of PAD on intestines.

Background

As an essential water-soluble vitamin, pantothenic acid serves as a component of two coenzymes, coenzyme A (CoA) and acyl-carrier-protein. The coenzymes of this vitamin participate in various metabolic reactions, such as glucose, fatty acids and amino acids enter into energy-yielding tricarboxylic acid (TCA) cycle, fatty acid oxidation and synthesis, cholesterol synthesis, acetylcholine synthesis, and heme synthesis etc. [1, 2]. Its importance is highlighted by the adverse effects of pantothenic acid deficiency (PAD) in mammals such as rats, cats, and pigs, including growth depression, skin lesions, diarrhea, loss of hair [2-6]. Also in poultry studies, PAD results in growth retardation, dermatosis, rough feathers, and high mortality in chicks, turkeys, geese, and ducks [7-13]. It has been demonstrated extensively that pantothenic acid can keep the structure of intestine integrity and maintain the intestinal function of animals [3, 14-16]. Intestinal hypofunction was shown to be a major consequence of PAD in rats, dogs, cats, chicks, and fish, such as intestinal ulceration, diarrhea, and colitis [4, 5, 17-21]. Rats deficient in pantothenic acid exhibit duodenitis and duodenal ulcers [18], as well as duodenal changes including eventual atrophy of crypts, diminution in size of villi [16]. In addition, previous studies in fish showed that PAD decreased intestinal digestive and absorptive capacities by reducing the activities of both intestinal brush border enzymes and digestive enzymes [14, 21].

Besides, it is shown that pantothenic acid could protect the cell membrane against damage caused by lipid peroxidation [22-24]. Previous studies have shown that dietary PAD could lead to oxidative stress in geese [9] and fish [21]. It is proposed that oxidative stress induced by PAD leads to intestinal injury and hypofunction. While it is clear that PAD affects growth and intestinal function, an experimental overview of underlying processes has not been presented and the extend of changes induced by PAD have not been investigated. Therefore, the objective of the present study was to investigate the effects of PAD on mucosal protein levels of starter Pekin ducks using a proteomic approach.

Results

Mortality and growth performance

The mortality of ducks was greater in the PAD group than in the CON group ($P < 0.001$; Table 2). In comparison with the CON group, ADG and ADFI were declined in the PAD group, while FCR was increased ($P < 0.001$; Table 2).

Plasma parameters

Plasma pantothenic acid concentration decreased by 85%, while plasma ALP activity decreased by approximately 70% as a result of PAD when compared to the CON group ($P < 0.001$; Table 3). Compared to the CON group, plasma glucose and insulin content were declined in the PAD group ($P < 0.01$; Table 3). Plasma glucagon did not differ between the PAD and CON group ($P > 0.05$; Table 3). Compared to the CON group, plasma MDA content was increased, while plasma T-SOD activity was decreased in the PAD group ($P < 0.001$; Table 3).

Intestinal morphology analyses

Dietary PAD resulted in morphological alterations of the small intestine of Pekin ducks. Compared to the CON group, PAD reduced villus height and villus surface area of duodenum, jejunum, and ileum ($P < 0.001$; Table 4). Villus width and crypt depth of both duodenum and jejunum were not affected by PAD in ducks ($P > 0.05$; Table 4). Compared to the CON group, PAD decreased crypt depth of ileum in ducks ($P < 0.001$; Table 4) but not villus width of ileum ($P > 0.05$; Table 4).

Changes in the intestinal mucosal proteomics of duck in response to PAD

A total of 22,973 peptide spectral matches were found, from which 3345 proteins were identified in the duodenum mucosa of two groups. Comparisons of the relative abundance of proteins from mucosa of PAD ducks with those of CON ducks showed that a total of 421 proteins showed a FC > 1.5 , of which 198 proteins were upregulated and 223 proteins were downregulated. The complete list of proteins regulated by PAD is presented in Additional file 1.

We performed GO categories of biological process, cellular component, and molecular function, and pathway analysis on the set of 421 differentially expressed proteins in mucosa from the PAD group

compared with those in the CON group. As shown in Fig. 1, the top 15 enriched terms under biological process included oxidation-reduction process, small molecule metabolic process, generation of precursor metabolites and energy, purine nucleoside triphosphate metabolic process, monocarboxylic acid metabolic process, nucleoside triphosphate metabolic process, carboxylic acid metabolic process, ATP metabolic process, oxoacid metabolic process, ribonucleoside monophosphate metabolic process, organic acid metabolic process, nucleoside monophosphate metabolic process, ribonucleoside triphosphate metabolic process, coenzyme metabolic process, and cellular respiration. The top 15 enriched terms under cellular component included extracellular exosome, extracellular vesicle, extracellular organelle, vesicle, mitochondrion, cytoplasmic part, myelin sheath, cytoplasm, brush border, adherens junction, organelle inner membrane, focal adhesion, cell-substrate adherens junction, mitochondrial inner membrane, and cluster of actin-based cell projections. The top 15 enriched terms under molecular function included cell adhesion molecule binding, oxidoreductase activity, coenzyme binding, identical protein binding, cadherin binding, actin filament binding, actin binding, aminopeptidase activity, RNA binding, fatty-acyl-CoA binding, exopeptidase activity, cytoskeletal protein binding, hydrolyase activity, glutathione transferase activity, and S100 protein binding.

The pathway analysis by KEGG on differentially expressed proteins suggested that the significantly affected pathways were glycolysis and gluconeogenesis, amino acid metabolism, cori cycle, fatty acid beta oxidation, striated muscle contraction, TCA cycle, trans-sulfuration pathway, PPAR signaling pathway, cytoplasmic ribosomal proteins, vitamin A and carotenoid metabolism, oxidative stress, regulation of actin cytoskeleton, glutathione metabolism, oxidative phosphorylation (Figure 2). Among them, energy metabolism pathways (glycolysis and gluconeogenesis, fatty acid beta oxidation, TCA cycle, and oxidative phosphorylation), regulation of actin cytoskeleton, and oxidative stress were selected in the present study and the proteins associated with these processes are listed in Table 5.

Of the proteins in the enriched glycolysis and gluconeogenesis, eleven proteins were downregulated (fructose-bisphosphate aldolase (ALDOB), hexokinase domain containing 1 (HKDC1), alpha-enolase (ENO1), fructose-bisphosphatase 1 (FBP1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyruvate kinase (PKM), ATP-dependent 6-phosphofructokinase (PFKP), triosephosphate isomerase (TPI1), phosphoglycerate kinase (PGK1), dihydrolipoyl dehydrogenase (DLD), and pyruvate dehydrogenase E1 beta subunit (PDHB)), while two proteins were upregulated (fructose-bisphosphate aldolase (ALDOA) and hexokinase 1 (HK1)).

Nine proteins were involved in fatty acid beta oxidation, which were all downregulated in the PAD group (acyl-CoA synthetase long chain family member 5 (ACSL5), acyl-CoA dehydrogenase family member 11 (ACAD11), medium-chain specific acyl-CoA dehydrogenase (ACADM), carnitine O-acetyltransferase (CRAT), DLD, hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta (HADHB), enoyl-CoA delta isomerase 2 (ECI2), acyl-coenzyme A oxidase (ACOX1), and acyl-coenzyme A oxidase (ACOX2)).

Of the proteins enriched in TCA cycle, six proteins were downregulated (DLD, aconitase 1 (Fragment) (ACO1), aconitate hydratase, mitochondrial (ACO2), malate dehydrogenase (fragment) (MDH1), malate dehydrogenase (MDH2), and pyruvate dehydrogenase E1 beta subunit (PDHB)), while one protein was upregulated (isocitrate dehydrogenase [NADP] (IDH1)).

