KRMIVA

THE EFFECT OF L-CARNITINE SUPPLEMENTATION INTO FEED MIXTURES UPON PRODUCTION AND OVERWINTERING RESULTS IN COMMON CARP (CYPRINUS CARPIO) CULTURE

UČINAK DODATKA L-KARNITINA U KRMNE SMJESE NA REZULTATE PROIZVODNJE I PREZIMLJENJA KULTURE OBIČNOG ŠARANA (CYPRINUS CARPIO)

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SUMMARY

The experiments aimed at the investigation of L-Carnitine prospects in carp fry feeding were arranged under pond conditions in foil enclosures 3 x 3 m using mirror carp (strain C 866) in 1994-96. Alma 6208 feed mixture with various concentrations (0, 250, 500 and 1000 mg kg^{-1}) of L-Carnitine was supplied to fish.

No significant differences were found between the control and experimental groups as the final individual weight is concerned. However, the highest individual weight gain (26.31 g) was achieved in the group fed 500 mg kg^{-1} L-Carnitine supplementation, followed by fish fed 250 mg kg^{-1} supplementation (25.83 g), whilst only 24.26 g were achieved in the control. The highest survival rates (95.33 and 94.67%) were found in the control and 1000 mg kg^{-1}, respectively. In the last group mentioned, the lowest feeding quotient (2.41) was found. During overwintering, the most favorable results were also achieved in groups fed the feed supplemented with L-Carnitine in concentrations of 500 and 1000 mg kg^{-1}. The individual weight differences and survival rates after the period of overwintering amounted to -8.08% and 68.07%, and +3.92 and 56.94% in these groups, respectively. Based on the preliminary results, the attention was concentrated upon the effect of L-Carnitine in concentration of 250 mg kg^{-1} in the following growing season. However, no significant promoting impact of this level of supplementation was registered, although the parameters of relative weight gain, specific growth rate and feed conversion were better in comparison with the control group.

INTRODUCTION

Prospects of probiotic preparations supplementation into fish feed mixtures were investigated and found promising in many studies earlier. Predominantly, their impact upon fish growth and health condition was described in these conneions. The application of the probiotic Lactiferm and its results in experimental carp (Cyprinus carpio) and

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tench (Tinca tinca) culture were presented by Párová et al., (1987), in elvers (Anguilla anguilla) fattening by Párová and Pár (1986) and Peňáz et al., (1988). Hamáčková et al., (1992) described the effect of microbiotic addition in to feed mixtures for wels (Silurus glanis). Adámek et al., (1996) summarized the results of experimental supplementation of rainbow trout (Oncorhynchus mykiss) and wels feeds with the probiotic Ascogen. This probiotic was investigated also, in some other fishes - e. g. in splake, Salvelinus namaycush x Salvelinus fontinalis (Durant, Cho, 1979), male tilapia, Oreochromis niloticus x Oreochromis aureus (Ramadan et al., 1990/91) and goldfish, Carassius auratus (Adámek, 1994).

The aim of the study presented was to evaluate the possibilities of L-Carnitine addition into carp feeds for the improvement of fish growth and survival rates during the growing season and overwintering period in particular.

MATERIAL AND METHODS

The basic design of the experimental culture consisted of the initial growth testing of fish fed 4 various L- Carnitine concentrations which was grown in foil enclosures. This experiment was followed by their evaluation from the point of view of fish performance during the period of overwintering. Finally, two groups were tested for growth performance in cages during the following growing season.

Feed Composition and Feeding Regime

Feed mixture Alma 6208 for carp fry with declared 36% protein and 10% fat contents was used as a carrier. The essential mixture was supplied with 1000 mg kg^{-1} L-Carnitine (mixture D). Mixtures B with 250 and C with 500 mg kg^{-1} were formulated by mixing the mixture D with control (mixture A - 0 mg kg^{-1}). Mixing was performed manually in small amounts in order to provide an appropriate dispersion of all feed particles.

Dally feeding dose was calculated on the basis of weekly control weighing of fish and precisely determined for the following week with respect to its results. The original RIFCH computer program was applied for these purposes. Daily feed dose was given to fish manually in 1-3 partial doses according to water temperature. Fish were not supplied with feed during the overwintering period.

