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Growth performance of common carp (*Cyprinus carpio* L.) fingerlings fed with various protein levels

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Abstract. Common carp *(Cyprinus carpio* L.*)* is one of the most used freshwater species in aquaculture worldwide. The species and its subspecies have very good skills for farm production system breeding. Although nutritional aspects of energy and protein needs were fairly well studied and enshrined, new trends proposes a "live food" nutrition and sustainable breeding of the species, with the best capitalization of the local resources. Food and Agricultural Organization of the United Nations (FAO) proposed two decades ago a manual on the production of live food for aquaculture, but few applications were recorded, mainly because of competition with intensive and semi-intensive breeding systems. Romania is natural range of *C. carpio* distribution and has a long tradition in carp breeding. Moreover, traditional fish farms which practice an extensive breeding could easily use natural resources of live food. Three types of fodder have been used to feed indigenous carp fingerlings, from which one is 100% natural live food – *Musca domestica* and *Eisenia fetida*, a mixture one of those two species (50%) with maize (*Zea mays*) 50%, respectively a classic granulated fodder with fish meal. Crude protein level ranged from 30 to 54.77%. Best food conversion rate and average daily gains were obtained by using 100% live food, followed by mixture of live food with maize and at the last place was recorded the classical fodder. Biochemical and hematological sanguine parameters were in normal physiological limits for this age category and for all three types of fodder.
Key Words: *Cyprinus carpio* L., protein levels, fish nutrition, aquaculture, live food.

Introduction. Common carp *(Cyprinus carpio* L.*)* is one of the most popular freshwater species in aquaculture worldwide (Hasan at al 2007; FIGIS 2011). Asia reaches more than 70% of total production, while Europe decreased the carp production considerably in the last two decades with more than 50%, mainly because of political changes in Eastern Europe in late 80'. The European trend is now to grow again as the breeding systems performance do. Within the breeding technology, nutrition has been one of the main issues, and energy and protein needs seem to be enshrined in carp nutrition and for all categories (http://www.fao.org/fishery/affris/species-profiles/common-carp/nutritional-requirements/en/). Not only those two basic nutrients were established, but lipids, carbohydrates, vitamins and minerals also. Amino acids and fatty acids are of main interest, especially that some of those are limitation factors for grow and development.

Usually the main ingredients used for carp nutrition are composed by cereals and fish meal, supplemented with vitamins, minerals and possibly additives. Granulated fodder is largely used in intensive and semi-intensive producing systems, while in extensive systems local resources play a crucial role. In spite of increasing demand of fresh fish on world market, local resources of primary ingredients for carp feeding did not much diversified or quantitatively increase. By contrary, the natural sources of fish used in fish meal are decreasing (Deutsch et al 2007). Substitute of fish meal with soybean provided good results in carp growth (Pongmaneerat et al 1993), but other ingredients which have been tested are promising, such: single-cell proteins such bacteria (Atack et al 1997) or krill tested in salmons (Albrektsen 2007 cited by Stanković et al 2011).

Two extreme feeding systems are largely debated: traditional and intensive one. Within those two systems, nutrition philosophy is heading towards those aspects of best return in terms of economy, but also durable development for the traditional breeding system. The two concepts have different approaches in regard with the source of primarily ingredients used in rations for carps. One major constrain is given by meat quality of slaughtered fishes, especially on European markets, but should not be neglected the issue of providing a more "natural" food for farmed fish. Meat quality from natural ingredients fed individuals has at least comparable organoleptic and physic-chemical characteristics with intensive produced one and probably more health benefits.

Within the traditional aquaculture, variation in nutritional aspects of feeding is present mainly because of local resources availability, efficiency being one major constrain. New insights into natural source and easy to produce food are becoming increasingly interrogated for best optimize of durable exploitation of the species.

