

The influence of feeding diets containing wheat gluten supplemented with dipeptides or free amino acids on structure and development of the skeletal muscle of carp (*Cyprinus carpio*)

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Abstract A 28-day feeding trial was conducted to evaluate the influence of feeding diets containing plant protein wheat gluten supplemented with dipeptides or free amino acids on structure and development of the skeletal muscles of carp (*Cyprinus carpio*). Common carp fingerlings (1 month old) having an of average weight of 0.07 ± 0.02 g and total length of 17.79 ± 1.79 mm were fed three formulated diets—wheat gluten protein-based diets supplemented with Lys–Gly dipeptide (PP), free lysine and glycine (AA), control diet without lysine supplemented (CON)—and two other diets: restrictive diet—frozen zooplankton (Z) and commercial diet Aglo Norse (AN). After 28 days of experimental feeding, statistically significant higher survival was observed among fish fed AN and Z diets (99.5 ± 1.0 %; $P \leq 0.05$). The feeding AN diet has had also a positive influence on weight and growth rate as well as on development and growth of skeletal muscles. Furthermore, carps fed AN diet had the largest area of red and white muscle as compared with the other feeding groups, and the differences were statistically significant ($P \leq 0.05$). The increase in the number of proliferating cells (proliferating cell nuclear antigen) was observed on the last day of the experiment among carps fed PP, AA and CON. Moreover, fish fed PP significantly had the greatest number of MyoD- and myogenin-positive nucleus ($P \leq 0.05$). Among the experimental diets based on wheat gluten, a positive impact on structure and development of muscles has been observed in carps fed PP diet.

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Introduction

Common carp is one of the most important freshwater fish species in Poland's aquaculture (Kucharczyk et al. 2008). Carp larvae, during the first 2 weeks of feeding, may increase the volume of red and white muscles from 20 to even 30 % of the larval body volume (Alami-Durante et al. 1997). The growth of muscles is influenced by temperature, oxygen availability (Johnston et al. 2011), the quantity and quality of given food (Ostaszewska et al. 2008a; Alami-Durante et al. 2010). According to Chapalamadugu et al. (2009), the nutrition affects the molecular changes in the metabolism of the muscle cells and the myogenic progenitor cells. Myogenic progenitor cells play a key role in the processes of growth and development of the muscle tissue. The nutrition also affects the level of molecular factors regulating the transcription of PCNA, MyoD, myogenin and Pax 3/7 (Brodeur et al. 2002).

In response to the rising prices of fishmeal, new sources of protein and lipids that will allow the creation of appropriate artificial diets are being searched (Coyle et al. 2004). Currently, studies being conducted on the use of soybean meal, casein, wheat gluten, and others as a source of protein in diets for various species of fish (Ostaszewska et al. 2005, 2010a; Kamaszewski et al. 2010). The plant proteins contain antinutritional components and unbalanced amount of amino acids in diet (Kolkovski and Dabrowski 1999). Therefore, research is conducted on supplementation of amino acid to fish diets formulated based on the plant protein. One of the interesting concepts of digestive physiology at present is the role of dipeptides in accretion of indispensable amino acids in animal nutrition. According to Dabrowski et al. (2005, 2010), amino acids given entirely as dipeptides can sustain fish growth through eliminating the negative effects of indispensable amino acids deficiency. One of the important amino acid is lysine. This amino acid limits the growth of fish (Small and Soares 2000). Ostaszewska et al. (2010a) reported that the plant protein-based diets supplemented with the Lys–Gly dipeptide have a positive impact on the carp's development. Similar results confirming the positive influence of the supplementation with the Lys–Gly dipeptide diet based on the wheat gluten on survival, growth and morphology of digestive tract of rainbow trout (*Oncorhynchus mykiss*) and yellow perch (*Perca flavescens*) were obtained by Ostaszewska et al. (2010b, 2013).

Therefore, the aim of this work was to determine the influence of feeding diets containing plant protein wheat gluten supplemented with the Lys–Gly dipeptide or free amino acid Lys and Gly on the development of red and white muscles of carp.

