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THE USABILITY OF BAROTHERMALLY MODIFIED CASEIN IN FEED MIXES FOR SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT) JUVENILES

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ABSTRACT. Experimental tests were conducted on six Siberian sturgeon feed mixes containing from 0-35% sodium caseinate. A 50-day growth test of Siberian sturgeon fry with an individual body weight of 6.77 g was performed under controlled conditions in an aquarium hall in 60 l flowthrough aquaria. The extrusion method was applied to prepare experimental feeds for isonitric and isocaloric diets. The composition of the protein amino acids and the fatty acids profiles of the lipids in the feeds were identified. The following rearing effectiveness indices were used in the final evaluation of the feeding test: Specific Growth Rate (SGR); Food Conversion Ratio (FCR); apparent Net Protein Utilisation (aNPU); Protein Efficiency Ratio (PER). Conclusions were drawn based on statistical analysis using the SAS computer program. The best rearing results were obtained with feed mixes containing 7-21% sodium caseinate - final individual fish mass of 64.57-67.57 g, food conversion ratio of 0.88-0.90 and a protein efficiency ratio of 2.19-2.24.

Key words: SIBERIAN STURGEON (*ACIPENSER BAERI*), FEEDING, FEED MIXES, CASEIN, EXTRUSION

INTRODUCTION

Modern intensive fish rearing is not possible without the use of fully balanced and effective feeds that guarantee quick growth rates, high survival rates and the good health condition of the stock. Laboratory studies have confirmed that the fry of some acipenserid fish, e.g. star sturgeon (*Acipencer stellatus*), have a negative reaction to the sensoric properties of fish meal, a component commonly used in commercial feed mixes for fish (Kasumyan et al. 1992). The search for alternative protein sources that meet the specific food requirements of sturgeon has led to the use of unconventional components including milk protein (Gawęcki 1998), i.e. casein, which has a high biological value and is highly assimilable. Casein, and to an even greater degree casein salts, have very good functional properties such as viscosity, water absorbency and the ability to emulsify fat, all of which significantly extends the possibility of using them in the food industry (Szpendowski 1991).

The basic objective of the present studies was to determine the usability of barothermally modified casein wastes as a substitute for fish meal in extruded feed mixes for Siberian sturgeon, *Acipenser baeri* Brandt juveniles.

MATERIAL AND METHODS

EXPERIMENTAL FEEDS

The recipes for the experimental diets were calculated using a computer program written with the linear Simplex method in Turbo Pascal 5.0. The experimental feeds were made in the Feed Laboratory of the Experimental Plant of Feed Production Technology and Aquaculture in Muchocin, Poland.

The feed mixes were produced by the barothermal method in an N-60 endogenous single-start worm extruder manufactured by Metalchem, Gliwice. The extrudates were crushed in a RUT-10 cylinder mill and then forced through percussion sieves to obtain two granulate fractions: particles from 1.6 to 2.0 mm for sturgeon juveniles up to 20 g of individual body weight; particles from 2.0 to 3.15 mm for sturgeon juveniles of over 20 g of individual body weight.

The following components were used to prepare the experimental diets - fish meal, blood meal, sodium caseinate, soya seeds, wheat flour and low-erucic rape oil. The basic feed mixes were supplemented with Polfamix W and Vitazol AD_3EC mineral-vitamin mixes and cod liver oil. In feed mixes K7, K14, K21, K28 and K35, fish meal was substituted with sodium caseinate produced at the Lacpol Casein Production Plant in Murowana Goślina, Poland (Table 1).

The feeds were prepared according to the recipes and the following procedure. The animal meals and soya seeds were ground in a percussion mill into very fine particles which were passed through a sieve with 1 mm mesh diameter. A premix was made by supplementing the carriers (i.e. soya and blood meal) with vitamin and mineral components, choline chloride and binder and then mixing for 10 minutes in a cubic mixer. The premixes were combined with the remaining components in a drum mixer and a mixture of rape oil, cod-liver oil and soya lecithin was added and heated to 50°C. The mixture was then conditioned in a mixer by adding hot water and steam to reach a temperature of 65-70°C and a moisture level of 19-21%.

