Int. J. Aquat. Biol. (2013) 1(2): 42-47
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Effects of dietary free L-Lysine on growth performance and muscle composition of Beluga *Huso huso* (Linnaeus 1785) juveniles

Seyyed Morteza Hoseini *[1], Seyed Abbas Hosseini[1], Mohammad Soudagar[1]

[1] Department of Fisheries, Faculty of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: Effect of dietary free L-Lysine on growth, food intake, and muscle composition of beluga juveniles were investigated over 6 weeks. Control diet lysine content was 2.1% of dry matter (4.4% of dietary protein). Three experimental diets were prepared by adding lysine (0.5, 1 and 1.5%) to control diet to obtain diets containing 2.6, 3.1 and 3.6% of dry matter lysine (corresponding to 5.5, 6.6 and 7.6 of dietary protein). Fish were fed 2.6% of dry matter lysine showed significantly higher final weight, weight gain and SGR and lower FCR compared to other treatments. There was no significant change in food intake and survival between treatments. Lysine supplementation resulted significantly in increase and decrease in muscle protein and lipid, respectively. Dietary lysine has no effect on muscle ash and moisture content. Results showed that lysine supplementation had no significant effect on food intake in beluga juveniles. It seems that dietary lysine level of 2.6% of dry matter (corresponding to 5.5% of dietary protein) is suitable for growth of beluga juveniles.

Article history:
Received 2 March 2013
Accepted 14 April 2013
Available online 25 April 2013

Keywords:
L-Lysine
Huso huso
Food intake
Muscle composition

Introduction

Sturgeon populations are under pressure due to over-fishing, pollution and habitat degradation (Birstein, 1993). Beluga is one of the most important species inhabits in Caspian Sea. This species is included as endangered species in IUCN Red List and in 1997 in the CIIES Appendices. Aquaculture is a critical technique to decrease pressure on Beluga wild population and increase caviar and meat production. Fishes require the same ten indispensable amino acids as other animals for growth (Wilson, 1985). Indispensable amino acids deficiency will result in reduced growth performance and feed utilization (Wilson and Halver, 1986). It is important to fulfill the indispensable amino acid requirements of fish by formulating balanced nutrients in fish feeds. Lysine is one of the indispensable amino acids (Wilson, 2002) which is often the limiting amino acid in commercial feeds (Harris, 1980; Forster and Ogata, 1998; Small and Soares, 2000; Tantikitti and Chimsung, 2001). An important function of lysine is to serve as the precursor of carnitine which carries long chain fatty acids into the mitochondria for β-oxidation of lipids (Walton et al., 1984). Researchers had reported the lysine requirements of commonly cultured fish species to range from 3.2 to 6.2% dietary protein (Wilson, 2002).

Lysine deficiency causes poor growth performance in number fish species (Zhou et al., 2007; Mai et al., 2006 and Wang et al., 2005). But no information is available about effect of different levels of dietary lysine on beluga juveniles' growth performance.

Amino acids are one of the major classes of feeding stimulants (Jones, 1992) which can maximize food consumption and reduce food waste by improving initial feeding and food palatability (Lee and

* Corresponding author: Seyyed Morteza Hoseini
E-mail address: seyyedmorteza.hoseini@gmail.com
Tel: +989112750713

Table 1. Basal diet ingredient.

Ingredients	%
Fish meal	49
Soybean meal	15.5
Wheat meal	4
Corn meal	3.2
Wheat gluten	2.2
Barely meal	2.5
Meat meal	7.5
Cottonseed	2.5
Antioxidant	0.1
Salt	0.5
Mineral mix	1
Vitamin mix	1
Binder	1
Fish oil	5
Soybean oil	5
Crude protein	46.8
Crude lipid	12.4
Carbohydrate	17.1
Ash	4
Moisture	15

Table 2. Basal diet amino acids profile.

