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Influence of the protein quantity from combined fodder on the corporal development of guppy (*Poecilia reticulata*)

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Abstract. This paper aims to highlight the effects of various concentrations of protein premixtures on the growth in length and weight of adult specimens of ornamental fish *Poecilia reticulata*, bred in captivity. Of this consideration, the experiment was set up with six groups: R1, R2, R3, TG, TP and TD, which were fed differently as follows: R1 with mixed fodder containing 30% CP (crude protein), own recipe; R2 with mixed fodder containing 30% GP, own recipe; R3 with mixed fodder containing 30% CP, own recipe; TG with Troco Grower fodder containing 43% CP; TP with Troco Prim fodder containing 44% CP; TD with Tetra Discus fodder containing 48% CP. The research was conducted over a period of three months in which there have been followed the length and weight, to determine body indices (average increase of the weight gain, Fulton index and average weight ratio multiplier).
Key Words: Guppy, *Poecilia reticulata*, mixed fodder, crude protein, body development, body indices.

Rezumat. Lucrarea are drept scop punerea în evidenţă a efectelor diferitelor concentraţii de proteină din nutreţurile combinate administrate asupra creşterii în lungime şi greutate a exemplarelor adulte din specia de peşti ornamentali *Poecilia reticulata* crescute în captivitate. În acest sens, s-au format şase loturi experimentale: R1, R2, R3, TG, TP şi TD care au fost hrănite diferenţiat după cum urmează: R1 cu nutreţ combinat după reţetă proprie cu 30% PB; R2 cu nutreţ combinat după reţetă proprie cu 30% PB; R3 cu nutreţ combinat după reţetă proprie cu 30% PB; TG cu nutreţ Troco Grower cu 43% PB; TP cu nutreţ Troco Prim cu 44% PB; TD cu nutreţ Tetra Discus cu 48% PB. Cercetările s-au derulat pe o perioadă de trei luni în care s-au urmărit: lungimea şi greutatea corporală pe baza cărora s-au determinat indicii corporali (sporul mediu de creştere în greutate, indicele Fulton şi raportul de multiplicare a greutăţii medii).
Cuvinte cheie: Guppy, *Poecilia reticulata*, nutreţ combinat, proteină brută, dezvoltare corporală, indici corporali.

Introduction. Guppy (*Poecilia reticulata*) is one of the best model organisms used in various studies of sex-determination (Lindholm & Breden 2002; Lozinsky 2011; Mousavi-Sabet et al 2012), classical and molecular genetics (Dreyer et al 2007), color patterns (Petrescu-Mag & Bourne 2008), evolution of the color patterns (Hughes et al 1999), speciation and evolution (Alexander & Breden 2004), behavioral ecology (Houde 1997), spinal deformities (Gorman & Breden 2007), anatomical, physiological, embryological (Martyn et al 2006), ecotoxicological (Georgescu & Georgescu 2012; Talu et al 2012) and nutrition.

P. reticulata is a common exotic species raised in captivity conditions because it's high prolificacy (Bud 2006), low biological cycle (reaches sexual maturity at three months) and does not require special conditions for growth (Oprea 2000).

If when we talk about the consumption fish, it is mainly followed the relationship between body mass and skeletal weight, for aquarium fish the appearance and the body shape presents more interest. All this is determined by the quantity and quality of the given mixed fodder (Petrescu-Mag 2007).

This paper aims to highlight how the amount of protein in the fodder influences the body development of *P. reticulata*, one of the most common ornamental fish in

aquariums throughout the world. Proteins are substances of particular importance, they are only found in the highest proportion in organs and muscle tissue, performing biological functions that can not be met by another group of nutrients (Halga et al 2005).

Material and Method. Biological material on which the investigations were carried out was formed of 90 adults, aged 87-93 days, of the *P. reticulata* species, that were divided into six batches of 15 samples each. Each group was fed differently, as follows:
- group R1 with mixed fodder with 30% CP, own recipe;
- group R2 with mixed fodder with 30% CP, own recipe;
- group R3 with mixed fodder with 30% CP, own recipe;
- group TP with Troco Prim fodder with 43% CP;
- group TG with Troco Grower fodder with 44% CP;
- group TD with Tetra Discus fodder with 48% CP.

