RESEARCH ARTICLE

Fishmeal replacement using housefly larvae meal as protein ingredient in balanced feeds for bullfrog tadpoles and froglets (*Lithobates catesbeianus*)

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HIGHLIGHTS

- **The substitution of housefly larvae meal for fishmeal in bullfrog feed is feasible in different percentages depending on the cultivation stage.**
- **In tadpoles substitution by up to 50% does not limit growth and metamorphosis**
- **In froglet, inclusion up to 25% does not affect productive performance.**

KEYWORDS **alternative feed, frog culture, productive performance**

Abstract

This research evaluates the use of housefly larvae meal (HLM) as an alternative protein replacing fishmeal (FM) present in feeds for bullfrog tadpoles and froglets. The treatments consisted of the formulation of four feeds for tadpole stage with 30% of protein and four inclusion percentages of HLM ($T30_0$ – 0%; $T30_{25}$ – 25%; $T30_{50}$ – 50%; $T30_{75}$ – 75%). Likewise, for pre-fattening stage (froglet), four feeds with 40% of protein and the same inclusion percentages of HLM were managed ($T40_0$ – 0%; $T40_{25}$ – 25%; $T40_{50}$ – 50%; $T40_{75}$ – 75%). Weight gain (WG), survival rate (SR), feed conversion rate (FCR), protein efficiency rate (PER) and metamorphosis process (start and duration) were established as response variables. Statistical analyses were performed using ANOVA and Tukey's test. The results suggest that in the tadpole stage $T30_{25}$ contributes more to weight gain (4401.39 ± 36.66%) and metamorphosis process (started at 35 ± 0.5 and duration of 169 ± 7 hours). On the other hand, $T30_{50}$ did not show differences with respect to $T30_0$ for WG and start of metamorphosis. In the pre-fattening stage, treatments $T40_0$ and $T40_{25}$ presented outstanding values in WG (154.13 ± 5.91 and 149.80 ± 6.33%, respectively) and SR (88.3 ± 1.2 and 87.6 ± 1.5%, respectively). Finally, considering the productive performance at the end of both stages, the diets with 0 and 25% inclusion of HLM did not show differences for the variables of WG, FCR and PER. The values obtained suggest that HLM has nutritional characteristics that allow it to replace fishmeal between 25–50% in the formulation of balanced feeds for bullfrog culture.

1 Introduction

In the formulation of balanced feeds, the protein part is a determining factor in the growth of organisms (Mansano et al., 2013). Within aquaculture feeding, fishmeal is the protein source par excellence; however, its use has ecological and monetary disadvantages; being that its production requires conventional fishing, causing variation in its availability and cost (FAO, 2014).

Due to the above, in recent years the potential of some species of insects as an alternative protein in the formulation of balanced feeds has been studied (Sanchez-Muros et al., 2013; Henry et al., 2014). As an example of it, the housefly stands out for its characteristics: a short life cycle, high reproduction rate, growth capacity in different substrates,

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and its nutritional composition in the larval stage (Aniebo and Owen, 2010; Odesanya et al., 2011; Sanchez-Arroyo and Capinera, 2014). Related studies on the use of housefly larvae in the formulation of balanced feeds for aquaculture species show acceptable results after the incorporation of 15 to 45 % (Ogunji et al. 2008; Ogunji et al., 2011; Li et al., 2019).

The bullfrog (*Lithobates catesbeianus*) is one of the species used in frog culture, reporting a production that exceeds 3,000 tons per year worldwide (Pahor-Filho et al., 2019; FAO, 2020). The duration of the production cycle of the bullfrog is five to seven months and includes seven stages: reproduction, embryonic development, tadpole, metamorphosis, pre-fattening and fattening (Ferreira et al., 2002). Although feeding is a factor important throughout the production cycle, in the tadpole stage its relevance increases, being the period of growth and energy storage prior to metamorphosis. The same occurs at the beginning stage of pre-fattening since feeding impacts the time of growth and subsequent sexual development (Seixas-Filho et al., 2012; Pinto et al., 2015).