Five proteins were involved in the oxidative phosphorylation, which were all downregulated in the PAD group (NADH:ubiquinone oxidoreductase subunit A5 (NDUFA5), NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6 (Fragment) (NDUFA6), ATP synthase, H⁺ transporting, mitochondrial Fo complex subunit B1 (ATP5F1), ATP synthase subunit d, mitochondrial (Fragment) (ATP5H), and ATP synthase subunit O, mitochondrial (Fragment) (ATP5O)).

Of the proteins involved in regulation of actin cytoskeleton, two proteins were downregulated (Villin 1 (VIL1) and Ezrin (EZR)), while nine proteins were upregulated (vimentin (VIM), tropomyosin 3 (TPM3), tropomyosin alpha-1 chain (TPM1), myosin light chain 1 (MYL1), myosin light chain kinase (Fragment) (MYLK), actinin alpha 1 (ACTN1), fibronectin 1 (FN1), vinculin (VCL), and moesin (MSN)).

Of the proteins involved in oxidative stress, three proteins were all downregulated (amine oxidase (MAOA), catalase (CAT), and microsomal glutathione S-transferase 1 (Fragment) (MGST1)), while one protein was upregulated (glutamate-cysteine ligase catalytic subunit (GCLC)).

Besides, we found that four proteins were involved in intestinal absorption, which were all downregulated by PAD, including solute carrier family 2 member 2 (SLC2A2), VIL1, EZR, and monoacylglycerol O-acyltransferase 2 (MOGAT2).

Discussion

Previous studies described that dietary PAD causes deficiency symptoms such as growth retardation, dermatosis, diarrhea, and even death in both mammals [2-6] and poultry [7-13, 19, 25]. In ducks, decreased weight gain and excessive exudate from the eyes were exhibited in response to PAD [13]. In agreement with previous studies, the ducks fed the basal diet without pantothenic acid supplementation showed growth depression, exudate on eyelids, dermatosis, poor feathering, and a high mortality rate in the present study, indicating these ducks were pantothenic acid deficient. Furthermore, poor pantothenic acid status was found in PAD ducks indicated by a marked reduction of plasma pantothenic acid concentration, as tissue pantothenic acid is a useful biomarker for pantothenic acid status [21, 26, 27]. These results demonstrate that a severe PAD animal model was successfully established.

It has been shown that pantothenic acid can keep the structure of intestine integrity and keep the function of intestine normal of animals [3, 14-16]. Insufficient of pantothenic acid would cause intestinal hypofunction in rats, dogs, cats, chicks and fish [4, 5, 17-21]. And a feature common to experimental PAD in different animals is ulceration of the intestine [4, 5, 17-20]. Duodenitis and duodenal ulcers [18], as well as duodenal changes including eventual atrophy of crypts, diminution in size of villi, were observed in PAD rats [16]. In agreement with the previous studies, we observed small intestine morphological changes

indicated by the reduced villus height and villus surface area in PAD ducks in the present study, indicating damage to the intestinal epithelium.

Furthermore, PAD leads to abnormalities in carbohydrate metabolism. Low fasting blood glucose levels and increased sensitivity to insulin were found in PAD rats and dogs [28-32]. In agreement with previous studies, PAD caused fasting hypoglycemia and decreased plasma insulin level in ducks in the present study, indicating abnormal glucose metabolism.

Together, dietary PAD of ducks resulted in growth retardation, alterations of intestinal morphology and function, and abnormal glucose metabolism. However, limited data are available currently concerning the molecular mechanisms behind. Therefore, we used a proteomic approach, for the first time, to investigate the metabolic disorder of small intestine induced by PAD to explain intestinal hypofunction and growth depression. Proteomic analysis revealed 421 differentially expressed proteins in the mucosa of PAD ducks compared to those that were adequately supplied with pantothenic acid, indicating an important impact of pantothenic acid on intestinal function. The identical proteins are mainly enriched in in glycolysis and gluconeogenesis, fatty acid beta oxidation, oxidative phosphorylation, TCA cycle, intestinal absorption, regulation of actin cytoskeleton, and oxidative stress. It is indicated that these processes probably underlie the intestinal mucosa metabolic disorder and poor growth. Notably, the CoA-binding proteins were reduced in the intestinal mucosa of PAD ducks, such as ACSL5, ACAD11, ACADM, HADHB, ECI2, ACOX1, and ACOX2. This finding is in line with the hypothesis that CoA-binding protein may be depressed due to a reduced supply of pantothenic acid in the diet.

Glycolysis and gluconeogenesis

A total of thirteen proteins participating in the glycolysis and gluconeogenesis pathway were differentially expressed after PAD, making it the largest category of identified proteins. Of these, twelve proteins were involved in glycolysis, two enhanced (ALDOA and HK1) and ten diminished (ALDOB, HKDC1, ENO1, GAPDH, PKM, PFKP, TPI1, PGK1, DLD, and PDHB). HKDC1 and HK1 are two isozymes of hexokinases, mediate the initial step of glycolysis by catalyzing phosphorylation of D-glucose to D-glucose 6-phosphate [33]. PFKP catalyzes the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate by ATP [34]. ALDOA and ALDOB are two isoforms of [aldolase](#) family which are located in skeletal muscle and liver tissue respectively, cleaves fructose-1,6-bisphosphate to triose phosphates [35]. TPI1 catalyzes the interconversion between dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate [36]. GAPDH catalyzes the conversion of glyceraldehyde 3-phosphate into 1,3-diphosphoglycerate [37]. PGK1 catalyzes one of the two ATP producing reactions in the glycolytic pathway via the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate [38]. ENO1 catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate [39]. PKM catalyzes the second ATP generation reaction in the glycolytic pathway via conversion of phosphoenolpyruvate to pyruvate [40]. PDHB is a subunit of pyruvate dehydrogenase (E1). As the E3 component of [pyruvate dehydrogenase](#) complex, DLD oxidizes dihydrolipoic acid to lipoic acid [41]. Pyruvate dehydrogenase complex irreversibly decarboxylates pyruvate to acetyl-CoA, thereby linking glycolysis to

the TCA cycle and fatty acid synthesis [42, 43]. Therefore, ten out of twelve proteins (ALDOB, HKDC1, ENO1, GAPDH, PKM, PFKF, TPI1, PGK1, DLD, and PDHB) were downregulated in the PAD group suggests that the rate of glycolysis in the intestinal mucosa may be impaired. Besides, one protein (FBP1) involved in gluconeogenesis process was downregulated by PAD. FBP1, a rate-limiting enzyme in gluconeogenesis, catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate. The reduced expression of FBP1 in the PAD group probably indicates a decreased gluconeogenesis in the intestinal mucosa.

Fatty acid beta oxidation

In the present study, PAD downregulated 9 proteins involved in fatty acid beta oxidation, including ACSL5, ACAD11, ACADM, CRAT, DLD, HADHB, ECI2, ACOX1, and ACOX2. Notably, among these, ACSL5, ACAD11, ACADM, HADHB, ECI2, ACOX1, and ACOX2 are belong to CoA-binding proteins, which were all reduced due to PAD. ACSL5 belongs to the acyl-CoA synthetase family, catalyzing free fatty acids into fatty acyl-CoA esters, which plays a key role in lipid biosynthesis and fatty acid degradation [44]. ACAD11 and ACADM belong to the family of fatty acyl-CoA dehydrogenases that catalyze the initial step in each cycle of fatty acid beta-oxidation [45]. CRAT catalyzes the reversible transfer of an acetyl group from acyl-CoA to carnitine [46]. ECI2 is involved in the beta oxidation of unsaturated fatty acids, converting 3-cis or trans-enoyl-CoA to 2-trans-enoyl-CoA [47]. HADHB catalyzes the final step of beta-oxidation, in which 3-ketoacyl CoA is cleaved by the thiol group of another molecule of Coenzyme A [48]. ACOX1 and ACOX2 catalyze the first and rate-limiting step of peroxisomal [fatty acid beta oxidation](#), the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs. ACOX1 catalyzes medium to very long straight-chain fatty acids [49], while ACOX2 catalyze the CoA-esters of very long-chain fatty acids, branched-chain fatty acids and the C27-bile acid intermediates [50]. The decreased expression of all these proteins involved in the fatty acid beta oxidation process may imply that fatty acid beta oxidation is impaired by PAD. This implication is supported by previous finding in rats that PAD reduced CoA and short-chain acyl-CoA contents [51], as well as hepatic peroxisomal fatty acid beta oxidation [6].