Materials and methods

Fish and diets

The experiment was carried out in Division of Ichthyobiology and Fisheries, Warsaw University of Life Sciences. One-month-old carp (*Cyprinus carpio*) fingerlings having an average weight of 0.07 ± 0.02 g and total length of 17.79 ± 1.79 mm procured from

Inland Fisheries Institute in Olsztyn, Experimental Fisheries Institute in Zabieniec. The experiment was carried out in fish hatchery with the water recirculation system (biological filter and UV sterilization). The fish were reared in 20 tanks with a capacity of 20 L, at the density of 50 individuals per tank. An average water temperature was 20.91 ± 0.87 °C, pH 6.9 ± 0.09 , the level of the water oxygenation 8.07 ± 0.34 mg/L, the level of NH_4^+ 0.03 ± 0.01 mg/L, the level of PO_4^{3-} 0.2 ± 0.17 mg/L, the water flow rate was 40 L/h, and 12 h of lighting and 12 h of darkness were maintained during the experiment.

The fish were reared for 28 days. The fish were divided into 5 feeding groups. There were 4 replications for each feeding group. The fish were fed three formulated diets, wheat gluten protein-based diets supplemented with lysine–glycine (Lys–Gly) dipeptide (PP), free lysine and free glycine (AA) and control diet without lysine supplementation (CON), and two restrictive diets, frozen zooplankton (*Daphnia magna*) (Z) (IchthoTrophic Sp. z o.o., Poland) and commercial fodder Aglo Norse (AN) (Larvae feed Ewos, Bergen, Norway). Formulated diets were made in Aquaculture Laboratory, Ohio State University. The composition of formulated diets is shown in Table 1. The chemical composition of the diets used in experiment is shown in Table 2. During the experiment, the fish were being fed every 2 h, from 8 am to 8 pm. The daily ration of fish fed AN, PP, AA and CON diets was 10 % in the first 2 weeks of the experiment and 8 % of fish biomass in the following 2 weeks. Fish fed Z were being starved and received 3 % (the first 2 weeks) and 2 % (the following 2 weeks) of biomass.

Sample collection

The fish from each feeding group ($5 \times 4 \times 5$ groups) were taken out on 1st, 14th and 28th day of the experiment. After taking out, the fish were anaesthetized using MS-222 preparation (1:5000 MS-222, pH 7.5 adjust with NaHCO_3 , Sigma, Steinheim, Germany); then, the fish were weighed with 1 mg accuracy, and length measured with an accuracy of 0.01 mm. The fish were then fixed in Bouin's solution (picric acid, formaldehyde, glacial acetic acid) (Sigma, Steinheim, Germany) and used for histological and immunohistochemical purposes.

Histology and immunohistochemistry

Fixed samples were dehydrated in a graded series of ethanol, embedded in Paraplast and sectioned crosswise to 5- μm longitudinal section with microtome (Leica RM 2265, Leica Microsystems, Nussloch, Germany). The obtained histological slides were stained with hematoxylin and eosin (H&E).

The following histomorphometric measurements were taken within the upper right quarter of cross-section: white muscles area (CSAw), red muscles area (CSAr), the total number of muscle fibers (TFN), the total number of skeletal muscle nucleus (TNN) and the muscle fiber area (FA).

Immunohistochemical analyses were performed on histological preparations of five fish from each group on the 1st, 14th and 28th day of the experiment. To detect proliferating cells in the muscles, was performed PCNA (proliferating cell nuclear antigen) according to the method described by Ostaszewska et al. (2008b).

In order to detect myogenic regulatory factors (MyoD and myogenin) and Pax3/7, the histological slides were deparaffinized in xylene and rehydrated using a gradient of ethanol. Endogenous peroxidase was being blocked with the help of 3 % hydrogen peroxide. The slides were rinsed in Tris buffer (pH 8.0) (T-6664, Sigma). The characteristics of used

Table 1 The composition (%) of the ingredients used for the preparation of experimental diet

Ingredients	Diets		
	PP	AA	CON
Fish meal	12.4	12.4	12.4
Wheat gluten ^a	37	37	37
Wheat meal	17.18	17.18	17.18
Fish oil	5	5	5
Lecithin	15	15	15
Mineral mix	3	3	3
Vitamin mix	4	4	4
Lysine–Glycine ^b	2.9	–	–
Lysine ^c	–	1.8	–
Glycine ^c	–	1.1	1.1
Glutamate ^d	–	–	1.8
Cysteine ^e	0.2	0.2	0.2
Arginine	0.65	0.65	0.65
Methionine	0.33	0.33	0.33
Threonine	0.29	0.29	0.29
Ca-mono P	1.5	1.5	1.5
Vitamin C	1.5	1.5	1.5
Gross energy (MJ/kg)	19.6	19.6	19.6

