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Determination of Seasonal Differences in the Composition of Protein and Lipids of *Mytilus Galloprovincialis* (Lamarck, 1819) in Gulluk Gulf, Turkey

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Running title: **Range of Protein And Lipids of *Mytilus galloprovincialis* (Lamarck, 1819) in Gulluk Gulf**

Abstract
Mussels are filter feeding organisms. The main factor in the growth and the quality of their meat is the abundance of feeds and quality of the water.
In this study, the biochemical compositions of different sizes of *Mytillus galloprovincialis* (Lamarck, 1819) at different seasonal time which were cultured in two different environmental conditions at Kazıklı Bay, Gulluk Gulf, Turkey were investigated. The first station is located nearly 5 km far from the coastline in a place where a private aquaculture company cultivates fish in cages. The second station is located near the coast but nearly 4 km far from the fish cages. The mussels located on the ropes of anchoring systems and were stabled in the determined stations in nets in November 2013. The mussel samples were seasonally collected and the protein and lipid compositions were determined with respect to season and compared between the stations.
According to the results, the maximum crude lipid value (2,6 %) was found in the station near to the fish cages in summer; while the minimal values of lipids (1,2 %) were found in the same place in winter.
According to the protein measurement, the maximum value (11,3 %) was measured in the station far from the aquaculture cages in summer, but the minimum value (6,6 %) was determined in the station near to the aquaculture cages again in summer.

Practical applications
In this study we have investigated the changes in the biochemical compositions of the mussels in different sizes which were grown near the coast and near the fish farm. The obtained low values of protein content and high values of lipid contents is being nowadays associated with the application of fish feed extrusion technology. The lipid ratio in fish feeds are raised by using this technology. Naturally, the lipid content of the food which was given to the system is high. In this study, it was determined that the active substance which presents in fish feeds was effective on the mussels in this region.

Key words: Mussel, *Mytilus galloprovincialis,* lipid and protein, Gulluk Gulf, Turkey

Introduction

People tend to pay attention to their diets in developed countries. The mussels are preferred as a food by humans to obtain the nutritional needs in recent years. Also, mussels take place in diet products because they contain high protein. Biochemical composition of mussels have been widely investigated by many researchers in many parts of the world (Kocataş,1980 and Bongiorno et. al., 2015). Mussels have the comparable nutritional contents with other animal protein sources (Schormuller, 1968). Amino acid content of mussel is similar to shrimp and fish. The meat of mussels is especially rich with calcium, phosphorus, iron and iodine. The biochemical composition of mussel meat's quality has changed according to environmental conditions and reproduction periods (Bakan and Büyükgüngör, 2000). The biochemical composition of protein in mussel tissue is very high. During the gametogenesis period, proteins are regarded as alternative energy resources in some bivalve species. In general, energy is stored prior to gametogenesis when food is abundant in the form of glycogen, lipid, and protein. Lipids represent an important energy reserve because of their high caloric content (Çelik et. al., 2012).

Mytilus galloprovincialis (Lamarck, 1819) and *Mytilus edulis* (Linnaeus,1758) take important place in the world production of mussels (Lök, 2001). In Turkey, mussel consumption is lower than the other countries. The cultivation of mussels differs from in aquaculture, if you want to get the spat, you should not use the labor and feeding, because you can get new spat in natural ways (Lutz,1987, Yıldız and Lök 2005). In recent years, in Turkey, especially in the Black Sea, some researches were made on mussel cultivation and positive results were obtained (Koral and Süleyman, 2016). In recent years, integrated systems in aquaculture have been developed. Integrated systems prevent the decreases of organic and inorganic nutritional caused by fish farms. Furthermore, the farming symbiosis species together provides the increasing the energy efficiency and productivity.

Although there are many marine aquaculture fish farms in Gulluk Gulf, till to this study there were no published studies about mussel production in this region. The main objective of this study is to investigate the biochemical compositions such as protein and lipid content of varied sized mussels *Mytilus galloprovincialis* in different aquatic environments by integrated growing method in Gulluk Gulf, Kazıklı Bay.

