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Utilization of domestic plant components in diets for common carp *Cyprinus carpio* L.

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Abstract. The aim of this work was to determine the possibilities of substituting domestic plant protein ingredients for fish meal in complete diets in rearing different age/size groups of carp, *Cyprinus carpio* L. at high intensification. The mixture of legume seeds and extracted rapeseed meal used in the studies can partially replace fish meal in complete diets for carp, while the effective level of fish meal replacement was varied and depended on the carp age/size group. Introducing mixtures of plant components up to 26 and 22%, respectively, to the diets of carp early juveniles and juveniles did not adversely affect rearing results. The best results in rearing two-year old and market carp were obtained using diets that contained mixtures of plant components of 32 and 27%, respectively. Analyses were performed on the dependence between the share of plant protein in the dietary protein of the individual diets and the mean individual weight of carp and the values of the feed conversion ratio. This provided the basis for the conclusion that plant protein can comprise from 30-35% of the total protein pool in the diets of carp early juveniles and juveniles, while the maximum allowable share in the diets of two-year old and market carp is 45%. The experimental diet used in the feeding of market carp did not have an impact on the sensory properties of the fish meat.

Keywords: common carp, fish nutrition, diets, plant protein components, fish meal replacement

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1. Introduction

In recent decades, global aquaculture has been the fastest growing food production sector, which currently supplements and may someday replace fishing as the source of consumer fish (Frankic and Hershner 2003, Tacon 2004). The most common aquaculture products are freshwater, omnivorous fish, most of which come from the cyprinid family. Rapid development in aquaculture that has resulted from continuous improvement in existing technologies and the introduction of new biotechniques, has stimulated the demand for effective feeds that meet the nutritional needs of individual species (Sargent and Tacon 1999). For the past several years, one of the main directions in improving fish feeds has been the search for protein source alternatives to fish meal and determining their nutritional suitability in diets (Watanabe 2002). This trend is, firstly, a response to growing demands for formulated diets, and secondly, a response to limited resources of fish meal that will soon hit the upper threshold of exploitation. Additionally, according to Hardy (2008) one of methods to develop less expensive and effective formulations is lowering fish meal levels in diets. In Europe, an additional argument supporting the search for plant protein sources for feed is the increasing danger that components of animal origin can transmit pathogens. The appearance of Bovine Spongiform Encephalopathy (BSE) commonly known as

Mad-Cow Disease resulted in the limited application of animal meals in the fodder industry (Commission Regulation (EC) 2005).

To date, the most widely applied fish meal replacement that is confirmed nutritionally suitable is soy bean, *Glycine* sp. seeds and products obtained from them (extracted meal, protein concentrates). The partial substitution of soy bean meal for fish meal in feeds at quantities of up to 50-60%, or of its products, has been applied in practice for many years (Fowley 1980, Murai et al. 1982, Nour et al. 1989, Alexis 1990, Shiau et al. 1990, Reigh and Ellis 1992, Webster et al. 1992, Fontainhas-Fernandes et al. 1999, Elangovan and Shim 2000, Kotzamanis et al. 2008). The use of such a high percentage of these components is possible thanks to their nutritional (total protein content, amino acid composition) and palatable properties (Akiyama 1988, Pongmaneerat and Watanabe 1993, Hasan et al. 1997). The total replacement of fish meal with soy bean protein was successful only in a few cases (Kaushik et al. 1995, 2004, Adelizi et al. 1998).

Limitations stemming from climate conditions required for soy bean cultivation and the relatively high cost of extracted soy bean meal are the reasons why less expensive, and sometimes even local, alternative protein sources of plant origin are being sought (Gomes and Kaushik 1989, Booth et al. 2001, Hossain et al. 2001, Siddhuraju and Becker 2001, Mundheim et al. 2004, Glencross et al. 2007). Plant protein components for feed that are available on the Polish market include seeds from the legumes: lupines, *Lupinus* spp., pea, *Pisum* sp., and faba bean, *Vicia faba* L. Lupin seeds have the widest possibilities for application in carp diet because they contain the highest amounts of crude protein of this group of feed raw materials (mean: narrow leaf lupin, *L. angustifolius* L. 30% crude protein, white lupin, *L. alba* L. 31% crude protein, yellow lupin, *L. luteus* L. 39% crude protein). In comparison to extracted soy bean meal, lupin seeds contain less protein, more fiber, and more non-starch polysaccharides, and, depending on the variety, varied amounts of antinutritional components (Hughes 1988, Evans et al. 1993). They do not, however, require thermal processing (toasting) before they are added to feeds because of the lack of lectins and low levels of protease inhibitors (De la Higuera et al. 1988).

The second group of alternative protein components used in fish diets are by-products from the oil industry, such as oilcake or extracted meals from rape, sunflower, peanut, or cotton (Tacon and Jackson 1985, Higgs et al. 1988). Among these, the most important in Poland is rapeseed meal since demand for it is increasing as these seeds are finding ever widening applications in the production of biofuels. Rapeseed meal contains from 35-37% total protein with an advantageous amino acid profile, but their application in fish nutrition is limited by the relatively high content of raw fiber. Nevertheless, due to the low cost of rapeseed meal, it can be an important dietary component, especially for omnivorous fish.

From a nutritional standpoint, the undoubted disadvantage of feed components of plant origin is their load of antinutritional factors. Among these, the most significant include protease inhibitors, phytins, glucosinolates, saponines, tannins, pectins, oligosaccharides, non-starch polysaccharides, phytoestrogens, alkaloids, cyanogenics, antivitamins, feed fiber, and allergenic storage proteins, which will require additional confirmation (Francis et al. 2001). The impact of these factors on fish has been the subject of many studies mainly on salmonids. Experiments on these in reference to carp have focused on the use of thermally and hydro thermally modified grains of soy bean (Abel et al. 1984), tapioca and rice meal (Ufodike and Matty 1983), mustard seed oilcake and flax and sesame meal (Hossain and Jauncey 1989a, 1989b, 1990), and purified antinutritional substances such as sodium phytate (Hossain and Jauncey 1991), phorbol esters (Becker and Makkar 1998), or tannic acids and concentrated tannin (Becker and Makkar 1999). The concentrations of antinutritional substances that resulted from the use of protein raw materials in the diets studied did not result in higher fish mortality, but they did have a decisively negative impact on growth performance.

Individual plant raw materials used in feeds usually contain a few species-specific antinutritional factors. Lupin seeds contain protease inhibitors,

saponins, phytoestrogens, and alkaloids, while extracted rapeseed meal contains protease inhibitors, glucosinolate, phytic acid, and tannins. The negative impact of these can be curbed by limiting the share of these raw materials in diets, and by applying the appropriate technologies for processing them or manufacturing ready feeds (Drew et al. 2007). The most widely applied method of producing feed for aquatic animals is extrusion, which combines the impact of high temperature and pressure at raised raw material moisture content. Thermal processing effectively mitigates or eliminates the negative impact of thermolabile antinutritional factors, including protease inhibitors in lupin seeds, or glucosinolate in rapeseed meal by deactivating myrosinase, the enzyme that activates their transformation into toxic isothiocyanate (Bell 1984). The elimination of thermostable antinutritional substances is achieved by introducing into cultivation varieties of plants that have low levels of these compounds, such as sweet lupins with low levels of alkaloids (Roemer 1993) or the so-called the double zero rape variety with decreased levels of erucic acid and glucosinolates or the triple zero variety with additionally reduced tannin levels (Jamroz 2001). The elimination of thermostable antinutritional factors with a simultaneous increase in the amount of nutritional components might also be achieved by seed fractionation. These include methods ranging from simple procedures (dehulling), to more complicated ones (air separation), to highly advanced technologies such as protein isolation in water or solvents (Drew et al. 2007).

In recent years, increasing attention has been paid to the issue of fish farms releasing high concentrations of nitrogen, phosphorous, and organic compounds into waters. Nutrients that cause water eutrophication can come either directly from feeds or from fish excrement (Weismann et al. 1988, Seyour and Bergheim 1991, Watanabe et al. 1999). The simplest way to reduce the input of such substances into waters is to prepare feeds that are sufficiently water stable and contain nutritional components that fish are able to assimilate to the maximum (McGoogan and Gatlin 2000, Cho and Bureau 2001). The physical character of the feed used in rearing fish has a fundamental impact on the degree to which post-production pond waters are loaded. Studies by Jezierska-Madziar (1995) indicated that the lowest pollution loads were achieved by feeding fish extruded feeds. There was also a distinct correlation between increasing carp stocking density and the pollutant load in post-production waters. Reducing the level of pollution generated by intense fish farming is possible by using a balanced diet that ensures the maximum assimilation of dietary components, in particular phosphorus and nitrogen, which are the elements that stimulate eutrophication to the greatest degree. In the case of phosphorus, this can be achieved by choosing raw components for feed production that contain phosphorus that is highly bioassimilable (Riche and Brown 1996, Cho and Bureau 1997), or by the addition to the feed of an enzyme supplement, such as phytase that releases phosphorus from phytins (Mayer and McLean 1995, Forster et al. 1999, Vielma et al. 2000, Sugiura et al. 2001, Cheng et al. 2004, Nwanna et al. 2007). Reduction of the nitrogen load in post-production waters is possible by applying components with highly digestible protein and a diet with balanced energy content to protein quantity (Wilson and Poe 1985, Jahan et al. 2003).

In countries where aquaculture is of great importance, which entails increased environmental risks, legislation is introduced to minimize or reduce potential threats stemming from intensified production (Tacon and Forster 2003). The most widely-applied mechanisms include limiting concentrations of certain dissolved substances or ones that create suspensions in post-production waters from fish farms. Exceeding norms can lead to monetary fines, the requirement to perform initial cleaning of post-production waters originating from fish farms, limiting the number of permits granted to conduct fish farming in particular aquatic systems, determining the maximum allowable quantities of certain feed components, limiting the amount of feed permitted to be used, or allowing only certain feeds to be used that meet clear criteria (digestibility and assimilability, the degree to which feed components are utilized, feeding effectiveness as expressed by the feed conversion ratio (Hardy 2000). Various criteria are

applied in European countries that proposed or existing aquaculture facilities must meet; these can include attaining a required environmental impact score, imposing production quotas, limiting nitrogen and phosphorus loads, determining feed composition, determining the maximum feed conversion ratio for feed applied, or requiring post-production waters to be cleaned (European Commission (EC) 1995). Polish environmental protection legislation recognizes post-production waters from fish breeding or rearing as sewage subject to additional charges in two cases: (1) waters used for the breeding and rearing of salmonid fish and released to waters or land if they contain designated pollutant substances in excess of the allowable limit; or (2) waters directed away from breeding or rearing facilities of fish other than salmonids or other aquatic animals if the annual production of these fish or animals, understood as the mean annual weight gain in particular years of the production cycle, exceeds 1500 kg per ha of usable fish pond area of this facility in one year of a given cycle (Environmental Protection Legislation 2001).

According to the above principles, so far in Poland, the only way to avoid charges for the releasing of post-production waste waters was to limit the scale of fish production. There is no doubt that in the nearest future, the increasing restrictions referring to environmental protection will force the fish farmers make to major changes in fish rearing methods and to find new solutions for waste reduction. Some positive effects can be achieved in the limitation of waste emission by the implementation of balanced and effective feeds, or by decreasing the supplied feed doses. Such procedure would at the same time satisfy the environmental protection requirements and ensure fish welfare.

The application of alternative protein components should not have a negative impact on the nutritional effects of the diet. The most common cause of worse results when using a diet with a high share of plant protein is the deficiency of essential amino acids, especially sulphuric. Covering the amino acid requirements of fish is possible by choosing raw materials of the appropriate content or by using mixtures of several components that have different amino acid profiles. This was confirmed in recent experiments performed on various fish species in which the tested diets were manufactures based on a mix of several plant protein components (Gomes and Kaushik 1989, Moyano et al. 1992, Gomes et al. 1993, 1995, Watanabe et al. 1995, Regost et al. 1999, Carter and Hauler 2000, Xie et al. 2001, Fournier et al. 2004, Przybył et al. 2006, Barrows et al. 2007, Adamidou et al. 2008, Mazurkiewicz et al. 2009). The study performed by Mazurkiewicz et al. (2004) on feeding two-year old carp confirmed that it is possible to completely replace animal meal with a mixture of a few plant protein components (extracted soy bean and rapeseed meal, lupin seeds, and soy bean protein concentrate). A properly balanced diet based on plant protein alone requires supplementation with the crystalline amino acids (lysine and methionine).

The results of the above experiments provided the inspiration to undertake the research that is the topic of this thesis; namely, to determine the possibility of introducing domestic plant protein components to the diets for all ontogenetic stages of carp. From an applied point of view, studies of using alternative protein components should determine their optimal share in diets.

2. Aim of the studies

The aim of the studies was to determine the possibility of replacing fish meal with a mixture comprised of legume seeds (white and yellow lupin, faba bean) and extracted rapeseed meal in extruded complete diets for rearing particular ontogenetic stages of carp at high production intensity in a full production cycle.

3. Materials and methods

3.1. Experimental pattern and technical conditions of the study

The studies were conducted in the 2004-2006 period in the Experimental Plant of Feed Production

Technology and Aquaculture in Muchocin of the PoznańUniversity of Life Sciences. Four feeding experiments were conducted using different sizes of carp stocking material: early juveniles (in Polish aquaculture practice known as *narybek letni*), juveniles (in Polish aquaculture practice known as *narybek*), two-year old and market fish. The general plan of the experiments is presented in Table 1.

Table 1
Scheme of conducted experiments

Experiment code	Study period	Carp stocking material	IBW* (g)	Stocking density indiv. pond^{-1} (indiv. ha^{-1})	Number of	
					Variants	Replicates
EJ	14.07-04.09.2006	Early juveniles	2.7 ± 0.4	160 (40 000)	4	3
J	23.06-20.08.2004	Juveniles	125 ± 3.9	18 (4500)	4	3
TO	03.06-23.07.2005	Two-year old	360 ± 7.5	18 (4500)	4	3
M	25.07-12.09.2005	Market	969 ± 5.9	10 (2500)	4	3

*IBW – mean initial fish body weight ± SD.