The studies on bullfrog feeding have not addressed the incorporation of insects to obtain feeds for bullfrog culture, despite the fact that various insects are part of the natural diet of this organism (Howe et al., 2014). Therefore, the objective of this research was to determine the effect of the incorporation of housefly larvae meal (HLM) as a source of protein in balanced feeds for bullfrog tadpoles and froglets replacing fishmeal.

2 Materials and Methods

2.1 Production and processing of housefly larvae

The production and processing of housefly larvae began with the capture of adult houseflies. The flies were introduced into an isolated module with temperature (25-30 °C), light (350 lx) and humidity (50–60 %) control, thus generating a favorable microenvironment for reproduction; inside the module plastic trays filled with wheat bran were placed to feed the adult house flies and serve as deposit of the eggs, which were collected daily and incubated for 4 days to obtain the desired larvae, which were separated from the substrate for processing. For it, the selected larvae were cooled for 24 hours to -10 °C, then they were placed in an electric dehydrator at 65°C for 24 hours (Pieterse and Pretorius, 2014), then they were milled to obtain a meal (0.2 mm). Finally the HLM was stored to 5°C until analysis of proximate composition (*Table 1*) and mixing in experimental feeds.

2.2 Formulation of experimental feeds

Eight experimental diets were formulated, having two levels of protein content (40 and 30 %) each with different inclusion of HLM (0, 25, 50, 75 %) in replacement of fishmeal. The substitution of fishmeal by HLM was carried out based on weight, intending that each food maintain the necessary percentage of protein required; in addition soybean meal was also used as a protein source. Wheat and corn flour was used as a carbohydrate supply, and fish oil was used together with soybean lecithin for lipid supply. The formulation and proximal composition of the experimental diets are shown in *table 2*. The calculation of the amino acid profile for 100 grams of feed, as well as the required value of essential amino acids for tadpoles and frogs of *L. catesbeianus* is presented in the *table 3*. In the case of the feed for the tadpole stage, the ingredients were mixed then pressed into granules of 1.5 mm in diameter and finally they were grounded to obtain the final presentation of the feed (0.6 mm meal). The feeds for the tadpole stage had a protein level of 30%, reported as the ideal value in the feeding of tadpoles of *L. catesbeianus* (Mansano et al., 2013; Pinto et al., 2015). For the pre-fattening (froglet) stage feeds, the ingredients were mixed, and then pressed into 1.5 mm diameter granules. The feeds for the pre-fattening stage had a protein level of 40 %, a percentage recommended as optimal in the stage (De Castro et al., 2014).

TABLE 1
Values means ± SD of the proximate composition of the meals used as a source of protein for the formulation of experimental diets.

Composition (%)	FM	HLM	SM
Dry matter	91.5 ± 2.5 ^{a}	90.8 ± 2.9 ^{a}	91.1 ± 2.3 ^{a}
Proteins	52.8 ± 1.1 ^{a}	45.8 ± 1.6 ^{b}	46.9 ± 0.9 ^{b}
Lipids	9.9 ± 0.4 ^{a}	9.2 ± 0.2 ^{b}	8.9 ± 0.3 ^{b}
Nitrogen free extract	13.5 ± 0.5 ^{b}	16.9 ± 0.4 ^{a}	16.8 ± 0.4 ^{a}
Fiber	7.8 ± 0.3 ^{a}	9.6 ± 0.4 ^{a}	10.1 ± 0.3 ^{a}
Ash	7.5 ± 0.2 ^{c}	9.3 ± 0.3 ^{a}	8.4 ± 0.4 ^{b}

Fishmeal (FM), housefly larvae meal (HLM), and soybean meal (SM)
Values with the same superscripts do not show significant differences (P<0.05)
Analytical methods (Official Methods of Analysis of AOAC INTERNATIONAL 2019): Moisture: 925.23, protein: 925.15, lipids Soxhlet: 920.23, fiber: 991.42, ash: 945.46, nitrogen free extract: by diffence from the other components

