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Effect of Protein Deficiency Diet on Gastric Histology and Histomorphometric Indices in Mice Animal Model

Running title: Gastric Effects of Protein Deficiency Diet

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Abstract

BACKGROUND: Diets in various organisms in the long run can affect the histological structure and histomorphometry of the gastrointestinal tract to adapt to the diet in order to be able to provide its main role in providing the necessary materials and energy for the survival of the organism.

OBJECTIVES: The aim of this study is to investigate the effect of dietary protein deficiency on histology and histomorphometric indices of stomach and blood chemical parameters and liver enzymes.

METHODS: Twelve immature female Balb/C mice at the age of three weeks were divided into a control group that received a complete protein diet and an experimental group that received a protein-deficient diet. Three months later, the serum levels of calcium, phosphorus, glucose, cholesterol, triglycerides, urea, creatinine, and liver enzymes were evaluated and the thickness of the layers of the stomach wall, as well as pit depth, the number of parietal cells was measured in stomach tissue.

RESULTS: The results of comparing the thickness of stomach layers showed that the thickness of the mucosa, muscle layer, depth of pits and the number of parietal cells of the stomach wall increased significantly in the experimental group($P<0.05$). Also, the serum levels of phosphorus, glucose, cholesterol, triglyceride, urea, creatinine, AST and ALT in the experimental group showed a significant decrease, and the serum level of ALP in the experimental group showed a significant increase ($P<0.05$).

CONCLUSION: The results of the present study showed that changes in diet in the long term can cause changes in the histology and histomorphometry of the stomach wall as well as blood parameters, which may be unfavorable for people's health in some parameters.

KEYWORDS: Histology, Histomorphometry, Stomach, Protein deficiency, Mice

Introduction

The human digestive system consists of the alimentary canal (oral cavity, esophagus, stomach, small and large intestines and anus) and its attached glands (salivary glands, liver and pancreas). It is responsible for providing energy and survival of the living organism from the consumed materials. In the digestive process, proteins, complex carbohydrates, nucleic acids and fats are broken into smaller molecular subunits and absorbed by the small intestine. In addition, along the entire length of the alimentary canal, the inner layer forms a protective barrier between the contents of the alimentary canal and the internal environment of the connective tissue and vascular system of the body (Li *et al*., 2020; Mackie *et al*., 2020; Mescher, 2021). The present research was conducted on mice that have a stomach almost similar to the human digestive tract, with the difference that the mouse stomach consists of two parts, non-glandular and glandular, and has four regions: cardia, fundus, body and pylorus and has two small and big curvatures. From the small curvature, the lesser omentum connects the stomach to the visceral surface of the liver and from the greater curvature, the greater omentum, which has two layers, connects the stomach to the spleen and intestines. In mice, the border between non-glandular and glandular parts is clearly defined (Scudamore, 2014; Yang *et al*., 2022; Amalia *et al*., 2023).

Undoubtedly, the histological characteristics and histomorphometric indicators of the digestive system and especially the mucous layer of the stomach adapt in the long term according to the type of food consumed by each animal, so that it can fulfill its role in providing the materials and energy needed for the survival of the animal (Fazelipour *et al*., 2016a). So far, a lot of research has been done in order to know the components, physiology, histology of the digestive system, the function and the effect of diet on its development and growth (Fazelipour *et al*., 2016a; Amer *et al*., 2021; Choudhury *et al*., 2021; Ravindran and Abdollahi, 2021). A large part of the research related to the effect of diet on the digestive system is aimed at answering the questions raised in meat animal breeding centers. In these centers, which are economic enterprises, most of the current cost is the cost of providing feed for livestock, poultry, fish, etc. Therefore, there is always this concern that how can you get the most profit by changing the composition of diet ingredients and using cheaper ingredients while maintaining efficiency as much as possible? To answer this question, it is necessary to know the effect of increasing and decreasing the amount of different foods in the feed of animals kept in these centers. Therefore, a lot of research has been done in this direction, mainly related to meat breeding animals (Vermeulen *et al*., 2020; Erickson *et al*., 2020; Te Pas *et al*., 2021; Morach *et al*., 2021). Although the results of most of these researches have generally been in agreement with each other, sometimes differences are also seen, and in some cases, the reasons for the changes in the digestive system are not clearly known.

In addition to the changes in the histology and histomorphometry of digestive tract tissues, blood parameters and liver enzymes are also affected by diet (Kozeniecki *et al*., 2020; İçil *et al*., 2020; Ahmed and Ahmad, 2020; Traub *et al*., 2021; Kalas *et al*., 2021; Wang *et al*., 2023). Considering that conducting research in this field on humans faces many obstacles and problems, in this research the effect of diet on the mouse animal model was chosen due to its proximity to humans and less research records for it. This research is focused on the effect of reducing protein in the diet on the histology and histomorphometric indices of the stomach, as well as the chemical parameters of the blood and liver enzymes of mice.

Materials and Methods

Animals

This experimental research carried on twelve immature female Balb/C mice weighing 10 to 12 grams. The animals were obtained from Razi Institute and were kept in suitable living conditions for one week in the animal house of the university. Optimal conditions included a 12-hour light/dark cycle at an ambient temperature between 20 and 24°C with relative humidity between 40 and 60%. After the adaptation period, the mice were randomly divided into control group received complete protein diet (23%) and experimental group received protein-deficient diet (13.5 %). The diet prepared by the nutritionist.

Ethical rights regarding animals were equal to "Ethical Guidelines for Research on Animals, Policy Council of the Ministry of Health and Medical Education, Research Center for Ethics and History of Medicine, Tehran University of Medical Sciences" and "Guidelines for the Care and Use of Laboratory Animals in Scientific Affairs - Year 2019" it was observed (Ethical code: IR.IAU.TMU.REC.1402.166).