Materials and Methods

This study was done at Gulluk Gulf, Kazıklı Bay. There are two stations determined for the realization of the research. The distance between 2 stations was measured approximately 4km. (Figure 1). The research was made between November 2013- May 2014.

I. Station; The place situated on 37° 15′ 18″ N, 27° 26′ 93″ E coordinates, showed by an off-shore marine fish farm. *Sparus aurata* and *Dicentrarchus labrax* were grown with 1000 ton/year capacity. Total depth in this station was measured by 52 meters.

II. Station; Second station was located 37° 19′ 54″ N, 27° 29′ 38″ E and this station is nearby shore. It has 8 meters' depth. There were no human interactions, settlement and production facilities in this area.

In this research, *Mytilus galloprovincialis* (Lamarck, 1819) species was studied. In November 2013, the mussels were collected by hand with divers. Approximetly 480 mussels were classified as small and large according to their sizes.

The samples were divided into two groups and the small sized mussels were gathered in "first sized mussels" group and the large sized mussels were gathered in "second sized mussels". The minimum, maximum and the average width, length, height and weight were indicated in Table 1.

Divided mussel groups in order to determine the quality of meat in mussel samples were taken in November 2013 and May 2014. After that, by using these samples, lipid and protein analysis were done. Analyzes were repeated for three times.

In the analysis of raw lipid, the Bligh and Dyer's (1959) method was used. About 5 mg of mussel samples homogenized with homogenizer were taken for the analysis. 120 mL methanol: chloroform (1:2) were added on the samples and homogenized. Then 20 mL of 0,4% $CaCl_2$ solution were poured and the samples were filtered out. In the liquid, methanol phase was removed by separation funnel. The phase consisting of lipid and chloroform was distilled at 60^{o}C in rotary evaporator in order to remove chloroform, and last product was dried at 60^{o}C and stored in a desiccator. Then the lipid amounts were calculated by weighing the flask with lipids and extracting the empty vessel's weight. Lipid percentage of the samples was calculated according to equation 1. W_2 is corresponding to raw lipid's weight and W_1 is corresponding to the sample's weight.

$$\%\ lipid = \frac{w_2}{w_1} \times 100 \quad (1)$$

Raw protein content was analyzed via Kjeldahl method (AOAC 2006). The mussel samples which weighed 1g were put into Kjeldahl tube after homogenization. Catalyst (1 Kjeldahl tablet) were added into the tube, then 10 mL 98% H_2SO_4 was added and burned at 420°C until the sample's color changed into green-yellow. After cooling samples to room temperature, 75 mL of distilled water was poured and into distillation unit where automatically added 50 mL 40% NaOH and 25mL 3% boric acid into the tube. After distillation, indicator was added to 200mL of distilled solution and N percentage was calculated via titration with 0,2 N HCl. Calculations were made according to equation 2.

$$N\% = \frac{[14.01 \times (A-B) \times N]}{W \times 100} \quad (2)$$

Where A, B, N and W are volume of HCl titrated, acid volume used in model study, normality of HCl solution and weight of the sample respectively. The protein factor in animal products is 6.25 and raw protein percentage was determined by multiplying N% by 6,25.

Results

The maximum value of the crude lipid was measured as a 2.6% for size I group in first station at summer time. This group was located near by the fish farm. The minimum value of crude lipid was measured at the same station as 1.2% for size II group in winter period.

The maximum value of protein quantity for size I group was measured as 11.3% in the second station at summer period. The minimum value of protein quantity for size I group was measured as a 6.6% in first station at summer period (Table 2 and Table 3).