Experiment Design

Growth tests (experiment I) were performed in two experimental ponds (800 m^2), each with 8 experimental foil divisions 3 x 3 m, from 5 Aug to 10 Oct 1994. Every experimental group A-D was replicated 4-times in order to arrange the installation of each feeding variant in all positions with respect to the water depth in ponds.

After the autumnal harvest, all groups of fish were marked individually by cryogenic method and transferred into one overwintering pond of the acreage of 800 m^2 (experiment II). The overwintering period lasted from 13 Oct 1994 to 14 Apr 1995.

The tests continued (experiment III) in two variants (A and B) with 4 replications in cages 1.5 x 1.5 x 1.0 m during the growing season 1995 (20 Jul - 29 Sept/13 Oct).

Water analyses

Water temperature and oxygen concentration during the growing seasons (experiments I and III) were monitored 2-3-times a day at the beginning of the experiment and once a day later on. No daily monitoring of temperature and oxygen conditions was performed during the overwintering period in order to avoid disturbing fish.

Water samples for hydrochemical analyses were taken weekly in the first foil division of each row during the experiments I and III. Monthly sampling of outflow water was performed during overwintering (experiment II). Following parameters were investigated in the laboratory: pH, alkalinity, acidity, COD_{Cr}, COD_{Mn}, BOD_5, NH_4^+-N, $N0_3^-$-N, NO_2^--N, N_{org}-N, PO_4^3-P and total P.

The results of water quality monitoring are presented in table 1. No physic-chemical determinants of water can be considered as inconvenient from the point of view of young carp prosperity.

Fish

The advanced mirror carp fry of uniform origin - C866 strain - was used for testing. Before the initial stocking on 5 Aug 1994, fry was cultured under pond conditions.

An initial sample of 100 individuals was taken before stocking for the assessment of biometric determinants. On the day of water sampling, a fish sample captured by casting a net was also weighed for the assessment of the daily feeding dose during the following week. All fish from individual divisions were measured and weighed at the end of the experiment I. Initial and final pooled samples of randomly selected fingerling from each variant were taken and deep-frozen for biochemical evaluation. The same was performed at the beginning and end of experiment III.

Table 1. Mean (± StD) values of water quality and their range in individual experiments
Tablica 1. Srednje (± StD) vrijednosti kakvoće vode i njihov raspon u pojedinm pokusima

Experiment Pokus		I		II		III	
		Avg ± StD Prosjek	Range Raspon	Avg ± StD Prosjek	Range Raspon	Avg ± StD Prosjek	Range Raspon
Temperature °C	min	18.4±3.1	12.0	x	x	19.1±5.1	12.3
Temperatura °C	max	19.0±3.4	27.2			19.3±4.9	26.7
DO	min	8.7± 4.7	1.5	x	x	6.3±2.0	2.6
mg^{-1}	max	11.0±3.7	20.0			7.1±3.1	10.9
pH	min	7.33±0.18	7.00	7.95±0.99	6.80	7.31±0.38	6.89
	max	7.81±0.57	8.75		9.00		7.95
Alkalinity mmol l^{-1}	min	1.29±0.10	1.10	0.66±0.14	0.50	1.43±0.25	I.18
Lužnatost	max	1.41±0.18	1.65		0.80		1.86
Acidity mmol l^{-1}	min	0.05±0.04	0.00	0.06±0.03	-0.10	0.07±0.03	0.03
Kiselost mmol l^{-1}	max	0.09± 0.03	0.15		0.05		0.10
COD_{Cr}	min	33.9±8.9	10.1	8.0±0.6	7.4	9.8±1.9	7.8
mg l^{-1} O_2	max	47.5±19.8	75.6		8.8		13.6
COD_{Mn}	min	10.2±1.9	7.2	x	x	x	x
mg l^{-1} O_2	max	15.0±3.5	20.3				
BOD_5	min	5.0±1.7	2.5	x	x	x	x
mg l^{-1} O_2	max	7.5 ±2.4	12.8				
NH_4^-N	min	0.12±0.11	0.02	0.07±0.03	0.04	0.07±0.04	0.00
mg l^{-1}	max	0.36±0.30	1.03		0.10		0.11
NO_3^--N	min	0.20±0.13	0.02	1.64±0.12	1.47	0.31±0.18	0.09
mg l^{-1}	max	0.25±0.26	0.74		1.76		0.64
NO_2^--N	min	0.01±0.01	0.00	x	x	x	x
mg l^{-1}	max	0.02±0.01	0.04				
N_{org} - N	min	1.89±0.22	1.46	x	x	x	x
mg l^{-1}	max	2.36± 0.26	2.59				
PO_4^{3-}-P	min	0.03±0.01	0.01	0.05±0.02	0.02	0.05±0.03	0.01
mg l^{-1}	max	0.11±0.11	0.34		0.07		0.10
P total mg l^{-1}	min	0.20±0.05	0.10	x	x	x	x
P ukupni mg l^{-1}	max	0.25±0.04	0.44				