Preparing animal diet

The diet of protein deficiency and complete protein was prepared by a nutritionist, then the food items according to table 1 were purchased and transferred to the Faculty of Veterinary Medicine of Tehran University. The food ingredients were weighed according to the table and turned into powder by the mill, and then it was made into a paste, made into a plate, dried, and given to the animals.

Implementation of the plan

Mice were kept under the determined dietary conditions of each group for three months according to the period of growth and development of the mice. After the treatment period, the animals were anesthetized, and blood was taken from their hearts to determine the serum levels of calcium, phosphorus, glucose, cholesterol, triglycerides, urea, creatinine, and liver enzymes. The sera were sent to the pathobiology laboratory and the serum levels of the mentioned cases were measured by the Radioimmunoassay method (Badi *et al*., 2022; Chahnaz Hamza *et al*., 2024).

Then, the abdominal cavity of the animals was dissected and the stomach of the animals were removed, and after washing with normal saline, they were placed in 10% formalin. From each sample, serial sections with a thickness of 5 µm were prepared and stained by H&E method (Badi *et al*., 2022; Chukwu *et al*., 2023). Four sections of each sample and four fields of view were taken using a photomicroscope equipped with Axiovision and Dino-Capture software. The

required images were prepared and the histological and histomorphometric features (the thickness of the layers of the stomach wall, as well as pit depth, the number of parietal cells and the coordinates of the villi) were analyzed according to the parameters of the research variable table (Vajed Ebrahimi *et al*., 2024).

Data analysis

For data analysis in this study, SPSS software (ver. 26) was used and quantitative data results were presented as mean ± SD. Data analysis was done by one-way analysis of variance (ANOVA) to compare the average data between the control and experimental groups, and the significance level was $P<0.05$.

Results

Histological findings

The results showed that the stomach consists of two non-glandular and glandular parts. The non-glandular part, which is located immediately after the esophagus, has a squamous type of mucosal epithelium with fine keratin layers in the most superficial part. The thickness of the epithelium is more in some areas and less in some areas, which includes several layers of cells from the basement membrane to the surface, which consists of a basal layer of cubic to short cylindrical cells and on it, one to three cell layers of spherical to polyhedral with large spherical and euchromatic nuclei were placed. Then, a layer of granular cells and thin keratin sheets on it form the most superficial part of the epithelium. The mucous lining in the non-glandular part is not very thick, because it does not have any gastric glands. The mucosa and submucosa lining consists of loose to semi-loose connective tissue, and the muscular layer consists of two to three thin layers of smooth muscle, which is surrounded by a thin serous layer from the outside (Figs. 1 and 2).

The gastric mucosa is composed of three components: an epithelial tissue that lines the internal cavity and a connective tissue (lamina propria) and smooth muscle layers forming the mucosa. A simple cylinder composed of surface lining cells is furnished, which produce a thick mucus layer. In the histological examination of the stomach, no abnormal histological observations were observed between the control and experimental groups (Figs. 1 and 2).

Histomorphometrical findings

The thickness of the mucous layer of the stomach wall

The mouse stomach has two glandular and non-glandular regions, and in the experiment, the thickness of both regions was measured in micrometers (Table 2). The average thickness of the mucus in the sample of gastric mucosa tissues shows that the lack of dietary protein caused the thickness of the gastric mucosa to significant increase from 615.29 to 1298.03 micrometers in the glandular region (P=0.003). The average thickness of gastric mucosa in the non-glandular low protein group also increased significantly from 427.16 micrometers to 859.65 (P=0.009) (Table 2).

The thickness of the submucous layer of the stomach wall

The morphometrical analysis of the thickness of the submucous layer in glandular and non-glandular part of the stomach indicated that deficiency of dietary protein increased significantly the average submucosa thickness from 93.42 µm in the control group to 114.47 µm in the experimental group (P =0.029). In the non-glandular area, the average thickness of the submucosa increased significantly from 100.64 micrometers in the control group to 126.72

micrometers in the experimental group (*P* =0.01). So, according to the results, the thickness of the stomach submucosa in the glandular and non-glandular area was significantly increased in the group of rats that received a low protein diet (Table 2).

The thickness of muscular layer of the stomach wall

The thickness of the muscle layer of both glandular and non-glandular regions of the stomach tissue was listed in Table 2. Due to the lack of dietary protein, the average thickness of the muscle layer of the non-glandular area of the stomach has increased from 155.74 µm in the control group to 201.55 µm in the experimental group (p=0.11), which is far from the significant level. In the glandular region of the stomach, the average thickness of the muscle layer increased significantly from 104.05 µm in the control group to 246.38 µm in the experimental group (p=0.002). Therefore, a low protein diet caused a significant increase in the thickness of the stomach muscles in the glandular area, and the non-significant increase in thickness of the non-glandular area muscles (Table 3).

The stomach pit depth

According to table 3, due to lack of dietary protein, the stomach pit depth was significantly increased from 94.80 µm in the control group to 185.30 µm in the experimental group (*P*=0.0001) (Table 3).

The parietal cells count in stomach pit

The result of counting the number of parietal cells in the stomach pit in both control and experimental groups showed that the average number of cells in the control group increased significantly from about 9.83 to about 25.67 in the field of vision in the experimental group (P<0.0001) (Table 3).