Discussion

The biochemical composition of aquatic livings is varied with nutrition, seasons, geographical region, size, sex and reproduction cycle. Therefore, these parameters certainly have to be considered for a healthy observation of livings' biochemical composition. (Koral and Süleyman, 2016). In this study the changes in the biochemical compositions of the mussels in different aquatic environments and having different sizes; the lipid values were found at maximum in summer at the station (Station 1) near a fish farm. The minimum lipid values were observed in winter at Station 1. Koral and Süleyman's (2016) previous study the lipid values were found at maximum (2.59%) in summer, and the minimum value (1.32%) was observed at autumn season. Bongiorno et al.(2015), observed the highest lipid value (2.2%) in summer and observed the lowest value (1.0%) of lipid in winter in the study on *Mytilus galloprovincialis*. Fuentes et al. (2009), reported that the lipid contents of mussels were at between 1,40-2,10%. Çelik (2006) and Erkan (1996) determined that, the lipid contents in the mussels were 2.6% and 1.02% at average, respectively. The lipid values found in this study were in good agreement with these previous studies.

According to the protein analysis results, maximum protein values were seen at regions far from the fish farm (Station 2) in the summer and the minimum values were determined at the regions near to the cages (Station) in summer. The protein content of mussels was found %6.6- %11.3 in this study. This value is between the values (%7.8, %8.9, %9.46, %10.30) reported by Erüstün and Şentürk (1991), Şentürk (1994), Ölmez et al. (2002), Turan et al. (2008), respectively. Bongiorno et. al. (2015) also reported the highest value for protein (11,6%) in summer at the study on *Mytilus galloprovincialis*.

Although, the lipid and protein levels at gametogenesis stages increases, afterwards these levels decrease. Nutritional value of mussel meat is at the highest level in the autumn, winter and at the beginning of spring (Schormuller, 1968; Krzynowek and Wiggin, 1979). In this study, it was seen that, lipid and protein levels were at maximum in gametogenesis in the spring term. But also minimum lipid and protein levels were measured in autumn term.

In summer, low amounts of protein and high amounts of lipid were determined near the fish farm. These results were attributed to the rise of the temperature and the food needs of the fish resulting the excess amounts of feeds. According to Koral and Süleyman (2016), increasing in protein amounts was seen at the regions where the nutrition is high. Whereas, the lipid ratios in fish feeds were increased by application of extrusion technology. Therefore, in the areas where fish farming was done, mussels were affected by the feeds which the lipids were raised.

As a result of biochemical nutritional levels analysis on mussel, their lipid and protein levels were reached the highest value in summer, however lowest values were seen in winter. We can explain the occurrence of seasonal differences; nutritional needs of fish increased with the changing of the water temperature. Therefore, the amount of fish feed was increasing in the cage system. In Station I, the protein value was low but the lipid value was high on harvesting time in the summer. There were fish feed in the environment at the station I. Extrusion technology is applied to the fish feed nowadays. Lipid ratio were increased in fish feed using this technology. In this research, we determined that the fish feed given to the cage system is effective on mussels. According to our result, mussels were found useful for the healthy diet because of consisting high protein and lipid.

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Table 1. The distribution according to the size of mussel groups used in the study

		STATION I		STATION II	
		Size Group I	**Size Group II**	**Size Group I**	**Size Group II**
Width (mm)	Min-Max.	15,25 – 24,02	26,97 – 33,98	16,18 – 21,39	27,15 – 36,35
	Average	19,91 (±1,8)	30,37 (±1,8)	19,07 (±1,2)	31,80 (±1,8)
Lenght (mm)	Min-Max.	34,07 – 40,59	54,25 – 59,99	32,00 – 38,94	57,06 – 62,95
	Average	37,75 (±1,9)	56,82 (±1,7)	35,14 (±1,9)	60,37 (±1,9)
Height (mm)	Min-Max.	9,34 – 18,84	19,14 – 26,96	10,53- 14,92	19,22 – 25,83
	Average	13,22 (±1,7)	22,81(±1,7)	12,43 (±1,0)	23,55 (±1,4)
Weight (g)	Min-Max.	6,01 – 7,16	19,39 – 21,44	3,29 - 4,00	22,88 – 25,24
	Average	6,66(±0,3)	20,31 (±0,6)	3,61 (±0,2)	24,21 (±0,7)

Table 2. Lipid and protein values in winter season of the mussels

November 2013		**Lipid (%)**	**Protein (%)**
Station I	**Size Group I**	1,4	10,1
	Size Group II	1,2	9,3
Station II	**Size Group I**	1,5	9,9
	Size Group II	1,6	9,1

