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RESEARCH PAPER

Plant Oil Impact on Growth and Health in Juvenile Bullfrog

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Abstract

This study evaluated the effects of dietary plant oils on liver health, biochemical parameters, and growth performance in juvenile American bullfrogs (*Aquarana catesbeiana*). Ninety-six frogs were housed in 12 boxes and fed diets containing sunflower, olive, corn, or soybean oil for 61 days. A commercial feed (40% protein) was supplemented with 2% of each oil source. At the end of the experiment, animals were analyzed for biometric data, blood and liver biochemistry, meat composition, and fatty acid profiles. The hepatosomatic index (HSI) and visceral fat index (VFI) were similar across treatments, though frogs fed soybean oil showed a higher intestinal quotient (IQ) than those fed olive oil ($P<0.05$). Liver histology was unaffected by diet, but significant differences were observed in moisture, protein, and lipid levels ($P<0.05$), while mineral content remained constant. Frogs fed olive oil exhibited higher concentrations of total saturated, monounsaturated, and polyunsaturated fatty acids. This study recommends sunflower and olive oil as beneficial dietary inclusions to optimize growth and fatty acid profiles in juvenile bullfrogs, contributing to sustainable aquaculture practices.

Introduction

Brazil is one of the main aquaculture producers in the world (FAO, 2021), with fish production as the primary focus, reaching almost 887 thousand tons in 2023 (PeixeBR, 2024). Another segment, frog farming, is undergoing rapid development (FAO, 2021). Originally from North America, the American bullfrog (*Aquarana catesbeiana*) entered the Brazilian frog farming industry in 1935 and has gained popularity since then (Cribb et al., 2013). The bullfrog is the most widely produced species in the world (FAO, 2021), with Brazil ranking as the fourth largest producer (Ribeiro & Toledo, 2022). *A. catesbeiana* is ideal for production due to its adaptability to climate, rapid growth, high fecundity, and ease of handling (Lima & Agostinho, 1992; Cribb, 2009). For the industry to enhance its production chain, several obstacles need to be overcome, with nutrition being a key factor that must be prioritized.

Currently, there is no specific commercial feed for frogs in the Brazilian market, as the dietary requirements of bullfrogs are still not fully understood. According to previous studies, bullfrogs require diets rich in proteins (28-40%) (Seixas Filho et al., 2013), metabolizable energy (2600 Kcal kg^{-1}) (Castro et al., 2008), essential amino acids such as lysine and

methionine, as well as vitamins A, D, E, and minerals, including calcium and phosphorus. It is known, however, that nutritional imbalances can lead to various issues, including increased excretion, higher visceral fat, reduced protein deposition, and macroscopic and histological changes in the liver and intestine (Queiroz et al., 2021). For efficient and healthy growth, it is essential to provide a diet that meets the animals' nutritional needs, including both macro and micronutrients, appropriate to their developmental stages (Navarro et al., 2007).

Lipids are a major nutrient group, providing metabolic energy with a high gross energy value (9.5 kcal g^{-1}) (Tacon, 1987; Sorgeloos & Léger, 1992), as well as essential fatty acids and phospholipids (Sargent et al., 2002). Fatty acids are crucial for growth, reproduction, and improving feed conversion ratios in many species (Brett & Müller-Navarra, 1997). Different oil sources used as an energy source in feed have varied fatty acid profiles, which can influence the proximate composition and fatty acid profile of the organisms consuming the diet. Consequently, the oil source can modulate the fatty acid profile of the meat, contributing to the concentration of n-3 fatty acids in the final product (de Souza et al., 2014). Sunflower oil, olive oil, corn oil, and soybean oil differ significantly in their fatty acid compositions, with olive oil being rich in monounsaturated fats, sunflower oil containing high linoleic acid, and soybean oil providing a balance of linolenic and linoleic acids (Bobo et al., 2021; Rabail et al., 2021; Suárez et al., 2021). As poikilothermic animals, frogs have limited ability to metabolize energy from carbohydrates but are highly efficient at metabolizing fats (Tacon, 1987), thus allowing dietary proteins to be utilized for body synthesis.

Given the diverse oil profiles, this study aims to determine how different plant oil sources affect growth, health, and the biochemical composition of bullfrogs, providing practical recommendations for the aquaculture industry. The objective of this experiment was to evaluate the growth performance, biochemical parameters, proximate composition, and fatty acid content of thigh muscles, as well as to assess the liver histology of bullfrogs (*A. catesbeiana*) fed diets containing different plant lipid sources.

Materials and Methods

Experimental Design and Diets

This trial was conducted at the Laboratório de Produção Aquícola da Universidade Federal da Grande Dourados (UFGD), State of Mato Grosso do Sul, Brazil, and was approved by the Animal Ethics Committee of the same institution under protocol No. 18/2020. The experiment lasted 61 days, involving 96 bullfrogs (*Aquarana catesbeiana*) with an initial average weight of 50.00±1.32 g and 9.86±0.26 cm in length. The frogs were randomly distributed into 12 plastic boxes, each containing eight animals. The boxes measured 86.0×36.0×35.0 cm with a capacity of 108 L (Figure 1). A completely randomized design was used with four treatments and three replications. The treatments consisted of four commercial diets with 40% crude protein (Table 1), enriched with 2% sunflower, corn, olive, or soybean oils. The animals were fed at a rate of 3.5% of their live weight (de Castro et al., 2012) four times daily (08:00 AM, 11:00 AM, 2:00 PM, and 5:00 PM), with feed rate calculations updated every 20 days.

Figure 1. Photograph of the experimental environment.

Growth Performance Indices

Production data were collected for all animals used in the experiment, including:

Final Average Weight (FW) (g);

Final Average Length (FL) (cm);

Weight Gain (WG)=(final body weight−initial body weight);

Survival Rate (SU)=(number of animals at trial end/number at trial beginning)×100;

Feed Conversion (FC)=(diet consumed/weight gain);

Hepatosomatic Index (HI)=((liver weight, g/final body weight, g)×100);

Visceral Fat Index (VF) = ((visceral fat weight, g/final body weight, g) × 100);

Intestinal Quotient (IQ) = (gut length/final animal length);

Specific Growth Rate (SGR) = (((ln(final weight) − ln(initial weight))/experiment days) × 100);

Protein Efficiency Ratio (PER) = (weight gain/protein intake) × 100;

Feed Efficiency Ratio (FER) = ((final biomass − initial biomass)/feed consumed) × 100.

Biochemical Parameters of Blood, Liver, and Intestine

Three animals were randomly selected from each experimental unit (nine per treatment) for biochemical blood parameter analysis, with 3.0 mL of blood drawn per animal. Plasma glucose, triglycerides, total cholesterol, and creatinine were analyzed using enzymatic-colorimetric methods with specific kits (Gold Análises Diagnóstica, Belo Horizonte, Minas Gerais, Brazil).

Table 1. Proximate composition of diets enriched with soybean oil (control), sunflower oil, olive oil, and corn oil

Variables	Analyzed values				p-value
	Feeds enriched with oil				
	Soybean oil (control)	Sunflower oil	Olive oil	Corn oil	
	Bromatological values (mg kg^{-1})				
Moisture	80.63 ±1.08	80.12 ±1.12	82.03 ±1.54	81.23 ±1.66	0.76
Crude protein	400.99 ±6.41	400.23 ±5.36	398.57 ±6.38	401.11 ±6.54	0.64
Crude fat	118.61 ±1.33	120.28 ±1.26	119.68 ±1.99	119.26 ±1.28	0.79
Ash	69.33 ±0.71	68.32 ±0.66	67.78 ±0.57	66.98 ±0.74	0.33
	Fatty acid values (mg g^{-1})				
14:0	14.63 ±0.52^{a}	12.97 ±0.19^{b}	12.13 ±0.66^{b}	12.75 ±0.50^{b}	0.04
16:0	176.34 ±1.99^{a}	176.00 ±0.67^{a}	174.36 ±0.36^{b}	167.50 ±0.90^{c}	0.03
18:0	88.61 ±0.57^{c}	110.45 ±0.38^{a}	100.53 ±0.63^{b}	88.12 ±0.42^{c}	<0.01
20:0	6.79 ±0.31^{a}	1.56 ±0.01^{d}	4.37 ±0.07^{b}	2.46 ±0.03^{c}	<0.01
21:0	2.78 ±0.04^{c}	2.88 ±0.04^{b}	3.10 ±0.15^{a}	2.67 ±0.04^{d}	<0.01
22:0	1.38 ±0.10^{b}	2.36 ±0.06^{a}	1.37 ±0.09^{b}	0.63 ±0.01^{d}	<0.01
SFA	287.75 ±3.49^{c}	306.22 ±1.35^{a}	295.86 ±1.96^{b}	274.13 ±1.88^{d}	<0.01
16:1	6.02 ±0.20^{a}	4.35 ±0.09^{b}	2.92 ±0.30^{c}	6.50 ±0.30^{a}	<0.01
18:1n-9	227.75 ±0.74^{b}	246.94 ±0.90^{a}	228.50 ±1.65^{b}	218.90 ±0.53^{d}	<0.01
18:1n-7	15.36 ±0.11^{d}	19.61 ±0.12^{b}	21.09 ±1.07^{a}	16.57 ±0.37^{c}	<0.01
20:1n-9	-	-	4.40 ±0.24	-	-
MUFA	249.13 ±1.05^{c}	270.9 ±1.11^{a}	256.91 ±3.33^{b}	241.97 ±1.29^{d}	<0.01
18:2n-6	85.24 ±0.20^{b}	63.48 ±0.26^{d}	98.97 ±0.39^{a}	78.41 ±0.23^{c}	<0.01
18:3n-6	6.80 ±0.06^{b}	6.09 ±0.07^{c}	11.22 ±0.58^{a}	4.14 ±0.18^{d}	<0.01
18:3n-3	4.80 ±0.09^{b}	5.80 ±0.12^{a}	5.58 ±0.33^{a}	2.69 ±0.06^{c}	<0.01
22:6n-3	3.28 ±0.37^{a}	2.43 ±0.47^{b}	2.78 ±0.42^{b}	2.75 ±0.42^{b}	<0.01
PUFA	96.84 ±3.92^{b}	75.37 ±0.43^{d}	118.45 ±1.62^{a}	85.24 ±0.47^{c}	<0.01

