Turkish Journal of Zoology

Volume 46 | Number 3 Article 3

1-1-2022

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EMRAH ŞİMŞEK

AHMET ALKAYA

HÜLYA ŞEREFLİŞAN

AYŞE ÖZYILMAZ

TÜBİTAK

**Turkish Journal of Zoology**

**http://journals.tubitak.gov.tr/zoology/**

**Research Article**

Turk J Zool
(2022) 46: 261-269

doi:10.55730/1300-0179.3054

# Comparisons of biochemical compositions in marsh frog (*Pelophylax ridibundus*) (Anura; Ranidae) grown in different conditions; wild, semicultured and cultured ones

**Emrah ŞİMŞEK[1,*], Ahmet ALKAYA[2], Hülya ŞEREFLİŞAN[2], Ayşe ÖZYILMAZ[1]**

[1]Department of Marine Technologies, Faculty of Marine Sciences and Technology, İskenderun Technical University, Hatay, Turkey
[2]Department of Aquaculture, Faculty of Marine Sciences and Technology, İskenderun Technical University, Hatay, Turkey

**Received:** 05.01.2022 • **Accepted/Published Online:** 07.04.2022 • **Final Version:** 09.05.2022

**Abstract:** Frogs can be added to a list of different raw materials which have sustainable economic value to their producers. Therefore, the biochemical attributes of the frogs require further scientific scrutiny. The major objective of the current study is to investigate some biochemical compositions of edible portions of *Pelophylax ridibundus* (Pallas, 1771) grown in different conditions. The wild frogs, semicultured frogs, and cultured frogs were investigated regarding female and male specimens, leading to six groups that were determined to investigate the biochemical features of frogs. The results show that they (all frogs in all six groups) have low lipid contents ($p < 0.05$). The protein contents of the frogs varied in the range of 10.54%–16.10%. The lowest ash contents were found in semicultured male and female frogs. The average moisture contents were roughly 80% for all specimens. The major fatty acids were C16:0, C18:0, C18:1n9, C18:2n6, and C20:4n6 for wild, semicultured, and cultured frogs, respectively, for both sexes, showing their respective amounts varied among groups. All in all, the results show that there exist some differences in the nutritional content of frogs grown in different conditions in terms of the analyzed parameters.

**Key words**: Frog, *Pelophylax ridibundus*, carbohydrate, lipid, fatty acid

## 1. Introduction

Anurans are the amphibians which are called tailless amphibians or simply frogs and toads. The edible parts of the frogs are mainly their legs, which are described as resembling chicken taste but a little harder in muscle tissue. Meat is one of the most important food items for people and should be consumed by various sources due to its nutrients and vitamins contents. Because people are used to consuming red meat which costs a lot, and red meat may be associated with health problems when consumed more than enough, current research now focuses on alternative food resources for consumption. Frogs have the potential to be cheaper and safer alternative food resources (Stuart et al., 2004). Because meats contain proteins, fat, vitamins, and minerals in their structure, they are able to provide the required nutritional value to their consumers.

The wild frogs that belong to the Ranidae family generally prey on motile creatures (Itamies and Koskela, 1970; Houston, 1973; Blackith and Speight, 1974; Hodar et al., 1990; Hirai and Matsui, 1999). In addition, it has been previously reported that because water frogs generally feed on invertebrates and carnivores, they can choose their own food according to their body size and can feed on fish, reptiles, and mammals of their own size[1] (Başoğlu et al., 1994; Duellman and Trueb, 1994; Browne, 2009). Frogs, which are generally dependent on water, are widely found in many regions of all countries around the world (Tok et al., 2011; Dönmez and Şişman 2021; Zhelev et al., 2022).

Some of the frog species are considered to be of good nutritional value e.g., *Pelophylax ridibundus* (Pallas, 1771), *Rana esculenta* (Linnaeus, 1758), *Rana ridibunda* (Pallas, 1771) (Çaklı et al., 2009; Çağıltay et al., 2014; Alkaya et al., 2018; Büyükdeveci et al., 2019). The consumable anuran meats are getting known as a new food product in many countries. Frog meat not only has a significant amount of protein and minerals but is also low in fat content. It has been previously reported that frog legs are preferred food in large quantities by many European countries (Özoğul et al., 2008; Mohneke et al., 2010; Onadeko et al., 2011; Şereflişan and Alkaya, 2016). According to published reports, European countries generally meet the demand for frog meat from supply sources available in such