^a MP Biomedicals–Solon-OH

^b MP Biomedicals

^c Bahen, NY

^d Hughes (1985) non-toxic level of glutamate

^e Hara (2006) most potent olfactory stimulating amino acid

antibodies and the details concerning incubation of preparations with antibodies are shown in Table 3. The visualization process was completed according to the manufacturer's instructions using DAKO EnVision+System-HRP (DAKO K4010). The cell nucleus was stained using Harris hematoxylin. The immunohistochemical analysis was used a negative control (without antibody). Slides were dehydrated in a series of ethanol and rinsed in xylene, and then they were mounted with a DPX (Sigma). The number of

Table 2 Particle size, chemical composition (% dry weight basis) and caloric value of commercial diet AN, frozen zooplankton Z and formulated diets PP, AA, CON fed to *Cyprinus carpio*

Parameters (%)	Diets				
	AN	Z	PP	AA	CON
Crude protein	59	64.5	46	46	46
Crude lipid	21	12	6	6	6
Ash	11	3.2	7	7	7
Moisture	7	88.2	8	8	8
Gross energy (MJ kg ⁻¹)	23.2	20.7	19.6	19.6	19.6

PCNA-, MyoD-, myogenin- and Pax 3/7-positive cells were determined on cross-section area of one epaxial quadrante of white muscle.

Microscopic observations were carried out using a microscope Nikon Eclipse 90i and a camera Nikon Digital Sight DS-U1 (Nikon Corporation, Tokyo, Japan). Histomorphometric measurements and photographs were taken using the program NIS-Elements AR 2.10 (Nikon Corporation, Tokyo, Japan).

Statistical analysis

The received results were elaborated using statistical software Statgraphics Plus 4.1 and Statistica 10.0. Mean and standard deviation for histomorphometric measurements and for number of positive nucleus in immunohistochemical studies were calculated for all feeding groups. Statistical analysis was performed using the two-way analysis of variance (multifactor ANOVA). Significant differences between the experimental groups were analyzed using Tukey's test (HSD) ($P \leq 0.05$).

Results

Growth performance

On the twenty-eighth day of the experiment, the fish fed AN and Z had the highest survival rate (99.5 ± 1.0 %) compared to fish fed PP, AA and CON (85.3 ± 1.2 , 83.3 ± 5.8 , 85.05 ± 5.8 %, respectively), and the differences were statistically significant ($P \leq 0.05$). The highest body mass was found in fish fed AN (0.61 ± 0.28 g), while fish fed PP, AA and CON diets had a statistically lower body weight (0.23 ± 0.006 , 0.22 ± 0.006 , 0.19 ± 0.006 g, respectively) ($P \leq 0.05$). The lowest body weight was found in Z feeding group (0.12 ± 0.04 g) ($P \leq 0.05$).

Histology and immunohistochemistry

On the first day of the experiment, the morphological analysis of carps' cross-sections revealed the presence of red and white muscles. Cross-section area of white muscle was $272,557 \pm 56,294 \mu\text{m}^2$ and red muscle $15,735 \pm 2,137 \mu\text{m}^2$. During the experiment together with the growth of fish, the area of red and white muscles was increasing (Table 4).

Table 3 Antibodies and dilutions used in immunohistochemical analyses

Antibodies	Origin	Dilution	Incubation conditions
Monoclonal antibody of mouse PCNA klon PC10	DAKO, M0879	1:200	1-h room temperature
Polyclonal antibody of rabbit MyoD	Santa Cruz Biotechnology, sc-760	1:200	Overnight, 4 °C
Polyclonal antibody of rabbit Myogenin	Santa Cruz Biotechnology, sc-576	1:200	Overnight, 4 °C
Polyclonal antibody of rabbit Pax 3/7	Santa Cruz Biotechnology, sc-25409	1:200	Overnight, 4 °C

Table 4 Histomorfometry of the white muscles area (CSA_w), red muscles area (CSA_r), the total number of muscle fibers (TFN), the total number muscle nucleus (TNN) and muscle fiber area (FA) of carp fed experimental diets on 1st, 14th and 28th day of the experiment