Amino acid	Band	Free
Trp	0.9	0
Asp	31.7	1.3
Glu	74.2	0.3
Ser	14.8	0.8
Gly	28	0.6
His	8.4	1.2
Arg	26.2	6.1
Thr	13.4	0.6
Ala	22.1	1.5
Pro	25.7	1
Tyr	6.7	0.3
Val	22.1	0.7
Met	7.1	0.3
Cys	1.9	0.7
Ileu	17.4	1.2
Leu	28.7	0.9
Phe	15.8	0.3
Lys	20	1

Meyers, 1996). Sturgeons are virtually blind and olfaction is one of the fundamental senses for feeding behavior of them (Kasumyan, 1999). When the food odor (like as Chironomid larvae extract) is released in water body, sturgeons will receive it and move along circular and S-shaped trajectories or loops around food place (Kasumyan, 1999). Therefore, the main objective of this study was to investigate the effects of adding free lysine to beluga juveniles' diet, on food intake, growth performance, survival and muscle composition.

Materials and methods

Fish and maintenance condition: 240 beluga juveniles (17.71±2.68 g) were randomly distributed in 12 tanks (500 l.) and were allowed to acclimate for one week. All tanks were supplied by dechlorinated tap water. All tanks were aerated continuously over the experiment. Water exchange was 50% daily. During acclimation week, all fish were fed control diet until apparent satiation once a day. Thereafter, a six-week trial carried out with four triplicate treatments (one control and three lysine treatments). During whole experiment, fecal and food waste were siphoned every day. Dissolved oxygen, pH and water temperature were measured daily (by digital dissolved oxygen meter + pH meter apparatus) over the six weeks, which ranged 5.5±0.5 mg/L, 8.04 ± 0.05 and 24 ± 1 (mean ± SD) °C, respectively.

Diet preparation: The diet ingredients and approximate composition of the control group are shown in Table 1. It contained 2.1% of diet (correspond to 4.4% of dietary protein) lysine. Other experimental diets were prepared by adding crystalline L-Lysine at levels of 0.5, 1 and 1.5% of diet. Thus, we reached four treatments contained 2.1, 2.6, 3.1 and 3.6% of diet total lysine. Free and protein banded amino acid profiles is shown in Table 2. All diets were pelleted 3 mm in diameter.

Experimental protocol: Each treatment was fed corresponding diet until 6th week. Feeding was performed by hand, once a day. In each meal, exceed food was offered to fish and allowed to eat until

Table 3. Effects of dietary L-Lysine (%) on Weight Gain (%), FCR, SGR, Food intake (% of body weight per day) and survival (%) of *H. huso* juveniles.

Dietary lysine	IW	FW	WG	FCR	SGR	FI	SUR
2.1	16.3±1.0a	43.0±1.8a	263±5.3a	1.0±0.02a	2.3±0.04a	2.8±0.2a	91.6±10a
2.6	17.5±3.2a	57.0±7.1b	328±24b	0.8±0.06b	2.8±0.20b	3.0±0.5a	98.3±2.9a
3.1	18.5±1.5a	47.6±6.6a	256±18a	1.0±0.04a	2.2±0.10a	2.8±0.2a	98.3±2.9a
3.6	18.1±3.1a	40.2±2.9a	224±32a	1.1±0.16a	1.9±0.30a	2.4±0.3a	98.3±2.9a
P_{Value}	0.71	0.017	0.003	0.016	0.006	0.11	0.44

IW = Initial weight, FW = Final weight, WG = Weight gain, FCR = Food Conservation Ratio, SGR = Specific Growth Rate, FI = Food Intake, SUR = Survival

satiation and then remaining pellets were counted and food intake was calculated. At the end of trial all fish were weighed and growth factors were calculated. For determination of approximate muscle composition, dorsal muscle of 15 fish in each treatment were collected and stored in -20 °C until further analyses.

Analyses: At the end of the trial, all fish were weighed and percent weight gain (WG%), feed conversion ratio (FCR), specific growth rate (SGR), average food intake (FI) and percent survival were calculated using the following equations:

WG% = final weight × 100/ initial weight
FCR = dry feed fed / body weight gain
SGR = (Ln final weight - Ln initial weight) × 100/ days
FI = total consumed food / days

Muscles and diet dry matter (at 105 ˚C for 24 h), crude protein (Kjeldahl apparatus, Gerhardt, Königswinter, Germany. Nitrogen* 6.25), crude fat (extraction with petroleum ether by Soxhlet apparatus, Behr, Düsseldorf, Germany) and ash (incineration at 600 1C for 6 h) were determined (AOAC, 1995). Diet carbohydrate was measured using the method by Lane – Eynon method (AOAC, 1978).

