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## Length, weight and condition factor comparison of *Carassius auratus* (Linnaeus, 1758) juveniles cultured in biofloc system

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### Abstract

*Carassius auratus* is an omnivorous organism resistant to their handling, so it is a popular specie that accept a widely food variety like leaflets, live food or pellets. The Biofloc technique system was considered as efficiency alternative, which supply nutrients that can be reused or recycled. One hundred and twenty juvenile organisms of *C. auratus* were used with a mean length of  $5.9 \pm 0.95$  cm and weight of  $4.2 \pm 1.08$  g. The juveniles were fed with commercial diet (Wardley), with a protein content of 45% and were supply 10% of their body weight. The food quantity was adjusted every 15 days. Also, were supply a different carbohydrates sources: molasses, coffees remain and dry moringa in C/N ratio of 20:1. Every 15 days, biometry (weight and length) were taken to obtain absolute and instantaneous growth rates and condition factor until 120 days. At end of experiment it was observed that carbon source molasses had the best results in weight/length ratio with respect to coffee and moringa. With respect to condition factor (KM), all experiment diets presented highest values than initial KM. Control diet showed better weight/length ratio, although this diet showed lowest values in length and weight.

**Keywords:** *Carassius auratus*, Biofloc, growth rates, condition factor

### 1. Introduction

Condition factor (KM) and weight/length ratio are important tools for biology, ecology and physiology of many fishes and to advice regulation of fisheries and maintenance for wild populations (Froese, 1998; Oscoz *et al.* 2005; De Giosa *et al.* 2014) [1-3]. Also, they are useful tools to compare well-being, corpulence and pregnancy (Tesch 1971) [4]. The weight/length ratio is necessary to estimate growth range, length and age, as well as another component of population dynamics (Beyer 1987; Oscoz *et al.* 2005) [5, 2].

Few information about condition factor and weight/length ratio at different species of *Carassius sp.* genus exists. In this topic, was only found the study with *C. gibelio* in Lake Leszczynskie, Poland (De Giosa *et al.* 2014) [3], which studied seasonal changes in wild population. With respect *C. aureatus* in captivity, the last study was made by Priestly *et al.* (2006) [6], which studied the influence of food frequency and body condition of this specie. Moreover, it is convenient to consider, that these characteristics were different depending developing stage, type and quality food, and environmental conditions.

*Carassius sp.* genus in some countries was an important fishery resource like Poland with *C. gibelios* specie (De Giosa *et al.* 2014) [3] and Lake Trasimeno (Italy) (Lorenzoni and Ghetti 2010) [7]. It is a popular specie because it accepts leaflets, live foods and pellets. For general, it is common to fed this organism with only one ratio diet to satiety, but this provoke that not consumed food modify culture water quality and food waste (Priestley *et al.* 2006) [6].

Biofloc culture technology was considered as an efficient alternative, which apply nutrients to system that were reused or recycled. This Biofloc system was supported in production and growing of microorganism in culture medium, which participate in two ways: a) maintenance of water quality using nitrogen components generated *in situ* for bacteria action over proteins and; b) like supply of live food, increasing culture economic feasibility reducing feed cost and water supply, because of water recirculation in biofloc system (Avnimelech 2012) [8].

For all this, this study applies Biofloc system to culture *C. auratus* with different carbon resources: molasses, coffee and moringa to observe the effect on condition factor (KM) and absolute and instantaneous growth rates.

**2. Material and Methods**

**2.1 Obtainment of fish**

A lot of 120 juvenile organisms of *C. auratus* were obtained from Ornamental Fish Production center from Mexico City, with a mean length of 5.0±0.95 cm and mean weight of 4.2 ±1.08 g. The juveniles were acclimated previously during 15 days.

**2.2 Biofloc production**

After acclimation period, juveniles of *C. auratus* were divided in circular beakers of 750 L capacity (30 juveniles each one), with air diffuser in middle of beaker to assure continuous water movement and suspended particles. Every day the juveniles were fed with commercial diet (Wardley ®) with 45% protein content and particle size of 0.6-0.8 mm. This diet was supplied considering 10% of total body weight of juvenile population from each beaker. Every 15 days, food quantity was adjusted, until 120 cultured days.