The basic formulation for fish feed with 400 g kg^{-1} crude protein was provided by the manufacturer. The formulation considers ingredients with high protein content, balanced energy, and essential nutrients: fish meal (300-400 g kg^{-1}), soybean meal (250-300 g kg^{-1}), corn gluten meal (100-150 g kg^{-1}), and wheat bran (100-150 g kg^{-1}).

Supplementation levels per kg of feed: Vit. A 12,000 IU; Vit. D3 3,000 IU; Vit. K3 15 mg kg^{-1}; Vit. B1 20 mg kg^{-1}; Vit. B2 20 mg kg^{-1}; Vit. B6 18 mg kg^{-1}; Vit. B12 0.04 mg kg^{-1}; Vit. C 300 mg kg^{-1}; Niacin 100 mg kg^{-1}; Calcium pantothenate 50 mg kg^{-1}; Biotin 1 mg kg^{-1}; Folic acid 6 mg kg^{-1}; Inositol 150 mg kg^{-1}; Choline 500 mg kg^{-1}; Sulfate copper 18 mg kg^{-1}; Sulfate iron 80 mg kg^{-1}; Sulfate manganese 50 mg kg^{-1}; Sulfate zinc 120 mg kg^{-1}; Calcium iodate 0.8 mg kg^{-1}; Sulfate cobalt 0.6 mg kg^{-1}; Selenium 4 mg kg^{-1}; Vit. E 200 mg kg^{-1}.

Results expressed as mean ± standard deviation of three replicates. ;Values in the same column followed by a different letter differ statistically ($p < 0.05$) by the Tukey test. SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids

For liver triglyceride analysis, 100 mg of liver tissue was homogenized with a Potter–Elvehjem homogenizer, diluted in distilled water (1:10), and centrifuged at 13,400×g for three minutes. The supernatant was used for triglyceride quantification using a BioPlus S-200 spectrophotometer.

For digestive enzyme activity, 100 mg of tissue from the anterior intestine was homogenized in 20 mM sodium phosphate buffer and 10 mM Tris (with glycerol v/v, pH 7.0) using a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 600×g (4°C, 3 min), and the supernatant was centrifuged again at 6,000×g (4°C, 8 min). This final supernatant was used as the crude enzyme extract.

Lipase activity was determined following Albro et al. (1985). The reaction mixture, containing an aliquot of the crude enzyme extract and 0.4 mM p-nitrophenyl myristate in 24 mM ammonium bicarbonate (pH 7.8) with 0.5% Triton X-100, was incubated for 30 min at 25°C. The reaction was stopped by adding 25 mM NaOH and placing it in an ice bath for 15 min. Optical density was recorded at 405 nm.

Histological Studies

The livers were dissected into approximately 1.0 cm and immediately fixed in Bouin solution overnight. The fixed samples were sliced into appropriate sizes and dehydrated in an increasing graded ethanol series (70% to 100%). Tissues were then passed through xylene before being embedded in molten paraffin wax. Each tissue sample was sectioned (6.0 µm) using a microtome, mounted on slides and afterwards stained with haematoxylin and eosin (H&E rapid stain). Three slides or sections per replicate were prepared for the histological analyses of the liver samples. The slides were examined using a microscope at 40x magnification (model Precision P207). Pictures were taken with a digital camera (BEL model Eurekam 1.3). For image analysis, the Image J software was used, where morphometric analyzes of nuclei and hepatocytes were performed, verifying the following parameters: hepatocyte diameter, hepatocyte area, hepatocyte perimeter, hepatocyte nucleus diameter, hepatocyte nucleus area, hepatocyte nucleus volume, hepatocyte nucleus perimeter , hepatocyte nuclear circularity , and nucleus/cytoplasm perimeter ratio. Measurement of the perimeter ratio of nucleus and cytoplasm (Rpcn), ratio of nucleus and cytoplasm area (Racn), nuclear volume and circularity of nucleus (Rn), according to the following equations displayed at Table 2.

Chemical Composition Frog Legs Meat

For the chemical composition analysis of diets and tissues, the methodology proposed by the (AOAC, 2016) was used in which the moisture content was determined bydrying the sample in an air circulation oven at 105ºC until a constant weight was reached (Method number 950.46). The ashes were determined by calcination of samples in a muffle furnace (Model 2000B, Belo Horizonte - Minas Gerais, Brazil) at 600ºC, using method number 920.153, and protein by the Kjeldahl method (Model MA-036, Piracicaba - São Paulo, Brazil) using method number 981.10. The total lipids were determined following the Bligh & Dyer (1959) methodology. All analyses were performed in triplicate. The values are displayed in the Table 1.

Fatty Acids Analysis

The fatty acid composition of the feeds were determined following the extraction method by (Bligh & Dyer, 1959) and, derivatization process as described in (ISO, 1978). The results are presented in Table 1. For the analysis of fatty acids in the tissues, we followed the extraction and derivatization process described by Figueiredo et al. (2016). After, the fatty acid methyl esters (FAME) were separated by gas chromatography on a TRACETM Ultra chromatograph (Thermo ScientificTM, USA) equipped with a fused silica capillary column (100 mx0.25 mm di, 25 µm cyanopropyl, CP-7420 select FAME) and equipped with flame ionization detector (FID). The injector and detector temper- atures were 230 and 250°C, respectively. The column temperature was maintained at 165◦C for 18 min, programmed at 4◦C min−1 at 235◦C and held at that temperature for 20 minutes. H2 was used as the carrier gas with a flow of 1.2 mL min−1, N2 was used for the auxiliary gas with a flow of 30 m min−1, FID flame was

Table 2. Equations used for calculation of histological studies

Variable	Equation	Unit
Hepatocyte area (H_a)	$H_a=\pi r_{cell}^2$	µm²
Hepatocyte perimeter (H_p)	$H_p=2\pi r_{cell}$	µm
Hepatocyte nucleus perimeter (H_{np})	$H_{np}=2\pi r_{nucleus}$	µm
Hepatocyte nucleus area (H_{na})	$H_{na}=\pi r_{nucleus}^2$	µm²
Hepatocyte nucleus volume (H_{nv})	$H_{nv}=4/3\pi r_{nucleus}^3$	µm³
Nucleus/cytoplasm area ratio (R_{acn})	$\%R_{acn}=H_a/H_{na}\times100$	%
Nucleus/cytoplasm perimeter ratio (R_{pcn})	$\%R_{pcn}=H_{np}/H_p\times100$	%
Circularity of hepatocyte (R_n)	$R_n=H_a/H^{p2}=4\pi$	none

r_{cell} = average cell radius; $r_{nucleus}$ = average nucleus radius

produced with H2 (30 mL min−1) and synthetic air (300 mL min−1). The injected volume was at a ratio of 1:40.31. Retention times and peak areas were determined using Chrom-QuestTM software (Thermo ScientificTM, USA). For the identification of fatty acids, the retention times obtained were compared to the methyl esters standards (Sigma, USA) and quantification was performed using tricosanoic acid methyl ester (Sigma, USA) as internal standard (IS) according to the Visentainer & Franco (2012).

