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EVALUATION OF BAROTHERMALLY PROCESSED CASEIN AS A COMPONENT OF TROUT STARTERS. I. OPTIMISATION OF THE COMPOSITION AND PRODUCTION OF EXPERIMENTAL FEEDS

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ABSTRACT. Six experimental rainbow trout extruded starters were produced. Fish meal, feather meal, and fowl meal as a source of animal protein were supplemented with barothermally processed casein in amounts of 8-40% in particular experimental groups. Protein content in the feeds was 50%, and E/P coefficient was 8.8 kcal of digestible energy per 1 g of protein.
Physical properties of the starters were evaluated - water stability and buoyancy; chemical composition was analysed – Ca and P content, amino acid composition (CS and EAAI), fatty acid profile; and concentrations of toxic metals (mercury, lead, cadmium) were measured.

Key words: RAINBOW TROUT, FEEDING, STARTERS, CASEIN, EXTRUSION

INTRODUCTION

Trout artificial feeds are expensive due to their high total protein content (40-50%), mainly of animal origin, usually fish meal. Fish meal is hardly available, so there are many attempts to find substitutes having similar nitrogen content and nutritive value.

Fish meal in trout feeds was usually replaced with fowl or feather meals (Klik, Trzebiatowski 1982), or krill meal (Steffens, Albrecht 1980). Meat-and-bone meal (Pokorny 1982), acidified and raw cattle blood (Ausgreng 1986), pig cracklings (Wojno 1987), dried whey (Koops et al. 1981), fish silage (Stone 1989), pig bristles, and activated sludge (Gropp et al. 1981) were also tested. Usefulness of barothermally processed pig bristle in trout feeding was also evaluated by Przybył et al. (1995a, 1995b).

Other alternative animal protein sources are milk proteins, such as casein of high biological value and readily assimilated. Casein, and especially its salts – caseinates, show very good viscosity, water absorbing capacity, ability to emulsify fats which makes them very useful in feed production (Szpendowski 1991).

The main objective of the present study was to evaluate biothermally processed casein offal as an alternative component of extruded rainbow trout starters.

MATERIAL AND METHODS

Feed formulas were calculated using computer software written with the Simplex method in Turbo Pascal 5.0. The experimental feeds were made in the Feed Laboratory of the Experimental Centre of Feed Production Technology and Aquaculture in Muchocin.

The feeds were composed of fish meal, feather meal hydrolyzate, fowl meal, sodium caseinate, soybean powder, yeast, and ground corn. Formulas B, C, D, E, and F contained 8-40% of casein as a substitute for animal meal, especially the fish meal (Tab. 1).

TABLE 1

Feed composition (%)

Components	Feeds					
	A	B	C	D	E	F
Fish meal	45,0	37,0	29,0	21,0	13,0	6,0
Feather meal	8,0	8,0	7,0	5,0	3,0	2,0
Fowl meal	8,0	7,0	5,0	6,0	5,0	4,0
Na caseinate	**-**	**8,0**	**16,0**	**24,0**	**32,0**	**40,0**
Soybean powder	10,0	9,0	8,0	7,0	5,5	4,5
Maze	10,6	11,6	14,6	15,6	15,6	15,6
Candidayeast + vitamins	4,0	4,0	4,0	4,0	4,0	4,0
Soybean + rapeseed oil	10,0	11,0	12,0	13,0	13,6	14,0
Soybean lecithin	0,5	0,5	0,5	0,5	0,5	0,5
Polfamix W	1,5	1,5	1,5	1,5	1,5	1,5
Vitazol AD$_3$EC	0,1	0,1	0,1	0,1	0,1	0,1
Vitamin C + glucose	0,1	0,1	0,1	0,1	0,1	0,1
Choline chloride	0,2	0,2	0,2	0,2	0,2	0,2
Potato starch	0,0	0,0	0,0	0,0	3,9	5,5
Binding agent „Biodone AQ"	2,0	2,0	2,0	2,0	2,0	2,0
Totally	100,0	100,0	100,0	100,0	100,0	100,0

Offal casein from dairy industry was processed in the Casein Processing Plant „Lacpol" in Murowana Goślina. Processed casein (sodium caseinate) was produced by moistening casein to 15%, adding 30% NaOH solution, and barothermal processing at 140^{o}C in a French extruder Clextral.