To assure biofloc development in cultured system, a C/N ratio of 20:1 (Avnimelech 2012) [8] was maintained using a three-carbon source: molasses, coffee and moringa. A control diet was considered without carbon source. To adjust C/N ratio, requirements calculations was made according Emerenciano *et al.* (2011) [9].

**2.3 Biometric parameters**

Every 15 days, from each beaker, all juveniles were measured obtaining weight with a digital balance Ohaus (0.001 g). Total length, wide, and high values were taken with a digital Vernier (0.001 cm).

**2.4. Data processing**

Biometric values were introduced in database in Excel 2010 program to obtain descriptive analysis (mean values ±S.D.). With these data, it was obtained:

Absolute Growth Rate (AGR) with formula:

$$AGR = \frac{Bv \text{ final value} - Bv \text{ initial value}}{\text{Total experimental days}}$$

Where:

Bv = Biometric value (length, high, wide or weight)

Instantaneous Growth Rate (IGR) with formula:

$$IGR = \frac{NL Bv \text{ final} - NL Bv \text{ initial}}{\text{Total experimental days}} \times 100$$

Where:

Bv = Biometric value (length, high, wide or weight)

NL = natural logarithm

Condition factor (KM)

$$KM = \frac{\text{Weight}}{\text{Length}^3} \times 100$$

Gain (G) with formula:

$$G = Bv \text{ final} - Bv \text{ initial}$$

Where:

Bv = Biometric value (length, high, wide or weight)

**2.6 Statistical analysis**

One way ANOVA analysis was made to determined significant differences ( $P<0.05$ ) between juvenile's biometric values from each carbon source and control diets. When significant differences were found, multiple means value techniques were apply using a Tukey's test.

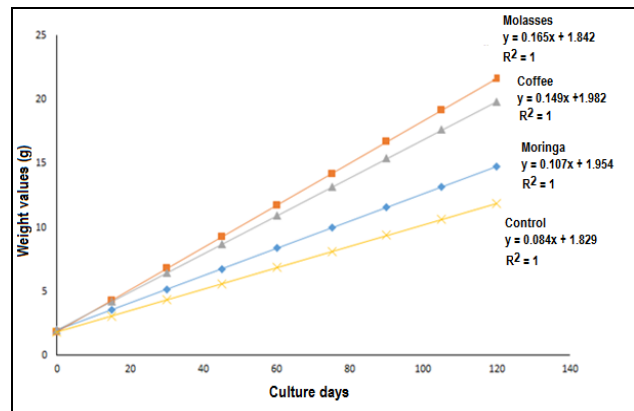
**3. Results**

**3.1 Weight**

Table 1 and Fig. 1 shows weight biometric values (g). The juvenile carps fed with biofloc with molasses carbon source reach highest weight values (21.64 ±0.19 g) during 120 experimental cultured days. The coffee carbon source diet carbon obtained 19.86±0.18 g and moringa 14.794±0.19 g. Control diet showed lowest values with 11.90±0.19 g.

**Table 1:** Weight biometric values (g) of *C. auratus* juveniles during 120 cultured days using different carbon source diets.

Sample day	Experimental carbon source			
	Moringa	Molasses	Coffee	Control
0	1.95 ± 0.19	1.84 ± 0.18	1.98 ± 0.18	1.82 ± 0.18
15	3.55 ± 0.19	4.31 ± 0.18	4.21 ± 0.19	3.08 ± 0.19
30	5.16 ± 0.18	6.79 ± 0.18	6.45 ± 0.18	4.34 ± 0.18
45	6.76 ± 0.18	9.26 ± 0.19	8.68 ± 0.18	5.60 ± 0.19
60	8.37 ± 0.19	11.74 ± 0.18	10.92 ± 0.19	6.86 ± 0.18
75	9.97 ± 0.19	14.21 ± 0.19	13.15 ± 0.18	8.12 ± 0.19
90	11.58 ± 0.18	16.69 ± 0.18	15.39 ± 0.18	9.38 ± 0.19
105	13.18 ± 0.19	19.16 ± 0.19	17.62 ± 0.18	10.64 ± 0.18
120	14.79 ± 0.19	21.64 ± 0.19	19.86 ± 0.18	11.90 ± 0.19



