

Effects of incorporating in diets cold-pressed rapeseed cake on the growth performance, nutrient utilization, and body composition of common carp (*Cyprinus carpio* L.)

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Abstract. Alternative proteins from vegetal sources are being studied, because of the high costs and limited resources of fish meal. The aim of this study was to determine the possibility of including cold-pressed rape cake (CPRC) as a partial protein substitute in diets for common carp, Cyprinus carpio L. Common carp fry were stocked into experimental ponds at a density of 30 fish per pond. The effects on growth, feeding efficiency, and fish body composition were studied for four amounts of CPRC (0, 70, 130, 200 g \times kg⁻¹). Statistically significant higher final weights (528-530 g) were obtained with fish fed diets with 130 and 200 g \times kg⁻¹ CPRC. The fish growth rate was nearly identical in all variants (SGR of 3.3-3.4 % d⁻¹). Similar results were presented in FCR at 1.3, and in PER at 2.2. Our results suggest that it is possible to include up to 200 g \times kg $^{\text{-1}}$ of CPRC in diets for two-year old common carp without significant effects on growth, nutritive efficiency, or the proximate composition of the fish.

Keywords: carp fish feed, chemical composition of fish body, cold pressed rapeseed, growth trial, plant inclusion

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Introduction

One of the most important directions in aquaculture for several years has been the search for alternative, plant protein source replacements for fish meal (Watanabe 2002, Hardy 2008, Kraugerud and Svihus 2011). The most common plant feedstuff is soybean meal (SBM) largely thanks to its high protein level, balanced amino acid profile, and widespread availability (Gatlin et al. 2007). Alternative protein components are also by-products of the oil industry, such as oilcake or extracted meals from rape, sunflower, peanut, or cotton (Higgs et al. 1988, Davies and Gouveia 2010, Nogales et al. 2011). Detoxified Jatropha curacas kernel meal has been studied as an alternative dietary protein source in common carp feeds (Kumar et al. 2010). Rapeseed products contain from 35-37% total protein with advantageous amino acid profiles (Leming and Lember 2005), and at very low costs. However, using rapeseed in fish nutrition is limited because of the relatively high content of antinutritional factors, e.g., fiber, phytins, tannins, and glucosinolates (Francis et al. 2001). The aim of the study was to determine the effects of including various amounts of cold-pressed rapeseed cake (CPRC) in diets for common carp fry and their effect on growth performance, feeding efficiency, and fish body composition.

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Study area

The growth trial was conducted for 50 days (May 22 – July 10, 2006) in the Experimental Plant of Feed Production Technology and Aquaculture in Muchocin, Poznań University of Life Sciences. Experimental ponds, with a surface area of 40 m² each (constructed of concrete with sand on the bottom) were supplied individually with water from an open system. The ponds held a maximum water level, and water flow was constant. The individual water inlet and outflow systems allowed conducting cyclic control catches of all fish.

Materials and methods

Technical conditions

During the tests, water temperature (°C) and dissolved oxygen content (mg $O_2 \text{ dm}^{-3}$) were measured daily at 09:00 with a microcomputer oxymeter (ELMETRON CO 315, Elsent Wrocław, Poland). Water pH was measured once per week with a pH meter (WTW Multi Line P3, Weilheim, Germany). Dissolved oxygen, pH, and water temperature in the ponds were maintained within recommended ranges for common carp (Szumiec 1998). The average daily water temperature during the test ranged from 15.2 to 24.5°C. Extreme values of dissolved oxygen were highly variable from 3.9 to 8.0 (mg $O_2 \text{ dm}^{-3}$) (Fig. 1). The water pH during the trial was close to neutral and fluctuated within the range of 6.9 to 7.5.