The tests were conducted in experimental ponds with a surface area of 40 m each that were supplied with water individually in an open system. The construction of the ponds permitted maintaining the maximum water level with constant water flow, while the individual water inlet and outflow systems allowed conducting cyclic control catches of all fish. During the tests, water temperature (°C) and dissolved oxygen content (mg O_2 dm^{-3}) (microcomputer oxymeter ELMETRON CO 315, Elsent Wrocław, Poland) were measured daily at 09:00. Water pH was measured once per week with a WTW Multi Line P3 pH meter (WTW Weilheim, Germany).

The diet was delivered everyday with an automatic band feeder for 12 hours per day (09:00-21:00). The daily diet ration was calculated based on the carp feeding key developed by Miyatake (1997) while taking into consideration the water temperature and the current fish weight. The size of the ration was determined every ten days based on monitoring weight measurements, which also served to calculate the values of the rearing indices. During the experiment with carp early juveniles, no monitoring weight measurements were made at ten-day intervals due to the technical difficulties involved with catching fish of this size.

Prior to commencing each of the tests and immediately following them, fish samples were taken for determining their proximal body composition. The fish were anesthetized with a solution of the Propiscin anesthetic (Siwicki 1984), decapitated and ground (KNIFETEC 1095 Sample Mill, FOSS TECATOR, Höganäs, Sweden), and homogenized (Laboratory homogenizer H500, POLEKOLAB, Warsaw, Poland). This material was then used to determine the following: dry matter, total protein, raw fat and ash (methodology for determinations was the same as for the diets).

3.2. Experimental diets

The diets were manufactured at the Feed Laboratory of the Experimental Plant of Feed Production Technology and Aquaculture in Muchocin. The recipes of the legume-rapeseed mixture and the experimental diets were calculated with a computer program designed to maximize the composition of the fish diets.

For each experiment four diets were prepared according to the procedure below:

1. Preparation of the legume-rapeseed mix: individual components weighed out; mixed; ground in a percussion mill until very fine (mesh size 1 mm).
2. Preparation of components of the diets: individual components weighed out; ground in a percussion mill until very fine (mesh size 1 mm).
3. Preparation of the premix: vitamin and mineral components, and choline chloride added to the

carrier (cereal component); mixed for 5 min in a cubic mixer.

4. Preparation of the diets: all ingredients and the premix mixed in a drum mixer for 5 min; mixture of rapeseed oil and soy bean lecithin heated to 50°C added; mixed in blade mixer for 5 min.
5. Conditioning the diets: hot water added; mixed in blade mixer for 5 min.
6. Extrusion: Metalchem S-60 single screw warm extruder (Gliwice, Poland) (extrusion parameters for experimental diets are presented in Table 2).
7. Drying: on mesh under a stream of heated air.
8. Sifting: the dust fraction sifted off in a percussion sifter.
9. Oiling: rapeseed oil heated to 50°C in quantities of 2% for diets EJ and J and 1% for diets TO and M was used to coat extruded diet in a pelletizing drum.

Table 2
Extrusion parameters of experimental diets

Parameter	Diet			
	EJ	J	TO	M
Moisture content (%)	10	10	12	14
Cylinder temperature in the zone of increasing pressure (^oC)	85	90	90	80
Cylinder temperature in the zone of high pressure (^oC)	100	100	100	90
Head temperature (^oC)	110	110	110	100
Time of passage through extruder (s)	56	54	63	72
Nozzle diameter (mm)	6.0	3.0	6.0	6.0

Manufacturing a diet for carp early juveniles required extra preparation to obtain the appropriate granulate fraction. The extruded diet was crushed with a crumblier, and then sifted through percussion sieves to separate out two experimental diet size fractions:

- Granulate from 0.8 to 2.0 mm for carp early juveniles weighing up to 20.0 g;
- Granulate from 2.0 to 3.0 mm for carp early juveniles weighing more than 20.0 g.

The ingredients of the diets tested in experiments EJ, J, TO, and M are presented in Tables 3, 4, 5, and 6. The main source of protein in diets EJ1, J1, TO1, and M1 was fish meal, and this was gradually replaced in subsequent diets with plant protein components in the form of legume-rapeseed meal mixes, the maximum amount of which was limited by the level of raw fiber (≤5.5%) in the diets. The legume--rapeseed mix used to manufacture the diet for juveniles (experiments EJ and J), and two-year old carp (experiment TO) had the following composition: white lupin 35%, yellow lupin 45%, extracted rapeseed meal 20%. The diets for market carp (experiment M) containing a legume-rapeseed mixture: white lupin 25%, yellow lupin 15%, faba bean 45%, extracted rapeseed meal 15%.

Table 3
Ingredients (%) in experimental diets for carp early juveniles

Ingredient	Diet			
	EJ1	EJ2	EJ3	EJ4
Fish meal	21.5	20.0	16.0	12.0
Erythrocyte meal	12.0	10.0	10.0	10.0
Legume-rapeseed mixture	-	10.0	18.0	26.0
Fish protein hydrolysate[1]	4.0	4.0	4.0	4.0
Yeast	8.0	8.0	8.0	8.0
Wheat meal	15.7	19.2	23.2	31.2
Rye bran	30.0	20.0	12.0	-
Rapeseed oil	5.0	5.0	5.0	5.0
Soy bean lecithin	0.5	0.5	0.5	0.5
Premix[2]	1.5	1.5	1.5	1.5
Vitazol AD_3EC[3]	0.1	0.1	0.1	0.1
Chalk	1.5	1.5	1.5	1.5
Choline chloride	0.2	0.2	0.2	0.2

[1]C.P.S.P. 90, Sopropeche, Boulogne-Sur-Mer, France.
[2]Polfamix W, BASF Polska Ltd. Kutno, Poland – containing per 1 kg: 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 – contains in 1 kg: vitamin A 50 000 IU, vitamin D_3 5 000 IU, vitamin E 30.0 mg, vitamin C 100.0 mg.

A 4% supplement of fish protein hydrolysate was added to the carp early juveniles and juveniles diets

Table 4
Ingredients (%) in experimental diets for carp juveniles

Ingredient	Diet			
	J1	J2	J3	J4
Fish meal	16.2	16.5	12.0	8.0
Erythrocyte meal	12.0	8.0	8.0	8.0
Legume-rapeseed mixture	-	12.0	22.0	32.0
Fish protein hydrolysate[1]	4.0	4.0	4.0	4.0
Yeast	8.0	8.0	8.0	8.0
Triticale meal	22.0	23.9	25.7	31.7
Wheat bran	30.0	20.0	12.0	-
Rapeseed oil	4.5	4.3	4.5	4.5
Soy bean lecithin	0.5	0.5	0.5	0.5
Premix[2]	1.5	1.5	1.5	1.5
Vitazol AD_3EC[3]	0.1	0.1	0.1	0.1
Chalk	1.0	1.0	1.5	1.5
Choline chloride	0.2	0.2	0.2	0.2

[1]C.P.S.P. 90, Sopropeche, Boulogne-Sur-Mer, France.
[2]Polfamix W, BASF Ltd, Kutno, Poland; see Table 3.
[3]BIOWET Drwalew, Poland; see Table 3.

Table 5
Ingredients (%) in experimental diets for two-year old carp

Ingredient	Diet			
	TO1	TO2	TO3	TO4
Fish meal	17.5	12.3	8.9	5.0
Erythrocyte meal	8.0	8.0	8.0	8.0
Legume-rapeseed mixture	-	12.0	22.0	32.0
Yeast	8.0	8.0	8.0	8.0
Triticale meal	22.2	27.4	35.8	41.0
Rye bran	40.0	28.0	12.0	-
Rapeseed oil	1.0	1.0	1.0	1.0
Soy bean lecithin	0.5	0.5	0.5	0.5
Premix[1]	1.5	1.5	1.5	1.5
Vitazol AD_3EC[2]	0.1	0.1	0.1	0.1
Chalk	1.0	1.0	1.3	1.1
Monocalcium phosphate	-	-	0.7	1.6
Choline chloride	0.2	0.2	0.2	0.2

[1]Polfamix W, BASF Ltd, Kutno, Poland; see Table 3.
[2]BIOWET Drwalew, Poland; see Table 3.

in order to obtain well agglomerated granules. The content of yeast in all of the diets was identical (8%). The carbohydrate component was either wheat or triticale. Wheat or rye bran was added to equalize the amount of raw fiber in the individual diets.

Table 6
Ingredients (%) in experimental diets for market carp

Ingredient	Diet			
	M1	M2	M3	M4
Fish meal	12.2	7.7	5.1	2.5
Erythrocyte meal	5.0	5.0	5.0	5.0
Legume-rapeseed mixture	-	17.0	27.0	37.0
Yeast	8.0	8.0	8.0	8.0
Triticale meal	25.2	31.9	36.9	41.3
Rye bran	45.0	24.0	12.0	-
Rapeseed oil	1.0	1.0	1.0	1.0
Soy bean lecithin	0.5	0.5	0.5	0.5
Premix[1]	1.5	1.5	1.5	1.5
Vitazol AD_3EC[2]	0.1	0.1	0.1	0.1
Chalk	1.3	1.3	1.3	1.3
Monocalcium phosphate	-	0.8	1.4	1.6
Choline chloride	0.2	0.2	0.2	0.2

[1]Polfamix W, BASF Ltd, Kutno, Poland; see Table 3.
[2]BIOWET Drwalew, Poland; see Table 3.

Rapeseed oil was added to balance the energy levels of the diets, which also served as a hydrophobic film on the granule surface. The carriers of the vitamin and mineral compounds were Premix, Vitazol, fodder chalk, and calcium monophosphate. Choline chloride was used as the lipotropic component, and the emulsifier was soy bean lecithin.

3.3. Determining the composition of diet and fish

Chemical analyses were performed in accordance with AOAC (1996). The total protein content was determined on a Kjel-Foss Automatic 16210 analyzer (AISN Foss Electric, Denmark). Raw fat was determined with the Soxhlet method (extraction with ethyl ether for 12 h). The amount of raw fiber was determined with a Fibertec System M 1020 Hot Extractor (Tecator Flawil, Switzerland). Ash was determined by incinerating dried sample (5 g) at a temperature of 550°C for 12 h (furnace by Linn High Therm GmbH,

Eschenfelden, Germany). The quantity of nitrogen-free extract was determined as the difference between the amount of dry matter in the sample, and the sum of the remaining nutritional components: total protein, raw fat, raw fiber and ash. The level of total phosphorus and calcium was determined on an atomic absorption spectrophotometer (ASS3 by Carl Zeiss Jena, Germany) according to the methods described by Gawęcki (1988).

The amino acids in the dietary protein were separated after being hydrolyzed in 6n HCl at a temperature of 106°C for 24 h on an AAT 339 analyzer (Microtechna Prague, Czech Republic). Sulfur amino acids, methionine and cystine, were determined after they had been oxidized and fixed with formic acid. Tryptophan was determined with the colorimetric method. On the basis of the amino acids analysis, the chemical values of the experimental diets were determined by calculating the indexes of limiting amino acid Chemical Score (CS) and the Indispensable Amino Acids Index (IAAI). The gross energy of the diets was calculated from the proximate composition using the energy conversion factors for fish: carbohydrates 17, protein 24, and fat 39 J g^{-1} (Jobling 1994).

The analysis of the proximate composition of the fish included determining the dry matter, total protein, raw fat, and ash according to methods applied in the analysis of diet composition.

3.4. Sensory analysis of fish meat

After the termination of the test, the meat of market carp was subjected to a comparative sensory analysis. Three carp were chosen at random from each experimental variant; these were stunned and then decapitated, and then fillets with skin were obtained through manual processing. The carp fillets were packed in air-tight plastic bags to protect the meat from drying, and then frozen at -18°C. After 10 days, the fillets were defrosted and steamed without seasonings. The evaluation was conducted using the scale method to rate the following characteristics: color, smell, firmness, juiciness, taste, overall impression (Baryłko-Pikielna 1975, Gawęcka and Jędryka 2001). Ten respondents participated in the evaluation, and each always received the same part of the fillet to evaluate. The individual characteristics were evaluated on a scale of 1 to 9, and then the evaluations were recalculated to a scale of 0.0 to 1.0, with 0 as the lowest and 1 as the highest scores for a given characteristic and a given evaluator.

3.5. Evaluation of effects

The effects of rearing procedures were evaluated on the basis of stock biomass and diet utilization; equations recommended by Hardy and Barrows (2002) were used.

- Specific Growth Rate (SGR), with the equation:

$$SGR = (100 \times (\ln w_t - \ln w_o) \times t^{-1}) \qquad (1)$$

where: w_o – initial mean body weight (g); w_t – final mean body weight; t – number of study days.

- mean absolute Feed Conversion Ratio (FCR), with the equation:

$$FCR = D_d \times (W_t - W_o)^{-1} \qquad (2)$$

where: D_d – weight of diet delivered (g), W_t – final fish weight (g), W_o – initial fish weight (g).

- Protein Efficiency Ratio (PER), from the equation:

$$PER = (W_t - W_o) \times P^{-1} \qquad (3)$$

where: P – weight of protein in diet fed to fish during the experiment (g), remaining symbols as in equation 2.

- Protein Retention index (PR), with the equation:

$$PR = (P_t - P_o) \times P^{-1} \qquad (4)$$

where: P_t – total protein weight of fish at the end of the experiment (g), P_o – total protein weight of fish before beginning the experiment (g), P – as in equation 3.

- Fat Retention index (FR), from the equation:

$$FR = (F_t - F_o) \times F^{-1} \qquad (5)$$

where: F_t – raw fat weight of fish at the end of the experiment (g), F_o – raw fat weight of fish before the beginning of the experiment (g), F – raw fat weight in diet fed to fish (g).

- Survival Rate (SR), from the equation:

$$SR = (N_t \times N_o^{-1}) \times 100 \qquad (6)$$

where: N_t – final number of fish (indiv.), N_o – initial number of fish (indiv.).

3.6. Statistical analysis

The results were evaluated using the Microsoft Excel spreadsheet, and analyzed with the statistical package Statistica 5 PL (StatSoft 2001). In experiments J, TO, and M, the weight of the stock and the values of the SGR, FCR, and PER indices were calculated for each of the four experimental variants and each for five terms, while the values of the PR and FR indices were calculated for only one term. In experiment EJ, statistical analyses were performed for the stock weight and the values of the SGR, FCR, PER, PR, and FR in all four variants for one term. In experiment M the results of sensory evaluations of carp meat were statistically analysed.