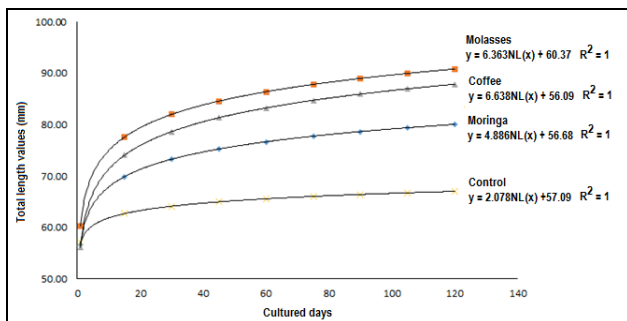
**Fig 1:** Growth tendency weight curve of *C. auratus* during 120 experimental cultured days using three different carbon sources.

**3.2 Total length**

With respect to total length reached for juvenile carps, it was observed that biofloc diet with carbon molasses source showed better result than other diets with 90.83±0.75 mm; followed by coffee carbon source diet with 87.87±0.73 mm, and moringa carbon source diet with 80.07±0.70 mm. Control diet showed lowest length value during 120 cultured days with 67.04±0.78 (Table 2 and Fig. 2).

**Table 2:** Biometric length values (mm) during 120 cultured days in *C. auratus* juveniles using three different carbon source experimental diets.

Sample day	Experimental carbon source diet			
	Moringa	Molasses	Coffee	Control
0	56.68 ± 0.67	60.37 ± 0.68	56.09 ± 0.70	57.09 ± 0.76
15	69.91 ± 0.72	77.60 ± 0.72	74.07 ± 0.79	62.72 ± 0.76
30	73.30 ± 0.70	82.01 ± 0.67	78.67 ± 0.74	64.16 ± 0.71
45	75.28 ± 0.69	84.59 ± 0.69	81.36 ± 0.70	65.00 ± 0.67
60	76.68 ± 0.69	86.42 ± 0.71	83.27 ± 0.71	65.60 ± 0.80
75	77.78 ± 0.77	87.84 ± 0.70	84.75 ± 0.78	66.06 ± 0.78
90	78.67 ± 0.68	89.00 ± 0.73	85.96 ± 0.72	66.44 ± 0.69
105	79.42 ± 0.70	89.98 ± 0.74	86.98 ± 0.76	66.76 ± 0.75
120	80.07 ± 0.70	90.83 ± 0.75	87.87 ± 0.73	67.04 ± 0.78



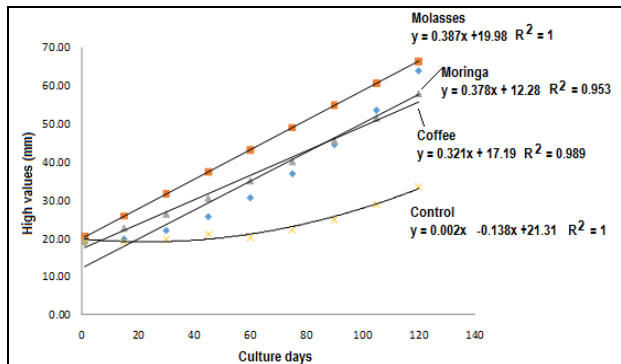
**Fig 2:** Tendency curve of total length values (mm) of *C. auratus* during 120 cultured days with three different experimental carbon source diets.

**3.3 High**

Table 3 and Fig. 3 show high mean values (mm) of *C. auratus* during 120 cultured days molasses carbon source diet showed the highest value with 66.42±0.40 mm; moringa and coffee with 63.87±0.32 mm and 57.87±0.39 mm respectively. Control diet showed lowest value with 33.55±0.45 mm.

**Table 3:** High mean biometric values of *C. auratus* juveniles during 120 experimental culture days, with three different carbon source diets.