Fish, diets, and feeding

Common carp fry with a mean weight of 100 g were obtained from a commercial farm and stocked into experimental ponds at a density of 30 specimens each. Four isonitrogenous (369-380 g \times kg $^{-1}$ CP) and isoenergetic (18.3-18.4 MJ \times kg⁻¹ GE) diets were formulated using commercial ingredients. The amounts of CPRC included were 0, 70, 130, and 200 g \times kg⁻¹ in diets RC0, RC7, RC13 and RC20, respectively. When the CPRC content was increased in the diets, the SBM level was reduced (Table 1). The yeast content in all of the diets was the same (80 g \times kg⁻¹). The carbohydrate component of wheat meal and rye bran was added to equalize the amount of raw fiber in the diets. Rapeseed oil was added to balance the energy levels of the diets and to form a hydrophobic film on the granule surface. The carriers of the vitamin and mineral compounds were Premix, Vitazol, and fodder chalk. Choline chloride was used as the lipotropic component, and the emulsifier was soy bean lecithin. The



Figure 1. Daily changes in water temperature and dissolved oxygen concentrations during growth tests.

Table 1

Ingredients and composition of experimental diets

	Diet				
	RC0	RC7	RC13	RC20	
Ingredients (g kg ⁻¹)					
Fish meal ^a	107	126	142	161	
Erythrocyte meal ^b	100	100	100	100	
CPRC ^c	0	70	130	200	
Soybean meal ^d	250	180	120	50	
Yeast	80	80	80	80	
Wheat meal	210	258	305	346	
Rye bran	150	100	50	0	
Rapeseed oil	65	53	40	30	
Soybean lecithin	5	5	5	5	
Premix ^e	15	15	15	15	
Vitamin premix ^f	2	2	2	2	
Chalk	15	10	10	10	
Choline Cl	1	1	1	1	
Analysed composition (g kg ⁻¹ dry mat	ter)				
Crude protein (CP)	380	377	373	369	
Crude lipid	95	93	88	88	
Crude fiber	41	41	40	39	
Ash	82	79	80	82	
Total phosphorus	8.6	9.6	10	11	
Calcium	13	12	13	14	
Essential amino acids (g 100 g ⁻¹ protein	.)				
Arginine	5.6	5.5	5.5	5.4	
Histidine	3.0	3.0	3.0	3.0	
Lysine	6.0	6.0	5.9	5.9	
Tryptophan	1.2	1.2	1.2	1.1	
Phenylalanine +Tyrosine	5.5	5.4	5.3	5.2	
Methionine + Cystine	2.4	2.6	2.7	2.8	
Threonine	3.8	3.7	3.7	3.6	
Leucine	7.8	7.7	7.6	7.5	
Isoleucine	4.5	4.3	4.1	3.9	
Valine	5.5	5.5	5.5	5.5	
Calculated values					
N-free extract,(g kg-1)	320	336	350	362	
Gross energy (GE) (MJ kg ⁻¹)	18.3	18.4	18.3	18.4	
GE/CP (kJ g ⁻¹ protein)	48.0	48.8	49.1	49.9	
CS	28.0	27.7	27.6	27.2	
IAAI	Met+Cys 16.0	Met+Cys 16.9	Met+Cys 17.7	Met+Cys 18.6	

^aDanish fishmeal, type F, 72% protein, 12% fat, FFSkagen, Denmark; ^bSpray dried, 90% protein, APC Europe, Spain; ^cCold-pressed rapeseed cake, 32% protein, 9% fat, 12% fiber, Semco Co., Poland; ^dSolvent extracted, 45% protein; ^ePolfamix W, BASF Polska Ltd. Kutno, Poland – containing per 1 kg: vitamin A 1000000 IU., vitamin D₃ 200000 IU, vitamin E 1.5 g, vitamin K 0.2 g, vitamin B₁ 0.05 g, vitamin B₂ 0.4 g, vitamin B₁₂ 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; ^fVitazol AD₃EC, BIOWET Drwalew, Poland – contains in 1 kg: vitamin A 50000 IU, vitamin D₃ 5000 IU, vitamin E 30.0 mg., vitamin C 100.0 mg

diets were prepared by extrusion processing with a single-screw warm extruder (Metalchem S-60 Gliwice, Poland). Processing conditions were 90°C cylinder temperature in the zone of increasing pressure, 100°C cylinder temperature in the zone of high pressure, 110°C head temperature, and 52 rpm speed screw and 6 mm nozzle diameter.