TCA cycle

PAD downregulated six proteins involved in the TCA cycle, including DLD, ACO1, ACO2, MDH1, MDH2, and PDHB, and upregulated one protein, IDH1. PDHB is a subunit of pyruvate dehydrogenase (E1). As the E3 component of [pyruvatedehydrogenase](#) complex and [α-ketoglutaratedehydrogenase](#) complex, DLD oxidizes dihydrolipoic acid to lipoic acid. [41]. Pyruvate dehydrogenase complex irreversibly decarboxylates pyruvate to acetyl-CoA, thereby linking glycolysis to the TCA cycle and fatty acid synthesis [42, 43]. [α-ketoglutaratedehydrogenase](#) is a rate-limiting enzyme of the TCA cycle, catalyzing the conversion of α-ketoglutarate to succinyl-CoA and [NADH](#) [52, 53]. ACO1 and ACO2 are two isozymes of aconitate hydratase located in cytoplasmic and mitochondrial respectively, which catalyzes the isomerization of citrate to isocitrate via cis-aconitate. MDH1 and MDH2 are two isozymes of malate dehydrogenase located in cytoplasmic and mitochondrial respectively, which catalyzes the reversible interconversion of malate and oxaloacetate [54]. IDH1 is a subunit of isocitrate dehydrogenase,

catalyzing the oxidative decarboxylation of isocitrate into α -ketoglutarate [55]. Six out of seven proteins (DLD, ACO1, ACO2, MDH1, MDH2, and PDHB) were downregulated in the PAD group, which likely indicates a decreased mucosa TCA cycle.

Oxidative phosphorylation

PAD downregulated 5 proteins involved in the oxidative phosphorylation process, including NDUFA5, NDUFA6, ATP5F1, ATP5H, and ATP5PO. NDUFA5 and NDUFA6 are two subunits of complex I, which play a direct role in complex I assembly [56, 57]. ATP5F1, ATP5H, and ATP5PO are three subunits of complex V, which play a direct role in complex V assembly [58-60]. The downregulated expression of proteins involved in the oxidative phosphorylation process, including complex I and complex V, probably indicates that this process is impaired by PAD.

Together, our duodenum mucosal proteomic analysis revealed glycolysis and gluconeogenesis, fatty acid beta oxidation, TCA cycle, and oxidative phosphorylation processes are probably impaired in response to PAD, which may consequently leads to insufficient ATP production in the small intestine and subsequent growth retardation.

Regulation of actin cytoskeleton

Eleven proteins altered by PAD have actin-binding domains and play a direct role in the organization of structure of the cytoskeleton, including two downregulated proteins (VIL1 and EZR) and nine upregulated proteins (ACTN1, FN1, MSN, MYL1, MYLK, VCL, TPM3, TPM1, and VIM). VIL1 and EZR (also known as villin 2; VIL2) are microvillar proteins in intestinal epithelial cells [61]. VIL1 is an epithelial cell-specific actin-binding protein that regulates cell migration, cell death, cell morphology, and epithelial-to-mesenchymal transition [62, 63]. EZR is critical for the de novo lumen formation and expansion during villus morphogenesis, and EZR absence resulted in abnormal villus morphogenesis [64, 65].

Actinin is a component of stress fibers and links the cytoskeleton to adherens-type junctions. As one isoform of actinin, ACTN1 plays a major role in cell migration and adhesion [66]. Besides, ACTN1 can directly binds to VCL, and the two proteins cooperate to organize the cytoskeleton at adhesion junctions [67]. FN1, a glycoprotein component of the extracellular matrix, has a role in cell adhesion and migration [68]. VIM induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition [69]. MYLK induces contraction of the perijunctional actomyosin ring through myosin II regulatory light chain phosphorylation, and thereby increases intestinal epithelial permeability when activated [70]. TPM3 and TPM1 are two isoforms of tropomyosins which play important roles in the regulation of assembly, stability, and motility of the intestinal epithelial cells [71]. Collectively, the over expression of nine proteins (ACTN1, FN1, MSN, MYL1, MYLK, VCL, TPM3, TPM1, and VIM) and downregulation of two proteins (VIL1 and EZR) in response to PAD probably indicates orchestrated regulation of actin cytoskeleton dynamics and the negative impact on intestinal integrity. Remodeling of the cytoskeleton is fundamental in proliferation, apoptosis, cell invasion and metastasis [72]. Therefore, these altered proteins involved in regulation of actin cytoskeleton in response to PAD probably resulted in

morphological changes of small intestine, such as atrophy of their intestinal villus. Furthermore, it is reported that ATP depletion uncouples the gate and fence functions of the tight junction and induces actin network dissolution of epithelial cells [73]. The alterations of intestinal morphology and actin cytoskeleton due to PAD in the present study may be attributed to ATP depletion indicated by impaired glycolysis, fatty acid beta oxidation, TCA cycle, and oxidative phosphorylation processes.

Oxidative Stress

PAD downregulated three proteins involved in the oxidative stress, including MAOA, CAT, and MGST1, and upregulated one protein, GCLC. MAOA metabolizes dopamine to dihydroxyphenylacetic acid and H_2O_2 , a potential source of reactive oxygen species [74]. Both CAT and MGST1 take part in the oxidative stress defense as its scavenging of H_2O_2 [75, 76]. GCLC is a subunit of glutamate cysteine ligase which catalyzes the rate-limiting step in reduced glutathione (GSH) synthesis. And glutamate cysteine ligase is often activated to increase cellular GSH content in response to oxidative stress [77]. Collectively, the reduction of MAOA, CAT, and MGST1 in PAD ducks, as well as the enhanced GCLC, indicates small intestinal oxidative stress was induced. This is supported by the results of increased plasma MDA content and decreased T-SOD activity in the present study and is in line with previous studies in geese [9] and fish [21]. It has been shown that pantothenic acid can protect cells against oxidative stress by increasing the levels of glutathione and promoting cellular repair mechanisms by potentiating synthesis of membrane phospholipids [22-24]. Furthermore, PAD induced oxidative stress in ducks, which may be associated with intestinal injury and morphological alterations.

Intestinal absorption

A novel and important finding of this study is that four proteins (SLC2A2, VIL1, EZR, and MOGAT2) involved in intestinal absorption were downregulated in duodenum mucosa as a result of PAD. Intestinal SLC2A2 (GLUT2) is known as a means to transfer glucose and fructose from the lumen to the bloodstream and, thereby, to provide sugar to tissue. And intestinal SLC2A2 deletion in mice induced glucose malabsorption visualized by the delay in the distribution of oral sugar in tissues, as well as decreased microvillus length and body weight gain [78]. It is reported pantothenic acid appears to be part of a glucose carrier system [79], therefore, PAD may direct reduce SLC2A2 and resulted in abnormal glucose absorption and hypoglycemia in ducks. Villin (VIL1 and EZR) is also involved in the absorptive and secretory function of epithelial cells by modulating F-actin polymerization/depolymerization. Specifically, villin-depleted mice showed a reduction in intestinal glucose absorption [80]. Decreased protein expression of SLC2A2, VIL1, and EZR due to PAD probably impair glucose absorption system, which may provide a possible explanation for fasting hypoglycemia that is seen. MOGAT2 plays a central role in absorption of dietary fat in the small intestine by catalyzing the resynthesis of triacylglycerol in enterocytes [81]. MGAT2 deficient specifically in the small intestine showed a delay in fat absorption in mice [82]. Therefore, a reduction of SLC2A2, VIL1, EZR, and MOGAT2 suggests that glucose and fat malabsorption in the small intestine may be induced by PAD, which probably leads to growth depression that is seen. This is in line with the previous findings in fish that PAD decreased the digestive and

absorptive capacities indicated by the reduced the activities of both intestinal brush border enzymes and digestive enzymes [14, 21].