Sample day	Experimental carbon source diet			
	Moringa	Molasses	Coffee	Control
0	18.77 ± 0.28	20.37 ± 0.44	19.55 ± 0.41	18.97 ± 0.40
15	19.67 ± 0.32	25.79 ± 0.36	22.59 ± 0.38	19.15 ± 0.38
30	21.93 ± 0.43	31.59 ± 0.47	26.28 ± 0.48	19.69 ± 0.37
45	25.55 ± 0.22	37.40 ± 0.42	30.42 ± 0.35	21.17 ± 0.46
60	30.51 ± 0.44	43.20 ± 0.34	35.01 ± 0.37	20.23 ± 0.28
75	36.83 ± 0.34	49.01 ± 0.47	40.05 ± 0.41	22.21 ± 0.25
90	44.49 ± 0.36	54.81 ± 0.47	45.54 ± 0.26	25.09 ± 0.50
105	53.51 ± 0.25	60.62 ± 0.40	51.48 ± 0.49	28.87 ± 0.26
120	63.87 ± 0.32	66.42 ± 0.40	57.87 ± 0.39	33.55 ± 0.45



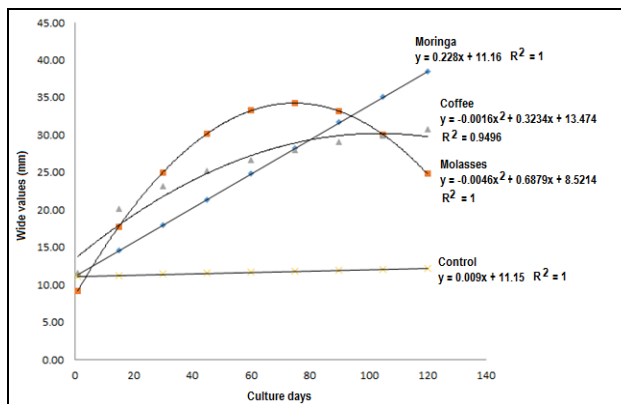
**Fig 3:** Tendency curve of high values (mm) of *C. auratus* juveniles during 120 experimental culture days with three different carbon sources.

**3.4 Wide**

In wide biometric values, Moringa carbon source diet showed highest values with 38.52±0.27 mm, followed for coffee carbon source diet with 30.79±0.29 mm. During first experimental days, molasses carbon source diet was enough to increase wide values, but after 80 cultured days it only reached 24.83±0.24 mm. Control diet obtained lowest value with 12.23±0.27 mm (Table 4 and Fig.4).

**Table 4:** Wide biometric mean values (mm) of *C. auratus* juveniles during 120 experimental culture days with three different carbon source diet.

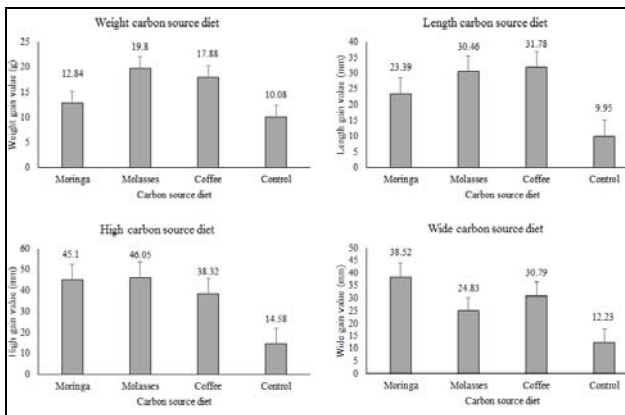
Sample day	Experimental carbon source diet			
	Moringa	Molasses	Coffee	Control
0	11.39 ± 0.29	9.20 ± 0.28	11.54 ± 0.24	11.16 ± 0.11
15	14.58 ± 0.32	17.80 ± 0.21	20.11 ± 0.20	11.29 ± 0.26
30	18.00 ± 0.26	25.02 ± 0.31	23.17 ± 0.22	11.42 ± 0.33
45	21.42 ± 0.29	30.16 ± 0.33	25.18 ± 0.29	11.56 ± 0.26
60	24.84 ± 0.25	33.24 ± 0.30	26.71 ± 0.22	11.69 ± 0.27
75	28.26 ± 0.30	34.24 ± 0.28	27.96 ± 0.32	11.83 ± 0.30
90	31.68 ± 0.20	33.17 ± 0.31	29.03 ± 0.19	11.96 ± 0.27
105	35.10 ± 0.31	30.04 ± 0.21	29.96 ± 0.32	12.10 ± 0.24
120	38.52 ± 0.27	24.83 ± 0.24	30.79 ± 0.29	12.23 ± 0.27



**Fig 4:** Tendency curve of wide values (mm) of *C. auratus* juveniles during 120 experimental culture days with three different carbon source diets.

