ClinicSearch
International Journal of Clinical Case Studies
Seyed Davood Sharifi *
Open Access
Research Article

The histological and pathological changes of liver and testis of Japanese quails fed different levels of dietary L-valine

Ali Rashidi [1], Seyed Davood Sharifi [1*], Abozar Najafi [1], and AliReza Alizadeh [2]

[1] University of Sulaymaniyah, Republic of Iraq.

[2] Maternity Teaching Hospital, Directorate of Health, 0046 Sulaimaniyah, Republic of Iraq.

***Correspondence Author:** Seyed Davood Sharifi, University of Tehran,Tehran, Iran

Received Date: September 03 2024 | Accepted Date: September 16 2024 | Published Date: September 20 2024

Citation: Ali Rashidi, Seyed Davood Sharifi, Abozar Najafi, AliReza Alizadeh; (2024), The histological and pathological changes of liver and testis of Japanese quails fed different levels of dietary L-valine, *International Journal of Clinical Case Studies*,3(5); **DOI:**10.31579/2835-9291/022

Abstract:

While the roles of dietary L-valine (VAL) in body weight gain were reported, reinforcing roles of this BCAA in oxidative stress and the side effects in sensitive organs such as liver and testis is an undertreated issue. This experiment was carried out to investigate the histological changes of liver and testis of Japanese quail fed different levels of dietary VAL. Japanese quail chicks (male and female) were used in a completely randomized design with five experimental groups (five levels of VAL), for 42 days. Experimental diets including 0.75 (Control), 0.85, 0.95, 1.05 and 1.15%VAL in diet were formulated to be isoenergetic and isonitrogenous to meet nutrients recommendation of growing quails. At 42 d of age, quails were slaughtered and tissue samples were collected and fixed to evaluate the histological indices of liver and testis. Increase of VAL in low protein diet (17% crude protein (CP)), increased diameter of liver cell nucleus and liver hepatocytes ($P < 0.01$) in both male and female. Bile duct hyperplasia was observed in treatment 1.05% VAL, and treatment 1.15% VAL showed mild hepatosteatosis. In 0.75% and 0.85% VAL groups, there was no negative effects on the liver histology. The level of 0.85% VAL in the diet improved reproductive indexes in male (Tubular differentiation index (TDI) and spermatic index (SI); $P < 0.05$). In conclusion, the use of high levels of VAL (more than 0.85%) in a low protein diet during at 1–42 d of age can lead to histological damage in the liver and testis of quails.

Keywords: histological response; Japanese quail; liver; L-valine; testis; diet

Introduction

High quality protein with adequate amino acid balance is one of the most important nutrients for quails (Soares et al., 2003). Insufficient levels of amino acids can lead to lower performance because deficiency of amino acids restricts protein synthesis (Lima et al., 2016). Branched-chain amino acids (BCAAs), including leucine, isoleucine, and VAL, are essential amino acids for the growth and development of animals, which participate in the synthesis of proteins and precursor of other amino acids. The BCAAs have various biological effects, including the promotion of protein synthesis and hepatocyte proliferation, simulation of immune systems, improvement of insulin resistance, inhibition of liver cancer cell proliferation and neovascularization (Tajiri & Shimizu, 2018). Protective effects of BCAA were shown in previous studies. In a rat model with CCl4-induced liver injury, the supplementation of BCAA was shown to suppress hepatocyte apoptosis leading to retardation of the progression of the injury (Kuwahata et al., 2012). Interestingly, several studies have reported that supplying animals with a BCAA deficient diet increased lipolysis (Zhang et al., 2017). However, the roles of each member of BCAA's family in diet is an undertreated issue (Sefidabi et al., 2022).

Unlike the other BCAA, VAL is a limiting AA in the corn-soybean diet, and must often be supplemented in a low CP diet (Kim et al., 2022). Harms & Russell (2001) reported an improvement in commercial layers' performance by adding VAL to a corn-soybean meal diet containing supplemental methionine, lysine, tryptophan, isoleucine and threonine. They reported that Japanese quail required CP slightly higher than 16% with 0.83% VAL in deit. Similarly, 16% CP laying quail diets supplemented with Thr., Trp. and VAL or their mixture was suitable for better performance, egg quality, lower feeding cost and reduction of nitrogen excretion compared to control (20% CP) diet (Alagawany et al., 2014). Jian et al. (2021) reported that dietary supplemented VAL enhanced the trypsin activity of duodenum chime and promoted the mRNA expression levels of amino acid transporter, in the jejunum and corresponding serum free Ile, Lys, Phe, Val, and Tyr level in laying hens. Recently, Emadinia et al. (2020) pointed out that the gene expression of VAL transporter increased with incremental levels of CP and VAL supplementation in Japanese quail. More recently, it was suggested that dietary VAL supplementation exerts positive effects on performance of the broiler by promoting amino acid nutrient uptake and utilization (Jian et al., 2021). However, not only the responses and mechanisms regarding positive/negative effects of VAL high levels were not reported, but also the histology changes in sensitive tissues such as liver and testis were ignored in previous studies.

