



MINERAL COMPOSITION OF VUNDU CATFISH LARVAE FED DIETS CONTAINING SOYBEAN AND COTTONSEED MEALS DURING WEANING

*¹IMOROU TOKO, I., ²S.F.X. BACHABI, ³A. ADITE, ¹M. TIDJANI, ³E. D. FIOGBE & ⁴P. KESTEMONT

1. Research Unit in Aquaculture and Aquatic Ecotoxicology (URAEaq), Faculty of Agronomy, University of Parakou, 03 BP 61, Parakou-Université, Benin.

2. Ecole Nationale des Sciences et Techniques Agronomiques de Djougou, ENSTA-Djougou, BP 788, Parakou

3. Department of Zoology and Genetic, FAST, University of Abomey-Calavi, 01BP526 Cotonou, Benin.

4. Research Unit in Environmental Biology, URBE, University of Namur (FUNDP), 61 rue de Bruxelles, 5000 Namur, Belgium.

*Corresponding author: iimorou_toko@hotmail.com, (+229) 95182936 / 96132150

ABSTRACT

Soybean and cottonseed meals were used to replace fish meal in practical diets for vundu catfish (*Heterobranchus longifilis*) larvae. Three isonitrogenous (430 g kg⁻¹ crude protein) practical diets (SBM: 40% soybean meal based diet, CSM: 40% cottonseed meal based diet and SBM+CSM: 20% soybean meal + 20 % Cottonseed meal based diet) were fed to duplicate groups of 300 larvae (initial mean weight: 7 mg) in each tank (20 L). A commercial African catfish fry diet was used as control. Larvae were hand fed in excess, 7-days a week from days 8 to 26 posthatch (p.h.). At the end of this trial, the effects on growth, survival and body Ca, P, Mg, Fe, Zn and Mn contents were examined. The final mean weight and specific growth rate of larvae fed SBM (129.2 mg and 16.2% day⁻¹) were significantly better ($P < 0.05$) than those fed CSM (75.2 mg and 13.2% day⁻¹) or SBM+CSM (90.9 mg and 14.2% day⁻¹). No significant differences ($P > 0.05$) were found for survival, cannibalism and condition factor between fish fed tested diets. Furthermore, only Fe, Zn and Mn were higher in fish fed CSM or SBM+CSM than those fed SBM, while their dietary level was similar.

Keywords: larviculture; African catfish; antinutritional factors; body mineral content; phytic acid

INTRODUCTION

Growth and survival rates are decisive factors which mainly depend on food quality and suitability. In vundu catfish (*Heterobranchus longifilis*), commercial artificial diets are often used to supplement the live food requirements in larviculture (Imorou Toko *et al.*, 2008a). However, the available diets in most developing countries are not designed specially for this species, and fail to meet the nutrient requirements of the larvae. Fish meal is an excellent but costly and scarce protein source for fish feed formulation, and is generally incorporated at more than 50% in commercial feeds for carnivorous fish species, like vundu (Hertrampf and Piedad-Pascual, 2000). Therefore, to develop viable vundu catfish production, alternate sources of high quality proteins will have to be identified to replace scarce fish meal in the diets.

The nutritional value of proteins is primarily based on the amount of amino acids present in the protein source, particularly the essential amino acid content and bioavailability. According to Lovell (1989), in plant proteins sources used in aquaculture, soybean meal and cottonseed meal have one of the best amino acid profiles that meet the essential amino acid requirements of fish. However, sulphur amino acids (methionine + cystine) and lysine have

generally been identified as the most limiting essential amino acids in these plant proteins. Moreover, soybean and cottonseed meals have been reported to contain high fibre (Hertrampf and Piedad-Pascual, 2000) and a number of antinutritional factors which could be inactivated by heat treatment (105°C for 10 to 20 min) (Bollini *et al.*, 1999; Elmaki *et al.*, 2007), soaking (Duhan *et al.*, 2002; Elmaki *et al.*, 2007) or supplementation of diets with specific hydrolysing enzymes, amino acids or minerals (Southern *et al.*, 1990; Sandberg and Svanberg, 1991; Portz and Liebert, 2004; El-Saidy and Gaber, 2004). The most commonly known and studied antinutritional factors in soybean and cottonseed meals are the inhibitor of trypsin and gossypol, respectively. Nevertheless, phytic acid is another kind of antinutritional factor in most oilseed products that is heat-stable and binds phosphorus as phytin, unavailable to fish (Hertrampf and Piedad-Pascual, 2000). Moreover, phytic acid forms complex with other minerals such as calcium, magnesium, iron, zinc and manganese at intestinal pH, thereby reducing the availability of these minerals (Liener, 1981) which are critical in skeleton formation, regulation of acid-base equilibrium, carbohydrate, lipid, and amino acid metabolism and as cofactors for

biologically important compounds such as hormones and enzymes (Watanabe *et al.*, 1997; Lall, 2002).