Biochemical parameters of blood serum

The assessment of the phosphorus, glucose, cholesterol, triglycerides, urea, and creatinine levels in blood serum of experimental and control groups indicated that the reduction of dietary protein caused mentioned blood chemical parameters to decrease in the experimental group compared to the control group, but the calcium parameter was non-significantly increased in experimental group (*P*> 0.05) (Table 4, and 5).

Liver enzymes

The evaluation of ALT, AST, and ALP levels in blood serum of experimental and control group, showed that the ALT level was decreased significantly in experimental group in comparison to control group. The AST and ALP levels were significantly increased in experimental group ($P < 0.01$) (Table 6).

Discussion

Previous studies have shown that various diets or medications can have long-term effects on the histological and histomorphometric structure, and consequently on the function of different layers of the stomach walls. These studies have also indicated that the growth and survival of organisms are significantly dependent on essential nutrients such as protein (Fazelipour *et al*., 2016a; Limbach *et al*., 2021).

The present study investigated the effects of adequate protein intake or protein deficiency on the tissue layers of the stomach walls, which is fundamental components of the gastrointestinal tract. The results indicated that a protein-deficient diet led to a significant increase in the layers of the mucosa, submucosa, and muscles of the stomach wall, as well as an increase in the number of parietal cells and the depth of the gastric pits. In this study, the thickness of the mucosal layer in both the non-glandular and glandular parts of the stomach showed a significant increase in the protein-deficient group compared to the control group.

In a study, it was observed that a diet containing soy, which replaced animal protein, also resulted in a significant increase in the thickness of the mucosal layer in both the glandular and non-glandular parts of the stomach (Fazelipour *et al*., 2016a). In line with the present study, the number of parietal cells in the protein-deficient group was higher than in the control group. It can be suggested that the increase in these cells leads to an increase in stomach acid, and the reason for the increased thickness of the mucosa may be due to the increased stomach acid. It can be said that the mucosal layer of the stomach wall increases in thickness to protect the wall and prevent damage from excessive acid. This is achieved through the secretion of mucus by all epithelial cells of the glandular part of the stomach, providing a greater protective role. The present study also showed that the increase in the depth of the pits could be another protective mechanism against acid, allowing for longer-term protection by preventing acid penetration into the stomach.

In a study by Karam, it was observed that ranitidine, which was administered to mice, was able to reduce the secretion of parietal cells responsible for stomach acid production. This study showed that the longer the treatment duration with ranitidine, the less acid was secreted, as the drug both reduced acid secretion and caused some parietal cells to be destroyed, thereby decreasing acid secretion (Karam & Alexander, 2001). The examination of parietal cells in this study indicated that the vacuolization and dilation of the canaliculi, and possibly their destruction, were effective in reducing acid levels, aligning with the present study. Therefore, the increase in the mucosal layer observed in this study is a compensatory response of the stomach to protect the wall against increased acid.

In another study, it was shown that long-term consumption of aspartame, an artificial sweetener in beverages, led to the destruction and disorganization of the epithelial mucosa of the stomach wall and atrophy of some glandular cells in the glandular part of the stomach lining. This study demonstrated that high doses of aspartame could cause an increase in the thickness of the glandular part of the stomach wall in mice, which could be attributed to the long-term effect of aspartame on the epithelial cells of the glandular part of the stomach. Another result of this study was an increase in the thickness of the muscular layer of the stomach wall in the glandular part of the mouse stomach, which is similar to the human stomach. The increase in the muscular layer may lead to increased mechanical activity of the animal's stomach, possibly due to a digestive disorder and the compensatory response of the stomach wall by increasing the muscular layer

and enhancing mechanical activity in response to digestive disturbances. In this context, the presence of an extensive vascular system in the stomach wall should also be considered related to these reactions, as some drugs with high doses can influence blood flow in the stomach wall, affecting the thickness of the layers and the activity of the muscular layer. Additionally, in a study on the muscular layer and stomach wall of diabetic mice, the observed increase in the muscular layer thickness supports the findings of the present study (Tootian *et al*., 2022).

Masuoka *et al*. found that mice on a protein-deficient diet experienced weight loss and decreased urea levels (Masuoka *et al*., 2020). The present study also found that a protein-deficient diet significantly reduced serum urea levels compared to the control group. This can be attributed to the fact that urea is formed from the deamination of amino acids in the liver and excreted by the kidneys; thus, protein deficiency leads to fewer amino acids and consequently lower urea levels. Fazelipour *et al*. also observed that dietary changes could affect serum urea levels, reducing them (Fazelipour *et al*., 2016b).

Additionally, the study indicated that a protein-deficient diet could affect liver enzymes, with serum ALT levels decreasing and AST and ALP levels increasing in the experimental group. Morovvati *et al*. found that administration of Dianabol in rats increased serum AST, ALT, and ALP levels, aligning with this study's findings (Morovvati *et al*., 2018).

The present study also showed significant reductions in serum glucose, triglycerides, cholesterol, and creatinine in the experimental group compared to the control group. Fazelipour *et al*. found that nanoparticles affected blood factors, increasing glucose, triglycerides, cholesterol, and creatinine levels compared to the control group (Fazelipour *et al*., 2020).

Thus, it can be concluded that dietary changes and the use of various medications can rapidly alter serum blood factor levels. Mousaie *et al*. also reported a significant decrease in triglycerides in the protein-deficient diet group compared to the control group (Mousaie *et al*., 2011).