Diets	Parameter				
	CSA _w	CSA _r	TFN	FA	TNN
<i>1st day of the experiment</i>					
	272,557 ± 56,294 ^a	15,735 ± 2,137 ^a	564 ± 50 ^{ab}	465.0 ± 251.1 ^{bc}	1,222 ± 177 ^a
<i>14th day of the experiment</i>					
AN	2,512,764 ± 272,359 ^{cd}	165,124 ± 5,477 ^e	1,053 ± 51 ^{de}	401.5 ± 381.7 ^{bc}	1,910 ± 226 ^{bc}
Z	497,262 ± 51,637 ^a	83,070 ± 10,105 ^{cd}	699 ± 98 ^{ab}	304.6 ± 377.3 ^a	2,016 ± 159 ^c
PP	780,667 ± 45,936 ^{ab}	61,404 ± 2,993 ^{abc}	442 ± 19 ^a	585.1 ± 512.1 ^d	1,297 ± 293 ^a
AA	692,646 ± 20,274 ^{ab}	72,516 ± 14,857 ^{bcd}	819 ± 134 ^{bcd}	383.5 ± 343.2 ^{ab}	1,652 ± 128 ^{ab}
CON	1,940,438 ± 51,202 ^c	58,814 ± 13,399 ^{abc}	581 ± 46 ^{ab}	423.9 ± 385.1 ^{bc}	1,997 ± 105 ^{abc}
<i>28th day of the experiment</i>					
AN	3,058,150 ± 691,721 ^d	164,980 ± 50,600 ^e	1,898 ± 288 ^f	848.1 ± 519.0 ^e	2,948 ± 483 ^d
Z	512,143 ± 53,184 ^a	40,249 ± 23,198 ^{abc}	726 ± 28 ^{abc}	481.0 ± 362.2 ^c	1,244 ± 81 ^a
PP	1,269,166 ± 30,602 ^b	112,045 ± 15,609 ^d	1,228 ± 42 ^c	1,302.2 ± 659.8 ^f	1,796 ± 34 ^{bc}
AA	759,699 ± 22,361 ^{ab}	57,658 ± 3,989 ^{abc}	1,032 ± 65 ^{cde}	825.5 ± 533.1 ^e	1,644 ± 144 ^{abc}
CON	2,104,677 ± 56,049 ^c	31,611 ± 5,741 ^{ab}	745 ± 26 ^{abcd}	477.1 ± 325.5 ^c	1,906 ± 232 ^{abc}

Different letters indicate statistically significant differences ($P \leq 0.05$)

On the fourteenth day of experiment did not observe any pathological changes in white muscles of fish from each experimental group (data not shown). The largest area of white and red muscle was observed in fish fed AN, whereas the smallest in fish fed Z, and the differences were statistically significant ($P \leq 0.05$) (Table 4). The largest TFN was observed in fish fed AN, while the smallest was observed in fish fed PP diet, and differences were statistically significant ($P \leq 0.05$) (Table 4). On the fourteenth day of experiment, the largest muscle FA was found in fish fed PP, whereas the smallest in fish fed Z, and the differences were statistically significant ($P \leq 0.05$) (Table 4). The largest TNN was found on 14th day of experiment in fish fed Z, while the least was noticed in fish fed PP, and the differences were statistically significant ($P \leq 0.05$) (Table 4).

On the twenty-eighth day of the experiment, morphological observations of the cross-sections of white muscles of fish fed PP, AA, CON and AN revealed that muscle fibers were tightly connected and separated by connective tissue (Fig. 1a), whereas fish fed Z exhibited wide empty spaces within the fibers, which may suggest either muscular degeneration or delayed myofibril synthesis (Fig. 1b). On the last day of the experiment, the largest area of white muscle was observed in fish fed AN, whereas the smallest in fish fed Z, and the differences were statistically significant ($P \leq 0.05$) (Table 4). Fish fed AN diet had the largest area of CSA_r, while the smallest was observed in fish fed CON diet (Table 4). The greatest TFN was observed in fish fed AN, while the least number in fish fed Z, and differences were statistically significant ($P \leq 0.05$) (Table 4). On the twenty-eighth day of the experiment, the largest muscle FA was found in fish fed PP, whereas the smallest in fish fed CON diet, and the differences were statistically significant ($P \leq 0.05$) (Table 4). The largest number of the muscle cells nucleus were found on 28th day of the experiment in fish fed AN, while the least was noticed in fish fed Z, and the differences were statistically significant ($P \leq 0.05$) (Table 4).