Amino acid profile of diet with exception of protein banded tryptophan was determined using HPLC as described by Ovissipour et al. (2009). For protein banded tryptophan determination, protein was extracted according to the method of Concon (1975) and tryptophan was estimated by the method described by Szakacs and Perl (1990).

Statistical analysis: Data were analyzed by one-way ANOVA using the statistical software SPSS version 9.0. Subsequent significance among groups was delineated by LSD test. Data are presented as treatment mean ± SD. The values of $P<0.05$ were considered significantly different.

Results

2.6% lysine treatment exhibited the best WG, SGR and FCR than other groups (Table 3). There was no significant difference in food intake and survival among the treatments (Table 3). There were no differences in moisture and ash content of muscle

Table 4. Effects of dietary L-Lysine (%) on muscle composition (%) of *H. huso* juveniles.

Dietary lysine	Moisture	Protein	Lipid	Ash
2.1	77.96±0.62a	14.45±0.53a	6.27±0.19a	1.13±0.04a
2.6	78.35±0.55a	15.83±0.37b	4.58±0.15b	1.08±0.02a
3.1	78.21±0.28a	15.98±0.14b	4.58±0.23b	1.08±0.09a
3.6	78.06±0.33a	16.64±0.26bc	4.03±0.1c	1.13±0.04a
P_{Value}	0.76	< 0.001	< 0.001	0.62

samples among the groups. Increasing of dietary lysine resulted in increase and decrease in muscles protein and lipid, respectively (Table 4; $P < 0.05$).

Discussion

One of the major classes of olfactory stimulants are amino acids that cause exhibition of food searching behavior in a variety of fishes (Jones, 1992). Thus, diet supplementation with free amino acid can improve food intake and growth. Our results showed that lysine failed to stimulate food intake in beluga juveniles. Based on our knowledge, there is no study on stimulatory characteristic of amino acids on beluga juveniles. Previous study on other sturgeon species have shown stimulatory effect of amino acids is species specific (Kasumyan, 1999; Kuzmin et al., 1999). However, present results demonstrated lysine, at least in our experimental conditions, was not a stimulator amino acid in beluga juveniles.

High growth performance in 2.6% lysine group seems to be as a result of optimization of dietary total lysine (5.5% of dietary protein) that is approximately similar to the data published for cobia, *Rachycentron canadum* (5.3%, Zhou et al., 2007) white sturgeon, *Acipenser transmontanus* (5.5%, Ng and Hung, 1995), Grass carp, *Ctenopharyngodon idella* (5.89%, Wang et al., 2005) and Japanese sea bass, *Lateolabrax japonicus* (5.8%, Mai et al., 2006) but higher than those reported for rainbow trout, 3.7% (Kim et al., 1992), and Coho salmon, 3.8% (Arai and Ogata, 1991), and lower than that for catla, 6.2% (Ravi and Devaraj, 1991), or Rainbow trout, 6.1% (Ketola, 1983). The wide variation observed in the requirements for lysine among fish species may be due to the differences in dietary protein sources, the reference protein which amino acid pattern is being imitated (Forster and Ogata, 1998), diet formulation, size and age of fish, genetic differences, feeding practices and rearing conditions (Ruchimat et al., 1997). Digestibility, amino acid profile and energy content may also bring variable effects in amino acid requirement studies (Simmons et al., 1999; De Silva et al., 2000).

In present study, fish which fed higher lysine than 2.6 (3.1 and 3.6% group) showed slightly lower SGR, WG and final weight. It was suggested that decrease of growth of Indian carp fed high amount of lysine (2.78% dry diet) may be due to the negative effects (lysine–arginine interaction) of excessive amount of free lysine at this level (Ahmed and Khan, 2004). Dietary lysine–arginine antagonism has been well known in poultry and rats (Jones, 1964; Harper et al., 1970; Fico et al., 1982), but there are few studies on fishes. Kaushik and Fauconneau (1984) have offered some biochemistry evidences indicating that some metabolic antagonism may exist between lysine and arginine in Rainbow trout. The increasing dietary lysine intake affected plasma arginine and urea levels, and ammonia excretion. Similarly, some studies showed that some metabolic interactions occur between arginine and lysine when elevated levels of either were fed to Atlantic salmon (Berge et al., 1997, 1998). Mai et al. (2006) also reported lower SGR of Japanese sea bass fed high amount of lysine (3.66% and 4.25%).