Conclusion

PAD caused growth retardation, fasting hypoglycemia, morphological alterations of the small intestine, and oxidative stress in ducks. We have performed a proteomic profiling further to investigate the effects of PAD on duodenum mucosal proteome of ducks. The results indicated that PAD may suppress energy generation processes such as glycolysis and gluconeogenesis, fatty acid beta oxidation, TCA cycle, and oxidative phosphorylation, leading to impaired ATP generation. Furthermore, PAD may induce glucose and fat malabsorption in the small intestine indicated by four diminished proteins involved. Besides, PAD probably leads to negative impacts on intestinal integrity and morphology because most of proteins involved in regulation of actin cytoskeleton were upregulated. To be sum, PAD causes intestinal hypofunction and growth depression probably by impairing glycolysis and gluconeogenesis, fatty acid beta oxidation, TCA cycle, oxidative phosphorylation, actin cytoskeleton, and intestinal absorption processes. These findings significantly expand our knowledge on effects of PAD on intestines.

Methods

Animals ethics statement

All experimental procedures with ducks were performed according to the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). Ethical approval on animal survival was given by the animal ethics committee of the Institute of Animal Sciences (IAS), Chinese Academy of Agricultural Sciences (CAAS, Beijing, China) with the following reference number: IASCAAS-2019-19.

Animals and housing

A total of 128 one-day-old male white Pekin ducks (*Anas platyrhynchos*) were obtained from the Pekin duck breeding center (Chinese Academy of Agricultural Sciences) and randomly assigned to 16 raised plastic-floor pens with 8 birds per pen. All ducks were divided into two experimental groups, each containing 8 replicates with 8 birds per replicate. The ducks were either *ad libitum* fed with a PAD diet or a control diet (CON). All ducks were fed their respective diets and had free access to water from hatch to 16 days of age. During this period, the light was continuously on. The temperature was kept at 33 °C from 1 to 3 days of age, after which it was gradually reduced to approximately 25 °C until 14 days of age and kept at approximately 16 to 22 °C for the remainder of the experiment.

Diet

The basal diet during the experimental period was pantothenic acid-deficient containing 4.65 mg pantothenic acid /kg of diet (Table 1). The PAD diet and control diet were produced from the basal diet,

and were supplemented with respectively 0 and 8 mg crystalline calcium pantothenate /kg diet (purity, 99%; Xinfu Technology Co. Ltd, Hangzhou, China). The pantothenic acid concentration for the control diet met the recommendations for starter ducks provided by NRC (1994) [83].

Sampling

At 16 days of age, after overnight fasting, the ducks and residual diet from each pen were weighed to determine average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). ADFI and FCR were all corrected for mortality. Two ducks from each pen were randomly selected. Blood samples were collected from each bird via jugular vein puncture in 5 ML heparin sodium-containing tubes, then centrifuged at 1500× *g* for 20 min to separate plasma. The plasma samples were stored at -20 °C until further analysis. Thereafter, these selected ducks were sacrificed by CO₂ inhalation and intestinal tissues were obtained immediately. The intestinal sections were divided into the duodenum, jejunum, and ileum. And they were rinsed with physiological saline and then cut into 1 cm length segments, fixed in 10% neutral formalin used for histological analysis. Duodenum mucosa from the remaining segment was obtained as described previously [84], snap frozen in liquid nitrogen, and stored at -80 °C until further analysis.

Pantothenic acid content

Pantothenic acid concentrations in feed and plasma were determined by HPLC coupled with triple quadrupole mass spectrometry (Agilent 6470) according to the methods described previously [85]. Agilent 1290 HPLC system consisting of ZORBAX Eclipse Plus C18 column (3.0 × 150 mm i.d., 1.8μm) was applied for pantothenic acid separation. The column oven was maintained at 35 °C, the flow rate of the mobile phase was 0.2 mL/min. The binary mobile phase used consisted of acetonitrile and water containing 0.1% formic acid. Before LC/MS analysis, feed samples were prepared according to the methods described previously [86]. Plasma samples were prepared according to the method described previously [87]. The peak was identified and quantified by using the pure authentic standards purchased from (Sigma-Aldrich, St. Louis, MO, USA).

Plasma parameters

Plasma glucose and alkaline phosphatase (ALP) were determined using commercial kits according to manufacturer's instructions (BioSino Bio-technology and Science Inc., Beijing, China). Plasma malondialdehyde (MDA) content, total superoxide dismutase (T-SOD) activity, insulin, and glucagon were measured by commercial kits according to manufacturer's protocols (Nanjing Jiancheng Institute of Bioengineering, Nanjing, Jiangsu, China).

Intestinal morphology assessment

The duodenum, jejunum, and ileum sections were embedded in paraffin and transversely sectioned in (4 μm thick) and stained with hematoxylin and eosin following deparaffinization and dehydration. Intestinal

tissues and structures were observed using a BH2 Olympus microscope (Olympus, Tokyo, Japan) and analyzed using an image analysis system (Olympus 6.0). Villus height, villus width, crypt depth, and villus surface area were assessed following the method as described previously [84].

Duodenum mucosal proteomics

Three individual duodenum mucosa samples were randomly chosen from each group to conduct the isobaric tags for relative and absolute quantification (iTRAQ) assays. Proteins were extracted and digested as described previously [88]. The digested samples were labelled according to the manufacturer's protocol for 8-plex iTRAQ reagents (AB SCIEX, Foster City, USA) as follows: the PAD samples were labelled with iTRAQ tags 113, 114, and 115, and the CON samples were labelled with tags 116, 117, and 118. Labelled samples were mixed and fractionated into 20 fractions by HPLC (DINOEX Ultimate 3000 BioRS, Thermo Fisher, Waltham, MA, USA) using a Durashell C18 column (5 µm, 100 Å, 4.6 × 250 mm). LC-electrospray ionization-MS/MS analysis was carried out with a Triple TOF 5600 plus system (AB SCIEX, Framingham, USA). The original MS/MS file data for identification and quantitation were analysed against the database *UniProt_Mallard_8839* using ProteinPilot Software version 4.5 (AB SCIEX). To minimize false-positive results, we counted only peptides at the 95% CI and a false discovery rate < 0.01, containing at least one unique peptide. For protein quantitation, a differentially expressed protein containing at least two unique spectra was used with a fold change (FC) value > 1.5 or < 0.67 with $P < 0.05$ between the PAD and the CON groups. Gene Ontology (GO) and pathway enrichment analysis of the differentially expressed proteins were conducted using ClueGo software as described previously [88].

Statistical analyses

For results of growth performance, plasma parameters, and intestinal mucosal histomorphology, data were analysed using the Student's *t* test procedures of SAS software (SAS Institute Inc., 2011). Each pen was considered the experimental unit for analysis. The variability in the data was expressed as the standard error of the means (SEM). Differences between means were considered statistically significant at $P < 0.05$. For analysis of proteomic results, the relative expression of identified proteins was based on the ratio of the reporter ions of the peptides in the PAD group to the CON group. Compared with the CON group, proteins with a threshold of > 1.5- or < 0.67-fold and $P < 0.05$ were considered differentially expressed proteins.