The research was conducted in the Laboratory of Aquaculture of USAMV Iasi, Romania, on a period of three months, time in which was followed the body development indices (increase in body weight and length).

For this purpose we used two tanks of the same capacity (15 liters) divided into three compartments each. Both tanks were fitted with the lighting (fluorescent lamp), air filter (electric indoor filter Hailea), portable aeration (aeration pump equipped with a nozzle Hailea), plant substrate (plants of the genus *Vallisneria* and *Elodea*), mineral substrate (quartz gravel). The two tanks were introduced in two aquariums, larger both in width and in length. The heating was performed with one electric 50W Kandge heater with thermostat, mounted outside the aquarium. The temperature reading was made using alcohol thermometers, one for each tank.

The followed parameters were average body length and average weight with which were calculated:

- Fulton index (maintenance index):

It was determined with the formula (Păsărin & Stan 2002):

$$L_i = \frac{g \times 100}{l^3}$$

where:
Li = maintenance index;
g = weight (g);
l = standard body length (cm).

- Average increase weight gain:

It has been determined using the formula (Grether et al 2004):

$$S_p = G_f - G_i$$

where:
Sp = average gain of weight (mg);
Gf = average final weight (mg);
Gi = average initial weight (mg).

- The multiplying average weight ratio:

It was calculated with the formula (Misăilă et al 1990):

$$R.M.G.M. = \frac{G_f}{G_i}$$

where:
R.M.G.M. = the average weight multiplier;
Gf = average final weight;
Gi = average initial weight.

The parameters were determined at the end of each month throughout experiment. Body length was measured using an electronic calliper and weight using the Denver analytical balance type.

Results and Discussion. The gross chemical composition of the mixed fodder was given importance in the development of the fish's body. Each specie has specific nutrient requirements that must be provided by the administered combined fodder.

Table 1

The gross chemical composition of the used feeds

Combined fodder	*DM (%)*	*A (%)*	*CP (%)*	*CF (%)*	*CC (%)*
R1	93	13	30	7,0	4,4
R2	93	13	30	7,5	3,4
R3	93	13	30	8,0	4,0
Troco Prim	95	5	43	15,0	2,5
Troco Grower	93	10	44	22,0	1,1
Tetra Discus	94	11	48	6,5	2,0

DM – dry matter, A – ash, CP – crude protein, CF – crude fat, CC - crude cellulose.

The combined premixtures R1, R2 and R3 have the same amount of protein, respectively 30%, being obtained in the Quality Control Feed Laboratory of USAMV Iasi as were the nutritional requirements of the species *P. reticulata*. Commercial feeds have a higher percentage of protein which varied between 43-48%.

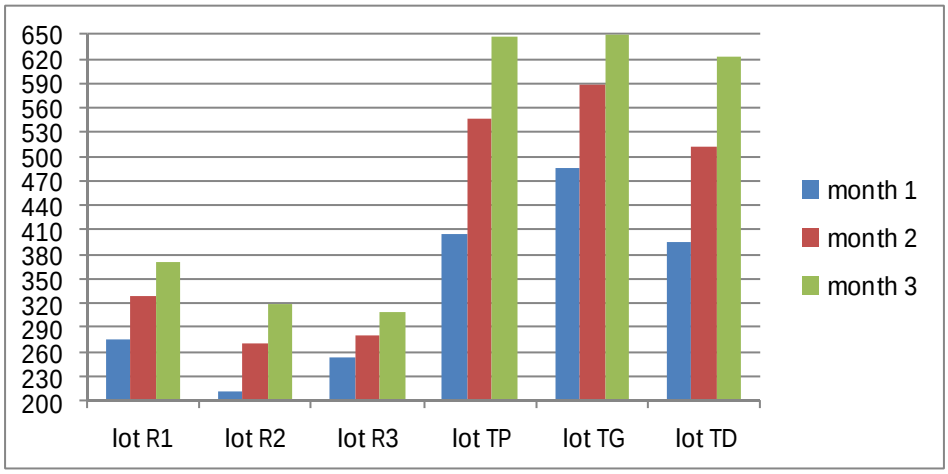

Figure 1. Evolution of average weight of the experimental groups.