2.3 Biological material and culture system

A total of 600 bullfrog tadpoles were used, all from the same spawning and fertilised with the semen of a single male. Tadpoles used were at the beginning of Gosner stage 25 with 28 days of age, mean weight and length and standard deviations were 0.143 ± 0.03 g and 11.26 ± 0.41 mm, respectively. Tadpoles were distributed in 12 fishbowls (75 cm long x 30 cm wide x 30 cm high), with a volume of 50 liters and a density of 1 L^{-1} tadpole (Bellakhal et al., 2014). After metamorphosis, the froglets were transferred to 12 ponds (120 cm long x 80 cm wide) equipped with vibrating feeders and water troughs to evaluate the productive stage of pre-fattening (froglet) using a density of 40 frogs m^{-2} (Pereira et al., 2014).

The fishbowls used in the tadpole stage were divided into 4 recirculation systems that had a replacement rate of 480 L h^{-1}. Each system consisted of: 3 fishbowls (each one provided with a thermostat to maintain the water temperature in the range of 22–30 °C), a sedimentation tank (with a capacity of 75 L) and a canister filter equipped with an UV lamp; likewise, each system had a daily replacement of 5% of the water volume. In the pre-fattening stage (froglet), the ponds did not have a recirculation system; however, each one of them had a change of 50% of the volume of water every third day.

TABLE 2
Formulation, proximate composition of the feeds used for bullfrog tadpoles and froglets

Ingredients (%)	$T30_0$	$T30_{25}$	$T30_{50}$	$T30_{75}$	$T40_0$	$T40_{25}$	$T40_{50}$	$T40_{75}$
FM	40.0	30.0	20.0	10.0	60.0	45.0	30.0	15.0
HLM	0	10.0	20.0	30.0	0	15.0	30.0	45.0
SM	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wheat flour	16.0	16.0	16.0	16.0	10.0	10.0	10.0	10.0
Corn flour	16.0	16.0	16.0	16.0	10.0	10.0	10.0	10.0
Fish oil	6.5	6.5	6.5	6.5	2.5	2.5	2.5	2.5
Lecithin	6.5	6.5	6.5	6.5	2.5	2.5	2.5	2.5
Mineral premix[a]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[b]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Proteins	31.0	30.3	29.6	28.9	40.2	39.1	38.1	37.0
Lipids	16.2	16.2	16.1	16.0	11.7	11.6	11.5	11.4
Nitrogen free extract	29.4	29.8	30.1	30.5	23.8	24.3	24.8	25.5
Fiber	8.9	9.0	9.2	9.4	8.8	9.1	9.4	9.6
Ash	7.2	7.4	7.6	7.8	7.6	7.9	8.2	8.4
Moisture	7.3	7.3	7.4	7.5	7.9	8.0	8.1	8.2
Gross energy (kJ 100 g^{-1})	1956.4	1943.4	1930.9	1917.9	1886.1	1867.3	1848.0	1828.8
P:E	66.3	65.2	64.1	63.0	89.1	87.7	86.2	84.7

Fishmeal (FM), Housefly larvae meal (HLM) and soybean meal (SM)
[a] Vitamin premix: vitamin A(6.5 g), vitamin D3 (1 g), vitamin C (1 g), vitamin E (300 mg), vitamin K3 (12 mg), vitamin B1 (30 mg), vitamin B2 (24 mg), vitamin B6 (15 mg) vitamin B12 (40 mg), folic acid (10 mg), panthotenic acid (100 mg)
[b] Mineral premix: iron (150 mg), zinc (140 mg), manganese (75 mg), copper (25 mg), selenium (1 mg)

TABLE 3
Essential amino-acid calculation in experimental feeds, tadpole body and froglet legs