The fish were fed with an automatic band feeder for 12 hours per day (09:00-21:00). The daily diet ration was that of the carp feeding key developed by Miyatake (1997) taking into consideration water temperature and current fish weight. The size of the ration was determined every ten days based on monitoring weight measurements, which also served to calculate the rearing indices. The fish were sampled every 10 days, and the feed ration was adjusted accordingly.

Evaluation of effects

The effects of rearing procedures were evaluated based on stock biomass and diet utilization. Equations recommended by Hardy and Barrows (2002) were used.

• Specific growth rate (SGR, % day⁻¹)

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

 w_o – initial mean individual fish weight (g); w_t – final mean individual fish weight; t – number of study days.

• Mean absolute feed conversion ratio (FCR)

$$FCR = F_d \times (W_t - W_o)^{-1}$$

 F_{d} - weight of diet delivered (g), W_t – final fish weight (g), W_o – initial fish weight (g).

• Protein efficiency ratio (PER)

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

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

• Protein retention index (PR)

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

 P_t – total protein weight of fish at the end of the experiment (g), P_o – total protein weight of fish before the experiment (g), P – total weight of protein in diet fed to fish (g).

• Fat retention index (FR)

$$FR = (F_t - F_o) \times F^1$$

F_t - raw fat weight of fish at the end of the experiment (g), F_o - raw fat weight of fish before the experiment (g), F - raw fat weight in diet fed to fish (g).
Survival rate (SR)

 $SR = (N_t \times N_o - 1) \times 100$

 N_t – final number of fish (individuals), N_o – initial number of fish (individuals).

Chemical analysis

Chemical analyses were performed in three replicates. Diets and fish body composition were determined in accordance with AOAC (1996) procedures: dry matter (105°C to constant weight), crude protein with the Kjeldahl method (Kjel-Foss Automatic 16210 analyzer, AISN Foss Electric, Denmark), crude lipid with the Soxhlet method (extraction with ethyl ether for 12 h), raw fiber (Fibertec System M 1020 Hot Extractor, Tecator Flawil, Switzerland, ash by incinerating dried sample (5 g) at a temperature of 550°C for 12 h (furnace by Linn High Therm GmbH, Eschenfelden, Germany). 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).

Total amino acids were analyzed after hydrolyzation in 6N HCl at a temperature of 106°C for 24 h on an AAT 339 analyzer (Microtechna Prague, Czech Republic). Methionine and cystine were determined after they had been oxidized and fixed with formic acid. Tryptophan was determined with the colorimetric method.

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 gross energy of the diets was calculated from the proximate composition using the energy conversion factors for fish: 17.6 kJ × g⁻¹ carbohydrates, 23.9 kJ × g⁻¹ protein and 39.8 kJ × g⁻¹ lipid (Jobling 1994).

Statistical analysis

The data were evaluated using the Microsoft Excel spreadsheet, and analyzed with the statistical package Statistica 5 PL (StatSoft 2001). 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 sampling, while the values of the PR and FR indices were calculated only once. The normality of distribution was tested with the Kołmogorow-Smirnov test (the P-value for statistical significance was 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 time and diet type. Their interactions were also estimated (with the exception of PR and FR indices). Following the analysis of variance, post-hoc group analysis was also performed. Homogeneous groups were determined with the T-Tukey test.

