A USABILITY TRIAL OF ERYTHROCYTE MEAL IN FEEDING JUVENILES OF COMMON CARP, CYPRINUS CARPIO L.

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Background. Constant improvement of animal feed formulas calls for new raw materials. In the feeding of fish, one of diet recipe optimization methods is to search for feed protein sources alternative to fish meal. The presently report ed study focuses on an unconventional protein source—the erythrocyte meal—for feeding fry of common carp.

Materials and Methods. The barothermal method (extrusion) was applied for the formulation of experimental feeds prepared as isonitrogenous and isocaloric diets. In the feeds tested, the erythrocyte meal was used as fish meal substitutes at different substitution levels (control diet C = 0%, E5 diet = 5%, E10 diet = 10%, and E15 diet = 15%). The feeds were evaluated on the basis of their physical and chemical characteristics. The feeding trial was carried out on the fry of carp (3.8 ± 0.1 g). The experiments were carried out under controlled conditions in an indoor fish tank facility in twelve 60-L flow-through aquaria. Each aquarium was stocked with 14 fish. In the final evaluation of the feeding tests, the following rearing effectiveness indices were used: food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and survival rate (SR). The data obtained were statistically processed using Statistica computer software.

Results. At the end of the experiment, the fish body weight from treatment C was significantly (P ≤ 0.05) higher than the weights of treatments E5 and E15. Significant body weight differences occurred also between treatments E5 and E15. Values of specific growth rate (SGR) depended on the type of applied feed. The minimal value (1.86% · d⁻¹) was reached in treatment E15, while the maximal value (3.34% · d⁻¹) was recorded in treatment C. The most favourable FCR and PER values were recorded for C feed and they differed significantly from the values obtained with other experimental feeds. The application of experimental feeds to carp resulted in an increase in dry matter content in all treatments, and total protein and fat in the fish bodies from groups C and E5. However, there was no change in body ash content at feeding trial.

Conclusion. Experimental results showed that the erythrocyte meal addition at 5%, 10%, and 15% rate as replacement for 15% of fish meal did not improve the rearing parameters of common carp fry.

Keywords: feeding, erythrocyte meal, fish meal substitution, diets, carp, Cyprinus carpio

INTRODUCTION

Constant improvement of animal feed formulas calls for new raw materials. In the feeding of fish, one of the methods diet recipe optimization is to search for feed protein sources alternative to fish meal. According to Gawęcki (1998), proteinaceous blood preparations, like erythrocyte meal, can be included as unconventional proteinaceous component.

In the technological process of blood preparation manufacturing, the spray-drying method is used (18 s at ≤90°C), yielding amino-acids of high digestibility. Additional sterilization (≤20 min at 145°C), guaranties that the products are free of microbiological hazards.

Spray-dried red blood cells have the form of red-brown powder with a neutral smell containing at least 92% of total protein characterized by high content of exogenous a-
mino-acids and their metabolic energy value ranges between 15.08–17.86 MJ · kg\(^{-1}\) (Ewan 1989). In feed for farm animals, the optimal level of erythrocyte meal has been determined for 5%–10% (Gatnau and Zimmerman 1990, Kats et al. 1994, van der Peet-Schwering and Binndi 1997, Kamyczek 2000).

The basic purpose of the present experiment was to determine the usability of erythrocyte meal as a replacement for the traditionally used meals of animal origin (e.g., fish meal) in extruded feeds for carp fry.

**MATERIALS AND METHODS**

Recipes of experimental feeds were calculated with the use of a computer program, according to the purpose, written in the form of a linear program by Simplex method in Turbo Pascal 5.0 language. Experimental feeds were made in the Feed Laboratory of the Experimental Plant of Feed Production Technology and Aquaculture in Muchocin.

The ingredient composition of experimental diets is presented in Table 1. In control feed (C) the main protein sources were fish meal and blood meal. In E5, E10, and E15 feeds fish meal was substituted by meal of erythrocytes at 5%, 10%, and 15% levels, respectively. Feeds were prepared by barothermal method in an endogenous single-start worm extruder, type N-60, produced by Metalchem (Gliwice, Poland). Feeds were conditioned by adding to a mixer hot water and water steam to attain the temperature of 65–70°C and 19%–21% moisture level and extruded by applying the following technological parameters:

- Moisture content of feed 18%
- Cylinder temperature in the zone of increasing pressure 90°C
- Cylinder temperature in the zone of high pressure 100°C
- Head temperature 110°C
- Speed of worm revolutions 63 rev · min\(^{-1}\)
- Time of passage through extruder 52 s
- Nozzle diameter 6.0 mm

After drying, the obtained granules were crushed on a cylinder crusher and the pellets were segregated into two granulometric groups:

- 1.6–2.0 mm for carp of average weight up to 10 g;
- 2.0–3.15 mm for carp of average weight above 10 g.

