Journal of Applied Biological Sciences
E-ISSN: 2146-0108 13 (3): 138-141, 2019

The Effect of Different Levels of Niacin in Diet on Immunological and Blood Biochemical Parameters of Juvenile Common carp (*Cyprinus carpio*)

Kheyri MOHAMMADI[1], Hamid MOHAMMADIAZARM[1]*, Amirparviz SALATI[1], Ebrahim RAJABZADEH[1]

Department of Fisheries, Faculty of Marine Natural Resources, Khorramshar University of Marine Science and Technology, Khorramshar, Iran[1]

*Corresponding Author
E-mail: azarmhamid@kmsu.ac.ir

Received: August 10, 2019
Accepted: October 30, 2019

Abstract

This experiment was conducted to examine the effect of different levels of niacin on some immunological and blood biochemical parameters of juvenile carp. A control diet (containing 30 mg niacin kg $^{-1}$ commercial diet) and four other diets were prepared to contain 50, 70, 90 and 110 mg niacin per kg of commercial diet. Fish with initial average weight of 25 ± 0.28 g were allotted to 15 circular tanks of 300 L capacity at a density of 12 fish per tank and fed the experimental diets to satiation triple daily for 56 days. At the end of feeding trial, lysozyme activity, lymphocyte, monocyte, neutrophil and basophil percentage were not significantly different from the control. The blood protein, albumin and glucose values of fish fed 90 and 110 mg niacin kg $^{-1}$ diet were significantly higher than control group. The triglyceride and total cholesterol values of fish fed different levels of niacin were not significantly different compared to control group. Fish fed 70 up to 110 mg niacin kg $^{-1}$ diet showed significantly higher high density lipoprotein (HDL) than 50 mg niacin $^{-1}$ kg diet and control. The results confirmed that 90 mg niacin kg $^{-1}$ affected performance of juvenile common carp through effect on total protein and glucose values.

Keywords: Blood, Common carp, Lysozyme, Niacin, Vitamin

INTRODUCTION

Today, feed cost generally constitutes the largest changeable cost in intensive fish production; therefore, formulation of cost-effective feeds can significantly influence the profitability. Also, the role of nutrition in fish health and metabolism is known and major efforts are being made to recognize the interrelationship between nutrient levels in the diet, the absorption of nutrients and health status of fishes reared in aquaculture **[9]**. Therefore, development of nutritious and cost-effective diet is dependent on knowing the species nutritional requirement with balanced feed formulation.

Vitamins are required in trace amounts and essential for maintenance, growth, health and reproduction of all animals. The niacin is also known as vitamin B_3 is essential due to its significance role in normal body growth and other metabolic function in fish. It is required for the synthesis of nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+). Both of them are cofactor for numerous dehydrogenases, e.g., lactate and malate dehydrogenases. Niacin is also involved in lipid, protein and carbohydrate metabolism. De Silva and Anderson 1995 **[6]** reported that nicotinic acid can be synthesized by most animals from amino acid tryptophan. But, the conversion of tryptophan to niacin is limited in fish, and it must be fed to prevent deficiency and impaired growth **[13]**. However, too much niacin inhibits growth **[16]** as a result, defined information of niacin requirement is essential for the formulation of diet of common carp.

The Common carp (*Cyprinus carpio*) is one of the most important species in Iran. It is suitable for aquaculture because of its fast growth, easy reproduction and high tolerance to poor environmental conditions. Therefore, further detection of commercially available dietary additives to improve performance and health of it is still highly most wanted. So, the purpose of the present study was to evaluate effect of different levels of niacin on immune response and blood biochemical parameters of juveniles carp.

MATERIAL AND METHODS

Diet preparation

For preparing the experimental diets, a commercial common carp diet (21Beiza, Shiraz, Iran) containing a complete vitamin mixture which provided 30 mg niacin per kg of diet, was used as a basal feed or control. A nicotinic acid (NA) supplement (Purity 98%, Sigma Aldrich) was used to provide other final levels of 50, 70, 90 and 110 mg niacin per kg of diet. The proximate composition of the experimental diets was shown in Table 1. The basal diet was crushed, mixed with the supplement nicotinic acid and then water was added to create stiff dough then dough was homogenized completely in a mixer for 15 min. After that, the dough was pelleted with meat grinder to get pellets with size of 2 mm. Pellets were dried in room temperature at 25 °C and stored in plastic bags at -20 °C until their use. Each dietary treatment was tested by triplicate.