Muscle protein content increased while diet lysine increased that was reported by Mai et al., (2006). This can be attributed to an increase of net protein synthesis with the increase in dietary lysine level (Ruchimat et al., 1997) or optimum nutritional status which corresponded to the optimum dietary lysine level. Muscle lipid decreased while dietary lysine increased that is partially in agreement with other studies (Kim et al., 1992; Zarate and Lovell, 1997; Ahmed and Khan, 2004; Luo et al., 2006).

In conclusion, lysine supplementation has no significant effect on food intake in beluga juveniles. It seems that dietary lysine level of 2.6% of dry matter (corresponding to 5.5% of dietary protein) is suitable for growth of beluga juveniles.

Acknowledgements

The authors wish to thank aquaculture research center of Gorgan University or agricultural sciences and natural resources for providing necessary facilities for the study and Shahid Marjani Sturgeon Propagation and Culture Complex for Sturgeon fish.

References

Ahmed I., Khan M.A. (2004). Dietary lysine requirement of fingerling Indian major carp, *Cirrhinus mrigala*. Aquaculture, 235: 499–511.

Arai S., Ogata H. (1991). Quantitative amino acid requirements of fingerling coho salmon. In: M.R. Collie, J.P. McVey (Eds.). Proceedings of the Twentieth US–Japan Symposium on Aquaculture Nutrition. UJNR Department of Commerce, Newport, OR, USA. pp: 19–28.

AOAC (Association of Official Analytical Chemists) (1995). Official Methods of Analysis, 16th edn. AOAC, Arlington, VA, USA.

AOAC (Association of Official Analytical Chemists) (1978). Official Methods of Analysis of the Association of Official Agricultural Chemists, 13th Edition. AOAC, Arlington, VA, USA.

Berge G.E., Lied E., Sveier H. (1997). Nutrition of Atlantic salmon (*Salmo salar*): the requirement and metabolism of arginine. Comparative Biochemisrty and Physiology, 117A: 501–509.

Berge G.E., Sveier H., Lied E. (1998). Nutrition of Atlantic salmon (*Salmo salar*): the requirement and metabolic effect of lysine. Comparative Biochemistry and Physiolology, 120A: 447–485.

Birstein V.J. (1993). Sturgeons and paddlefishes: threatened fishes in need of conservation. Conservation Biology, 7: 773– 787.

Concon J.M. (1975). Rapid and simple method for the determination of tryptophan in cereal grains. Analytical Biochemistry, 67: 206-219.

De Silva S.S., Gunasekera R.M., Gooley G. (2000). Digestibility and amino acid availability of three protein-rich ingredient-incorporated diets by Murray cod *Maccullochella peelii peelii* (Mitchell) and the Australian shortfin eel *Anguilla australis* Richardson. Aquaculture Research, 31: 19–205.

Fico M.E., Hassan A.S., Milner J.A. (1982). The influence of excess lysine on urea cycle operation and pyrimidine biosynthesis. Journal of Nutrition, 112: 1854–1861.

Forster I., Ogata H.Y. (1998). Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea bream *Pagrus major*. Aquaculture, 161: 131-142.

Harper A.E., Benevenga N.G., Wohlhuetre R.M. (1970). Effects of ingestion of disproportionate amount of amino acid. Physiological Review, 50: 428–558.

Harris L.E. (1980). Feedstuffs. In: Fish Feed Technology. T.V.R. Pillay (Ed.). UNDP/FAO, Rome, Italy. pp: 111–168.

Jones J.D. (1964). Lysine–arginine antagonism in the chick. Journal of Nutrition, 84: 313–321.

Jones K.A. (1992). Food search behavior in fish and the use of chemical lures in commercial and sports fishing. In: T.J. Hara (Ed.) Fish Chemoreception. Chapman and Hall, London. pp: 288–320.

Kaushik S.J., Fauconneau B. (1984). Effects of lysine administration on plasma arginine and on some nitrogenous catabolites in rainbow trout. Comparative Biochemistry and Physiolology, 79A: 459–462.

Kasumyan A.O. (1999). Olfaction and taste senses in sturgeon behaviour. Journal of Applied Ichthyology, 15: 228-232.

