Reduction of feed protein levels and of nitrogenous N-excretions by lysine supplementation in intensive carp culture

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Abstract

Four experiments were performed with carp (*Cyprinus carpio*) in ponds of 200 m^2 to test the effects of lysine supplementation of feeds with a low protein content. The initial mean weight of the carp and their daily rations were equal in all the ponds of the same experiment. In experiments 1 (400 fish/pond, 3 replicates) and 2 (200 fish/pond, 4 replicates), three diets were tested: A. 30% crude protein (1.7% total lysine); B. 25% crude protein + 0.5% lysine-HCl (1.7% lysine); C. 25% crude protein (1.3% lysine). In both experiments diet B resulted in weight gains equal to diet A, 20% higher protein retention and 20% less nitrogen discharge per kilogramme gain. Diet C lagged 10% in gain and excreted 10% more nitrogen than B. In experiments 3 and 4 (200 fish/pond, 4 replicates) the feed protein was reduced further and 5 diets were tested: A, B, C, similar to the former experiments, D. 20% crude protein + 1.0% lysine-HCl (1.8% total lysine), E 20% crude protein + 0.5% lysine-HCl (1.4% lysine). In both experiments the results of groups A, B and C were parallel to experiments 1 and 2. Diets D and E resulted in lower growth compared to diet C (significantly in experiment 4) and increased body fat content. In conclusion, 30% protein in carp feeds, of which half the protein was soybean protein, could be decreased to 25%, by replacing soybean meal by grains, and supplementing lysine and methionine to the level of the 30% control. Growth was not impaired and nitrogen discharge into the environment was reduced approximately by 20%. Further feed protein reduction to 20%, with lysine supplementation, impaired growth rate below that of the unsupplemented 25% control diet, although total N-excretion was reduced even more.

Keywords: carp, feed protein reduction, nitrogen excretion, lysine.

Diminution du niveau de protéines et d'excrétions azotées au moyen d'une supplémentation de lysine en élevage intensif de carpe.

Résumé

Quatre expériences ont été conduites avec la carpe (*Cyprinus carpio*) dans des bassins de 200 m^2 afin de tester les effets d'une supplémentation en lysine dans les aliments avec un faible taux de protéines. Le poids moyen initial des carpes et leurs rations journalières étaient égales dans tous les bassins d'une même expérience. Dans les expériences 1 (400 poissons/bassin, 3 réplicats) et 2 (200 poissons/bassin, 4 réplicats), trois régimes alimentaires ont été testés : A. 30 % de protéine brute (lysine : 1,7 %); B. 25 % de protéine brute + 0,5 % HCl-lysine (lysine : 1,7 %); C. 25 % de protéine brute (lysine : 1,3 %). Dans les deux expériences, les gains en poids avec le régime B ont été identiques à ceux obtenus avec le régime A, avec une augmentation de la rétention azotée de l'ordre de 20 % et 20 % de moins de déchets azotés par kilogramme de gain de poids. Dans les expériences 3 et 4 (200 poissons/bassin, 4 réplicats) le taux en protéine a été réduit et cinq régimes ont été testés : A, B et C identiques à la précédente expérimentation; D. 20 % de protéine brute + 1 % d'HCl-lysine (lysine :

1,4 %); E. 20 % de protéine brute + 0,5 % HCl-lysine (lysine : 1,4 %). Les résultats des groupes A, B et C étaient similaires aux expérimentations 1 et 2. Les régimes D et E entraînèrent une croissance inférieure comparée au régime C (particulièrement dans l'expérience 4) et une augmentation en graisse. En conclusion, 30 % de protéine dans l'alimentation des carpes, dont la moitié était à base de protéines de soja, peuvent tomber à 25 %, en remplaçant le soja par des graines, et en ajoutant de la lysine et de la méthionine, au niveau des 30 % du régime témoin. La croissance n'a pas été affectée et les déchets azotés ont été réduits d'environ 20 %. Une diminution de 20 % de protéine brute, accompagnée d'une supplémentation en lysine, affecte le taux de croissance au-dessous du régime témoin, 25 % sans supplémentation, bien que l'excrétion en azote totale soit davantage réduite.