Abbreviations

ADG, average daily weight gain; ADFI, average daily feed intake; ALP, alkaline phosphatase; CoA, coenzyme A; CON, control; FC, fold change; FCR, feed conversion ratio; GO, Gene Ontology; HPLC, high performance liquid chromatography; iTRAQ, isobaric tags for relative and absolute quantification; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDA, malondialdehyde; PAD pantothenic acid deficiency; SEM, standard error of the mean; TCA, tricarboxylic acid; T-SOD, total superoxide dismutase.

Declarations

Ethics approval and consent to participate

All experimental procedures with ducks were performed according to the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). Ethical approval on animal survival was given by the animal ethics committee of the Institute of Animal Sciences (IAS), Chinese Academy of Agricultural Sciences (CAAS, Beijing, China) with the following reference number: IASCAAS-2019-19.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during the current study are available from the corresponding author on reasonable request. The datasets supporting the conclusions of this article are included within the article and its additional file.

Competing interests

All authors declare that they have no conflict of interests.

Funding

This work was supported by the earmarked fund for the China Agriculture Research System (CARS-42), the science and technology innovation project of the Chinese Academy of Agricultural Sciences (CXGC-IAS-09). The funding bodys had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

JT performed the study, was involved in all aspects of analysis and drafted the manuscript. YF, MX, ZZ and SH were involved in experimental design. BZ, YW, ZG performed data analysis and sample analysis. JT, YF, SL and SH were involved in the data interpretation and writing. All authors read and approved the final manuscript.

Acknowledgments

We thank the members of our laboratory for their helpful comments on the manuscript.

References

1. Miller JW, Rucker RB. Pantothenic acid. In Present Knowledge in Nutrition, 10th ed. In: Erdman JW, Macdonald IA, Zeisel SH, editors. Washington, DC, USA: Wiley-Blackwell; 2012. p. 375-390.
2. Smith CM, Song WO. Comparative nutrition of pantothenic acid. *J Nutr Biochem*. 1996;7:312-321.
3. Nelson RA. Intestinal transport, coenzyme A, and colitis in pantothenic acid deficiency. *Am J Clin Nutr*. 1968;21:495-501.
4. Zucker TF. Pantothenic acid deficiency and its effect on the integrity and functions of the intestines. *Am J Clin Nutr*. 1958;6:65-74.
5. Gershoff SN, Gottlieb LS. Pantothenic acid deficiency in cats. *J Nutr*. 1964;82:135-138.
6. Youssef JA, Song WO, Badr MZ. Mitochondrial, but not peroxisomal, β -oxidation of fatty acids is conserved in coenzyme A-deficient rat liver. *Mol Cell Biochem*. 1997;175:37-42.
7. Bauernfeind JC, Norris LC, Heuser GF. The pantothenic acid requirement of chicks. *Poult Sci*. 1942;21:142-146.
8. Jukes TH. The pantothenic acid requirement of the chick. *J Biol Chem*. 1939;129:225-231.
9. Wang B, Zhang X, Yue B, Ge W, Zhang M, Ma C, et al. Effects of pantothenic acid on growth performance, slaughter performance, lipid metabolism, and antioxidant function of Wulong geese aged one to four weeks. *Anim Nutr*. 2016;2:312-317.
10. Kratzer FH, Williams D. The pantothenic acid requirement of poult for early growth. *Poult Sci*. 1948;27:518-523.
11. Hegsted DM, Riggs TR. The pantothenic acid requirements of chicks receiving a purified diet. *J Nutr*. 1949;37:361-367.
12. Lepkovsky S, Bird FH, Kratzer FH, Asmundson VS. The comparative requirements of chicks and turkey poult for pantothenic acid. *Poult Sci*. 1945;24:335-339.
13. Hegsted DM, Perry RL. Nutritional studies with the duck. I. riboflavin and pantothenic acid requirements. *J Nutr*. 1948;35:411-417.
14. Wen ZP, Zhou XQ, Feng L, Jiang J, Liu Y. Effect of dietary pantothenic acid supplement on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquacult Nutr*. 2009;15:470-476.
15. Sabui S, Kapadia R, Ghosal A, Schneider M, Lambrecht NWG, Said HM. Biotin and pantothenic acid oversupplementation to conditional SLC5A6 KO mice prevents the development of intestinal mucosal abnormalities and growth defects. *Am J Physiol Cell Physiol*. 2018;315:C73-C79.
16. Seronde J. The pathogenesis of duodenal ulcer disease in the pantothenate-deficient rat. *Yale J BioMed*. 1963;36:141-156.
17. Ellestad-Sayed JJ, Nelson RA, Adson MA, Palmer WM, Soule EH. Pantothenic acid, coenzyme A, and human chronic ulcerative and granulomatous colitis. *Am J Clin Nutr*. 1976;29:1333-1338.
18. Berg B. Duodenitis and duodenal ulcers produced in rats by pantothenic acid deficiency. *Br J Exp Pathol*. 1959;40:371-374.

19. Gries CL, Scott ML. The pathology of thiamin, riboflavin, pantothenic acid and niacin deficiencies in the chick. *J Nutr.* 1972;102:1269-1285.
20. McKibbin JM, Black S, Elvehjem CA. The essential nature of pantothenic acid and another alkali labile factor in the nutrition of the dog. *Am J Physiology.* 1940;130:365-372.
21. Qian Y, Li XF, Zhang DD, Cai DS, Tian HY, Liu WB. Effects of dietary pantothenic acid on growth, intestinal function, anti-oxidative status and fatty acids synthesis of juvenile blunt snout bream *Megalobrama amblycephala*. *PloS one.* 2015;10:e0119518.
22. Slyshenkov VS, Piwocka K, Sikora E, Wojtczak L. Pantothenic acid protects Jurkat cells against ultraviolet light-induced apoptosis. *Free Radical Bio Med.* 2001;30:1303-1310.
23. Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. *Cent Eur J Urol.* 2013;66:60-67.
24. Slyshenkov VS, Rakowska M, Moiseenok AG, Wojtczak L. Pantothenic acid and its derivatives protect Ehrlich ascites tumor cells against lipid peroxidation. *Free Radical Bio Med.* 1995;19:767-772.
25. Beer AE, Scott ML, Nesheim MC. The effects of graded levels of pantothenic acid on the breeding performance of white leghorn pullets. *Br Poult Sci.* 1963;4:243-253.
26. Lin YH, Lin HY, Shiau SY. Estimation of dietary pantothenic acid requirement of grouper, *Epinephelus malabaricus* according to physiological and biochemical parameters. *Aquaculture.* 2012;324:92-96.
27. Shiau SY, Hsu CW. Dietary pantothenic acid requirement of juvenile grass shrimp, *Penaeus monodon*. *J Nutr.* 1999;129:718-721.
28. Arnrich L, Hurley LS, Forker BR, Morgan AF. Response to stress by riboflavin-deficient and pantothenic acid-deficient dogs. *Am J Physiol.* 1956;184:515-520.
29. Arnrich L, Nelson MR, Gram MR, Morgan AF. Effect of adrenal hormones on carbohydrate metabolism in riboflavin and pantothenic acid-deficient dogs. *Am J Physiol.* 1956;186:427-434.
30. Hurley LS, Morgan AF. Carbohydrate metabolism and adrenal cortical function in the pantothenic acid-deficient rat. *J Biol Chem.* 1952;195:583-590.
31. Schultz RB, Winters RW, Krehl WA. The adrenal cortex of the pantothenic acid deficient rat: modification of the lesion by ACTH and cortisone treatment. *Endocrinology.* 1952;51:336-343.
32. Winters RW, Schultz RB, Krehl WA. The adrenal cortex of the pantothenic acid-deficient rat: carbohydrate metabolism. *Endocrinology.* 1952;50:388-398.
33. Ludvik AE, Pusec CM, Priyadarshini M, Angueira AR, Guo C, Lo A, et al. HKDC1 is a novel hexokinase involved in whole-body glucose use. *Endocrinology.* 2016;157:3452-3461.
34. Lee JH, Liu R, Li J, Zhang C, Wang Y, Cai Q, et al. Stabilization of phosphofructokinase 1 platelet isoform by AKT promotes tumorigenesis. *Nat Commun.* 2017;8:949.
35. Dawson NJ, Biggar KK, Storey KB. Characterization of fructose-1, 6-bisphosphate aldolase during anoxia in the tolerant turtle, *Trachemys scripta elegans*: an assessment of enzyme activity, expression and structure. *PloS one.* 2013;8:e68830.