[1] Browne RK (2009). Amphibian diet and nutrition. AArk Science and Research [online]. Website http://portal.isis.org/partners/AARK/ResearchGuide/Amphibian%20husbandry/Amphibian%20diet%20%and%20nutrition.pdf [accessed 05 January 2022]

* Correspondence: emrah.simsek@iste.edu.tr

countries as Turkey, Bangladesh, Albania, China, Malaysia, and Indonesia (Negroni, 1997; Neveu, 2004; Neveu, 2009; Altherr et al., 2011). For example, France imports about 4000 tons of frozen frog legs and 800 tons of live frogs annually (Çağıltay et al., 2014). Turkey exports on average 642 tons of edible frogs.[2] Commercial-scale frog farming has a great potential as a food source, and, therefore, the number of farms has been increasing over the years. Cultured frog even in an area limited to little water yields a higher market value than cultured freshwater fish that requires a lot of water and investment (Somsueb and Boonyaratpalin, 2001).

Worldwide, more than 50 species of frogs are known to be harvested from nature for human consumption (Neveu, 2004). Larger and bigger leg weighting frog species such as American bullfrog (*Rana catesbeiana* Shaw, 1862), Indian bullfrog (*Rana tigrina* Daudin, 1802), and green frog (*R. esculenta*) are mostly consumed in Central and South America and Asian countries. The cultivation of these species is completely based on wild and culture (Alvarez and Real, 2006). Çağıltay et al. (2014) reported that frog meat has been an attractive food option in some high-end restaurants in European countries.

The wild and cultured frogs are imported from Turkey and other countries such as Indonesia, China, Thailand, India, and Vietnam (Sardava and Srikar, 1982; Fugler, 1985; Martin, 2000). The demand for frogs has been increasing due to the decrease in the number of frogs in the world, and, as a result, there has been a tendency towards frog culture in the world (Chardonnet et al., 2002; Miles et al., 2003; Tokur et al., 2008). Therefore, according to the Food and Agriculture Organization of the United Nations, the annual aquaculture production of frogs has increased in the world.[3]

A few studies have shown that frog meat had good nutritional composition e.g., lipids (Özoğul et al., 2008; Çağıltay et al., 2014; Büyükdeveci et al., 2019). The lipids contain fatty acids omega 3 and 6 which have an important role in human health (Davis and Kris-Etherton, 2003). The frogs have been investigated by limited studies, therefore there is little information on the biochemical compositions of edible frog e.g., *Rana galamensis* (Dumeril & Bibron, 1841), *P. ridibundus*, *R. esculenta, R. ridibunda* (Özoğul et al., 2008; Çaklı et al., 2009; Muhammad and Ajiboye, 2011; Büyükdeveci et al., 2019). In addition, the studies on the cultivation of the frog in farm areas have been very limited over the last decade (Çağıltay et al., 2014; Alkaya et al., 2018; Silla et al., 2021). To the best of the authors' knowledge, there is no study yet conducted about biochemical aspects of wild, semicultured, and cultured frogs for both sexes. Therefore, the objective of this current study is to determine proximate, carbohydrate, and fatty acid compositions of wild, semicultured, and cultured frogs for males and females.

## 2. Materials and methods

### 2.1. Design of the experiment

We wanted to investigate the difference between the nutritional content of frogs grown in different conditions such as lipid, protein, ash, moisture carbonhydrate, and fatty acid composition. The wild frogs that belong to *Pelophylax ridibundus* (Pallas, 1771) were hunted from the wild called "wild frogs". They were adopted and fed in a cultural environment called "semicultured frogs". The eggs of the semicultured frogs hatched in the fully controlled cultured area. These offsprings that belong to the semicultured frogs were fed and raised under fully cultured environments, and, therefore, they have been called "cultured frogs".

In summary, the hunted frogs in the wild are called wild frogs, which are our first group. The frogs that were adopted and fed in the cultured area are called semicultured frogs, which are our second group. The eggs that belong to the second group were hatched and reared in a fully controlled cultural environment until they are mature specimens, which is our third group.

Finally, all three groups were separated as male and female groups. We had six individual groups in the trials; 1) wild female frogs, 2) wild male frogs, 3) semicultured female frogs, 4) semicultured male frogs, 5) cultured female frogs, and 6) cultured male frogs.