The morphological study of the liver, allows to evaluate objectively the main parameters of metabolism, to reveal organ pathology, which develops as a result of the supplementation (Fletcher, 2016; Skovorodin et al., 2019). To our knowledge, little information is available about the effects of dietary VAL on liver histology of Japanese quail (1–42 days).

On the other hand, it was pointed out the differences between males and females are evident only from 35 days of age in Japanese quails when the production phase begins (NRC, 1994). Highest testosterone and estrogen levels in Japanese quails were recorded on 8 and 10 weeks of age. Hence, histological parameter of testes sections indicated a complete development for a seminiferous tubule beginning 8 to 10 weeks (Chillab & AL-Salhie, 2018). Hanafy & Attia (2018) reported that no significant differences related to the effect of CP, VAL or their interaction on the reproductive parameters of male quail, except for cloacal gland area (CGA) and semen ejaculate volume (EV). CGA and EV of quail were significantly improved by 18% CP level compared to control (20% CP) diet. They also revealed that VAL was considered a limiting amino acid in corn-soybean diets when CP was reduced by 2%. Thus, adjusting the CP content in the diet is fundamental viewpoint to guaranteeing profitability in a production system. Additionally, reduce dietary CP is an approach in quails, so it is important to know the adequate dietary requirements of CP for quails to formulate diets, based on the ideal protein concept. Therefore, we hypothesized that the provision of low protein diets supplemented with VAL during the rearing period can have a favorable effect on histological changes of liver and testis in Japanese quails. The objective was to verify the influence of 17% CP alongside with 0.75 (Control), 0.85, 0.95, 1.05 and 1.15%VAL in diet on the histology of the liver and testis in Japanese quails and to determine the best level of VAL in low protein diet.

Materials And Methods

Feeding and bird management

All the experimental procedures for the care and use of animals in the present study were approved by the Animal Care Committee of University of Tehran.

A total of 1000 one-day-old Japanese quail chicks (male and female) were used in completely randomized design with 5 treatments, 5 replicates and 40 birds per replicate, for 42 days. The quails were allocated among 25 brick cages with water troughs. The birds received 17 h of light per day. Diet and fresh water were provided ad libitum. Experimental diets were formulated to meet nutrients recommendation of growing quails with different levels of dietary VAL concentration (0.75 (Control), 0.85, 0.95, 1.05 and 1.15% of diet). Washed builder's sand was used as an inert filler to complete diet formulations to 100%. Filler was replaced with L-valine to produce diets with different levels of digestible VAL (0.85, 0.95, 1.05 and 1.15% of diet). Corn-soybean meal diets were formulated to be isocaloric and iso-nitrogenous. The ingredients and chemical composition of the diets are given in Table 1.

Ingredients (%)	1–21 days	22–42 days
Corn Grain	70.3923	67.4087
Soybean Meal-44	23.4345	23.1566
Wheat bran		3.0000
Dicalcium Phosphate	1.6137	1.2789
Limestone	1.2595	1.1849
Vegetable Oil	0.6493	2.0186
Mineral mix[1]	0.2500	0.2500
Vitamin mix[2]	0.2500	0.2500
Salt	0.2500	0.2500
L-Lysine HCL	0.4743	0.3216
DL-Methionine	0.3041	0.2239
L-Arginine	0.2521	0.0236
L-Threonine	0.2320	0.1333
L-Isoleucine	0.1178	
L-Triptophan	0.0205	
Inert [3]	0.5000	0.5000
Chemical composition (calculated)		
AMEn[4] (Kcal/Kg)	2.9	2.95
CP (%)	17.7	17.0
Fat (%)	2.88	2.90
Fiber (%)	2.91	3.17
Calcium (%)	0.90	0.80
Available Phosphorus (%)	0.39	

Ingredients (%)	1–21 days	22–42 days
Digestible Lys (%)	1.20	1.08
Digestible Met (%)	0.537	0.457
Digestible Met + Cys (%)	0.76	0.68
Digestible Val(%)	0.75	0.75
Note: [1]Mineral premix per kilogram of feed: Mn, 60 g; Fe, 80 g; Zn, 50 g; Cu, 10 g; Co, 2 g; I, 1 g, Se, 250 mg; and vehicle quantity sufficient to 500 g. [2]Vitamin premix per kilogram of feed: vitamin A, 15,000,000 IU; vitamin D3, 1,500,000 IU; vitamin E, 15,000 IU; vitamin B1, 2.0 g; vitamin B2, 4.0 g; vitamin B6, 3.0 g; vitamin B12, 0.015 g; nicotinic acid, 25 g; pantothenic acid, 10 g; vitamin K3, 3.0 g; and folic acid, 1.0 g. Washed builder's sand was used as an inert filler to complete diet formulations to 100%. [3]Filler was replaced with L-valine to produce diets with different levels of digestible Val (0.85, 0.95, 1.05 and 1.15% of diet). [4]AMEn: Nitrogen-corrected apparent metabolisable energy.		