The aspect of "natural" fodder feeding is directly linked with carp metabolism and physiology and integrates basic aspects of species alimentary behavior and ecology. Within natural ecosystem, the common carp dispose of various nutritional resources, both vegetal and animal origins (Stanković et al 2011). Starting from these aspects, new insights into carp nutrition have focused on "live food" benefits, such as insects, larvae, worms and pupae of various species (Nandeesha et al 1990; Lavens & Sorgeloos 1996; Tacon 1997; Fadaee 2012). Growing and developing performances of different age and categories of fish fed with these "live food" were encouraging (Nandeesha et al 1990). But there are still aspects to complete, such industrial producing of "live food", storage possibilities, economical competition with intensive and semi-intensive industrial farms, more studies on feeding efficiency or best feed rations of the mixture. One more advantage of naturally fed fish is proper maintaining of best adapted individuals to this natural food, in spite of higher concentrations of individuals comparing with natural ecosystems.

Natural food for carp in fish ponds is mainly represented by zooplankton (*Rotifers*, *Cladocerans* and *Copepods*) and zoobenthos (mostly *Chironomids* and *Oligochaeta*) (Stanković et al 2011). Nutritional behavior of carp also changes with age (Anton-Pardo et al 2014).

Natural food is a valuable source of proteins, free amino acids and oligopeptides, fat and fatty acids and vitamins that are essential substances for growth and development of fish (Kibria et al 1997). Various "live foods" have been tested in fish nutrition and all concluded real benefits for growing performances and meat quality. In common carp nutrition there have been used several species to replace fish meal: *Eudrilus eugeniae* (Kinberg, 1867) (Nandeesha et al 1988), *Perionyx excavatus* (Ngoc et al 2015), silkworm (*Bombyx mori*) pupae (Nandeesha et al 1990) and other species, in various forms, either alive or dried. The main challenge is that rotifer (*Brachionus plicatilis*) and brine shrimp (*Artemia*) are the only zooplankters produced in mass quantities (CAN 1993) and thus seems to be the most limiting factor of "live food" feeding.

Multiple mixtures of natural resources could complete common carp nutrition. One of the main issues is to identify and promote those which sustain long term development, correlated with species nutrition behavior.

An optimal mixture is probably one composed of both animal and vegetal origin ingredients. To avoid refusals, "live food" required a mixture with vegetal origin nutrients, a process which is surely not simple. Moreover, those two types of ingredients have different buoyancy and cannot be granular or encapsulated together.

Although Romanian rivers are native ecosystems for species evolution (*C. carpio,* River Danube subpopulation - Kottelat 1996), carp breeding constantly decreased livestock production over the past two decades (http://www.fao.org/fishery/culturedspecies/Cyprinus_carpio/en.). Instead, Romania has great possibilities to ensure low cost vegetal nutrients such cereals, cakes rich in oil and other raw ingredients for common carp nutrition, while producing "live food" is a matter of specific technology and even local resources when a limited small farms demand. One major challenge for a good quality of carp meat production is therefore to optimize a feeding technology which ensures that. A good food conversion ensures better average daily gain, survival rate, growth rate, conformation, optimal hematological parameters and meat quality.

We propose here to compare three types of feed ratios: a standard one based on granulated food, one entirely composed of “live food”, respectively a mixture of “live food” and cereals handled to fingerlings of indigenous carp *C. carpio*. We have used this subspecies for its good performance in artificial breeding, but also as the best adapted to ecological conditions.

Material and Method

Biological material. 183 individuals of indigenous *C. carpio* were randomly allocated into three plots. Starting live weight was between 10.43±0.345 and 10.75±0.453 grams. The fingerlings were all in a good health condition and homogeneous as conformation.

Water quality. Water parameters were optimized according with age category of biological material. Temperature (T °C), pH, water salinity and dissolved oxygen (DO) were daily recorded with portable multi-parameter HANNA HI 9828. Temperature variation ranged between 18 and 20°C, water oxygen level ranged between 7.28 and 9.15 mg/L and oxygen saturation ranged between 88.7 and 96.7 dissolved oxygen (DO) and salinity between 0.04 and 0.06 ‰.