The activities of a constellation of hepatic enzymes and enzymatic complexes have been found to be reduced in human and experimental protein deficiency. The hepatic enzymes are generally more severely affected by protein deprivation than the same enzymes in other tissues. Since most of the hepatic enzymes diminish either in proportion to the reduction in total hepatic protein or even to a greater extent, this would indicate that in protein deficiency, certain hepatic proteins are more affected than others. However, the special lability of hepatic enzymes is not a characteristic of the enzymes but rather of hepatic protein metabolism. The quality of the dietary protein appears to be important in the maintenance of the enzymatic profiles of the liver as indicated by the fact that a dietary deficiency of methionine, tryptophan, or histidine resulted in the reduction of xanthine oxidase and betaine transmethylase. Rats deprived of protein show a substantial increase in activity of hepatic amino acid activating enzymes and the same occurs in malnourished children. Controversial results have been reported in the case of hepatic

cytochrome oxidase activity in protein-deficient rats: being found increased in some studies and decreased or unaltered in others. Alkaline phosphatase activity was reported increased in livers of rats fed protein-free diets (Porta & Hartroft, 1970; Lenox, 2021). In this study, the level of ALP was decreased significantly in experimental group, which is aligned with Porta and Hartroft (1970).

It is difficult at present to determine from the available data if protein deficiency affects the enzymes of a particular intracellular location more extensively than those of other parts of the cell. Reduced enzymes have been found in practically all subcellular fractions but most of the results are derived from animals fed protein-free rather than low protein diets. On the other hand, the reported increases in amino acid activating enzymes, in alkaline phosphatase, and in cytochrome oxidase of rats and the increased levels of catalase, malic dehydrogenase, transaminase, and alkaline phosphatase found in livers of patients with protein malnutrition would suggest that some adaptive changes may have taken place under nutritional stress in order to spare the more vital enzymes. It is logical to assume that a low protein intake is less catastrophic than complete lack of dietary protein in relation to enzymatic lability (Porta & Hartroft, 1970; Lenox, 2021). In the present study the liver enzymes levels were significantly altered in experimental group. The AST and ALP levels were significantly increase in protein deficiency group, which it can be indicated to primary fatty liver due to protein metabolism disorders in liver.

Conclusion

As the results of the present research and other researches have shown regarding the change in the amount of protein in the diet, the lack of protein consumption in the long term causes changes and sometimes unfavorable changes in the histology and histomorphology of the tissues of the digestive system. Also, blood parameters and liver enzymes undergo changes as a result, which can have an adverse effect on the growth and health of living beings in the long run. Therefore, getting enough protein is important for good health.

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References

Ahmed, I., & Ahmad, I. (2020). Effect of dietary protein levels on growth performance, hematological profile and biochemical composition of fingerlings rainbow trout, Oncorhynchus mykiss reared in Indian himalayan region. *Aquaculture Reports*, *16*, 100268. [DOI: 10.1016/j.aqrep.2019.100268]

Amalia, R., Panenggak, N. S. R., Doohan, D., Rezkitha, Y. A. A., Waskito, L. A., Syam, A. F., & Miftahussurur, M. (2023). A comprehensive evaluation of an animal model for Helicobacter

pylori-associated stomach cancer: Fact and controversy. *Helicobacter*, *28*(1), e12943. [DOI: 10.1111/hel.12943] [PMID: 36627714]

Amer, S. A., Mohamed, W. A., Gharib, H. S., Al-Gabri, N. A., Gouda, A., Elabbasy, M. T., Abd El-Rahman, G. I., & Omar, A. E. (2021). Changes in the growth, ileal digestibility, intestinal histology, behavior, fatty acid composition of the breast muscles, and blood biochemical parameters of broiler chickens by dietary inclusion of safflower oil and vitamin C. *BMC Veterinary Research*, *17*, 1-18. [DOI: 10.1186/s12917-021-02773-5] [PMID: 33541348] [PMCID: PMC7863266]

Badi, N., Fazelipour, S., Naji, T., Babaei, M., Kalantari Hesari, A. (2022). 'Histomorphometric and Biochemical Study of Liver and Thyroid Hormones Following Administration of MoO3 Nanoparticles in Female Rats', *Iranian Journal of Veterinary Medicine*, 16(2), 188-201. [DOI: 10.22059/ijvm.2021.330872.1005196]

Chahnaz Hamza, M., Bourabah, A. (2024). 'Exploratory Study on the Relationship Between Age, Reproductive Stage, Body Condition Score, and Liver Biochemical Profiles in Rembi Breed Ewes', *Iranian Journal of Veterinary Medicine*, 18(2), 169-176. [DOI: 10.32598/ijvm.18.2.1005455]

Choudhury, R., Middelkoop, A., De Souza, J. G., Van Veen, L. A., Gerrits, W. J. J., Kemp, B., Bolhuis, J.E., & Kleerebezem, M. (2021). Impact of early-life feeding on local intestinal microbiota and digestive system development in piglets. *Scientific Reports*, *11*(1), 4213. [DOI: 10.1038/s41598-021-83756-2] [PMID: 33603087] [PMCID: PMC7892833]

Chukwu, O. O., Emelike, C. U., Konyefom, N. G., Ibekailo, S. N., Ekakitie, O., Ghasi, S., Iyare, E. E. (2023). 'Histological Studies of the Heart and Biochemical Changes Due to the Perinatal Consumption of Hibiscus sabdariffa (Flavonoid-rich Extract) to Feed-restricted Rats on Offspring', *Iranian Journal of Veterinary Medicine*, 17(1), 37-46. [DOI: 10.22059/ijvm.17.1.1005272]

Erickson, P. S., Anderson, J. L., Kalscheur, K. F., Lascano, G. J., Akins, M. S., & Heinrichs, A. J. (2020). Symposium review: Strategies to improve the efficiency and profitability of heifer raising. *Journal of dairy science*, *103*(6), 5700-5708. [DOI: 10.3168/jds.2019-17419] [PMID: 32147255]

Fazelipour, S., Assadi, F., Tootian, Z., Sheibani, M. T., Dahmardeh, M., Zehtabvar, O., Namdar, S. & Farshidfar, S. (2020). Effect of molybdenum trioxide nanoparticles on histological changes of uterus and biochemical parameters of blood serum in rat. *Comparative Clinical Pathology*, *29*(5), 991-999.