Mots-clés : Carpe, protéine, alimentation, excrétion azotée, lysine.

INTRODUCTION

The non-retained feed protein ([1]) in intensive fish culture constitutes a major management problem. For carp, protein levels of 40% were formerly recommended, based mainly on trials with fingerlings (NRC-NAS, 1977). However, in later studies the recommendations were lowered to 30-35%, based on practical feeding studies (Viola and Arieli, 1982; NRC-NAS, 1983; Watanabe *et al.*, 1987; Takeuchi *et al.*, 1989). Sparing protein by increasing energy from oil or carbohydrates has been found possible for several genera of fish (Hepher, 1988). In particular, Watanabe *et al.* (1977) reduced N-excretion of carp growing in cages in a lake by lowering the total feed protein to 32% with lower rations of high-energy feeds. This method is not feasible in Israeli pond culture for several reasons and feeding rates of 3-4% are required for optimal growth (Marek, 1975; Viola and Arieli, 1982) (*see* discussion).

The lysine requirement of carp is relatively high: 5.7% of the total protein (NRC-NAS, 1983). When fishmeals are cheap, they are the preferable source of protein and lysine. However, in many countries plant proteins have to be employed – at least partially – and among them soybean meal. In these feeds lysine is liable to become the limiting amino acid (Viola and Arieli, 1989; Viola *et al.*, 1992). Soybean meal is not a very efficient feedstuff for carp. The complex carbohydrates of the soybean meal – 25-30%, approximately – are barely digestible by the stomachless carp and reduce the available energy content of the feed (Viola *et al.*, 1982; Hepher, 1988, p. 308).

In addition, quite often the meal is not toasted sufficiently and antitrypsin residues hamper the protein digestion of the pepsin-deficient carp, which is largely dependent on trypsin (Viola *et al.*, 1983). Even in properly heated soybean meals part of the lysine is bound into Maillard compounds, reducing the lysine availability (Riesen *et al.*, 1947; Smith, 1977; Dabrowski, 1983; Abel *et al.*, 1984). Viola *et al.* (1982) found that lysine availability was 10-15% lower for carp than for poultry.

Watanabe *et al.* (1987) reduced the nitrogen discharge by reducing the total protein (mainly fishmeal) and increasing the gross energy by fat and carbohydrates to 4 500 kcal/kg. The present study tried a different approach: to replace a part of the soybean meal by cereal grains, while balancing the levels of lysine and methionine by synthetic amino-acids. Concomitantly, the easily digestible grains increased the available energy.

Amino-acid supplementation to half-synthetic or test diets has been tried with fingerlings of several fish species for total protein sparing – yet growth rates were impaired, compared to the positive control diets (Nose *et al.*, 1974; Andrews *et al.*, 1977; Fauconneau, 1988). In practical catfish culture lysine supplementation improved low-lysine feedstuffs – like cottonseed meal, peanut meal – without reducing total protein (Robinson, 1992). To the best of our knowledge, amino-acid supplementation in order to spare protein has not been tried with carp.

Four experiments were designed in the Intensive Fishculture Station during two growth seasons, to investigate the possibility and extent of reducing part of the soybean protein. In the first two experiments the first step of protein reduction from the standard 30% protein to 25% + lysine was examined. In the two following experiments the investigation was extended to diets containing 20% protein.

Fish and their management

In all four experiments Israeli carp were raised in earthen ponds of 200 m^2 during summer or early autumn. All the ponds were aerated by paddle wheels and oxygen levels were maintained above 5 ppm. All the fish were counted and weighed at the start, so that their number and average weight were equal in all the ponds in the same experiment. About half of the fish of the 30% protein group (positive control) were weighed and counted bi-weekly for the adjust-

([1]) Non-retained protein is used in this paper synonymously to N-excretion.

ment of the daily ration. At the end of each experiment all the fish in each pond were counted and weighed collectively. Two representative samples of fish, close to the average final weight (three to five fish according to size), were selected from each treatment for body analyses. Water loss due to evaporation and seepage was replenished daily from the nearby lake (pH 7.5-8.0) and also from a recirculation system.