Normality of distribution was tested with the Kołmogorow-Smirnov test (level of significance $\alpha = 0.05$). The parameters were also subjected to the Bartlett test of variance homogeneity, and the result was positive. Since the data base fulfilled all the necessary assumptions, it was also subjected to multidimensional analysis of variance. The main effects were the time and the type of diet; their interactions were also estimated (with the exception of the results of experiment EJ and indices PR and FR for the remaining experiments). Following the analysis of variance, *post-hoc* group analysis was also performed. Homogeneous groups were determined with the T-Tukey test.

4. Results

4.1. Environmental conditions of the tests

Extreme values that were noted in the individual experiments are presented in Table 7. Changes in the mean value of water temperature and the oxygen content are presented graphically in Figures 1, 2, 3, and 4 for experiments EJ, J, TO, and M, respectively. The highest average daily water temperature was observed during the test with carp early juveniles (ranged from 17.1 to 25.2°C), while the lowest mean

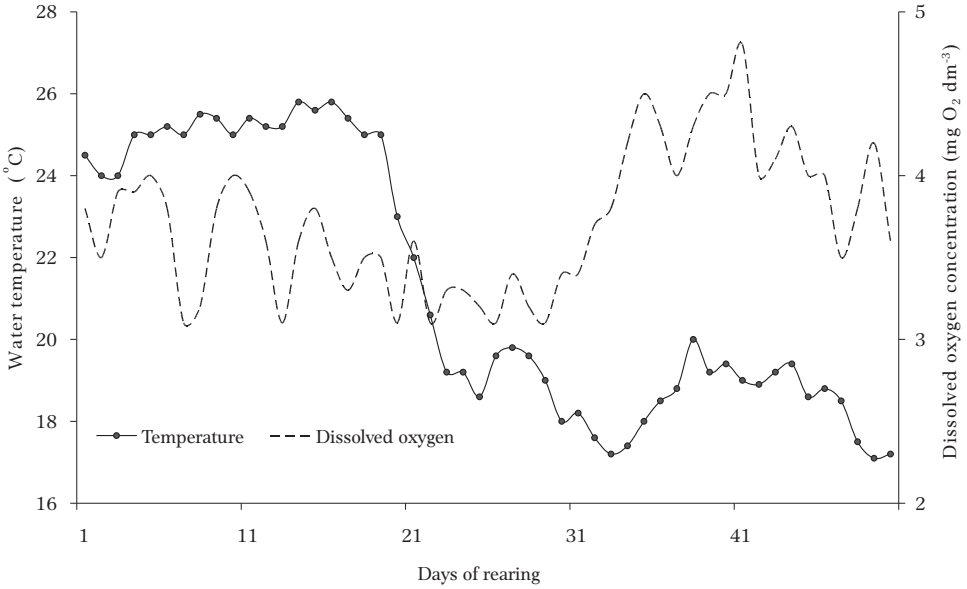

Figure 1. Diel changes of water temperature and dissolved oxygen concentration during test with carp early juveniles

Table 7
Extremal values of physical and chemical water properties during tests

Parameter	Test			
	EJ	J	TO	M
Water temperature (°C)				
Minimum	17.1	17.0	14.4	16.0
Maximum	25.8	22.0	23.5	22.0
Dissolved oxygen concentration (mg O_2 dm^{-3})				
Minimum	3.1	6.0	3.1	3.2
Maximum	4.8	8.3	10.0	6.8
pH value				
Minimum	6.8	7.0	6.9	7.0
Maximum	7.5	7.6	7.3	7.8

values of this parameter was recorded during the test with market fish (from 16.0 to 22.0°C). Content of extremal values of dissolved oxygen was very variable: from 3.1 (experiments EJ and TO) to 10.0 mg O_2 dm^{-3} (experiment TO). The water pH during all of the tests was close to neutral and fluctuated within the range from 6.8 to 7.8.

4.2. Chemical composition of the legume-rapeseed mixture

The chemical composition of legume-rapeseed mix used to prepare the experimental diets for early juveniles, juveniles and two-year old carp was identical

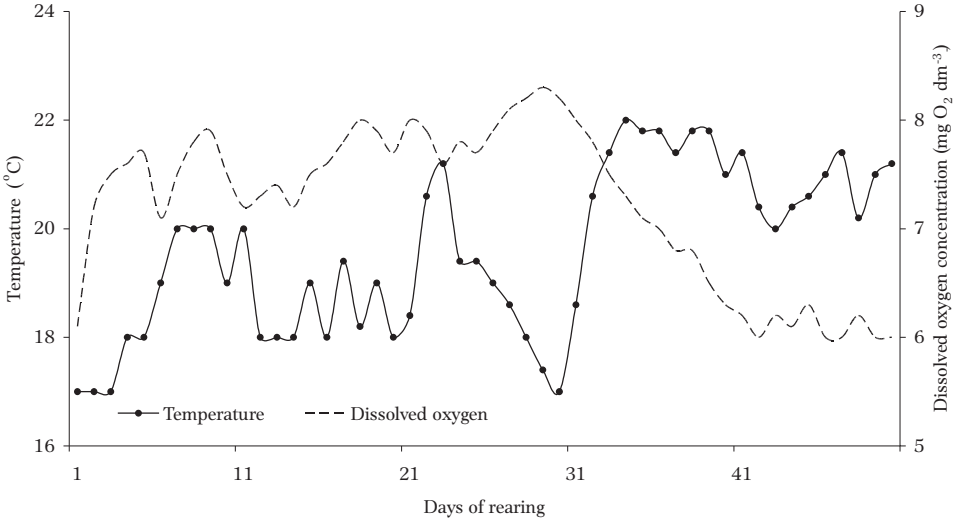

Figure 2. Diel changes of water temperature and dissolved oxygen concentration during test with carp juveniles

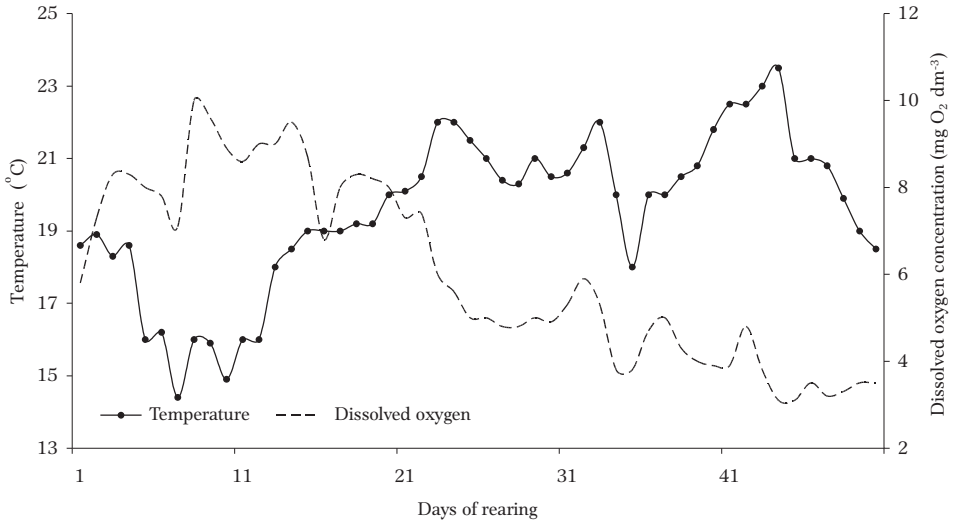

Figure 3. Diel changes of water temperature and dissolved oxygen concentration during test with two-year old carp

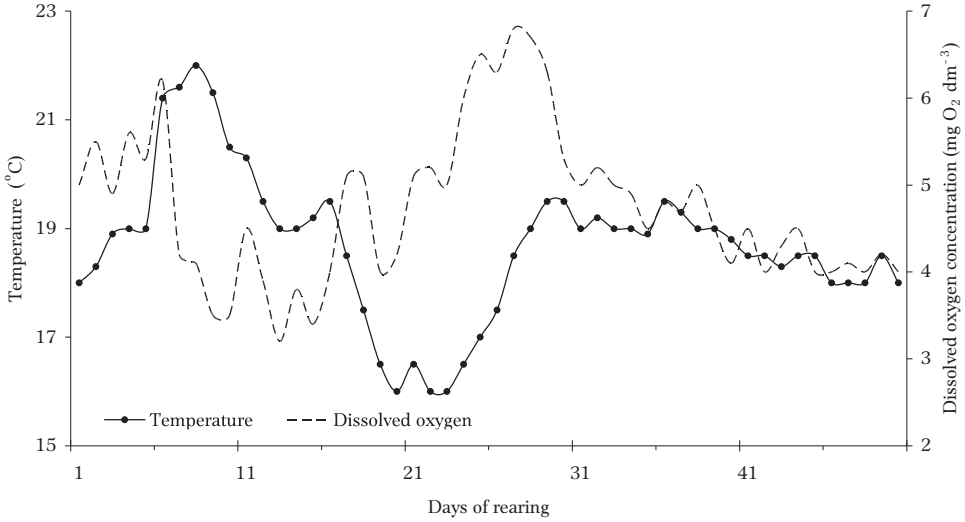

Figure 4. Diel changes of water temperature and dissolved oxygen concentration during test with market carp

(% of dry matter): crude protein 40.0; crude fat 5.3; nitrogen-free extract 29.9; crude fibre 13.3; ash 4.8; total phosphorus 0.6; calcium 0.4; lysine 2.4; methionine with cystine 1.8.

The chemical composition of the legume--rapeseed mix used to prepare the diet for market carp was the following one (% of dry matter): crude protein 32.0; crude fat 3.5; nitrogen-free extract 43.0; crude fibre 10.1; ash 4.0; total phosphorus 0.5; calcium 0.5; lysine 2.1; methionine with cystine 1.3.

4.3. Experiment EJ

The balance of the carp early juveniles diet was 38% total protein at a raw fat content range of 9.3 to 9.8% (Table 8). The quantity of raw fiber increased with increasing shares of plant components, and was the highest at 4.8% in diet EJ4. The ash content did not exceed 9.5%, at comparable levels of total phosphorus (about 1%) and quantities of calcium that ranged from 1.4 to 1.7%. The energy values of the diets were similar, as were the diet protein-energy relationships. The quantities of essential amino acids fulfilled the needs of the common carp, and the values of the indispensable amino acid indices ranged from 66.1 to 69.7. The limiting amino acid in diets EJ1, EJ2, and EJ3 was phenylalanine, while in diet EJ4 it was methionine with cystine (Table 8).

The diets applied with increasing shares of plant protein components did not have a significant effect on the final individual weight of the carp early juveniles (Table 9). The type of diet applied also had no impact on fish weight gain as expressed by the SGR or the conversion of diet components expressed by feed conversion (FCR) or protein efficiency (PER) ratios. Indicators of feed protein retention (PR) were comparable in all variants and ranged from 30 to 32%. The degree of fat retention (FR) in carp bodies varied, with a distinct tendency to increase along with increasing contents of plant protein components. In the variant EJ1 FR exceeded 20%, in variants EJ2 and EJ3 it was just under 33%, while the maximum values were noted in fish from variant EJ4 (46%). Only in the degree of fat retention (FR) was an inter-group difference confirmed statistically ($P \leq 0.05$). Fish mortality was noted in all variants, but it was low and did not exceed 6%, which permits excluding diet as having an effect on the health of the fish (Table 9). The proximate analysis of the carp early juveniles body indicated significantly higher dry matter and crude fat following the test (Table 10). The content of overall protein and ash did not change significantly following the test.

Table 8
Chemical and amino acid composition of dry matter (DM), caloric value and chemical nutritive value of protein (the Chemical Score CS, and the Indispensable Amino Acids Index IAAI) of the experimental diets for carp early juveniles

	Diet			
Properties	EJ1	EJ2	EJ3	EJ4
Crude protein (% DM)	38.0	38.2	38.1	38.0
Essential amino acids (g 100 g^{-1} protein)				
Arginine	5.0	5.3	5.5	5.6
Histidine	3.0	2.9	2.9	2.9
Lysine	6.0	5.8	5.7	5.7
Tryptophan	1.0	1.1	1.1	1.1
Phenylalanine +Tyrosine	4.2	4.8	5.1	5.5
Methionine + Cystine	3.2	3.1	3.3	3.1
Threonine	3.8	3.8	4.0	3.9
Leucine	7.5	7.4	7.5	7.7
Isoleucine	3.8	3.8	3.9	3.9
Valine	5.5	5.4	5.4	5.4
Crude fat (% DM)	9.7	9.8	9.6	9.3
Nitrogen-free extract (% DM)	31.5	31.7	32.6	34.0
Raw fibre (% DM)	4.0	4.4	4.7	4.8
Ash (% DM)	9.5	9.1	8.6	7.9
Total phosphorus (% DM)	1.1	1.0	0.9	0.9
Calcium (% DM)	1.7	1.7	1.5	1.4
Gross energy (MJ kg^{-1})	18.23	18.38	18.43	18.53
E/P (kJ g^{-1} protein)	48.05	48.11	48.37	48.75
IAAI	66.1	67.6	68.5	69.7
CS	Phen 38.6	Phen 42.1	Phen 44.7	Met + Cys 46.2

Table 9
Mean fish body weight (IBW–initial, FBW-final) and values of carp early juveniles rearing indices[1]

	Diet			
Parameters*	EJ1	EJ2	EJ3	EJ4
IBW (g)	2.8 ± 0.5^{a}	2.7 ± 0.6^{a}	2.7 ± 0.5^{a}	2.6 ± 0.5^{a}
FBW (g)	64.9 ± 3.4^{a}	68.1 ± 9.0^{a}	63.8 ± 5.5^{a}	68.3 ± 7.9^{a}
SGR (% d^{-1})	6.27 ± 0.31^{a}	6.44 ± 0.25^{a}	6.31 ± 0.14^{a}	6.53 ± 0.25^{a}
FCR	1.19 ± 0.21^{a}	1.12 ± 0.17^{a}	1.15 ± 0.07^{a}	1.05 ± 0.08^{a}
PER	2.44 ± 0.47^{a}	2.56 ± 0.35^{a}	2.48 ± 0.15^{a}	2.71 ± 0.20^{a}
PR (%)	30.5 ± 2.4^{a}	32.3 ± 3.1^{a}	30.1 ± 3.2^{a}	32.3 ± 2.9^{a}
FR (%)	22.5 ± 1.9^{a}	32.7 ± 3.5^{b}	32.9 ± 3.6^{b}	46.0 ± 3.8^{c}
SR (%)	97.5 ± 1.25^{a}	94.2 ± 2.90^{a}	93.7 ± 3.15^{a}	96.0 ± 2.0^{a}

[1]Values are mean ± SD from triplicate groups of fish. In rows mean values with different superscript are significantly different ($P<0.05$).
*see Material and Methods

Table 10
Chemical composition (% wet matter) of carp early juveniles body before and after the test[1]

Component		After the test in diet			
	Before the test	EJ1	EJ2	EJ3	EJ4
Dry matter	16.68 ± 0.3^{a}	20.18 ± 0.49^{b}	20.06 ± 0.45^{b}	19.27 ± 0.39^{b}	20.12 ± 0.41^{b}
Crude protein	11.86 ± 0.27^{a}	13.76 ± 0.42^{a}	13.32 ± 0.33^{a}	12.66 ± 0.28^{a}	12.47 ± 0.35^{a}
Crude fat	1.32 ± 0.12^{a}	2.87 ± 0.1^{b}	3.48 ± 0.13^{b}	3.48 ± 0.26^{b}	4.36 ± 0.31^{b}
Ash	2.52 ± 0.11^{a}	2.44 ± 0.09^{a}	2.27 ± 0.08^{a}	2.23 ± 0.15^{a}	2.11 ± 0.12^{a}

[1]Values are mean ± standard deviation (SD) from triplicate groups of fish. In rows mean values with different superscript are significantly different ($P<0.05$).