The pellets were covered with a film of rapeseed oil heated to 70°C (in the amount of 1.5% of the pellet weight). It was done by spraying method in a pelleting drum.

Water stability of experimental feeds was evaluated by Hastings-Hepher method (Hepher 1968) modified by Szmiec and Stanny (1975) on the basis of the percentage of...
feed particles weight lost during water bath simulating water movements, and after drying of the sample to a content weight at 105°C. The oxygen consumption by water used for testing was determined in an alkaline environment applying the method described by Gomółka and Szykowski (1973).

Chemical analysis of feeds was carried out according to AOAC (Anonymous 1996). The total protein content was determined using Kjel-Foss Automatic 16210 analyzer; raw fat was estimated by Soxhlet method (ethyl ether extraction for 12 h). The amount of raw fibre was determined using Tecator Fibertec System M 1020 Hot Extractor. Ash content was determined by sample combustion at 550°C for 12 h (Linn Electro-Therm furnace). The amount of N-free extract was estimated as the difference between the dry weight and the sum of the remaining components. Total calcium was determined in the feed by atomic absorption spectrophotometry, type ASS3 (Carl Zeiss, Jena). Total phosphorus was determined by flame ionization technique. Amino acids of the feeds protein were assayed in a Microtech AAAT 339 analyzer after hydrolysis of a sample (0.1 mL) in 6n HCl at 106°C for 24 h. Methionine and cystine were determined after previous oxidation in formic acid. Tryptophan was determined by colorimetric method (Votitsky and Gunkel 1989). On the basis of the results of amino acid analyses of protein, the chemical value of experimental diets was defined by calculating the chemical score (CS) and the indispensable amino acid index (IAAI) (Hardy and Barrows 2002).

Gross energy of the diets was calculated from the chemical composition using the conversion factors of gross energy for fish: 17.2 kJ · g⁻¹ (carbohydrates); 23.6 kJ · g⁻¹ (protein), and 39.5 kJ · g⁻¹ (fat) (Bureau et al. 2002).

The experiment was carried out in controlled conditions (aquarium hall of the Division of Inland Fisheries and Aquaculture, Agricultural University in Poznań) in an open supply system. Water was drawn from water-pipe network, and in order to reduce chlorine content, it was run through an active carbon filter. The main element of the water system was an equalizing tank of 2.4 m³ capacity in which water was heated to constant temperature and aerated with a HIBLOW HP-60 blower. During the experiment, the physicochemical parameters of the water were maintained at the relatively constant, optimal levels for carp fry of 22–23°C temperature and oxygen saturation above 70% (Steffens 1981). The physicochemical parameters of the water were monitored with an ELMETRON CO-315 oximeter microcomputer.

Carp juveniles were stocked in 60-L tanks in which a constant water flow was maintained at a total water exchange rate of five times per 24 h. Every day at 0800 h the tanks were cleaned with a water siphon to remove excrement and unconsumed feed. The experimental feeds were supplied around the clock (24 h) using automatic belt feeders with a clock drive. The daily feed rations were calculated according to the feeding standards given by Miyatake (1997) based on the actual fish body weight. The size of rations was determined every tenth day based on weight monitoring which also served for determining the values of the other rearing indices.

The growth experiment lasted 50 days (08 Oct–27 Nov 2002). The biological material consisted of carp bred at the facility with an average individual body weight of 3.8 ± 0.1 g. The experiment was conducted in four treatments (including a control group) of three replications each. Each tank was stocked with fourteen fish.

Before initiating of the experiment, the fish from each replicate were sampled randomly in order to determine the basic chemical body composition. The sampled carp were anesthetized using Propiscin (Siwicki 1984) and then decapitated. Subsequently, the fish were ground and homogenized, and then the dry weight, total protein, raw fat, and ash were determined using the same analytic methods applied to the feed.

The fish stocking biomass obtained for the four experimental treatments were analyzed statistically. Furthermore, the fish stocking biomass and feed consumption were used to calculate the following animal husbandry rearing indices: mean specific growth rate of fish (SGR, % · d⁻¹); mean absolute food conversion ratio (FCR); and protein efficiency ratio (PER).