Ammonia, nitrite, nitrate and organic nitrogen in the ponds were measured bi-weekly in the first experiment. Since the levels found were very low (see Results), these measurements were not continued in the following experiments.

The conditions for the four experiments were as follows:

	Fish Number	Initial weight (g)	Duration (days)	Replicates	Water temperature (±2°C)
1.	400	240	47	3	26
2.	200	395	57	4	24
3.	200	260	48	4	25
4.	200	425	58	4	23

MATERIALS AND METHODS

Feeds

All the feeds were mixed and pelleted (4 mm) *in situ* and distributed from clock feeders. Each pond in the same experiment received equal daily rations – 4% of the mean wet body weight of the positive control treatment.

In the first two experiments three feeds were compared (*table* 1): A. 30% crude protein (1.7% total lysine); B. 25% protein + 0.5% lysine-HCl (1.7% total lysine), further designated 25% + 0.5 L; C. 25% crude protein without supplementary lysine (1.3 total lysine), negative control. The feed batches for the two experiments had very similar compositions with insignificant differences.

In experiments 3 and 4 five diets were tested (*table* 2).

Analyses

Water: ammonia, nitrite and nitrate were measured weekly using commercial instruments (Merck).

Table 1. – Composition of feeds for experiments 1 and 2 (% as fed).

Diet code	A	B	C
	Positive control	Lysine supplemented	Negative control
Protein designation	30%	25% + 0.5% L	25%
Lysine (%)	1.7	1.7	1.3
Composition			
Fishmeal (62% protein)	10.0	10.0	10.0
Soybean meal (44% protein)	34.0	20.0	20.0
Poultry meatmeal (60% protein)	5.0	5.0	5.0
Rapeseed meal (34% protein)	5.0	5.0	5.0
Lysine-HCl (79% lysine)	–	0.5	
Milo (Sorghum)	5.6	19.0	19.5
Wheat	20.0	20.0	20.0
Wheat bran	10.0	10.0	10.0
Dicalcium phosphate	3.0	3.0	3.0
Methionine (98%)	0.2	0.3	0.3
Vitamin-mineral premix[1]	0.2	0.2	0.2
Nutribinder (Sorghum base)	5.0	5.0	5.0
Oil (blended)	2.0	2.0	2.0
Analysis[2]			
Protein	29.5	25.0	24.5
Fat	4.7	5.0	4.8
Fiber	4.8	4.5	5.0
Ash	8.5	8.0	7.9
Nitrogen free extract	40.5	45.5	45.8
Calcium	1.8	1.8	1.8
Phosphorus	1.45	1.3	1.3
Methionine and cystine[3]	1.0	1.0	1.0
Lysine[3]	1.7	1.7	1.3
Gross energy (kcal/g)[3]	4.0	3.9	3.9

[1] Viola *et al.*, 1982.

[2] The analyses of the feed batches prepared for the two experiments showed small insignificant differences.

[3] Calculated (NRC-NAS, 1977, 1983).

Table 2. – Composition of feeds for experiments 3 and 4 (% as fed).