TABLE 1

Composition of feed mixes tested (%)

Feed component	Feed mix					
	K0	K7	K14	K21	K28	K35
Fish meal	35.0	28.0	21.0	14.0	7.0	-
Blood meal	16.3	15.8	15.3	14.8	14.4	13.9
Na caseinate	-	7.0	14.0	21.0	28.0	35.0
Soya seed	15.0	15.0	15.0	15.0	15.0	15.0
Wheat flour	21.4	24.3	24.2	24.1	23.9	23.9
Rapeseed oil	1.5	2.1	2.7	3.3	3.9	4.4
Cod-liver oil[1]	2.0	2.0	2.0	2.0	2.0	2.0
Soyabean lecithin	0.5	0.5	0.5	0.5	0.5	0.5
Premix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[3]	0.1	0.1	0.1	0.1	0.1	0.1
Choline chloride[4]	0.2	0.2	0.2	0.2	0.2	0.2
Binder[5]	4.0	4.0	4.0	4.0	4.0	4.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1]*Jecoris Aselli ol. LYSI HF, Island - 1000 ml contains: vitamin A – 920 000 IU, vitamin D_3 – 92 000 IU, vitamin E – 276 IU;*
[2]*Polfamix W, BASF Polska Ltd. Kutno, Poland – 1 kg contains: vitamin A – 1 000 000 IU, vitamin D_3 – 200 000 IU, vitamin E – 1.5 g, vitamin K – 0.2 g, vitamin B_1 – 0.05 g, vitamin B_2 – 0.4 g, vitamin B_{12} – 0.001 g, nicotinic acid – 2.5 g, D-calcium pantothenate – 1.0 g, choline chloride 7.5 g, folic acid – 0.1 g, methionine – 150.0 g, lysine – 150.0 g, Fe – 2.5 g, Mn – 6.5 g, Cu – 0.8 g, Co – 0.04 g, Zn – 4.0 g, J – 0.008 g, carrier > 1000.0 g.;*
[3]*Vitazol AD_3EC BIOWET Drwalew, Poland – 1 kg contains: vitamin A – 50 000 IU, vitamin D_3 – 5 000 IU, vitamin E – 30.0 mg, vitamin C – 100.0 mg;*
[4]*UCB Chemicals Espana S.A., Spain;*
[5]*High Protein Binder, DAKA a.m.b.a. Losning, Danmark*

The most favorable extrusion conditions for the experimental mixes were obtained with the following technological parameters:

– moisture of feed mix - 20%
– cylinder temperature in the zone of increasing pressure - 79°C
– cylinder temperature in the zone of high pressure - 109°C
– head temperature - 124°C
– speed of worm revolutions - 63 rev min^{-1}
– time of passage through the extruder - 52 s
– nozzle diameter - 6.0 mm

The extrudate leaving the matrix was cut with a rotational knife into 9 mm pellets that were dried on sieves in a stream of heated air. The diameter of the pellets after drying was 7 mm, on average. After sieving, the granulate fractions were segregated according to an accepted key and sprayed in a pelletizing drum with a film of rape oil (not exceeding 1.5% of the granulate) heated to 70°C.

PHYSICAL METHODS OF FEED EVALUATION

The water stability of the experimental feeds was determined by the Hastings-Hepher method (Hepher 1968) and modified by Szumiec and Stanny (1975). This was determined during a water bath which simulated water movement; the percentage of feed mass loss after the bath and subsequent drying to a constant temperature of 105°C was determined. Another criterion of water stability assessment was the oxygen consumption of the water used for testing in a basic environment as described by Gomółka and Szypowski (1973).

CHEMICAL METHODS OF FEED EVALUATION

The chemical analysis of the feed was carried out according to Skulmowski (1974). The basic analysis included determining the dry mass, total nitrogen, crude fat and crude fibber. The total protein content was calculated by multiplying the amount of nitrogen determined by a coefficient of 6.25. The aminoacids were separated from the feed protein by hydrolyzing samples in 5n HCl at 106°C for 26 hours in an AAA-881 analyzer. Sulfuric aminoacids, methionine and cystine were determined after oxidation and fixation in formic acid. Tryptophan was determined using the colorimetric method (Votisky and Gunkel 1989). Based on the results of aminoacidic analyses of protein, the chemical value of experimental diets was defined by calculating the chemical score (CS) and the essential amino-acids index (EAAI).