### 2.2. Frog collecting area, measurements, and sample preparations of the chemical analysis

This study was carried out in accordance with animal welfare and the ethics of trial. The frog specimens were collected while they were alive from nature, indeed, from Kırıkhan Gölbaşı Lake, Hatay province in Turkey. After several investigations and observations had been carried out about the spawning period of the frog, it was decided to hunt them down early in March, which was the closest period to spawning time. We have measured frog body, legs with bones, and legs without bones. All measurements were recorded and reported in Table 1. The sex of the frogs was determined according to the secondary sex characters. Accordingly, males have swollen, which distinguishes them from females.

We have only worked with adult frogs [SVL > 60.0 mm, according to Bannikov et al. (1977)]. Biochemical analyses

[2] Turkstat (2019). Turkish Statistical Institute Fishery Statistics [online]. Website https://data.tuik.gov.tr/Bulten/Index?p=Su-Urunleri-2019-33734 [accessed 05 January 2022]

[3] FAO (2006). Cultured aquatic species information programme *Rana catesbeiana* [online]. Website https://www.fao.org/figis/servlet/static?xml=/Rana_catesbeiana.xml@@dom=culturespecies> [accessed 05 January 2022]

**Table 1.** Total weight, leg weight, and meat yields of wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs.

| Measurements | Wild | | Semicultured | | Cultured | |
|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male |
| **Body weight (g)** | 63.39 ± 10.06 | 31.16 ± 3.25 | 59.46 ± 18.51 | 40.82 ± 11.50 | 65.59 ± 20.89 | 48.88 ± 16.75 |
| **Leg weight(g)** | 22.76 ± 4.64 | 11.23 ± 1.56 | 20.49 ± 6.64 | 15.21 ± 4.31 | 21.16 ± 5.30 | 18.39 ± 6.82 |
| **Leg muscle weight (g)** | 13.56 ± 1.69 | 6.71 ± 0.65 | 12.94 ± 4.21 | 9.57 ± 3.00 | 12.53 ± 3.34 | 10.99 ± 4.11 |
| **Meat yield of leg( %)** | 35.70 ± 1.68 | 35.95 ± 2.34 | 34.50 ± 3.82 | 37.26 ± 0.40 | 32.68 ± 1.88 | 37.41 ± 1.32 |
| **Meat yield of leg muscle (%)** | 21.57 ± 2.34 | 21.65 ± 2.26 | 21.71 ± 1.52 | 23.31 ± 1.00 | 19.31 ± 1.05 | 22.35 ± 1.19 |

were carried out to explore the edible parts of the frogs, and proximate and fatty acid compositions were measured for nutritional quality. To prepare the edible parts, skins were removed, and the feet were clipped immediately. The legs of the frogs were removed from the body. The bones were removed from the legs. Only the muscle parts from the hind legs were gathered in a container for each group because this part was consumed as food. A total of six different muscle containers were formed for the biochemical analysis. Each container was chopped and mixed in order to obtain a homogeneous sample for the biochemical analysis.

**2.3. Adaptation of period**

We tried to handle hunting operations very carefully to reduce their stress factors as well as the welfare of the animals. The specimens that were caught and used in the study were placed in specifically designed proper bags for frogs right after the hunting operations. The frogs were regularly checked and moisturized through the trip from the hunted area to the adaptation and breeding facility in the Aydıncık district of Mersin.

**2.4. Pellets ingredients**

The semicultured frogs were placed in the cultivation area and fed with pellets which were prepared for them for the study's purposes. The feed ration required for the feeding of frogs was the previously created feed which was explained in greater detail in Alkaya and Şereflişan (2016). The feed contains protein (roughly fish meal 12% and chicken meal 25%) and fish oil (< 5%). The ingredients were mixed homogeneously and mixed into the pellet feed machine (LM 300 model). The size of each pellet ranged from 3 to 5 mm.

**2.5. From eggs to adult period**

This part of the experiment was carried out in a specially designed frog farm research and development center, which was established in Aydıncık district in Mersin. The hunted frogs were taken to an adaptation and breeding facility. The female and male specimens were placed in 1 × 1 $m^2$ spawning ponds. They have been fed for five months after the adaptation period. Their offspring were obtained from the eggs and raised until harvesting in fully cultured environments.

A total of nine growth ponds with concrete walls and floors (breeding floor, tadpole rearing unit, food unit) was designed (all area of 300 $m^2$) for the cultured frogs (female and male, our third groups). The ponds were located inside a metal structure covered with plastic to form a greenhouse. The frog farming ponds (6 $m^2$) were designed according to a semiwet system. Water depth was approximately 10 cm in ponds. The water troughs of the ponds were emptied and cleaned daily, and the water was refreshed daily. The temperature of the air and water was monitored daily.