For the specimen lots R1, R2 and R3, which were fed with compound feed with the same amount of protein (30%), the average weight had values close for each of the three months of experiment. Copies of lots TP, TG and TD achieved a higher growt in weight compared to previous batches due to higher amount of protein administered in the fodder (43%, 44% respectively 48%).

Table 2

The variation of the average body weight

Group	Specification	Month		
		1	2	3
R1	Variation interval (mg)	200 – 450	250 – 504	290 – 537
	$\bar{x}$	274.20	328.50	370.80
	$\bar{x} \pm s_{\bar{x}}$	274.20 ± 19.43	328.50 ± 19.22	370.80 ± 18.70
	C_V%	27.45	22.66	19.53
R2	Variation interval (mg)	120 – 420	178 – 478	228 – 525
	$\bar{x}$	210.80	269.00	318.50
	$\bar{x} \pm s_{\bar{x}}$	210.80 ± 19.34	269.00 ± 19.41	318.50 ± 19.16
	C_V%	35.53	27.94	23.30
R3	Variation interval (mg)	190 – 440	140 – 470	165 – 500
	$\bar{x}$	253.10	280.90	310.40
	$\bar{x} \pm s_{\bar{x}}$	253.10 ± 17.68	280.90 ± 20.32	310.40 ± 20.34
	C_V%	27.06	28.02	25.39
TG	Variation interval (mg)	210 – 980	310 – 1070	370 – 1110
	$\bar{x}$	484.30	588.60	649.10
	$\bar{x} \pm s_{\bar{x}}$	484.30 ± 64.14	588.60 ± 65.70	649.10 ± 64.72
	C_V%	51.29	43.23	38.62
TP	Variation interval (mg)	220 – 680	350 – 795	510 – 900
	$\bar{x}$	405	547.90	647.50
	$\bar{x} \pm s_{\bar{x}}$	405.00 ± 42.80	457.90 ± 37.47	647.50 ± 38.25
	C_V%	40.93	26.49	22.88
TD	Variation interval (mg)	220 – 610	340 – 725	445 – 830
	$\bar{x}$	395	513.10	621.90
	$\bar{x} \pm s_{\bar{x}}$	395.00 ± 32.88	513.10 ± 32.80	621.90 ± 32.79
	C_V%	32.23	24.76	20.42

The amount of protein directly influences the weight gain. The higher amount of protein in the feed, the greater is the increase of the weight (Figure 1, Table 2). However, the amount of the protein in the fodder should rational dosed, to avoid unjustified expenses with fodder, which would increase the final price and became economically onerously.

Statistics (Fisher and Tukey test) has been revealed that among the groups R1, R2 and R3 the differences in weight gain were insignificant throughout the experiment. Copies of these groups were fed with fodder obtained according to recipes R1, R2 or R3, containing the same amount of protein (30%). For lots TD, TP and TG the feed was with a higher amount of protein (43%, 44% and 48%), showing a greater increase in weight compared to groups R1, R2 and R3, these differences being distinctly significant. Between lots TD, TG and TP the statistical differences were insignificant.