4.4. Experiment J

The level of total protein in the carp juveniles diets was just over 35%, and the difference in the raw fat content (about 8%) was within the margin of error of the method (Table 11). The level of raw fiber increased with increasing share of plant components in the diet and was from 3.3% in diet J1 to 5.0% in diet J4. The ash content was similar in all the diets (about 6%), and the amounts of total phosphorus and calcium determined did not exceed 0.9% and 1.3%, respectively. The level of essential amino acids did not vary among diets. The limiting amino acid in all the diets was isoleucine; the calculated values of the IAAI ranged from 72.9 to 74.9. The energy-protein ratio of the experimental diets was nearly identical, and differences in the levels of gross energy were minimal (Table 11).

The mean individual weight of the carp juveniles did not differ significantly until day 40 of the test (Table 12). At the end of the fourth decade, a significantly lower individual body weight was noted in variant J2, while at the end of the test the highest individual weights were achieved by carp fed the diet based on animal protein components, but they did not differ statistically significantly from the fish weights in variant J3. It is noteworthy that no statistically significant inter-group differences were noted in the individual weights of carp fed diet with plant protein components. The daily weight gain of carp throughout the test was similar (without statistically significant differences) at an SGR of between 2.25 to 2.46% d^{-1}. Only during the last decade of the experiment significant inter-group differences were noted for this parameter. During this period, carp from variants J2 and J3 exhibited faster growth. The use of the nutritional components of the diet by the

Table 11
Chemical and amino acid composition of dry matter (DM), caloric value and chemical nutritive value of protein (the Chemical Score CS, and the Indispensable Amino Acids Index IAAI) of the experimental diets for carp juveniles

	Diet			
Properties	J1	J2	J3	J4
Crude protein (% DM)	35.2	35.2	35.1	35.2
Essential amino acids (g 100 g^{-1} protein)				
Arginine	5.2	5.6	5.7	5.8
Histidine	3.7	3.4	3.3	3.2
Lysine	7.3	7.0	6.6	6.2
Tryptophan	2.9	2.9	2.4	1.9
Phenylalanine +Tyrosine	6.1	6.8	6.9	7.0
Methionine + Cystine	3.2	3.3	3.1	3.2
Threonine	4.1	4.0	3.9	4.2
Leucine	8.0	7.7	7.7	7.8
Isoleucine	2.9	3.2	3.2	3.1
Valine	5.7	5.5	5.5	5.5
Crude fat (% DM)	8.2	8.3	8.4	8.2
Nitrogen-free extract (% DM)	36.2	36.0	36.0	36.9
Raw fibre (% DM)	3.3	4.0	4.6	5.0
Ash (% DM)	6.0	6.0	6.1	5.7
Total phosphorus (% DM)	0.9	0.9	0.8	0.6
Calcium (% DM)	1.3	1.3	1.3	1.1
Gross energy (MJ kg^{-1})	17.88	17.8	17.82	17.92
E/P (kJ g^{-1} protein)	50.57	50.58	50.77	50.9
IAAI	72.9	74.9	74.5	74.0
	Ileu	Ileu	Ileu	Ileu
CS	41.8	46.6	45.5	43.8

Table 12
Mean fish body weight and values of carp juveniles rearing indices[1]

	Diet			
Days of test	J1	J2	J3	J4
Mean fish body weight (g)				
start	125.0 ± 3.6^{a}	125.7 ± 4.7^{a}	125.4 ± 5.9^{a}	125.0 ± 4.8^{a}
10	192.2 ± 1.9^{a}	170.9 ± 12.8^{a}	188.1 ± 3.6^{a}	189.4 ± 6.4^{a}
20	235.0 ± 4.7^{a}	207.9 ± 15.0^{a}	226.5 ± 7.0^{a}	224.4 ± 2.9^{a}
30	264.0 ± 8.5^{a}	231.8 ± 11.5^{a}	254.8 ± 7.5^{a}	254.6 ± 11.9^{a}
40	331.3 ± 15.8^{b}	290.0 ± 11.5^{a}	310.2 ± 13.9^{b}	311.5 ± 12.8^{b}
50	427.0 ± 17.2^{b}	387.6 ± 21.2^{a}	407.0 ± 20.0^{ab}	385.9 ± 20.4^{a}
SGR (% d^{-1})				
1-10	4.31 ± 0.3^{a}	4.06 ± 0.38^{a}	4.07 ± 0.32^{a}	4.16 ± 0.13^{a}
10-20	2.01 ± 0.24^{a}	1.96 ± 0.1^{a}	1.85 ± 0.22^{a}	1.7 ± 0.24^{a}
20-30	1.16 ± 0.2^{a}	1.1 ± 0.23^{a}	1.18 ± 0.11^{a}	1.25 ± 0.35^{a}
30-40	2.26 ± 0.16^{a}	2.24 ± 0.11^{a}	1.96 ± 0.18^{a}	2.02 ± 0.20^{a}
40-50	2.54 ± 0.31^{a}	2.9 ± 0.22^{b}	2.72 ± 0.28^{b}	2.14 ± 0.57^{a}
1-50	2.46 ± 0.11^{a}	2.33 ± 0.07^{a}	2.39 ± 0.04^{a}	2.25 ± 0.03^{a}
FCR				
1-10	1.66 ± 0.09^{a}	1.61 ± 0.42^{a}	1.77 ± 0.09^{a}	1.73 ± 0.07^{a}
10-20	2.05 ± 0.29^{a}	2.06 ± 0.1^{a}	2.23 ± 0.31^{a}	2.29 ± 0.63^{a}
20-30	2.06 ± 0.34^{a}	2.4 ± 0.38^{a}	2.01 ± 0.19^{a}	1.97 ± 0.59^{a}
30-40	1.61 ± 0.12^{a}	1.63 ± 0.09^{a}	1.64 ± 0.12^{a}	1.85 ± 0.21^{a}
40-50	1.96 ± 0.29^{a}	1.67 ± 0.16^{a}	1.81 ± 0.22^{a}	2.5 ± 0.86^{b}
1-50	1.82 ± 0.08^{a}	1.92 ± 0.08^{a}	1.77 ± 0.14^{a}	2.01 ± 0.12^{a}
PER				
1-10	1.72 ± 0.1^{a}	1.61 ± 0.21^{a}	1.61 ± 0.09^{a}	1.65± 0.06^{a}
10-20	1.4 ± 0.18^{a}	1.38 ± 0.06^{a}	1.29 ± 0.17^{a}	1.3 ± 0.35^{a}
20-30	1.41 ± 0.25^{a}	1.2 ± 0.18^{a}	1.42 ± 0.14^{a}	1.53 ± 0.45^{a}
30-40	1.77 ± 0.13^{a}	1.74 ± 0.09^{a}	1.72 ± 0.12^{a}	1.55 ± 0.18^{a}
40-50	1.47 ± 0.2^{b}	1.71 ± 0.15^{b}	1.59 ± 0.19^{b}	1.21 ± 0.35^{a}
1-50	1.56 ± 0.07^{a}	1.48 ± 0.06^{a}	1.61 ± 0.13^{a}	1.41 ± 0.08^{a}
PR (%)				
1-50	22.25 ± 1.51^{b}	20.83 ± 1.21^{a}	23.46 ± 1.91^{a}	19.08 ± 0.96^{a}
FR (%)				
1-50	60.15 ± 3.19^{a}	78.82 ± 5.24^{b}	102.47 ± 8.11^{d}	93.19 ± 5.64^{c}
SR (%)				
1-50	100	100	100	100

[1]Values are mean ± SD from triplicate groups of fish. In rows mean values with different superscript are significantly different ($P<0.05$).

carp juveniles was similar. The mean value of the FCR ranged from 1.8 to 2.0, while the PER values ranged from 1.41 to 1.61 (Table 12). In both indices, significant inter-group differences were noted only in the last decade of the trial, when worse values were noted for the variants in which the carp received the diet with the highest level of plant protein components.

While protein retention in the bodies of carp juveniles was similar (with no statistically significant

inter-group differences), the fat retention varied depending on the type of diet applied. Much higher lipid levels were stored in the bodies of carp juveniles fed the diet with plant protein components; additionally, significant differences were noted among fish from the various experimental groups (Table 12).

After the end of the test, the bodies of the carp juveniles had significantly lower water and ash contents than at the start, but total protein content remained the same (Table 13). Significant differences were noted, however, in fat content. There were significant increases of two- to threefold in the quantity of this compound in fish from all variants, with a tendency to increase with diets that had higher contents of plant protein components. One immediate consequence of this was described earlier as the differences in the value of fat retention (FR).

ergy-protein ratio of just over 55 kJ g^{-1} protein (Table 14).

The results obtained during the test and at its conclusion are presented in Table 15. The impact of the type of diet on the mean individual weight of the fish became apparent from day 30 of the test. Significantly higher weights were obtained by two-year old carp fed the diet with plant protein components, and on the final day of the experiment the individual weight of the carp in variant TO4 exceeded one kilogram.

The mean daily weight gain of individual fish throughout the test was significantly varied, and reached SGR values of nearly 1.8% d^{-1} in variant TO1 to nearly 2.2% d^{-1} in variant TO4 (Table 15). The values of the SGR indicator in the first three decades of the experiment were higher for the fish receiving diet with the highest share of plant protein

Table 13
Chemical composition (% wet matter) of carp juveniles body before and after the test[1]

Component	Before the test	After the test in diet			
		J1	J2	J3	J4
Dry matter	20.2 ± 0.9^{a}	26.63 ± 0.52^{b}	27.54 ± 0.04^{b}	29.11 ± 1.94^{b}	28.98 ± 1.36^{b}
Crude protein	13.6 ± 0.55^{a}	14.06 ± 0.32^{a}	13.89 ± 0.66^{a}	14.32 ± 1.29^{a}	13.54 ± 0.01^{a}
Crude fat	3.51 ± 0.11^{a}	7.38 ± 1.07^{b}	9.63 ± 0.97^{c}	11.64 ± 0.62^{d}	11.54 ± 0.75^{d}
Ash	2.61 ± 0.13^{b}	2.22 ± 0.41^{a}	2.04 ± 0.29^{a}	1.72 ± 0.38^{a}	1.74 ± 0.18^{a}

[1]Values are mean ± SD from triplicate groups of fish. In rows mean values with different superscript are significantly different ($P<0.05$).

4.5. Experiment TO

The total protein of the diets of two-year old carp was about 30%, while crude fat comprised about 4.5% (Table 14). The level of raw fiber was between 4.3 and 5.3%. The amount of ash determined ranged from 5.8 to 7.3%. Calcium and total phosphorus were not variable at about 1.2% and 0.8%, respectively. The levels of essential amino acids did not vary much among diets. The value of Indispensable Amino Acid Index was about 72, and the limiting amino acid in all the diets was methionine with cystine. The amount of gross energy was nearly identical (about 16.6 MJ kg^{-1}) at an equalized en-

components, but this equalized in the fourth and fifth decades. The utilization of the nutritional components of the diet, as expressed by the FCR and PER indicators, varied in the first two decades of the test, and these parameters were significantly more advantageous with increasing shares of plant protein components in the diet. In the subsequent three decades of the experiment, the values of these indicators equalized. The mean values of the feed conversion ratio and protein efficiency ratio calculated for the entire experiment period was distinctly more advantageous in variants in which the two-year old carp received the diet with plant protein components. The type of diet delivered did not impact the retention of

TABLE 14
Chemical and amino acid composition of dry matter (DM), caloric value and chemical nutritive value of protein (the Chemical Score CS, and the Indispensable Amino Acids Index IAAI) of the experimental diets for two-year old carp

	Diet			
Properties	TO1	TO2	TO3	TO4
Crude protein (% DM)	30.2	30.1	30.1	30.1
Essential amino acids (g 100 g^{-1} protein)				
Arginine	5.4	5.4	5.5	5.5
Histidine	3.7	3.5	3.4	3.3
Lysine	7.2	6.6	6.2	5.8
Tryptophan	3.4	2.7	2.3	1.7
Phenylalanine +Tyrosine	5.5	5.6	5.8	6.0
Methionine + Cystine	3.2	3.1	3.0	3.1
Threonine	4.0	4.2	3.9	4.1
Leucine	7.7	7.8	7.9	8.0
Isoleucine	3.5	3.4	3.4	3.3
Valine	5.6	5.6	5.7	5.7
Crude fat (% DM)	4.6	4.3	4.4	4.5
Nitrogen-free extract (% DM)	45.3	45.7	45.4	44.8
Raw fibre (% DM)	4.3	4.9	4.9	5.3
Ash (% DM)	7.3	6.5	6.3	5.8
Total phosphorus (% DM)	0.9	0.8	0.8	0.8
Calcium (% DM)	1.3	1.1	1.2	1.1
Gross energy (MJ kg^{-1})	16.74	16.67	16.66	16.59
E/P (kJ g^{-1} protein)	55.44	55.38	55.34	55.13
IAAI	72.5	72.2	71.9	71.3
	Met+Cys	Met+Cys	Met+Cys	Met+Cys
CS	48.4	45.8	43.4	41.2

protein or fat in the fish bodies (no statistically significant inter-group differences). The values of the protein retention indicator ranged from just under 34% to an excess of 36%, while the fat retention value was high at above 150% (Table 15).