### 3.5 Gain values

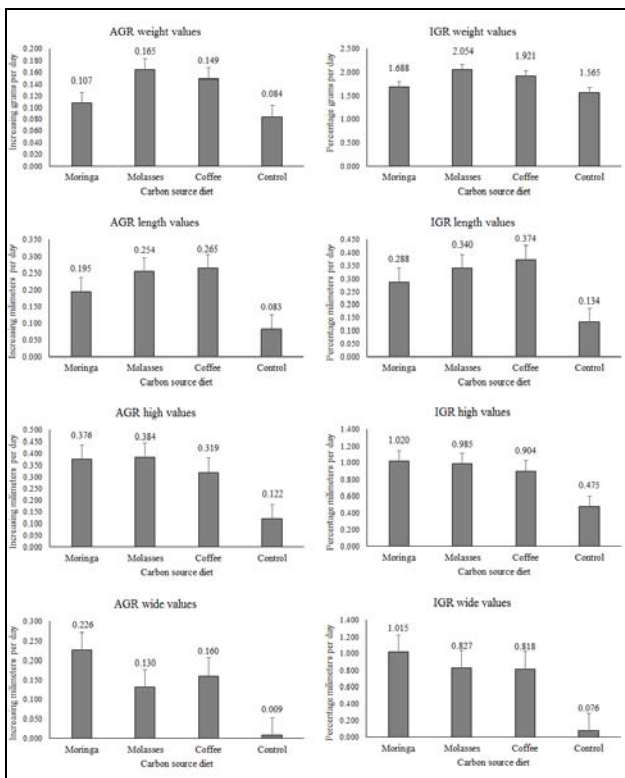
Fig. 5 show gain values with three different carbon source diets. With respect to weight values, molasses obtained highest values with 19.8 g and lowest values were presented in control diet (10.08 g). Length gain values shows better results with coffee carbon source diet with 31.78 mm and lowest gain values in control diet with 9.95 mm. In high biometric gain values showed better result in molasses diet with 46.05 mm and lowest values in control diet with 14.58 mm. Regarding too wide gain values, moringa carbon source diet showed better results with 38.52 mm and lowest gain values with 12.23 mm in control diet.



**Fig 5:** Gain values of weight (g), length (mm), high (mm) and wide (mm) of *C. auratus* juveniles at 120 experimental culture days with three different carbon source diets.

### 3.6 Absolute and instantaneous growth rates

Fig. 6 show AGR and IGR values obtained at 120 experimental culture days.

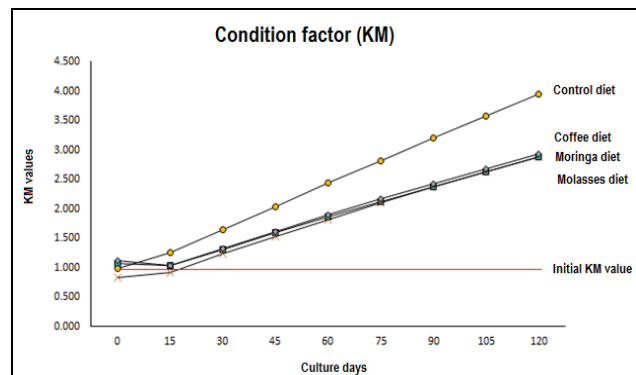


**Fig 6:** AGR and IGR obtained in *C. auratus* juveniles during 120 experimental culture days with three different carbon source diets.

Regarding too AGR values, better weight value showed with molasses diet (0.165 g per day), and lowest with control diet (0.084 g per day). In length values, better data was founded with coffee diet with 0.265 mm increase per day, lowest value founded in control diet with 0.083 mm per day. Higher high values were founded in moringa diet with 0.376 mm increase per day, lowest values were founded in control diet with 0.122 mm per day. With respect to wide biometric values, better results were founded with moringa diet (0.226 mm per day) and lowest values with control diet (0.009 mm per day). IGR percentage per day values showed with weight data better results with molasses diet (2.054%) and lower values in control diet (1.565%). Regarding to length values, highest values were obtained in coffee diet with 0.374% and lowest values in control diet (0.134%). Better high percentage values showed in moringa diet with 1.020% increasing per day, and lowest increase showed in control diet with 0.475%. In wide biometric values, better increase was presented in moringa diet with 1.015% per day and lowest increase showed in control diet with 0.076% per day.