The effects of soybean and cottonseed meals as partial replacement of fish meal in juvenile vundu and African catfish diets have been experimented in our previous study (Imorou Toko *et al.*, 2007; Imorou Toko *et al.*, 2008b). It results that the increase of dietary soybean or cottonseed meals from 30 to 60% reduced growth performances just as minerals such as P, Zn and Mn content in the body. This has been attributed to the dietary fibre and phytic acid, their levels increasing with the inclusion of these ingredients in the diets.

The current study is addressing the use of these oilseed meals in practical diets for vundu catfish larvae during weaning. It aims to determine, as for juvenile vundu, to what extent these plant proteins sources affect growth, survival and mineral content of larvae.

MATERIALS AND METHODS

Fish origin

The larvae were obtained through induced spawning (Ovaprim, 0.5 mL kg⁻¹, Syndel Laboratories, Ltd., Vancouver, British Columbia) of captive broodstock in an overall procedure similar to that described by Gilles *et al.*, (2001). Incubation lasted from 22.00 to 24.00 hours (at 30 °C) and hatching rates were higher than 65%. At hatching, larvae were fed *Artemia* nauplii (EG grade, INVE Aquaculture, Dendermonde, Belgium) from days 3 to 8 post hatch (p.h.) before allotted in the experimental facilities.

Experimental set-up

The experiment lasted from days 8 to 26 p.h. in eight plastic experimental tanks (20 L each). All tanks were linked to a 225 L recirculation system which received water from the experimental tanks. Water was recirculated through a system of mechanical and biological filters system before supplied in each tank at a flow rate of 0.35 L min⁻¹. Water temperature was maintained at 30°C throughout the experiment, and dissolved oxygen and ammonia were not significantly different between the

experimental tanks, ranging from 1.2 to 4.6 mg L⁻¹ and 0.002 and 0.08 mg L⁻¹, respectively.

Experimental procedure

Duplicate tanks of 300 larvae per tank (15 larvae L⁻¹) (initial mean weight: 7 mg) were used for each dietary treatment. Each duplicate group received a 400 g kg⁻¹ soybean meal-based diet (SBM), 400 g kg⁻¹ cottonseed meal-based diet (CSM), a combined diet of 200 g kg⁻¹ soybean meal plus 200 g kg⁻¹ cottonseed meal (SBM+CSM), or a commercial African catfish (*Clarias gariepinus*) fry diet. The formulation of locally made diets is shown in Table 1. The proximate composition of each diet is given in Table 2. The diets were sieved and particle sizes ranged from 500 to 800 µm throughout the experiment. Larvae were hand fed in excess, six times daily at 07:00, 10:00, 13:00, 16:00, 19:00 and 21:00 h, seven days a week. From day 9 to 12 p.h., the larvae were weaned on experimental diets, respectively. This was done by gradually decreasing the quantity of *Artemia* nauplii fed to catfish over a four day period, while increasing the amount of compounded feed, *i.e.* *Artemia* nauplii: dry feed in a ratio of 75:25, 50:50, 25:75 and 0:100 %, respectively. The daily feed ration was fixed at 100% biomass basis per tank and adjusted every three days after a representative sample of 50 larvae was netted from each tank and weighed together. To evaluate if larvae were fed to excess, each tank was carefully siphoned just before a new feeding to remove faeces and dead fish. Throughout the experiment, tanks were carefully checked, mortalities and delivered food daily recorded. Dead fish were checked under binocular microscopy to find out cannibalistic aggression signs. At the end of the experiment, fish biomass as well as individual measurements of weight and total length of 15 larvae per tank was taken. Moreover, to assess the resistance of larvae to stress test, 15 larvae per tank were placed in saline water (30 g L⁻¹ NaCl) during 10 min (at 30 °C) and survival was recorded. The samples of 50 larvae per tank were taken, kept in airtight plastic sealed bags and stored at -20 °C prior to analyses of dry matter, protein and minerals.