We have obtained larvae after we breed semicultured frogs. The larvae were fed with egg yolk for the first week and then fed with pellet feed prepared according to the nutritional needs of the frogs. We have formed the pellets that were discussed previously. The average water temperature of the pools where tadpoles were grown was measured as 24 ± 1 °C. Tadpoles metamorphosis after feeding for about 60 days and became juvenile individuals during the study. The juveniles became adult individuals approximately 8 months after they were started to be fed in concrete ponds designed as a semidry wet system. We have harvested them after they reached adult size. It took us 24 months to prepare the last two groups (third groups). We spent 2 years collecting the data for the present study.

**2.6. Biochemical analysis**

Proximate compositions included crude lipid, crude protein, crude moisture, crude, and ash. It has been used equal amount of muscle for each of the group. Determination of the levels of the crude lipid in edible proportions in frogs was carried out by the modified Bligh and Dyer method modified by Hanson and Olley (1963). The analyses of the crude protein were calculated by using nitrogen content obtained by the Kjeldahl method. A conversion factor of 6.25 was used for the calculation of protein content with the procedure described by

AOAC (Official Methods of Analysis 39.1.15) (AOAC, 2008). The crude moisture and crude ash contents of the edible proportions in frogs were assessed by using the recommended methods, CEC (2003) and AOAC (2005) with method no 938.08, respectively. Carbohydrate value was calculated by the method described in detail in Özyılmaz et al. (2016).

A GC-MS (gas chromatography-mass spectrometry) with a Hewlett Packard GC (model 6890) coupled with a Hewlett Packard model 5972A HP 6890 system MS detector was used to determine fatty acid profiles. Individual components of the fatty acids were separated using an HP-INNOWAX Polyethylene Glycol Capillary Column (60 m), model number: HP 19091N-133. An automatic injection system was used for the injections. Injection and detector temperatures were set at 250 °C and 270 °C, respectively. The injection was washed three times with FAME containing isobutane prior to injection. After injection, the postinjection schedule was also set to flush the injection three times for each sequential injection. The oven temperature was initially programmed at 120 °C and held for 3 min. The temperature was then increased to 180 °C at an incremental rate of 10 °C per minute and held at this temperature for 10 min. The temperature was then increased to 250 °C at a ramp rate of 10 °C per minute and held at this temperature for 10 min. Identification and quantification of the fatty acids were given in detail in Özyılmaz (2016).

### 2.7. Statistical analysis

All statistical analyses for results of crude lipid, protein, ash, moisture, carbonhydrate, and fatty acid compositions were performed using SPSS 22.0. Descriptive statistics (means and standard deviations) were calculated for wild, semicultured, and cultured frogs. The effects of different growing conditions (origin), sex, and interactions between them (origin × sex) were determined by two-way analysis of variance (ANOVA) at $p < 0.05$ level of significance. Normality and homogeneity were tested by using Kolmogorov–Smirnov and Levene's tests, respectively. Differences between female and male frogs for each growing condition were separately analyzed by using the Duncan test (one-way ANOVA) to investigate if there existed any statistically significant differences between groups. The homogeneity of variances was tested before ANOVA analysis was performed.

## 3. Results

### 3.1. The measurements of the frogs and meat yields

The measurements of the wild female frogs, semicultured female frogs, and cultured female frogs were found to be higher than those of the wild male frogs, semicultured male frogs, and cultured male frogs. All females were bigger than males regarding their body weight. The meat yield diverged among the groups (Table 1).

### 3.2. Proximate compositions and carbohydrate levels

Proximate and carbohydrate in edible portions of wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs were tabulated in Table 2. Although there were statistically significant differences in lipid contents between male and female wild frogs ($p < 0.05$), they all had lower amounts of lipid (less than 2%). In addition, the protein contents of the frogs differed from each other, and these differences were found to be statistically significant only in cultured groups. Sex had an effect on protein levels but the different growing conditions did not. The ash contents of the all groups did not change much as they did in protein levels. In addition, the ash contents of the wild frogs had the highest levels compared to the ash contents of semicultured and cultured frogs. The differences were found statistically nonsignificant ($p < 0.05$). Moreover, statistically significant differences were

**Table 2.** Proximate and carbohydrate contents in edible portions of wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs.