Note: x - no data

All fish were individually measured, weighed and cryogenically marked according to their origin from variants A-D before stocking for overwintering (experiment II). After this experimental period was finished, a pooled random samples of fish were taken and deep-frozen for the biochemical evaluation.

The biometric data obtained were elaborated for condition parameters (Fulton's condition factor - FCF) and all determinants were evaluated statistically.

The following formulas were used for the calculation of specific growth rate (SGR) and condition factor (FCF):

$$SGR = [EXP \cdot (\frac{\ln W_t - \ln W_o}{t}) - 1]100, \text{ where}$$

W_o - initial mean weight in g
W_t - final mean weight in g
t - number of days

$$FCF = (W / TL^3) .100, \text{ where}$$

W - weight in g
TL - total length in cm.

RESULTS

Experiment I

No significant differences were found between control and experimental groups in any parameter investigated (Table 2). The biggest final individual weight gain 26.31 g was achieved in the variant C, i. e. with 500 mg addition of L-Carnitine per 1 kg of feed, followed by groups B – 25.83 g (250 mg supplementation), D - 25.04 g (1000 mg supplementation) and the control group – 24.26 g. The highest comparable survival rates were registered in the control and D variant. Feeding quotients (feed conversion ratio - FCR) fluctuated between 2.41 and 2.65 with the best found in the group D. Values of the specific growth rate (SGR) were well balanced and ranged from 2.93 to 3.03 g. day^{-1}.

Growth determinants were higher in fish in all variants fed L-Carnitine supplemented feeds in comparison to control. The condition factor of fish in the control variant was slightly higher than those in experimental groups (Table 3). However, no above mentioned differences were statistically significant.

Table 2: Production parameters of carp fingerlings culture in foil divisions
Tablica 2. Proizvodni parametri kulture mladih šarana u pregradama s folijom

Variant - Varijanta	A	B	C	D
L-Carnitine content - Sadržaj L- carnitina mg. kg^{-1}	0	250	500	1000
Survival rate % - Stopa preživljavanja %	95.33	85.00	87.33	94.67
Individual weight gain g - Pojedinačni prirast težine g	24.26	25.83	26.31	25.04
Relative weight gain-total % - Relativni prirast težine-ukupno %	563	526	536	578
Relative weight gain-daily % - Relativni prirast težine-dnevno %	8.40	7.86	8.01	8.63
SGR % day^{-1} – Specifična stopa rasta % $dnevno^{-1}$	2.93	3.03	3.01	2.98
FCR g. g^{-1} – Iskorištenje hrane g/g^{-1}	2.56	2.65	2.59	2.41

Experiment II

Altogether, 532 fish marked according to their origin from particular variants, were stocked into the experimental overwintering pond. Stocking scheme and overwintering results are presented in Table 4.

Fish survival rates amounted to approx. 60% in average. The highest value was registered in the variant C (68.07%) whilst the lowest one in D (56.94%). However, highest total biomass losses of the stock were registered in variants B and A (46.41 and 46.38%, respectively). The individual weight losses ranged from 8.09 to 15 26% in groups A – C, whilst slight weight growth (3.95%) was found in variant D. Neither biometric evaluation established any statistically significant differences between the groups investigated.

Biochemical analyses of fish body before and after overwintering are compared in Table 5. In comparison with parameters determined at stocking, contents of dry matter, crude protein and fat increased during the growing season. Values of total energy content below 5 kJ g^{-1} dry matter were found in fish of the control variant, whilst those in experimental groups amounted to 5.61 - 5. 91 and 5.13 - 5. 80 before and after overwintering. No

considerable differences between individual experimental and control groups were registered the concerning total lipid energy of fat, and dry matter, crude protein, fat and crude ash contents.

