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# DIETARY EFFECT OF SUPPLEMENTATION WITH AMARANTH MEAL ON GROWTH PERFORMANCE AND APPARENT DIGESTIBILITY OF RAINBOW TROUT *ONCORHYNCHUS MYSKISS*

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K e y w o r d s: *Oncorhynchus mykiss*, *Amaranthus cruentus*, digestibility, chromic method.

A b s t r a c t

The aim of the this study was the to assessment of use the amaranth meal in feed for rainbow trout *Oncorhynchus mykiss*. Two experimental feeds contained 5.0% and 10.0% of amaranth meal were prepared and compared to reference diet (commercial feed) containing similar amounts of specific nutrients. Initial body wet weight of experimental trout was 524.8 ± 28.5 g and mean length of 35.2 ± 0.6 cm. Feed was offered in ration between 0.50–1.71% of fish biomass (calculated by software) of respective group for 21 days. The validation of chromic method to analyze digestibility of fish diet components was done and the linear regression formula $Y = 0.0142X + 0.075$ was determined for chromium oxide (VI) concentration. Significant differences ($p<0.05$) in crude protein and Nitrogen Free Extract digestibility were found between the experimental groups and the reference one. The present results indicated the feasibility of using of amaranth flour as plant component in feeding of rainbow trout.

## WPŁYW SUPLEMENTACJI MĄKI Z SZARŁATU NA WZROST I STRAWNOŚĆ POZORNĄ PSTRĄGA TĘCZOWEGO *ONCORHYNCHUS MYSKISS*

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S ł o w a k l u c z o w e: *Oncorhynchus mykiss*, *Amaranthus cruentus*, strawność, metoda chromowa.

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Abstrakt

Celem pracy było zastosowanie mąki z szarłatu (*Amaranthus cruentus*) w żywieniu pstrąga tęczowego *Oncorhynchus mykiss*. Przygotowano dwie eksperymentalne pasze z dodatkiem mąki z amarantusa na poziomie (5 i 10%) i jako referencyjną zastosowano paszę komercyjną o podobnej zawartości składników pokarmowych. Badania przeprowadzono na rybach o średniej masie 524,8 ± 28,5 g i długości 35,2 ± 0,6 cm. Doświadczenie trwało 21 dni, podczas których ryby karmiono paszą w dawkach 0,5–1,7% całkowitej biomasy wyliczanej przez hodowlany program komputerowy. Na potrzeby określenia strawności pozornej przygotowanych pasz wykonano walidację metody chromowej dla koncentracji tlenku chromu (VI), otrzymując krzywą korelacji o wzorze $Y = 0.0142X + 0.075$. Różnice istotne statystyczne ($p$<0,05) zaobserwowano w strawności białka surowego i bezazotowych związków wyciągowych w grupach eksperymentalnych w stosunku do grupy referencyjnej. Uzyskane wyniki wskazują na możliwość zastosowania mąki z szarłatu w żywieniu pstrąga tęczowego.

## Introduction

Annual worldwide production of rainbow trout reached more than 800 thousand at tones in 2014 (FAO 2016). Commercial fish farms use extruded fishmeal-based feeds in general (NAYLOR et al. 2000). The percentage of farms using commercial feeds varies from 100% for salmon and 83% for trout. Many aquaculture feed formulations still include fishmeal at levels in excess of 50% (GLENCROSS et al. 2007). Main source of fishmeal comes from marine ecosystems (DEUTSCH et al. 2007). Demand for fishmeal was increasing with the increase development of the aquaculture sector, despite the limited and already overfished marine ecosystems. The replacement of fishmeal as the major protein source with plant origin components is challenging the sustainability of aquaculture industry (VILHELMSSON et al. 2004). Many researchers in recent years tested vegetable: especially soya (KAUSHIK et al. 1995), rapeseed (BUREL et al. 2000), lupine (GOMES et al. 1995), faba bean (OURAJI et al. 2013), pea (THIESSEN et al. 2003), proteins as alternatives for fishmeal.

Amaranth (*Amaranthus cruentus*) grains contain about 15% of protein, and balanced amino acid profile with high level of lysine makes it attractive protein source (PEDERSEN et al. 1987). Main problem with plant components is anti-nutritional factors impact on performance of salmonid fish, with decreased digestion and reduced utilization of proteins followed by decreased growth rates (KROGDAHL et al. 1994). Chemical analysis of amaranth meal showed low level of anti-nutritional factors: saponins and phytic acid (ESCUDERO et al. 2004). VIRK and SAXENA (2003) used amaranth (seeds) in fish diet for *Labeo rohita*. POCZYCZYŃSKI et al. (2014) used amaranth oil as substitution of fish oil in experimental feed for rainbow trout.