36. Rodríguez-Almazán C, Arreola R, Rodríguez-Larrea D, Aguirre-López B, Gómez-Puyou MT, Pérez-Montfort R, et al. Structural basis of human triosephosphate isomerase deficiency mutation E104D is related to alterations of a conserved water network at the dimer interface. *J Biol Chem*. 2008;283:23254-23263.
37. Ercolani L, Florence B, Denaro M, Alexander M. Isolation and complete sequence of a functional human glyceraldehyde-3-phosphate dehydrogenase gene. *J Biol Chem*. 1988;263:15335-15341.
38. Li X, Jiang Y, Meisenhelder J, Yang W, Hawke DH, Zheng Y et al. Mitochondria-translocated PGK1 functions as a protein kinase to coordinate glycolysis and the TCA cycle in tumorigenesis. *Mol Cell*. 2016;61:705-719.
39. Ejeskär K, Krona C, Carén H, Zaibak F, Li L, Martinsson T, et al. Introduction of in vitro transcribed ENO1 mRNA into neuroblastoma cells induces cell death. *BMC Cancer*. 2005. doi:10.1186/1471-2407-5-161.
40. Christofk HR, Heiden MGV, Wu N, Asara JM, Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature*. 2008;452:181-186.
41. Carothers DJ, Pons G, Patel MS. Dihydrolipoamide dehydrogenase: functional similarities and divergent evolution of the pyridine nucleotide-disulfide oxidoreductases. *Arch Biochem Biophys*. 1989;268:409-425.
42. Sugden MC, Holness MJ. Recent advances in mechanisms regulating glucose oxidation at the level of the pyruvate dehydrogenase complex by PDKs. *Am J Physiol Endoc Metab*. 2003;284:E855-E862.
43. Holness M, Sugden M. Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. *Biochem Soc Trans*. 2003;31:1143-1151.
44. Watkins PA, Maiguel D, Jia Z, Pevsner J. Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genome. *J Lipid Res*. 2007;48:2736-2750.
45. Ghisla S, Thorpe C. Acyl-CoA dehydrogenases. *Eur J Biochem*. 2004;271:494-508.
46. Godárová A, Litzlbauer E, Brunner S, Agu A, Lohninger A, Hofbauer. L-Carnitine regulates mRNA expression levels of the carnitine acyltransferases - CPT1A, CPT2, and CRAT. *Monatsh Chem*. 2005;136: 1349-1363.
47. Weeghel MV, Brinke HT, Lenthe HV, Kulik W, Minkler PE, Stoll MSK, et al. Functional redundancy of mitochondrial enoyl-CoA isomerases in the oxidation of unsaturated fatty acids. *FASEB J*. 2012;26:4316-4326.
48. Das AM, Illsinger S, Lucke T, Hartmann H, Ruiter JPN, Steuerwald U, et al. Isolated mitochondrial long-chain ketoacyl-CoA thiolase deficiency resulting from mutations in the HADHB gene. *Clin Chem*. 2006;52:530-534.
49. Jiao Y, Zan LS, Liu YF, Wang HB. Molecular characterization, polymorphism of the ACOX1 gene and association with ultrasound traits in *Bos taurus*. *Genet Mol Res*. 2011;10:1948-1957.
50. Ferdinandusse S, Denis S, Roermund CWT, Preece MA, Koster J, Ebberink MS, et al. A novel case of ACOX2 deficiency leads to recognition of a third human peroxisomal acyl-CoA oxidase. *BBA-Mol Basis Dis*. 2018;1864:952-958.

51. Moiseenok AG, Sheibak VM, Gurinovich VA. Hepatic CoA, S-acyl-CoA, biosynthetic precursors of the coenzyme and pantothenate-protein complexes in dietary pantothenic acid deficiency. [Int J Vitam Nutr Res](#). 1987;57:71-77.
52. Gibson GE, Blass JP, Beal MF, Bunik V. The α -ketoglutarate-dehydrogenase complex. *Mol Neurobiol*. 2005;31:43-63.
53. Zündorf G, Kahlert S, Bunik V, Reiser G. α -Ketoglutarate dehydrogenase contributes to production of reactive oxygen species in glutamate-stimulated hippocampal neurons in situ. *Neuroscience*. 2009;158:610-616.
54. Lo AS, Liew CT, Ngai SM, Tsui SK, Fung KP, Lee CY, et al. Developmental regulation and cellular distribution of human cytosolic malate dehydrogenase (MDH1). [J Cell Biochem](#). 2005;94:763-773.
55. Grassian AR, Parker SJ, Davidson SM, Divakaruni AS, Green CR, Zhang X, et al. IDH1 mutations alter citric acid cycle metabolism and increase dependence on oxidative mitochondrial metabolism. *Cancer Res*. 2014;74: 3317-3331.
56. Lazarou M, McKenzie M, Ohtake A, Thorburn DR, Ryan MT. Analysis of the assembly profiles for mitochondrial-and nuclear-DNA-encoded subunits into complex I. *Mol Cell Biol*. 2007;27:4228-4237.
57. Stroud DA, Surgenor EE, Formosa LE, Reljic B, Frazier AE, Dibley MG, et al. Accessory subunits are integral for assembly and function of human mitochondrial complex I. *Nature*. 2016;538:123-126.
58. Rönn T, Poulsen P, Tuomi T, Isomaa B, Groop L, Vaag A, et al. Genetic variation in ATP5O is associated with skeletal muscle ATP5O mRNA expression and glucose uptake in young twins. *PLoS one*. 2009;4:e4793.
59. Chinopoulos C. ATP synthase complex and the mitochondrial permeability transition pore: poles of attraction. *EMBO Rep*. 2007;18:1041-1042.
60. Brüggemann M, Gromes A, Poss M, Schmidt D, Klümper N, Tolkach Y, et al. Systematic analysis of the expression of the mitochondrial ATP Synthase (Complex V) subunits in clear cell renal cell carcinoma. *Transl Oncol*. 2017;10:661-668.
61. Saleh HS, Merkel U, Geißler KJ, Sperka T, Sechi A, Breithaupt C, et al. Properties of an ezrin mutant defective in F-actin binding. *J Mol Biol*. 2009;385:1015-1031.
62. Khurana S, George SP. Regulation of cell structure and function by actin-binding proteins: villin's perspective. *FEBS Lett*. 2008;582:2128-2139.
63. Friederich E, Vancompernelle K, Louvard D, Vandekerckhove J. Villin Function in the Organization of the Actin Cytoskeleton correlation of in vivo effects to its biochemical activities in vitro. *J Biol Chem*. 1999;274:26751-26760.
64. Saotome I, Curto M, McClatchey AI. Ezrin is essential for epithelial organization and villus morphogenesis in the developing intestine. *Dev Cell*. 2004;6:855-864.
65. Casaletto JB, Saotome I, Curto M, McClatchey AI. Ezrin-mediated apical integrity is required for intestinal homeostasis. *PNAS*. 2011;108:11924-11929.