Table 1: Formulation of the locally made diets

Ingredients (g kg ⁻¹)	SBM	CSM	SBM+CSM	Price (Fcfa* kg ⁻¹)
Menhaden fish meal ¹	400	400	400	680
Soybean meal ²	400	0	200	250
Cottonseed meal ²	0	400	200	90
Maize meal ²	60	60	60	125
Palm oil ²	90	90	90	400
Vitamin mix ³	15	15	15	1600
Mineral mix ⁴	15	15	15	1500
L-lysine ⁵	10	10	10	2200
L-methionine ⁵	10	10	10	2400

* 1 Euro = 665.957 Fcfa ; ¹ Supplied by Coppens International bv, The Netherlands. ² Supplie by ‘Ovograin Feed depot’, Benin. ³ Vit Mix Fish 0.5%, INVE Aquaculture, Belgium (composition per kg: Vit A: 2500000 IU; Vit D3: 500000 IU; Vit E : 30000 mg ; Vit K3 : 2000 mg ; Vit B1 : 2000 mg ; Vit B2 : 5000 mg ; Panthotenic acid: 10000 mg ; Niacin 5000 mg; Vit B6: 4000 mg; Folic acid: 2000 mg; Vit B12: ‘ mg; Vit C: 20000 mg; Biotin: 200 mg and Inositol: 80000 mg). ⁴ INRA France, MLNP 763, (composition per kg: dibasic calcium phosphate: 500 g; calcium carbonate: 215 g; sodium chloride: 40 g; potassium chloride: 90 g; magnesium hydroxide: 124 g; iron sulfate: 20 g; zinc sulfate: 4 g; manganese sulfate: 3 g; cobalt sulfate: 0.02 g; potassium iodide: 0.04 g; sodium selenite: 0.03 g and sodium fluoride: 1 g). ⁵ Guaranteed and distributed by BASF Aktiengesellschaft, Ludwigshafen.

Table 2: Proximate composition and cost price of experimental diets

(g kg ⁻¹ dry matter basis)	Commercial	SBM	CSM	SBM+CSM
Dry matter (fresh matter basis)	922	894	891	892
Crude protein	560*	432	429	431
Crude lipid	150*	137	142	139
Fibre ¹	4*	25	57	41
Ash	109*	113	104	121
Phytic acid	0.7	5.8	2.7	7.9
Lysine (g kg ⁻¹ crude protein) ¹	nm	0.946	0.863	0.904
Methionine (g 100 g ⁻¹ crude protein) ¹	nm	0.469	0.471	0.470
Gross energy (kJ g ⁻¹) ²	20.9*	18.8	19.5	19.1
Cost price (Fcfa ³ kg ⁻¹)	1600	508	444	476

nm, not mentioned; *According to manufacturer; ¹Calculated from ingredients (including added lysine and methionine); ²Calculated from nutrient content: 23.01 kJ g protein⁻¹; 38.07 kJ g lipid⁻¹ and 17.15 kJ g⁻¹ carbohydrates; ³1Euro = 665.957 Fcfa.

Data analysis and statistics

Fish samples were analyzed by standard methods for moisture (oven drying at 105 °C for 24 h), and proteins (organic N x 6.25) were determined by analysing organic nitrogen in dried samples using a Carlo Erba NA 1500 element analyser (Rodana, Milan, Italy). Diets were analyzed for protein, lipid, ash, mineral composition (Ca, P, Mg, Fe, Zn and Mn) and phytic acid content according to the protocols described in Imorou Toko *et al.* (2007). Mineral content in fish was also analyzed using the same methods.

At the end of the experiments, variables such as growth, survival, cannibalism, coefficient of final weight variation, condition factor and osmotic shock resistance were evaluated as follows:

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = 100 (\text{LnFMW} - \text{LnIMW}) \Delta T^{-1}$$

$$\text{Survival (\%)} = 100 N_f N_i^{-1}$$

$$\text{Cannibalism (\%)} = 100 (N_c + N_m) N_i^{-1}$$

$$\text{Coefficient of variation (CV, \%)} = 100 \text{SD FMW}^{-1}$$

$$\text{Condition factor (K)} = \text{FIW L}^{-3}$$

$$\text{Osmotic shock resistance (\%)} = 100 N_s N_o^{-1}$$

Where: N_i and N_f = initial and final number of fish per tank, N_c = number of dead fish due to cannibalism (generally type I cannibalism, *i.e.* fish partly consumed by a cannibal), N_m = number of missing fish at the counting (at the end of experiment), N_s = number of survivors in saline water, N_o = initial number of larvae used in the osmotic resistance test, IMW and FMW = initial and final mean body weights (mg), ΔT = duration of the experiment (days), SD = standard deviation of FMW, FIW = final individual weight, L = fish length (cm).