Nutrition characteristics and chemical composition of the fodder. Each plot received a specific fodder. Plot 1 was fed with a granulated (2 mm) classic fodder with 30% crude protein (CP), 7% crude fat (CF), 43% water-soluble carbohydrates (WSC), 5% crude fiber (CFi) and 7% ash respectively. Ingredients: fish meal, soybean, blood meal, rape, wheat, fish oil, vegetable oils, minerals and vitamins. Plot 2 received an entirely live food mixture (mixture 1) of *Musca domestica* larvae (50%) and *Eisenia fetida* adults (50%). Chemical composition of the mixture was: 54.77% CP, 27.19% CF, 12.67% WSC, 0% CFi and 5.37% ash respectively. Plot 3 was fed with a mixture (mixture 2) of *M. domestica* larvae (25%), *E. fetida* adults (25%) and *Zea mays* spp. grains (50%). Chemical composition of the mixture was: 30.89% CP, 20.15% CF, 41.38% WSC, 3.12 % CFi and 4.46% ash respectively.

Chemical analyses were made using Kjeldahl method for crude proteins with automatic analyzer Gerhardt Turbotherm with Vapodest Distiler; Soxhlet method was used for crude fats based on automatic analyzer Velp Scientifica Ser 148 Solvent Extractor; automatic analyzer Dosi-Fiber for crude fiber. For Dry matter and water content classical method of 105°C drying in oven was applied. Ash content has been determined in a calciner.

Hematological blood parameters. Physiological status of growth was tested by measuring the main hematological blood parameters: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood ells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (Ht) (Ghergariu et al 1999). Total proteins (TP in g/dL), gamma-globulin (GG in g/dL) and albumins (Al in g/dL), total lipids (TL in mg/dL), triglycerides (TG in mg/dL) and cholesterol (Ch in mg/dL) were also determined (Ghergariu et al 1999).

Statistical analyses. Statistical analyses were computed using StatSoft Statistica version 10. T-test was performed. For equal or unequal variances assuming preliminary F-test were computed, for each comparison between variables.

Growth parameters. Average daily gain (ADG) was calculated as difference between final and initial live weight, all divided to number of experimental days (45 days). Intermediary controls of weight were performed, weekly. Total biomass plot was weight.

Feeding efficiency (FE), protein utilization efficiency (PUE) and food conversion rate (FCR) were calculated according to Oprea & Georgescu (2000). Based on preliminary estimates a certain quantity of fodder has been distributed to all plots and refusals were daily quantified.

Results and Discussion. Mixture of *M. domestica* larvae (50%) and *E. fetida* adults (50%) provided best ADG (Table 1), FE, PUE and FCR (Table 2) in common carp from plot 2, followed by plot 3 fed with mixture2 (50% live food), while classic fodder ensured the lowest efficiency. Significant differences (P<0.001) were observed between each plot compared with others and for all feed conversion characteristics.

Table 1

Variation of live weight and average daily gain (ADG) of *Cyprinus carpio* fingerlings fed with various protein levels

Specification	*Plot*	*UM*	*Variable*			*Statistical differences (plot vs. 2)*	*Statistical differences (plot vs. 3)*
			$X \pm s_x$	*s*	*V%*		
Initial weight (IW)	1	g	10.43±0.373	3.735	35.82	ns	ns
	2	g	10.75±0.453	4.212	45.30	-	ns
	3	g	10.43±0.345	3.452	33.11	-	-
Final weight (FW)	1	g	10.97±0.446	4.458	45.07	***	***
	2	g	24.16±1.134	11.342	46.95	-	***
	3	g	18.11±0.796	7.960	43.94	-	-
Total weight gain (TWG)	1	g	0.54±0.916	0.723	28.66	***	***
	2	g	13.41±1.221	7.130	36.24	-	***
	3	g	7.68±0.983	4.508	26.49		-
Average daily gain (ADG)	1	g/day	0.0120±0.023	0.016	36.06	***	***
	2	g/day	0.2980±0.086	0.158	37.56	-	***
	3	g/day	0.1706±0.054	0.100	35.15	-	-

*** = P<0.001; ns = P>0.05.

Table 2

Variation of protein utilization efficiency (PUE), feeding efficiency (FE) and food conversion rate (FCR) in *Cyprinus carpio* fingerlings fed with various protein levels