Fazelipour, S., Tootian, Z., Sheibani, M. T., Hooshmand abasi, R., Chegini, H., Chegini, M., Minaei, M., Brahman, G., Vazir, N. (2016a). Effect of soybean on histomorphometric parameters of stomach and biochemical factors of blood serum in animal model. *Research in Medicine*, 39,177-182.

Fazelipour, S., Tootian, Z., Sheibani, M. T., Razmyar, J., Hooshmand Abasi, R., Minaei, M., & Kianii, S. (2016b). Effect of diet with soybean on histology and histomorphometry of small intestine villi and serum level of Calcium, Phosphorus and Glucose in mice. *Journal of Gorgan University of Medical Sciences*, *18*(4), 62-68.

İçil, N. İ., Polat, E. S., & Coşkun, B. (2020). Effect of diet protein and energy levels on serum biochemical profile of fatty tailed sheep. *Livestock Studies*, *60*(1), 16-24. [DOI: 10.35864/uhaem.631045]

Kalas, M. A., Chavez, L., Leon, M., Taweesedt, P. T., & Surani, S. (2021). Abnormal liver enzymes: A review for clinicians. *World journal of hepatology*, *13*(11), 1688. [DOI: 10.4254/wjh.v13.i11.1688] [PMID: 34904038] [PMCID: PMC8637680]

Karam, S. M., & Alexander, G. (2001). Blocking of histamine H2 receptors enhances parietal cell degeneration in the mouse stomach. *Histology and histopathology*, *16*(2), 469-480. [DOI: 10.14670/HH-16.469] [PMID: 11332703]

Kozeniecki, M., Ludke, R., Kerner, J., & Patterson, B. (2020). Micronutrients in liver disease: roles, risk factors for deficiency, and recommendations for supplementation. *Nutrition in Clinical Practice*, *35*(1), 50-62. [DOI: 10.1002/ncp.10451] [PMID: 31840874]

Lenox, C. E. (2021). Nutritional management for dogs and cats with gastrointestinal diseases. *Veterinary Clinics: Small Animal Practice*, *51*(3), 669-684. [DOI: 10.1016/j.cvsm.2021.01.006] [PMID: 33653539]

Li, C., Yu, W., Wu, P., & Chen, X. D. (2020). Current in vitro digestion systems for understanding food digestion in human upper gastrointestinal tract. *Trends in Food Science & Technology*, *96*, 114-126. [DOI:10.1016/j.tifs.2019.12.015]

Limbach, J. R., Espinosa, C. D., Perez-Calvo, E., & Stein, H. H. (2021). Effect of dietary crude protein level on growth performance, blood characteristics, and indicators of intestinal health in weanling pigs. *Journal of animal science*, *99*(6), skab166. [DOI: 10.1093/jas/skab166] [PMID: 34019637] [PMCID: PMC8202089]

Mackie, A., Mulet-Cabero, A. I., & Torcello-Gómez, A. (2020). Simulating human digestion: developing our knowledge to create healthier and more sustainable foods. *Food & function*, *11*(11), 9397-9431. [DOI: 10.1039/D0FO01981J]

Masuoka, H., Suda, W., Tomitsuka, E., Shindo, C., Takayasu, L., Horwood, P., Greenhill, A.R., Hattori, M., Umezaki, M. & Hirayama, K. (2020). The influences of low protein diet on the intestinal microbiota of mice. *Scientific reports*, *10*(1), 17077. [DOI: 10.1038/s41598-020-74122-9] [PMID: 33051527] [PMCID: PMC7555506]

Mescher, A. L. (2021). Junqueira's basic histology: text and atlas, 16th edn. New York: McGraw-Hill Education.

Morach, B., Witte, B., Walker, D., von Koeller, E., Grosse-Holz, F., Rogg, J., Brigl, M., Dehnert, N., Obloj, P., Koktenturk, S., & Schulze, U. (2021). Food for thought: the protein transformation. *Industrial Biotechnology*, *17*(3), 125-133. [DOI: 10.1089/ind.2021.29245.b]

Morovvati, H., Babaei, M., Tootian, Z., Fazelipour, S., Anbara, H., & Akbarzadeh, A. (2018). The effects of vitamin E on Liver and kidney damage induced by Dianabol in Small Laboratory Mice. *Journal of Babol University of Medical Sciences*, *20*(9), 36-47. [DOI: 10.18869/acadpub.jbums.20.9.36]

Mousaie, A., Valizadeh, R., Nasserian, A. A., & Rassouli, M. B. (2011). Effect of Dietary Protein on blood Lipids and Lipoproteins and Female Wistar Rats. *Iranian Journal of Animal Science Research*, 3, 1 (2011). [DOI: 10.22067/IJASR.V3I1.10591]

Porta, E. A., & Hartroft, W. S. (1970). Protein deficiency and liver injury. *The American Journal of Clinical Nutrition*, *23*(4), 447-461. [DOI: 10.1093/ajcn/23.4.447] [PMID: 4985958]

Ravindran, V., & Abdollahi, M. R. (2021). Nutrition and digestive physiology of the broiler chick: state of the art and outlook. *Animals*, *11*(10), 2795. [DOI: 10.3390/ani11102795] [PMID: 34679817] [PMCID: PMC8532940]

Scudamore, C.L. (2014). A practical guide to the histology of the mouse. John Wiley & Sons.