| Fatty acids | Wild | | | Semicultured | | | Cultured | | | Two-way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | p | Female | Male | p | Female | Male | p | Sex | Origion | G × O |
| **Lipid** | 0.86 ± 0.03 | 1.21 ± 0.04 | 0.000 | 0.66 ± 0.03 | 0.88 ± 0.04 | 0.002 | 1.22 ± 0.04 | 1.52 ± 0.06 | 0.001 | 0.000 | 0.000 | 0.000 |
| **Protein** | 15.74 ± 0.58 | 12.76 ± 1.01 | 0.309 | 10.54 ± 0.60 | 16.10 ± 0.47 | 0.477 | 15.43 ± 0.19 | 12.08 ± 0.38 | 0.000 | 0.000 | 0.000 | 0.018 |
| **Ash** | 1.31 ± 0.07 | 1.26 ± 0.05 | 0.008 | 1.00 ± 0.05 | 0.97 ± 0.03 | 0.279 | 0.94 ± 0.02 | 1.20 ± 0.03 | 0.056 | 0.000 | 0.229 | 0.000 |
| **Moisture** | 82.10 ± 0.70 | 79.83 ± 0.40 | 0.011 | 82.83 ± 1.00 | 82.00 ± 0.44 | 0.000 | 81.50 ± 0.52 | 79.10 ± 1.44 | 0.000 | 0.100 | 0.037 | 0.000 |
| **Carbohydrate** | 0.00 ± 0.09 | 4.93 ± 1.45 | 0.004 | 4.97 ± 0.90 | 0.05 ± 0.06 | 0.001 | 0.92 ± 0.33 | 6.07 ± 1.14 | 0.002 | 0.034 | 0.000 | 0.001 |

Data represent means ± standard deviation (n = 3), S × O= Sex × Origin interaction.
Origin means different growing conditions in the table.
Changes determined to be significant were indicated in bold under p column.

observed in moisture and carbohydrate contents muscle of the frogs in all groups. Finally, the statistical analysis revealed that the effect of sex and the different growing conditions had strong effects on the frogs.

### 3.3. Fatty acid compositions

Fatty acid compositions of the muscle in wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs were shown in Table 3. The fatty acid compositions of each group ranged from 29.53% to 39.28% in saturated fatty acids (SFA), 21.15% to 30.59% in monounsaturated fatty acids (MUFA), and 31.84% to 47.28% polyunsaturated fatty acids (PUFA).

The average MUFA content of the wild frogs was lower than the semicultured and cultured ones. The reasons for that could be attributed to the environments and the pellets that we gave to them. In order to get benefits of the MUFA components, semicultured and cultured ones could be preferred. Among MUFA contents, oleic acid (C18:1n9) was the major fatty acid. In addition, the highest and the lowest average C18:1n9 were calculated to be 19.41% for cultured female frogs and 10.73% for semicultured male frogs. The mean C18:1n9 differed among groups. While these differences were found statistically significant for wild and semicultured frogs ($p < 0.05$), they did not for cultured ones ($p > 0.05$). The interactions among all groups in the present study were also found statistically significant ($p < 0.05$).

The average PUFA contents of all groups varied from one group to another. The level of total PUFA for wild frogs was found to be higher than that of total PUFA for semicultured and cultured ones. The average linoleic acid (C18:2n6, LA) was found to be the major fatty acid for all groups followed by arachidonic acid (C20:4n6, ARA). The amount of docosahexaenoic acid (C22:6n3, DHA) and eicosapentaenoic acid (C20:5n3, EPA) were lower than LA and ARA, among the n6 series of the fatty acids, in all groups throughout the trials in the present study.

## 4. Discussion

Based on the results of the present study, we revealed that changes in growing conditions had an effect on the lipid contents of frogs. This could be the result of frogs' ability to get used to dealing with the different living conditions. In addition, this could be attributed to the fact that frogs had a great capacity to struggle to cope with unfavorable conditions. Depending on the conditions that frogs were exposed to, they may have had to struggle a lot or have had better change capabilities. Moreover, this could be the result of the spawning period, a period that was very close to the one when we collected our data, indeed, March (Drohvalenko, 2021).

The levels of protein were higher in semicultured males compared to the frogs in other groups. The reason for this result could be environmental changes or feeding habits. In addition, the protein levels of the wild frogs were very similar to cultured ones. These similarities could be the result of pellets. Based on these results, pellets are suitable for frogs. This could be the result of a successful breeding time period regarding their protein contents.