Experiment fish and feeding conditions

Juveniles of carp were purchased from Shahid Maleky farm, Khouzestan, Iran. After transportation, fish in laboratory were kept in a 1000 L circular polyethylene tank, which was filled with 800 L fresh water. After two days, one hundred and eighty *C. carpio* juveniles with initial weight of 25 ± 0.28 g randomly were distributed into 15 cylindrical polyethylene tanks (250 volume) with 12 fish per each tank and adapted to the experimental condition for two weeks. Tanks were filled with 250 L of fresh tap water and almost 50% of water was changed every two days. Fish were manually fed to visual satiation three times a day (09:00 a.m, 13:00 and 18 p.m) for eight weeks. Mean values for water temperature, dissolved oxygen content and pH were 29 ± 1°C, 6.18 ± 0.11 mg L^{-1} and 7.4 ± 0.28 respectively and photoperiod was 16L: 8D (light:darkness) during the experimental period.

Chemical analysis

Proximate analyses of the diets were detected [**4**]. Crude protein content was determined by Kjeldahl method by using an Auto Kjeldahl System (Kjeltec ™2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105℃ for 24 h and ash by a furnace muffler (550℃ for 6 h). Also, before the preparing experimental diets with different levels of niacin, the base amount of niacin in basal diet was detected by measuring Liquid Chromatography (HPLC) (CECIL-Model 1100) with UV detector and ProntoSil column with flow rate 1/1 ml in minutes. So, according to the amount of niacin in basal diet, the desired amounts of niacin were added in experimental diets.

Tables 1, Biochemical composition of experimental diets were fed by juvenile carp for 56 days.

diet	Niacin	protein	Lipid	Ash	Moisture
Basal[a]	0.03	350.00	82.00	119.20	100.00
NA 50	0.05	349.00	78.30	122.20	98.10
NA 70	0.07	353.00	79.00	123.40	95.60
NA 90	0.09	351.00	81.00	119.00	101.20
NA 110	0.11	347.50	78.50	120.10	97.50

[a] 21Beiza Company, Shiraz, Iran

Biochemical assays

At the end of experiment, 10 fish from each tank were anesthetized with clove flower powder (300 mg L^{-1}) for bleeding from the caudal vein with un-heparinized syringes. Then, blood specimens were divided to two parts, one part was transferred into vials and allowed to clot at room temperature. After that, samples were centrifuged (room temperature, 3000 g, 10 min), sera were extracted and stored at -80 °C until analysis. Serum biochemical parameters were spectrophotometrically analyzed by means of an auto-analyzer *(*Eurolyser, Austria*)* using commercial kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were conducted for total protein (g dl^{-1})*,* albumin *(*g dl^{-1}*)* glucose (mg dl^{-1}), triglyceride (mg dl^{-1}), total cholesterol (mg dl^{-1}), HDL (mg dl^{-1}) and LDL (mg dl^{-1}) according to the procedures of the company. Also, the amount of globulin was calculated by reducing the amount of albumin from total protein. Furthermore hematocrit (Hct) was assayed in another part of blood specimens according to a micro hematocrit method, respectively as describe 18.

Lysozyme activity

Lysozyme activity in serum was determined according to the method of Demers and Bayne 1997 [**7**] based on the lysis of the lysozyme sensitive gram positive bacterium, *Micrococcus lysodeikticus* (Sigma). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 µL mL^{-1} (in 0.1 M phosphate citrate buffer, pH 5.8) were taken as the standard. This along with the undiluted serum sample (25 µL) was placed into wells of a 96-well plate in triplicate. One hundred and seventy-five microliters of *M. lysodeikticus* suspension (75 mg mL^{-1}) were prepared in the same buffer was then added to each well. After rapid mixing, the change in turbidity was measured every 30 s for 5 min at 450 nm at approximately 20 °C using a microplate reader.