Diet code	A	B	C	D	E
	Positive control	Lysine supplemented	Negative control	Low-protein experimental feeds	
Protein designation	30%	25% + 0.5% L	25%	20% + 1% L	20% + 0.5% L
Lysine (%)	1.8	1.8	1.4	1.8	1.4
Composition					
Fishmeal (63% protein)	10.0	10.0	10.0	10.0	10.0
Soybean meal (44% prot.)	34.0	21.0	21.0	8.0	8.0
Poultry meatmeal (60%)	5.0	5.0	5.0	5.0	5.0
Rapeseed meal (34%)	5.0	5.0	5.0	5.0	5.0
Corn	9.8	21.1	21.6	32.4	32.9
Lysine-HCl (79% lysine)	-	0.5	-	1.0	0.5
Wheat	20.0	20.0	20.0	20.0	20.0
Wheat bran	10.0	10.0	10.0	10.0	10.0
Dicalcium phosphate	4.0	4.0	4.0	4.0	4.0
Methionine (98%)	-	0.2	0.2	0.4	0.4
Vitamin-mineral premix[1]	0.2	0.2	0.2	0.2	0.2
Nutribinder (Sorghum)	5.0	5.0	5.0	5.0	5.0
Oil (blended)	2.0	3.0	2.0	4.0	4.0
Analysis[2]					
Protein	31.4	26.5	26.5	22.0	22.0
Fat	4.5	6.0	6.0	7.0	7.0
Fiber	4.1	3.3	3.1	2.4	2.8
Ash	10.0	10.0	10.5	11.0	11.5
Nitrogen free extract	37.0	42.2	41.9	45.6	44.7
Calcium	2.2	2.1	2.1	2.0	2.0
Phosphorus	1.3	1.2	1.2	1.2	1.1
Methionine and cystine[3]	1.0	1.0	1.0	1.05	1.05
Lysine[3]	1.8	1.8	1.4	1.8	1.4
Gross energy (kcal/g)[3]	3.9	3.9	3.9	3.9	3.9

[1] Viola *et al.*, 1982.

[2] The analyses of the feed batches prepared for the two experiments showed small insignificant differences.

[3] Calculated (NRC-NAS, 1977, 1983).

Organic nitrogen was assayed by oxidation with potassium persulfate, dried in an autoclave at 1.5 atmospheres, reduced by Devarda's alloy and estimated colorimetrically with indophenol (Raveh and Avnimeleh, 1980).

Feeds: all the feeds, as well as the homogenized samples of fish, were assayed for moisture (105°C, 24 h), protein (Kjelfoss apparatus), fat (Fosslet apparatus with perchloroethylene), fibre (Fibrotec), ash and minerals by Standard Methods (AOAC, 1984). Lysine in fish carcasses was determined by courtesy of the companies: Degussa (Hanau), Eurolysine (Amiens), Novus-Europe (Brussels) and Hoffman-La Roche (Basle), by amino-acid analysis after hydrolysis of the dried samples (each sample by two laboratories). The method used was disclosed by one of them: dried and finely ground samples (about 200 mg) were cooled to 0°C, oxidized by H_2O_2 + HCOOH + Phenol, incubated at 20-30°C and cooled. Flasks were sealed and refrigerated 16 h, hydrolysed by reflux with 6 N-HCl for 23 h and neutralized on ice with NaOH to pH 2.2. Amino-acids were determined in a Beckman 6300 after extraction, filtering and dilution, and compared with a standard solution.

Data processing and statistics

The primary data (mean individual weight gain per pond, body composition per sample) were subjected to ANOVA and Duncan's Multiple Range Test for significant differences at the 5% level (SAS/PC, 1986). Since all the ponds in the same experiment were offered equal daily rations, the feed conversion (FCR) became inversely related to gain and not suitable for independent statistical analysis.

The means of the primary data were processed further for the following feed utilization efficiency calculations – thus also unsuited for ANOVA: Specific Growth Rate (SGR) = 100 (Ln final weight-Ln initial weight)/days (%); Energy in fish was calculated by factors: protein – 5.7 kcal/g, fat – 9.5 kcal/g (NRC-NAS, 1977); Retention of energy, protein and lysine = Gain in fish/amount in offered feed (%); Non-retained protein/weight gain (g/kg) = (Protein intake – protein retained)/weight gain.

Part of the non-retained protein was excreted as different N-compounds.