Calculated EAA	Froglet leg	Tadpole body	Treatments							
(g 100 g^{-1})			$T30_0$	$T30_{25}$	$T30_{50}$	$T30_{75}$	$T40_0$	$T40_{25}$	$T40_{50}$	$T40_{75}$
Arg	4.65	4.10	2.57	2.41	2.25	2.09	3.53	3.29	2.21	2.8
His	2.09	2.96	1.76	1.8	1.83	1.87	2.35	2.4	1.78	2.51
Ile	3.07	2.74	1.12	1.16	1.2	1.24	1.55	1.61	1.18	1.72
Leu	4.98	3.38	1.89	1.79	1.7	1.6	2.59	2.44	1.65	2.14
Lys	5.25	6.05	3.31	3.07	2.83	2.58	4.52	4.15	2.74	3.43
Met	1.85	3.14	3.3	3.03	2.75	2.48	4.69	4.28	2.73	3.47
Phe	2.70	3.96	1.12	1.08	1.03	0.99	1.6	1.53	1.02	1.39
Thr	3.39	3.64	1.71	1.66	1.6	1.55	2.36	2.28	1.57	2.12
Trp	0.72	0.81	0.48	0.53	0.59	0.64	0.62	0.7	0.57	0.87
Val	3.21	3.43	2.12	2.04	1.96	1.88	2.92	2.79	1.91	2.55

Amino acids in froglet legs, tadpoles and ingredients were analyzed by reverse phase high performance liquid chromatography (HPLC).
The amino acids in the diets were calculated from the inclusion percentage of each ingredient.

The water in each system was monitored in the variables of: dissolved oxygen, pH and temperature using the Hach® HQ40d equipment (these variables were measured every day of the experiment directly in fish tanks and ponds) and the nitrogen compounds were determined by the Hach® brand DR6000 spectrophotometer through the method 8039 for nitrates, 8507 for nitrites and 8038 for non-ionized ammonia (water samples were collected weekly). During experimental period the physicochemical characteristics of the water were within the values established for the bullfrog culture (Table 4), excluding the influence of water quality as a limiting factor in the growth of the individuals present for each treatment.

TABLE 4
Water characteristics physicochemical.
Values are presented as means ±SD of samples collected during the experimental period.

Variable	$T30_0$ - $T40_0$	$T30_{25}$ - $T40_{25}$	$T30_{50}$ - $T40_{50}$	$T30_{75}$ - $T40_{75}$
Temperature (°C)	25.6 ±2.6 [a]	25.2 ±2.2 [a]	26.1 ±2.3 [a]	26.7 ±1.9 [a]
Dissolved oxygen (mg L^{-1})	6.54 ±1.12 [a]	6.79 ±1.26 [a]	6.65 ±1.55 [a]	6.76 ±1.33 [a]
pH (range)	6.9 ±1.1 [a]	7.4 ±0.7 [a]	7.3 ±0.9 [a]	7.1 ±1.2 [a]
Nitrate (mg L^{-1})	2.69 ±0.33 [a]	2.85 ±0.38 [a]	3.05 ±0.42 [a]	2.78 ±0.36 [a]
Nitrite (mg L^{-1})	0.68 ±0.14 [a]	0.61 ±0.09 [a]	0.64 ±0.12 [a]	0.67 ±0.07 [a]
Non-ionized ammonia (mg L^{-1})	0.27 ±0.05 [a]	0.25 ±0.03 [a]	0.22 ±0.07 [a]	0.31 ±0.06 [a]

Values with the same superscripts do not present significant differences (P<0.05). Values for culture: temperature (22 – 32 °C), dissolved oxygen (4 – 10 mg L^{-1}), pH (6 – 8.5), nitrate (< 10 mg L^{-1}), nitrite (< 2 mg L^{-1}), nitrate (<10 mg L^{-1}) and non-ionized ammonia (<10 mg L^{-1})

2.4 Experimental design

The experiment was carried out during a 112-day period in the aquaculture unit at the Universidad Autónoma de Querétaro, Facultad de Ingenieria Campus Amazcala (100° 26' W, 20° 73' N, 1920 m.a.s.l.). Inside the aquaculture unit there was a photoperiod of 12 L: 12 D, ambient temperature of 18–32 °C and humidity of 70–80 %. The evaluation in both stages (tadpole and froglet) was carried out with a randomized block experimental design. Four treatments with three repetitions were used; where the initial experimental unit in the tadpole stage was 50 individuals per fishbowl. Tadpoles were feeding three times a day (08:00, 12:00, and 16:00 hours) at a rate of 2% of the biomass in each of the schedules (Méndez et al., 2010). While the froglets were feeding twice a day (8:00 and 18:00 hours) with 3 % of the biomass at each time (De Castro et al., 2014). In each of the stages, the amount of feed supplied was adjusted according to the biometries carried out weekly.