While the average levels of protein were decreased in semicultured female ones, the average amount of protein was increased. The reason for this result could be environmental changes or feeding habits. In addition, the protein levels of the wild ones were very similar to cultured ones. These similarities could be the result of pellets that were prepared for them. Based on these results, pellets are suitable for them and work very well. This could be the result of successful breeding regarding their protein contents.

The cultured frogs, offsprings of the semicultured frogs of both males and females, have got higher lipid levels in both semicultured and wild frogs in the current study. These results were in accordance with the previously reported studies for *R. esculenta* (Çaklı et al., 2009; Alkaya et al., 2018), *Lithobates catesbeianus* (Shaw, 1802) (Ayres et al., 2015), and wild and cultured *R. ridibunda* (Çağıltay et al., 2014).

The protein levels of all groups were ranged from 12.08% to 16.10%. These results were in accordance with the values of protein levels reported by Büyükdeveci et al. (2019) whistle they were lower than those reported by Çağıltay et al. (2014), Baygar and Özgür (2010), Çaklı et al. (2009), and Özoğul et al. (2008). The differences between the data on wild frogs in the present study and the data provided by previous studies can be due to differences among species. In addition, Oyibo et al. (2020) reported earlier that the crude protein of *Hoplobatrachus occipitalis* (Günther, 1858) was 16.91%, which was higher than all crude protein levels of all groups in the present study, except for the semicultured males.

The female frogs were found to have higher moisture content than male ones. All groups of frogs have a considerable amount of moisture levels, which constitutes the major parts of the proximate compositions. The results related to their moisture levels for wild frog (*P. ridibundus*) were in parallel with the results reported in previous studies by *R. esculenta* (Özoğul et al., 2008) and *P. ridibundus* (Alkaya et al., 2018). Higher levels of moisture available in the edible parts of the frogs could be a distinctive feature of them.

The carbohydrate levels of all frog groups under investigation in the present study varied among groups (Table 2). Oyibo et al. (2020) reported that carbohydrate levels of *Hoplobatrachus occipitalis* (Günther, 1858) were

**Table 3.** Fatty acid profiles in edible portions of wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs (*Pelophylax ridibundus* Pallas, 1771).