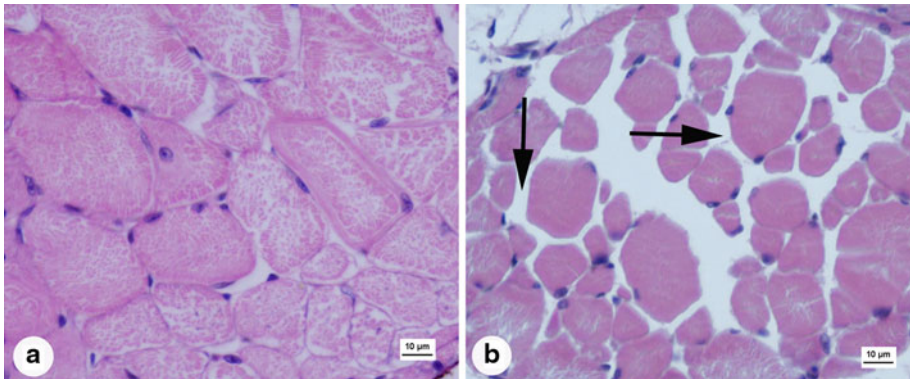


Fig. 1 The cross-section of carp white muscles on 28th day of the experiment fed: **a** AN; **b** Z. Arrows indicate fiber separation. Scale bars 10 μ m

On the fourteenth day of the experiment, the greatest number of PCNA-positive and myogenin-positive nucleus was found in fish fed AA, while the least number was found in fish fed Z, and differences were statistically significant ($P \leq 0.05$) (Table 5). The largest number of MyoD-positive nucleus was observed in fish fed PP, while the lowest number was observed in fish fed Z, and the differences were statistically significant ($P \leq 0.05$) (Table 5). The largest number of Pax 3/7-positive nucleus was found in fish fed AN, while the least number was found in the PP feeding group, and the differences were statistically significant ($P \leq 0.05$) (Table 5).

On the twenty-eighth day of the experiment, the greatest number of PCNA-positive nucleus was found in fish fed AA (Fig. 2a), while the least number was found in the Z feeding group (Fig. 2b), and the differences were statistically significant ($P \leq 0.05$) (Table 5). The largest number of MyoD (Fig. 2c)- and myogenin (Fig. 2d)-positive nucleus, compared to other feeding groups, was observed in fish fed PP diet ($P \leq 0.05$) (Table 5), while the largest number of Pax 3/7-positive nucleus was observed in fish fed Z diet (Fig. 2e) (Table 5). The least number of MyoD-positive nucleus was observed in fish fed AN (Fig. 2f), myogenin-positive nucleus in fish fed Z (Fig. 2g) and Pax 3/7-positive nucleus (Fig. 2h) in fish fed PP (Table 5).

Discussion

The growth of skeletal muscles in fish is a dynamic process involving hyperplasia and hypertrophy processes (Alami-Durante et al. 2010). In the present study, the histomorphometric results of red and white muscles indicate that the type of consumed food affects the growth of the muscles, as suggested by Alami-Durante et al. (2010). In recent years, many experiments were carried out to investigate the possibility of replacing fishmeal with plant protein, which is a cheaper source of protein (Urán et al. 2008; Ostaszewska et al. 2010a). Fontainhas-Fernandes et al. (1999) found that diets containing plant protein are worse absorbed and digested compared with diets containing fishmeal. In addition, diets containing a significant proportion of plant protein can cause a decrease in the diameter of muscle fibers and can affect the development of muscle tissue (Alami-Durante et al. 2010). Gómez-Requeni et al. (2004) found in sea bream (*Sparus aurata*) that