The one-way ANOVA and the Tukey Honest Significant Difference test (Winer, 1962) were used to compare differences between diets. The root mean square error (RMSE) analysis of group is made to measure the degree to which the value can be estimated accurately. The mean body weight improvements over time were analyzed by one-way repeated-measure ANOVA. Percentage data were normalized by arc-sine transformation prior analyses. For each element, \log_{10} body concentration was regressed against \log_{10} (dietary element concentration +1), \log_{10} (dietary phytic acid concentration +1) and \log_{10} (body mean weight), respectively (Shearer, 1984). The level for statistical significance was set at 5% and all statistical analyses were performed using the statistical software package, STATISTICA, version 4.1.

RESULTS

During the present experiment, the larvae showed active feeding behaviour and high feed consumption when fed commercial or soybean meal diet, compared to cottonseed meal-based diets. At the end of the trial, growth of vundu catfish larvae fed SBM was as good as ($P>0.05$) the one of fish fed the commercial diet (Table 3). The final mean weight and specific growth rate of vundu catfish larvae fed SBM were significantly higher than those of fish fed CSM or SBM+CSM. Larvae fed SBM+CSM have also a significantly better growth ($P<0.05$) than those fed CSM. However, there was no significant difference ($P>0.05$) for condition factor between fish fed the different diets. Initially, larvae fed CSM grew more slowly than those fed other diets (Fig. 1). From the third day of the experiment, the mean weight of fish fed commercial diet and diet including 40% soybean meal was significantly higher than that of groups fed the CSM or SBM+CSM. The survival and cannibalism rates were not significantly different ($P>0.05$) among fish fed the different diets (Table 3).

Table 3: Growth, survival, cannibalism, coefficient of weight variation, condition factor and osmotic shock resistance of vundu catfish larvae fed soybean or cottonseed meal based diets, $n = 2$

Diets	Commercial	SBM	CSM	SBM+CSM	RMSE	P
Final mean weight (mg)	127.4 ± 3.3 ^a	129.2 ± 1.9 ^a	75.2 ± 2.4 ^c	90.9 ± 3.3 ^b	2.79	0.0001
SGR (% day ⁻¹)	16.1 ± 0.1 ^a	16.2 ± 0.1 ^a	13.2 ± 0.2 ^c	14.2 ± 0.2 ^b	0.16	0.0001
Survival rate (%)	74.7 ± 7.1	72.2 ± 1.4	55.7 ± 12.3	53.5 ± 6.4	0.56	0.761
Cannibalism rate (%)	18.3 ± 7.1	21.0 ± 3.3	20.8 ± 5.4	35.0 ± 4.2	0.55	0.739
CV (%)	40.2 ± 0.3	47.5 ± 6.0	33.2 ± 15.1	56.4 ± 41.5	0.44	0.308
Condition factor (K)	0.81 ± 0.1	0.86 ± 0.0	0.78 ± 0.0	0.81 ± 0.0	0.04	0.474
Osmotic shock resistance rate (%)	30.0 ± 4.7 ^b	63.3 ± 4.7 ^a	86.7 ± 0.0 ^a	70.0 ± 14.1 ^a	0.34	0.195

RMSE, root mean square error; Value is mean ± SD; nm, not measured.

Numbers in the same row with the same superscripts are not significantly different ($P > 0.05$).