Table 1: Feed ingredients and chemical composition of basal diet

Sampling and tissue preparation

At the end of experiment (42 d of age), five males and five females' birds of each treatment were randomly selected. Birds were slaughtered by dislocation of the first cervical vertebra after 4 hours feed deprivation, and then the necropsy were applied to remove the liver and testis. Sections of the liver and testis were dissected and immediately fixed by formalin (10%). After dehydration with ethyl alcohol in increasing concentration (70–100%) and passed in two contents of xylol the samples were embedded in paraffin, sectioned by the rotary microtome at 5µm. After slides samples were passed through the decreasing concentration (100 − 70%) of ethylic alcohol and in xylol. The histological slides were stained by Hematoxylin and Eosin stain (Luna, 1968). An ocular micrometer was used to measure the diameter of liver cells nucleus and hepatocyte and the diameter of testis.

The photomicrographs were captured with the aid of a micro camera attached to a microscope (Olympus BX-51) and the images were digitalized on software KS 400.3 (Zeiss 4.3).

Statistical analysis

Effects of dietary treatments on histology of liver and testis were analyzed using a completely randomized design with 5 treatments, 5 replicates and 40 birds per replicate. The regression analysis was performed using linear, quadratic and cubic effects to estimate the effects of Valine levels on the biological response of the Japanese quail. When $p < 0.05$, Tukey's test was performed to determine the significance between mean values. The obtained data were statistically analyzed using the SAS 9.1.3 program.

Results

Histology of the liver

As shown in Table 2, the diameter of cell nucleus ($P < 0.05$) and hepatocytes ($P < 0.0001$) increased with increasing of dietary VAL levels, and the highest amount was at the level of 1.15% of VAL (8.47 and 13.32 µm, respectively). The highest percentage changes versus control were observed in the group containing 1.15% VAL (79 and 39%, respectively in the diameter of cell nucleus and the diameter of hepatocytes). However, the difference between control and treatment 0.85% VAL was not significant. The volume of sinusoids was not different among quails (Table 2).

Item	Diameter of liver cellnucleus (µm)	%Changes versuscontrol	Diameter of liver hepatocytes (µm)	%Changes versuscontrol	Volume ofsinusoids
Sex					
female	6.4[b]		10.32[b]		14.98[a]
male	6.64[a]		12.09[a]		10.62[b]
SEM	0.18		0.259		0.54
Val level (% diet)					
0.75	4.72[c]		9.6[b]		13.27
0.85	4.68[c]	-1	8.87[b]	-8	12.09
0.95	5.95[b]	26	12.65[a]	32	11.77
1.05	7.87[a]	67	12.97[a]	35	13.46
1.15	8.47[a]	79	13.32[a]	39	13.42
SEM	0.285		0.41		0.85
Val level× sex					
0.75× male	4.49[d]		9.66[b]		13.5
0.85× male	4.27[d]	-5	8.51[b]	-12	9.02

Item	Diameter of liver cellnucleus (µm)	%Changes versu scontrol	Diameter of liver hepatocytes (µm)	%Changes versu scontrol	Volume o fsinusoids
0.95× male	6.73^{bc}	50	14.63^{a}	51	8.73
1.05× male	8.99^{a}	100	13.96^{a}	45	11.03
1.15× male	8.71^{a}	104	13.71^{a}	42	10.83
0.75× female	4.95^{dc}		9.53^{b}		13.04
0.85× female	5.09^{dc}	3	9.25^{b}	-3	15.16
0.95× female	5.18^{dc}	5	9.9^{b}	7	14.8
1.05× female	6.75^{bc}	36	9.98^{b}	1	15.89
1.15× female	8.23^{ab}	66	12.93^{a}	30	16.0
SEM	0.403		0.58		1.209
p-value					
Sex	0.025		< 0.0001		< 0.0001
Val level	< 0.0001		< 0.0001		0.48
Val level× Sex	0.0027		< 0.0001		0.057

Note: Means with different superscripts within the same column differ significantly ($P < 0.05$).