The Kolmogorov–Smirnov test (significance level $P \leq 0.05$) revealed that the distribution of stocking biomass and the SGR coefficient was normal. The homogeneity of variance for the same parameters was checked with the Bartlett test and the result was positive. Since the sets of data satisfied all necessary assumptions, they were subject to analysis of variance. The main effects included time and feed type; also the interaction was estimated. Following analysis of variance, the post-hoc group of analyses was applied. Homogenous groups were determined with the Tukey test.

The remaining two parameters, feed conversion ratio (FCR) and protein efficiency ratio (PER) were subjected to time analysis (because of the absence of assumptions for multinomial analysis of variance) and the variability in time has been shown for the particular experimental treatments.

**RESULTS**

The experimental feeds were characterized by satisfactory water stability. All of the feeds were positively evaluated (good) as for the loss of granule weight during the water bath, while feeds C, E5, and E10 obtained good evaluation marks for oxygen demand by water used in the testing and feed E15 was evaluated as very good one (Table 2).

Total protein content of the feeds ranged between 37.98% and 38.04%. The level of crude lipid in experimental diets ranged from 9.40% to 9.56%, and the content of raw fibre amounted to 1.84%–1.87% (Table 3). The determined amounts of exogenous amino acids in feeds are shown in Table 4. Methionine and cystine were the first essential amino acids for diets C, E5 and E10, while isoleucine was for diet E15. The indices of exogenous
amino acids ranged from 69.16 to 76.93 (Table 5). The energy–protein ratio (E/P) in all experimental feeds was very similar amounting to 39.52–40.0 KJ · g⁻¹, while the digestible energy of the studied diets ranged between 15.02 and 15.24 MJ · kg⁻¹ (Table 5).

The use of feeds with different portions of erythrocyte meal had a significant effect on the growth of carp fry (Table 6). On day 35 of the growth test, fish from treatment C reached a significantly higher body weight in comparison with treatment E15. On day 42 of the test, significant differences in fish body weight were recorded among treatments C, E10 and E15. At the end of the experiment, the fish body weight from treatment C was significantly higher than that from treatments E5 and E15. Significant body weight differences occurred also between treatments E5 and E15.

The obtained values of specific growth rate (SGR) depended on the type of applied feed. The minimal value of SGR (1.86% · d⁻¹) was reached in treatment E15, while the maximal value (3.34% · d⁻¹) was shown in treatment C during the experimental period. Differences in SGR values were also statistically significant ($P < 0.05$) (Table 7).

### Table 2
**Water stability of feeds**

| Parameter                  | C     | E5    | E10   | E15   |
|----------------------------|-------|-------|-------|-------|
| Weight loss [%]            | 25.6  | 29.7  | 26.7  | 25.5  |
| Score                      | good  | good  | good  | good  |
| Oxygen demand [mg O₂ · L⁻³] | 50.2  | 58.6  | 55.2  | 48.9  |
| Score                      | good  | good  | good  | very good |

### Table 3
**Proximate composition of experimental diets, [% wet weight]***

| Component               | C       | E5      | E10     | E15     |
|-------------------------|---------|---------|---------|---------|
| Crude protein           | 37.98 ± 0.98  | 38.0 ± 1.04  | 38.04 ± 1.23  | 38.03 ± 1.67  |
| Crude fat               | 9.40 ± 0.11   | 9.45 ± 0.23  | 9.41 ± 0.22   | 9.56 ± 0.45   |
| Nitrogen-free extract   | 32.57 ± 0.76  | 33.08 ± 0.89  | 33.67 ± 1.38  | 34.17 ± 1.97  |
| Crude fibre             | 1.86 ± 0.09   | 1.86 ± 0.13   | 1.87 ± 0.12   | 1.84 ± 0.2    |
| Ash                     | 6.57 ± 0.1    | 6.18 ± 0.32   | 5.79 ± 0.23   | 5.39 ± 0.78   |
| Phosphorus              | 0.83 ± 0.05   | 0.77 ± 0.19   | 0.71 ± 0.08   | 0.65 ± 0.05   |
| Calcium                 | 1.58 ± 0.08   | 1.39 ± 0.2    | 1.21 ± 0.07   | 1.02 ± 0.09   |