RESULTS

Mortalities in all the ponds were 5±3%, in all experiments. The performances of the fish in experiments 1 and 2 and their feed efficiency evaluations are presented in *table* 3, the results of experiments 3 and 4 in *tables* 4 *and* 5. Weight gains and non-retained protein are shown in condensed form in *figure* 1 for experiments 1 and 2, and in *figure* 2 for experiments 3 and 4.

Experiments 1 and 2 resulted in very similar conclusions for diets A, B and C, notwithstanding the differences in size. The experimental feed B (25% + 0.5 L) enabled the fish to grow at the same rate as the positive control group A, whereas the negative control group C lagged by about 10%. Body compositions were not significantly different between the three diets in both trials. Protein retention was highest with the lysine supplemented feed B and lower by 15-20% in both control groups, in both experiments. Lysine retention was nearly equal for all three diets and somewhat higher in experiment 1. Costs of feed per kilogramme gain were 5% lower for feed B – at the prices in that year.

At the end of experiment 1, organic nitrogen in the water was highest in group A – 2,5 ppm (not significantly different) and 2.0 ppm in groups B and C. Total nitrogen also was highest in group A – 3.3 ppm, least in C and intermediate in B (not significantly). Subsequently, the measurements were discontinued, due to these low values.

The first two experiments showed, that a diet of 25% protein, containing 1.7% total lysine (6.8% of the protein) was adequate for maximal growth rate and superior with regard to feed costs and pollution.

One of the major implications is the diminished nitrogen discharge into the environment, which is of primary importance in intensive fish farming. Since feed protein was reduced, yet growth unimpaired – the excreted nitrogen per kilogramme gain was decreased by 20% with diet B, compared to the 30% recommended standard protein feed (*fig*. 1).

In experiments 3 and 4 the dietary protein was further reduced to 20%, by further replacement of 13% soybean meal by grains. Lysine, methionine and energy were fully supplemented, either to the level of 1.8% – as in the 30% protein diet – by 1.0% lysine-HCl (diet D), or to 1.4% – as in the negative control diet – by 0.5% lysine-HCl (diet E).

The results for the diets A, B and C fully corroborated those of experiments 1 and 2 (*fig*. 2).

The extension of the protein reduction to diets containing 20% protein with partial or full lysine supplementation – diets D and E – failed to achieve the performance of the negative control group C. In experiment 4 the growth retardation even surpassed the statistical significance level of 5% for both feeds, with insignificant differences between them. The body

Table 3. – The effects of lysine supplementation to a 25% protein carp feed in two pond experiments Nos 1 and 2.

	Experiment 1			Experiment 2		
Diet code Protein	A 30%	B 25% + 0.5 L	C 25%	A 30%	B 25% + 0.5 L	C 25%
Weight gain ± SEM (g)	$342^c \pm 17$	$342^c \pm 11$	$309^d \pm 21$	$560^a \pm 37$	$562^a \pm 40$	$490^b \pm 33$
SGR[3] (%)	1.9	1.9	1.75	1.55	1.55	1.42
Body composition[4]						
Protein (%)	15.6^a	16.0^a	15.7^a	15.5^a	15.8^a	15.3^a
Fat (%)	11.5^a	12.1^a	11.9^a	11.3^a	11.4^a	12.4^a
Ash (%)	2.3^a	2.2^a	2.4^a	2.4^a	2.3^a	2.4^a
Lysine/prot. (%)	7.4^a	7.5^a	7.4^a	7.35^a	7.55^a	7.45^a
FCR[5]	2.2	2.2	2.4	2.35	2.35	2.7
Retentions[6]						
Protein (%)	25.0	30.0	26.4	22.0	27.3	23.0
Lysine (%)	31.5	32.0	34.3	29.0	30.0	32.3
Energy (%)	25.0	26.3	23.4	29.0	29.3	27.0
Non-retained protein/weight gain (g/kg)	477	383	440	548	427	508
Feed cost/weight gain (NIS/kg) (1 U.S. $ = 2.5 NIS)	1.40	1.33	1.40	1.51	1.43	1.57

Different superscripts indicate statistically significant differences ($p < 5\%$).