Te Pas, M. F., Veldkamp, T., de Haas, Y., Bannink, A., & Ellen, E. D. (2021). Adaptation of livestock to new diets using feed components without competition with human edible protein sources-a review of the possibilities and recommendations. *Animals*, *11*(8), 2293. [DOI: 10.3390/ani11082293] [PMID: 34438751] [PMCID: PMC8388495]

Tootian, Z., Fazelipour, S., Sheibani, M. T., Erik-Aghaji, H., & Abbasi, R. H. (2022). Effect of Aspartame on Histology and Histomorphometry of Stomach in Balb/C Mice. *Journal of Ilam University of Medical Sciences: Volume*, *30*(4), 39-46.

Traub, J., Reiss, L., Aliwa, B., & Stadlbauer, V. (2021). Malnutrition in patients with liver cirrhosis. *Nutrients*, *13*(2), 540. [DOI: 10.3390/nu13020540] [PMID: 33562292] [PMCID: PMC7915767]

Vajed Ebrahimi, M. T., Mohammadi Gheshlagh, F., Parham, A. (2024). 'Using Black Carrot Extracts as an Alternative Biological Dye for Tissue Staining', *Iranian Journal of Veterinary Medicine*, 18(2), 279-290. [DOI: 10.32598/ijvm.18.2.1005381]

Vermeulen, S. J., Park, T., Khoury, C. K., & Béné, C. (2020). Changing diets and the transformation of the global food system. *Annals of the New York Academy of Sciences*, *1478*(1), 3-17. [DOI: 10.1111/nyas.14446] [PMID: 32713024] [PMCID: PMC7689688]

Wang, X., Wang, Y., Wang, Q., Dai, C., Li, J., Huang, P., Li, Y., Ding, X., Huang, J., Hussain, T., & Yang, H. (2023). Effect of dietary protein on growth performance, and serum biochemical index in late pregnant Hu ewes and their offspring. *Animal Biotechnology*, *34*(2), 97-105. [DOI: 10.1080/10495398.2021.1939042] [PMID: 34187316]

Yang, H., Yang, W. J., & Hu, B. (2022). Gastric epithelial histology and precancerous conditions. *World journal of gastrointestinal oncology*, *14*(2), 396-412. [DOI: 10.4251/wjgo.v14.i2.396] [PMC8919001]

تأثیر رژیم غذایی کمبود پروتئین بر بافت شناسی معده و شاخص های هیستومورفومتری در مدل حیوانی موش

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چکیده

زمینه مطالعه: رژیم‌های غذایی در موجودات مختلف در درازمدت می‌توانند ساختار بافت‌شناسی و هیستومورفومتری دستگاه گوارش را برای انطباق با رژیم غذایی تحت تأثیر قرار دهند تا بتوانند نقش اصلی خود را در تأمین مواد و انرژی لازم برای بقای ارگانیسم فراهم کنند.

هدف: هدف از این مطالعه بررسی اثر کمبود پروتئین جیره بر بافت شناسی و شاخص های هیستومورفومتری معده و پارامترهای بیوشیمیایی خون و آنزیم های کبدی می باشد.

روش کار: 12 موش ماده نابالغ Balb/C در سن سه هفته به یک گروه کنترل که یک رژیم غذایی کامل پروتئینی دریافت کردند و یک گروه تجربی که یک رژیم غذایی با کمبود پروتئین دریافت کردند، تقسیم شدند. سه ماه بعد، سطح سرمی کلسیم، فسفر، گلوکز، کلسترول، تری گلیسیرید، اوره، کراتینین و آنزیم های کبدی بررسی شد و ضخامت لایه های دیواره معده و همچنین عمق حفره، تعداد سلول های جداری در بافت معده اندازه گیری می شود.

یافته‌ها: نتایج مقایسه ضخامت لایه‌های معده نشان داد که ضخامت مخاط، لایه عضلانی، عمق حفره‌ها و تعداد سلول‌های جداری دیواره معده در گروه آزمایش افزایش معنی‌داری داشت ($P<0/05$). همچنین سطوح سرمی فسفر، گلوکز، کلسترول، تری گلیسیرید، اوره، کراتینین، AST و ALT در گروه آزمایش کاهش معنی‌داری و سطح سرمی ALP در گروه آزمایش افزایش معنی‌داری نشان داد ($P<0/05$).

نتیجه گیری نهایی: نتایج مطالعه حاضر نشان داد که تغییر رژیم غذایی در درازمدت می تواند باعث تغییراتی در بافت شناسی و هیستومورفومتری دیواره معده و همچنین پارامترهای خونی شود که ممکن است در برخی پارامترها برای سلامت افراد نامطلوب باشد.

واژگان کلیدی: بافت شناسی، هیستومورفومتری، معده، کمبود پروتئین، موش

Table 1. Diet in 1000 g of mouse food.

Food items	Protein deficiency (13.5 % protein)(gr)	Complete protein (23 % protein)(gr)
corn	700	430
rapeseed meal	150	420
Sunflower meal	112	112
oyster powder	17	17
Dicalcium phosphate	15	15
mineral supplement	2.5	2.5
vitamin supplement	2.5	2.5
Salt	1	1
Total	1000	1000

Table 2. Thickness of the mucous and submucous layers of the glandular and non-glandular parts of stomach in control and experimental groups.