Statistical Analysis

The data were grouped by the treatments and submitted to a normality of Bartlett test (Bartlett, 1937) and a homogeneity of Shapiro-Wilk test (Shapiro & Wilk, 1965). Normal results were submitted to an analysis of variance (ANOVA). Significant differences ($P<0.05$) were subjected to Tukey's mean comparison test (Tukey, 1949) and statistical analyses were carried out using package ExpDes.pt (Ferreira et al., 2021) on the R software (R Core Team, 2022).

Principal Components Analysis

The most applied methodology in agricultural and aquaculture studies is the principal component analysis (PCA), which performs an exploration of the correlation structure between the constituent variables of the data base (Olsen et al., 2012). PCA decomposes the original data performing a linear transformation, and this process produces a smaller number of more important variables that reflect the original set, providing a database that is useful for assessing the set data (Wise et al., 1999; Olsen et al., 2012). For performing the PCA analysis the data set was normalized following the Equation 1:

$$Xi = \frac{x_i - \bar{x}}{SD}$$

Where Xi is a normalized value, x_i individual value, $\bar{x}$ and, SD are the mean and, standard deviation of the variable, respectively.

Several variables were used to evaluate these diets, and a multi-factorial analysis method (PCA) was used in order to evaluate the parameters determined from the different diets with the intention of evaluating the diets more comprehensively. PCA analyzes were performed using the R software (R Core Team, 2022) with multcomp packages (Hothorn et al., 2008; Lê et al., 2008). The original parameters and the PCs are correlated by the loadings factor, explaining the weights of the PCs in the original parameters (Tabachnick & Fidell, 2013). For this PCA analysis, only PCs with eigenvalues greater than 1 (Kaiser, 1960) were taken into account. For statistical analysis, the statistical program R was used (R Core Team, 2022).

Results

Growth Performance Indices

The animals accepted the diets without signs of rejection, such as spitting out the pellets. The highest final weight (FW) (Figure 2A) was observed in bullfrogs fed diets enriched with sunflower oil (SFO - 138.33±1.22 g), olive oil (OO - 136.10±1.20 g), and soybean oil (SBO - 135.69±1.19 g). The lowest FW was found in the corn oil (CO) diet (132.29±1.16 g) ($P<0.05$). For weight gain (WG) (Figure 2A), the highest values were in diets with SFO (87.07±2.09 g), OO (84.50±2.03 g), and SBO (82.61±1.98 g), while the lowest was CO (80.94±1.94 g) ($P<0.05$). Evaluating the intestinal quotient (IQ) variable (Figure 2C), the highest value was observed for SBO (5.33±0.42) and the lowest for OO (4.28±0.33), with statistical differences noted. SFO and CO presented similar results compared to SBO and OO. No significant differences ($P>0.05$) were found in protein efficiency, feed efficiency rate, survival (Figure 2B), feed conversion (Figure 2C), visceral fat, hepatosomatic index, conversion efficiency rate (Figure 2D), or the final length of animals fed the plant oil diets (Figure 2E).

Biochemical Parameters of Blood, Liver, and Intestine

No significant differences were found in lipase activity ($P>0.05$) in the digestive index (Table 3). A similar situation was observed for blood metabolites, such as glucose, triglycerides, and cholesterol. However, creatinine showed a significant difference ($P<0.05$) between treatments, with the highest value observed in the SO treatment. Liver triglycerides also differed significantly, with the highest level in the CO-enriched diet.

Histology Studies

Histological analysis showed no significant differences ($P>0.05$) in the liver parameters analyzed for diets containing the tested plant oils (Table 4), including hepatocyte diameter (µm), hepatocyte area (µm²), perimeter of hepatocyte (µm), hepatocyte nucleus diameter (µm), hepatocyte nucleus area (µm²), hepatocyte nucleus volume (µm³), hepatocyte nucleus perimeter (µm), nuclear circularity (µm³), and core/cytoplasm perimeter ratio. Liver tissues did not exhibit fat deposits, as shown in Figure 3.

Chemical Composition of Frog Leg Meat

Diets with different oil inclusions significantly affected moisture, crude fat, and crude protein contents ($P<0.05$), but not ash content ($P>0.05$) (Figure 4). Moisture was highest in OO (777.32 g kg^{-1}) and lowest in CO (766.66 g kg^{-1}), while SFO and SBO had values similar to OO and CO. Crude fat ranged from 33.26 to 37.09 g kg^{-1} ($P<0.05$), with the highest value in animals fed OO.

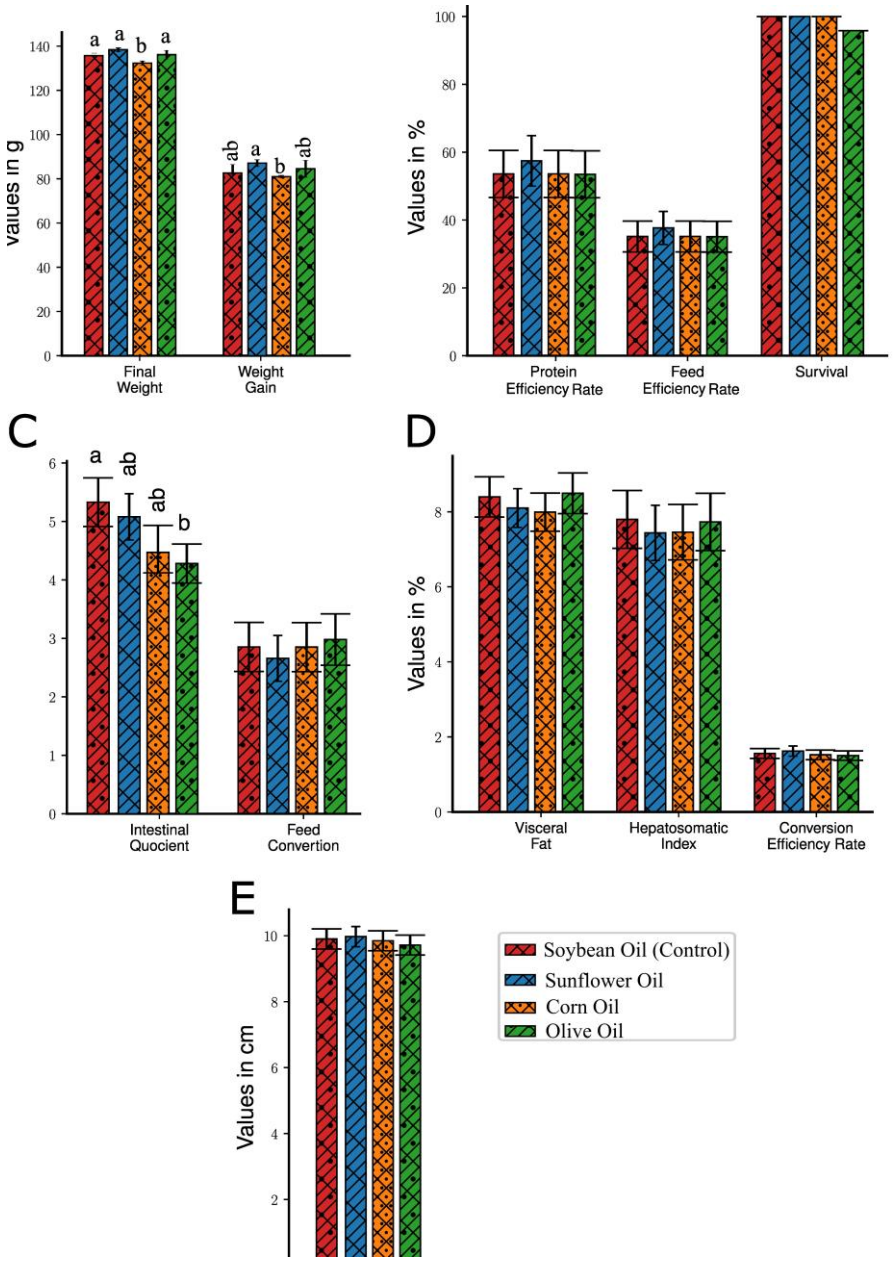


Figure 2. Growth performance of Aquarana catesbeiana with different treatments: Sunflower Oil, Corn Oil, Olive Oil and Soybean Oil. Different lowercase letters indicate a difference between the treatments according to Tukey's test (P<0.05).