The aim of the study was the assessment of use of amaranth meal digestibility crude component in fish feed for rainbow trout *Oncorhynchus mykiss*.

## Materials and methods

### Fish, feeding, experimental system

Rainbow trout with initial mean body weight of 524.8 ± 28.5 g and mean total length of 35.2 ± 0.6 cm were used in the experiment. Fish ($n$ = 144) were randomly distributed in 9 tanks (3 groups in triplicates: control and two experimental) and acclimated for 4 weeks to the experimental conditions. Each tank volume was 500 $dm^3$ and tanks were a part of a Recirculation Aquaculture System (RAS). Fish were exposed to a natural light regime of approximately 8 LD and 16 DD. Water quality parameters were measured every day (7.00 am) and the mean values were as follow (mean ± SD): dissolved oxygen 7.72 ± 0.52 mg $dm^{-3}$, temperature 16.58 ± 0.12°C, pH 7.93 ± 0.14, total ammonia < 0.005 mg $dm^{-3}$, nitrate < 0.20 mg $dm^{-3}$, nitrite < 0.001 mg $dm^{-3}$ and phosphates <0.005 mg $dm^{-3}$.

Every week fish were anaesthetised with propofol (7 mg $dm^{-3}$) (GOMUŁKA et al. 2014) and each fish was measured and weighted. Feed was offered at 0.5–1.71% of fish biomass of respective group. Feed rations were calculated with Djurnal 1.0 software (Denmark) depending on, biomass, feed gross energy content, water oxygen saturation and temperature. Every week fish were weighted for feed intake (FI) calculated. The experiment lasted 21 days.

### Diet preparation

Ingredients and nutrients composition of the experimental diets are showen in Table 1. Fish were fed with two experimental diets containing different levels of amaranth meal (5.0% EF5 and 10.0% EF10) and control feed (CF) – commercial trout pellet AllerAqua Silver (Danemark). Chromic oxide (III) was included in all diet at 1.0% level. Experimental feeds were extruded with a co-rotating twin screw extruder (METALCHEM, Poland) equipped with a Ø 4.5 mm pellet stencil. The entire oil content of the diets was added after the extrusion process. Then, feed remained room temperature until oils were completely absorbed and then keep in refrigerator (6°C).

### Chemical analysis

The contents of the basic chemical components in feed and faeces (dry matter, crude: protein, fat and ash) were determined according to standard methods of (AOAC, 2000). Dry matter was determined by drying in an oven at

Table 1

Feeding experiment diets

| Ingrediens [%] | EF5 | EF10 | CF |
|---|---|---|---|
| Fishmeal | 44.25 | 44.25 | NA |
| Wheat flour | 20.31 | 20.31 | NA |
| Soybean meal | 15.00 | 10.00 | NA |
| Amaranth meal | 5.00 | 10.00 | NA |
| Fish oil | 6.24 | 6.24 | NA |
| Soybean oil | 6.00 | 6.00 | NA |
| Vitamin premix* | 2.00 | 2.00 | NA |
| Mineral premix** | 0.10 | 0.10 | NA |
| Choline | 0.50 | 0.50 | NA |
| Ascorbic acid | 0.50 | 0.50 | NA |
| Chromic oxide | 1.00 | 1.00 | 1.00 |
| Nutrient analysis [%] | | | |
| Dry matter | 94.27 | 95.02 | 93.51 |
| Crude protein | 41.01 | 40.62 | 42.26 |
| Crude fat | 11.95 | 13.45 | 13.70 |
| Crude ash | 10.44 | 10.43 | 8.52 |
| Crude fibre | 3.27 | 3.25 | 3.22 |
| Chromic oxide | 1.00 | 1.00 | 1.00 |
| Gross energy (MJ $kg^{-1}$) | 18.04 | 15.41 | 17.42 |

NA – data not available

* Vitamin premix (IU $kg^{-1}$ or mg $kg^{-1}$ dry diet): witamin A – 15 000 UI $kg^{-1}$; witamin D – 6 000 UI $kg^{-1}$; witamin E – 15; witamin C – 70; witamin $B_1$ – 0.8; witamin $B_2$ – 3.0; witamin $B_6$ – 1.50; witamin $B_{12}$ – $8 \cdot 10^{-3}$; witamin K – 1,5; Biotin – 2,5.