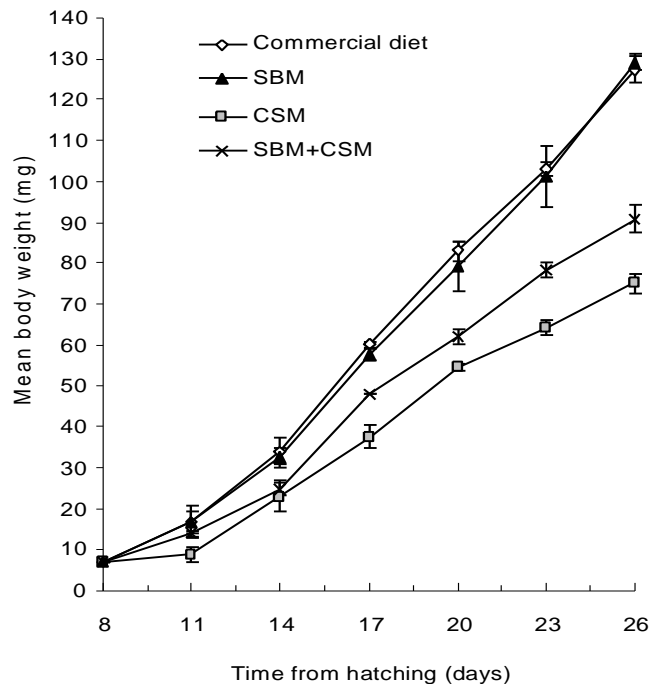


Fig. 1: Growth curve of vundu catfish larvae fed SBM, CSM, SBM+CSM or a commercial African catfish fry diet

The daily mortality variation (Fig. 2) indicated that most deaths occurred during the first three days of feeding compounded diet, due to habituation to inert food, but also to sibling aggressiveness which was observed in all groups during this period. Since this period, CSM and SBM+CSM caused more deaths than the commercial or the SBM. This is possibly due to feeds palatability which reduced feed intake as observed during experiment, in larvae fed these diets. At the end of the experiment, the survival of larvae to osmotic shock were significantly better ($P < 0.05$) in fish fed locally made diets (86.7, 70.0

and 63.3%, with CSM, SBM+CSM and SBM, respectively) than in those fed the commercial diet (30.0%).

The body dry matter content of fish did not show significant differences between groups ranging from 176 to 203 g kg⁻¹ fresh matter). However, body protein was lower ($P < 0.05$) in larvae fed commercial diet (654 g protein kg⁻¹ of dry matter) than in the other groups (746, 729 and 740 g protein kg⁻¹ of dry matter, for SBM, CSM and SBM+CSM, respectively).

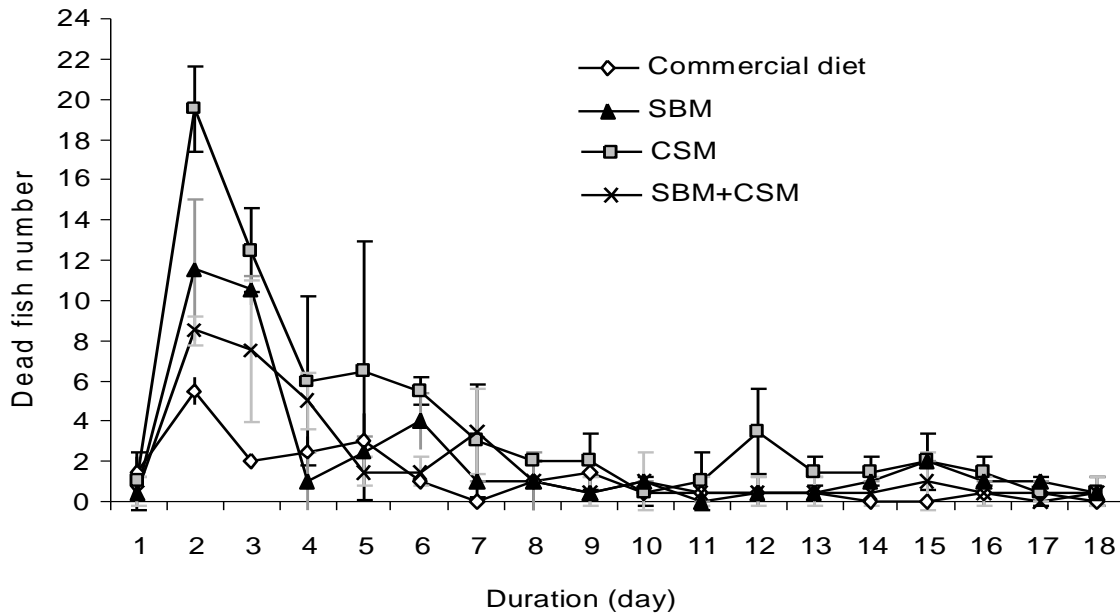


Fig. 2: Daily mortality of vundu larvae in response to SBM, CSM, SBM+CSM or commercial African catfish fry diet

The body mineral concentration varied between experimental groups (Fig. 3). The locally made diets were more concentrated in P, Mg, Fe, Zn and Mn than the commercial African catfish fry diet used. Therefore, larvae fed the commercial diet had reduced body element concentration than those fed SBM, CSM or SBM+CSM. Nevertheless, the concentrations of Fe, Zn and Mn were higher in fish fed CSM or SBM+CSM than in those fed SBM, while their dietary level was similar. Moreover, the content of these minerals in fish fed commercial diet were lower than in those fed CSM or SBM+CSM. Furthermore, Ca, P and Mg concentrations in fish

body did not differ ($P>0.05$) among fish fed locally made diets, although their dietary level increase as dietary cottonseed meal level increases.