Results

Growth performance and diet utilization

The mean individual weight of the carp did not differ significantly until day 30 of the growth test (Table 2). At the end of the third decade, the highest mean body weight was noted in variant RC20. It was significantly higher than the mean weight in groups RC0 and RC7. This tendency was observed until the end of the fourth decade. At the end of the trial, fish in both RC13 and RC20 groups had significantly higher mean body weights than did the fish in groups RC0 and RC7. The daily weight gain of carp throughout the test was quite similar at an SGR of 3.3 to 3.4% d^{-1} . Statistically significant inter-group differences

were found only in the second decade, during which the fish fed with diets RC13 and RC20 had higher SGR. The utilization of the nutritional components of the diet by the carp was very similar. The mean value of the FCR was 1.3, while the PER was 2.2. Significant inter-group differences were noted for each index during the test. In the second decade, FCR was significantly higher in groups RC0 and RC7. During the fifth decade, this parameter was higher in group RC20. PER was the highest in group RC20 in the second decade and the lowest in the same group in the fifth decade. Significant differences were not observed in fish survival during the growth test and values were in the 95-98% range (Table 2).

Protein retention in the fish bodies was similar, with no statistically significant inter-group differences. Lipid retention varied significantly; in group RC7 it was about 92% while in the other groups this value exceeded 100%. The highest lipid levels were stored in the bodies of fish fed diet RC13 (Table 2).

Fish body composition

The crude protein content did not change throughout the experiment (Table 3). Increased dry matter and crude lipid content were statistically significant in all groups, and there were nointer-group differences. A statistically significant decrease (P < 0.05) of ash content was found in the carp bodies in all variants.

Discussion

The lowest water temperature during the experiment was on days 14-19 of the trial, and after this it increased to 24°C. The dissolved oxygen content in the water was maintained within the optimal range for carp (min. 3.9 mg O₂ dm⁻³).Carp growth was relatively fast (SGR = 3.3-3.4% d⁻¹) and dietary nutrient conversion was effective (FCR = 1.3, PER = 2.2). This was achieved by using diets that were balanced correctly with regard to the content of nutritive components like total protein and fat (Ogino 1980), mineral components (Satoh 1991, NRC 1993), exogenous

Table 2
Effect of diet on growth performance, nutrient retention, and survival of carp (C. carpio)