Ketola H.G. (1983). Requirement for dietary lysine and arginine by fry of rainbow trout. Journal of Animal Science, 56: 101–107.

Kim K.I., Kayes T.B., Amundson C.H. (1992). Requirements for lysine and arginine by rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 106: 333–344.

Kuzmin S., Mironov S., Vostroushkin D., Shutov V. (1999). Behavioral responses to various chemical incentives in hybrid beluga x Russian sturgeon (*Huso huso* x *Acipenser gueldenstaedtii*) fry. Journal of Applied Ichthyology, 15: 233-236.

Lee P.G., Meyers S. (1996). Chemo attraction and feeding stimulation in crustacean. Aquaculture Nutrition, 2: 157-164.

Luo Z., Liu Y.J., Mai K.S., Tian L.X., Yang H.J., Liang G.Y., Liu D.H. (2006). Quantitative L-lysine requirement of juvenile grouper, *Epinephelus coioides*. Aquaculture Nutrition, 12: 165–172.

Mai K., Zhang L. , Ai Q., Duan Q., Zhang C., Li H., Wan J., Liufu Z. (2006). Dietary lysine requirement of juvenile Japanese seabass, *Lateolabrax japonicus*. Aquaculture, 258: 535–542.

Ng W.K., Hung S.S.O. (1995). Estimating the ideal dietary essential amino acid pattern for growth of white sturgeon, *Acipenser transmontanus*. Aquacultuyre Nutrition, 1: 85–94.

Ovissipour M., Abedian A., Motamedzadegan A., Rasco B., Safari R., Shahiri H. (2009). The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from Persian sturgeon, *Acipenser persicus* viscera. Food Chemistry, 115: 238–242.

Ravi J., Devaraj K.V. (1991). Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). Aquaculture, 96: 281–291.

Ruchimat T., Masumoto T., Hosokawa H., Itoh Y., Shimeno S. (1997). Quantitative lysine requirement of yellowtail, *Seriola quinqueradiata*. Aquaculture, 158: 331–339.

Simmons L., Moccia R.D., Bureau D.P., Sivak J.G., Herbert K. (1999). Dietary methionine requirement of juvenile Arctic charr *Salvelinus alpinus* (L.). Aquaculture Nutrition, 5: 93–100.

Small B.C., Soares Jr J.H. (2000). Quantitative dietary lysine requirement of juvenile striped bass *Morone saxatilis*. Aquaculture Nutrition, 6: 207–212.

Szakacs M.P., Perl I.M. (1990). Determination of tryptophan in unhydrolyzed food and feedstuffs by the acid ninhydrin method. Journal of Agricultural and Food Chemistry, 38: 720–726.

Tantikitti C., Chimsung N. (2001). Dietary lysine requirement of freshwater catfish (*Mystus nemurus* Cuv. & Val.). Aquaculture Research, 32: 135–141.

Walton M.J., Cowey C.B., Adron J.W. (1984). The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). British Journal of Nutrition, 52: 115–122.

Wang S., Liu Y.J., Tian L.X., Xie M.Q., Yang H.J., Wang Y. Liang G.Y. (2005). Quantitative dietary lysine requirement of juvenile grass carp, *Ctenopharyngodon idella*. Aquaculture, 249: 419– 429.

Wilson R.P. (1985). Amino acid and protein requirements of fish. In: C.B. Cowey, A.M. Mackie, J.G. (Eds.). Bell Nutrition and Feeding in Fish. Academic Press, London. pp: 1–15.

Wilson R.P. (2002). Amino acids and proteins, In: J.E. Halver, R.W. Hardy (Eds.). Fish Nutrition, 3rd ed. Academic Press, San Diego, CA. pp: 144–175.

Wilson R.P., Halver J.E. (1986). Protein and amino acid requirements of fishes. Annual Review of Nutrition, 6: 225–244.

Zarate D.D., Lovell R.T. (1997). Free lysine (L-lysine. HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish, *Ictalurus punctatus*. Aquaculture, 159: 87–100.

Zhou Q.C., Wu Z.H., Chi S.Y., Yang Q.H. (2007). Dietary lysine requirement of juvenile cobia, *Rachycentron canadum*. Aquaculture, 273: 634-640.