Plot	*Protein utilization efficiency (PUE)*	*Feeding efficiency (FE)*	*Food conversion rate (FCR)*
1	0.000621	1.8589	53.7947
2	0.008650	46.1625	2.1663
3	0.008559	26.4351	3.7782

Mixture 1 has provided a better efficiency of protein utilization, which is normal given that crude protein level is significantly higher that other two mixtures, being almost double as value (54.77% vs 30%). It was expected to have better protein conversion, given that protein composition from those larvae and land worms shows a very good metabolic compatibility with carp species, even in early growing phases, such post-larvae (Chaves et al 2014). Instead, the same mixture had lower energy in terms of carbohydrates (WSC) but higher fat content, which can replace carbohydrates energy if necessary. Compared with classical fodder which has 42% WSC, fully animal origin of mixture 1 has only 12.67 WSC, but significantly higher fat content (27.19% vs 7%). Minimal requirements for carp fingerlings in fats is 7-8% (Csengeri & Majoros 2004 cited by FAO http://www.fao.org/fishery/affris/species-profiles/common-carp/nutritional-requirments/en/), but there are no maximal values. Fat is a valuable energy source to metabolize proteins if there is a lack of carbohydrates. High protein level of mixture 1 is mainly given by *E. fetida*, which has around 60% CP reported to dry matter (Zhenjun et al 1997; Medina et al 2003). Amino acid composition is comparable to that of fish meal and hen egg and higher than that of cow milk powder and soybean meal (Zhenjun et al 1997). Moreover, *E. fetida* has been suggested as a valuable live food for animals in general not only for freshwater fishes because its proteins are safe for feeding animals intended for human consumption and have immunological properties (Medina et al 2003). But high fat level ensured the main energy source of protein utilization.

Classical fodder and mixture 2 both had around 30% crude protein content and similar values for WSC, but there were significant differences ($P<0.001$) in favor of mixture 2 for all ADG, FE, PUE and FCR. Fat content greatly varied in favor of mixture 2 (almost triple). The fat source of mixture 2 is primarily given by *M. domestica* larvae. *M. domestica* larvae has an average fat content of 14% (Pretorius 2011), which was completed by *E. fetida* worms.

Fats presents different metabolic rate, contains more energy per unit than carbohydrates and they are more efficiently used as energy for fish (De Silva & Anderson 1995) and in fingerlings the needs for carbohydrates are higher (Seenappa & Devaraj 1995). From this perspective, mixture 2 consisted in much more energy. Thus, plot 3 revealed an ADG of 0.1706 ± 0.054 g/day, significantly higher ($P<0.001$) than ADG of plot 1 which was only 0.0120 ± 0.023 g/day. Single fat content variation of the two fodders could not probably explain such a difference. Protein source from those two species which are naturally part of common carp diet into nature is more likely to potency the difference. Common carp diets usually do not consist of other fish consumption, but occasionally (Michel & Oberdorff 1995; http://www.fao.org/fishery/affris/species-profiles/common-carp/natural-food-and-feeding-habits/en/); thus fish meal does not necessarily consist of best protein source in the subspecies.

Analyzing protein utilization efficiency, once again mixture 1 revealed best results when compare with other two plots.

Analysis of sanguine profile of proteins revealed physiological limits (Velisek et al 2005; Talas et al 2012; Abedi et al 2012) and various levels of statistical differences between the three plots. Somehow expected, plots 1 and 3, who received similar levels of crude proteins recorded similar sanguine levels of TP, GG and Al. Higher variation in significance were revealed ($P<0.001$) when compared plot 1 with plot 2 and plot 2 with plot 3 respectively, for total proteins (Table 3) and lower ($P<0.05$) for the same comparisons for albumin. As expected, no significant variation ($P>0.05$) was recorded between plot 1 and 3.