Table 2: The effect of different VAL levels and bird sex (n = 35 each group) on the liver histology of Quails

The interaction of different levels of VAL and bird sex on the diameter of cell nucleus (P = 0.0027) and the diameter of hepatocytes (P < 0.0001) of quails were significant, and the highest percentage changes versus control was higher in males (104 and 51%, respectively) than in females (66 and 30%, respectively). The diameter of liver cell nucleus (6.64 µm; P = 0.025) and the diameter of hepatocytes (12.09 µm; P < 0.0001) of male were higher than the female (6.4 µm and 10.62 µm, respectively) quails. The volume of sinusoids of female (14.98) was higher than male (10.62) quails (P < 0.0001, Table 2).

During microscopic examination, accumulation of inflammatory cells was observed in treatments 0.95% VAL. Bile duct hyperplasia was observed in treatment 1.05% VAL, and treatment 1.15% VAL showed mild hepatosteatosis (Figure 1).

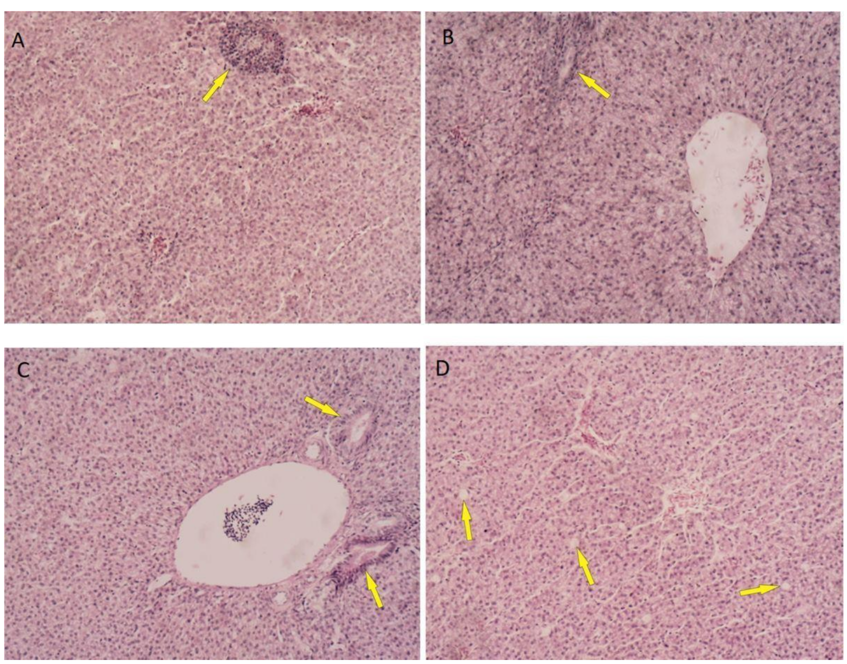

Figure 1: Section of liver tissue of quails. HXE, 100X. A. Accumulation of inflammatory cells in treatment 0.95% VAL. B, C.Bile duct hyperplasia in treatment 1.05% VAL. D. Mild hepatosteatosis in treatment 1.15% VAL; (shown by yellow arrow).

Testis histology

Data in Table 3 showed that tubular differentiation index (TDI; P = 0.029) and spermatic index (SI; P = 0.002(were altered by treatments, and the highest amount was at the level of 0.85% of VAL (59.4 and 62.4%, respectively in TDI and SI). Dietary inclusion of VAL did not affect the testicular capsule diameter, epithelium height, and Sertoli cell number (Table 3).

VAL level (% of ration)	**Tubular differentiationindex (TDI; %)**	**Spermatic index (SI; %)**	**Testicular capsul ediameter (μm)**	**Epithelium height (μm)**	**Sertoli cells (n)**
0.75	51.3[b]	59.8[ab]	218.4	54.4	22.7
0.85	59.4[a]	62.4[a]	138.5	58.2	24.2
0.95	58.9[a]	56.8[b]	136.3	61.4	23.4
1.05	58.1[ab]	56.4[b]	152.4	58.2	23.3
1.15	57.4[ab]	55.5[b]	145.1	59.3	23.5
SEM	1.73	1.14	34.6	3.74	1.15
p-value					
VAL level	0.029	0.002	0.449	0.766	0.925
Linear	0.062	0.001	0.244	0.420	0.853
Quadratic	0.013	0.650	0.226	0.414	0.681
Cubic	0.013	0.032	0.371	0.694	0.473
Note: Means with different superscripts within the same column differ significantly ($P < 0.05$).					

Table 3: The effect of different levels of VAL in the diet on the testis histology of Quails

The release of spermatogenic cells into the lumen of the seminiferous tubule was observed in treatment 1.15% VAL. In the germinal epithelium of the groups treated with the levels of 0.95%, 1.05% and 1.15% VAL, vacuole formation was observed between the germ cells (Figure 2).