Table 3

The evolution of the body weight in adults

Month	*Statical differences*	*Fisher / Tukey test*
1	SDD	$F_{a0,001} = 4.76 < F = 7.21$
	DS	R1 – TD $w_{0,05} = 117.80 < w = 120.83 < w_{0,01} = 141.30$
	ID	R1 – TP $w_{0,05} = 122.61 < w = 130.83 < w_{0,01} = 147.07$
	SDD	R1 – TG $w = 210.12 > w_{0,01} = 141.30$
	ID	R1 – R3 $w = 21.09 < w_{0,05} = 120.13$
	ID	R1 – R2 $w = 63.40 < w_{0,05} = 120.13$
	SDD	R2 – TD $w = 184.23 > w_{0,01} = 138.66$
	SDD	R2 – TP $w = 194.23 > w_{0,01} = 144.10$
	SDD	R2 – TG $w = 273.52 > w_{0,01} = 138.66$
	ID	R2 – R3 $w = 42.31 < w_{0,05} = 117.80$
	SDD	R3 – TD $w = 141.92 > w_{0,01} = 138.66$
	SDD	R3 – TP $w = 151.92 > w_{0,01} = 144.10$
	SDD	R3 – TG $w = 231.21 > w_{0,01} = 138.66$
	ID	TG – TD $w = 89.29 < w_{0,05} = 113.51$
	ID	TG – TP $w = 79.29 < w_{0,05} = 117.80$
	ID	TP – TG $w = 10.00 < w_{0,05} = 117.80$
2	SDD	$F_{a0,001} = 4.14 < F = 13.2$
	SDD	R1 – TD $w = 184.57 > w_{0,01} = 135.81$
	SDD	R1 – TP $w = 219.33 > w_{0,01} = 141.36$
	SDD	R1 – TG $w = 260.07 > w_{0,01} = 135.81$
	ID	R1 – R3 $w = 47.65 < w_{0,05} = 114.36$
	ID	R1 – R2 $w = 59.60 < w_{0,05} = 114.36$
	SDD	R2 – TD $w = 244.07 > w_{0,01} = 133.27$
	SDD	R2 – TP $w = 278.83 > w_{0,01} = 138.50$
	SDD	R2 – TG $w = 319.57 > w_{0,01} = 133.27$
	ID	R2 – R3 $w = 11.85 < w_{0,05} = 112.14$
	SDD	R3 – TD $w = 232.23 > w_{0,01} = 133.27$
	SDD	R3 – TP $w = 266.99 > w_{0,01} = 138.50$
	SDD	R3 – TG $w = 307.73 > w_{0,01} = 133.27$
	ID	TG – TD $w = 75.50 < w_{0,05} = 108.06$
	ID	TG – TP $w = 40.74 < w_{0,05} = 112.14$
	ID	TP – TG $w = 34.76 < w_{0,05} = 112.14$
3	SDD	$F_{a0,001} = 3.33 < F = 27.62$
	SDD	R1 – TD $w = 251.1 > w_{0,01} = 117.22$
	SDD	R1 – TP $w = 276.75 > w_{0,01} = 119.64$
	SDD	R1 – TG $w = 278.32 > w_{0,01} = 114.94$
	ID	R1 – R3 $w = 60.37 < w_{0,05} = 96.79$
	ID	R1 – R2 $w = 52.29 < w_{0,05} = 96.79$
	SDD	R2 – TD $w = 303.4 > w_{0,01} = 114.94$
	SDD	R2 – TP $w = 329.04 > w_{0,01} = 117.22$
	SDD	R2 – TG $w = 330.61 > w_{0,01} = 112.79$
	ID	R2 – R3 $w = 8.08 < w_{0,05} = 94.91$
	SDD	R3 – TD $w = 311.47 > w_{0,01} = 114.94$
	SDD	R3 – TP $w = 337.12 > w_{0,01} = 117.22$
	SDD	R3 – TG $w = 338.69 > w_{0,01} = 112.79$
	ID	TG – TD $w = 27.21 < w_{0,05} = 93.14$
	ID	TG – TP $w = 1.57 < w_{0,05} = 94.91$
	ID	TP – TG $w = 25.64 < w_{0,05} = 96.79$

SDD - significant distinct differences, DS - distinct differences, ID - insignificant differences.