** Mineral premix [mg $kg^{-1}$ dry diet]: calcium – $25 \cdot 10^3$; phosphorus – $27 \cdot 10^3$; sodium – $18 \cdot 10^3$; magnesium – $2 \cdot 10^3$; mangan – 720; iron (II) – 400; copper – 127; zinc – 800; Iodine – 23.

105°C for 24 h. Total protein was determined by Kjeldahl's method and crude fat by Soxhlet's method. NFE was calculated as formula: NFE(%) = 100 – (moisture% + protein% + lipid% + ash% + fibre%). Chromic oxide in diets and faeces was analyzed according to the method of FURUKAWA and TSUKAHARA (1966). The validation of this method for luminometer TECAN Infinity M200 Pro was done before the experiment. The linear regression formula $Y = 0.0142X + 0.075$ was determined for chromium oxide (VI) concentration ($R^2 = 0.997$). Validation parameter were as follow: CV = 2.306%, LOD = 2.302, LOQ = 6.904 for the test range 0.395 – 158 g $dm^{-3}$ chromium concentration. Repeatability was 0.7%.

## Sample collection

Fish were caught individually and immediately anaesthetized with excessive propofol concentration (20 mg $dm^{-3}$). Then fish were killed by brain destruction with sharp scissors. All fish were individually weighed and meas-

ured. Then internal organs were removed and faeces sample was obtained by manual stripping of the posterior part of the gut (AUSTRENG 1978). The samples were pooled for each group. Weight of liver was recorded for Heapato-somatic index (HSI) calculation.

### Digestibility determination

Dry matter digestibility was calculated as follow (WINDELL et al. 1978):

$$\text{dry matter digestibility (\% DM)} = 100[-[100\ (\%Cr_2O_3 \text{ in feed}/\%\ Cr_2O_3 \text{ in faeces})]]$$

Apparent nutrient (protein, lipid and energy) digestibility was calculated as follow (MAYNARD et al. 1979):

$$\text{apparent nutrient digestibility (\%)} = 100 - [(\%Cr_2O_3 \text{ in feed}/\%Cr_2O3 \text{ in faeces}) \cdot (\%\ \text{nutrient in faeces}/\%\text{nutrient in feed})] \cdot 100$$

### Growth measurements

Growth performance was determined as follow:

1. Percent weight gain [%] = [Final body weight [g] – Initial body weight [g]] · 100.
2. Specific growth rate (SGR) = [(ln final weight – ln initial weight)/time (days) · 100].
3. Feed conversion ratio (FCR) = (feed intake/wet weight gain).
4. Fulton Condition Factor (FCF) = (final weight/final total length$^{-3}$).
5. Hepato-somatic index (HSI) = (liver weight/body weight) · 100.

## Statistical analysis

Normality of data distribution was tested by Shapiro-Wilk test and variance homogeneity by Leven's test. When above assumptions were met, differences between means were analysed using ANOVA and *post hoc* Tuckey's test (TT). For the others, Kruskal-Wallis ANOVA and Mann-Whitney U test (UT) were used. Results were analysed with Statistica 10.0 (Statsoft, USA) software at significance level $p \leq 0.05$.

# Results

## Fish Growth

Growth performance of experimental fish are presented in Table 2. Different between initial and final measurement not statistic significantly for body length and body weight for all experimental groups (ANOVA, $p>0.05$). The highest fish weight gain (above 21%) was revealed in experimental groups. SGR for CF (0.64) was significantly lower ($p<0.05$), than for EF5 and EF10. FCR noted in experimental group EF5 (1.12) and EF10 (1.00) were better than for CF (1.39). HSI recorded in CF (1.31%) was lower than, those in experimental group EF5 (1.96) and EF10 (2.08). Significantly different ($p<0.05$) in FCF was observed between: CF (1.19) and EF5 (1.30).