66. Otey CA, Carpen O. α -actinin revisited: A fresh look at an old player. *Cell Motil Cytoskel.* 2004;58:104-111.
67. Bois PRJ, Borgon RA, Vornrhein C, Izard T. Structural dynamics of α -actinin-vinculin interactions. *Mol Cell Biol.* 2005;25:6112-6122.
68. Goossens K, Soom AV, Zeveren AV, Favoreel H, Peelman LJ. Quantification of fibronectin 1 (FN1) splice variants, including two novel ones, and analysis of integrins as candidate FN1 receptors in bovine preimplantation embryos. *BMC Dev Biol.* 2009. doi:10.1186/1471-213X-9-1.
69. Mendez MG, Kojima SI, Goldman RD. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *FASEB J.* 2010;24:1838-1851.
70. Cunningham KE & Turner JR. Myosin light chain kinase: pulling the strings of epithelial tight junction function. *Ann NY Acad Sci.* 2012 1258, 34-42.
71. Broschat K, Burgess DR. Low Mr tropomyosin isoforms from chicken brain and intestinal epithelium have distinct actin-binding properties. *J Biol Chem.* 1986;261:13350-13359.
72. Jaffe AB, Hall A. Rho GTPases in transformation and metastasis. *Adv Cancer Res.* 2002; 84:57-80.
73. Bacallao R, Garfinkel A, Monke S, Zampighi G, Mandel LJ. ATP depletion: a novel method to study junctional properties in epithelial tissues. I. Rearrangement of the actin cytoskeleton. *J Cell Sci.* 1994;107:3301-3313.
74. Maragos WF, Young KL, Altman CS, Pocernich CB, Drake J, Butterfield DA, et al. Striatal damage and oxidative stress induced by the mitochondrial toxin malonate are reduced in clorgyline-treated rats and MAO-A deficient mice. *Neurochem Res.* 2004;29:741-746.
75. Salvi M, Battaglia V, Brunati AM, Rocca NL, Tibaldi E, Pietrangeli P, et al. Catalase takes part in rat liver mitochondria oxidative stress defense. *J Biol Chem.* 2007;282:24407-24415.
76. Siritantikorn A, Johansson K, Åhlen K, Rinaldi R, Suthiphongchai T, Wilairat P, et al. Protection of cells from oxidative stress by microsomal glutathione transferase 1. *Biochem Bioph Res Co.* 2007;355:592-596.
77. Wild AC, Mulcahy RT. Regulation of γ -glutamylcysteine synthetase subunit gene expression: insights into transcriptional control of antioxidant defenses. *Free Radical Res.* 2000;32:281-301.
78. Schmitt CC, Aranas T, Viel T, Chateau D, Gall ML, Waligora-Dupriet AJ, et al. Intestinal invalidation of the glucose transporter GLUT2 delays tissue distribution of glucose and reveals an unexpected role in gut homeostasis. *Mol Metab.* 2017;6:61-72.
79. Huan PH, Hung LV. Use of the double Thiry-Vella loop in the study of the effects of pantothenic acid on intestinal absorption of glucose. *Br J Nutr.* 1972;28:405-408.
80. Mziaut H, Mulligan B, Hoboth P, Otto O, Ivanova A, Herbig M, et al. The F-actin modifier villin regulates insulin granule dynamics and exocytosis downstream of islet cell autoantigen 512. *Mol Metab.* 2016;5:656-668.
81. Gao Y, Nelson DW, Banh T, Yen MI, Yen CLE. Intestine-specific expression of MOGAT2 partially restores metabolic efficiency in Mogat2-deficient mice. *J Lipid Res.* 2013;54:1644-1652.

82. Nelson DW, Gao Y, Yen MI, Yen CLE. Intestine-specific deletion of acyl-CoA: monoacylglycerol acyltransferase (MGAT) 2 protects mice from diet-induced obesity and glucose intolerance. *J Biol Chem.* 2014; 289:17338-17349.
83. National Research Council. *Nutrient Requirements of Poultry*, 9th ed. Washington, DC: National Academies Press; 1994.
84. Tan J, Applegate TJ, Liu S, Guo Y, Eicher S. Supplemental dietary L-arginine attenuates intestinal mucosal disruption during a coccidial vaccine challenge in broiler chickens. *Br J Nutr.* 2014;112:1098-1109.
85. Lu B, Ren Y, Huang B, Liao W, Cai Z, Tie X. Simultaneous determination of four water-soluble vitamins in fortified infant foods by ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry. *J Chromatogr Sci.* 2008;46:225-232.
86. Woollard DC, Indyk HE, Christiansen SK. The analysis of pantothenic acid in milk and infant formulas by HPLC. *Food Chem.* 2000;69:201-208.
87. Petteys BJ, Frank EL. Rapid determination of vitamin B2 (riboflavin) in plasma by HPLC. *Clin Chim Acta.* 2011;412:38-43.
88. Tang J, Hu J, Xue M, Guo Z, Xie M, Zhang B, Zhou Z, Huang W, Hou S. Maternal diet deficient in riboflavin induces embryonic death associated with alterations in the hepatic proteome of duck embryos. *Nutr Metab.* 2019;16:19.

Tables

Table 1. Composition of pantothenic acid-deficient basal diet from hatch to 16 days of age (% as-fed).

Item	Value
Ingredient, %	
Corn	79.7
Soy isolate protein	16.0
Limestone	1.0
Dicalcium phosphate	1.6
Vitamin and trace mineral premix ^a	1.0
Sodium chloride	0.3
DL-Methionine	0.3
L-Lysine·HCl	0.1
Total	100.0
Calculated composition	
Metabolizable energy ^b , MJ/kg	13.35
Crude protein	20.39
Calcium	0.93
Nonphytate phosphorus	0.43
Lysine	1.17
Methionine	0.57
Methionine + cysteine	0.80
Threonine	0.77
Tryptophan	0.19
Arginine	1.38
Pantothenic acid ^c , mg/kg	4.65

^a Supplied per kilogram of total diet: Cu (CuSO₄·5H₂O), 10 mg; Fe (FeSO₄·7H₂O), 60 mg; Zn (ZnO), 60 mg; Mn (MnSO₄·H₂O), 80 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.2 mg; choline chloride, 1,000 mg; vitamin A (retinyl acetate), 10,000 IU; vitamin D₃ (Cholcalciferol), 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K₃ (menadione sodium bisulfate), 2 mg; thiamin (thiamin mononitrate), 2 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 4 mg; cobalamin, 0.02 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

^b The value is calculated according to the AME of ducks (Ministry of Agriculture of China, 2012).

^c The value was based on high performance liquid chromatography coupled with triple quadrupole mass spectrometry.

Table 2. Growth performance on day 16 of ducks in the pantothenic acid-deficient (PAD) and control (CON) group.

Variable	PAD	CON	SEM	<i>P</i> -value
Mortality (%)	68.7 ^a	0 ^b	3.16	<0.001
ADG (g/d)	10.6 ^b	31.3 ^a	1.13	<0.001
ADFI (g/d)	16.8 ^b	40.1 ^b	1.30	<0.001
FCR (g:g)	1.66 ^a	1.28 ^b	0.03	<0.001

ADG, average daily weight gain; ADFI, average daily feed intake; FCR, feed conversion ratio; and SEM, standard error of the mean.

^{a, b} Mean values with unlike superscript letters were significantly different ($P < 0.05$). Data were analyzed by the Student's *t* test. Data are expressed as mean and SEM (n=8).

Table 3. Plasma parameters of 16-day-old ducks in the pantothenic acid-deficient (PAD) and control (CON) group.