Only Ca, P, Mg and Mn contents in the body were found to be significantly correlated ($P<0.05$) to the dietary amount of these elements ($r^2 = 0.821, 0.800, 0.652$ and 0.575 , respectively, Table 4). However, no significant trend ($P>0.05$) was observed with regard to the relationship between dietary phytic acid concentration and body mineral content. As observed in Table 4, mineral concentration in the body decreased as fish weight increased.

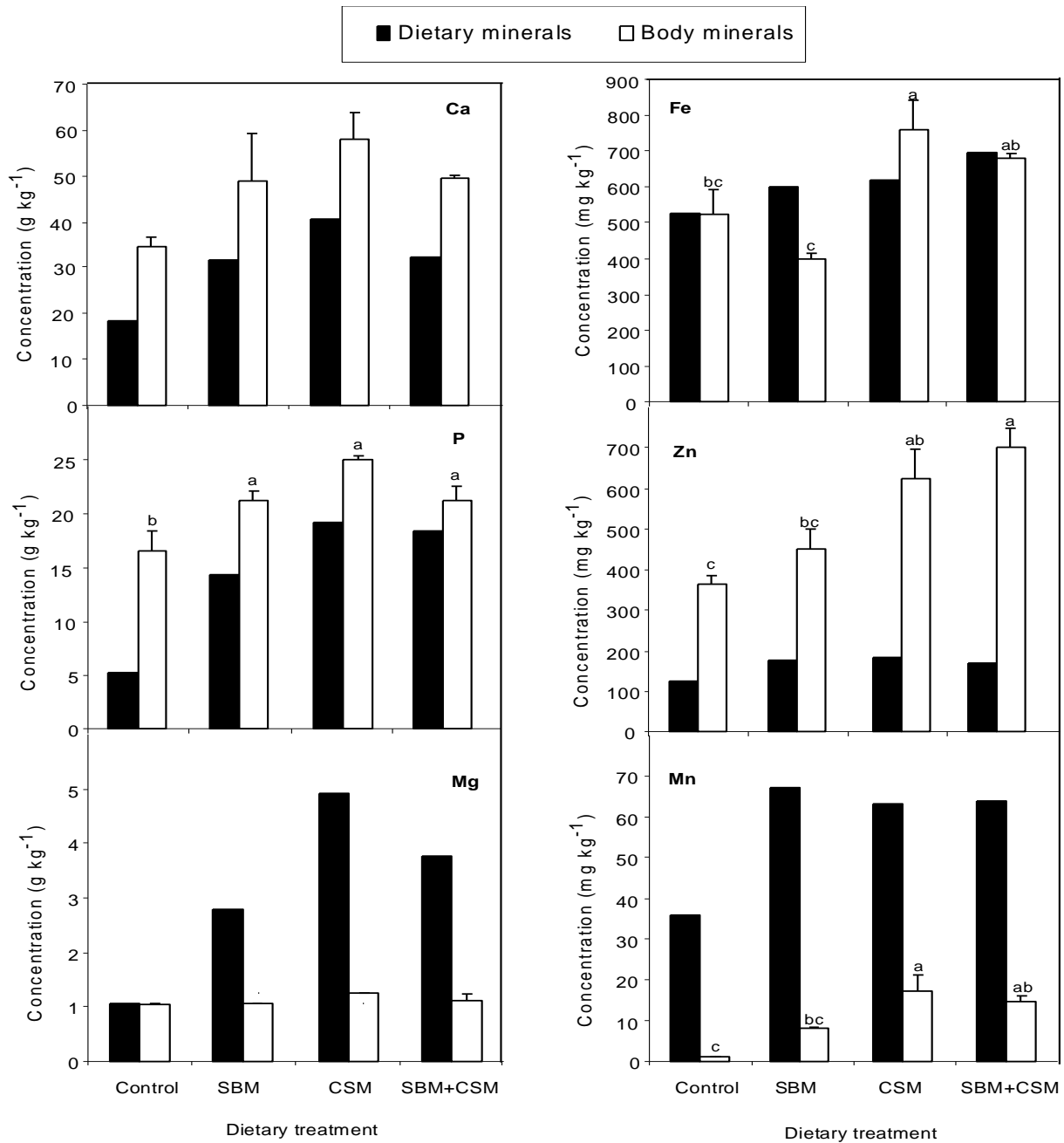


Fig. 3: Mineral contents (dry matter basis) in experimental diets and fish after 18 days feeding. Different letters indicate that means are significantly different from each other ($P < 0.05$).