2.5 Productive performance

In order to analyze the efficiency of the HLM as a protein source, the following response variables were established: survival rate (SR), weight gain (WG), feed conversion rate (FCR), protein efficiency rate (PER), metamorphosis rate (MR), and finish of the metamorphosis phase in each treatment.

$$SR\ (\%) = \frac{\text{initial number of animals}}{\text{final number of animals}} \times 100 \quad (1)$$

$$WG\ (\%) = \frac{\text{final weight - initial weight}}{\text{initial weight}} \times 100 \quad (2)$$

$$FCR = \frac{\text{grams of feed consumed}}{\text{grams of increase in weight}} \quad (3)$$

$$PER = \frac{\text{grams of increase in weight}}{\text{grams of protein ingestion}} \quad (4)$$

$$MR\ (\%) = \frac{\text{number of animals metamorphosed}}{\text{total number of animals}} \times 100 \quad (5)$$

2.5 Statistical analysis

Data analysis was performed using minitab18® software. The data collected for each of the variables were subjected to one-way ANOVA, expressing the results as mean ±standard deviation (SD). Likewise, the Tukey's test was performed to determine the significant differences between the means of the treatments, using a significance level of P<0.05.

3 Results

The results of productive performance in the tadpole stage presented significant differences (*Table 5*), the values of the WG showed that $T30_{25}$ had the greatest increase (4476.22 ±50.05%), while $T30_{50}$ (4381.81 ±43.33%) and $T30_0$ (4401.39 ±36.66%) did not show differences (P<0.05). The survival showed significant differences among all diets, $T30_0$ being the best valued (86.01 ±1.1%). Regarding FCR and PER, $T30_{25}$ was located with the best results below or above the other treatments (1.57 ±0.04 and 2.11 ±0.02), respectively. Meanwhile, the treatment that received a higher inclusion level of HLM ($T30_{75}$) produced the worst values for the variables established.

In the froglet stage (*Table 5*), the WG and SR variables did not show differences between $T40_{25}$ (WG = 149.80 ±6.33 % and SR = 87.6 ±1.5 %) and $T40_0$ (WG = 154.13 ±5.91% and 88.3 ±1.2 %). The diet including 50 % of HLM ($T40_{50}$) reduced its contribution to growth in relation to the control treatment, disagreeing with what was observed in the tadpole stage. Regarding FCR and PER in the froglet stage, $T40_0$ was located with the outstanding values about the other treatments (1.61 ±0.04 and 1.55 ±0.02, respectively).

At the end of the experiment, the total WG of individuals fed with the treatments that included 25% of HLM ($T30_{25}$ - $T40_{25}$) did not show differences in comparison to those individuals fed with the control diets ($T30_0$ - $T40_0$). However, the total SR of the 25% HLM diets was significantly lower than those of the control diets. Regarding the treatments with 50 and 75% inclusion of HLM, the values WG and SR decreased as the replacement of FM by HLM increased.

The metamorphosis process showed differences between the treatments (Table 6). $T30_{25}$ was the treatment that had the first incidence of metamorphosis (day 35). 100% of the individuals under $T30_{25}$ and $T30_{50}$ completed the metamorphosis in less time compared to $T30_0$ (17, 18 and 23 days, respectively). The duration of the metamorphosis phase (Gosner stage 42–46) was favored by $T30_{25}$ (169 ±7 hours) followed by $T30_{50}$ (181 ±4 hours).