Table 4

The evolution of body length in adults

Group	Specification	Month		
		1	2	3
R1	Variation interval (cm)	3.00 – 3.70	4.00 – 4.70	5.10 – 5.80
	$\bar{x}$	3.20	4.20	5.30
	$\bar{x} \pm s_{\bar{x}}$	3.20 ± 0.06	4.2 ± 0.05	5.3 ± 0.05
	C_V%	6.64	4.87	3.87
R2	Variation interval (cm)	2.90 – 3.60	3.90 – 4.50	5.10 – 5.70
	$\bar{x}$	3.10	4.10	5.30
	$\bar{x} \pm s_{\bar{x}}$	3.10 ± 0.05	4.10 ± 0.05	5.30 ± 0.05
	C_V%	5.75	4.36	3.38
R3	Variation interval (cm)	3.00 – 3.40	3.90 – 4.30	5.00 – 5.40
	$\bar{x}$	3.10	4.10	5.20
	$\bar{x} \pm s_{\bar{x}}$	3.10 ± 0.03	4.10 ± 0.03	5.20 ± 0.03
	C_V%	4.17	3.15	2.48
TG	Variation interval (cm)	3.00 – 4.30	4.00 – 5.30	5.00 – 6.30
	$\bar{x}$	3.50	4.50	5.50
	$\bar{x} \pm s_{\bar{x}}$	3.50 ± 0.11	4.50 ± 0.11	5.50 ± 0.11
	C_V%	12.57	9.50	7.79
TP	Variation interval (cm)	3.10 – 3.80	4.10 – 4.80	5.10 – 5.80
	$\bar{x}$	3.40	4.40	5.40
	$\bar{x} \pm s_{\bar{x}}$	3.40 ± 0.07	4.40 ± 0.07	5.40 ± 0.07
	C_V%	8.19	5.89	4.80
TD	Variation interval (cm)	3.00 – 3.80	4.00– 4.80	5.00 – 5.80
	$\bar{x}$	3.40	4.40	5.40
	$\bar{x} \pm s_{\bar{x}}$	3.40 ± 0.06	4.40 ± 0.06	5.40 ± 0.06
	C_V%	7.31	5.60	4.57

The increase in length it is less influenced by the amount of protein administered in the combined fodder compared to the weight gain. Average body length had similar values in each of the three months of experimentation for all lots. In the first month there had been a body length ranged from 3.10 to 4.10 cm, in the second between 4.10 cm and 5.10 cm and in the third between 5.20 cm and 5.50 cm.

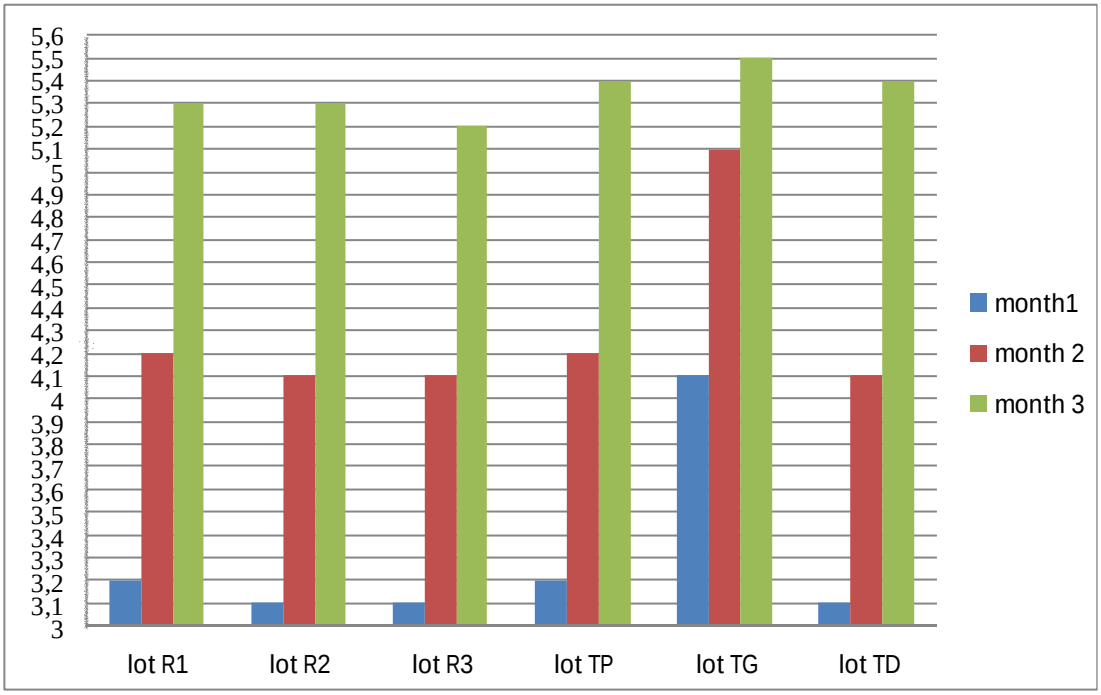

Figure 2. The evolution of the average body length.