	Mucous layer thickness (µm)		Submucous layer thickness (µm)	
	Glandular part	Non-glandular	Glandular part	Non-glandular
Control	615.29 ± 75.90 [a]	427.16 ± 70.78 [a]	93.42 ± 7.93 [a]	100.64 ± 7.59 a
Experimental	1298.03 ± 144.97 [b]	859.65 ± 79.52 [b]	114.47 ± 16.68 [b]	126.72 ± 13.26 [b]
Significancy	*P*=0.003	*P*=0.009	*P* =0.029	*P* =0.01

Dissimilar letters in each parameter indicate significant differences between the groups

Table 3. Thickness of the muscular layer of the glandular and non-glandular parts of stomach, Stomach pit depth, and the parietal cells count in stomach pit, in control and experimental groups.

	Muscular layer thickness (µm)		Stomach pit depth (µm)	The parietal cells count in stomach pit
	Glandular part	Non-glandular		

Control	104.05 ± 10.70 [a]	155.74 ± 25.77 [a]	94.80 ± 11.81 [a]	9.83 ± 1.34 [a]
Experimental	246.38 ± 76.54 [b]	201.55 ± 53.38 [a]	185.30 ± 31.17 [b]	25.67 ± 1.97 [b]
Significancy	P=0.002	P=0.11	P=0.0001	P<0.0001

Dissimilar letters in each parameter indicate significant differences between the groups

Table 4. The calcium, phosphorous, and glaucous levels of mice blood serum in control and experimental groups (n=6).

	Calcium (mg/dl)	Phosphorus (mg/dl)	Glaucous (mg/dl)
Control	5.57 ± 0.96 [a]	11.42 ± 3.37 [a]	78.00 ± 32.61 [a]
Experimental	6.17 ± 0.93 [a]	8.82 ± 1.85 [b]	38 ± 12.86 [b]
Significancy	$P < 0.05$	$P < 0.05$	$P < 0.05$

Dissimilar letters in each parameter indicate significant differences between the groups

Table 5. The Cholesterol, triglyceride, urea, and creatinine levels of mice blood serum in control and experimental groups (n=6).

	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	157.50 ± 14.22 [a]	155.66 ± 59.46 [a]	67.25 ± 7.38 [a]	0.53 ± 0.13 [a]
Experimental	123.83 ± 9.98 [b]	118.66 ± 5.27 [b]	48.78 ± 3.27 [b]	0.28 ± 0.7 [b]
Significancy	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Dissimilar letters in each parameter indicate significant differences between the groups

Table 6. The ALT, AST, and ALP levels of mice blood serum in control and experimental groups (n=6).

	ALT (IU/Lit)	AST (IU/Lit)	ALP (IU/Lit)
Control	6.17 ± 0.93 [a]	8.82 ± 1.85 [a]	38 ± 12.86 [a]
Experimental	5.57 ± 0.96 [b]	11.42 ± 3.37 [b]	78.00 ± 32.61 [b]
Significancy	$P < 0.01$	$P < 0.01$	$P < 0.01$

Dissimilar letters in each parameter indicate significant differences between the groups