The contents of water, total protein, and raw fat in the carp bodies did not vary following the conclusion of the experiment (Table 16). A significant decrease was noted, however, in ash in variants TO2 and TO3 in comparison to the quantities determined initially, as well as in all the variants on the day of the conclusion of the test.

4.6. Experiment M

The proximal composition and characteristic of the diets offered to market carp are presented in Table 17. The total protein level of all the diets slightly exceeded 25%, while the crude fat ranged from 2.8 to 4.0%. The level of raw fiber was nearly identical in all the diets (4.8-4.9%), and the content of ash was within the range of 5.5 to 6.9%. The levels of calcium and total phosphorous did not vary among diets (1.2 and 0.8%, respectively). The limiting amino acid in all the diets was methionine with cystine, and the IAAI value exceeded 72. The gross energy of the diets tested oscillated within the range of 15.67 to 16.06 MJ kg^{-1}, and the energy-protein ratio was varied (from 62.2 to 63.7 kJ g^{-1} protein).

In terms of the biological material, experiment M with market carp was the continuation of experiment TO, which was the further rearing of these carp using diets with lower protein and fat contents appropriate for the final stage of rearing. The mean individual

Table 15
Mean fish body weight and values of two-year old carp rearing indices[1]

	Diet			
Days of test	TO1	TO2	TO3	TO4
Mean fish body weight (g)				
start	366.7 ± 9.6^{a}	361.1 ± 9.6^{a}	359.3 ± 6.4^{a}	355.6 ± 5.6^{a}
10	408.7 ± 12.5^{a}	409.3 ± 11.2^{a}	413.3 ± 9.8^{a}	418.1 ± 5.0^{a}
20	477.7 ± 10.1^{a}	485.0 ± 15.1^{a}	489.3 ± 11.7^{a}	514.4 ± 8.6^{a}
30	622.2 ± 18.3^{a}	627.8 ± 5.7^{a}	646.3 ± 16.2^{ab}	695.4 ± 3.4^{b}
40	738.7 ± 14.9^{a}	754.8 ± 13.0^{ab}	788.9 ± 18.9^{b}	848.5 ± 13.2^{c}
50	887.4 ± 27.8^{a}	908.5 ± 7.5^{a}	954.3 ± 17.3^{b}	1058.3 ± 25.1^{c}
SGR (% d^{-1})				
1-10	1.08 ± 0.06^{a}	1.25 ± 0.01^{a}	1.4 ± 0.32^{a}	1.62 ± 0.12^{b}
10-20	1.56 ± 0.15^{a}	1.7 ± 0.1^{a}	1.69 ± 0.01^{a}	2.07 ± 0.07^{b}
20-30	2.64 ± 0.08^{ab}	2.58 ± 0.25^{a}	2.78 ± 0.1^{ab}	3.01 ± 0.12^{b}
30-40	1.72 ± 0.17^{a}	1.84 ± 0.11^{a}	1.99 ± 0.12^{a}	1.99 ± 0.18^{a}
40-50	1.83 ± 0.12^{a}	1.85 ± 0.1^{a}	1.9 ± 0.09^{a}	2.21 ± 0.09^{a}
1-50	1.77 ± 0.03^{a}	1.85 ± 0.04^{ab}	1.95 ± 0.06^{b}	2.18 ± 0.05^{c}
FCR				
1-10	1.66 ± 0.1^{b}	1.46 ± 0.06^{b}	1.31 ± 0.29^{b}	1.08 ± 0.09^{a}
10-20	1.61 ± 0.17^{b}	1.46 ± 0.09^{b}	1.47 ± 0.01^{b}	1.17 ± 0.04^{a}
20-30	1.46 ± 0.05^{a}	1.5 ± 0.17^{a}	1.37 ± 0.06^{a}	1.25 ± 0.06^{a}
30-40	1.88 ± 0.22^{a}	1.73 ± 0.11^{a}	1.61 ± 0.09^{a}	1.60 ± 0.16^{a}
40-50	1.85 ± 0.13^{a}	1.82 ± 0.12^{a}	1.78 ± 0.1^{a}	1.5 ± 0.07^{a}
1-50	1.69 ± 0.05^{c}	1.63 ± 0.04^{bc}	1.54 ± 0.03^{b}	1.37 ± 0.05^{a}
PER				
1-10	1.71 ± 0.1^{a}	1.96 ± 0.07^{ab}	2.26 ± 0.55^{bc}	2.63± 0.21^{c}
10-20	1.78 ± 0.18^{a}	1.95 ± 0.12^{ab}	1.94 ± 0.01^{ab}	2.42 ± 0.09^{b}
20-30	1.95 ± 0.07^{a}	1.91± 0.21^{a}	2.08 ± 0.08^{a}	2.27 ± 0.11^{a}
30-40	1.52 ± 0.17^{a}	1.64 ± 0.11^{a}	1.77 ± 0.1^{a}	1.79 ± 0.17^{a}
40-50	1.55 ± 0.11^{a}	1.57 ± 0.1^{a}	1.61 ± 0.09^{a}	1.9 ± 0.09^{a}
1-50	1.68 ± 0.05^{a}	1.74 ± 0.05^{ab}	1.85 ± 0.03^{b}	2.07 ± 0.07^{c}
PR (%)				
1-50	34.16 ± 2.11^{a}	36.45 ± 1.92^{a}	34.74 ± 2.55^{a}	33.73 ± 4.06^{a}
FR (%)				
1-50	154.7 ± 13.45^{a}	179.4 ± 25.88^{a}	162.19 ± 18.74^{a}	158.56 ± 25.0^{a}
SR (%)				
1-50	100	100	100	100

[1]Values are mean ± SD from triplicate groups of fish. In rows mean values with different superscript are significantly different ($P<0.05$).

weight of fish did not differ statistically significantly in the first three decades of the experiment (Table 18). In the subsequent two decades, an individual weight in excess of 1650 g was attained by fish in variant M3, while the final weight of fish from the other variants ranged from 1520 to 1590 g.

The daily weight increase of carp throughout the test did not differ significantly among the feeding group (Table 18). The value of Specific Growth Rate calculated for subsequent decades of the study was at a similar level, while lower levels, and in the third decade a statistically significantly lower one, were

Table 16
Chemical composition (% wet matter) of two-year old carp body before and after the test[1]

Component	Before the test	After the test in diet			
		TO1	TO2	TO3	TO4
Dry matter	28.94 ± 2.1[a]	31.65 ± 1.72[a]	31.35 ± 1.84[a]	29.47 ± 1.14[a]	28.94 ± 2.06[a]
Crude protein	11.34 ± 0.65[a]	14.93 ± 1.92[a]	15.31 ± 1.78[a]	14.33 ± 1.19[a]	13.06 ± 1.11[a]
Crude fat	13.64 ± 0.11[a]	12.78 ± 1.17[a]	13.02 ± 1.35[a]	11.93 ± 0.99[a]	11.14 ± 1.14[a]
Ash	2.22 ± 0.17[b]	1.66 ± 0.21[b]	1.57 ± 0.2[a]	1.59 ± 0.18[a]	2.17 ± 0.35[b]

[1]Values are mean ± standard deviation (SD) from triplicate groups of fish. In rows mean values with different superscript are significantly different (P<0.05).

Table 17
Chemical and amino acid composition of dry matter (DM), caloric value and chemical nutritive value of protein (the Chemical Score CS, and the Indispensable Amino Acids Index IAAI) of the experimental diets for market carp

Properties	Diet			
	M1	M2	M3	M4
Crude protein (% DM)	25.2	25.1	25.2	25.2
Essential amino acids (g 100 g^{-1} protein)				
Arginine	5.4	5.5	5.6	5.7
Histidine	3.4	3.3	3.2	3.2
Lysine	6.7	6.2	5.9	5.7
Tryptophan	3.0	2.3	1.9	1.5
Phenylalanine +Tyrosine	5.5	5.8	5.9	6.1
Methionine + Cystine	2.9	3.0	3.2	3.0
Threonine	4.1	4.1	3.9	4.0
Leucine	7.4	7.6	7.8	7.9
Isoleucine	3.7	3.8	3.8	3.9
Valine	5.3	5.4	5.5	5.5
Crude fat (% DM)	4.0	3.4	3.1	2.8
Nitrogen-free extract (% DM)	49.7	49.9	49.9	50.2
Raw fibre (% DM)	4.8	4.8	4.8	4.9
Ash (% DM)	6.9	6.3	5.9	5.5
Total phosphorus (% DM)	0.8	0.8	0.8	0.7
Calcium (% DM)	1.2	1.2	1.2	1.2
Gross energy (MJ kg^{-1})	16.06	15.83	15.74	15.67
E/P (kJ g^{-1} protein)	63.72	63.08	62.46	62.2
IAAI	72.5	72.6	72.6	72.4
	Met+Cys	Met+Cys	Met+Cys	Met+Cys
CS	50.7	46.5	44.1	41.9

noted in the group that received diet based on fish meal. The value of the feeding coefficient in the various decades of the test, and also during the entirety of the study, was equal and ranged from 2 to 2.5. Relatively high values of the feeding coefficient resulted from the low levels of protein (25%) in the diets. However, the nutritional value of the diets confirms a high coefficient of protein utilization (PER) that was within the range of 1.19 to 1.43. The equalization of these parameters (lacking statistically significant inter-group differences) indicates that a large share of plant protein components can be used in diets for market carp.

The indicators for protein and fat retention also demonstrated that the nutritional components of the diets were well used. Protein retention values did not differ significantly among the feeding group and were within the range of about 26-34%. The level of fat retention in the carp in variant M4 was significantly lower than all the others, at about 190%, while in the other feeding groups this value exceeded 200%. These high levels of fat retention confirm the natural tendency of older carp to store a large amount of lipids. This was also confirmed by the high amount of fat in the bodies of fish at the end of the experiment (11.8 to 15.6%) as compared to the values on the day the test began (12.2%) (Table 19). Carp on diets M1 and M2 had elevated lipids at the end of the test. A significant increase in ash content in carp bodies after the end of the experiment was also noted (with the exception of fish from variant M2), while the dry matter and protein levels did not change (Table 19).

Table 18
Mean fish body weight and values of market carp rearing indices[1]

	Diet			
Days of test	M1	M2	M3	M4
Mean fish body weight (g)				
start	970.7 ± 1.1[a]	969.3 ± 5.8[a]	972.3 ± 7.2[a]	965.7 ± 9.3[a]
10	1125.3 ± 4.0[a]	1124.7 ± 15.9[a]	1146.0 ± 18.7[a]	1115.0 ± 26.2[a]
20	1235.7 ± 2.3[a]	1246.0 ± 16.5[a]	1282.7 ± 18.7[a]	1252.0 ± 14.8[a]
30	1307.0 ± 19.2[a]	1350.7 ± 32.1[a]	1386.3 ± 29.4[a]	1364.0 ± 27.1[a]
40	1425.0 ± 17.9[a]	1483.1 ± 28.1[a]	1539.0 ± 50.2[b]	1490.7 ± 51.2[a]
50	1522.0 ± 29.6[a]	1568.3 ± 42.6[a]	1650.7 ± 40.3[b]	1592.7 ± 50.1[a]
SGR (% d^{-1})				
1-10	1.48 ± 0.05[a]	1.49 ± 0.08[a]	1.64 ± 0.12[a]	1.44 ± 0.22[a]
10-20	0.94 ± 0.05[a]	1.02 ± 0.02[a]	1.13 ± 0.02[a]	1.16 ± 0.16[a]
20-30	0.56 ± 0.14[a]	0.81 ± 0.1[b]	0.78 ± 0.08[b]	0.86 ± 0.14[b]
30-40	0.86 ± 0.07[a]	1.28 ± 0.56[b]	1.04 ± 0.12[a]	0.89 ± 0.23[a]
40-50	0.66 ± 0.09[a]	0.91 ± 0.7[a]	0.7 ± 0.19[a]	0.66 ± 0.05[a]
1-50	0.9 ± 0.04[a]	0.96 ± 0.04[a]	1.06 ± 0.04[a]	1.0 ± 0.07[a]
FCR				
1-10	2.2 ± 0.08[a]	2.19 ± 0.13[a]	1.97 ± 0.15[a]	2.31 ± 0.4[a]
10-20	2.15 ± 0.13[a]	1.95 ± 0.04[a]	1.76 ± 0.03[a]	1.73 ± 0.27[a]
20-30	2.59 ± 0.79[a]	2.43 ± 0.21[a]	2.49 ± 0.25[a]	2.28 ± 0.41[a]
30-40	2.23 ± 0.19[a]	2.06 ± 0.23[a]	1.83 ± 0.2[a]	2.28 ± 0.69[a]
40-50	2.23 ± 0.3[a]	2.66 ± 0.48[a]	2.16 ± 0.56[a]	2.2 ± 0.19[a]
1-50	2.36 ± 0.12[a]	2.21 ± 0.09[a]	1.99 ± 0.07[a]	2.11 ± 0.17[a]
PER				
1-10	1.29 ± 0.04[a]	1.30 ± 0.08[a]	1.45 ± 0.11[a]	1.25± 0.21[a]
10-20	1.33 ± 0.08[a]	1.46 ± 0.03[a]	1.61 ± 0.03[a]	1.66 ± 0.24[a]
20-30	0.82 ± 0.21[ab]	0.69± 0.04[a]	1.15 ± 0.12[bc]	1.27 ± 0.23[c]
30-40	1.28 ± 0.12[a]	1.39 ± 0.16[a]	1.56 ± 0.18[a]	1.32 ± 0.36[a]
40-50	1.29 ± 0.18[a]	1.09 ± 0.2[a]	1.38 ± 0.4[a]	1.3 ± 0.11[a]
1-50	1.21 ± 0.06[a]	1.19 ± 0.12[a]	1.43 ± 0.05[a]	1.35 ± 0.1[a]
PR (%)				
1-50	26.14 ± 3.01[a]	28.9 ± 2.59[a]	33.77 ± 2.93[a]	27.22 ± 3.15[a]
FR (%)				
1-50	226.79 ± 23.44[b]	208.3 ± 28.0[b]	217.0 ± 28.34[b]	189.5 ± 19.52[a]
SR (%)				
1-50	100	100	100	100

[1]Values are mean ± SD from triplicate groups of fish. In rows mean values with different superscript are significantly different ($P<0.05$).