TABLE 5
Productive performance that presents the means ±SD of weight gain (WG), survival rate (SR), feed conversion rate (FCR) and protein efficiency rate (PER) for the tadpole stage, pre-fattening stage (froglet), and total values after 112 days of experimentation

	Treatments			
Tadpole stage	**$T30_0$**	**$T30_{25}$**	**$T30_{50}$**	**$T30_{75}$**
WG tadpole (%)	4401.39 ±36.66 ^{b}	4476.22 ±50.05 ^{a}	4381.81 ±43.33 ^{b}	4193.10 ±53.33 ^{c}
SR tadpole (%)	86.0 ±1.1 ^{a}	80.6 ±0.8 ^{b}	78.3 ±1.1 ^{c}	72.6 ±1.5 ^{d}
FCR tadpole	1.61 ±0.02 ^{b}	1.57 ±0.04 ^{b}	1.72 ±0.03 ^{a}	1.76 ±0.04 ^{a}
PER tadpole	2.03 ±0.03 ^{b}	2.11 ±0.02 ^{a}	1.76 ±0.04 ^{c}	1.73 ±0.03 ^{c}
Pre-fattening	**$T40_0$**	**$T40_{25}$**	**$T40_{50}$**	**$T40_{75}$**
WG froglet (%)	154.13 ±5.91 ^{a}	149.80 ±6.33 ^{a}	117.68 ±4.94 ^{b}	106.65 ±5.12 ^{c}
SR froglet (%)	88.3 ±1.2 ^{a}	87.6 ±1.5 ^{a}	73.1 ±0.8 ^{b}	70.6 ±1.2 ^{c}
FCR froglet	1.61 ±0.04 ^{d}	1.78 ±0.03 ^{c}	1.94 ±0.04 ^{b}	2.07 ±0.02 ^{a}
PER froglet	1.55 ±0.02 ^{a}	1.42 ±0.03 ^{b}	1.29 ±0.02 ^{c}	1.21 ±0.04 ^{d}
Tot^{al}	**$T30_0$ -$T40_0$**	**$T30_{25}$-$T40_{25}$**	**$T30_{50}$-$T40_{50}$**	**$T30_{75}$-$T40_{75}$**
WG total (%)	11185.31 ±41.65 ^{a}	11181.81 ±47.11 ^{a}	9538.46 ±52.66 ^{b}	9042.65 ±37.33 ^{c}
SR total (%)	76.0 ±2.1 ^{a}	70.6 ±1.6 ^{b}	58.0 ±1.2 ^{c}	51.3 ±1.5 ^{d}
FCR total	1.97 ±0.04 ^{b}	2.00 ±0.05 ^{b}	2.13 ±0.03 ^{a}	2.18 ±0.04 ^{a}
PER total	1.95 ±0.03 ^{a}	2.01 ±0.04 ^{a}	1.19 ±0.04 ^{b}	1.21 ±0.05 ^{b}

Values with the same superscripts do not present significant differences (P<0.05).

TABLE 6
Values means ±SD of initiation, duration and progress of metamorphosis (50 and 100%) of the frog tapdoles in each treatment

	Treatments			
Tadpole stage	**$T30_0$**	**$T30_{25}$**	**$T30_{50}$**	**$T30_{75}$**
Start of metamorphosis (days)	36 ±0.5 ^{a}	35 ±0.5 ^{a}	36 ±1.0 ^{a}	39 ±1.5 ^{b}
50% metamorphosis (days)	45 ±1.0 ^{b}	41 ±1.0 ^{a}	42 ±1.5 ^{a}	50 ±1.5 ^{c}
100% metamorphosis (days)	59 ±1.0 ^{b}	52 ±1.5 ^{a}	54 ±1.5 ^{a}	76 ±2.0 ^{c}
Duration of the metamorphosis phase (hours)	206 ±8 ^{c}	169 ±7 ^{a}	181 ±4 ^{b}	210 ±1 ^{c}

Values with the same superscripts do not present significant differences (P<0.05).

4 Discussion

The results suggest that bullfrog tadpoles and froglets can be fed with feed containing housefly larvae meal instead of fishmeal; however, productive performance is affected as the percentage of substitution increases. The treatments were formulated to contain equal amounts of macronutrients at both protein levels (30% for tadpoles and 40% for froglets); however, the analysis proximate composition showed slight differences, mainly in the content of protein and gross energy, which could have contributed to the productive performance.