Table 4: Regression parameters of \log_{10} (body mineral content + 1) vs. \log_{10} (dietary element concentration + 1) (1), \log_{10} (dietary phytic acid + 1) (2) or \log_{10} (fish mean weight) (3), respectively, $n = 8$

Elements	Factors	Regression parameters		
		Equation	R^2	P
Ca	1	$Y = 0.65X + 0.72$	0.821	0.002**
	2	$Y = 0.47X + 1.61$	0.274	0.183
	3	$Y = -0.60X + 2.89$	0.475	0.059
P	1	$Y = 0.27X + 1.03$	0.800	0.003**
	2	$Y = 0.32X + 1.29$	0.237	0.221
	3	$Y = -0.46X + 2.27$	0.533	0.040*
Mg	1	$Y = 0.09X + 0.27$	0.652	0.015*
	2	$Y = 0.03X + 0.32$	0.029	0.687
	3	$Y = -0.16X + 0.65$	0.755	0.005**
Fe	1	$Y = 1.10X - 0.03$	0.203	0.263
	2	$Y = 0.01X + 0.20$	0.001	0.946
	3	$Y = -0.37X + 0.94$	0.853	0.001**
Zn	1	$Y = 3.11X - 0.02$	0.460	0.064
	2	$Y = 0.27X + 0.15$	0.432	0.076
	3	$Y = -0.32X + 0.82$	0.634	0.018*
Mn	1	$Y = 0.40X - 0.01$	0.575	0.029*
	2	$Y = 0.02X + 0.00$	0.311	0.151
	3	$Y = -0.02X + 0.05$	0.769	0.004**

*significant ($P < 0.05$); **highly significant ($P < 0.001$)

DISCUSSION

In the present study, the growth and survival of vundu larvae fed both commercial and locally made diets were better than those obtained by Legendre *et al.* (1995) from first feeding with various dry diets, after 15 days (from 70 to 114 mg with diets containing different lipid sources). The similar growth rate obtained from SBM and commercial diet indicated that the protein utilization was similar in these two diets. This suggest that the biological value of SBM proteins was improved by using a heated soybean meal or by supplementing a diet with methionine and lysine, possibly in excess to what is needed for protein synthesis. This is also corroborated by the highest protein content (746 g kg^{-1}) in fish fed SBM compared to those reared on commercial diet (654 g kg^{-1}), despite initial difference in dietary protein level. The present study also reveals that growth was poorer in fish fed CSM or SBM+CSM than those receiving SBM. This may be attributed to poor utilization of cottonseed meal proteins by vundu larvae, possibly due to various

reasons such as improper balance of essential nutrients, especially amino acids, and/or presence of fibre. Little is known about the specific amino acids requirements of vundu catfish. However, in African catfish larvae, nutritionally close to vundu (Kerdchuen, 1992), Conceição *et al.* (1998) found that whole fish amino acid profiles change considerably during larval development, and are different from the adult profiles reported by Hoffman *et al.* (1995). According to these authors, methionine and lysine (which were more critical in soybean and cottonseed proteins) requirement in African catfish larvae was close to 0.248 and 0.862 g kg^{-1} crude protein, respectively, on day 7 of exogenous feeding. Based on this finding, we can assume that in the present study, the amount of these amino acids in the locally made diets (ranging from 0.469 to $0.471 \text{ g methionine kg}^{-1}$ crude protein and 0.86 to $0.946 \text{ g lysine kg}^{-1}$ crude protein) was sufficient to meet the requirements of vundu catfish larvae.