* $\bar{x} ± s$ from analysis of three samples of each feed

### Table 4
**Essential amino acid composition of experimental diets, [g 100g⁻¹ protein]***

| Amino acid              | C       | E5      | E10     | E15     |
|-------------------------|---------|---------|---------|---------|
| Arginine                | 5.32 ± 0.23  | 5.01 ± 0.12  | 4.70 ± 0.15  | 4.38 ± 0.23  |
| Histidine               | 3.89 ± 0.09  | 3.96 ± 0.16  | 4.02 ± 0.07  | 4.12 ± 0.08  |
| Lysine                  | 7.53 ± 0.08  | 7.30 ± 0.09  | 7.06 ± 0.24  | 6.85 ± 0.19  |
| Tryptophan              | 2.84 ± 0.19  | 2.37 ± 0.06  | 1.89 ± 0.08  | 1.41 ± 0.1   |
| Phenylalanine+Tyrosine  | 7.18 ± 0.23  | 6.69 ± 0.21  | 6.20 ± 0.15  | 5.68 ± 0.21  |
| Methionine+Cystine      | 2.62 ± 0.06  | 2.49 ± 0.08  | 2.36 ± 0.04  | 2.22 ± 0.08  |
| Tyrosine                | 3.98 ± 0.07  | 3.86 ± 0.18  | 3.75 ± 0.18  | 3.63 ± 0.18  |
| Leucine                 | 8.59 ± 0.19  | 8.80 ± 0.17  | 9.00 ± 0.23  | 9.24 ± 0.31  |
| Isoleucine              | 3.51 ± 0.21  | 3.19 ± 0.09  | 2.87 ± 0.18  | 2.52 ± 0.07  |
| Valine                  | 5.57 ± 0.14  | 5.83 ± 0.2   | 6.09 ± 0.09  | 6.37 ± 0.171 |

* $\bar{x} ± s$ from analysis of three samples of each feed
The best FCR and PER values were recorded for C feed and they differed significantly from the values of the remaining feeds (Table 7). During the growth test, no mortality of fish was recorded in any of the treatments.

The application of experimental feeds to carps had an effect on the proximate composition of their carcass (Table 8). The comparison of dry weight content of the fish bodies on day 1 and on the last day of experiment showed an increase in all treatments. Furthermore, there was also an increase in the amount of total protein and fat in the fish carcass of groups C and E5. On the other hand, in the remaining groups, the levels of these components decreased. The determined amounts of ash in carp carcass did not change after the termination of the growth test.

### Table 5

Characteristics of experimental diets

| Parameter | C               | Treatment (diet) | E5       | E10       | E15       |
|-----------|-----------------|------------------|----------|-----------|-----------|
| CS        | Met + Cys = 45.28 | Met + Cys = 42.98 | Met + Cys = 40.71 | Ile = 36.52 |
| IAAI      | 76.93           | 75.05            | 72.83    | 69.16     |
| DE [MJ kg⁻¹] | 15.02           | 15.09            | 15.14    | 15.24     |
| E/P [kJ g⁻¹] | 39.52           | 39.71            | 39.81    | 40.10     |

CS, chemical score; IAAI, indispensable amino acid index; DE, digestible energy level; E/P, energy–protein ratio; Met, methionine; Cys, cysteine; Ile, isoleucine

### Table 6

Changes of fish biomass [g] during the growth test*

| Days of growth test | C                  | E5                  | Treatment (diet) | E10 | E15                  |
|---------------------|--------------------|---------------------|------------------|-----|----------------------|
| start               | 64.4 ± 3.67a       | 65.5 ± 1.69a        | 63.7 ± 3.39a     | 67.9 ± 1.98a     |
| 7                   | 80.1 ± 3.96a       | 80.75 ± 3.32a       | 80.2 ± 5.52a     | 89.0 ± 0.28a     |
| 14                  | 102.3 ± 3.11a      | 99.25 ± 0.07a       | 95.75 ± 4.45a    | 100.75 ± 2.62a   |
| 21                  | 130.2 ± 8.34a      | 121.85 ± 0.49a      | 112.8 ± 14.14a   | 110.95 ± 5.30a   |
| 28                  | 165.25 ± 9.12a     | 148.65 ± 0.78a      | 135.85 ± 14.35a  | 123.5 ± 0.85a    |
| 35                  | 215.8 ± 13.15a     | 186.1 ± 1.27ab      | 172.75 ± 19.59ab | 146.45 ± 11.10b  |
| 42                  | 274.55 ± 19.87a    | 224.55 ± 0.92ab     | 201.15 ± 27.93bc | 159.1 ± 7.21c    |
| 50                  | 342.7 ± 23.48a     | 278.45 ± 4.31b      | 242.5 ± 44.83bc  | 172.6 ± 14.57c   |