[1] Means of 3 ponds × 400 fish; at start: 240 g, 15.4% protein, 9.3% fat; 47 days.

[2] Means of 4 ponds × 200 fish: at start: 395 g, 15.3% protein, 10.0% fat; 57 days.

[3] Specific Growth Rare = 100 (Ln final weight – Ln initial weight)/days.

[4] Means of two pooled samples.

[5] Feed Conversion Ratio = Feed offered/weight gained (g/g wet weight).

[6] Retentions = Amount gained/amount in offered feed.

Table 4. – The effects of lysine supplementation of low protein carp feeds in pond experiment 3[1].

Diet	A	B	C	D	E
Protein	30%	25% + 0.5% L	25%	20% + 1.0% L	20% + 0.5% L
Weight gain ± SEM (g)	167[a] ± 6.0	170[a] ± 5.4	155.5[b] ± 7.7	147.5[b] ± 12.9	146.5[b] ± 3.2
SGR[2] (%)	1.9	1.9	1.75	1.55	1.55
Body composition[3]					
Protein (%)	16.3[a]	16.4[a]	16.2[a]	16.5[a]	15.7[b]
Fat (%)	6.1[c]	6.0[c]	8.2[b]	8.1[b]	10.1[a]
Ash (%)	2.75[a]	2.7[a]	2.65[a]	2.5[a]	2.55[a]
Lysine/prot. (%)	7.35[a]	7.3[a]	7.4[a]	6.9[a]	7.0[a]
FCR[4]	3.0	2.95	3.2	3.4	3.4
Retentions[5]					
Protein (%)	17.8	21.7	19.2	22.3	20.2
Lysine (%)	25.0	26.3	23.4	29.0	29.3
Energy (%)	13.7	15.3	16.5	15.9	18.6
Non-retained protein/weight gain (g/kg)	672	511	690	580	600
Feed cost/gain (NIS/kg) (1 U.S. $ = 2.5 NIS)	2.20	2.03	2.12	2.27	2.18

Different superscripts indicate statistically significant differences ($p < 5\%$).

[1] Means of 4 ponds × 200 fish; at start: 260 g, 15,1% protein, 6.0% fat; 48 days.

[2] Specific Growth Rate = 100 (Ln final weight – Ln initial weight)/days.

[3] Means of two pooled samples.

[4] Feed Conversion Ratio = Feed offered/weight gained (g/g wet weight).

[5] Retentions = Amount gained/amount in offered feed.

Table 5. – The effects of lysine supplementation of low protein carp feeds in pond experiment 4[1]

Diet code	A	B	C	D	E
Protein	30%	25% + 0.5% L	25%	20% + 1.0% L	20% + 0.5% L
Weight gain ± SEM (g)	349[a] ± 6.3	351[a] ± 5.7	316[b] ± 3.6	293[c] ± 4.8	285.5[c] ± 6.6
SGR[2] (%)	1.05	1.06	0.98	0.92	0.90
Body composition[3]					
Protein (%)	16.8[a]	16.4[ab]	16.3[ab]	16.4[ab]	15.9[b]
Fat (%)	6.7[c]	7.6[bc]	9.0[abc]	9.6[ab]	10.8[a]
Ash (%)	2.7[a]	2.8[a]	2.9[a]	2.6[a]	2.7[a]
Lysine/prot. (%)	7.2[a]	7.2[a]	7.3[a]	7.25[a]	7.3[a]
FCR[4]	3.15	3.13	3.5	3.75	3.85
Retentions[5]					
Protein (%)	18.5	20.5	18.3	20.3	18.2
Lysine (%)	23.3	22.2	25.3	17.6	20.6
Energy (%)	13.6	15.1	15.4	15.8	16.8
Non-retained protein/gain (g/kg)	785	644	740	660	700
Feed cost/gain (NIS/kg) (1 U.S. $ = 2.5 NIS)	2.33	2.22	2.30	2.50	2.47

Different superscripts indicate statistically significant differences ($p < 5\%$).