Table 3. Liver index, digestive index, and blood metabolites of bullfrogs fed with different dietary sources of plant oils

Variables	Oil source				p-value
	Soybean (control)	Sunflower	Olive	Corn	
Liver index					
Triglycerides (mg dL^{-1})	1.17 ±0.49^{ab}	1.04 ±0.43^{b}	1.26 ±0.17^{ab}	1.68 ±0.45^{a}	< 0.01
Digestive index					
Lipase (UI mg^{-1})	126.38 ±3.70	130.33 ±7.97	124.83 ±4.51	126.08 ±5.936	0.561
Blood metabolites (mg dL^{-1})					
Glucose	65.64 ±19.70	55.66 ±9.23	71.14 ±15.35	56.53 ±9.21	0.341
Triglycerides	163.05 ±10.89	158.93 ±42.48	161.46 ±4.76	159.58 ±2.55	0.125
Cholesterol	214.13 ±4.14	213.15 ±3.52	215.05 ±4.146	215.1 ±5.38	0.221
Creatinine	0.239 ±0.184^{a}	0.113 ±0.019^{b}	0.1298 ±0.032^{ab}	0.111 ±0.040^{b}	< 0.01

Values in the same column followed by a different letter differ statistically (P<0.05) by the Tukey *test.*

Table 4. Morphometric analysis of hepatocytes and hepatocyte nuclei of bullfrogs fed different dietary lipids

Variables	Type of oil in the diets				p-value
	Soybean (Control)	Sunflower	Olive	Corn	
H_a	10519.60 ±1075.80	9709.10 ±1510.20	10228.40 ±1921.80	10306.20 ±949.60	0.91
H_p	394.86 ±17.09	386.37 ±30.80	389.79 ±37.84	391.64 ±21.65	0.98
Hepatocyte diameter	125.70 ±5.46	123.00 ±9.83	124.11 ±12.03	124.69 ±6.93	0.98
H_na	523.92 ±59.92	514.32 ±41.53	499.74 ±54.70	485.57 ±42.81	0.80
H_np	84.60 ±4.60	83.63 ±2.96	82.67 ±4.12	81.29 ±3.78	0.76
Hepatocyte nucleus diameter	26.93 ±1.47	26.62 ±0.95	26.32 ±1.31	25.88 ±1.21	0.76
$R_a$cn	5.48 ±0.35	5.96 ±0.46	5.54 ±0.62	5.17 ±0.36	0.29
$R_p$cn	21.92 ±0.93	22.29 ±1.14	21.85 ±1.34	21.24 ±0.76	0.68
H_nv	10654.10 ±1757.50	10296.39 ±1236.90	9332.60 ±1530.00	9392.60 ±1342.20	0.76
R_n	0.90 ±0.01	0.91 ±0.01	0.90 ±0.01	0.91 ±0.01	0.87

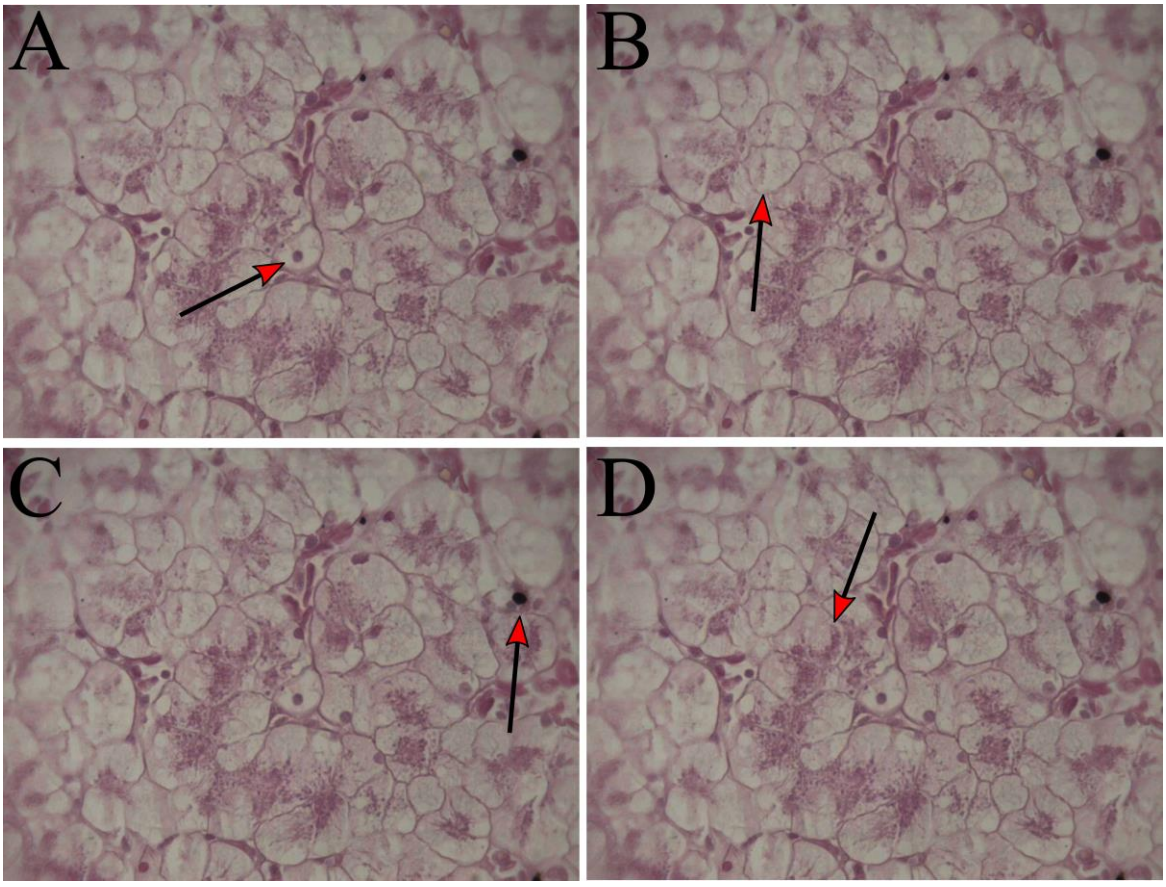


Figure 3. Histological examination images in livers of grouper fed diets containing plant oil source (A Sunflower; B Corn oil; C Olive oil; D Soybean oil)in the diets.

The highest crude protein content was found in diets with SFO, SBO, and CO (181.34 - 186.33 g kg^{-1}), and the lowest in the OO diet.

Fatty Acids Analysis

The fatty acid (FA) profile in bullfrog leg muscles was influenced by plant oil-enriched diets, as shown in Table 5. The OO diet had the highest values for several saturated fatty acids (SFA) such as C12:0, C16:0, and C18:0 ($P<0.05$). The highest levels of monounsaturated fatty acids (MUFA) (C18:1n−7, C18:1n−9) were also found in animals fed OO. There was no significant difference ($P>0.05$) for n−3 polyunsaturated fatty acids (PUFA) between treatments. For n−6 PUFA, only C18:2n−6 showed differences ($P<0.05$), with the highest value in the OO diet. Animals fed OO-enriched diets had the best ΣSFA, ΣMUFA, ΣPUFA, and ΣFA values (Figure 5).

Principal Components Analysis (PCA)

PCA was conducted to determine the origin of variables for animals fed with plant oil-enriched diets. PCA scores are shown in Figure 6A, with ellipses separating groups. Figure 6B shows the eigenvalues and loadings for each PCA component. The first two components (PC1 and PC2) accounted for 60.95% of the total data variance (Figure 6B).

Only principal components with eigenvalues ≥1 were considered. PC1 had an eigenvalue of 11.69, explaining 34.89% of data variation, while PC2 had an eigenvalue of 8.74, representing 26.08%. PC1 loadings ≥±0.90 were: FA-12:00 (0.95); FA-21:00 (0.92); FA-16:1n−7 (0.91). In PC2, loadings ≥±0.90 were FA-20:00 (0.94) and FA-17:1n−7 (0.92). Diets with OO and SBO were closely related, indicating similar growth performance, chemical composition of frog leg meat, liver histology, and biochemical evaluations in blood, intestine, and liver.

Discussions

Growth Performance Indices

Plant oils have been recognized as an essential lipid source for aquatic animals (Wang et al., 2005). However, there is limited information on the impact of sunflower oil, olive oil, corn oil, and soybean oil on the growth and health of bullfrogs (*A. catesbeiana*). The increased final weight and weight gain performance in diets enriched with sunflower oil, olive oil, and soybean oil indicates nutritional enhancements, as well as a protein-sparing effect. This suggests that dietary proteins are used for muscle development, while lipids support metabolic functions, similar to findings in other aquatic organisms, such as *Centropomus undecimalis* juveniles (Arenas et al., 2021), the hybrid fish *tambatinga* juveniles (Welengane et al., 2019), and *Megalobrama amblycephala* juveniles (Li et al., 2012). Notably, the reference diet was a high-quality commercial diet for fish, and this study showed that growth performance of *A. catesbeiana* fed the enriched diet did not significantly differ across all growth performance parameters, indicating that all diets had sufficient nutrients for frog development.