Total white blood cell count

Leucocyte differentiation was determined in blood extension stained with May-Grunwald-Giemsa-Wright. Differential counting was performed under microscope at 100Ð in immersion oil. One hundred cells were counted to establish percentages for each cellular component of interest.

STATICAL ANALYSIS

Data were subjected to one-way ANOVA to test treatments effects on fish. When significant difference was found in one-way ANOVA, Duncan·s multiple range test was used to rank the groups. All statistical analyses were performed using SPSS version 16 (SPSS, Chicago, IL USA) with a significant level of ($p < 0.05$). The values presented are mean ± standard error (SE).

RESULTS AND DISCUSSION

The result of different levels of dietary niacin on some immunological and blood biochemical parameters of juvenile carp are shown in Tables 2 and 3.

Hematocrit values of fish fed different levels of niacin in diet were not significantly different ($P > 0.05$) compared to the control group. The blood protein and albumin values of fish fed 90 and 110 mg niacin kg $^{-1}$ diet were significantly higher ($P < 0.05$) than control group. Also, glucose values of fish fed 90 and 110 mg niacin kg $^{-1}$ diet were significantly higher ($P < 0.05$) than control group. The triglyceride and total cholesterol values of fish fed different levels of niacin were not significantly different compared to ($P > 0.05$) control group. Fish fed 70 up to 110 mg niacin kg $^{-1}$ showed significantly higher ($P < 0.05$) HDL than 50 mg niacin $^{-1}$ kg and control. LDL values of fish fed different levels of niacin were not significantly different ($P < 0.05$) compared to control group.

Table 2, Effects of experimental diets on some serum biochemical parameters of common carp (mean ± SEM, n=3)

Parameters	Control	NA50	NA70	NA90	NA110
Hematocrit %	28[n.s].3±86.29	52.1±75.33	92.1±46.35	70.1±27.30	96.1±55.32
Total protein, g/dl	19[a].0±40.3	25[ab].0±90.3	25[ab].0±04.4	21[b].0±15.4	20[b].0±21.4
Albumin, g/dl	22[a].0±93.1	15[ab].0±14.2	17[abc].0±50.2	22[bc].0±69.2	19[bc].0±79.2
Globulin, g/dl	36 [n.s].0±46.1	32.0±75.1	35.0±42.1	26.0±46.1	40.0±53.1
Glucose, g/dl	77[a].3±25.54	23[a].11±75.63	52[ab].13±00.74	58[b].7±50.103	55[ab].11±00.81
Triacylglycerol, mg/dl	20[n.s].15±00.203	08.30±25.206	07.41±25.205	18.24±50.209	21.26±50.207
Cholesterol, mg/dl	11[n.s].11±00.108	83.8±25.106	84.13±50.104	33.7±75.102	70.3±25.101
HDL, mg/dl	25[a].1±25.21	86[a].0±50.23	64[b].0±50.24	47[bc].0±75.25	25[c].1±25.28
LDL, mg/dl	38 [n.s].7±25.59	80.7±75.58	49.10±75.54	37.3±75.53	75.5±00.50

HDL: high-density lipoproteins, LDL: low-density lipoproteins
A different letter in the same line means significantly different ($P < 0.05$). NS: Non significant ($P > 0.05$). SEM: Standard error of mean.
Also, Lysozyme activity, lymphocyte, monocyte, neutrophil and basophil percentage were not significantly different ($P > 0.05$) compared to the control.

Table 3, Effects of experimental diets on lysozyme activity and white blood cell count of common carp (mean ± SEM, n=3)

Parameters	Control	NA50	NA70	NA90	NA110
Lysozyme ,U/mgprotein/min	33$^{n.s}$.0±66.32	50.5±50.34	64.2±00.32	63.4±33.37	31.3±33.31
Lymphocyte %	66^{ab}.0±44.92	61^{b}.0±22.93	33^{ab}.1±33.91	13^{ab}.1±66.90	66^{a}.0±22.90
Monocyte %	60 $^{n.s}$.0±44.4	42.0±11.4	70.0±33.4	74.0±83.4	58.0±88.5
Neutrophil %	47^{ab}.0±00.3	52^{a}.0±44.2	80^{ab}.0±66.3	67^{a}.0±77.4	40^{ab}.0±77.3
Basophil %	11$^{n.s}$.0±11.0	14.0±22.0	16.0±16.0	14.0±22.0	11.0±11.0

A different letter in the same line means significantly different ($P < 0.05$). NS: Non significant ($P > 0.05$). SEM: Standard error of mean.