From the previous chart we can see that the groups R1, R2 and R3 had similar values in the increase in length, the difference between them being 0.10 cm, while between lots TG, TP and TD the differences were larger (1 cm).

In the second month the increase in length was 1 cm for all the experimental groups compared to the first month, while in the third month the growth varied between 1.10 cm and 1.30 cm, slightly higher values for the groups TP, TG and TD.

The amount of protein in the given fodder is one of the factors determining the growth in length, with environmental conditions (temperature, pH, water chemistry) and social factors (stocking density, relations between samples in the group).

At the end of the three months of experimentation there was a varied value of the average length in the lots fed with the laboratory recipes from 5.20 cm for group R3 and to 5.30 cm for R1 group, while the groups fed with commercial feed had body length values between 5.40 cm and 5.60 cm.

The average body length for lots R1, R2 and R3 had similar values at the end of each experiment; he differences between groups were not significant, a possible explanation could be that the feeds used had the same concentration of protein, of 30%. For the groups fed with Troco Prim and Tetra Discus, the average body length value at the end of three months of experimentation was 5.40 cm, although they received fodder with different concentrations of protein (43% for Troco Prim and 48% for Tetra Discus). The average body length for the TG group, whose copies were fed with Troco Grower (44% CP) was with 0.20 cm higher than the TD group, which was fed with Tetra Discus (48% CP).

From this we conclude that the value of the body length is influenced in a lesser extent of the amount of protein in the administered fodder, compared to the weight gain.

Table 5

The evolution of body length in adults

Month	*Statistical differences*	Fisher / Tukey test
1	SDD	$F_{a0,01} = 3.34 < F = 3.95 < F_{a0,001} = 4.76$
	ID	R1 – TD $w = 0.14 < w_{0,05} = 0.22$
	ID	R1 – TP $w = 0.15 < w_{0,05} = 0.23$
	SD	R1 – TG $w_{0,05} = 0.22 < w = 0.24 < w_{0,01} = 0.26$
	ID	R1 – R3 $w = 0.12 < w_{0,05} = 0.22$
	ID	R1 – R2 $w = 0.10 < w_{0,05} = 0.22$
	SD	R2 – TD $w_{0,05} = 0.22 < w = 0.25 < w_{0,01} = 0.26$
	SD	R2 – TP $w_{0,05} = 0.22 < w = 0.25 < w_{0,01} = 0.27$
	SDD	R2 – TG $w = 0.34 > w_{0,01} = 0.26$
	ID	R2 – R3 $w = 0.02 < w_{0,05} = 0.22$
	SD	R3 – TD $w_{0,05} = 0.22 < w = 0.26 < w_{0,01} = 0.27$
	SD	R3 – TP $w_{0,05} = 0.22 < w = 0.27 < w_{0,01} = 0.28$
	SDD	R3 – TG $w = 0.36 > w_{0,01} = 0.26$
	ID	TG – TD $w = 0.09 < w_{0,05} = 0.21$
	ID	TG – TP $w = 0.09 < w_{0,05} = 0.22$
	ID	TP – TD $w = 0.01 < w_{0,05} = 0.22$
2	SDD	$F_{a0,001} = 4.76 < F = 5.39$
	ID	R1 – TD $w = 0.15 < w_{0,05} = 0.21$
	ID	R1 – TP $w = 0.16 < w_{0,05} = 0.21$
	SDD	R1 – TG $w = 0.29 > w_{0,01} = 0.25$
	ID	R1 – R3 $w = 0.14 < w_{0,05} = 0.21$
	ID	R1 – R2 $w = 0.10 < w_{0,05} = 0.21$
	SDD	R2 – TD $w = 0.25 > w_{0,01} = 0.24$
	SDD	R2 – TP $w = 0.26 < w_{0,01} = 0.25$
	SDD	R2 – TG $w = 0.39 > w_{0,01} = 0.24$
	ID	R2 – R3 $w = 0.04 < w_{0,05} = 0.21$
	SDD	R3 – TD $w = 0.29 > w_{0,01} = 0.24$
	SDD	R3 – TP $w = 0.30 > w_{0,01} = 0.25$
	SDD	R3 – TG $w = 0.43 > w_{0,01} = 0.24$
	ID	TG – TD $w = 0.14 < w_{0,05} = 0.20$
	ID	TG – TP $w = 0.13 < w_{0,05} = 0.21$
	ID	TP – TD $w = 0.01 < w_{0,05} = 0.21$
3	ID	$F = 1.5 < F_{a0,05} = 2.37$