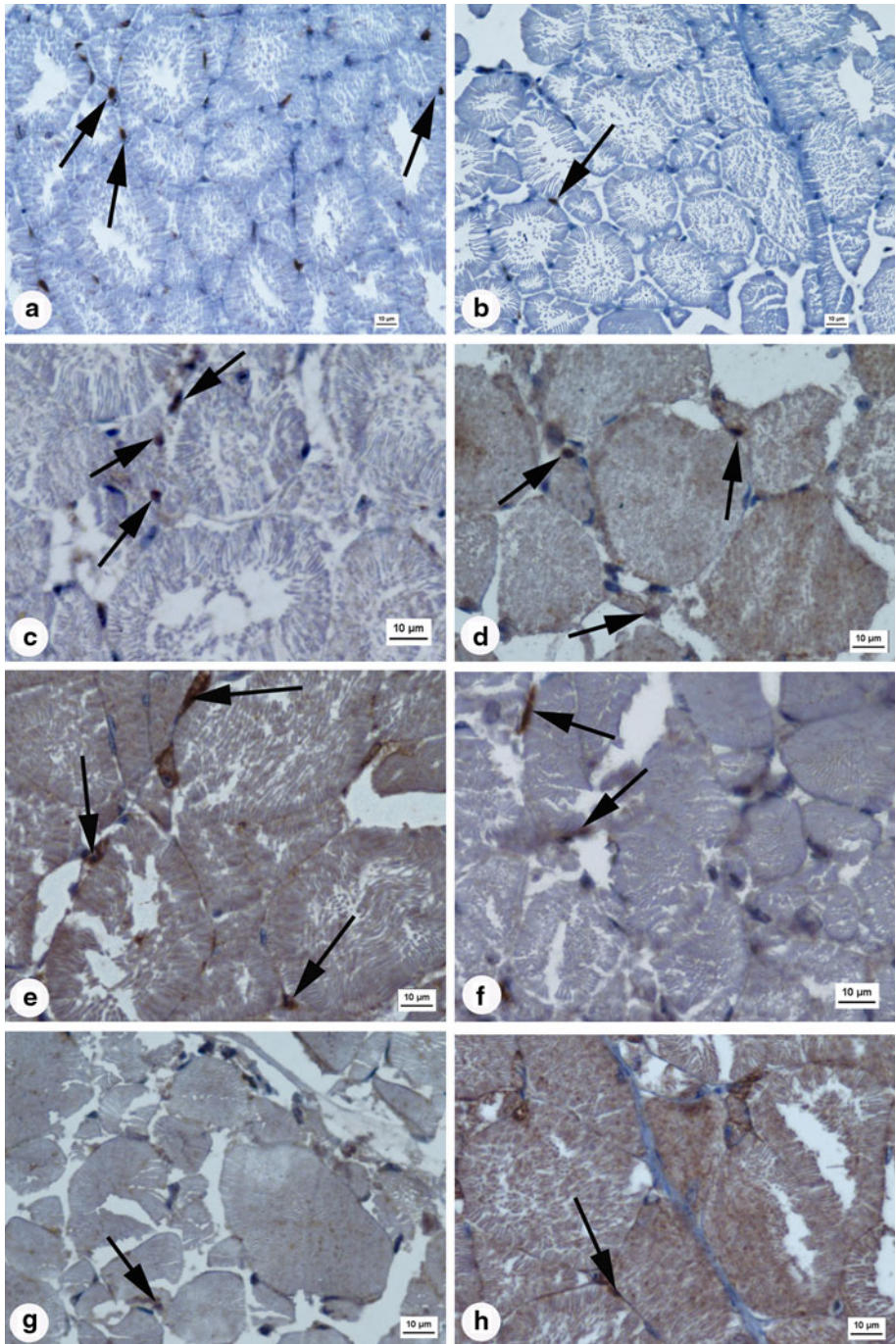
Fig. 2 Transverse section of myotomal muscle of carp on 28th day of the experiment. Immunohistochemical staining of the PCNA-positive nucleus (*arrows*) in muscles of carps fed: **a** AA; **b** Z. The greatest number of MyoD-positive nucleus (*arrows*) (**c**) and myogenin-positive nucleus (*arrows*) (**d**) in muscles of carps fed PP. The greatest number of Pax 3/7-positive nucleus (*arrows*) (**e**) in muscles of carps fed Z. **f** The least number of MyoD-positive nucleus (*arrows*) in the cross-section of white muscles of fish fed AN. The few myogenin-positive nucleus (*arrows*) (**g**) in the cross-section of white muscles of fish fed Z. The few Pax 3/7-positive nucleus (*arrows*) (**h**) in the cross-section of white muscles of fish fed PP. Scale bars 10 μ m

the proportion of plant protein in diet can reach up to 60–75 % without any negative influence on the growth of fish. However, according to Sitjá-Bobadilla et al. (2005), the complete replacement of fishmeal in diet is not recommended due to the significant decline in the growth of fish. The research from recent years suggests that it is possible completely replacing the fishmeal with plant protein, like canola protein isolate, without any negative influences on the growth of fish (Slawski et al. 2013). Replacing the fishmeal with plant protein has a positive influence on cell metabolism, due to the content of antioxidant compounds (Adom and Liu 2002) and antiviral and anti-inflammatory substances in plant protein (Sitjá-Bobadilla et al. 2005). The results of this study indicate that AN diet has a positive impact on the growth of red and white muscles, the area and the number of muscle fibers and the number of muscle nucleus. Among the formulated diets, composed of plant protein wheat gluten, the best growth of muscles was observed in carps fed, the diet containing wheat gluten supplemented with Lys–Gly dipeptide. Carps from this feeding group had a higher value of some histomorphometric parameters, such as CSAr, TFN, FA and TNN compared to fish fed AA and CON diets. This indicates that lysine given entirely as dipeptide was good absorption via carp's intestinal epithelium (Ostaszewska et al. 2010a) and eliminates the negative effects of indispensable amino acid deficiency (Dabrowski et al. 2010). Similar results were obtained by Aguiar et al. (2005) in Nile tilapia (*Oreochromis niloticus*). Tilapias fed a commercial diet had faster