Tadpoles fed with the $T30_{50}$ diet culminated with a growth rate similar to the control diet, both treatments were located below the $T30_{25}$ diet which had the highest growth rate. The biomass generated in the tadpole stage has been related to the duration and energy expenditure in metamorphosis. Larger organisms have been reported to metamorphose in less time than those of smaller size (Downie et al., 2004; Scott et al., 2007). It has also been mentioned that a larger size favors the percentage reduction of the energy expenditure destined for metamorphosis, which allows a better distribution of metabolic energy between that required for development and maintenance (Orlofske and Hopkins, 2009). The above is consistent with the results of this work, where the organisms that finished the tadpole stage with the greatest increase in biomass ($T30_{25}$) completed their metamorphosis in less time in relation to the other treatments. This suggests that the $T30_{25}$ diet, in addition to promoting growth, allows the adequate accumulation of energy required for the metamorphosis process.

On the other hand, the survival for the HLM diets showed a decrease as the inclusion percentage increased. The possible reason could be related to the content of chitin; chitin is a polysaccharide that is part of the exoskeleton of insects (Sanchez-Muros et al., 2014) and its presence is reported in a range of 6.5 to 9.1 % in housefly larvae (Zhang et al., 2011; Kim et al., 2016). Tadpoles and frogs have the ability to degrade

chitin, this by having chitinolytic bacteria in their intestinal tract (Warne et al., 2017; Zhang et al., 2020). It has been reported that chitin may act as a prebiotic, contributing to the digestion of nutrients (Chen et al., 2014; Najafabad et al., 2016). However, the chitin content in the 75% HLM treatment could have been higher than that degradable by these organisms; being able to affect the adsorption of some nutrients (Olsen et al., 2006).

In the froglet feeding, $T40_{25}$ showed no differences in relation to the control treatment in WG and SR, this could be firstly due to the increase in biomass obtained by $T30_{25}$ in the tadpole stage, being that, the weight at the beginning of pre-fattening (froglet) has been linked to the viability of growth and survival (Orlofske and Hopkins, 2009). In addition, the amino acid composition of feeds $T40_0$ and $T40_{25}$ is close to the contents reported with higher digestibility in bullfrog (arginine 5.2%; histidine 1.7%; leucine 4%; Lysine 4.1%; phenylalanine 2%; threonine 2.8% ; valine 3.3%) (Mansano et al., 2017). During the two stages evaluated (tadpole and froglet) the feeds with 25% inclusion of HLM were constant in their contribution to growth, without showing differences to the control diets at the end of the 16 weeks of experimentation. The results obtained in the tadpoles fed with $T30_{25}$ and $T30_{50}$ resemble the HLM inclusion values reported as appropriate in the tadpole stage (Li et al., 2019). This suggests that depending on the stage of the production cycle in which the bullfrog organisms are found (tadpole, pre-fattening, fattening, etc.) the inclusion percentage of HLM can be adjusted to a greater or lesser amount. The above is consistent with studies that report the variation in the capacity for digestion and assimilation of proteins throughout the growth of the bullfrog (Mansano et al., 2017). Although the results suggest that the substitution of FM by HLM could vary in the range of 25 to 50%; feed must be evaluated at all stages of the production cycle to confirm this postulate

5 Conclusions

In this research, replacing parts of fishmeal (FM) by housefly larvae meal (HLM) as a source of protein in the feeding of tadpoles and froglet of bullfrog was proposed. The results showed the feasibility of replacing 25% FM by HLM without affecting the variables of productive relevance. However, the percentage of FM replacement by HLM could be adjusted in a range of 25 to 50% according to each stage of the frog culture. The use of HLM and other insect meals requires further research on their production cycle, particularly in the composition of growth substrates, with the aim of enhancing the nutritional characteristics of insect meals, increasing its interest as a source of protein in animal feed.

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