Biochemical Parameters of Blood, Liver, and Intestine

Oils in animal feed serve multiple roles, such as stimulating digestive enzyme secretion, enhancing gastric and intestinal motility, endocrine stimulation, antibacterial and antiviral activity, immune response stimulation, anti-inflammatory effects, antioxidant activity, and pigmenting action (Basmacioglu Malayoğlu et al., 2010; Brenes & Roura, 2010; Zhang et al., 2016). Levy et al. (2004) suggested that intrahepatic triglyceride concentration depends on triglyceride uptake, secretion, synthesis, and degradation balance. In this study, hepatic triglycerides were higher in frogs fed corn oil compared to those fed soybean oil. This may be attributed to corn oil's lower unsaturated fatty acid content (Table 1), leading to increased hepatic triglycerides. This effect was also observed in rats (Levy et al., 2004; Bargut et al., 2014). Though the triglyceride levels were low, the higher hepatic triglycerides in frogs fed with corn oil might reflect polyunsaturated fatty acids of the n−3 series, which are linked to reduced hepatic triglyceride synthesis via inhibition of SREPB-1c and increased lipid beta-oxidation in the liver through PPAR-α activation (Levy et al., 2004; Popescu et al., 2013), modulating lipid metabolism (de Almeida, 2014). The elevated creatinine level may indicate a possible toxicity due to soybean oil in the diet (Ohtake et al.,

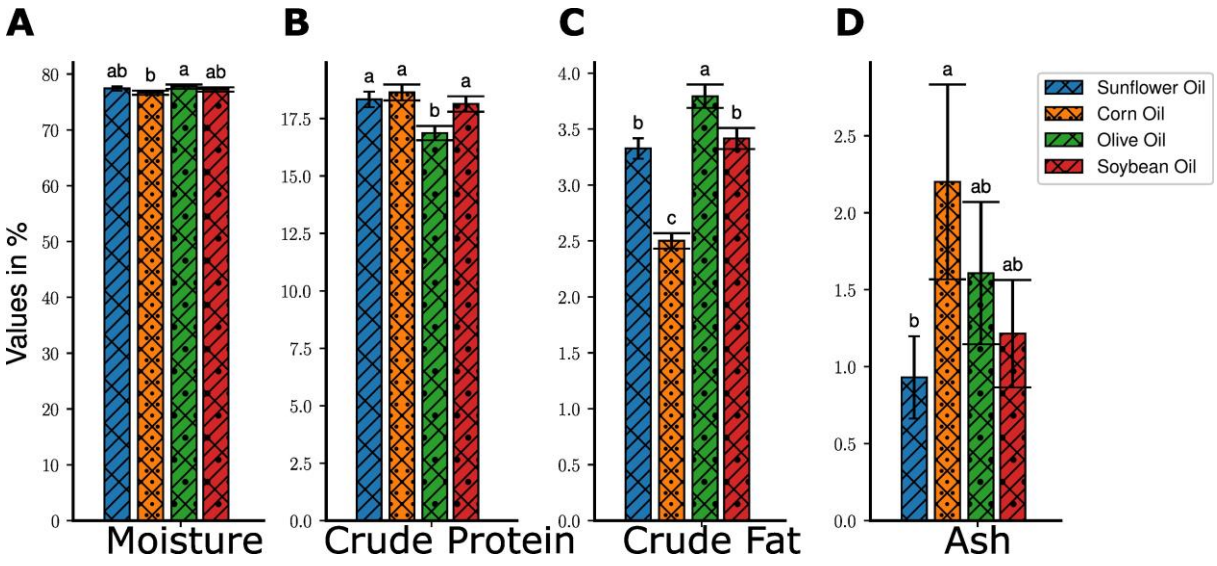


Figure 4. The chemical composition in Aquarana catesbeiana tissue at different treatments: and Soybean Oil (Control), Sunflower Oil, Corn Oil and, Olive Oil. Different lowercase letters indicate a difference between the treatments according to Tukey's test (P<0.05).

Table 5. The composition of fatty acids (mg g^{-1} of tissue) of *Aquarana catesbeiana* fed with feed enriched with sunflower oil, olive oil, corn oil, and soybean oil

Variables	Type of oil in the diets				p-value
	Soybean Oil (Control)	Sunflower Oil	Corn Oil	Olive Oil	
C12:0	0.27 ±0.05^{b}	0.47 ±0.08^{ab}	0.59 ±0.10^{a}	0.65 ±0.11^{a}	3.07E^{-3}
C14:0	0.68 ±0.15	0.56 ±0.13	0.73 ±0.16	0.63 ±0.14	0.56
C16:0	6.18 ±0.53^{b}	6.34 ±0.59^{b}	7.26 ±0.62^{b}	8.49 ±0.73^{a}	4.13E^{-6}
C18:0	1.93 ±0.21^{b}	1.89 ±0.21^{b}	1.84 ±0.20^{b}	2.61 ±0.29^{a}	9.48E^{-3}
C20:0	0.55 ±0.16	0.65 ±0.16	0.75 ±0.21	0.70 ±0.20	0.65
C21:0	2.84 ±0.50^{a}	1.99 ±0.35^{ab}	1.72 ±0.30^{b}	1.95 ±0.34^{ab}	0.031
C22:0	1.18 ±0.13^{b}	1.78 ±0.20^{a}	1.52 ±0.17^{ab}	1.19 ±0.13^{a}	4.22E^{-2}
∑ SFA	13.64 ±0.93^{b}	13.69 ±0.93^{ab}	14.41 ±0.98^{ab}	16.23 ±1.10^{a}	3.87E^{-2}
C16:1n−7	1.99 ±0.13^{a}	0.96 ±0.06^{b}	1.10 ±0.07^{b}	0.94 ±0.06^{b}	7.11E^{-2}
C16:1n−9	0.16 ±0.09^{b}	0.83 ±0.15^{ab}	3.76 ±0.32^{a}	2.90 ±0.35^{a}	5.26E^{-6}
C17:1n−7	1.47 ±0.29^{a}	0.83 ±0.16^{b}	1.46 ±0.29^{b}	1.26 ±0.48^{ab}	0.039
C18:1n−7	0.78 ±0.09^{c}	1.07 ±0.12^{bc}	1.27 ±0.15^{b}	1.71 ±0.20^{a}	2.59E^{-4}
C18:1n−9	10.32 ±1.25^{ab}	7.77 ±0.94^{b}	8.75±1.06^{ab}	10.85 ±1.31^{a}	0.036
∑ MUFA	15.72 ±1.37^{ab}	13.69 ±1.19^{b}	16.33 ±1.42^{b}	17.67 ±1.54^{a}	0.043
C18:3n−3	0.24	nd	0.36	0.19	-
C18:3n−6	nd	nd	nd	nd	-
C18:2n−6	2.45 ±0.25^{b}	2.10 ±0.22^{b}	3.94 ±0.41^{a}	3.75 ±0.39^{bc}	2.00E^{-4}
∑ PUFA	3.17 ±0.33^{b}	2.10 ±0.22^{c}	4.30 ±0.44^{a}	3.93 ±0.41^{a}	5.21E^{-5}
∑ Fatty acids	32.34 ±1.73^{bc}	29.49 ±1.57^{c}	35.04 ±1.87^{ab}	37.83 ±2.02^{a}	2.54E^{-3}

CXY:n-Z; *XY* = Number of Carbon and n-Z = the double bond position in the fatty acid; nd = Not detected; Values in the same column followed by the different letter differ statistically (p <0.05) by the Tukey test.