SDD - significant distinct differences, SD - significant differences, ID - insignificant differences.

In the first month, the maintenance index had values around 1, the specimens having a good maintenance. In the next two months, the relationship between body weight and length was reduced because some females entered in the gestation period and therefore had a greater weight gain compared to the increase in length. Weight gain of the females is illustrated also by the increase of weight gain, being bigger for the lots TG, TP and TD.

In the second month the maintenance index decreased for all the experimental groups, being minimum for lot R2 (0.31) and maximum for TP and TD groups (0.74). The length and weight growth decreased with age. Therefore, also the Fulton index's values were lower than the first month.

The multiplication average weight ratio had similar values for all groups, ranging between 1.11 and 1.35.

In the third month, the maintenance index declined further for all the experimental groups, being minimum for lot R2 (0.21) and maximum for the TP group (0.41). Weight gain during the gestation period at females resulted in a greater weight gain for lots TG, TP and TD, compared to the groups R1, R2 and R3. The multiplication of the average weight ratio for all groups had similar values, ranging between 1.11 and 1.21.

Table 6

Corporeal indices in adults

Specification	*Month*	*Experimental group*					
		R1	*R2*	*R3*	*TP*	*TG*	*TD*
Average body length (cm)	1	3.20	3.10	3.10	3.20	4.10	3.10
	2	4.20	4.10	4.10	4.20	5.10	4.10
	3	5.30	5.30	5.20	5.40	5.50	5.40
Average initial weight (mg)	1	216.0	157.8	228.5	278.8	333.3	273.3
	2	274.2	210.8	253.1	405.0	484.3	395.0
	3	328.5	269.0	280.9	547.9	588.6	513.1
Average final weight (mg)	1	274.2	210.8	253.1	405.0	484.3	395.0
	2	328.5	269.0	280.9	547.9	588.6	513.1
	3	370.8	318.5	310.4	647.5	649.1	621.9
Maintenance index (Fulton)	1	0.84	0.71	0.85	1.03	1.13	1.01
	2	0.44	0.31	0.41	0.74	0.44	0.74
	3	0.25	0.21	0.22	0.41	0.39	0.40
Average weight gain (mg)	1	58.2	53.0	24.6	126.2	151.0	125.2
	2	54.3	58.2	27.8	142.9	104.3	118.1
	3	42.3	49.5	29.5	99.6	60.5	118.8
Multiplication ratio of average weight	1	1.27	1.34	0.90	1.45	1.45	1.45
	2	1.20	1.28	1.11	1.35	1.22	1.30
	3	1.13	1.18	1.11	1.18	1.10	1.21

Conclusions

1. The weight gain is influenced directly proportional by the percentage of protein in the given combined fodder;
2. The percentage of protein in combined fodder used for feeding the fish of the species *P. reticulata* causes small changes in body length;
3. The maintenance index's values increase proportionally to the percentage of the protein in the used combined fodder;
4. Average value of the weight gain is directly dependent on the protein amount of the used food;
5. Body development of the species of *P. reticulata* depends on the gross content in protein of the used mixed fodder.

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