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Determination of the optimal feed ration for best growth of *Hoplobatrachus occipitalis* (Günther, 1858) tadpoles reared in controlled medium

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Abstract
The experiment was carried out for 24 days in open circuit made of 18 cemented ponds containing 50 L water each. The current study aims to estimate the optimal feed ration in *Hoplobatrachus occipitalis* tadpoles (0.097 ± 0.026 g). Six rations (5, 10, 15, 20, 25 and 30% of biomass/day) were tested in triplicate to evaluate their effect on the growth, survival and the carcass composition of these latter. Results showed Specific Growth Rate and Feed Efficiency was not significantly influenced by feed ration (P>0.05). Highest Specific Growth Rates were observed in tadpoles fed on rations of 15 and 20 % of biomass or 9.059 ± 0.209 %/Day and 9.027±0.142 %/Day respectively. Besides, highest mean values of Feed Efficiency were obtained in tadpoles fed on rations of 10 and 15% of biomass or 0.778±0.383 and 0.757±0.052. Optimal and maximum feed rations in tadpoles of *H. occipitalis* are respectively 6.02 and 21.37 % of biomass per day.

Keywords: *Hoplobatrachus occipitalis*, tadpoles, optimal feed ration

1. Introduction

Tadpoles are important in most of fresh water ecological communities, excellent indicators of environment quality and biodiversity conservation [1-6]. Due to the destruction of their habitat, overexploitation, pollution and lack of management program, many amphibians' populations are in decrease [7]. A part from their ecological importance, frog meat is an attractive food in European big restaurants since the 16th century [8]. It represents a very important source of protein that can be affiliated to chicken meat with a good taste [9]. It is so necessary to rear them not only for species conservation but to satisfy human protein demand. Feed diet is a capital part of rearing requiring an optimization even though we don't mention what tadpoles feed on in the wildlife [10]. Feed ration can influence larvae growth and survival [11]. Similarly, the density, temperature, water quality, photoperiod, feed quality, physical characteristics of the feed etc… are factors influencing the growth of frog tadpoles [12]. The optimal feeding level improves not only growth performances but enable a reduction of production cost [13] as the price of feed represents between 50% and 70% of the production cost [14]. Several studies showed the influence of artificial feed on survival and growth of tadpoles of *Rana catesbeiana* [15], *Litoria ewingii* [16], *Rana temporaria* [17], but a few studies were focused on *H. occipitalis* tadpoles. By the same way, a few studies examined how feeding level can influence growth of amphibians' tadpoles [18]. This study aims to determine the optimal ration of *H. occipitalis*.

2. Materials and methods

2.1 Tadpoles province and experimental design

Tadpoles of *H. occipitalis* used in this study were obtained by semi-artificial reproduction on the site of the Research Laboratory on Wetlands (LRZH) of the University of Abomey-Calavi. Mean value of water temperature, pH and dissolved oxygen in rearing ponds are respectively 28.33 ± 0.09 °C, 7.01 ± 0.01 and 3.98 ± 0.02 mg/l.

Experiment was led for 24 days (from September 25th to October 18th, 2017). Three days after eggs hatching, tadpoles were collected, counted and distributed in an open circuit made of 18 cemented circular ponds containing 70 tadpoles each in 50 L water.

2.2 Feeding

Experiment was preceded of a progressive acclimation of tadpoles to physico-chemical conditions where they fed only on live zooplankton and *Artemia salina* nauplii. Starving was progressive as indicated in Table 1. Coppens was the artificial feed used (diameter 0.5-0.8 mm, 56% protein rate and 15% of lipid). Six rations (5, 10, 15, 20, 25 and 30% of tadpoles biomass) were tested on tadpoles (initial mean weight = 0.099 ± 0.002 g) for a 24 days period. Feed was distributed manually every three (03) hours from 8 a.m to 5 p.m.