Analysis of sanguine profile of lipids profile revealed more variation between the plots. Plots 2 and 3 had higher cholesterol levels ($P<0.001$ for all between plots comparisons), but lower triglycerides and total lipids values. Higher cholesterol is easily explained by more than three times bigger values of crude fats in mixtures 2 and 3. Lower TG in plots 2 and 3 is partially explained by higher carbohydrates content of classic mixture (plot 1), although mixture 2 had similar content, similar for protein content too, but much more total fats. Given that ADG of plot 1 was surprisingly low, higher sanguine values for total lipids are explained by a better carbohydrates conversion rate into lipids, but deficiency of proteins (even of lipids when compared with plots 2 and 3) disordered protein level of synthesis.

We have not analyzed essential and non-essential amino acids content of classic fodder, we just instead presume that larvae and adults used for mixtures 2 and 3 are more compatible with carp fingerlings and a valuable source of EAA and NEAA.

Protein deficiency (if report to those kindly suggested by FAO http://www.fao.org/fishery/affris/species -profiles /common-carp/natural-food-and-feeding-habits/en/) of 10 percent in plot 1 nutrition was highly felt, but not the same happened in plot 3. One possible cause is “natural food” nutrition, which provided at least theoretic better food composition for fingerlings growth. The other argument is higher fat content of live food which consisted as valuable energy source for optimal protein metabolism.

When compare a 100% animal origin mixture with far more levels of protein and fat of mixture 1 with mixture 2 (“well” balanced in terms of carbohydrates source), biochemical sanguine parameters revealed best protein utilization in plot 2 and lower TL and Ch and comparable TG. Lower carbohydrates content of plot 2 probably maintained lowest level in TG.

Table 3

Variation of biochemical parameters of protein and lipid metabolism in *Cyprinus carpio* fingerlings fed with various live foods and classic fodder

Blood parameters	UM	Plot	N	$X \pm s_x$	s	V%	Statistical differences (plot vs. 2)	Statistical differences (plot vs. 3)
Total proteins	g/dL	1	5	3.99±0.065	0.651	16.32	***	ns
		2	5	3.48±0.061	0.615	17.67	-	***
		3	5	3.97±0.061	0.614	15.47	-	-
Gama-globulin	g/dL	1	5	0.27±0.005	0.046	17.17	ns	ns
		2	5	0.27±0.004	0.038	13.85	-	ns
		3	5	0.26±0.006	0.059	22.18	-	-
Albumin	g/dL	1	5	1.10±0.018	0.182	16.58	*	ns
		2	5	0.98±0.025	0.246	25.08	-	*
		3	5	1.08±0.024	0.243	22.41	-	-
Total lipids	mg/dL	1	5	1537.00±18.346	183.458	11.94	***	***
		2	5	1202.78 ± 32.393	323.929	26.93	-	***
		3	5	1393.20 ± 25.329	253.290	18.18		-
Triglyceride	mg/dL	1	5	406.55±7.634	76.342	18.78	***	***
		2	5	352.60±14.096	140.958	39.98	-	ns
		3	5	348.06±10.468	104.679	30.08	-	-
Cholesterol	mg/dL	1	5	83.78±4.015	40.153	47.93	***	***
		2	5	102.10±4.537	45.369	44.44	-	***
		3	5	133.24±4.087	40.872	30.68	-	-

*** = P<0.001; ns = P>0.05.

FE and FCR of the fodder were at best for plot 2, in spite of lower carbohydrate level. In case of plot 1 failure to gain weight, besides low protein level, fat content could be also insufficient, because fat is known to spare proteins in fish nutrition when necessary (Manjappa et al 2002). For comparison, plot 3 which has three times more fats at the same protein level and similar carbohydrates presented very good ADG, also FE, PUE and FCR. For mixture 1 where 50% is *M. domestica* and 50% is *E. fetida*, FCR is the best, although in similar experiments conducted on *Clarias gariepinus* a proportions of 25% *M. domestica* has provided best feed conversion, but when used as a supplement in the mixture (Idowu et al 2003). Similar results were obtained by Dedeke et al (2013) which observed that fish (*C. gariepinus*) diets exceeding 25% replacement of fish meal with earthworm (*Libyodrilus violaceus*) meal had depressed growth, while feed conversion ratio was highest in fish fed with 35% replacement of fish meal.