### 3.7 Condition factor (KM)

Fig. 7 show condition factor values (KM) of *C. auratus* juveniles fed with Biofloc produced with different carbon sources. All diets showed better results with respect initial KM value. This condition allows that all diets cover juvenile's nutritional requirements to growth. Control diet allows better weight/length ratio with respect other diets although these organisms showed lowest weight and length values. Moringa, coffee and molasses diets showed highest values of weight and length biometric data with respect to control diet, but did not showed significant differences between their weight/length ratio.



**Fig 7:** KM values of *C. auratus* juveniles in 120 experimental culture days with three different carbon source diets.

### 3.8 Statistical analysis

One way ANOVA analysis showed significant differences ( $P < 0.001$ ) between biometric values (weight, length, high and wide) with respect to all experimental carbon source diets.

### 4. Discussion

The obtained results in this research showed when *C. auratus* was cultivated in Biofloc system, total length, high, wide and weight were increased with respect to traditional culture system. Authors as Crab *et al.* (2007, 2012) [10, 11] showed that biofloc is a good food low cost strategy which was better than traditional culture system because formed flocs that have high protein, lipids, carbohydrates and ashes content for their use as food in aquaculture industry. Some studies showed that

using biofloc, farmers can substitute dry pellet food to aquatic organisms and promoted growth in some aquatic species like *Oreochromis mossambicus* (Avnimelech 1999, 2007) [12, 13], *Macrobrachium rosenbergii* (Asaduzzaman *et al.* 2008) [14] and *Litopenaeus vannamei* (Burford *et al.* 2004; Xu and Pan 2012) [15, 16].

During the experiment, C: N = 20:1 ratio was maintained with experimental carbon sources diets and the values were higher than control diet. Wang *et al.* (2015) [17], have found the same results in weight gain, absolute and instantaneous grow rates of *C. auratus* using a C: N ratio of 20:1 and 25:1 in comparison with control diet. These authors used dextrose as carbon source and obtained in 52 experimental days a weight gain of 5 g, while in this research with molasses, as carbon source, reached in 60 experimental days 9.9 g weight gain, with moringa and coffee values upper 6 g weight gain in same period.

Castro *et al.* (2011) [18] have made a growth experimental study with *C. auratus* during 120 days with dry diet enriched with probiotic *Lactobacillus casei* (commercial product Yakult®) and obtained a weight gain of 2.520 g and total length gain of 2.160 cm. In comparison with our experiment, after 120 experimental culture days, weight gain was 19.80 g and 4.60 cm of total length gain with molasses carbon source diet. Coffee and moringa carbon source diets also reached high values with respect to only use of probiotics in *C. auratus* diet.

Avnimelech (2006, 2007) [19, 13] and De Schryver (2008) [20], have mentioned that carbon source used in biofloc growth promotion, directly influence over nutritional content in development flocs, because many of carbon sources reach concentration range between 40 to 50% content and cause different growth results, that agree with this study, where was found better results of AGR and IGR in weight values with molasses, better length rate of AGR and IGR with coffee and respect wide values, was better with moringa.

No matter what carbon sources were used, the increase of length and weight were high with respect control group. Authors as Emerenciano (2012, 2013) [21, 22] and Kuhn and Lawrence (2012) [23] mentioned that molasses carbon source is most used to produce biofloc, because it was constituted with simple carbohydrates, easily assimilation for microorganisms founded in biofloc, so this system can promote better growth results in aquatic cultured species and, molasses carbon source was accessible and low cost. It is possible that biofloc stimulate digestive enzyme activity as shown in Moss *et al.* (2001) [24] and Xu *et al.* (2012a,b) [25, 26] studies which contribute growth increasing in aquatic organisms, as it can be observed in this study with three different carbon sources, with respect to control diet. However, in many studies where sub products were used, obtained from human or animal industry, of easily acquisition and handling with good results (Asaduzzaman *et al.* 2008, Samochoa *et al.* 2007) [14, 27].

Biofloc can be used to produce and growth better fishes as *C. auratus* in laboratory conditions, because this system can produce their own food and herbivorous and omnivorous fishes can be maintained with good growth gain values.