2.3 Growth control

Control fishing happened every three (03) days following by ponds emptying and cleaning. Number of tadpoles and biomass were determined in order to adjust feed ration. After 24 days of rearing, tadpoles were collected from ponds and weighted individually. Zootechnical performances such as Specific Growth Rate (SGR), Survival Rate (SR) and Feed Conversion Rate (FCR) were calculated according to the following formula:

$$SGR\ (\%) = 100 \times \frac{\text{Ln(final body weight)} - \text{Ln(initial body weight)}}{\text{Duration of rearing period (days)}}$$

$$SR\ (\%) = 100 \times \frac{\text{Final nomber of tadpoles}}{\text{Initial nomber of tadpoles}}$$

$$FCR = 100 \times \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}}$$

2.4 Water quality monitoring

Water quality monitoring was carried out by measuring temperature, dissolved oxygen and pH. These parameters were measured twice a week (morning: 7:30 a.m and evening: 04:30 p.m) in each pool at 5 cm depth of water using the portable multi-meter (Calypso ORCHIDIS SN-ODEOA 2138). Water renewing was carried out everyday (7 a.m and 6p.m) before and after feeding with 0.5 L/min water flow.

2.5 Chemical analyses

Samples were taken before and after experiment for chemical composition analyses (crude protein, fat, dry matter and crude ash) of tadpoles carcass according to the method of [19].

2.6 Statistical analysis

Data were encoded in Microsoft Excel 2013. They served to zootechnical parameters calculation. Mean and range were calculated for each parameter. For each repetition, mean data were considered as an observation.

Statistical software R (version 3.4.2) was used for comparisons with 5 % significance. A one way analysis of variance helped to compare zootechnical parameters in different treatments. The Student-Newman-Keuls test (SNK test) was used for different means pair comparisons in case of significant differences among treatments to determine ideal ration, growth and survival rate of *H. occipitalis* tadpoles. The model of Brett & Grove [20] was applied with second order polynomial regression between specific growth rate and feed ration to determine the optimal and maximum feed ration of *H. occipitalis*.

Table 1: Feed substitution for *H. occipitalis* tadpoles.

Duration (Days)	Feed
2	100% zooplankton
2	*Artemia salina* 50% + zooplankton 50%
1	*Arthemia salina* 75% + zooplankton 25%
1	*Arthemia salina* 100%
1	*Arthemia salina 50%* + artificial feed 50%
1	*Arthemia salina* 25% + artificial feed 75%
16	Artificial feed 100%

Artificial feed = Coppens 0.5 – 0.8 mm, 56 % protein rate and 15 % lipid

3. Results

Physico-chemical parameters in different ponds are mentioned in Table 2. During experiment period, the highest pH mean value was obtained with ration of 20 % biomass/day. Although, there were no significant differences among pH values (P > 0.05). Dissolved oxygen in rations R1 (5%), R2 (10%) and R3 (15%) was significantly upper than those in R4 (20%), R5 (25%) and R6 (30%).

Growth performances, rations and survival rates of tadpoles are mentioned in Table 3. Survival rate of tadpoles varied between 79.45 ± 7.24 % and 94.97 ± 2.09 %. There were no significant differences between these values (P > 0.05).

The *H. occipitalis* carcass composition at the end of experiment is mentioned in Table 4. Crude proteins, lipids, ash and dry matters were significantly affected by the different feeding rates (P > 0.05). The highest protein rate was observed in tadpoles fed on the R6 (30 %) ration. Concerning ash and dry matter rates, the highest values were observed in tadpoles fed on 10 % (R2) of biomass per day. The highest value of lipid was obtained in individuals fed on the R3 (15%) ration while the lower was observed in those fed on R1. Generally, dry matter, lipid and crude protein of the initial sample are similar to the values obtained after tadpoles feeding with experimental diet.

According to the model of Brett, the optimal and maximum rations of *H. occipitalis* are approximately 6.02%/day and 21.37%/day of tadpoles biomass (Figure 2).

Table 2: Mean ± range values of dissolved oxygen, pH and water temperature in ponds during the tadpoles rearing period with the different feed rations.