The digestible energy of the model diets was calculated from the chemical composition using conversion factors for digestible energy for fish - extruded carbohydrates – 2.5, protein – 5.2 and fat – 8.5 kcal g^{-1} (Halver 1988).

Mineral components P and Ca were determined in the feed with an ASS3 atomic absorption spectrophotometer (Carl Zeiss, Jena) according to the method presented in Gawęcki (1988).

The profile of fatty acids in the total lipids of experimental feeds was also determined. Fat extraction was done using the Folch's method described by Pie et al. (1991).

EVALUATION METHODS OF GROWTH RATE TESTS

The following indices of rearing effectiveness were used in order to evaluate the final results of growth studies: survival rate (%); specific growth rate of individual body weight (SGR - %) (De Silva and Anderson 1995); apparent net protein utilization (aNPU - %) (Zeitoun et al. 1973); mean absolute food conversion ratio (FCR); protein efficiency ratio (PER) (Steffens 1985). Fish samples were collected randomly for the basic chemical determinations both prior to and following the completion of the growth tests. The fish were anaesthetized with Propiscin, (IFI Olsztyn, Poland) then ground and homogenized. The material obtained was used to determine the following parameters - dry mass, total protein, raw fat and raw ash.

TECHNICAL CONDITIONS OF GROWTH TESTS

The experiment was carried out under controlled conditions in the aquarium hall of the Department of Inland Fisheries and Aquaculture, Agricultural University of Poznań. The experimental material consisted of Siberian sturgeon juveniles with a mean individual body weight of 6.77 g. The aquaria were stocked on 2 June 1999, and the growth test proper was carried out for 50 days from 10 June to 29 July 1999. The fish were purchased from the Fish Hatching and Rearing Facility in Wąsosze. The experiment was carried out in six variants (including one control) in two replications of each. Each aquarium was stocked with 12 fish. The fish were placed in glass aquaria with a capacity of about 60 dm^3 and dimensions of $0.35 \times 0.35 \times 0.50$ m. The walls and the bottom of the aquaria were painted with grey paint. During the experiment, the physico-chemical parameters of the water - temperature (°C) and dissolved oxygen content (mg O_2 dm^{-3}) - were checked daily. The aquaria were cleaned every morning by removing excrement and unconsumed feed with a water siphon.

Experimental feeds were delivered day and night by automatic band feeders with a clock-drive (FIAP Fischtechnik Gmbh). The daily feed doses were calculated according to the feeding curves in Kolman (1998) and according to the actual fish weight. The dose was adjusted every seven days based on weight checks, which also served to determine the fish growth rate dynamics and other rearing indices.

STATISTICAL ANALYSIS OF EXPERIMENT RESULTS

The biotechnical results of the growth tests and the values of conversion indices of the nutritive components of the feeds were calculated using Microsoft Excel. In order to find significant differences between the variants, the rearing effectiveness indices and the feed conversion indices were statistically analyzed using the procedure described in the statistical program SAS – General Linear Model with regression to the initial body weight of the fish (SAS Institute 1996).

RESULTS

In the Hastings-Hepher tests, the loss of extrudate mass oscillated between 19.4% (K14 mix) and 29.8% (K0 mix). Oxygen demand indices ranged from 38.1 (K7 mix) to 49.9 mg O_2 dm^{-3} (K35 mix) (Table 2).

TABLE 2

Water stability of feed mixes tested

Parameter	Feed mix					
	K0	K7	K14	K21	K28	K35
Weight loss (%)	29.8	19.8	19.4	26.6	21.6	21.8
Score	good	good	good	good	good	good
Oxygen demand mg (O_2 dm^{-3})	49.1	38.1	38.5	44.7	48.1	49.9
Score	v. good	v. good	v. good	v. good	v. good	v. good

Table 3 presents the chemical composition of the fry feeds, while Table 4 shows the aminoacid composition. The experimental diets were prepared at isonitric and isocaloric levels. The total protein content oscillated between 49.98 and 50.07%. The raw fat level in the experimental diets ranged from 9.96 – 10.05%, and the crude fibre content was the same in all mixes at 0.76%.