| Fatty acids | Wild | | | Semicultured | | | Cultured | | | Two-way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | p | Female | Male | p | Female | Male | p | Sex | Origin | S × O |
| C12:0 | 0.41 ± 0.03 | 0.47 ± 0.02 | 0.049 | 0.60 ± 0.03 | 0.20 ± 0.09 | 0.002 | 0.20 ± 0.01 | 0.66 ± 0.13 | 0.003 | 0.001 | 0.000 | 0.003 |
| C14:0 | 2.08 ± 0.09 | 1.82 ± 0.00 | 0.008 | 2.90 ± 0.25 | 1.67 ± 0.21 | 0.003 | 1.35 ± 0.19 | 1.32 ± 0.77 | 0.962 | 0.000 | 0.068 | 0.023 |
| C16:0 | 19.95 ± 0.43 | 20.21 ± 0.10 | 0.369 | 20.89 ± 0.41 | 23.71 ± 0.34 | 0.001 | 21.22 ± 0.97 | 21.23 ± 1.41 | 0.990 | 0.000 | 0.066 | 0.005 |
| C17:0 | 0.80 ± 0.03 | 0.66 ± 0.03 | 0.003 | 1.53 ± 0.34 | 0.17 ± 0.05 | 0.002 | 0.10 ± 0.01 | ND | | 0.000 | 0.000 | 0.753 |
| C18:0 | 7.79 ± 0.06 | 6.37 ± 0.09 | 0.000 | 8.62 ± 0.22 | 13.53 ± 0.06 | 0.000 | 6.82 ± 0.14 | 7.17 ± 0.09 | 0.020 | 0.000 | 0.000 | 0.000 |
| **Σ SFA** | **31.04** | **29.53** | | **34.54** | **39.28** | | **29.68** | **30.39** | | | | |
| C16:1n9 | 0.31 ± 0.00 | 0.48 ± 0.02 | 0.002 | 0.78 ± 0.21 | 5.06 ± 0.26 | 0.000 | 0.63 ± 0.01 | 0.59 ± 0.15 | 0.689 | 0.000 | 0.000 | 0.000 |
| C16:1n7 | 1.32 ± 0.03 | 1.72 ± 0.02 | 0.000 | 2.45 ± 0.12 | 1.50 ± 0.27 | 0.005 | 1.94 ± 0.02 | 1.91 ± 0.88 | 0.966 | 0.795 | 0.015 | 0.199 |
| C17:1 | 2.57 ± 0.05 | 2.16 ± 0.03 | 0.000 | 3.68 ± 0.40 | 3.63 ± 0.20 | 0.865 | 3.46 ± 0.04 | 1.53 ± 0.03 | 0.000 | 0.428 | 0.002 | 0.000 |
| C18:1n9 | 12.85 ± 0.10 | 16.58 ± 0.22 | 0.000 | 15.32 ± 0.30 | 10.73 ± 0.40 | 0.000 | 19.41 ± 0.34 | 18.87 ± 0.19 | 0.072 | 0.000 | 0.000 | 0.000 |
| C18:1n7 | 4.10 ± 0.03 | 3.08 ± 0.06 | 0.000 | 5.71 ± 0.10 | 3.75 ± 0.33 | 0.001 | 5.15 ± 0.29 | 5.12 ± 0.08 | 0.869 | 0.001 | 0.000 | 0.000 |
| **Σ MUFA** | **21.15** | **24.02** | | **27.95** | **24.68** | | **30.59** | **28.02** | | | | |
| C18:2n6 | 20.56 ± 0.12 | 23.45 ± 0.23 | 0.000 | 13.18 ± 0.03 | 12.60 ± 0.27 | 0.022 | 21.69 ± 0.27 | 23.90 ± 0.40 | 0.001 | 0.015 | 0.000 | 0.000 |
| C18:3n6 | 2.21 ± 0.01 | 2.64 ± 0.03 | 0.000 | 1.61 ± 0.29 | 0.37 ± 0.24 | 0.005 | 0.94 ± 0.02 | 1.02 ± 0.03 | 0.018 | 0.000 | 0.000 | 0.000 |
| C20:3n6 | 1.37 ± 0.09 | 1.24 ± 0.12 | 0.209 | ND | ND | 0.025 | 0.75 ± 0.07 | ND | 0.593 | 0.001 | 0.145 | 0.000 |
| C20:4n6 | 11.13 ± 0.51 | 8.25 ± 0.43 | 0.002 | 10.25 ± 0.41 | 11.27 ± 0.29 | 0.407 | 3.74 ± 0.21 | 3.66 ± 0.08 | 0.088 | 0.000 | 0.000 | 0.000 |
| C22:5n6 | 0.49 ± | ND | | 0.17 ± 0.02 | 0.55 ± 0.03 | | 0.89 ± 0.06 | 0.15 ± 0.03 | | 0.433 | 0.000 | 0.219 |
| **Σ n6** | **35.76** | **35.58** | | **25.21** | **24.79** | | **28.00** | **28.73** | | | | |
| C18:3n3 | 0.54 ± 0.03 | 0.49 ± 0.03 | 0.108 | 0.49 ± 0.09 | ND | | ND | ND | | | | |
| C20:5n3 | 4.03 ± 0.17 | 3.13 ± 0.08 | 0.001 | 2.61 ± 0.03 | 2.94 ± 0.63 | 0.000 | 3.73 ± 0.39 | 3.22 ± 0.04 | 0.000 | 0.790 | 0.015 | 0.001 |
| C22:5n3 | 2.80 ± 0.04 | 2.22 ± 0.08 | 0.000 | 0.44 ± 0.06 | 0.90 ± 0.01 | 0.000 | 0.66 ± 0.10 | 0.60 ± 0.18 | 0.654 | 0.000 | 0.000 | 0.000 |
| C22:6n3 | 4.14 ± 0.06 | 4.02 ± 0.08 | 0.102 | 3.10 ± 0.09 | 3.73 ± 0.14 | 0.003 | 3.67 ± 0.42 | 4.27 ± 0.06 | 0.073 | 0.150 | 0.112 | 0.000 |
| **Σ n3** | **11.51** | **9.87** | | **6.63** | **7.57** | | **8.06** | **8.09** | | | | |
| **Σ PUFA** | **47.28** | **45.45** | | **31.84** | **32.36** | | **36.06** | **36.82** | | | | |
| **n3/n6** | **0.32** | **0.28** | | **0.26** | **0.31** | | **0.29** | **0.28** | | | | |
| **n6/n3** | **3.11** | **3.61** | | **3.80** | **3.28** | | **3.47** | **3.55** | | | | |
| **PUFA / SFA** | **1.52** | **1.54** | | **0.92** | **0.82** | | **1.22** | **1.21** | | | | |

Data represent means ± standard deviation (n = 3), ND, not detected; S × O = Sex × Origin interaction. Origin means different growing conditions in the table.
The bold values refer to Σ SFA = Total Saturated Fatty Acids, Σ MUFA = Total Monounsaturated Fatty Acids, and Σ PUFA = Total Polyunsaturated Fatty Acids.
Changes determined to be significant were indicated in bold under p column.