Table 5 The number of PCNA-, MyoD-, myogenin- and Pax 3/7-positive nucleus on cross-section of epaxial quadrante of carp white muscle during the experiment

Diets	Parameter			
	PCNA	MyoD	Myogenin	Pax 3/7
<i>1st day of the experiment</i>				
	203 \pm 11 ^b	290 \pm 23 ^{bc}	265 \pm 23 ^{ab}	368 \pm 30 ^b
<i>14th day of the experiment</i>				
AN	206 \pm 9 ^b	185 \pm 12 ^{ab}	522 \pm 31 ^c	720 \pm 33 ^g
Z	152 \pm 12 ^a	166 \pm 4 ^a	343 \pm 22 ^c	612 \pm 17 ^{ef}
PP	361 \pm 10 ^{cd}	315 \pm 10 ^c	441 \pm 8 ^d	343 \pm 10 ^b
AA	422 \pm 37 ^{ef}	199 \pm 16 ^{ab}	672 \pm 21 ^f	671 \pm 20 ^{fg}
CON	346 \pm 28 ^c	196 \pm 8 ^{ab}	460 \pm 11 ^{de}	503 \pm 14 ^d
<i>28th day of the experiment</i>				
AN	371 \pm 27 ^{cde}	226 \pm 20 ^{abc}	437 \pm 18 ^d	422 \pm 27 ^c
Z	203 \pm 6 ^{ab}	236 \pm 34 ^{bc}	263 \pm 23 ^a	675 \pm 20 ^h
PP	403 \pm 14 ^{def}	339 \pm 30 ^c	471 \pm 12 ^{de}	251 \pm 16 ^a
AA	443 \pm 21 ^f	306 \pm 16 ^c	361 \pm 27 ^c	543 \pm 23 ^{de}
CON	418 \pm 17 ^{ef}	322 \pm 25 ^c	334 \pm 19 ^{bc}	612 \pm 25 ^{ef}

Different letters indicate statistically significant differences ($P \leq 0.05$)



growth and higher number of muscle fibers in the myotomal count area and the larger diameter of fibers in the red and white muscles compared to fish fed diets containing corn gluten supplemented with different doses of lysine. Feeding with diets rich in plant

protein provides the energy used to build muscle proteins, antibody hormones and enzymes (Gómez-Requeni et al. 2011). Inappropriate amino acid composition in plant protein is a factor limiting the use of such proteins in the fish feeding (Finn and Fyhn 2010). According to Small and Soares (2000) shortages of amino acid, lysine limits the growth of the fish in the highest degree and can affect the protein synthesis. According to Ostaszewska et al. (2010a), lysine deficiencies in carps' diet can cause poor growth of fish and pathological changes in the digestive system. Similarly, in the present study, carps fed lysine deficiency diets had a low value of TFN and FA, which may indicate an adverse changes in the muscle of fish resulting from the indispensable amino acid deficiency.

The natural food, zooplankton, administered in the present study at a restriction dose has caused an increase in the rate of growth and pathological changes in the muscle tissue. Starvation may result in a damage of the structure and pathological changes in the muscles (Green and McCormick 1999). These changes, in the case of carp, were mainly for the white muscles and caused the separation of the muscle fibers. However, as suggested by Margulies (1993), the pathological changes in muscles observed later than those appearing in other organs. This may be the result of the body's defense reaction subjected to the stress of starvation, when observed glucose influx from the liver to the muscles takes place in order to maintain the tissue metabolism (Love 1980).

The type of administered food also affects the molecular changes in the metabolism of muscle cells and myogenic progenitor cells that play a key role in the growth and muscle tissue development processes (Chapalamadugu et al. 2009). New myocytes can be merged with the existing muscle fibers (hypertrophy) or by connecting with each other, create new muscle fibers (hyperplasia) (Rowlerson and Veggetti 2001). Myogenic progenitor cells are responsible for the processes of regeneration in muscle tissue, and their deficiency may result in pathological changes in the muscles (Jejurikar and Kuzon 2003). In the case of fish reaching the large size, such as carp, rainbow trout (*Oncorhynchus mykiss*) (Chapalamadugu et al. 2009) and Atlantic salmon (*Salmo salar*) (Johnston et al. 1998), myogenic progenitor cells are also activated in the processes of muscle cell hyperplasia (Alami-Durante et al. 2010).