Hematological values are within physiological parameters (Table 4) but overall the plot 3 revealed higher Hb, MCV and MCHC values than plot 1, while RBC and WBC has higher values in plot 1. Overall plot 2 has lower values than those of plots 1 and 3 and for all parameters, except for RBC (Table 4). Physiological limits were observed for all parameters.

Table 4

Variation of hematological and eritrocitar parameters of *Ciyprinus carpio* fingerlings

Parameter	*UM*	*plot*	*N*	$X \pm s_x$	*s*	*V%*	*References to compare with*
RBC	x10^6/mm^3	1	5	4.12 ± 0.014	0.140	3.41	Witeska et al 2010 (2.32±0.35;2.58±0.58) Abedi et al 2012 (2.86±0.05)
		2	5	4.02 ± 0.034	0.342	8.52	
		3	5	3.97 ± 0.061	0.614	15.47	
WBC	x10^3/mm^3	1	5	52.10 ± 0.339	3.388	6.50	Witeska et al 2010 (40.1±16.9; 52.1±14.1; 64.5±25.3)
		2	5	48.75 ± 0.446	4.456	9.14	
		3	5	49.20 ± 0.458	4.575	9.30	
WBC/RBC	x10^{-3}	1	5	12.65 ± 0.081	0.811	6.41	-
		2	5	12.23 ± 0.173	1.725	14.11	
		3	5	12.62 ± 0.222	2.216	17.56	
Hb	g/dL	1	5	8.80 ± 0.034	0.339	3.85	Bozorgnia et al 2011 (7.4±0.40; 7.5±0.43; 7.7±0.49;9.0±0.35); Talas et al 2011 (8.25±0.21)
		2	5	8.26 ± 0.104	1.041	12.60	
		3	5	9.42 ± 0.159	1.594	16.93	
Ht	%	1	5	28.00 ± 0.138	1.377	4.92	Bozorgnia et al 2011(21.20±1.19); Kumar et al 2010 (27.50±5.00; 29.75±2.73; 31.75±1.71)
		2	5	26.60 ± 0.114	1.140	4.29	
		3	5	26.80 ± 0.192	1.924	7.18	
MCV	fl	1	5	68.03 ± 0.218	2.182	3.21	Kondera & Witeska 2012 (106.00±21; 109.00±23); Al-Ghanim 2011 (60.00±0.1-140.00±0.1)
		2	5	66.50 ± 0.516	5.159	7.76	
		3	5	68.50 ± 0.824	8.236	12.02	
MCH	pg	1	5	21.37 ± 0.085	0.847	3.96	Ahmad 2011 (26.91±2.15; 27.11±2.15; 29.01±2.13) Ajani and Akpoilih 2010 (28.89±2.46)
		2	5	20.55 ± 0.175	1.749	8.51	
		3	5	23.76 ± 0.217	2.168	9.13	
MCHC	g/100mL	1	5	31.42 ± 0.169	1.688	5.37	Bozorgnia et al 2011 (34.6±0.66; 35±0.68;34.7±0.53; 38.6±1.04)

Conclusions. Live food used to feed *C. carpio* fingerlings revealed high rates of feed/food conversion rate and average daily gain. When fingerlings of common carp were fed with similar protein and carbohydrate levels but various levels of fat, live food leaded to incomparable better results for growth and feeding efficiency. From this perspective, it seems that I. higher fats level substituted protein deficiency and/or II. Protein “quality” from live food to better metabolize, given that an optimal content of energy (here fats) is naturally present in this food. When compare two live food mixtures, one having 100% live composition (*M. domestica* larvae and *E. fetida* adults) and the second only 50% from the same species, in spite of low carbohydrates (main energy in here) level of 100% live food, the fingerlings have best harnessed this mixture. Live food seems to have strong beneficial effects of common carp growth, while biochemical and sanguine parameters ranged in between physiological values for all experimental plots. Live food represents a viable nutritional alternative to classic fodder in common carp breeding, at least for extensive farms and where live food naturally occurs. One major challenge is mass production of live food and finding best mixtures between those and vegetal origin ingredients as cheaper energy sources.

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