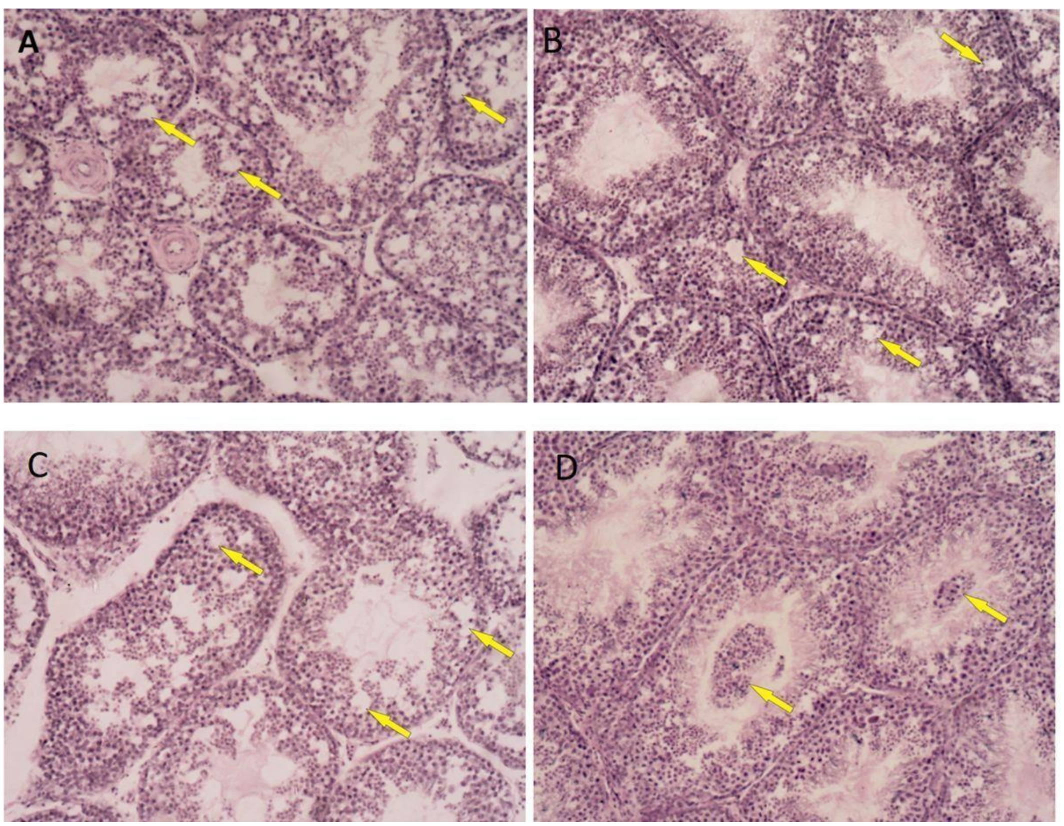

Figure 2: Optical photomicrograph of seminiferous tubules of male quails. HXE, 100X. Formation of vacuoles between germ cells in the germinal epithelium in treatments 0.95% (A), 1.05% (B), and 1.15% (C) of VAL, respectively (shown by yellow arrow). D. the release of spermatogenic cells into the lumen of the seminiferous tubule in 1.15% VAL group (shown by yellow arrow).

Discussion

In general, in our experiment, different levels of dietary VAL were effective on the histology of quail liver and testis. With increasing VAL level, the highest diameter of liver cell nucleus and hepatocytes, were observed with the consumption of 1.05% and 1.15% of VAL in both female and male, and the percentage of these changes versus control were high, which means a decrease in the hepatocytes' functional activity through the reduction of nuclear plasma ratio in higher levels of VAL in diet. At high levels of VAL consumption (1.05 and 1.15% VAL), bile duct hyperplasia and mild hepatosteatosis were also observed. Pathomorphological liver changes consist in dystrophic and necrotic changes in hepatocytes and bile duct epithelial hyperplasia (Zao et al., 2010). In broilers, hyperplasia and fibroplasia of the bile ducts are classified as nonspecific lesions and are associated with changes in hepatic metabolism that occur systematically after lesion of the liver parenchyma (Hochleithner et al., 2005). Altogether, the use of high levels of VAL (more than 0.85%) in a low protein diet can lead to histological damage in the liver of quails.