Fish diets were supplemented with mineral and vitamin mixture Polfamix W, Vitazol AD_3EC, and vitamin C with glucose. Formulas E and F were additionally supplemented with potato starch used as a proteinless energy source.

Soybean oil was used as the major energetic substrate. It was stabilised with Rendox containing two antioxidants – butylated hydroxyanisole (BHA) and etoxyquinoline (EQ), added in amount of 0.1% of fat content.

Digestibility and assimilability of fats were improved with soybean lecithin as an emulsifier, and choline chloride as a lipotropic factor counteracting fatty liver degeneration.

The feeds were prepared according to the formulas, and extruded in an endogenous mono-worm food extruder N-60 made by „Metalchem" Gliwice, under the following conditions:

- Temperatures: I section – 105^{o}C, II section – 120^{o}C, head – 112^{o}C
- Rotational worm speed – 52 rpm
- Nozzle diameter – 4.15 mm
- Processing time – 42 s

Expanding pellets were cut into 5 mm pieces with a rotating tool, spread on sieves, cooled, and air dried. Diameter of dried pellets was 5.5-5.8 mm. Then, the pellets were ground to obtain 3 fractions of feed particles:

- 0.8-1.25 mm for 2 g trouts,
- 1.25-1.60 mm for trouts up to 10 g,
- 1.60-2.50 mm for trout fingerlings up to 25 g.

All fractions were sprayed with rape oil heated to 70^{0}C to produce a film (up to 4% of pellet weight).

Number of granules per 1 kg and water buoyancy were evaluated. The latter was performed using an electronic meter of sinking velocity of the particles.

Water stability of the feeds was also measured according to Hasting - Hepher method (Hepher 1968), based on per cent loss of pellet weight in water (with pond water movement simulation), and oxygen demand (in mg O_2/dm^3) being an indicator of environment pollution with soluble and easily decomposing feed components.

Chemical analysis of the feeds was done according to Skulmowski (1974). The following parameters were measured: dry mass, total nitrogen, crude fat, and crude fiber. Total protein content was calculated as nitrogen content multiplied by 6.25.

Digestible energy of the feeds was calculated from their chemical composition using conversion factors for fish: extruded carbohydrates – 2.5 kcal/g, protein – 5.2 kcal/g, and fat – 8.5 kcal/g (Halver 1988).

Amino acids were analysed in feed protein hydrolysed with 5N HCl, at 106°C for 2 h, in AAA-881 analyzer. Sulphur-containing amino acids: methionine and cystine were oxidised and preserved with formic acid before analysis. Tryptophan content was measured with colorimetric method (Voitsky, Gunkel 1989). Amino acid composition was used to calculate chemical value of the feeds. Chemical score was calculated (CS), and essential amino-acids index (EAAI).

Concentrations of mineral phosphorus and calcium compounds were measured using atomic absorption spectrophotometer AAS3, Carl Zeiss Jena, according to Gawęcki (1988).

Fatty acid profile was also determined in total lipids. Fat was extracted according to Folch's method (Pie et al. 1991).

Heavy metal content (copper, zinc, chromium, mercury, lead, and cadmium) was also measured in the feeds, using atomic absorption spectrophotometer Varian (Spectr AA100/200). The samples were mineralised in microwave mineralizer Star 6 made by CEM*, and analysed in atomic absorption spectrophotometer Varian (Spectr AA100/200)*, according to the methods described by Beaty (1988). For copper, zinc, chromium, and lead, flame atomic absorption was applied, and for mercury and cadmium – atomic absorption with atomization in graphite furnace.