Variable	PAD	CON	SEM	<i>P</i> -value
Pantothenic acid (nmol/L)	121 ^b	800 ^a	54.5	<0.001
Glucose (mmol/L)	7.33 ^b	9.91 ^a	0.14	<0.001
ALP (U/L)	236 ^b	740 ^a	27.7	<0.001
Insulin (μIU/mL)	6.11 ^b	8.84 ^a	0.38	0.008
Glucagon (pg/mL)	147	147	5.72	0.971
MDA	6.47 ^a	4.18 ^b	0.21	<0.001
T-SOD	80.2 ^b	103 ^a	2.24	0.020

ALP, alkaline phosphatase; MDA, malondialdehyde; T-SOD, total superoxide dismutase; SEM, standard error of the mean.

^{a, b} Mean values with unlike superscript letters were significantly different ($P < 0.05$). Data were analyzed by the Student's t test. Data are expressed as mean and SEM (n=8).

Table 4. Intestinal mucosal histomorphology of 16-day-old ducks in the pantothenic acid-deficient (PAD) and control (CON) group.

Items	PAD	CON	SEM	P -value
Duodenum				
Villus height (μm)	666 ^b	893 ^a	27.7	<0.001
Villus width (μm)	108	130	5.60	0.093
Crypt depth (μm)	175	214	9.90	0.153
Villus surface area (mm^2)	0.23 ^b	0.36 ^a	0.018	0.001
Jejunum				
Villus height (μm)	467 ^b	678 ^a	42.4	0.033
Villus width (μm)	121	134	7.20	0.249
Crypt depth (μm)	123	158	8.30	0.076
Villus surface area (mm^2)	0.18 ^b	0.28 ^a	0.019	0.010
Ileum				
Villus height (μm)	305 ^b	534 ^a	32.4	<0.001
Villus width (μm)	118	122	3.40	0.618
Crypt depth (μm)	126 ^b	194 ^a	10.5	0.002
Villus surface area (mm^2)	0.11 ^b	0.20 ^a	0.014	<0.001

SEM, standard error of the mean.

^{a, b} Mean values with unlike superscript letters were significantly different ($P < 0.05$). Data were analyzed by the Student's t test. Data are expressed as mean and SEM (n=8).

Table 5. Selected differentially expressed proteins in mucosal caused by pantothenic acid deficiency.

UniProtKB ID	Protein name	Short name	Fold change*	P-Value
Glycolysis and gluconeogenesis				
U3IHG8	Fructose-bisphosphate aldolase	ALDOB	-5.47	5.90E-09
U3IVG9	Hexokinase domain containing 1	HKDC1	-4.34	1.13E-13
U3IR52	Alpha-enolase	ENO1	-3.75	6.19E-08
U3J2H8	Fructose-bisphosphatase 1	FBP1	-2.42	1.35E-10
U3J1L1	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	-3.02	9.49E-06
U3I0F9	Pyruvate kinase	PKM	-3.28	1.94E-07
U3IZA5	ATP-dependent 6-phosphofructokinase	PFKP	-2.33	3.93E-05
U3I8D8	Triosephosphate isomerase	TPI1	-2.93	9.45E-05
U3ILF5	Phosphoglycerate kinase	PGK1	-2.01	1.71E-06
U3IR48	Dihydrolipoyl dehydrogenase	DLD	-1.66	2.09E-02
U3IEW2	Pyruvate dehydrogenase E1 beta subunit	PDHB	-1.58	1.79E-02
U3I939	Fructose-bisphosphate aldolase	ALDOA	1.89	1.34E-03
U3IR68	Hexokinase 1	HK1	3.31	1.12E-02
Fatty acid beta oxidation				
U3J4Z9	Acyl-CoA synthetase long chain family member 5	ACSL5	-3.06	6.74E-09
U3I9A1	Acyl-CoA dehydrogenase family member 11	ACAD11	-1.96	1.34E-02
U3ITA9	Medium-chain specific acyl-CoA dehydrogenase	ACADM	-1.85	1.79E-05
U3IHS8	Carnitine O-acetyltransferase	CRAT	-1.78	6.54E-03

U3IR48	Dihydrolipoyl dehydrogenase	DLD	-1.66	2.09E-02
U3I6S1	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta	HADHB	-1.65	1.60E-03
U3IEF4	Enoyl-CoA delta isomerase 2	ECI2	-1.65	4.61E-02
U3J928	Acyl-coenzyme A oxidase	ACOX1	-1.51	1.68E-05
U3IDQ1	Acyl-coenzyme A oxidase	ACOX2	-1.74	3.50E-05
TCA cycle				
U3IR48	Dihydrolipoyl dehydrogenase	DLD	-1.66	2.09E-02
R0J775	Aconitase 1 (Fragment)	ACO1	-1.64	2.75E-10
U3IC15	Aconitate hydratase, mitochondrial	ACO2	-2.13	9.22E-11
R0JXM5	Malate dehydrogenase (Fragment)	MDH1	-1.78	2.25E-05
U3IA60	Malate dehydrogenase	MDH2	-2.89	4.45E-08
U3IEW2	Pyruvate dehydrogenase E1 beta subunit	PDHB	-1.58	1.79E-02
U3J597	Isocitrate dehydrogenase [NADP]	IDH1	1.58	5.05E-06
Oxidative phosphorylation				
U3J532	NADH:ubiquinone oxidoreductase subunit A5	NDUFA5	-1.58	1.94E-02
R0LLX6	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6 (Fragment)	NDUFA6	-1.56	4.97E-02
U3J175	ATP synthase, H ⁺ transporting, mitochondrial Fo complex subunit B1	ATP5F1	-1.80	2.12E-03
R0LYJ7	ATP synthase subunit d, mitochondrial (Fragment)	ATP5H	-1.79	2.69E-04
R0LIL9	ATP synthase subunit O, mitochondrial (Fragment)	ATP5O	-1.60	2.16E-03
Regulation of actin cytoskeleton				
U3IFN5	Villin 1	VIL1	-3.12	1.13E-

				08
U3IY96	Ezrin	EZR	-2.00	1.61E-05
U3IRY0	Vimentin	VIM	2.66	5.54E-04
U3J6G2	Tropomyosin 3	TPM3	4.51	1.36E-02
U3I7J8	Tropomyosin alpha-1 chain	TPM1	9.58	6.76E-04
U3IA79	Myosin light chain 1	MYL1	11.47	2.50E-06
R0LM85	Myosin light chain kinase (Fragment)	MYLK	2.88	1.83E-02
U3I4I9	Actinin alpha 1	ACTN1	2.13	5.89E-07
U3IZ83	Fibronectin 1	FN1	2.28	4.31E-02
U3IR26	Vinculin	VCL	1.87	4.54E-11
U3I935	Moesin	MSN	2.07	5.48E-03
Oxidative stress				
U3J0T0	Amine oxidase	MAOA	-2.56	5.04E-08
A0A172QNN4	Catalase	CAT	-1.84	1.49E-05
R0JZP2	Microsomal glutathione S-transferase 1 (Fragment)	MGST1	-1.57	2.66E-02
U3I5T1	Glutamate-cysteine ligase catalytic subunit	GCLC	1.57	9.62E-03
Intestinal absorption				
U3IRP7	Solute carrier family 2 member 2	SLC2A2	-5.11	3.17E-02
U3IFN5	Villin 1	VIL1	-3.12	1.13E-08
U3IY96	Ezrin	EZR	-2.00	1.61E-05
U3IUS6	Monoacylglycerol O-acyltransferase 2	MOGAT2	-2.28	5.35E-03

TCA, tricarboxylic acid.

* Fold change is expressed as the ratio of the pantothenic acid-deficient to the control group. For the downregulated proteins, the fold change was transformed to the corresponding negative value.

Figures

Figure 1

Top 15 significantly terms enriched biological processes, cellular components, and molecular functions.

Figure 2

The pathway analysis by KEGG on differentially expressed proteins.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile.docx](#)
- [msNC3RsARRIVEGuidelinesChecklist.docx](#)