1.76%, which is lower than those levels of males of wild and cultured frogs but higher than that of semicultured males.

The palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0) in SFA were determined to be the major fatty acids in wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs. While sex and origin seemed to have a strong effect on levels of C18:0 of the groups ($p < 0.05$), they do not on the other two fatty acids ($p > 0.05$). In addition, the average level of C16:0 in wild male and female frogs (*P. ridibundus*) in the present study was found to be lower than that of C16:0 in the cultured frogs. Çağıltay et al. (2014) reported higher levels of C16:0 in wild frogs and lower levels of C16:0 in cultured frogs (*R. ridibunda*). The results related to C16:0 levels reported in previous studies are different from the results presented in the current study, highly likely due to different pellet ingredients and environmental conditions.

The mean levels of C18:2n6 in all groups were the highest level in PUFA in this current study. Similarly, Özyurt and Etyemez (2015) found out that levels of C18:2n6 in *R. esculenta* as the highest fatty acid in the PUFA component. In addition, Çağıltay et al. (2014) reported that wild frogs (*R. ridibunda*) have the highest level of C18:2n6 in PUFA. Having higher levels of C18:2n6 could be one distinctive attribute for frogs. On the other hand, the semicultured frogs and cultured frogs also had higher amounts of C18:2n6 in both males and females. Contrarily, Çağıltay et al. (2014) reported that cultured frogs (*R. ridibunda*) have got highest percentage as C20:4n6 instead of C18:2n6 in PUFA. The difference can be or cannot be the result of different pellets given to frogs as meals. Therefore, further studies should be carried out on this matter to find out the real reason.

The PUFA contents of all groups were found to be statistically different ($p < 0.05$). The reasons for the differences can be attributed to many reasons or conditions e.g., environmental changes, lifetime stages for frogs, and the spawning period. According to Mathieu-Resuge et al. (2021), taxon-specific also influences the differences in PUFA contents.

The level of n6 was higher than that of n3 in all groups (*P. ridibundus*) in the present study. The previous research reported higher n6 levels for wild *R. ridibunda* (Çağıltay et al., 2014) and wild *R. esculenta* (Büyükdeveci et al., 2019). In addition, the ratio of n6/n3 is an important issue in the food we consume. According to the UK Department of Health, an ideal ratio of n - 6/n - 3 should be lower than 4.0 (at a maximum) (HMSO, 1994). All frog groups in this study were in the range of 3.11 to 3.80 which were all lower than the cut-off value of 4.0. These values agree with the values presented in the studies provided before for *R. esculenta* (Özoğul et al., 2008).

The ratio is another important feature of the food we consume. A recommended minimum value for the PUFA / SFA ratio should be 0.45 (HMSO, 1994). The PUFA/SFA of wild female frogs, wild male frogs, semicultured female frogs, semicultured male frogs, cultured female frogs, and cultured male frogs (*P. ridibundus*) in this current study were found to be higher than recommended levels for PUFA / SFA. These values were found to be in accordance with the results presented in the previously reported studies associated with *R. esculenta* (Özoğul et al., 2008; Büyükdeveci et al., 2019).

## 5. Conclusion

Frogs in the present study have lean meat because of their relatively low lipid contents. However, their lipid contents are rich in n3 fatty acids, which are found to be in the range of recommended levels. Different growing conditions (wild, semicultured, and cultured) and sex have effects on lipid, protein, carbohydrate, and fatty acid compositions of frogs. We believe that fixing their diets and their cultured environmental conditions could help better results for cultivating this specimen in order to catch success and determine the best cultivating conditions. Although this present study is one of the few studies carried out in this field and has given useful information and clues for future studies, more future studies are required in this field to improve frog culturing in many different ways.

## Conflict of interest

The authors declare that there is no conflict of interest. The authors contributed equally to this paper.

## Statement on the welfare of animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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