Parameter	Rations (%)					
	5 (R_1)	**10 (R_2)**	**15 (R_3)**	**20 (R_4)**	**25 (R_5)**	**30 (R_6)**
DO, mg/l	5,280 ±0,330^a	5,297±0,149^a	5,323±0,422^a	4,141±0,308^b	3,938±0,414^b	2,791±0,208^c
pH	6,833±0,104^a	6,783±0,152^a	6,838± 0,058^a	6,900±0,133^a	6,800±0,086^a	6,605±0,091^a
T, °C	27,538±0,149^a	27,630±0,186^a	27,632±0,092^a	27,433±0,157^a	27,518±0,127^a	27,596±0,224^a

^{abc} mean values on the same ligne that are not affected by same letter are significantly different (P< 0.05).

Table 3: Initial Biomass (IB), Final Biomass (FB), Survival Rate (SR), Specific Growth Rate (SGR), Feed conversion Rate (FCR) of *Hoplobatrachus occipitalis* tadpoles reared for 24 days in ponds open circuit and fed on different feed rations.

Parameter	Rations (%)					
	5 (R_1)	10 (R_2)	15 (R_3)	20 (R_4)	25 (R_5)	30 (R_6)
IBW (g)	7,076±0,025[a]	7,180±0,147[a]	7,260±0,173[a]	7,283±0,185[a]	7,390±0,017[a]	7,377±0,040[a]
FBW (g)	28,727±4,542[b]	47,997±22,245[a]	58,707±4,411[a]	60,427±3,555[a]	57,717±3,014[a]	59,577±1,701a
SR (%)	79,452±7,249[a]	84,474±13,305[a]	91,781±1,370[a]	94,977±2,093[a]	94,107±3,089[a]	93,150 ±2,373[a]
SGR (%/days)	6,772±1,009[b]	8,253±1,704[a]	9,059±0,209[a]	9,027±0,142[a]	8,814±0,747[a]	8,999±0,076[a]
FCR	1,733±0,348[a]	1,657±1,139[a]	1,324±0,089[a]	1,536±0,088[a]	1,902±0,038[a]	2,098±0,071[a]

[abcdef] mean values on the same ligne that are not affected by same letter are significantly different ($P< 0.05$).

Table 4: The composition of *Hoplobatrachus occipitalis* tadpoles' carcass reared for 24 days and fed on six different rations (%/day) with a commercial feed (Coppens).

Analysis	Feeding rate						
	Initial	5%	10%	15%	20%	25%	30%
Dry Matter	89,754±0,001[b]	87,640±0,004[f]	90,388±0,001[a]	89,343±0,001[c]	85,190±0,004[g]	89,217±0,002[d]	88,357±0,005[f]
Crude ash	10,885±0,003[b]	7,584±0,003[d]	11,066±0,002[a]	7,751±0,003[c]	6,356±0,007[g]	7,043±0,001[e]	6,857±0,006[f]
Crude lipid	34,604±0,002[b]	22,709±0,005[f]	24,743±0,001[e]	37,550±0,001[a]	22,183±0,001[g]	34,483±0,003[c]	34,280±0,004[d]
Crude protein	43,789±0,001[b]	36,423±0,002[f]	40,758±0,004[e]	31,457±0,005[g]	43,160±0,003[c]	41,706±0,002[d]	43,868±0,001[a]

[abcdefg] mean values on the same ligne that are not affected by same letter are significantly different ($P< 0.05$).