TABLE 3

Chemical composition of feed mixes tested (%)

Component	Feed mix					
	K0	K7	K14	K21	K28	K35
Crude protein	50.07	50.04	50.01	49.98	50.02	50.01
Crude fat	10.00	10.02	10.03	10.04	10.05	9.96
Nitrogen-free extractable compounds	24.45	25.66	25.88	26.10	26.23	26.53
Crude fibre	0.76	0.76	0.76	0.76	0.76	0.76
Ash	5.91	5.47	5.03	4.60	4.16	3.73
Phosphorus	1.42	1.24	1.07	0.89	0.71	0.54
Calcium	2.02	1.68	1.33	0.99	0.65	0.30

The limiting aminoacid for K0, K7 and K14 feeds was isoleucine, and for mixes K21, K28 and K35, it was methionine with cystine. Values of exogenous aminoacid indices oscillated between 70.64 and 74.31 (Table 5).

The energy to protein ratio (E/P) was similar in all the experimental diets and ranged from 8.17 to 8.22. The digestible energy of the diets was between 4089.9 and 4111.1 kcal kg^{-1} (Table 5).

TABLE 4

Essential amino acid composition (g $100g^{-1}$ protein) in feed mixes tested

Amino acid	Feed mix					
	K0	K7	K14	K21	K28	K35
Arginine	5.73	5.37	5.02	4.66	4.30	3.94
Histidine	4.51	4.31	4.11	3.91	3.71	3.51
Lysine	8.48	8.21	7.93	7.66	7.39	7.11
Tryptophan	4.11	3.47	2.83	2.19	1.54	0.91
Phenyloalanine +tyrosine	6.28	6.48	6.68	6.87	7.07	7.26
Methionine + cystine	2.59	2.53	2.47	2.41	2.35	2.29
Treonine	3.97	3.90	3.82	3.75	3.68	3.61
Leucine	8.85	8.93	9.01	9.09	9.18	9.26
Isoleucine	2.70	2.81	2.93	3.04	3.16	3.27
Valine	5.70	5.78	5.87	5.95	6.04	6.13

TABLE 5

Chemical nutritive value of protein and digestible energy level in feed mixes tested

Item	Feed mix					
	K0	K7	K14	K21	K28	K35
CS*	I. Izoleu 39.13 II. Met +Cys 44.66 III. Tyr 55.61	I. Izoleu 40.72 II. Met +Cys 43.62 III. Tyr 60.99	I. Izoleu 42.46 II. Met +Cys 42.59 III. Tyr 65.37	I. Met +Cys 41.55 II. Izoleu 44.06 III. Arg 67.54	I. Met +Cys 40.52 II.Izoleu 45.80 III. Arg 62.32	I. Met +Cys 39.48 II. Izoleu 47.39 III. Arg 57.10
EAAI**	73.82	74.09	74.30	74.31	74.29	70.84
Digestible energy kcal kg^{-1}	4089.9	4095.3	4100.1	4104.9	4111.1	4110.4
E/P	8.17	8.18	8.20	8.21	8.22	8.22

**CS - Chemical Score*
***EAAI - Essential Amino Acid Index*

The percentage of fatty acids in the experimental mix compositions (from capronic acid C 10:0 to decosahexaenic acid C 22:6) is shown in Table 6. Of the monoenic acids, the percentage of oleic acid was the highest (24.18 – 41.14%). Of the polyenic acids, which are essential unsaturated fatty acids, linoleic acid (19.40 – 26.07%) and linolenic acid (3.31 – 5.87%) had the highest percentages.

TABLE 6

Fatty acids content in feed mixes tested (%)