From a practical viewpoint, an important element in evaluating the suitability of a diet for rearing market size fish, in addition to the proximal analysis of the body, is to evaluate the sensory properties of the meat. The results of sensory tests of market carp meat are presented in Table 20. No statistically significant inter-group differences were noted among any of the characteristics analyzed. This confirms the conclusion that the diets fed to fish does not affect the sensory properties of its meat.

Table 19
Chemical composition (% wet matter) of market carp body before and after the test[1]

Component	Before the test	After the test in diet			
		M1	M2	M3	M4
Dry matter	30.35 ± 1.9[a]	34.08 ± 2.42[a]	31.35 ± 1.84[a]	32.6 ± 1.88[a]	30.7 ± 2.54[a]
Crude protein	14.41 ± 1.05[a]	14.81 ± 1.75[a]	15.31 ± 1.78[a]	15.42 ± 1.66[a]	14.41 ± 1.58[a]
Crude fat	12.22 ± 0.98[a]	15.61 ± 2.23[c]	13.02 ± 1.35[bc]	12.7 ± 1.91[a]	11.76 ± 1.36[a]
Ash	1.74 ± 0.21[a]	2.61 ± 0.19[b]	1.57 ± 0.2[a]	2.62 ± 0.15[b]	2.8 ± 0.25[b]

[1]Values are mean ± SD from triplicate groups of fish. In rows mean values with different superscript are significantly different (P<0.05).

Table 20
Results of sensory analysis of market carp meat after the growth test (n=10)[1]

Evaluated characteristic	Diet			
	M1	M2	M3	M4
Colour	0.60 ± 0.15[a]	0.49 ± 0.16[a]	0.47 ± 0.11[a]	0.52 ± 0.19[a]
Smell	0.56 ± 0.20[a]	0.51 ± 0.12[a]	0.56 ± 0.20[a]	0.48 ± 0.15[a]
Firmness	0.60 ± 0.18[a]	0.51 ± 0.06[a]	0.54 ± 0.08[a]	0.56 ± 0.16[a]
Juiciness	0.53 ± 0.18[a]	0.42 ± 0.09[a]	0.44 ± 0.12[a]	0.52 ± 0.17[a]
Taste	0.53 ± 0.15[a]	0.52 ± 0.23[a]	0.49 ± 0.19[a]	0.58 ± 0.23[a]
Overall impression	0.52 ± 0.15[a]	0.49 ± 0.06[a]	0.46 ± 0.13[a]	0.51 ± 0.18[a]

[1]Values are mean ± SD. In rows mean values with different superscript are significantly different (P<0.05).

4.7. Effect of the share of plant protein in the dietary protein on carp growth and diet utilization

When mixtures of plant protein are used in diets, it is essential to determine which part of the protein pool actually comes from these sources. Bearing this in mind, the dependence between the share of plant protein in the overall protein pool in the various diets needs to be determined (calculated from its content in the components and their share of the diet) and the mean individual weight of carp and the value of the feed conversion ratio obtained during the experiments. In feeding tests with carp early juveniles, no distinct trend was noted concerning the impact of the quantity of plant protein on the individual weight of fish or on the utilization of the diet (Fig. 5). There were small, non significant differences among the experimental variants, which showed that diets for this carp stadium can contain up to 35% plant protein (maximum level applied in the experimental diets).

In carp juveniles rearing (Fig. 6), while the quantity of plant protein components had a more distinct impact on the individual weight of fish, no significant differences were noted in the values of the FCR. Similar body weights were obtained with diets comprised only of animal meal and that with a 31% share of plant protein. The comparison of the results obtained with carp early juveniles and juveniles indicate that plant protein can comprise from 30-35% of the protein pool of diets for these fish in the first year of rearing.

In the experiments with two-year old and market carp, the dependence between the results of rearing and the share of plant protein were distinct. In the case of two-year old carp, the most effective was the diet with a 45% share of plant protein (significance of differences confirmed statistically; Fig. 7). In the test with market fish, the highest individual carp weight

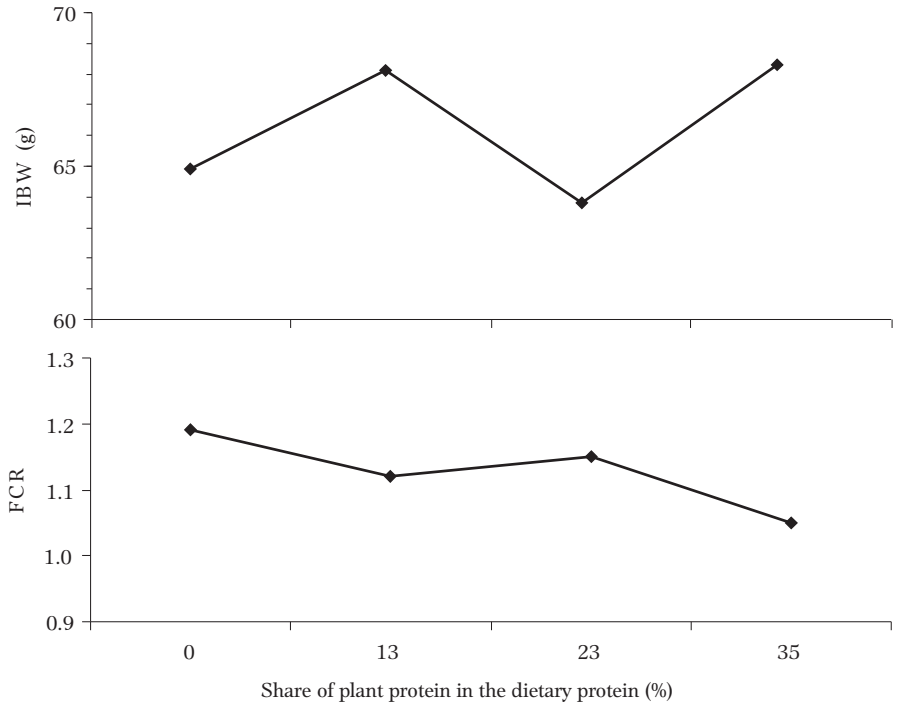

Figure 5. The dependence between the share of plant protein in the dietary protein and the mean individual fish body weight (IBW) and the value of the feed conversion ratio (FCR) obtained during the experiment with carp early juveniles

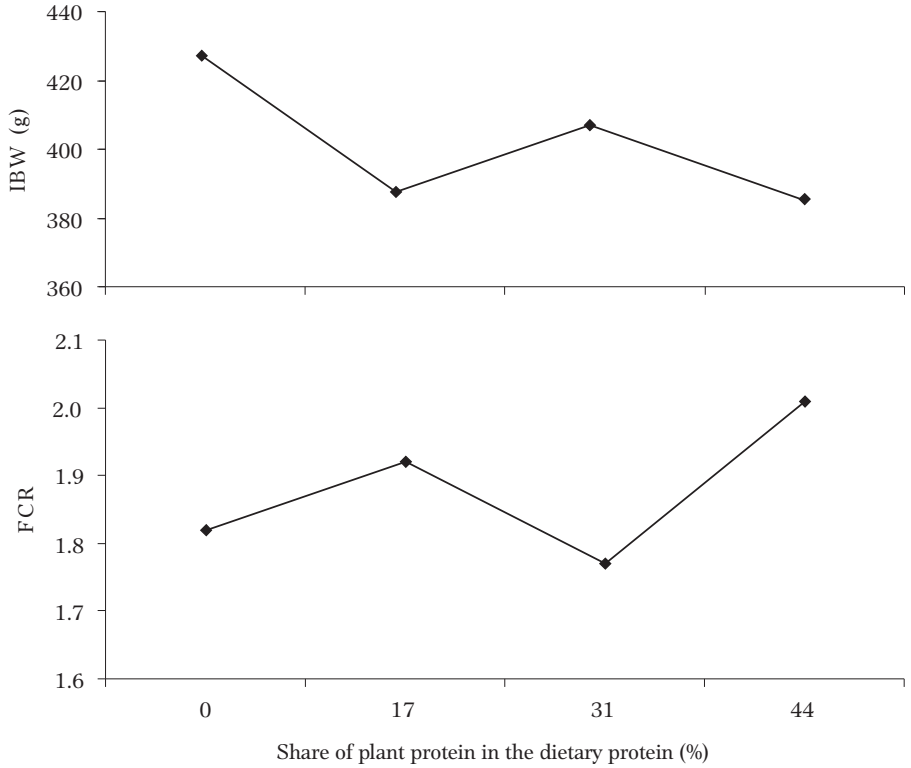

Figure 6. The dependence between the share of plant protein in the in the dietary protein and the mean individual fish body weight (IBW) and the value of the feed conversion ratio (FCR) obtained during the experiment with carp juveniles

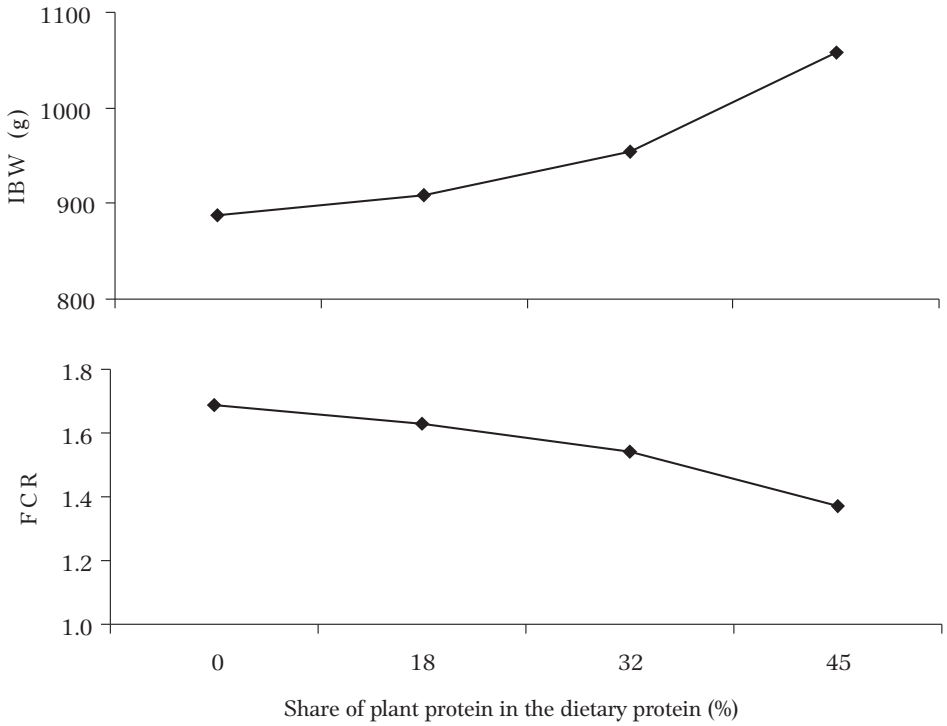

Figure 7. The dependence between the share of plant protein in the dietary protein and the mean individual fish body weight (IBW) and the value of the feed conversion ratio (FCR) obtained during the experiment with two-year old carp

and the most advantageous FCR, as in the experiment with two-year old carp, was obtained with the diet that had a 44% share of plant protein (Fig. 8). The maximum share of plant protein in diets for older carp age classes is 45%.

5. Discussion

5.1. Environmental conditions

Significant abiotic factors that determine growth during the rearing of carp in ponds are water temperature and water oxygen content. The optimal temperature for carp, determined in the laboratory as that at which there is maximum weight gain when feeding *ad libitum,* is relatively high (28°C, Aston and Brown 1978). According to Adelman (1978), this temperature is even higher, 29.6°C. The range of the thermal optimum for carp reared in ponds has been determined in many studies. As reported by Wolny (1974), Karpiński (1994), and Wojda (2006), the optimum water temperature for carp growth is within the range from 23°C to 32°C; at the latter temperature growth slows down (Jauncey 1982). According to Backiel (1964), rearing results are determined by the number of days during the rearing season on which water temperatures exceed 20°C, which was taken to be the threshold value of the optimal temperature range. According to Szumiec and Szumiec (1985), the low threshold of optimum temperature is 19°C. During the tests with early juveniles, juveniles, and two-year old carp, the number of days with water temperatures exceeding 20°C was 23, 25, and 27, respectively (Figs 1, 2 and 3), and the number of days at just one degree lower (above 19°C) was higher, 33, 34 and 33 respectively. In turn, during the test with market carp, the water temperature

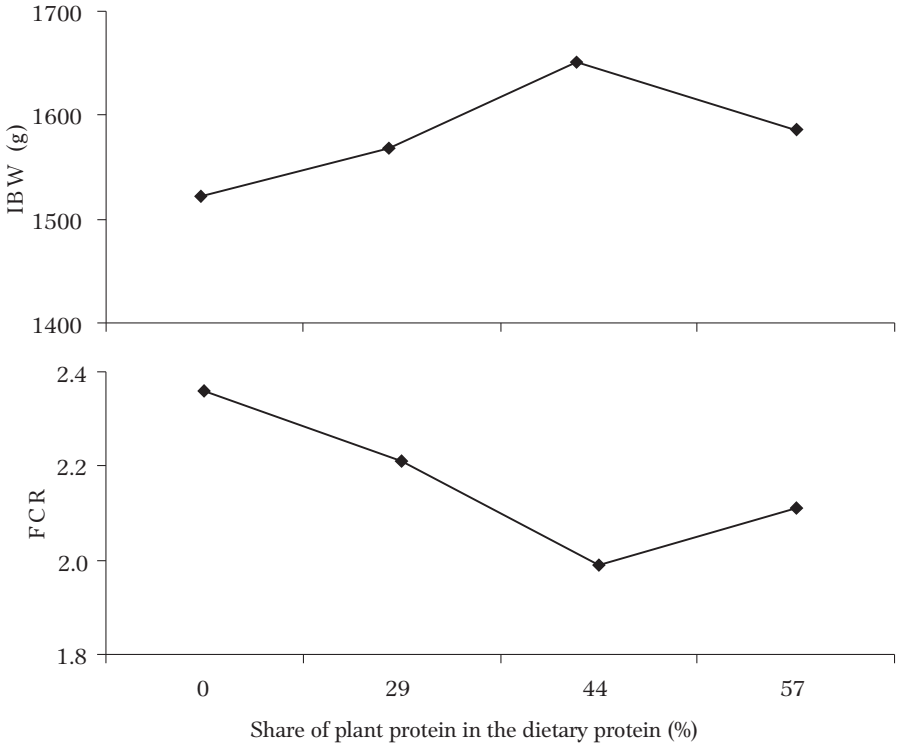

Figure 8. The dependence between the share of plant protein in the dietary protein and the mean individual fish body weight (IBW) and the value of the feed conversion ratio (FCR) obtained during the experiment with market carp

was lower and only on six days did it exceed 20°C, while for 24 days it was above 19°C (Fig. 4).