Liver hepatocytes are any of the polygonal epithelial parenchymatous cells of the liver that secrete bile. The BCAAs enhanced hepatocyte regeneration in a rat hepatectomy model (Kim et al., 2011) and were shown to increase the secretion of hepatocyte growth factor (Tomiya et al., 2004). BCAA has been associated with cell proliferation through activation of mechanistic target of rapamycin complex 1 (mTORC1). BCAAs are also shown to suppress oxidative stress by stimulating the expression of Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-α or Sirtuin-1 (SIRT-1), or by activating the genes involved in antioxidant defenses (Tajiri & Shimizu, 2018). Those mechanisms could also contribute to promote hepatocyte proliferation (Tajiri & Shimizu, 2018). It is surprising to note that among BCAA, VAL stimulates the lymphogenesis of granular and agranular lymphocytes as well as increases natural killer cells (Kim et al., 2022). In this regard, the highest level of superoxide dismutase was observed by 0.1 and 0.2% VAL with 18% CP diet in quail, which may result in a superior antioxidative status (Hanafy & Attia., 2018). Based on our findings, it seems that the level of 0.85% VAL with 17% CP diet was suitable for improving the functional activity of the liver, without any negative histological effects. Since BCAA administration is used in the treatment of hepatic encephalopathy, the results of the present experiment can also be proposed to investigation in human.

In our experiment, differences between the two sexes were also observed. The diameter of liver cell nucleus and the diameter of hepatocytes of male were higher than the female quails. While the volume of sinusoids was no different among treatments, in female was higher than male. Sinusoids, small blood vessels between the radiating rows of hepatocytes, convey oxygen-rich hepatic arterial blood and nutrient-rich portal venous blood to the hepatocytes and eventually drain into the central vein, which drains into the hepatic vein. In general, in adulthood, there is a weight difference between two sexes of quails. Female quail birds attained higher weight than the male quails at 4-weeks (Khaldari et al., 2010). Body weight is one of the main factors that affects the birds' maintenance requirements (NRC, 1994). Therefore, it is assumed that the maintenance requirements for males may also be lower than those of females. Thus, the use of similar diets for both sexes could exceed the nutrient needs for males (Retes et al., 2019). Therefore, the difference between the two sexes may be due to the difference in their body size and, as a result, the difference in their maintenance requirements and body metabolism.

With increasing VAL level in the diet, TDI and SI increased linearly. TDI is the percentage of seminiferous tubules containing at least three differentiated germ cells and SI is the number of spermatozoa per 100 spermatogenic cells. According to the Zamir-Nasta et al. (2021) a reduction in the percentage of spermatogenic tubules with TDI, tube replacement (RI) and negative spermatogenesis coefficient (SPI) in testicular tissue, disrupted the process of cell division, which in turn could disrupt the operation of spermatogenesis. Based on the fact that fertilization in avian species depends on the release of spermatozoa from sperm storage tubules (SST), active ciliary movement as well as structural integrity of glandular cells are important (Kimaro, 2016). Our study showed that the release of spermatogenic cells into the lumen of the seminiferous tubule was observed in treatment 1.15% VAL; and also, in the germinal epithelium of the groups treated with the levels of 0.95%, 1.05% and 1.15% VAL, vacuole formation was observed between the germ cells (Figure 2). In fact, these vacuoles can indicate the loss of cell connections or the reduction of adhesive molecules such as cadherins and can be considered as one of the signs of apoptosis (MohamadGhasemi, et al., 2010). In the present study, the use of high levels of VAL (0.95, 1.05 and 1.15%) with 17% CP in the diet lead to histological damage in the testis of quails.

In our experiment, the effect of VAL level on testicular capsule diameter, epithelium height and Sertoli cell number at 42 d of age were not significant between treatments. Retes et al. (2021) showed that the Sertoli cell number at 60 days of age increased linearly with increasing dietary CP. Increases in the number of Sertoli cells and spermatogonia were not associated with increases in the sperm concentration of the birds (Retes et al., 2022). Also, they showed that the testis size, seminiferous tubular area, number of spermatogonia, and germinal epithelial height at 35 days of age increased linearly with increasing dietary CP, while the number of Leydig cells decreased. Hanafy & Attia (2018) indicated that dietary 18% CP with 0.2% VAL was suitable for breeder quails at 14–28 weeks of age. They showed that cloacal gland area and semen ejaculate volume of male quail were significantly improved by 18% CP level. Dietary protein concentration affected body and testicular development in male Japanese quails but did not affect reproductive efficiency (Retes et al., 2022). During the growth phase, rapid body development is directly related to reproductive organ development (Sarabia et al., 2013). Thus, the supply of amino acids during this phase may affect bird growth. Based on the obtained results, it could be concluded that the dietary protein requirements for breeder quails could be reduced during 1 to 42 d of age. Supplementation of low protein diet (17% CP) with extra VAL (0.85% of diet) can improve reproductive performance of male quails by increasing TDI and SI, without any negative effect on testicular histology.

Conclusion

Our study demonstrated that increasing the level of dietary VAL to 0.85%, leads to significantly improved histological indexes of liver and testis in Japanese quail fed low-protein diets. But at high levels of VAL (0.95, 1.05 and 1.15%), negative effects were observed on both liver and testis histology which warrants further studies.