RESULTS

Fraction 0.8-1.25 mm of feed A contained the highest number of granules (1392 10^3 kg^{-1}), and showed the lowest sinking velocity (37.9 m s^{-1}) (Tab. 2).

Hastings-Hepher test revealed feed weight loss from 15.6% in F formula to 34.7% in A formula (Tab. 3). Oxygen demand ranged from 42.2 for F to 66.1 mg O_2 dm^{-1} for A.

Total protein content in the diets ranged from 50.0 to 50.4% which indicates similar nitrogen content. Crude fibre content did not exceed 0.7%, and crude fat was from 17.4 to 18.6% (Tab. 4).

TABLE 2

Physical properties of the feeds

Feeds	Pellet number in thousand ind. kg^{-1}			Sinking velocity $s \cdot m^{-1}$		
	fraktion			fraktion		
	0,8 - 1,25	1,25 - 1,60	1,60 - 2,50	0,8 - 1,25	1,25 - 1,60	1,60 - 2,50
A	1392	419	158	37,9	26,2	20,9
B	1379	404	156	36,2	24,7	19,4
C	1332	398	149	35,5	24,1	18,6
D	1294	395	146	35,1	23,1	18,4
E	1222	378	145	31,8	22,5	18,1
F	1197	361	141	29,7	22,2	17,1

TABLE 3

Water stability of the feeds

Parameter	Feeds					
	A	B	C	D	E	F
Weight loss %	34,7	29,8	24,4	20,5	17,3	15,6
Score	dst	dobra	dobra	dobra	dobra	dobra
Oxygen demand $mgO_2 \cdot dm^{-3}$	66,1	51,2	47,9	46,6	44,9	42,2
Score	good	good	very good	very good	very good	very good

TABLE 4

Chemical composition of the feeds (%)

Parameter	Feeds					
	A	B	C	D	E	F
Total protein	50,0	50,3	50,0	50,4	50,2	50,0
Crude fat	18,6	18,6	18,4	18,6	18,0	17,4
ZBAW	13,4	13,6	15,2	15,4	17,4	19,1
Crude fiber	0,6	0,6	0,7	0,7	0,6	0,6
Ash	8,3	7,6	6,6	6,3	5,5	4,8
Phosphorus	2,0	1,8	1,5	1,4	1,1	0,9
Calcium	3,3	2,9	2,4	2,1	1,7	1,3

Level of essential amino acids, especially of arginine, tryptophan, isoleucine, leucine, and phenylalanine in the diets differed considerably. Content of lysine, methionine and cystine was similar in all feeds (Tab. 5).

TABLE 5

Essential amino acids in the feeds (in g per 100 g of protein).

Amino acids	Feeds					
	A	B	C	D	E	F
Arginine	6,66	6,24	5,79	5,42	5,00	4,58
Histidine	3,28	3,18	3,11	3,07	3,01	2,99
Lysine	7,41	7,34	7,35	7,40	7,42	7,54
Tryptophan	4,40	3,76	3,19	2,62	2,06	1,57
Fenyloalanine + Tyrosine	7,94	8,49	9,06	9,59	10,16	10,74
Methionine + Cystine	3,40	3,46	3,48	3,41	3,43	3,39
Threonine	4,21	4,22	4,22	4,20	4,20	1,19
Leucine	7,38	7,76	8,13	8,43	8,79	9,13
Isoleucine	4,15	4,49	4,84	5,13	5,46	5,79
Valine	5,16	5,50	5,80	6,09	6,31	6,57

Methionine and cystine were the limiting amino acids in all feed formulas. Value of essential amino acid index (EAAI) was high and ranged from 82.16 to 87.19.

Energy – protein ratio (E/P) was similar for all diets, and ranged from 8.73 to 8.82 (Tab. 6).