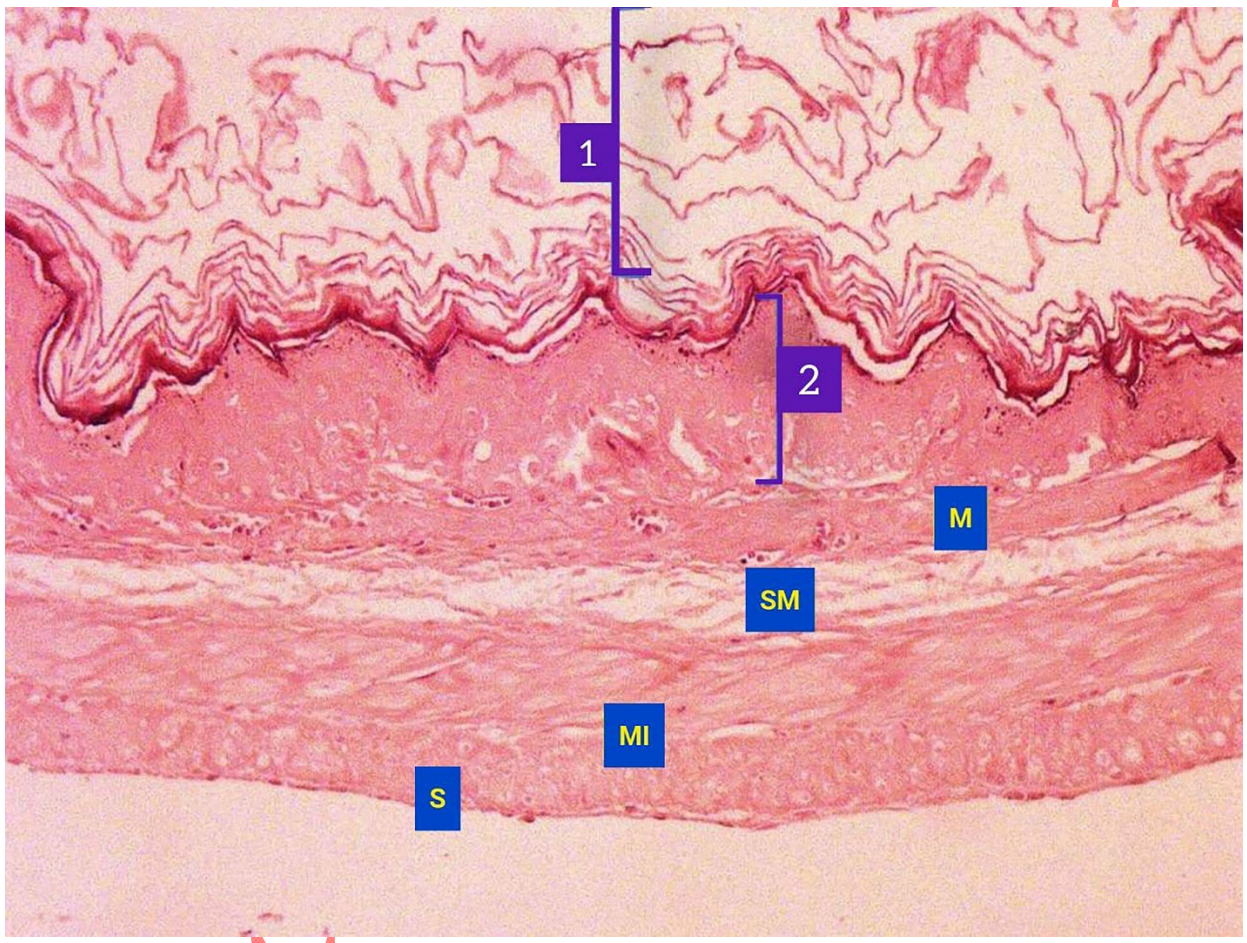

Figure 1. Photomicrograph of the non-glandular part of the stomach in the experimental group. In this image, the non-glandular part of the stomach can be seen with its different layers. **1**: keratinized stratified squamous epithelium **2**: Mucous lining: connective tissue. **M**, Mucous muscle: smooth muscle. **SM,** Submucosa layer. **MI**, Muscular layer is visible, which is seen inside the circular muscles and outside the longitudinal muscles. **S**, serous (H&E X 100)

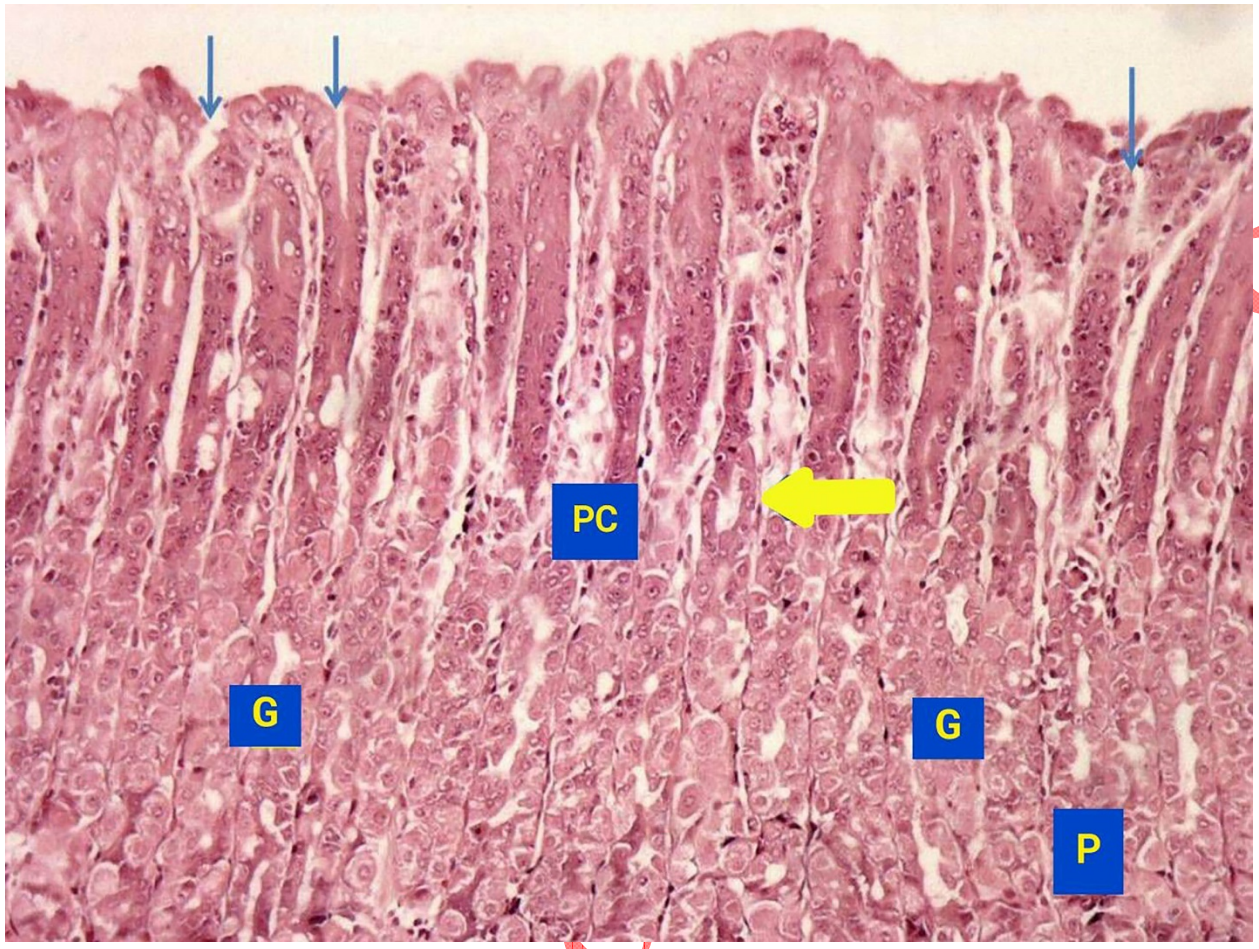

Figure 2. Photomicrograph of the glandular part of the stomach in the experimental group. In this picture, blue arrows: pits, yellow arrow: mucous lining. **PC**, parietal cells. **G**, gastric glands **P**, zymogenic cells. (H&E x 100)