Niacin is involved in the synthesis of high-energy phosphate in glycolysis, pyruvate metabolism and in pentose synthesis. The essentially of dietary niacin for growth of different fish species is obviously demonstrated in NRC 2011**[13]**. The optimum niacin requirement for African catfish *Clarias gariepinus* (33 mg kg^{-1}) **[11]**, channel catfish *Ictalurus punctatus* (7.4 mg kg^{-1}) **[14]**, gilthead seabream *Sparus aurata* (63±83 mg kg^{-1}) (12), brook trout *Salvelinus fontinalis* (95mg kg^{-1}) **[15]**, rainbow trout *Oncorhynchus mykiss* (10 mg kg^{-1}) **[17]**, Pacific salmon *Oncorhynchus gorbuscha* (150-200 mg kg^{-1}) **[8]** and yellowtail *Seriola quinqueradiata* (12 mg kg^{-1}) **[19]**. Reasons for different requirements is maybe related to leaching or diet composition, age of fish, disparities in methodology and assessment criteria **[11, 13]**. For example, it was reported that complex carbohydrate in the diet may be induce to increase requirement for niacin by fish. Therefore, it was reported that 26 and 121 mg niacin kg^{-1} diet were required for hybrid tilapia fed diets with glucose and dextrin as the carbohydrate source, respectively **[13]**. Generally, fish in earlier stage of life has high requirement for nutrients because of high metabolism rate. On the other hand, it was reported fish fed niacin deficient diets showed feed refusal, listlessness, weight loss, poor feed utilization, high mortality, skin haemorrhage and anaemia in different fish species such as; African catfish **[11]**, eel **[1]**, rainbow trout **[17]**, channel catfish **[3, 14]**, gilthead seabream **[12]** and Asian catfish **[5]**. Also, malnutrition of the deficient fish was contributed with low hemoglobin and red blood cell values as demonstrated previously in a study on starved Nile tilapia *Oreochromis niloticus* **[2]**. But in the present study, lysozyme activity, lymphocyte, monocyte, neutrophil, basophil percentage and also hematocrite values of fish fed different levels of niacin were same. So, it seems that 30 mg niacin kg^{-1} of diet is sufficient to maintain a normal Ht level, leucocyte number and prevent malnutrition. But, the blood protein and albumin values of fish fed 90 and 110 mg niacin kg^{-1} diet were significantly improved. Since there is a close relationship between the level of protein synthesized in liver tissue and plasma protein pools, total protein levels in plasma maybe elevated due to the increased levels of protein synthesis in liver tissue of fish. Also, it was reported that the increase in the levels of serum protein in fish is thought to be associated with a stronger innate immunity response **[21]**. Because, Serum total protein of fish which contains antibacterial peptides and immunoglobulins **[20]**.

On the other hand Fish fed 70 up to 110 mg niacin kg^{-1} showed significantly higher HDL than 50 mg niacin $^{-1}$ kg and control. It was stated **[10]**niacin also had significant effect on serum HDL content. Furthermore, glucose values of fish fed 90 and 110 mg niacin kg^{-1} diet were significantly increased compared to control group. So, it seems that dietary niacin through effect on energy metabolism leads to increase values of glucose and HDL content in blood of fish.

CONCLUSION

Based on the result obtained from the present study it was concluded that 90 mg niacin kg^{-1} diet improves total protein which contains antibacterial peptides and immunoglobulins. On the other hand 90 mg niacin kg^{-1} diet improves glucose and HDL values of fish probably through energy metabolism. So, the optimum inclusion of dietary niacin to achieve good health status and energy access is 90 mg niacin kg^{-1} diet.

ACKOWLEDEGMENTS

This work was supported by the funds of the Khorramshar University of Marine Science and Technology, Iran.