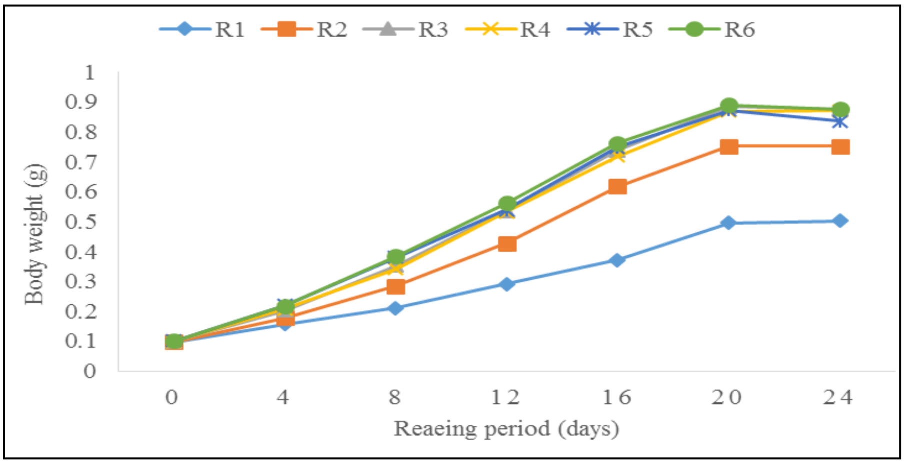

Fig 1: Variation of body weight of *Hoplobatrachus occipitalis* tadpoles reared for 24 days with different feed rations.

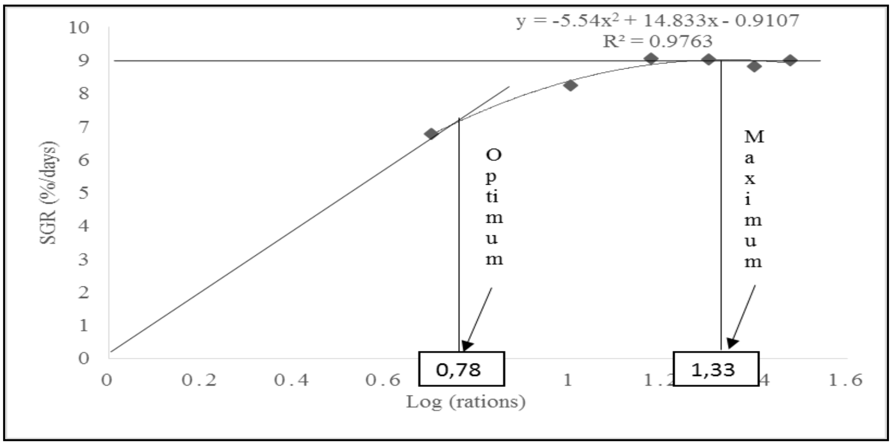

Fig 2: Determination of the optimal and maximum ration of *Hoplobatrachus occipitalis* tadpoles by the model of Brett and Grove after semi-logarithmic transformation of the different feed rations.

4. Discussion

Temperature is the major factor that interferes with frogs' growth due to its direct influence on the metabolism of the subject [18]. Mean value of temperature (27.632 ± 0.092) recorded during the experiment was similar to 26.52 °C obtained by Borges *et al.* [22] in Taurus frog tadpoles. Moreover, these values were upper than 24.68 °C recorded by Ferreira *et al.* [23] in *Lithobates catesbeianus* tadpoles. Besides, temperatures recorded during this study were situated in the range of 15 – 35 °C recommended for a good frog growth [21]. Mean values of pH and dissolved oxygen recorded during the experiment were in accordance with those obtained by certain authors [23, 24, 22] in *Lithobates catesbeianus* tadpoles.

Tadpoles' growth increases with feed ration. The final biomass recorded ranged from 28.727 ± 4.54 g and 60.427 ±