Declarations

Acknowledgments:

This project was part of a PhD dissertation and supported by Tehran University, Tehran, Iran. Additionally, this study was supported and performed by the Quail breeding Center of Agricultural Education and Natural Resources Training Center of Rasul Akram, Damghan, Iran. The authors appreciate the employees of the Quail breeding Center of Agricultural Education and Natural Resources Training Center of Rasul Akram for their contributions to all laboratory procedures and tireless efforts.

Author Contributions:

S.D. and A.A. designed and conducted the experiment. A.R. performed the research. A.R. and A.N. collected the samples and conducted the liver and testes histology. S.D. had the primary responsibility for the manuscript's content. A.R. performed the statistical analysis under guidance of S.D. A.R. and A.A. wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest:

The authors declare no conflict of interest.

Ethics approval:

This article does not contain any studies with human participants or animals.

References

1. Alagawany, M., Abd El-Hack, M.E., Laudadio, V., & Tufarelli, V. (2014). Effect of low-protein diets with crystalline amino acid supplementation on egg production, blood parameters and nitrogen balance in laying Japanese quail. Avian Biology Research, 7, 235–243
2. Artoni, S.M.B., Carneiro, A.P.M., & Giacomini, G. (2001). Macroscopic and Morphometric Evaluation of Japanese Quail (Coturnix coturnix japonica) Oviduct When Fed Diets with Different Protein Levels. Brazilian Journal of Poultry Science, 3, 225-231.
3. Bernal, B., Iglesias-Cabeza, N., Sanchez-Rivera, U., Toledano-Díaz,A., Castaño, C., Perez-Cerezales, S., Gutierrez-Adan, A., Lopez-Sebastian, A., García-Casado, P., Gil, M.G., Woelders, H., Blesbois, E., & Santiago-Moreno, J. (2020). Effect of supplementation of valine to chicken extender on sperm cryoresistance and post-thaw fertilization capacity. Poultry Science, 99, 7133–7141.
4. Birkhead T.R, & Fletcher F. (1994). Sperm storage and release of sperm from the sperm storage tubules in the Japanese quail Coturnix japonica. International Journal of Avian Science, 136, 101-105.
5. Chillab, K., & AL-Salhie, K. (2018). The effect of age on growth and development of the gonads pre- to post sexual maturity of Japanese quail (Coturnix japonica). Kufa Journal For Agricultural Sciences, 10, 39-55.
6. Cojocaru, E., Filip, N., Ungureanu, C., Filip, C., & Danciu, M. (2014). Effects of valine and leucine on some antioxidant enzymes in hypercholesterolemic rats. Health, 06, 2313–2321.
7. Emadinia, A., Toghyania, M., Foroozandeh, A.D., Tabeidian, S.A., & Ostadsharif, M. (2020). Effect of protein reduction and valine levels on growth performance, carcass characteristics, protein digestibility and SLC71 gene expression in Japanese quail. Livestock Science, 235, 103998.
8. Fletcher, O. (2016). Avian Histopathology. 4rd ed. AAAP, Jacksonville, FL.
9. Hanafy, A.M., & Attia, F.A.M. (2018). Productive and reproductive responses of breeder japanese quails to different dietary crude protein and l-valine levels. Egyptian Poultry Science Journal, 38, 735-753.
10. Harms, R. H. & Russell, G. B. (2001). Evaluation of valine requirement of the commercial layer using a corn-soybean meal basal diet. Poultry Science, 80, 215-218.
11. Jian, H., Miao, S., Liu, Y., Li, H., Zhou, W., Wang, X., Dong, X., & Zou, X. (2021). Effects of Dietary Valine Levels on Production Performance, Egg Quality, Antioxidant Capacity, Immunity, and Intestinal Amino Acid Absorption of Laying Hens during the Peak Lay Period. Animals, 11, 1972.
12. Khaldari, M., Pakdel, A., Mehrabani Yegane, H., Nejati Javaremi, A., & Berg, P. (2010). Response to selection and genetic parameters of body and carcass weights in Japanese quail selected for 4-week body weight. Poultry Science, 89, 1834-1841.
13. Kim, S.J., Kim, D.G., & Lee, M.D. (2011). Effects of branched-chain amino acid infusions on liver regeneration and plasma amino acid patterns in partially hepatectomized rats. Hepatogastroenterology, 58, 1280-5.
14. Kim, W.K., Singh, A.K., Wang, J., & Applegate, T. (2022). Functional role of branched chain amino acids in poultry: a review. Poultry Science, 101, 101715