	Diet					
Days of test	RC0	RC7	RC13	RC20		
Mean fish body we	eight (g indiv. ⁻¹)					
start	102.0 ± 2.1^{a}	103.0 ± 1.7^{a}	101.0 ± 2.0^{a}	102.0 ± 2.3^{a}		
10	151.9 ± 0.5^{a}	149.3 ± 3.9^{a}	149.6 ± 3.7^{a}	151.0 ± 5.4^{a}		
20	205.3 ± 1.3^{a}	203.8 ± 7.3^{a}	217.5 ± 9.8^{a}	221.3 ± 5.2^{a}		
30	284.4 ± 2.8^{a}	288.8 ± 6.1^{a}	303.6 ± 11.4^{ab}	312.3 ± 8.7^{b}		
40	394.1 ± 7.7^{a}	402.5 ± 8.5^{a}	421.4 ± 16.6^{ab}	443.1 ± 13.3^{b}		
50	497.7 ± 6.8^{a}	506.8 ± 6.5^{a}	$528.0 \pm 28.0^{\mathrm{b}}$	530.2 ± 9.3^{b}		
SGR ($\% d^{-1}$)						
1-10	4.2 ± 0.03^{a}	4.0 ± 0.26^{a}	4.0 ± 0.25^{a}	4.1 ± 0.35^{a}		
10-20	3.0 ± 0.08^{a}	3.1 ± 0.49^{a}	3.7 ± 0.21^{b}	$3.8 \pm 0.24^{\rm b}$		
20-30	3.2 ± 0.06^{a}	3.5 ± 0.20^{a}	3.3 ± 0.20^{a}	3.4 ± 0.16^{a}		
30-40	3.3 ± 0.10^{a}	3.3 ± 0.04^{a}	3.3 ± 0.40^{a}	3.5 ± 0.25^{a}		
40-50	2.3 ± 0.10^{a}	2.3 ± 0.08^{a}	2.3 ± 0.73^{a}	2.8 ± 0.40^{a}		
1-50	3.3 ± 0.03^{a}	3.4 ± 0.03^{a}	3.4 ± 0.07^{a}	3.4 ± 0.09^{a}		
FCR						
1-10	1.0 ± 0.01^{a}	1.1 ± 0.09^{a}	1.1 ± 0.08^{a}	1.1 ± 0.11^{a}		
10-20	$1.6 \pm 0.04^{ m b}$	1.5 ± 0.29^{b}	1.2 ± 0.08^{a}	1.2 ± 0.09^{a}		
20-30	1.4 ± 0.03^{a}	1.3 ± 0.09^{a}	1.4 ± 0.09^{a}	1.3 ± 0.07^{a}		
30-40	1.4 ± 0.08^{a}	1.4 ± 0.02^{a}	1.4 ± 0.22^{a}	1.3 ± 0.11^{a}		
40-50	1.1 ± 0.06^{a}	1.2 ± 0.05^{a}	1.3 ± 0.39^{a}	$1.6 \pm 0.41^{ m b}$		
1-50	1.3 ± 0.03^{a}	1.3 ± 0.01^{a}	1.3 ± 0.07^{a}	1.3 ± 0.05^{a}		
PER						
1-10	2.7 ± 0.03^{a}	2.6 ± 0.21^{a}	2.6 ± 0.19^{a}	2.7 ± 0.28^{a}		
10-20	1.7 ± 0.18^{a}	1.9 ± 0.35^{a}	2.2 ± 0.32^{ab}	2.4 ± 0.18^{b}		
20-30	1.9 ± 0.12^{a}	2.1 ± 0.21^{a}	2.0 ± 0.09^{a}	2.0 ± 0.06^{a}		
30-40	2.0 ± 0.12^{a}	2.0 ± 0.03^{a}	1.9 ± 0.25^{a}	2.2 ± 0.19^{a}		
40-50	2.5 ± 0.12^{b}	2.5 ± 0.10^{b}	2.4 ± 0.89^{b}	1.9 ± 0.45^{a}		
1-50	2.2 ± 0.05^{a}	2.2 ± 0.04^{a}	2.2 ± 0.09^{a}	2.2 ± 0.06^{a}		
PR (%)						
1-50	21.5 ± 1.3^{a}	22.1 ± 0.9^{a}	21.9 ± 1.9^{a}	22.8 ± 1.8^{a}		
FR (%)						
1-50	$105.8 \pm 10.0^{\rm b}$	91.9 ± 8.9^{a}	$108.2 \pm 7.7^{\rm b}$	$106.0 \pm 8.2^{\rm b}$		
SR (%)						
1-50	97.8 ^a	98.9 ^a	95.6 ^a	97.8 ^a		

Values are means \pm standard deviation (SD) from three replicate groups of fish. Mean values in each row with different superscripts are significantly different (P < 0.05)

Table 3

Effect of diet on chemical composition (g kg⁻¹ of wet matter) of carp (C. carpio) body before and at the end of the trial

		After the trial in diet				
Component	Initial	RC0	RC7	RC13	RC20	
Dry matter	222 ± 9.1^{a}	298 ± 5.5^{b}	285 ± 8.3^{b}	311 ± 11.2^{b}	293 ± 13.1^{b}	
Crude protein	124 ± 5.6^{a}	126 ± 6.6^{a}	126 ± 3.2^{a}	129 ± 5.0^{a}	124 ± 5.2^{a}	
Crude lipid	39 ± 1.1^{a}	131 ± 11.4^{b}	111 ± 13.0^{b}	$129 \pm 9.7^{\rm b}$	118 ± 15.2^{b}	
Ash	26 ± 1.3^{b}	18 ± 4.1^{a}	20 ± 2.9^{a}	20 ± 3.8^{a}	20 ± 1.9^{a}	

Values are mean \pm standard deviation (SD) from triplicate groups of fish. Mean values in rows with different superscripts are significantly different (P < 0.05)

amino acids for carp (Nose 1979), and the amount of energy and its relation to the content of total protein (Ohta and Watanabe 1996).