Furthermore, larvae fed SBM displayed a feeding behaviour more active than those fed CSM or

SBM+CSM. This suggests that the palatability was a major factor responsible for the lower feed consumption of cottonseed-based diets in vundu larvae. During our previous study on juvenile vundu catfish, fish fed soybean meal-based diet (at 30 and 60% inclusion level) have higher growth rate and better feed utilization than fish fed cottonseed meal-based diet at corresponding inclusion level (Imorou Toko *et al.*, 2007). Indeed, the inclusion of cottonseed meal in the diets resulted in an increase of fibre content that possibly reduces feed palatability and digestibility in fish, thereby reducing feed intake, transit time of intestinal contents, and therefore growth (Arnesen *et al.*, 1989; Krogdahl, 1989; Hertrampf and Piedad-Pascual, 2000; Imorou Toko *et al.*, 2008b). That is probably what occurs in this study with cottonseed meal-based diets which were possibly less digestible than the SBM. This might also be related to the poor development of digestive system during the first 3 days of weaning, since the mortalities increased during this period, especially in fish fed cottonseed meal based diets. In African catfish, Verreth *et al.* (1992) also noted that the stomach only become functional in larvae at approximately 20 mg wet weight. Hence, with respect to growth and survival, SBM is more suitable to replace commercial feed currently used in the production of vundu catfish larvae.

On the other hand, care must be taken when using oilseed meals, as soybean or cottonseed meals, in the diets for fish, particularly for the larvae, since they are known to contain several antinutritional factors which may impair nutrient availability. In our previous study on juvenile vundu and African catfish fed soybean or cottonseed meals-based diets, dietary phytic acid was found to reduce some minerals such as P, Zn and Mn availability as indicated by the drop in the content of these elements in the whole body or fillet (Imorou Toko *et al.*, 2007; Imorou Toko *et al.*, 2008b). However, in the present study, larvae fed CSM or SBM+CSM were generally more concentrated in minerals compared with those fed a 40% soybean meal-based diet, albeit the relatively similar dietary amount of these elements. Information about vundu dietary minerals requirement are unavailable. Moreover, studies on the mineral requirements of catfishes have focused on juveniles (Wilson *et al.*, 1982; Gatlin and Wilson, 1984; Imorou Toko *et al.*, 2007; Imorou Toko *et al.*, 2008b) and information regarding the optimum mineral level in larval diets is scarce. The present study reveals that mineral content in larvae depend on the dietary amount of these minerals or the growth rate of the larvae (Table 4). This was also corroborated by the good correlations observed between the contents of some minerals such as Ca, P, Mg and Mn in fish

body and the diets (Table 4). In fish, Watanabe *et al.* (1997) also reported that the availability of dietary elements depends not only on their chemical form or interactions with other nutrients, but also on their concentration. In salmonids or tilapia (*Oreochromis niloticus*), it has also been noted that Ca, P and Mg concentrations in fish body were responsive to graded levels of their dietary concentrations (Skonberg *et al.*, 1997; Mbahinzireki *et al.*, 2001). Moreover, Shearer (1995) found that body elemental content in fish was homeostatically regulated when the supply of dietary elements was sufficient. However, the lowest Mn content in the body of fish fed SBM or commercial diet compared to the others could also be due to an interaction between Fe and Mn concentrations, since these elements compete for the same binding sites for absorption (Andersen *et al.*, 1996; Lorentzen and Maage, 1999).

The regression equations described in Table 3 indicate that body Ca, P, Mg, Fe, Zn and Mn contents decreased with growth as the slopes for these elements were negative. Fast growing fish (fed SBM) display lower concentrations of Ca, P, Mg, Fe, Zn and Mn, than fish fed CSM or SBM+CSM. Indeed, several studies on fish showed that whole body contents of some elements change with growth and life cycle stage (Shearer, 1984; Shearer *et al.*, 1994; Lorentzen and Maage, 1999). Shearer (1984) reported that rates of whole body elemental accumulation of Ca, P, Mg, Fe, Zn and Mn were high in juvenile rainbow trout (*Oncorhynchus mykiss*) than in adult fish. Otherwise, Maage *et al.* (1993) also suggested that the fast growing fish have a higher requirement than the mean of the population. This might be due to rapid utilization of body minerals in fast-growing fish, especially when the dietary supply was not sufficient or available. Therefore, although mineral requirement of vundu is not known, the present study reveals that requirement might be higher in larvae, as the growth rate in the early life stages is higher.

ACKNOWLEDGEMENTS

This study was funded by the CUD-CIUF (University Cooperation towards Development - Interuniversity Council of French Speaking Universities of Belgium), the University of Namur and the University of Abomey-Calavi (UAC). The authors are grateful to Jean-Baptiste Atchamou, Ebenezer Abioga, Alexis Flimatin and Pierre Cambier for their technical assistance.

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