Table 3. Lipid and protein values in summer season of the mussels

May. 2014		**Lipid (%)**	**Protein (%)**
Station I	**Size Group I**	2,6	6,6
	Size Group II	1,5	7,1
Station II	**Size Group I**	2,5	7,7
	Size Group II	1,6	11,3

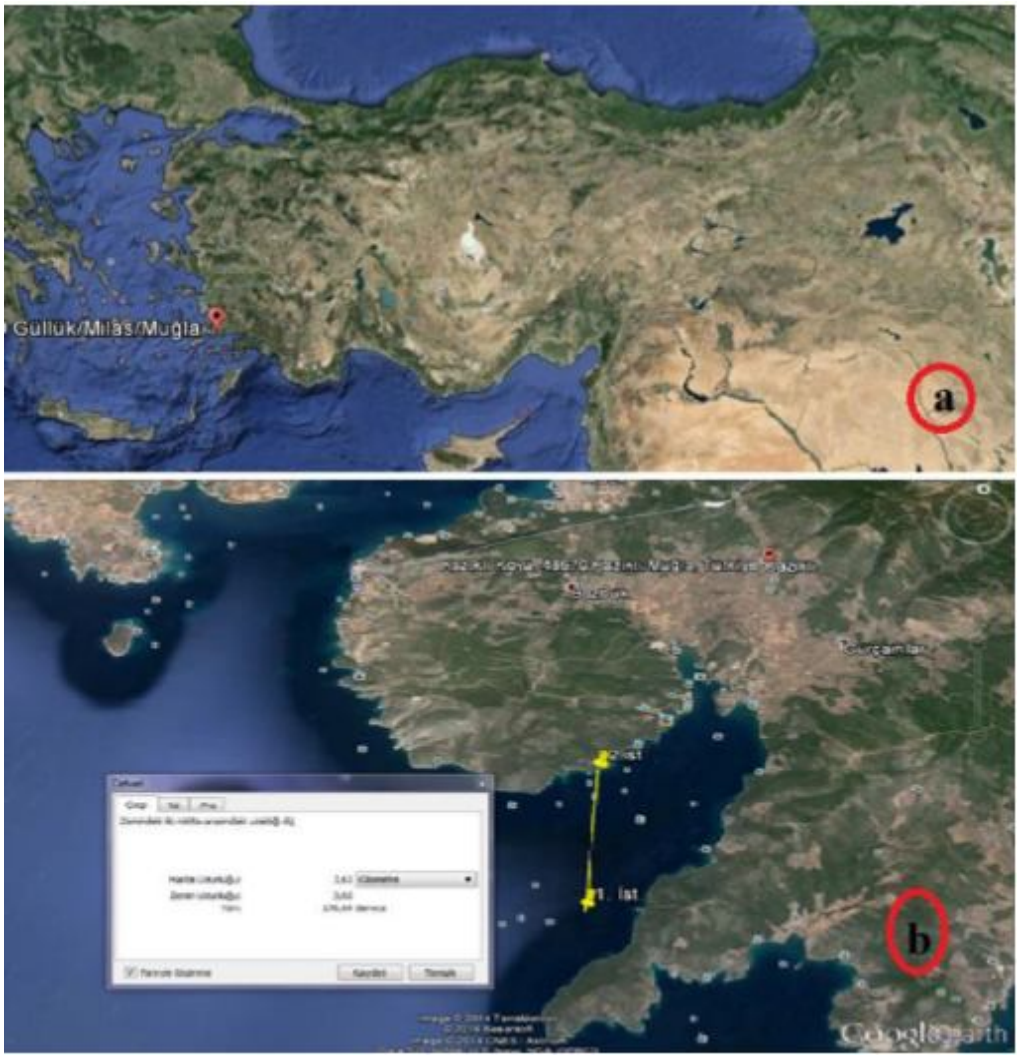

Figure 1. Working area a) The study area located west of Turkey b) Kazıklı Bay work stations