The concept introduced by Szumiec (1984) of an effective rearing temperature for carp at which an advantageous growth rate is maintained, is significant for practical reasons. The range is from 14 to 28°C, while feeding carp a full-portion diet increases the growth rate and decreases the lower threshold of effective temperature to 13°C (Szumiec and Szumiec 1985). This is also why the application of complete diet stimulates similar carp growth at lower temperatures to that at higher temperatures when the fish are fed cereal grains. The temperature range in all the experiments performed was within the effective range for carp.

The quantity of dissolved oxygen in the water is the second factor of key importance in fish rearing. According to Starmach (1963), carp have low oxygen requirements with a minimum of 2 mg dm^{-3} and an optimum of 3-5 mg dm^{-3}. These values concur with the recommendations of Karpiński (1994). The oxygen requirements of fish change with age, and usually decrease with growth. For example, the requirements of two-year old carp are 50-70% less than that of juveniles, while they are 30-40% less for market carp (Guziur et al. 2003). In fish culture the amount of oxygen dissolved in the water determines the possibilities of consuming and assimilating food. Carp exhibit full physiological capacity, as expressed by metabolic rate, in water that is over 70% saturation. At levels below 30% saturation (or below 3 mg O_2 dm^{-3}), it is recommended that feeding is either limited or discontinued until the oxygen conditions improve (Krüger 2000). According to the recommendations of Zobel (1982), the optimum oxygen content in water during carp rearing ranges from 6 to 8 mg O_2 dm^{-3}, which allows for short-term decreases to 3.5 mg O_2 dm^{-3}. During

the present tests, the water oxygen level did not fall beneath 3.1 mg O_2 dm^{-3}.

The optimal water pH used in carp rearing should be close to neutral (pH 6.8-7.5; Wolny 1974). According to Zobel (1982), the optimal water pH range in carp ponds is a little wider, 6.5 to 8.5. The water pH in ponds fluctuates, especially in those that are used intensively. The amount of ammonia in the water is linked strictly with water pH; when water pH is high (above 9), it is transformed into the undissociated form that is toxic to fish. The pH values registered during the feeding tests (Table 8) did not exceed the threshold recognized as optimal for carp. This was also confirmed in the high carp assimilation noted during the present tests.

5.2. Experimental diets composition

Obtaining the desired effects of fish rearing is conditioned by the application of diets that have the optimum balance in terms of the quantity and quality of nutritional components. Over the years, research of carp nutrition has provided the foundation for identifying the specific nutritional requirements of the species as well as many aspects that are related to feeding, including behavior and chemoreception. The nutritional requirements of fish are species specific and change with age (size), but they also depend on the physiological state of the fish (Steffens 1986, Jobling 1994). Defining the nutritional requirements of carp for protein was the focus of research undertaken in the late 1960s by Nose (1967) and Ogino and Saito (1970). Further many papers were published that reported the quantity of total protein required by carp. Ogino and Chen (1973) and Ogino (1980a) stated that the daily carp requirement for protein was about 1 g per kilogram of body weight to cover metabolic processes and about 12 g per kilogram of body weight to attain maximum protein retention. The optimal total protein content in carp diets should range from 30 to 38% wet weight according to Jauncey (1982) and Watanabe (1988). These quantities were determined with the application of a semi-synthetic diet containing single sources of high quality protein such as casein, egg protein, or fish meal. A diet that fully met the energy requirements of the carp had an optimum protein share of 30-35% Watanabe (1982). The American recommendations for animal feeding (NRC 1993) differed with regard to the optimum quantity of protein in carp diets depending on the developmental stage of the fish (it decreased with age). In comparison to the authors cited earlier, it is higher, as follows: larvae and early juveniles 43-47%; juveniles and two-year old fish 37-42%; market size fish and brood stock 28-32%. Kaushik (1995) reported that the optimal protein content in carp diet can reach even as high as 50% (in dry weight). This opinion appears to be confirmed by the fact that the protein content of dry matter in the natural carp diet is from 42 to 47% (Steffens 1986). The quantities of total protein in the experimental diets tested in the current study were adapted to the nutritional requirements for this component by the various age groups of carp. The highest protein content was in the experimental diet for carp early juveniles (38%), followed by that for juveniles (35%), and two-year olds (30%). The diet for the market carp had the lowest protein content (25%); the so-called protein sparing effect, which was observed in older fish stages, was taken into account. The older carp are able to have diets with lower protein content by meeting energy requirements with carbohydrates and fat without having a negative impact on growth (Watanabe et al. 1987, Shiau and Peng 1993, Steffens 1996).

The nutritional value of dietary protein is, to a significant degree, dependent on the quantity and quality of the amino acids composition. Increasingly, the term “protein requirement” is being replaced by the term “amino acid requirement” (Wilson and Halver 1986). Like most fish species, carp lack the ability to synthesize ten amino acids, the so-called essential ones, as follows: lysine, methionine, tryptophan, leucine, isolucine, valine, phenylalanine, tyrosine, threonine, histidine, and cysteine. The first three of these amino acids are most frequently limiting in carp diet. The carp requirement for essential amino acids was determined in tests performed by Nose (1979) and Ogino (1980b). Based on the profile

of amino acids in the fish bodies and the daily protein retention requirement, these authors estimated the threshold quantities of various essential amino acids expressed as their percentage share in the dietary protein. Later Dabrowski (1983a) assessed carp requirements for exogenous amino acids; he expressed them in quantities that should be delivered in the diet per day calculated by fish body weight. Dabrowski (1983b, 1986) demonstrated that the absorption of individual amino acids differs significantly depending on the protein source and the time that elapses from the moment the feed is consumed. The standard evaluation of fish diet quality should take into consideration meeting the requirements of all ten essential amino acids. All of the experimental diets tested in the current study met the carp requirement for essential amino acids with regard to the recommended quantity.

The effective utilization of the nutritional components of diets is closely linked to an appropriately balanced energy level, which, in practice, is most frequently expressed at the energy-protein ratio (E/P). Fish diets should contain from 33.5 to 42 kJ of digestible energy per 1 g of total protein since this guarantees that the dietary protein will be used for fish growth, and not to cover requirements for energy (Ogino et al. 1976, Takeuchi et al. 1979a). As cold-blooded animals, fish have far lower energy requirements in comparison to warm-blooded animals. In the instance of carp, the requirement for metabolic energy at water temperatures under 17°C is small. Chakraborti et al. (1992) and Kaushik (1995) demonstrated that there is a linear dependence between nitrogen retention and the energy level in the diet. The value of this is about 42 kJ per g of nitrogen. Diets with energy levels that are too low result in increased individual protein utilization, reduced growth rates, and a decrease of the feeding coefficient. The energy-protein ratio in the experimental diets EJ, J, and TO was very similar and oscillated within the limits of 48.05 to 55.44 kJ gross energy per 1 g of crude protein. In the experiment with market carp, the diet applied had a slightly higher energy-protein ratio (62-64 kJ g^{-1} protein) in relation to the "protein sparing effect", which was discussed above. The increase in fish weight obtained and the advantageous values of the feed nutritional component assimilation index confirms that the energy sources in the experimental diets were well balanced.

Carp is an omnivorous fish in which fat and carbohydrates are the dietary components that effectively cover energy requirements. This is why the level of digestible energy is more important than the quantity of fat. This was confirmed by Takeuchi et al. (1979b), who demonstrated that increase of the amount of digestible energy in diets from 13 to 15 MJ kg^{-1} with a 5-15% fat supplement did not result in better fish growth or higher protein retention. Excessive increases in the fat content of diets has a negative impact on carp meat quality since this species tends to store fats in the body mainly as fat deposits surrounding the organs (Zeitler et al. 1984, Murai et al. 1985). The quantity of crude fat in the diets applied in this experiment varied; the highest fat content was in the carp early juveniles diet (over 9%) and this decreased with carp age/size (over 8% for juveniles, about 4.5% for two-year old, and 2.8-4% for market carp). These differences were the immediate consequence of balancing the individual diets at a level appropriate to the energy-protein ratio; however, none of these fat contents reached that recommended for carp by Kaushik (1995): 12%. Earlier Ogino et al. (1976) and Takeuchi et al. (1979a) found that high carbohydrate values can serve as sources of energy in carp diets. The results of many tests indicate that the optimum range of carbohydrates in carp diets is from 30 to 40%. The high effectiveness of carbohydrate utilization (mainly starch) as a source of energy for carp stems from the activity of amylolytic enzymes, which is much higher in carp than in predatory fish (Murai et al. 1983). Additionally, producing carp feed with extrusion methods significantly increases the assimilability of the starch, which increases the digestible energy pool of the diet (Takeuchi et al. 1990).

In animal nutrition, including that of fish, the quality of the fat is considerably more important than the quantity. This stems from the functional properties of fats, and the role they play in many metabolic

processes. Determining the quantitative requirements of carp for essential fatty acids is fairly difficult, because the first symptoms of the deficiency of a given fatty acid do not become apparent until the diet deficient in this component is consumed for a long period of time. To date, the requirements of carp have been determined for only two fatty acids; linoleic (18:2 n-6) and linolenic (18:3 n-3), which should comprise 1% each of the dietary lipids (Takeuchi and Watanabe 1977). A later study by Kaushik (1995) indicated that carp requirements for essential fatty acids is lower. Recent studies indicated that phospholipids play a significant role in the nutrition of juvenile carp stages (Coutteau et al. 1997). These fats not only impact the physical properties of the diet (texture, resistance to oxidation, water stability), but they also impact fish health (deficiencies in them result in fat droplets gathering in enterocytes, increased epithelial cell height, and a decrease in the mean capacity of hepatocytes; Geurden et al. 1995). This is why, in feed production, a phospholipids supplement in the form of soybean lecithin is added, which is also an emulsifier (Geurden et al. 1998).

Some species of C*yprinidae* family fed on plant matter in the natural environment, which may indicate their physiological capability to digest and assimilate structural carbohydrates (cellulose). This hypothesis was not confirmed in the case of common carp, because neither significant cellulolytic enzyme activity nor the presence of cellulose digesting bacterial flora were found in the digestive tract of this fish (Bergot 1981, Ufodike and Matty 1983). It is commonly accepted that carp diets cannot contain quantities of fiber in excess of 5.5%, but according to American animal feeding recommendations (NRC 1993) the share cannot exceed 8%. Bearing this in mind, the introduction of plant protein components to carp diets is limited mainly by the content of structural carbohydrates. The quantity of raw fiber in the experimental diets tested in the current study did not exceed 5.5%, which certainly did not impact carp growth or the conversion of the nutritional components of the diet. While developing the formulas for the experimental diets, with low shares of legume-rapeseed mix, additional wheat or rye bran was added to increase and standardize the amount of crude fiber.

Determining the nutritional requirements of carp for vitamins and minerals has been the subject of many feeding experiments, and is currently well documented. The quantitative requirement of these components is expressed in the amount of ash, but it is much more important to determine the quantity of the various vitamins (especially the essential ones) and minerals. In practice, prepared vitamin-mineral mixes (premix) are used since they are formulated to cover the requirements of a specific fish species. With extrusion, a safety margin is set to compensate for losses during the technological process of feed manufacture; generally, the quantity of vitamin-mineral premix is two to five times higher than the recommended dose. In the present study all of the diets were supplemented with Polfamix W (in quantities of 1.5%) vitamin-mineral preparation, and the vitamin mix Vitazol AD_3EC (in quantities of 0.1%), which fully met the requirements of carp according to Satoh (1991), NRC (1993), and Kim et al. (1998). In carp nutrition, special attention is paid to the phosphorus content in a diet, not only because of its function in metabolic changes, but also its role in the eutrophication of waters, especially in pond rearing. The optimal quantity of this element in the diet was determined to be within a range of 0.6-0.8%, but it is important from which feed material it originates. The phosphorus in feed components occurs most frequently as mono-, di- or tricalcium phosphate. Tricalcium phophate from fish meal (white or brown) is much less assimilable for fish than is dicalcium phosphate (Satoh et al. 1997). In practice, phosphorus supplementation in fish diets is done with monocalcium phosphate. Viola et al. (1986) reported that carp diets with a high share of plant protein components require supplementation with monocalcium phosphate in quantities as high as 3%. This is due to the low assimilability of this nutrient, because in grains and seeds it is bound in phytate, and the digestive system of fish is incapable of hydrolyzing it. The diets used in the present study were supplemented with monocalcium phosphate to meet carp requirements for phosphorus, while chalk supplementation

was used to maintain the proper quantitative proportions of phosphorus to calcium.