Table 3. Biometric parameters of fish at stocking and harvesting
Tablica 3. Biometrički parametri ribe pri uzgoju i izlovu

Variant - Varijanta	Stocking - Nasad	A	B	C	D
L-Carnitine content mg kg^{-1} Sadržaj L-karnitina mg kg^{-1}		0	250	500	1000
Weight g Težina g	4.04±0.68 2.53-6.39	28.3±2.8 14.7-50.1	29.8±6.5 13.2-52.8	30.2±7.0 9.2-50.6	28.8±3.3 17.4-42.7
Total length mm Ukupna dužina mm	62±4 55-74	108±4 92-133	111±8 82-134	110±12 79-130	110±4 93-125
Body length mm Dužina tijela mm	51±3 45-61	88±3 73-111	90±7 62-109	90±6 67-108	89±3 78-105
Height mm Visina mm	18±1 16-22	34±1 27-43	34±3 26-43	35±5 21-48	34±2 28-40
FCF – Fultonov koeficijent	3.05	4.15	4.09	4.14	4.09

Table 4. Results of experimental overwintering with respect to individual variants
Tablica 4. Rezultati pokusnog prezimljenja s obzirom na pojedine varijante

Variant - Varijanta		A	B	C	D
L-Carnitine content mg kg^{-1} - Sadržaj L-karnitina mg kg^{-1}		0	250	500	1000
Stocking Uzgoj	ind g ind^{-1}	128 29.8	141 32.1	119 30.9	144 25.3
Harvesting ind g ind^{-1} Izlov	ind g ind^{-1}	78 26.3	89 27.2	81 28.4	82 26.3
Survival rate % - Stopa preživljavanja %		60.94	63.12	68.07	56.94
Stocking biomass loss % - Gubitak biomase u uzgoju %		46.38	46.41	37.43	40.83
Individual weight loss % - Pojedinačni gubitak težine %		11.74	15.26	8.09	+3.95

Table 5. Biochemical composition of fish body
Tablica 5. Biokemijski sastav tijela ribe

Variant - Varijanta	Stocking Uzgoj	A		B		C		D	
L-Carnitine content mg kg^{-1} Sadržaj L-karnitina mg kg^{-1}		0		250		500		1000	
Sampling period – Razdoblje uzimanja uzoraka		G	W	G	W	G	W	G	W
Total energy kJ g^{-1} DM Ukupna energija kJ g^{-1} ST	4.97	4.90	4.49	5.61	5.80	5.91	5.80	5.90	5.13
Total lipid energy kJ g^{-1} Ukupna energija lipida kJ g^{-1}	9.68	9.70	9.03	9.50	9.82	9.37	9.32	9.19	10.10
Dry matter % - Suha tvar %	16.12	24.90	23.01	24.39	22.98	25.31	22.27	23.82	22.41
Crude protein % - Sirove bjelančevine %	10.76	13.79	12.87	13.27	12.93	13.50	12.59	13.13	12.56
Fat % - Masnoća %	1.88	7.05	6.31	7.06	6.06	7.50	5.75	6.63	5.97
Crude ash % - Sirovi pepeo %	2.23	2.31	2.41	2.30	2.53	2.35	2.44	2.22	2.48

Note: Sampling period G – after growing season, W – after overwintering
Bilješka: Razdoblje uzimanja G – nakon sezone rasta W – nakon prezimljavanja

Experiment III

After overwintering, the experimental culture continued to be grown in cages, two variants were investigated – control and experimental D group (259 mg kg^{-1}). The results are presented in table 6. Although the individual weight gain was lower in the experimental group, the relative weight gain for both the whole period of culture and per day were higher in fish fed supplemented feed with L-Carnitine. When evaluating the specific growth rate for the whole experimental period, higher SGR (1.71% day^{-1}) was recognized in B group in comparison with control fish (1.52% day^{-1}). Feed conversion ratio was slightly lower in B group (2.43) in comparison to the control (2.46).