[1] Means of 4 ponds × 200 fish; at start: 425 g, 16.1% protein, 6.5% fat; 58 days.

[2] Specific Growth Rate = 100 (Ln final weight – Ln initial weight)/days.

[3] Means of two pooled samples.

[4] Feed Conversion Ratio = Feed offered/weight gained (g/g wet weight).

[5] Retentions = Amount gained/amount in offered feed.

fat content of group E was significantly higher, a phenomenon well known from previous experiences with low-protein carp feeds.

Lysine/protein ratios in the carcasses were not different for any group.

DISCUSSION

Reduction of N-excretion

Watanabe *et al.* (1987) reduced N-excretion in cage culture in a lake by diminishing total protein levels

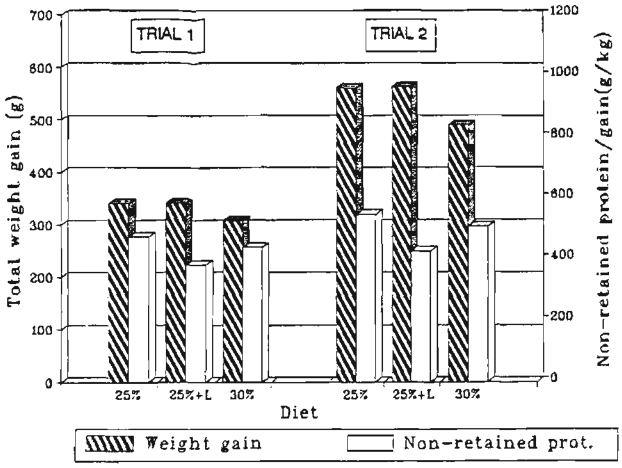

Figure 1. — Total mean weight gain (g) and non-retained protein gain (g/kg) in experiments 1 and 2, with 3 diet groups: A. 30% proteins; B. 25% protein + 0.5% lysine-HCl; C. 25% protein.

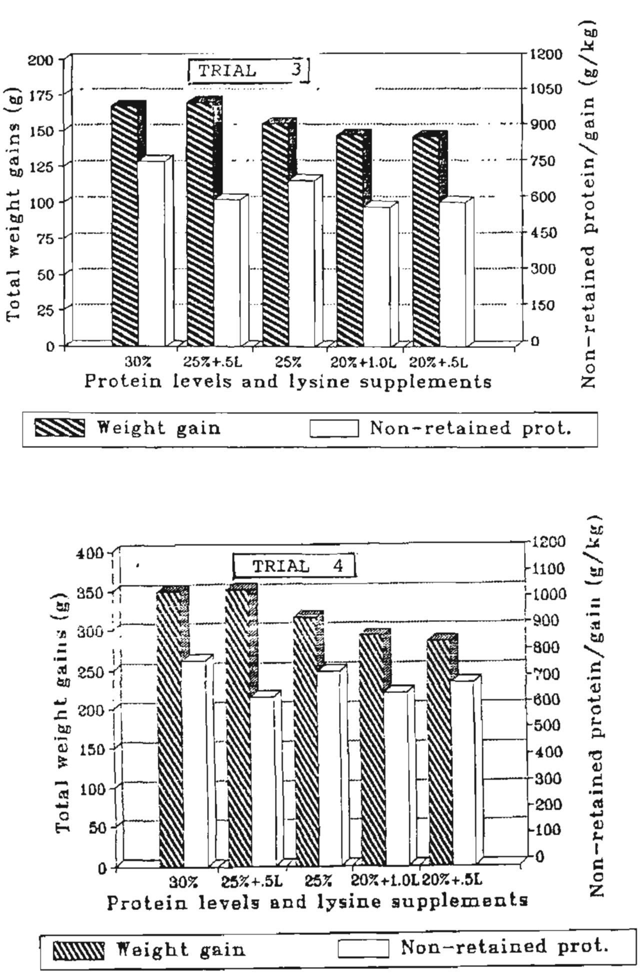

Figure 2. — Total mean weight gains (g) and non-retained protein/gain (g/kg) in experiments 3 and 4, with 5 diet groups: A. 30% protein; B. 25% protein + 0.5% lysine-HCl; C. 25% protein; D. 20% protein + 1.0% lysine-HCl; E. 20% protein + 0.5% lysine-HCl.