* ± s from triplicate groups of fish; values in each row, with the same superscript, are not significantly different (P ≤ 0.05)

### Table 7

Specific growth rate (SGR), food conversion rate (FCR), protein efficiency rate (PER), and survival rate (SR) in common carp fry fed experimental diets for 50 days*

| Parameter | C                  | Treatment (diet) | E10 | E15 |
|-----------|--------------------|------------------|-----|-----|
| SGR [% d⁻¹]# | 3.34 ± 0.21a      | 2.89 ± 0.77ab    | 2.66 ± 0.26b | 1.86 ± 0.11c |
| FCR$ | 1.29 ± 0.21       | 1.51 ± 0.49      | 1.69 ± 0.20 | 2.66 ± 0.27 |
| PER$ | 2.04 ± 0.03       | 1.74 ± 0.05      | 1.56 ± 0.19 | 0.99 ± 0.01 |
| SR [%] | 100.0             | 100.0            | 100.0 | 100.0 |

* ± s from triplicate groups of fish, values in each row, with the same superscript, are not significantly different (P ≤ 0.05); #SGR = (ln final wt. – ln initial wt.) d⁻¹; $FCR = (dry feed intake [g]) · (weight gain [g])⁻¹; $PER = (wet weight gain) · (protein intake)⁻¹
Table 8

Proximate composition of fish carcass before and after the growth test [% wet weight]*

| Parameter                  | Dry weight   | Ash          | Crude protein | Crude fat    |
|----------------------------|--------------|--------------|---------------|--------------|
| Before the growth test     |              |              |               |              |
| C                          | 30.75 ± 1.02c| 1.96 ± 0.25a | 15.56 ± 0.40c | 2.55 ± 0.15c |
| E5                         | 28.70 ± 0.98b| 1.89 ± 0.20a | 14.17 ± 0.23a | 2.04 ± 0.15c |
| E10                        | 26.91 ± 1.69a| 1.80 ± 0.11a | 13.59 ± 0.15a | 1.07 ± 0.18b |
| E15                        | 28.66 ± 1.25b| 1.90 ± 0.21a | 14.17 ± 0.23a | 2.04 ± 0.15c |
| After the growth test      |              |              |               |              |
| C                          | 30.75 ± 1.02c| 1.96 ± 0.25a | 15.56 ± 0.40c | 2.55 ± 0.15c |
| E5                         | 28.70 ± 0.98b| 1.89 ± 0.20a | 14.17 ± 0.23a | 2.04 ± 0.15c |
| E10                        | 26.91 ± 1.69a| 1.80 ± 0.11a | 13.59 ± 0.15a | 1.07 ± 0.18b |
| E15                        | 28.66 ± 1.25b| 1.90 ± 0.21a | 14.17 ± 0.23a | 2.04 ± 0.15c |

*± s from triplicate groups of fish, values in each column, with the same superscript, are not significantly different (P≤0.05)

DISCUSSION

In Hastings-Hepher tests (Hepher 1968), all the feeds received satisfactory evaluations indicating that their water stability had no essential effect on the carp rearing results. The experimental diets were correctly balanced regarding the content of total protein and raw fat (Ogino 1980a, Jauncey 1982, Watanabe 1982, 1988), mineral components (Satoh et al. 1991, Anonymous 1993, Kim et al. 1998), exogenous amino acids (Nose 1979, Ogino 1980b), and energy level in the diet and in its relation to the amount of protein (Ohta and Watanabe 1996) for carp fry.

The relevant publications emphasize that the use of erythrocyte meal in the nutrition of farm animals resulted in better body weight increments (by 35–40 percentage points), increased the feed uptake (by 10–30 percentage points), and gave a better FCR values (by 10–15 percentage points) (Kamyczek 2000). Positive results of erythrocyte meal utilization, observed in the rearing of farm animals, have not been confirmed in fish rearing. In case of Siberian sturgeon, the feeds containing this component (5%–15%) did not improve the results, in comparison with feed containing fish meal (Mazurkiewicz and Przybył 2002). Also the results of the present experiment did not bring any clear improvement in carp rearing caused by the erythrocyte meal feed supplementation.

The results of the presently described growth trial revealed that the most effective was the diet without erythrocyte meal. The addition of this feed supplement at different levels (5%, 10%, and 15% of diet) did not have a significant effect on the fish body weight increments nor nutrient conversion indices. The erythrocyte meal turned out to be a deficient isnitrogenous replacement of fish meal in carp fry nutrition.

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