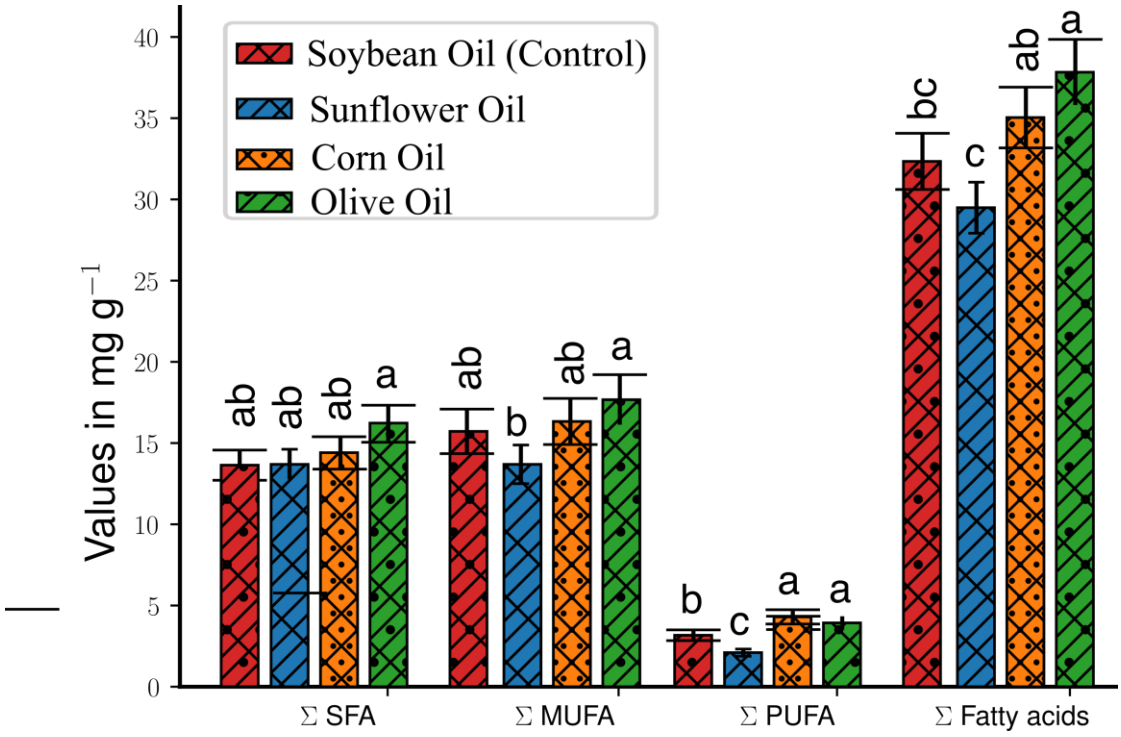
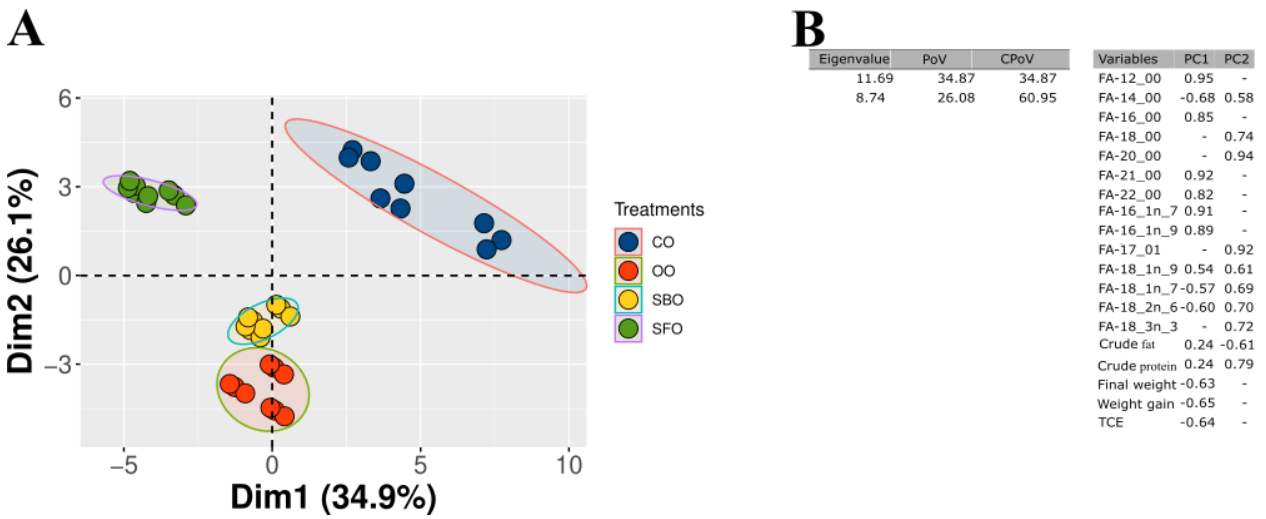


Figure.5. Fatty acid composition in Aquarana catesbeiana tissue at different treatments, with the inclusion of: Sunflower Oil, Corn Oil, Olive Oil and Soybean Oil. Different letters indicate a difference between treatments according to Tukey's average test (P<0.05). Σ SFA: Sum of saturated fatty acids; Σ MUFA: Sum of mono-unsaturated fatty acids; Σ PUFA: Sum of poly-unsaturated fatty acids; Σ Fatty acids: Sum of all fatty acids.



Eigenvalue	PoV	CPoV
11.69	34.87	34.87
8.74	26.08	60.95

Variables	PC1	PC2
FA-12_00	0.95	-
FA-14_00	-0.68	0.58
FA-16_00	0.85	-
FA-18_00	-	0.74
FA-20_00	-	0.94
FA-21_00	0.92	-
FA-22_00	0.82	-
FA-16_1n_7	0.91	-
FA-16_1n_9	0.89	-
FA-17_01	-	0.92
FA-18_1n_9	0.54	0.61
FA-18_1n_7	-0.57	0.69
FA-18_2n_6	-0.60	0.70
FA-18_3n_3	-	0.72
Crude fat	0.24	-0.61
Crude protein	0.24	0.79
Final weight	-0.63	-
Weight gain	-0.65	-
TCE	-0.64	-

Figure 6. A) Biplots of the 1st and 2nd PCs, where: Blue dots represent Corn Oil; Red dots represent Olive Oil; Yellow and dots represent Soybean Oil; Green dots represent Sunflower Oil; rings correspond to the 95% confidence ellipses estimated using the mean of the treatments. The variance explained for each PC is shown between parentheses. B) Loadings of the assessed treatments variables in the first two principal components, only ±≥0.50 loading are shown. PoV=Percentage of Variance; CPoV=Cumulative Percentage of Variance).

2002), likely due to antinutritional factors like saponins in soybeans (Thakur et al., 2019). Soybean oil is also known to elevate the hepatosomatic index, potentially leading to liver cell damage and reduced disease resistance in fish (Godoy et al., 2019; Ng et al., 2013; Li et al., 2016).

Histology Studies

The liver, which plays a critical role in detoxifying substances in aquatic organisms, is responsible for several vital functions. Liver tissue changes, such as cellular morphology shifts, necrosis, and vacuolization, vary with exposure time to causative agents (Bombonato et al., 2007). Liver histology has increasingly served as a biomarker for assessing aquatic nutrition knowledge (Rodrigues et al., 2017). This insight is used to tailor diet quality, quantity, and breeding practices to the developmental stage of aquatic animals (Gisbert et al., 2008, 2014). Nutritional imbalances from unbalanced diets may lead to lipid accumulation in hepatocyte cytoplasm, potentially causing fat degeneration and steatosis (Li et al., 2014; Zhang et al., 2014). However, this study found no signs of hepatic steatosis (Figure 2), which is important since steatosis can impair lipid metabolism and antioxidant and immune defense mechanisms (Ye et al., 2019). Although liver morphometry showed no significant differences, this result should be considered alongside biochemical parameters (see section 4.2). Triglyceride levels in hepatic tissue (evident in increased vacuolization of hepatocytes in Figure 2) and elevated creatinine suggest possible liver degeneration in some animals.

Chemical Composition of Frog Leg Meat

Feed quality, including protein and lipid levels, influences frog meat composition. Using oils as a lipid source can reduce feed costs (Vargas et al., 2007) while supplying essential fatty acids for proper animal development (Losekann et al., 2008). This study demonstrates that dietary fatty acids affect lipid metabolism, with the liver as the central lipid metabolism site (Zhang et al., 2016). The observed fat percentage (3.80%) was higher than reported by Martins et al. (2016) and Fragoso et al. (2013), who documented 0.79% and 0.34%, respectively, in the thigh, and Noll & Lindau (1987), who found 0.30% in the carcass. This discrepancy likely reflects the oils incorporated into the commercial feeds.

Fatty Acids Analysis

Major fatty acids in bullfrog muscle from various diets included palmitic (16:0), oleic (18:1n−9), and linoleic acids (18:2n−6). Bullfrogs rely on diet for polyunsaturated fatty acids (PUFAs), as they lack significant PUFA synthesis capacity. Frogs fed olive oil had higher saturated, monounsaturated, and polyunsaturated fatty acids in leg tissue, mirroring the tested oil compositions and animal retention capacity. The presence of 18:2n−6 is particularly important since it is essential and not synthesized metabolically. Corn oil diet frogs showed 4.30 mg/g (0.43%) of PUFA, compared to olive oil diet frogs with 3.93 mg/g (0.39%). FA levels in frog meat are closely linked to oil type and its composition, similar to studies on other species (Wang et al., 2005; Losekann et al., 2008; Chatzifotis et al., 2010). Olive oil, high in MUFA, led to increased MUFA in muscle, while corn and soybean oils, with similar PUFA amounts, had different tissue deposition rates. Navarro et al. (2012) reported that PUFAs in carcasses might be affected by vitamin E's antioxidant activity in diets. Although growth is unaffected, plant oils in frog diets influence tissue composition and fatty acid metabolism (Losekann et al., 2008).

The essential fatty acid requirements for bullfrogs remain unknown. Frogs on olive and corn oil diets, which are rich in oleic and linolenic acids, accumulated monounsaturated and polyunsaturated fatty acids in meat. However, frogs fed soybean oil did not show similar meat FA accumulation, despite the diet's high essential fatty acid content. This suggests that FA in the diet may alter lipid metabolism, with the liver playing a central role (Zhang et al., 2016).