## 5. References

1. Froese R. Length-weight relationships for 18 less-studied fish species. *Journal of Applied Ichthyology*. 1998; 14:117-118.
2. Oscoz J, Campos F, Escala MC. Weight-length relationships of some fish species of the Iberian Peninsula. *Journal of Applied Ichthyology*. 2005; 21(1):73-74.
3. De Giosa M, Czerniejewski P, Rybczyk A. Seasonal changes in condition factor and weight length relationship of invasive *Carassius gibelio* (Bloch, 1782) from Leszczynskie Lakeland, Poland. *Advances in Zoology*. 2014, 1-7.
4. Tesch FW. Age and growth in Fish Production in Fresh Waters, W. E. Ricker, Ed., Blackwell, Oxford, UK; 1971, 98-130.
5. Beyer JE. On length-weight relationship. Computing the mean weight of the fish of a given length class. *Fish Byte* 1987; 5:11-13.
6. Priestley SM, Stevenson AE, Alexander LG. The influence of feeding frequency on growth and body condition of the common goldfish (*Carassius auratus*). *The Journal of Nutrition*. 2006; 136:1979S-1981S.
7. Lorenzoni M, Gehtti L. Analysis of the biological features of the goldfish *Carassius auratus auratus* in lake Trasimeno (Umbria, Italy) with a view to drawing up plans for population control. *Inst. Vertebrate Biology AS CR*. 2010; 59(2):142-156.
8. Avnimelech Y. Biofloc technology-a practical guide book, The World Aquaculture Society, Baton Rouge, 2012.
9. Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W. Effect of biofloc technology (BFT) on the early post larval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance. *Aquaculture International*. 2011; 19:891-901.
10. Crab R, Avnimelech Y, Defoirdt T, Bossier P, Verstraete W. Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*. 2007; 270:1-14.
11. Crab R, Defoirdt T, Bossier P, Verstraete W. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*. 2012, 356-357, 351-356.
12. Avnimelech Y. Carbon and nitrogen ratio as a control element in aquaculture systems. *Aquaculture*. 1999; 176:227-235.
13. Avnimelech Y. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture*. 2007; 264:140-147.
14. Asaduzzaman M, Wahab MA, Verdegem MCJ. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*. 2008; 280(1):117-123.
15. Burford MA, Thompson PJ, McIntosh RP, Bauman RH, Pearson DC. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero exchange system. *Aquaculture*. 2004; 232:525-537.
16. Xu WJ, Pan LQ. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture*. 2012; 356-357:147-152.
17. Wang G, Ermeng Y, Xie J, Yua D, Li Z, Luoa W *et al.* Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture*. 2015; 443:98-104.
18. Castro BT, Monroy MCD, Castro JM, De Lara RA, Castro GM. Efecto de cuatro probióticos en el

- crecimiento y la sobrevivencia de *Carassius auratus*. *Ciencia Pesquera*. 2011; 19(1):21-28.
19. Avnimelech Y. Bio-filters: the need for a new comprehensive approach. *Aquaculture Engineering*. 2006; 34:172-178.
  20. De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W. The basics of bio-flocs technology: the added value for aquaculture. *Aquaculture*. 2008; 277:125-137.
  21. Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Engineering*. 2012; 43:447-457.
  22. Emerenciano M, Cuzon G, Arevalo M, Gaxiola G. Biofloc technology in intensive broodstock farming of the pink shrimp *Farfantepenaeus duorarum*: spawning performance, biochemical composition and fatty acid profile of eggs. *Aquaculture Research*. 2013, <doi: 10.1111/are.12117>.
  23. Kuhn DD, Lawrence A. Ex-situ biofloc technology. In: Avnimelech Y, editor. *Biofloc Technology-a practical guide book*, 2nd ed., The World Aquaculture Society, Baton Rouge, Louisiana, USA, 2012.
  24. Moss SM, Divakaran S, Kim BG. Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). *Aquaculture Research*. 2001; 32:125-131.
  25. Xu WJ, Pan LQ, Sun X, Huang J. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. *Aquaculture Research*. 2012a; 44:1093-1102.
  26. Xu WJ, Pan LQ, Zhao D, Huang J. Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture* 2012b; 350–353: 147-153.
  27. Samocha TM, Patnaik S, Speed M, Ali AM, Burger JM, Almeida RV *et al*. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering*. 2007; 36:184-191.