Fatty acid	Feed mix					
	K0	K7	K14	K21	K28	K35
C 10:0 capronic	0.05	0.03	0.04	0.04	-	-
C 12:0 lauric	0.06	0.08	0.06	0.06	-	-
C 14:0 mirystic	1.41	1.62	1.47	1.43	1.39	1.16
C 15:0 pentadecanic	0.63	0.33	0.09	0.07	0.08	0.07
C 16:0 palmitic	10.65	11.89	11.76	11.85	12.0	10.53
C 16:1ω9 oleopalmitic	6.32	3.95	2.89	1.99	1.82	1.54
C 17:0 margarinic	0.12	0.15	0.16	0.13	0.17	0.16
C 18:0 stearic	4.13	4.53	4.43	4.49	4.58	4.06
C 18:1ω9 oleic	24.18	29.69	32.94	35.48	37.04	41.14
C 18:1ω7 waccenic	3.88	3.33	3.06	2.93	3.01	3.01
C 18:2ω6 linoleic	19.40	22.31	24.30	25.18	26.07	23.85
Not identified	7.62	2.71	1,32	-	-	-
C 18:3ω3 linolenic	3.31	4.16	4.49	5.45	5.77	5.87
Not identified	1.71	0.91	0.66	0.15	0.02	0.20
C 20:0 arachidic	0.28	0.42	0.41	0.41	0.44	0.50
C 20:1ω9 gadoleic	4.00	4.04	3.45	3.27	3.05	2.87
C 20:5ω3 eikosapentadienic	2.00	2.10	1.91	1.80	1.54	1.19
C 22:0 behenic	0.20	0.23	0.27	-	-	-
Not identified	4.31	3.49	2.66	2.14	-	1.42
C 22:1ω9 erucic	0.54	0.62	0.65	0.68	0.73	0.80
C 22:6 decosahecsaenic	2.96	3.05	2.63	2.42	2.25	1.60
Not identified	1.13	0.75	-	-	-	-

The results presented in Table 7 indicate that the best final results were achieved with variant K14 with a final fish body weight of 67.57 g pc^{-1}, an individual increment of 60.80 g pc^{-1} and a specific growth rate (SGR) of 4.59%; the values of these indices did not differ significantly from those in variants K7, K21 and K28. Worse results were recorded for variant K0, which differed significantly from those obtained in variants K14 and K21.

The survival rate of the sturgeon in all the experimental variants was relatively high at 83.86 – 96.36% and did not differ significantly.

The lowest values of food conversion ratio (FCR) were recorded for variants K7, K14 and K21 at 0.89, 0.90 and 0.88, respectively. They did not differ significantly from

TABLE 7

Results of siberian sturgeon feeding test[1]

Parameter	Variants					
	K0	K7	K14	K21	K28	K35
Initial number of fish (pc)	24	24	24	24	24	24
Mean individual fish weight (g)	6.62	6.92	6.83	6.95	6.66	6.66
	Final data					
Final number of fish (pc)	20	23	22	23	23	20
Mean individual fish weight (g)	52.69 a	64.57 bc	67.57 b	66.99 b	61.39 c	53.28 a
Mean individual weight increment (g)	45.91 a	57.81 bc	60.80 b	60.21 b	54.61 c	47.04 a
SGR (%)	4.09 a	4.50 b	4.59 c	4.58 c	4.40 d	4.14 a
Survival rate (%)	84.04 a	95.16 a	91.39 a	95.00 a	96.36 a	83.86 a

[1]*The values are means of triplicate group of a fish and means in each row denoted by the same letters are not significantly different (p > 0.05).*

the values obtained in variants K28 and K35. A similar range was obtained with the PER coefficients and the most favorable were for variants K7, K14 and K21. Lower values, which differed significantly, were found for variants K28 and K35. The calculated values of apparent net protein utilization (aNPU) ranged from 16.08 to 22.63% and did not differ significantly (Table 8).

TABLE 8

Utilisation of feed nutritive component[1]

Index	Variants					
	K0	K7	K14	K21	K28	K35
FCR	0.99 a	0.89 b	0.90 b	0.88 b	1.08 c	1.18 d
PER	1.95 a	2.22 b	2.19 b	2.24 b	1.84 c	1.63 d
aNPU %	22.63	16.08	16.53	17.70	16.36	20.00

[1]*The values are means of a triplicate group of fish (except aNPU) and means in each row denoted by the same letters are not significantly different (p > 0.05).*

Table 9 contains results of the chemical analysis of the fish prior to and following the conclusion of the experiment. Similar amounts of total protein, crude fat and crude ash were noted in all variants. This indicates that the effect of feed on the chemical composition of the fish was insignificant.