Principal Components Analysis–PCA

Due to the varied behavior of multiple variables, PCA provided a clearer understanding of the dietary differentiation. The entire dataset was visualized using a biplot (combined scores and loadings for two components) in Figure 5. Essential FA intake is important for human health, helping prevent cardiovascular disease (Enser et al., 1998; Onk et al., 2019). This study enriches *A. catesbeiana* research by shedding light on plant oil diet impacts. Further research on frog nutrition, particularly post-metamorphosis, is recommended, with different fatty acid sources and levels. Experiments with restricted n−3 and/or n−6 can help clarify *A. catesbeiana* requirements. Protein-energy balance should also be evaluated to optimize growth without excessive fat deposition.

Conclusion

Plant oil-enriched diets effectively support bullfrog (*A. catesbeiana*) growth, body composition, and fatty acid profiles without harming liver health. Olive and sunflower oils offer the most benefits, and we recommend a 2% inclusion level to optimize growth and meat quality. Future studies should assess long-term health effects in amphibian farming.

Ethical Statement

The experiment was conducted under Animal Ethics Committee of Universidade Federal da Grande Dourados under protocol No. 18/2020.

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Author Contribution

Antonio Cesar Godoy: Conceptualization, Writing - review and editing; Shelby Maura Walker: Writing and English review; Patricia Daniele da Silva: Writing -review and editing; Rômulo Batista Rodrigues: Writing - review and editing. Daniel F. Rodrigues de Oliveira: Writing - review; Gustavo Teixeira da Silva: Writing – review; Layara Santos Morais: Writing - review and editing; Paulo Vitor Liuti Da Silva: Writing – review; Claucia Aparecida Honorato: Funding Acquisition, Project Administration; Dacley Hertes Neu: Funding Acquisition, Project Administration, Resources, Writing -review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

Albro, P. W., Hall, R. D., Corbett, J. T., & Schroeder, J. (1985). Activation of nonspecific lipase (EC 3.1.1.-) by bile salts. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 835, 477–490. https://doi.org/10.1016/0005-2760(85)90117-1

de Almeida, B. B. (2014). Efeitos metabólicos da combinação de triglicerídeos de cadeia média e óleo de peixe na esteatose hepática e estresse oxidativo induzidos pela dieta hiperlipídica termolizada em ratos (Doctoral dissertation). Universidade de São Paulo. https://doi.org/10.11606/T.17.2014.tde-20122014-103613

AOAC. (2016). Official Methods of Analysis of the Association of Official Analytical Chemists (20th ed.). John Wiley & Sons, Inc.

Arenas, M., Álvarez-González, C. A., Barreto, A., Sánchez-Zamora, A., Suárez-Bautista, J., Cuzon, G., & Gaxiola, G. (2021). Physiological and metabolic protein-sparing effects of dietary lipids on common snook Centropomus undecimalis (Bloch, 1792) juveniles. Aquaculture Nutrition, 27, 1089–1102. https://doi.org/10.1111/anu.13250

Bargut, T. C. L., Frantz, E. D., Mandarim-de Lacerda, C. A., & Aguila, M. B. (2014). Effects of a diet rich in n-3 polyunsaturated fatty acids on hepatic lipogenesis and beta-oxidation in mice. Lipids, 49, 431–444.

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences, 160, 268–282.

Basmacioglu Malayoğlu, H., Baysal, Ş., Misirlioğlu, Z., Polat, M., Yilmaz, H., & Turan, N. (2010). Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism, and immune response of broilers fed on wheat–soybean meal diets. British Poultry Science, 51, 67–80.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911–917.

Bobo, G., Nicolau-Lapeña, I., & Aguiló-Aguayo, I. (2021). Conventional oils and oilseeds: composition and nutritional importance. *Oil and oilseed processing: opportunities and challenges*, 23-40.

Bombonato, M. T. S., Rochel, S. S., Vicentini, C. A., & Vicentini, I. B. F. (2007). Estudo morfológico do tecido hepático de Leporinus macrocephalus. Acta Scientiarum. Biological Sciences, 29, 81–85.

Brenes, A., & Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. Animal Feed Science and Technology, 158, 1–14.

Brett, M., & Müller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology, 38, 483–499.

CASTRO, J. C., BARBOZA, W. A., SILVA, K. K. P., & PIRES, S. C. (2008). Níveis de energia metabolizável para rações de rã touro. *Boletim do Instituto de Pesca*, *34*(4), 519-525.

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., & Mylonas, C. C. (2010). Effect of dietary lipid levels on growth, feed utilization, body composition, and serum metabolites of meagre (Argyrosomus regius) juveniles. Aquaculture, 307, 65–70.

Cribb, A. (2009). O consumo de carne de rã: Caracterização, tendências e perspectivas. Embrapa Agroindústria de Alimentos.

Cribb, A. Y., Afonso, A. M., & Mostério, C. M. F. (2013). Manual técnico de ranicultura. Embrapa. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/160735/1/Manual-Ranicultura-Versao-Final-com-capa.pdf

de Castro, C. S., Agostinho, C. A., Argentim, D., de Alexandre, J. S., de Oliveira, L. C., de Sousa, R. M. R., & de Magalhães Padilha, P. (2012). Feed digestibility and productive performance of bullfrogs fed in high and low frequency. Aquaculture, 326-329, 123–128. https://doi.org/10.1016/j.aquaculture.2011.11.010

Enser, M., Hallett, K., Hewett, B., Fursey, G., Wood, J., & Harrington, G. (1998). Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Science, 49, 329–341.

FAO. (2021). Fisheries and Aquaculture Information and Statistics Branch. Technical Report. http://www.fao.org-/figis/servlet/SQServlet?file=/usr/local/tomcat/8.5.16/figis/webapps/figis/%0Atemp/hqp_4838169164528210977.xml&outtype=html

Ferreira, E. B., Cavalcanti, P. P., & Nogueira, D. A. (2021). ExpDes.pt: Pacote Experimental Designs (Portugues). https://CRAN.R-project.org/package=ExpDes.pt

Figueiredo, I. L., Claus, T., Júnior, O. O. S., Almeida, V. C., Magon, T., & Visentainer, J. V. (2016). Fast derivatization of fatty acids in different meat samples for gas chromatography analysis. Journal of Chromatography A, 1456, 235–241. https://doi.org/10.1016/j.chroma.2016.06.012

Fragoso, S., Ferreira, V., Araújo, I., Oliveira, E., Silva, F., & Moura, O. (2013). Características físicas e químicas de diferentes cortes da carne liofilizada de rã-touro (Lithobates catesbeianus) pigmentada e albina. Higiene Alimentar, 27, 951–955.

Gisbert, E., Moreira, C., Castro-Ruiz, D., Öztürk, S., Fernández, C., Gilles, S., Núñez, J., Duponchelle, F., Tello, S., & Renno, J.-F. (2014). Histological development of the digestive system of the Amazonian pimelodid catfish Pseudoplatystoma punctifer. Animal, 8, 1765–1776.

Gisbert, E., Ortiz-Delgado, J. B., & Sarasquete, C. (2008). Nutritional cellular biomarkers in early life stages of fish. Histology and Histopathology.

Godoy, A. C., Santos, O. O., Oxford, J. H., de Amorim Melo, I. W., Rodrigues, R. B., Neu, D., Nunes, R. V., & Boscolo, W. R. (2019). Soybean oil for Nile tilapia (Oreochromis niloticus) in finishing diets: Economic, zootechnical, and nutritional meat improvements. Aquaculture, 512, 734324.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. Biometrical Journal, 50, 346–363. https://doi.org/10.1002/bimj.200810425

ISO. (1978). Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids (ISO 5509). International Organization for Standardization.

Kaiser, H. F. (1960). The application of electronic computers to factor analysis. Educational and Psychological Measurement, 20, 141–151. https://doi.org/10.1177/001316446002000116

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: A package for multivariate analysis. Journal of Statistical Software, 25, 1–18. https://doi.org/10.18637/jss.v025.i01

Levy, J. R., Clore, J. N., & Stevens, W. (2004). Dietary n-3 polyunsaturated fatty acids decrease hepatic triglycerides in Fischer 344 rats. Hepatology, 39, 608–616. https://doi.org/10.1002/hep.20093

Li, J.-y., Zhang, D.-d., Xu, W.-n., Jiang, G.-z., Zhang, C.-n., Li, X.-f., & Liu, W.-b. (2014). Effects of dietary choline supplementation on growth performance and hepatic lipid transport in blunt snout bream (Megalobrama amblycephala) fed high-fat diets. Aquaculture, 434, 340–347.