TABLE 6

Chemical value of protein, and the level of digestible energy of the feeds

Parameter	Feeds					
	A	B	C	D	E	F
CS	Met+Cys 58,62	Met+Cys 59,66	Met+Cys 60,00	Met+Cys 58,79	Met+Cys 59,14	Met+Cys 58,45
EAAI	82,16	83,57	84,37	84,77	87,02	87,19
Digestible energy $kcal \cdot kg^{-1}$	4345,0	4397,1	4390,9	4430,9	4570,7	4556,2
E/P	8,82	8,75	8,78	8,79	8,76	8,73

Table 7 shows fatty acid profile in total lipid content of the feeds, from myristic acid ($C^0{}_{14}$), to erucic acid ($C^1{}_{22}$). In unsaturated acid group, oleic acid predominated ($C^1{}_{18}$), comprising 44.38 to 52.05%. Among the essential unsaturated fatty acids, linoleic acid was the most abundant ($C^2{}_{18}$) – 15.87-19.52%.

TABLE 7

Fatty acid content in the feeds (%)

Fatty acid	Feeds					
	A	B	C	D	E	F
C^0_{14}- myristic	1,40	1,23	0,94	0,83	0,66	0,46
C^0_{14}- palmitic	9,11	8,62	7,65	7,52	6,92	6,39
C^1_{16}– oleopalmitic	1,72	1,52	1,13	1,06	0,84	0,65
C^0_{18} - stearic	2,81	2,70	2,48	2,47	2,32	2,18
$C^1_{18\ \omega9}$ - oleic	44,38	45,91	48,17	49,33	50,46	52,05
$C^1_{18\ \omega7}$ - waccenic	3,71	3,54	3,55	3,50	3,83	3,73
$C^2_{18\ \omega6}$ - linoleic	15,87	16,66	18,06	18,49	18,91	19,52
Cis/trans	0,42	0,47	0,50	0,52	0,54	0,57
$C^3_{18\ \omega3}$ - linolenic	5,63	6,05	6,50	6,53	6,94	7,20
Cis/trans	0,41	0,44	0,49	0,49	0,52	0,55
C^0_{20} - arachidonic	0,54	0,54	0,56	0,56	0,57	0,58
$C^1_{20\ \omega9}$ - eicosanoic	2,83	2,64	2,40	2,26	2,17	2,02
C^0_{22} - behenic	1,28	1,10	0,30	0,59	0,39	0,20
$C^1_{22\omega9}$ – erucic	1,24	1,24	1,62	1,30	1,32	1,35

Metal concentrations in the diets are shown in Tab. 8. Among toxic elements, lead concentration ranged from 1.54 to 2.54 ppm, and content of mercury from 0.26 to 0.61 ppb. No traces of cadmium were detected.

TABLE 8

Concentrations of metals in the feeds

Metals	Feeds (ppm)					
	A	B	C	D	E	F
Copper Cu	0,41	0,44	0,46	0,40	0,29	0,40
Zinc Zn	2,56	2,53	2,35	1,98	1,91	2,08
Lead Pb	1,90	2,12	2,26	1,54	1,97	2,54
Chromium Cr	0,49	0,44	0,13	0,32	0,18	0,10
Cadmium Cd	-	-	-	-	-	-
	(ppb)					
Mercury Hg	0,61	0,53	0,42	0,26	0,30	0,27

DISCUSSION

Taking into consideration the physical properties – number of granules per kg, and buoyancy (s m^{-1}) (Tab. 2) – all experimental feeds met the requirements recommended by a Norwegian company FK Norsk Bioakva (1992/93).

According to the criteria set by Szumiec and Stanny (1975), the feeds showed good (A and B) or very good (C, D, E, and F) oxygen demand. Except diet A, which showed the highest weight loss (satisfactory), all other feeds were scored as good. Water stability of the diets is affected by various factors such as composition, production technology, binding additives, or covering films (Stanny 1987). In all tested feeds, binding agent Boidone AQ made by International Speciality Products was used, and the granules were coated with hydrophobic film of heated soybean and rape oil mixture. Despite this, not all diets showed good water stability in Hastings-Hepher test. It seems, however, that water stability did not considerably affect final results of fish feeding.