Table 2
Growth performance and feed efficiency of rainbow trout fed on experimental diets for 21 days. Values are presented as mean ± SD (range)

| | CF | EF5 | EF10 |
|---|---|---|---|
| Initial total lenght [cm fish$^{-1}$] | $35.41^{a}$ ± 1.87 | $35.26^{a}$ ± 2.29 | $34.84^{a}$ ± 2.26 |
| Final total lenght [cm fish$^{-1}$] | $37.05^{a}$ ± 1.97 | $36.80^{a}$ ± 2.42 | $36.70^{a}$ ± 2.58 |
| Initial weight [g fish$^{-1}$] | $528.94^{a}$ ± 110.54 | $533.44^{a}$ ± 134.75 | $512.02^{a}$ ± 124.11 |
| Final weight [g fish$^{-1}$] | $604.71^{a}$ ± 110.48 | $648.96^{a}$ ± 162.21 | $623.08^{a}$ ± 165.24 |
| Weight gain [%] | $14.33^{a}$ ± 1.51 | $21.66^{b}$ ± 0.14 | $21.69^{b}$ ± 0.64 |
| SGR [%/d] | $0.64^{a}$ ± 0.21 | $0.89^{b}$ ± 0.03 | $0.92^{b}$ ± 0.09 |
| HSI [%] | $1.31^{a}$ ± 0.09 | $1.96^{b}$ ± 0.02 | $2.08^{b}$ ± 0.10 |
| FCR (1) | $1.39^{a}$ ± 0.07 | $1.12^{b}$ ± 0.02 | $1.00^{b}$ ± 0.04 |
| FCF [g cm$^{-3}$] | $1.19^{a}$ ± 0.05 | $1.30^{b}$ ± 0.02 | $1.26^{ab}$ ± 0.10 |

Number indexes show columns with significantly different results ($p\leq0.05$).

## Digestibility

Apparent digestibility for dry matter, crude protein, crude lipid and NFE in the test ingredients consumed by rainbow trout are summarized in Table 3. For crude protein, digestibility coefficients exceeding 95% were noted for EF5 and EF10 and were higher than those for commercial feed ($p<0.05$). Apparent digestibility of NFE was higher in feeds with amaranth meal, when compared to control groups ($p<0.05$). No different in apparent digestibility of crude lipid was found. Assimilation of ash was significantly higher in EF5 when compared to CF ($p<0.05$).

Table 3

Table 3. Apparent digestibility [%] of nutrient diet fed to rainbow trout. Results are presented as mean ± SD (range)

| Nutrient | CF | EF5 | EF10 |
|---|---|---|---|
| Crude protein | $91.84^{a}$ ± 1.69 | $95.86^{b}$ ± 0.49 | $95.03^{b}$ ± 0.74 |
| Crude fat | $95.20^{a}$ ± 3.36 | $91.31^{a}$ ± 2.65 | $88.22^{a}$ ± 4.73 |
| NFE | $47.41^{a}$ ± 2.71 | $75.49^{b}$ ± 2.55 | $78.40^{b}$ ± 0.47 |
| Crude ash | $80.18^{a}$ ± 3.21 | $89.67^{b}$ ± 1.00 | $85.66^{ab}$ ± 2.85 |

Number indexes show columns with significantly different results ($p \leq 0.05$)

## Discussion

The present studies proved that amaranth meal is promising component of feeds for rainbow trout. Amaranth meal addition resulted in better growth indices; higher weight gain, SGR, and Fulton condition factor and lower FCR. However, increased HSI suggest possible negative influence on fish metabolism. This can result from different reasons: excessive energy load; anti-nutritional factors, incorrect balanced feed composition or nutritional deficiency.

Higher values of SGR indicate for better assimilation of feed given to fish. SGR found in the experimental fish (0.89–0.92) was lower than those for soya meal (1.09) and pea protein concentrate (1.21–1.23) reported by ØVERLAND et al. (2009) and noted by REFTSIE et al. (1997) for soy protein concentrate (1.49) and pea meal (1.3–1.6) pea meal concentrate (1.2–1.3) reported by COLLINS et al. (2012) and noted by BORQUEZ et al. (2011) of lupine flour (1.00–1.02) at 10–20% for rainbow trout. Similar SGR in the EF5 results (0.96) was noted by ØVERLAND et al. (2009) of soybean flour at 20% (0.995). However, in all cited above experiments, authors used smaller fish (between 33.5 and 160 g of weight) when compared to fish used in our experiment (initial body wet weight 524.8 ± 28.5g). According to STOREBAKKEN and AUSTRENG (1987) and SGR decreases with increasing fish size.