Li, X., Jiang, Y., Liu, W., & Ge, X. (2012). Protein-sparing effect of dietary lipid in practical diets for blunt snout bream (Megalobrama amblycephala) fingerlings: Effects on digestive and metabolic responses. Fish Physiology and Biochemistry, 38, 529–541. https://doi.org/10.1007/s10695-011-9533-9

Li, Y., Liang, X., Zhang, Y., & Gao, J. (2016). Effects of different dietary soybean oil levels on growth, lipid deposition, tissues fatty acid composition, and hepatic lipid metabolism related gene expressions in blunt snout bream (Megalobrama amblycephala) juvenile. Aquaculture, 451, 16–23.

Lima, S., & Agostinho, C. (1992). A tecnologia de criação de rãs. Universidade Federal de Viçosa, Imprensa Universitária.

Losekann, M. E., Raduž Neto, J., Emanuelli, T., Pedron, F. D. A., Lazzari, R., Bergamin, G. T., Corrêia, V., & Simões, R. S. (2008). Alimentação do jundiá com dietas contendo óleos de arroz, canola ou soja. Ciência Rural, 38, 225–230.

Martins, P. P., Costa, F. A. A., & Andrade, P. L. (2016). Caracterização da carne de rã touro, composição centesimal e cor. In XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos. Gramado - RS.

Navarro, R. D., Lanna, E. A. T., Donzele, J. L., da Matta, S. L. P., & de Souza, M. A. (2007). Níveis de energia digestível da dieta sobre o desempenho de piauçu (Leporinus macrocephalus) em fase pós-larval. Acta Scientiarum. Animal Sciences, 29, 109–114.

Navarro, R. D., Navarro, F. K. S. P., Ribeiro Filho, O. P., Ferreira, W. M., Pereira, M. M., & Seixas Filho, J. T. (2012). Quality of polyunsaturated fatty acids in Nile tilapias (Oreochromis niloticus) fed with vitamin E supplementation. Food Chemistry, 134, 215–218.

Ng, W.-K., Chong, C.-Y., Wang, Y., & Romano, N. (2013). Effects of dietary fish and vegetable oils on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia and efficacy of using fish oil

finishing diets. Aquaculture, 372, 97–110.

Noll, I. B., & Lindau, C. F. (1987). Aspectos da composição em nutrientes da carne de rã touro-gigante (Rana catesbiana). Caderno de Farmácia, 3, 29–36.

Ohtake, T., Kimura, M., Takemura, H., & Hishida, A. (2002). Effects of dietary lipids on daunomycin-induced nephropathy in mice: Comparison between cod liver oil and soybean oil. Lipids, 37, 359–366.

Olsen, R. L., Chappell, R. W., & Loftis, J. C. (2012). Water quality sample collection, data treatment and results presentation for principal components analysis – literature review and Illinois River watershed case study. Water Research, 46, 3110–3122. https://doi.org/10.1016/j.watres.2012.03.028

Onk, K., Yalcintan, H., Sari, M., Isik, S. A., Yakan, A., & Ekiz, B. (2019). Effects of genotype and sex on technological properties and fatty acid composition of duck meat. Poultry Science, 98, 491–499.

PeixeBR. (2024). Anuário PEIXE BR da Piscicultura 2024. Technical Report. https://www.peixebr.com.br/anuario-2024/

Popescu, L. A., Virgolici, B., Lixandru, D., Miricescu, D., Condruţ, E., Timnea, O., Ranetti, A., Militaru, M., Mohora, M., & Zagrean, L. (2013). Effect of diet and omega-3 fatty acids in NAFLD. Romanian Journal of Morphology and Embryology, 54, 785–790.

Queiroz, J. D., Alves, J., Losekann, M., Frasca-Scorvo, C., Scorvo Filho, J., Ferri, G., & Ishikawa, M. (2021). Manejo alimentar e da qualidade da água na produção de tilápia-do-nilo (Oreochromis niloticus). São Paulo: Embrapa Meio Ambiente.

Rabail, R., Shabbir, M. A., Sahar, A., Miecznikowski, A., Kieliszek, M., & Aadil, R. M. (2021). An intricate review on nutritional and analytical profiling of coconut, flaxseed, olive, and sunflower oil blends. *Molecules*, *26*(23), 7187.

R Core Team. (2022). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Ribeiro, L. P., & Toledo, L. F. (2022). An overview of the Brazilian frog farming. Aquaculture, 548, 737623.

Rodrigues, R. A., Saturnino, K. C., & Fernandes, C. E. (2017). Liver histology and histomorphometry in hybrid sorubim (Pseudoplatystoma reticulatum × Pseudoplatystoma corruscans) reared on intensive fish farming. Aquaculture Research, 48, 5083–5093. https://doi.org/10.1111/are.13325.

Sargent, J., Tocher, D., & Bell, J. (2002). The lipids. In J. Halver, & R. Hardy (Eds.), Fish Nutrition (pp. 181–257). New York, NY: Academic Press. (3rd ed.).

Seixas Filho, J. T. D., Navarro, R. D., Garcia, S. L. R., & Santos, A. C. D. S. (2013). Regime alimentar de girinos de rã touro. *Ciência Animal Brasileira*, *14*, 17-22.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). Biometrika, 52, 591. https://doi.org/10.2307/2333709.

Sorgeloos, P., & Léger, P. (1992). Improved larviculture outputs of marine fish, shrimp and prawn. Journal of the World Aquaculture Society, 23, 251–264.

de Souza, S. M. G., Anido, R. J. V., & Tognan, F. C. (2014). Ácidos graxos ômega-3 e ômega-6 na nutrição de peixes – fontes e relações. Revista de Ciências Agroveterinárias, 6, 63–71.

Suárez, M., Gual-Grau, A., Ávila-Román, J., Torres-Fuentes, C., Mulero, M., Aragonès, G., ... & Muguerza, B. (2021). Oils and oilseeds in the nutraceutical and functional food industries. *Oil and Oilseed Processing: Opportunities and Challenges*, 219-243.

Tabachnick, B., & Fidell, L. (2013). Using Multivariate Statistics. Always Learning. Pearson Education.

Tacon, A. G. J. (1987). The nutrition and feeding of farmed fish and shrimp: A training manual: The essential nutrients. Brasília: FAO.

Thakur, A., Sharma, V., & Thakur, A. (2019). An overview of anti-nutritional factors in food. Int. J. Chem. Stud, 7, 2472–2479.

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. Biometrics, 99–114.

Vargas, R. J., de Souza, S. M. G., Tognon, F. C., Gomes, M. E. C., & de Mello Kessler, A. (2007). Desempenho de alevinos de tilápias do Nilo (Oreochromis niloticus), alimentados com dietas contendo diferentes fontes de lipídios. Revista Brasileira de Agrociencia, 13, 377–381.

Visentainer, J. V., & Franco, M. R. (2012). Ácidos graxos em óleos e gorduras: Identificação e quantificação. (2nd ed.). São Paulo: Varela.

Wang, J.-T., Liu, Y.-J., Tian, L.-X., Mai, K.-S., Du, Z.-Y., Wang, Y., & Yang, H.-J. (2005). Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (Rachycentron canadum). Aquaculture, 249, 439–447.

Welengane, E., Sado, R. Y., & Bicudo, A. J. D. A. (2019). Protein-sparing effect by dietary lipid increase in juveniles of the hybrid fish tambatinga (♀ Colossoma macropomum × ♂ Piaractus brachypomus). Aquaculture Nutrition, 25, 1272–1280. https://doi.org/10.1111/anu.12941.

Wise, B. M., Gallagher, N. B., Butler, S. W., White Jr, D. D., & Barna, G. G. (1999). A comparison of principal component analysis, multiway principal component analysis, trilinear decomposition and parallel factor analysis for fault detection in a semiconductor etch process. Journal of Chemometrics, 13, 379–396. https://doi.org/10.1002/(SICI)1099-128X(199905/08)13:3/4<379:AID-CEM556>3.0.CO;2-N.

Ye, H., Zhou, Y., Su, N., Wang, A., Tan, X., Sun, Z., Zou, C., Liu, Q., & Ye, C. (2019). Effects of replacing fish meal with rendered animal protein blend on growth performance, hepatic steatosis and immune status in hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂). Aquaculture, 511, 734203.

Zhang, C.-x., Huang, K.-k., Le Lu, K., Wang, L., Song, K., Zhang, L., & Li, P. (2016). Effects of different lipid sources on growth performance, body composition and lipid metabolism of bullfrog (Lithobates catesbeiana). Aquaculture, 457, 104–108.

Zhang, D., Lu, K., Dong, Z., Jiang, G., Xu, W., & Liu, W. (2014). The effect of exposure to a high-fat diet on microRNA expression in the liver of blunt snout bream (Megalobrama amblycephala). PLoS One, 9, e96132.