(with a high fishmeal content) from 38 to 32% and feeding rates to 1.5-2.0%, while raising the gross energy to 4.5 kcal/g with carbohydrates or/and fats. In later laboratory trials the protein sparing effect of high energy diets was verified, but feeding rates were raised to 3.5% (Takeuchi *et al.*, 1989).

This method is not feasible in Israeli pond culture: A. The natural feed organisms in the ponds are very scarce (rain water or wells) and rations of 3-5% have been found necessary (Marek, 1975; Viola and Arieli, 1982). Similarly, other researchers found 3-4% rations optimal for bigger carp in absence of natural food (Huisman *et al.*, 1979). B. Fishmeal is very expensive and must be replaced, at least partially, by low-energy plant proteins. Thus the maximal gross energy levels are about 4.0 kcal/g, including fat spray-coated at 5-6%. Higher fat coatings create problems of chemical and physical stability of the pellets. Therefore, other approaches to protein sparing had to be explored, such as reduction of protein and supplementing critical amino-acids, a practice used for farm animals in many countries. A preliminary report on the first stage of this study has been presented elsewhere (Viola and Lahav, 1991).

Utilization of free amino-acids

The utilization of free amino-acids has troubled researchers in the 1970s, due to the different absorption rates of free or bound amino-acids (Aoe *et al.*, 1970; Nose *et al.*, 1974; Andrews *et al.*, 1977). Further studies with several species of fish showed, that free amino-acids are utilized, particularly with multiple feedings: carp (Viola *et al.*, 1982; D'Mello *et al.*, 1988), catfish (Robinson *et al.*, 1980), tilapia (Jackson and Capper, 1982), trout (Rumsey and Ketola, 1975; Fauconneau, 1988).

Variability of nutrient availability of soybean meals

Soybean meal has to be toasted properly. Insufficient toasting leaves antitrypsin residues, which impair protein digestion, in particular for the stomachless and therefore trypsin-dependent carp. Proper toasting values, as measured by the Cresol-red method (Olomucki and Bornstein, 1960) are 3.8-4.2. In the course of our experiments several batches with lower values were received. Overtoasting, on the other hand, produces Maillard compounds of lysine, which are digested with difficulty. Measured by protein solubility in NaOH (Lyman *et al.*, 1953) values below 35% indicate overheating. In previous studies the availability of lysine was found 10-15% lower for carp than for poultry (Viola *et al.*, 1982).

Feed costs

The economy of amino-acid supplementation depends, of course, on local conditions. Where fish-meal and soybean meal are expensive, amino-acid supplementation might yield important savings, which would amount to 5% in Israel. Plant proteins with lower lysine contents (cottonseed meal, peanut meal) would require higher feed protein levels and could possibly save even more protein by amino-acid supplementation.

Range of applicability

Two explanations are possible for the impaired performance of groups D and E in our experiments:

a. Another amino-acid had become critical, or

b. The level of carbohydrates had reached the limit of the digestive capacity of the carp (Nagai and Ikeda, 1973; Hertz *et al.*, 1989) – although the nitrogen-free-extract in feed E was only 45%, which is below the acceptable level of 48%, according to Chiou and Ogino (1975).

CONCLUSION

All four experiments corroborate the conclusions: when about 13% of soybean meal in carp feeds of 30% protein were replaced by grains and properly supplemented by lysine and methionine:

a. Neither growth nor body composition were impaired;

b. Nitrogen discharge was diminished by 20%;

c. Feed costs were reduced by 5% (at prevailing price conditions);

d. Further protein reduction to 20% impaired growth, increased body fat and had no benefit for N-discharge per weight gain.

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