The presence of PCNA protein is correlated with the synthesis of DNA and reaches its maximum in the S phase of the cell cycle (Baserga 1991). Cell activation and PCNA expression are significantly faster than the activation of MyoD factor, which reaches its maximum in the G1 phase of the cell cycle (Kitzmann et al. 1998). The number of PCNA-positive nucleus may be the result of the myogenic progenitor cells activation, as they are the main source of muscle cells in the myogenesis process (Brodeur et al. 2002). On frequency of PCNA positive nucleus influenced amount and quality of administered food (Brodeur et al. 2002; Ostaszewska et al. 2008a). The results of the present study are comparable with the results by Brodeur et al. (2002) in *Notothenia coriiceps*. Carp and *Notothenia coriiceps* had larger PCNA-positive nucleus number in muscle cells than MyoD at the same time. This is probably the result of the increase in DNA synthesis needed for the development of the muscle tissue. The largest PCNA-positive nucleus number at 14th and 28th of experiment was observed in carp fed AA. These results may suggest that feeding fish wheat gluten protein-based diets supplemented free lysine has a beneficial effect on proliferation of muscle cells. Similarly, in Nile tilapia, fed diets with different levels of lysine supplementation proliferation of muscle cells were higher compared to fish fed commercial diets (Aguiar et al. 2005). The least PCNA-positive nucleus number was observed in carp fed Z at a restriction dose, which may confirm that the diet does not fulfill the nutritional requirements of fish causes slow growth rate and reduced muscle growth (Ostaszewska et al. 2008a).

The evaluation of the activity of progenitor cells is carried out on the basis of the study results that determine the number of nucleus in which there are active myogenic factors regulating the transcription (MRFs). It was found that MyoD is activated at the time of initiation of the muscle cells, while myogenin at the moment of final differentiation and fusion of muscle cells (Sabourin and Rudnicki 2000). MyoD, as a factor regulating the transcription process, plays a key role in the process of specification and differentiation of the myogenic cells (Weitraub et al. 1994). Tan and Du (2002) have found that MyoD is activated in the early stages of myogenesis, while myogenin is activated much later and is responsible for joining the muscle cells into the process of differentiation (Rudnicki et al. 1992). Supplementation of the diets with plant protein influences on the expression change in MyoD and myogenin (Chapalamadugu et al. 2009; Alami-Durante et al. 2010) as it was observed in tested fish fed diet composed of plant protein wheat gluten. The largest number of MyoD-positive nucleus in carps fed PP diet indicates the intense proliferation of myoblasts causing the hyperplastic of white muscles (Johansen and Overtruf 2005; de Almeida et al. 2010). Moreover, the largest number of the myogenin-positive nucleus in the PP feeding group may suggest that apart from the hyperplastic muscle growth, in these fish, an intensive muscle growth occurs by hypertrophy (de Almeida et al. 2010), whereas a small number of PCNA nucleus, MyoD and myogenin-positive nucleus observed in carps fed Z may be the result of nutritional factors, as it happens in pike-perch larva fed with a diet, which does not fulfill the nutritional requirements (Ostaszewska et al. 2008a).

MRFs play a key role in myogenesis that regulate the transcription, but Pax proteins play an important role as well. Pax 3 and Pax 7 proteins affect the transcription of MRF genes (Przewozniak and Brzózka 2008). In addition, Pax 3 and Pax 7 protein are responsible for the proliferation and migration of progenitor cells during the myogenesis (Ludolph and Konieczny 1995). The results of studies concerning Pax 3/7 protein clearly showed that the highest Pax 3/7-positive nucleus frequency was found in carps fed a restrictive diet Z. This may be due to the function Pax 3/7 proteins perform in the development and growth of muscle cells. They are responsible for the growth and differentiation of cells in the embryonic and postembryonic phase, including the differentiation of muscle precursor cells (Przewozniak and Brzózka 2008). A large number of Pax 3/7-positive nucleus in the muscles of carps fed Z may indicate that, numerous present in fish from this feeding group, myogenic precursors cells might be implicated in muscle growth (Kacperczyk et al. 2011).

Feeding carps Aglo Norse commercial diet had a positive impact on the development and growth of red and white muscles. The weakest growth was observed in fish fed Z at a reduced dose, expressed as the smallest area of red and white muscles, but also the pathological changes in muscles. Among formulated diets, based on the plant protein wheat gluten, a positive impact on the structure and muscle development was observed in carps fed diet supplemented with Lys–Gly dipeptide (PP). This diet stimulates the growth of muscles and the development of muscle fibers. It has a positive influence on recruiting new muscle fibers needed for the growth of skeletal muscles of fish.

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