15. Kimaro. W. (2016). Morphological changes in the sperm storage tubules of the japanese quail exposed to methy-2-benzimidazole carbamate. Anatomy Journal of Africa, 5, 713-720.
16. Kuwahata, M., Kubota, H., Kanouchi, H., Ito, S., Ogawa, A., Kobayashi, Y., & Kido, Y. (2012). Supplementation with branched-chain amino acids attenuates hepatic apoptosis in rats with chronic liver disease. Nutrition Research, 32, 522-9.
17. Li, M.J., Zhang, Z.M., Fan, F., Ma, P., Wang, Y., & Lu, H.M. (2019). Exploring asthenozoospermia seminal plasma amino acid disorder based on GC-SIM-MS combined with chemometrics methods. Analytical Methods, 11, 2895–2902.
18. Lima H.J.D, Barreto S.L.T, Donzele J.L, Souza G.S, Almeida R.L, Tinoco I.F.F, & Albino, L.F.T. (2016). Digestible lysine requirement for growing Japanese quails. Applied Poultry Research. 25, 483-491.
19. Luna, L.G. (1968). Manual of histologic staining methods of 5th armed forces institute of pathology. 3rd McGraw-Hill book Company-New York.
20. MohamadGhasemi, F., Faghani, M., Khajehjahromi, S., Bahadori, M., Nasiri, E., & Hemadi, M. (2010). Effect of Melatonin on Proliferative Activity and Apoptosis in Spermatogenic Cells in Mouse under Chemotherapy. Reproduction and Contraception, 21, 79-94.
21. NRC (National Research Council). (1994). Nutrient requirements of poultry. 9th Rev. Ed. National Academy Press. Washington, DC. 176 Pages.
22. Retes, P.L., Neves, D.G., Bernardes, L.F., Alves1, V.V., Gonçalves, N.C., Lima, D.R., Alvarenga, R.R., Pereira, B.A., Seidavi, A., & Zangeronimo, M.G. (2022). Dietary crude protein levels during growth phase affects reproductive characteristics but not reproductive efficiency of adult male Japanese quails. animal bioscience, 3, 385-398.
23. Retes, P.L., Neves, D.G., Bernardes, L.F., de Rezende Lima, D., Ribeiro, C.B., de Castro Gonçalves, N., Alvarenga, R.R., Fassani, E.J., & Zangeronimo, M.G. (2019). Reproductive characteristics of male and female Japanese quails (Coturnix coturnix japonica) fed diets with different levels of crude protein during the growth and production phases. Livestock Science, 223, 124-132.
24. Sadeghzadeh, S.S., Daneshyar, M., Farhomand, P., Yazdian, M.R., & Hashemi, S.M. (2019). Effects of different levels of valine amino acid on performance, carcass traits, meat quality and insulin like growth factor-1 and insulin genes expretion in male Ross 308 broiler chickens. Animal Science Journal (Pajouhesh and Sazandegi), 125, 89-108.
25. Sarabia Fragoso, J., Pizarro Díaz, M., Abad Moreno, J.C., Casanovas Infesta, P., Rodriguez-Bertos, A., & Barger, K. (2013). Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). Reproduction in Domestic Animals, 48, 345-52.
26. SAS. Inc. (2010). SAS Online Doc® Version 9.1.3. *SAS Institute*, Inc., Cary, NC, USA.
27. Sefidabi, R., Kamali, R., Shahverdi, A., Pezeshki, A., Ghasemi, Z., Ghaleno, L.R. & Alizadeh, A. (2022). The effects of dietary branched-chain amino acids on mice semen parameters and plasma amino acids profile. *Human Reproduction*, 37, 210-210. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND: OXFORD UNIV PRESS.
28. Skovorodin, E., Bronnikova, G., Bazekin, G., Dyudbin, O., & Khokhlov, R. (2019). Antioxidant influence on poultry liver morphology and hepatocyte ultrastructure. *Veterinary World*. 12, 1716-1728.
29. Soares, R da TRN., Fonseca, J.B., Santos, A.S. de O dos., & Mercandante, M.B. (2003). Protein requirement of Japanese quail (Coturnix coturnix japonica) during rearing and laying periods. *Brazilian Journal of Poultry Science*, 5, 153-156.
30. Tomiya, T., Omata, M., & Fujiwara, K. (2004). Significance of branched chain amino acids as possible stimulators of hepatocyte growth factor. *Biochemical and Biophysical Research Communications*, 313, 411-6.
31. Tajiri, K., & Shimizu, Y. (2018). Branched-chain amino acids in liver diseases. *Translational Gastroenterology and Hepatology*, 3, 47.

32. Zamir-Nasta, T., Pazhouhi, M., Ghanbari, A., Abdolmaleki, A., & Jalili, C. (2021). Expression of cyclin D1, p21, and estrogen receptor alpha in aflatoxin G1-induced disturbance in testicular tissue of albino mice. *Research in Pharmaceutical Sciences*, 16, 182-192.
33. Zhang, S., Zeng, X., Ren, M., Mao, X., & Qiao, S. (2017). Novel metabolic and physiological functions of branched chain amino acids: a review. *Animal Science and Biotechnology*, 8, 10.