Studies on using rapeseed meal (RM) as a replacement for fish meal in fish diets were initiated by Yurkowski et al. (1978). This experiment showed it is possible to include up to 221 g \times kg⁻¹ of rapeseed meal in rainbow trout diets. However, there are few publications on using rapeseed and its derivative products in carp feeds. The dietary content of RM in fish diets was determined to be 200 to 300 g \times kg⁻¹ (Gomes et al. 1993). In the presented study, the growth rate, FCR, and PER were similar in all three groups fed experimental diets, but the highest final individual weight was obtained in fish fed diets RC13 and RC20 (130 and 200 g \times kg⁻¹ CPRC, respectively). In a study conducted by Trzebiatowski and Filipiak (1992), carp juveniles (initial average body weight 176 g) were fed diets containing different doses of RM. The most favorable results were obtained with the diet containing 80 g \times kg $^{-1}$ RM. However, the inclusion of up to 240 g \times kg⁻¹ RM did not result in a significant decrease of FCR, while SGR was similar to that obtained on the diet without RM. In a study conducted by Slawski et al. (2012) fish meal was replaced by rapeseed protein concentrate (RPC). Fish (initial average body weight 26.7 ± 0.8 g) were fed four diets with 0, 33, 66, and 100% fish meal replaced by RPC (diet R0, R33, R66, R100, respectively). No significant differences in growth parameters between fish fed diets R0 and R33 were noted. Higher RPC content led to reduced diet acceptance and growth performance, probably because the taste of the feed was affected by the rapeseed concentrate. The growth performance of common carp fry fed diets with various levels of pea seed meal varied (Davies and Gouveia 2010). Pea seed meal diets were prepared in three ways: with no treatment (NAT Diet), processed by autoclaving (AC Diet), and by dry roasting (DC Diet). The carp fed the NAT Diet had significantly lower final mean weights, SGR rates, and feed intake, while better results were noted in groups fed the thermally treated AC and DC diets.

The main limitations to adding greater amounts of RM or CPRC to fish diets are their antinutritive factors, primarily glucosinolans (GLS), the metabolites of which (thiocyanates, isothiocyanates) have goitrogenic activity in fish. Phytic acid, which is a reserve form of phosphorus occurring in seeds, significantly influences the digestibility and assimilability of dietary nutrients. Sensitivity to the antinutritive factors in rapeseed meal decreases with fish age (size) (Higgs et al. 1989). Nevertheless, because of its advantages, rapeseed products can be an important dietary component, especially for omnivorous fish. In the present study, the common carp used had an initial weight of about 100 g. Despite their small size and theoretically higher sensitivity to antinutritional factors, no negative effects on fish growth or survival were recorded.

The retention indices of protein and fat, which are distinctly correlated with their amounts in diets and with the degree of their utilization, are important indicators of diet nutritive effectiveness (Jobling 2001). In our study there were no statistically significant differences in the protein retention in fish bodies among groups (21.5-22.8%). This confirms the possibility of including RM in carp diets. Fat retention was correlated significantly with the amount of CPRC in the diet, and it was the highest in groups RC13 and RC20. The tendency to accumulate lipids in the body, mainly in the form of deposits of reserve fat accumulated around the organs, has a negative impact on the quality of carp meat (Murai et al. 1985).

The results of this study indicated that using CPRC in common carp fry diets had a positive effect on growth and feed utilization. In our previous study, a CRPC content of 330 g × kg⁻¹ in diets for common carp had no negative influence on the factors mentioned above or on meat quality (Mazurkiewicz et al. 2011). Moreover, adding CPRC to diets had no negative impact on fish body composition. We suggested than effective CPRC supplementation is up to 200 g × kg⁻¹ in practical diets.

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