5.3. Plant protein components in carp nutrition

Studies of the possibility of using plant protein in carp nutrition have mainly referred to soy bean and its products (isolates and protein concentrates) and locally available plant seeds (mainly from legumes) or by-products from the oil refining industry (oilcakes and extracted meals). The results of many experiments indicated that adding considerable amounts of soy bean meal, globally the most common replacement for fish meal, had a negative impact on fish growth. The probable causes cited as responsible for this phenomenon most frequently include the low digestibility of soy protein (Lei et al. 1996, Masumoto et al. 1996), the decreased organoleptic values of diets with high contents of soy bean or its product (Kim 1974, Mohsen and Lovell 1990), low energy levels (Viola et al. 1981), and the deficit of some amino acids (Jackson et al. 1982, Murai et al. 1986). The last of these causes was verified as early as in the first attempts to replace fish meal with soy bean meal in the diets of juveniles and two-year old carp (Viola et al. 1982). The partial (40%) replacement of fish meal required the addition of methionine and the supplementation of the energy pool with a 5% supplement of oil. The growth rate achieved then and the assimilation of the nutritional components of the diet were comparable to those of carp from the control group that was fed diet with fish meal as the only source of protein. Replacing fish meal in quantities of 40 to 100% by soy bean meal the diet must be supplemented with methionine and lysine (0.4-0.5%) and oil (10%). The study by Pongmaneerat and Watanabe (1993), in which the protein source for carp was a varying amount of soy bean meal, also confirmed this. As early as twenty days into the experiment, it appeared that despite the high digestibility of the protein, fish growth and diet utilization were both relatively low. The authors explained this as the result of a methionine deficiency in the diet.

The suitability of soy protein concentrates (SPC) as a basic source of protein in starters diets for carp was determined in the studies by Escaffre et al. (1997). One of the additional issues focused on in this study was to describe the impact soy bean trypsin inhibitor (SBTI) had on fish growth and the activity of the digestive enzymes. Carp larvae were fed for 21 days on diets containing 20, 40, 60, and 70% soy protein concentrate (SPC) or diets formulated using casein in the same proportions as SPC. It was revealed that introducing 40% SPC to the diet did not have a negative impact on either fish growth or survival. When applying higher levels of SPC, fish growth was observed to slow, and supplementing the diet with sulfur amino acids did not improve it. Studies by Escaffre et al. (1997) also show that limited growth of carp larvae fed diets with high quantities of SPC was probably caused not only by SBTI, but also by other unidentified antinutritional factors.

The impact of using plant protein components to fish diets has been studied primarily in short feeding tests. Long-term nutritional experiments were conducted by Noble et al. (1998), who fed the carp juveniles a diet with low amounts of protein (25%), but containing only raw materials of plant origin, and a commercial carp diet (32% protein), which contained 50% animal meal. Both of the diets were given to the fish for a year under laboratory conditions (aquaria) and in another experiment for seven months under natural conditions (ponds). The weight gain of carp fed diets based on soy bean meal was relatively low in the laboratory test (128%), while in the ponds the fish increased their body weight substantially (1190%). The advantageous results obtained in the ponds indicate that carp diet based on plant protein sources can be applied to advantage in semi-intensive rearing systems as a supplement to natural food resources (zooplankton).

The application of defatted soy bean meal in combination with corn gluten and meat meal as partial or total fish meal replacements in carp juveniles diet was the subject of a study by Pongmaneerat et al. (1993). The fish meal applied in the control diet was substituted with the components mentioned at levels of 56, 78, 89, and 100%. The effect of supplementing

diets without fish meal with crystalline amino acid was also determined. The highest carp weight gain and the most advantageous FCR and PER were obtained in the control group. Fish growth and feed utilization in the groups that received a diet in which 56% of the fish meal was substituted with soy bean meal and corn gluten was similar to that in the control group. Replacing larger amounts of fish meal with plant substitutes resulted in lowered carp growth rates and lower values of rearing indices. Interestingly, supplementing the diet without fish meal with essential amino acids to levels comparable to or double of those in the diet with fish meal resulted in fish growth elevated to 87 and 94%, respectively, of the results obtained in the control group.

Viola et al. (1988) studied the suitability of sweet lupin seeds for complete diets (with 30% protein) for carp reared in ponds. The weight gain in the fish obtained on this diet exceeded by a quarter growth in the control group fed a commercial diet with the same protein content. Carp fed a diet with a 45% share of lupin exhibited similar growth to that in the control group, even though the designated essential amino acid content in this diet was lower than that recommended for carp by the NRC (1993).

In addition to its impact on carp growth and the conversion of nutritional components in the diet, the type of raw plant materials used in diets can impact the slaughter yield, and, above all, the proximate composition of the fish (Steffens and Wirth 2007). Oberle et al. (1997) performed comparative studies in which two-year old carp were fed four isoprotein and isoenergetic diets with high participation of wheat (diet W), rice (diet R), maize (diet M), and lupine (diet L). The control group of carp received only frozen zooplankton (diet Z). After 105 days of the test, the weights of carp fed the cereal and lupin diets were similar (from 916 to 995 g); however, the fish fed zooplankton had significantly lower weights (659 g). The share of the skinless fillet (mean 38.7%), viscera (mean 13.2%), and the other parts of the carcass (mean 48.2%) were similar in carp fed the cereal and lupin diets, while the fillet of the fish fed zooplankton comprised a significantly lower share of the skinless fillet (33.6%) that was combined with an increased share of the other parts of the carcass (53%). The fish fed a diet with maize had the highest body fat content (14.5%), but it was slightly lower for the fish fed diets with wheat (13.3%) and rice (12.9%), while in the fish fed diets L and Z the fat content was the lowest at 10.7 and 8.2%, respectively. The protein content in the whole body was similar on the cereal and lupin diets (16.2 to 16.7%), while it was much lower in the carp fed zooplankton (15.5%).

Studies on the use of plants as a source of protein focused on introducing extracted rape meal to the diets of carp were carried out by Dąbrowski and Kozłowska (1981), and Mejza and Mejza (1986). The results of these indicated that it was possible to use this component effectively in quantities of up to 28%. Beginning in the 1990s, studies on optimizing dietary composition based on a wide variety of diet components, including plant proteins, were performed by Przybył et al. (1991, 1999a), and Przybył and Madziar (1997). For intensive carp rearing, the best results were obtained with diet that had a 10% share of sweet lupin (Przybył et al. 1999b). In an experiment conducted the next year, Przybył and Mazurkiewicz (2000) demonstrated the high effectiveness of carp rearing using diet containing 15% soy bean seeds, 9% sweet lupin seeds, 6% bitter lupin seeds, and 9% extracted rapeseed meal. The suitability of narrow leaf lupin seeds, both low- and high-alkaloid varieties, for two-year old carp diets was the subject of research conducted by Mazurkiewicz et al. (2007). In a 60-day growth experiment, four isoprotein diets with 15% sweet lupin seeds or 9% sweet lupin and 6% bitter lupin seeds were tested. Additionally, the diet composition was comprised alternately of soy bean meal and soy bean seeds or rapeseed meal and rape seeds. The results obtained indicate that carp utilized the diet containing sweet or sweet and bitter lupin seeds equally effectively. Similarly focused nutritional studies were also conducted by Filipiak and Trzebiatowski (1992), and Trzebiatowski and Filipiak (1992). The first experiment confirmed the possibility to replace 20% fish meal with lupin meal at a quantity of 36% without negative effects on the rearing indices. The results of the second experiment indicated that

market carp can be produced using a diet that contains up to 24% of extracted rapeseed meal.

The results of the present experiments indicated that the effective level of replacing fish meal with a mixture of plant protein components varied with different carp age/size groups. In the carp early juveniles or juveniles introducing mixtures of plant protein components in up to 26% and 22%, respectively, did not have a negative impact on the rearing results in comparison to fish that had been fed diets based on fish meal. In turn, the most advantageous effects in rearing two-year old and market carp were obtained by feeding them diets with, respectively, 32% and 27% of the plant protein component mixture. The values of these are concurrent with the results reported in the papers cited above.

6. Conclusions

1. The mixture of legumes seeds (white and yellow lupin, faba bean) and extracted rapeseed meal can partially replace fish meal in complete diets for carp.
2. The effective level of replacing fish meal with the mixture of the studied plant protein components varied depending on the age/size of carp.
3. The substitution of fish meal by a mixture of plant protein components in the diets for carp early juveniles and juveniles (in quantities 26% and 22%, respectively) did not cause inferior rearing results.
4. In two-year old and market carp the most advantageous rearing effects were obtained with a diet that had the mixture of plant protein components in amounts of 32% and 27%, respectively.
5. In diets for carp early juveniles and juveniles, plant protein can comprise from 30-35% of the total protein.
6. The maximum share of plant protein in the protein pool of the diets of two-year old and market carp is 45%.
7. The experimental diets for market carp did not have an impact on the sensory quality of the meat.

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8. Streszczenie

Wykorzystanie krajowych komponentów białkowych pochodzenia roślinnego w paszach dla karpia *Cyprinus carpio* L.

Celem pracy było określenie możliwości substytucji mączki rybnej mieszanką składającą się z nasion roślin strączkowych (łubiny: biały i żółty, bobik) oraz poekstrakcyjnej śruty rzepakowej w pełnoporcjowych paszach stosowanych w chowie poszczególnych grup wiekowych karpi na wysokim poziomie intensyfikacji. Badania wykonano w latach 2004-2006 w należącym do Uniwersytetu Przyrodniczego w Poznaniu Zakładzie Doświadczalnym Technologii Produkcji Pasz i Akwakultury w Muchocinie. Przeprowadzono cztery doświadczenia żywieniowe z wykorzystaniem różnych wielkości materiału obsadowego karpia: narybek letni (masa początkowa 2,7 g szt.$^{-1}$), narybek (masa początkowa 125 g szt.$^{-1}$), kroczek (masa początkowa 360 g szt.$^{-1}$) oraz handlówka (masa początkowa 969 g szt.$^{-1}$). Testy wzrostowe przeprowadzono w stawach doświadczalnych o powierzchni 40 m^2 każdy, zasilanych wodą indywidualnie w układzie otwartym. Podczas trwania testów prowadzono kontrolę parametrów fizykochemicznych wody w stawach: temperatury (°C) i zawartości rozpuszczonego w niej tlenu (mg O_2 dm^{-3}) oraz odczynu pH. We wszystkich paszach głównym nośnikiem białka pochodzenia zwierzęcego była mączka rybna, stopniowo zastępowana roślinnymi komponentami białkowymi w postaci mieszanek strączkowo-rzepakowych. W paszach dla narybku letniego, narybku i kroczka zastosowano mieszankę strączkowo-rzepakową wykonaną z nasion łubinu białego i żółtego oraz poekstrakcyjnej śruty rzepakowej, a w paszach dla handlówki karpia wykonaną z nasion łubinu białego i żółtego, bobiku oraz poekstrakcyjnej śruty rzepakowej. Pasze zadawane były codziennie z automatycznych karmników taśmowych dla ryb przez 12 godzin na dobę (9.00 – 21.00). Dzienne dawki paszy wyliczano jako procent biomasy ryb przy uwzględnieniu temperatury wody oraz wielkości ryb. Dla każdego z doświadczeń wyliczono następujące wskaźniki chowu: średni dobowy przyrost masy jednostkowej ryb (SGR), współczynnik pokarmowy pasz (FCR), współczynnik wydajności wzrostowej białka (PER), wskaźniki retencji białka (PR) i tłuszczu (FR) oraz przeżywalność ryb (SR). Ponadto oznaczono podstawowy skład chemiczny ciała ryb oraz pasz doświadczalnych. Po zakończeniu testu wzrostowego z handlówką wykonana została porównawcza analiza sensoryczna mięsa karpi metodą skalowania uwzględniająca następujące cechy: barwa, zapach, jędrność, soczystość, smak, wrażenie ogólne. Mieszanki nasion roślin strączkowych oraz poekstrakcyjnej śruty rzepakowej mogą być częściowym zamiennikiem mączki rybnej w pełnoporcjowych paszach dla karpi. Efektywny poziom substytucji mączki rybnej mieszanką roślinnych komponentów białkowych był zróżnicowany w zależności od grupy wiekowej karpi. Wprowadzenie do pasz dla narybku letniego oraz narybku karpia mieszanki roślinnych komponentów białkowych w ilościach, odpowiednio: do 26% oraz do 22% nie powodowało wpływu na przyrosty masy ciała ryb oraz wykorzystanie pasz. Z kolei najwyższe masy ciała oraz najkorzystniejsze wartości wskaźników chowu kroczka oraz handlówki karpia uzyskano skarmiając pasze zawierające, odpowiednio: 32% oraz 27% mieszanki roślinnych komponentów białkowych. Analiza wyników doświadczenia z narybkiem letnim karpia nie wykazała wyraźnej tendencji wpływu zastosowanych poziomów białka pochodzenia roślinnego w puli białkowej diet na uzyskane masy jednostkowe ryb oraz wykorzystanie pasz. Minimalne różnice (niepotwierdzone statystycznie) pomiędzy poszczególnymi wariantami doświadczalnymi wskazują, że białko paszy dla tego stadium karpia może zawierać nawet 35% białka pochodzenia roślinnego (maksymalny poziom zastosowany w paszach doświadczalnych). W chowie narybku karpia zaznaczył się wyraźniejszy wpływ ilości białka pochodzenia roślinnego na masy jednostkowe ryb, nie odnotowano natomiast istotnych różnic w wartościach współczynnika pokarmowego. Zbliżone masy jednostkowe narybku uzyskano skarmiając paszę wykonaną wyłącznie na bazie mączek zwierzęcych oraz w grupie, w której stosowano dietę z 31% udziałem białka pochodzenia roślinnego w białku ogólnym. W doświadczeniu z kroczkiem i handlówką karpia zależności pomiędzy wynikami chowu a udziałem białka pochodzenia roślinnego w białku ogólnym paszy były wyraźne. W przypadku kroczka karpia najefektywniejsza okazała się dieta z 45% zawartością białka roślinnego (istotność różnic potwierdzona statystycznie). W teście z handlówką najwyższe masy jednostkowe karpi oraz najkorzystniejsze wartości współczynnika pokarmowego pasz, podobnie jak w doświadczeniu z kroczkiem, uzyskano skarmiając paszę z 44% udziałem białka roślinnego. Pasze doświadczalne stosowane w żywieniu handlówki karpia nie miały wpływu na właściwości sensoryczne ich mięsa.