3.55 g. These results are better than those observed by Pedro *et al*. [25] with the tadpoles of Calyptocephalella gayi. That could be explained by the different protein rates among feed. Indeed, Pedro and collaborators used a feed with a low protein level (40.11%) whereas that used in our study is Coppens which is a high protein level (56%). In addition, these results are lower to those obtained by *Rana catesbeiana* [26, 27].That could result from the difference among experiments duration. Indeed, our experiment lasted 24 days that is very less than 15 months of theirs. Mean Specific Growth Rates (SGR) recorded in all treatments (6.772 ± 1.009; 8.253 ± 1.704; 9.059 ± 0.209; 9.027 ± 0.142; 8.814 ± 0.747 and 8.999 ± 0.076 %/day) are better than those obtained in *Rana catesbeiana* [28, 29, 27] and *alyptocephalella gayi* [25]. These results could be explained by the fact that the growth of aquatic animals (fish, frogs...) depends on the quantity and quality of the feed consumed. Besides, the increase in Specific Growth Rate (SGR) is synonym of feed availability till a maximum consumption threshold [30]. The increase of tadpoles' height is subject to optimal feed consumption enabling remarkable changes in morphology, physiology and behavior, during the metamorphosis process [31, 32]. This life stage makes difference among frogs and other aquatic vertebrates and affects the nutritional experiments due to the animal weight loss during the metamorphosis [33]. In addition, metamorphosis in tadpoles could be achieved unless they satisfy nutrients needs that enable them to be transformed in a new animal totally different. This dependence on nutritive elements was also observed by Martìnez *et al*. [34] who led an experiment on *Rana perezi*, though inadequate feeding elongates the larval stage. Otherwise, tadpoles in natural media are subject to food competition. If this resource is scarce, a part of animals satisfy their nutritional need that leads to food selection in tadpoles that metamorphose during a certain season. Remaining tadpoles stay in the medium until they me*et al*. the requirements for metamorphosis.

In nutrition studies, evaluation of nutritive values index are important. Mean values of Feed conversion Rate in the current study are lower than those obtained by Flores-Nava *et al*. [29], and those in *R. catesbeiana* tadpoles [26]. That shows a low feed conversion by *H. occipitalis* tadpoles. These differences could result from the used feed quality (40.11% crude protein and 10.22% of lipid) that is less rich than Coppens (56% of protein and 15% of lipid) and also the difference in species. Besides, these feed conversion rates were similar to those of certain carnivorous fishes (0.93 – 3.23) in *S. intermedius* larvae [35] and *H. longifilis* larvae [36].

The influence of the different feeding rates on the tadpoles' carcass composition justify the significant differences observed among dry matter, crude ash, crude lipids and crude proteins rates of *H. occipitalis* tadpoles ($p<0.05$). Tadpoles fed on the 10 % ration showed values nearby those in the initial sample. These results are similar to those of Cagiltay *et al*. [8], who led a study on the chemical composition of *Rana ridibunda* in rearing. Nevertheless, these values are upper than those recorded by Ozogul *et al*. [37] in *Rana esculenta* and Cagiltay *et al*. [8] in *Rana ridibunda* from Adana, Bursa and Trakya regions in Turkey. That could result not only from the difference species but from the used feed quality. Moreover, Shearer *et al*. [38] explained that moisture, proteins, fats and ash rates in aquatic animals tend to vary according to species, climate, seasons, sexual maturity and food diet. Our crude proteins values are lower than those observed by Tokur *et al*. [9] in *Rana esculenta* while lipids values are upper. Concerning crude ash values, our results are similar to those of Tokur *et al*. [9]. This difference between crude proteins and lipids values could due to the different proportions of bones and flesh in species. By the same way, the proportions of proteins in tadpoles flesh can slightly be tied to feed and climate [9].

The determination of appropriate feed quantity to animals is capital because it determines nutrients and energy availability in the subject. In the current study, the optimal ration for *H. occipitaalis* tadpoles rearing is 6.02 %/day of the body weight while the maximum ration is 21.37%/day. These results are nearby those in *Rana catesbeiana* ranging from 8 to 14 % of body weight [26]. They are also in accordance with those recorded by Fontanello *et al*. [39] and Justo *et al*. [40], ranging from 3% to 13% of body weight in *Rana catesbeiana* tadpoles.

5. Conclusion
Globally, the current study showed that in order to obtain the best yield in *H. occipitalis* tadpoles growth, it worth an optimal ration of 6.02 % of biomass. This feeding rate will enable a rapid biomass growth, specific growth and a good feed conversion rate in tadpoles.

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