Many study showed that intensive feeding of fish can result in harmful load of liver in a short time period. HSI in the experimental groups was significantly higher than reported by ESCAFFERE et al. (2007), ØVERLAND et al. (2009) and BORQUEZ et al. (2011) for soya beans concentrate (1.30), soybean flour (1.18) and for lupine flour (1.10) respectively. However, KAUSHIK et al. (1995) reported values similar to our results for rainbow trout fed with feeds based on fishmeal (2.0) and casein (2.3).

Enlargement of liver size and glycogen concentration was increased with elevated levels of dietary carbohydrate in several fish (TIEN et al. 2012) present in amaranth meal (above 40%). Absorbed carbohydrate that is not used for

energy usually accumulated in the liver of fish both as lipid and as glycogen after being converted (BRAUGE 1994). Moreover, the effect of fat deposition in the liver resulting from carbohydrates in the lipogenesis process and source of oil (fatty acid profile) used in fish diet. HSI increased due to accumulation of fat in the liver was observed in fish fed with feeds contains on vegetable oil (OLSEN et al. 2000). CABALLERO et al. (2002) reported that some fatty acid (predominantly C16:0) tend to accumulate in rainbow trout liver. Amaranth oil contains 19.46% of C16:0 fatty acid (POCZYCZYŃSKI et al. 2014). High content of C16:0 acid can be one of possible reasons of high HSI value in experimental fish.

Usually FCF for trout ranges between to 0.80–1.60. The value of FCF is influenced by age and sex of fish, season of the year, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve, degree of muscular development (BARNHAM and BAXTER 1998). Pathological changes in body proportions caused by Myxosporea can result in very high value of FCF (1.62–1.88) in some cases (WŁASOW et al. 1997). FCF recorded in our experimental fish (1.26–1.30) was similar to those reported by STOREBAKKEN and AUSTRENG (1987) (1.26–1.64) for rainbow trout fed with 21 days period at 8°C and 12°C. In both cases, experimental feeding period was relatively short.

The increased of HSI can be a result of increased crude protein and NFE digestibility. Digestibility of amaranth crude protein is at a high level exceeding 95%. Similar Apparent digestibility was noted by SUGIURA et al. (2000) of the meal of animal origin (above 95%), and noted by CARTER and HADLER (2000) for soybean (95.31–95.86), lupine (95.65–95.90) and pea meal (95.22–95.48). The bioavailability of protein amaranth was comparable to the results obtained by OURAJLI et al. (2013) of beans (above 95%).

The main reason of low digestibility of NFE is hard to digest starch which is the main complex carbohydrate in vegetables seeds. Usually Apparent digestibility of NFE in trout feeds is 50%. Our results show that digestibility of amaranth NFE is much higher (74.5–78.4%). Digestion is thought to be the primary limiting step in the utilization of starch for growth (NRC 2011). The majority of commercially available starches have a medium (10 to 25 μm) or large (>25 μm) granule size, while amaranth seed is one of the few sources of small-granule starch, typically 1 to 3 μm in diameter, and having regular granule size (HOSENEY 1994). Amaranth seeds contain fine particle starch which easy to assimilate. In many fish species, including trout, increased availability of dietary carbohydrates can result in liver disturbances (WALTON 1986).

The relationship between digestibility of protein, fat and NFE were reported by GRISDALE-HELLAND and HELLAND (1997) for different levels of fat and carbohydrates in fish feed. They found that decrease of fat and starch level in feed resulted in increased protein digestibility. Our results suggest other

trend, protein digestibility was higher in fish offered with feed of higher NFE digestibility. At the same time digestibility of fat was decreased. Explanation of this phenomenon needs further research.

Apparent digestibility of fat in experimental groups was markedly lower than control (Table 3). It was shown that amaranth oil contain more than 23% of saturated fatty acids mainly C16:0, C18:0; C20:0 (ESCUDERO et al. 2004). CABALLERO et al. (2002) proved that digestibility of saturated fatty acids in rainbow trout is between 38 and 71% for stearic acid (C18:0) and 61 to 92% for palmitic acid (C16:0). High share of relatively hard to digest fatty acids in experimental feeds could results in worse digestibility of crude fat.

## Conclusions

The present study demonstrated the usefulness of amaranth meal as a component of commercial feeds for rainbow trout. The experiment revealed that 5% replacement of soybean meal with amaranth meal in feed improves growth of trout. However, higher level of amaranth meal induced negative influence on the growth performance and liver condition. Phenomena of higher digestibility of NFE accompanying with higher digestibility of protein and lower digestibility of fat needs further study.

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