Table 6. Production results of carp culture in cages
Tablica 6. Proizvodni rezultati kulture šarana u kavezima

Variant varijanta	A	B
L-Carnitine content mg kg^{-1} L-karnitin mg kg^{-1}	0	250
Survival rate % - Stopa preživljavanja %	100.0	98.00
Individual weight gain g Pojedinačni prirast težine g	184.0	167.7
Relative weight gain-total % Relativni prirast težine-ukupno %	192	233
Relative weight gain- daily % Relativni prirast težine dnevno %	2.70	3.28
SGR % day^{-1} - SGR % dan^{-1}	1.52	1.71
FCR g/g^{-1} – Iskorištenje hrane g/g^{-1}	2.46	2.43

DISCUSSION

Although fish growth performance was best in the variant fed highest supplemented feed mixture (D-1000 mg kg^{-1}), no statistically significant differences were found between the control and experimental groups. However, the highest individual weight and weight gain (30.2 and 26.31 g, respectively) were achieved in the group fed 500 mg kg^{-1} L-Carnitine supplementation, followed by fish fed 250 mg kg^{-1} supplementation (29.8 and 25.83 g, respectively), whilst only 28.3 and 24.26 g, respectively, were achieved in the control. Highest survival rates (95.33 and 94.67%) were found in the control and 1000 mg kg^{-1}, respectively. In the last group mentioned, the lowest feeding quotient (2.41) was found as well.

The probiotic Ascogen supplementation (Adámek et al., 1994) in goldfish was effective in lower concentrations (80 and 125 mg kg^{-1}) as compared to L-Carnitine. The concentrations used for carp fingerlings in our experiments (250 mg kg^{-1} and >) resulted in growth retardation in goldfish.

During overwintering, the most favorable results were achieved in groups feed the feed supplemented with L-Carnitine concentrations of 500 and 1000 mg kg^{-1}. The individual weight differences and survival rates after the period of overwintering amounted to -8.08% and 68.07%, and +3.92% and 56.94% in these groups, respectively. This was probably due to the positive promotion of L-Carnitine, because probiotics in general are supposed to have a considerable influence upon fish health condition, including stress resistance and immune response. A positive effect of Ascogen supplementation upon the immune response of tilapia was described in details by Ramadan et al., (1990/91 b).

Based on the preliminary results, the final attention was concentrated upon the effect of L-Carnitine supplementation in the concentration of 250 mg kg^{-1} in the following growing season. However, no significant promoting impact of this level of supplementation was registered in 1+ carp, although the parameters of both total and daily relative weight gains, specific growth rate and feed conversion rate were better in comparison with the control group by 21.4, 21.5, 12.5 and 1.2%, respectively.

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SAŽETAK

Pokusi kojima je cilj bio ispitati kakvi su izgledi L-Carnitina, u hranidbi mladih šarana bili su postavljeni u uvjetima ribnjaka u folijom ograđenim pregradama 3 x 3 m i to s veleljuskavim šaranom (Caprinus carpia rex cyprinorum) (soj C 866) god. 1994-1996. Riba je dobivala krmnu smjesu Alma 6208 s različitim koncentracijama L- Carnitina. (0, 250, 500 i l000 mg kg^{-1}).

Nisu nađene značajne razlike između kontrolne i pokusnih skupina što se tiče konačne pojedinačne težine. Međutim, najviši se pojedinačni prirast težine (26.31 g), postigao u skupini hranjenoj dodatkom od 500 mg kg^{-1} L-Carnitina, zatim u ribe hranjene dodatkom od 250 mg kg^{-1} (25.83 g), dok se prirast od samo 24.26 g postigao u kontrolnoj skupini. Najviše su stope preživljavanja (95.33 i 94.67%) nađene u kontrolnoj, odnosno skupini s 1000 mg kg^{-1}. U zadnjoj je navedenoj skupini nađen najniži koeficijent hranidbe (2.41). Za vrijeme prezimljenja najprobitačniji su se rezultati također postigli u skupinama koje su dobivale hranu kojoj je dodan L-Carnitin u koncentracijama od 500 i 1000 mg kg^{-1}. Pojedinačne razlike u težini i stope preživljavanja nakon razdoblja prezimljenja dosezale su u ovim skupinama do -8.08% i 68.07%, odnosno + 3.92% i 56.94%. Na temelju preliminarnih rezultata u sljedećoj je uzgojnoj sezoni pozornost bila posvećena učinku L-Carnitina u koncentraciji od 250 mg kg^{-1}. Međutim, nije zabilježen nikakav značajniji pozitivan utjecaj ove razine dodatka, premda su parametri relativnog prirasta težine, specifična stopa rasta i konverzija hrane bili bolji u usporedbi s kontrolnom skupinom.