TABLE 9

Chemical composition of fish body before and after the experiment (%)

Feed mix	Dry weigt	Ash	Crude protein	Crude fat
Before the experiment	11.26	2.40	6.88	3.11
After the experiment				
K0	18.26	2.48	11.94	4.07
K7	20.49	2.48	11.56	6.33
K14	19.89	2.46	11.79	5.44
K21	20.86	2.49	12.21	5.61
K28	21.87	2.56	12.48	4.43
K35	18.56	2.38	12.48	4.84

DISCUSSION

The values of the basic physico-chemical properties of the water used during the experiment were within optimum limits for Siberian sturgeon fry at a temperature of 21°C and oxygen saturation above 70% (Kolman 1993). The experimental feed mixes received satisfactory results in studies of water stability (Hastings-Hepher test). The low emission of nutrients into the aquatic environment confirms their high ecological value.

The values of PER and aNPU can vary significantly depending on the level and quality of nutritive components (mainly of protein levels in the feed), the length of the rearing period, fish age and even species and sex. To date, experiments conducted on the utilization of casein protein in fish feeds have focused on species from the salmonidae family. According to Zeitoun et al. (1973), the highest values of PER (2.1) and aNPU (34.0%) were obtained by feeding rainbow trout (*Oncorhynchus mykiss*) fry casein-based feed which contained 40% total protein. The very high nutritive value of casein protein (PER from 2.36 to 2.22) at a total protein level of 35% was obtained in fry feeds for rainbow trout (Nose 1971). Koligot and Przybył (1999) found that rainbow trout fry most effectively utilized feed which contained 32% sodium caseinate; the following results were obtained: FCR – 0.85; PER – 2.32; aNPU – 33.5%. During the present studies, diets K7, K14 and K21 were best utilized and their FCR was in the range of 0.88 – 0.90, while the PER values ranged from 2.19 to 2.24.

The values of the most frequently used indices for the evaluation of the nutritive value of diets (FCR, PER, aNPU) indicate that sturgeon most effectively utilized feeds containing

7, 14 and 21% sodium caseinate. This is evidence of its high nutritive value and its usability as a substitute for fish meal in the nutrition of Siberian sturgeon juveniles.

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STRESZCZENIE

PRZYDATNOŚĆ MODYFIKOWANEJ BAROTERMICZNIE KAZEINY W MIESZANKACH PASZOWYCH DLA NARYBKU JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT)

Celem badań było określenie przydatności mieszanek doświadczalnych w żywieniu jesiotra syberyjskiego (*Acipenser baeri* Brandt 1869). W przeprowadzonym teście wzrostowym materiałem doświadczalnym był narybek jesiotra syberyjskiego o średniej masie jednostkowej 6,77 g szt^{-1}. Badania przeprowadzono w warunkach kontrolowanych - w hali akwaryjnej, w basenach przepływowych o pojemności 60 l. Obsadę jednego basenu stanowiło 12 szt. ryb. Doświadczenie żywieniowe trwało 50 dni, w okresie 10.06.-29.07.1999 r. W badaniach oceniono możliwości substytuowania mączki rybnej modyfikowaną barotermicznie kazeiną na różnych poziomach (0, 7, 14, 21, 28 i 35% - tabela 1).

Do upostaciowania mieszanek doświadczalnych przygotowanych jako diety izoazotowe i izokaloryczne, wykorzystana została metoda obróbki barotermicznej, tj. ekstruzja. Pasze doświadczalne oceniano na podstawie cech fizycznych i chemicznych. Ponadto w mieszankach oznaczono skład aminokwasowy białka oraz profil kwasów tłuszczowych w lipidach (tabele 1, 2, 3, 4, 5, 6). Do oceny końcowych wyników testów żywieniowych wykorzystane zostały następujące wskaźniki efektywności chowu: współczynnik pokarmowy (FCR), średni dobowy przyrost średniej masy jednostkowej (SGR), wskaźnik retencji białka paszowego (aNPU) oraz współczynnik wydajności wzrostowej białka (PER). Podstawę wnioskowania stanowiła analiza statystyczna przeprowadzona w oparciu o procedury pakietu statystycznego SAS.

Przeprowadzony test żywieniowy potwierdził przydatność badanego preparatu białkowego w żywieniu narybku jesiotra syberyjskiego, a najlepsze wyniki chowu uzyskano przy poziomie 7-21% udziału w diecie (tabela 7, 8, 9).

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