Extrusion method has been recently widely used in fish feed production (Dreyer 1988). Trout are unable to digest carbohydrates, thus barothermal processing of feeds is beneficial due to starch destruction which increases digestibility (Mościcki 1982). For this reason, extruded feeds are particularly appropriate for trout. It is noteworthy that extrusion allows to incorporate up to 30% of fat into the feed (Hilton et al. 1981).

Additionally, extruded feeds release little nutrients to water, and this reduces water pollution (Przybył, Madziar 1991).

Total protein level in the diets did not considerably vary and was appropriate for trout feeding (Cowey 1992). The feeds were relatively rich in phosphorus – from 9.1 to 20.5 g per kg. It is known, however, that 10 g of phosphorus is sufficient for good growth of rainbow trout (Wiesmann et al. 1988). According to Lovell (1979), phosphorus content in trout feed should not exceed 8 g/kg.

Besides structural fat in feed components, the diets contained also rape and soybean oils. Total fat level (17.4-18.6%) was near optimum (Steffens 1987). Content of essential unsaturated fatty acids in the starters met rainbow trout requirement (Castell et al. 1972), equal to 8-17 g kg^{-1} for linoleic acid.

Fat was stabilised using Rendox containing two antioxidants, BHA and EQ. Use of these two antioxidants was recommended by Rehulka, Mac (1986) who demonstrated that optimum stabilisation of diet lipids was beneficial for fish growth.

Concentrations of toxic elements in the diets, such as lead and mercury, were relatively low and did not exceed maximum levels permitted for commercial feeds (English Feeding Stuffs Regulation 1988). Chromium, present in the diets in concentrations 0.10-0.49 ppm, is not mentioned as a toxic element in the European Union regulations concerning feed components.

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STRESZCZENIE

PRZYDATNOŚĆ MODYFIKOWANEJ BAROTERMICZNIE KAZEINY W MIESZANKACH PASZOWYCH DLA PSTRĄGA TĘCZOWEGO *(Oncorhynchus mykiss).* I. OPTYMALIZACJA SKŁADU I SPOSÓB WYTWARZANIA PASZ NARYBKOWYCH DLA PSTRĄGA TĘCZOWEGO

Celem badań było określenie wartości żywieniowej modyfikowanej barotermicznie kazeiny, jako zamiennika tradycyjnie używanych mączek zwierzęcych : mączki rybnej, mączki z pierza i maczki drobiowej w paszach narybkowych dla pstrąga tęczowego.

Pasze eksperymentalne wykonano w Laboratorium Paszowym - Zakładu Doświadczalnego Technologii Produkcji Pasz i Akwakultury w Muchocinie. W przygotowanych dietach źródłem białka zwierzęcego, obok mączki rybnej, mączki z pierza i mączki drobiowej była również modyfikowana barotermicznie kazeina, której udział w poszczególnych wariantach paszowych wynosił od 8 do 40 %. Pasze doświadczalne zbilansowane zostały na poziomie 50 % białka i współczynniku E/P = 8,8 kal. energii strawnej na 1 g białka. Sumaryczny poziom tłuszczu w dietach wynosił od 17,4 do 18,6 %, a oznaczona analitycznie zawartość niezbędnych nienasyconych kwasów tłuszczowych w testowanych paszach narybkowych w pełni pokrywała zapotrzebowanie pstrąga tęczowego.

Dla mieszanek paszowych pierwszym aminokwasem ograniczającym była metionina z cystyną. Wartości EAAI wahały się od 82,16 do 87,19. Poziom ołowiu i rtęci był stosunkowo niski i nie przekroczył dopuszczalnych zawartości przyjmowanych dla pełnoporcjowych mieszanek paszowych. Nie stwierdzono obecności kadmu.

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