REFRENCES

[1] Arai S, Nose T, Hashimoto T. 1972. Qualitative requirement of young eels (*Anguila japonica*) for water soluble vitamins and their deficiency symptoms. Bulletin Fresh water Fisheries Research Laboratory, 22: 69-83.

[2] Abdel-Tawwab M., Khattab YA, Ahmad MH, Shalaby AM. 2006. Compensatory growth, feed utilization, whole-body composition, and hematological changes in starved juvenile Nile Tilapia, (*Oreochromis niloticus* L.). Journal of Applied Aquaculture, 18:17-36.

[3] Andrews JW, Murai T. 1978. Dietary niacin requirements for channel catfish. Journal of Nutrition, 108: 1508-1511.

[4] AOAc. 1995. Official Methods of Analysis of AOAC International. In: Association of Official Analytical Chemists, (16th edn) (ed.by P.Cunniff). AOAC International Arlington, Virginia, USA.

[5] Butthep C, Sitasit P, Boonyaratpalin M. 1983. Water-soluble vitamins essential for the growth of Clarias. In: Cho CY, Cowey CB, Watanabe T, editor. Finfish nutrition in Asia. Methodology approaches to research and development (Proceedings of the Asian Finfish nutrition workshop, Singapore 23–26 August).1st ed. Ottawa, Canada: International Development Research Centre. 118-129.

[6] De Silva SS, Anderson TA. 1995. The effect of ration on growth ratio. London, UK: Chapman and Hall.

[7] Demers NE, Bayne CJ. 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Developmental and Comparative Immunolgy, 21: 363-373.

[8] Halver JE. 1989. The vitamins. In: Halver JE, editor. Fish nutrition, 2nd ed. New York, NY, USA: Academic Press. 32-102.

[9] Halver JE. 2001. My 50 years in fish nutrition, 1949–99. Aquaculture Research, 32: 615-622.

[10] Huang F, Wen H, Wu F, Jiang M, LiuW, Tian J, Shao H. 2013. The dietary niacin requirement of large GIFT tilapia. Journal of South China Agriculture University, 34: 235-240.

[11] Morris PC, Backer RTM, Davies SJ. 1998. Nicotinic acid supplementation of diets for the African catfish, *Clarias gariepinus* (*Burchell*). Aquaculture Research, 29: 791–799.

[12] Morris PC, Davies SJ. 1995. The requirement of the gilthead seabream (*Sparus aurata L.*) for nicotinic acid. Animal Science, 61: 437-443.

[13] National Research Council. 2011. Nutrient Requirements of Fish and Shrimp.Washington, DC, USA: National Academies Press.

[14] Ng WK, Serrini G, Zhang Z, Wilson RP. 1997. Niacin requirement and inability of tryptophan to act as a precursor of NAD+ in channel catfish, *Ictalurus punctatus*. Aquaculture, 152: 273-285.

[15] Phillips AM, Brockway DR. 1947. The niacin and biotin requirement of trout. Transactions of the American Fisheries Society, 77:152-159.

[16] Poston H A, Combs GFJ. 1980. Nutritional implications of tryptophan catabolizing enzymes in several species of trout. Proceedings of the Society for Experimental Biology and Medicine, 163(4):452-454.

[17] Poston H A, Wolfe M J. 1985. Niacin requirement for optimum growth, feed conversion and protection of rainbow trout (*Salmo gairdneri*) from ultraviolet-B irradiation. J Fish Dis. 8: 451-460.

[18] Sandnes K, Lie O,Waagbo R. 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. Journal of Fish Biology, 32 (1): 129-136.

[19] Shimeno S. 1991. Yellowtail, *Seriola quinqueradiata*. In: Wilson RP, editor. Handbook of nutrient requirements of finfish. Boca Raton, Florida, USA: CRC Press. 181-193.

[20] Uribe C, Folch H, Enriquez R, Moran G. 2011. Innate and adaptive immunity in teleost fish: A review. Veterinary Medicine, 56: 486-503.

[21] Wiegertjes GF, Stet RJM, Parmentier HK, Van Muiswinkel WB. 1996. Immunogenetic of disease resistance in fish: a comparative approach. Developmental and Comparative Immunology, 20: 365-381.