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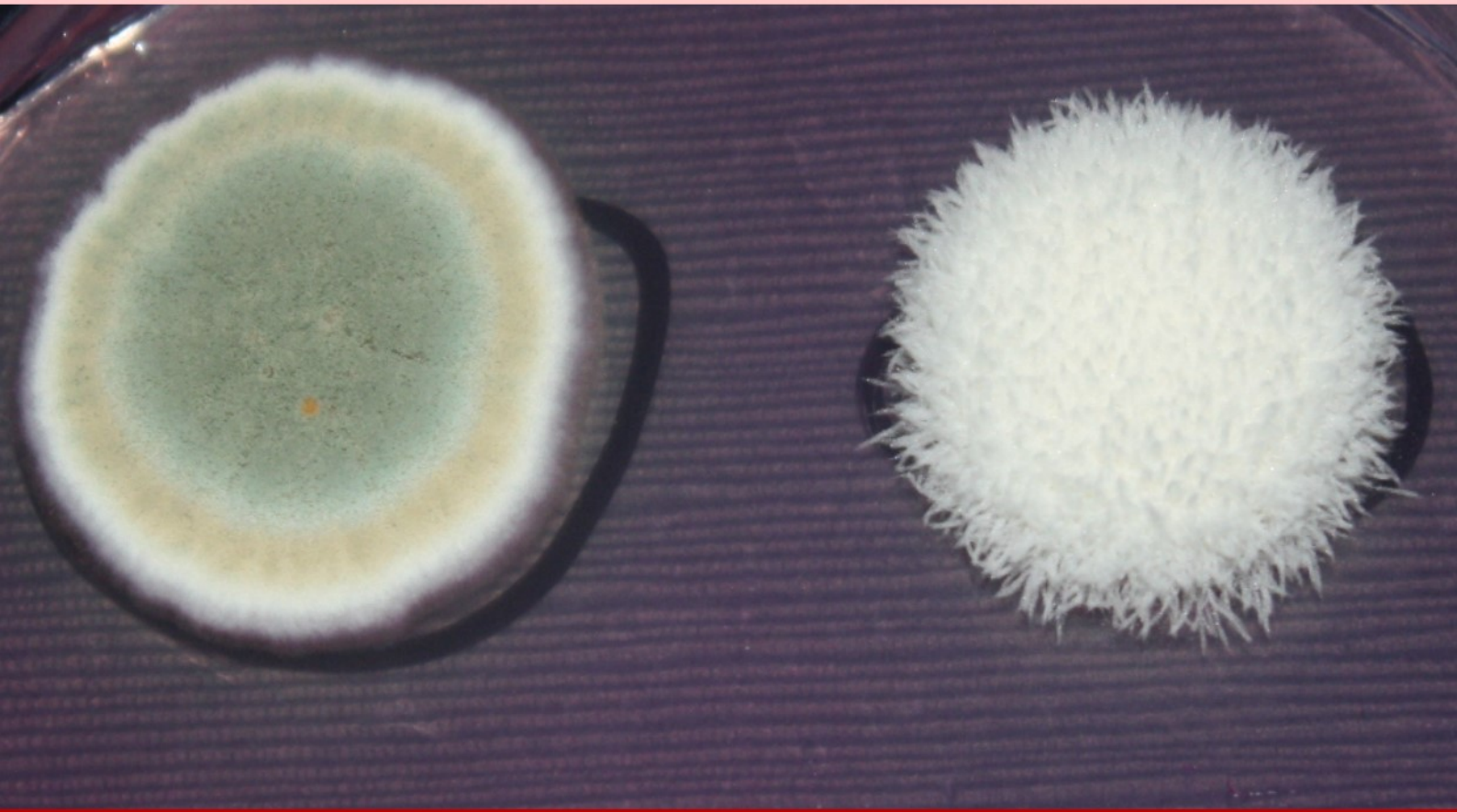

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"Journal of Food and Health Science" publishes peer-reviewed articles covering all aspects of **Food** and **Health science** in the form of review articles, original articles, and short communications. Peer-reviewed open access journal publishes articles in **English** or **Turkish** language.

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- Food Chemistry/Microbiology
- Food Packaging/Packaging Materials/Migration
- Food Safety/Hygiene/Quality Assurance/Control
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- Clinical Nutrition

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

HEAVY METALS IN EDIBLE TISSUES OF BENTHIC ORGANISMS FROM SAMSUN COASTS, SOUTH BLACK SEA, TURKEY AND THEIR POTENTIAL RISK TO HUMAN HEALTH

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Abstract:

The present study was aim to determine the concentrations of copper, cobalt, lead, zinc, cadmium, manganese, nickel and iron in the edible tissues of *Liocarcinus depurator* (decapoda), *Rapana venosa* (gastropoda) and *Mytilus galloprovincilais* (mollusca) collected from Samsun coasts of the Black Sea, Turkey in 2010. These biomonitor species are major food sources of benthic ecosystem. Exposing to heavy metals at higher concentrations might be toxic to demersal fish species and also humans. The concentrations of the metals were carried out using Flame Atomic Absorption Spectrophotometer (UNICAM 929). Metal concentrations in *L. depurator*, *R. venosa* and *M. galloprovincilais* decrease in the order: Fe > Zn > Mn > Cu > Ni > Pb > Co > Cd; Fe > Zn > Cu > Pb > Mn > Cd > Ni and Fe > Zn > Cu > Pb > Mn > Co > Ni > Cd, respectively. The results showed that the Fe and Zn concentrations were the highest in edible tissues of the three benthic organisms, respectively. Mn was higher in the edible tissues of *L. depurator* than those in other species, while *R. venosa* and *M. galloprovincilais* shows more of Cu and Pb levels. The estimates of EWI and EDI indicated no health risk as values are lower than the allowed tolerable levels cited by internationals committees. Based on the above results of this study, metal accumulation in the biomonitor demersal species study did not exceed the permissible limits set for heavy metals by FAO/WHO.

Keywords: *Liocarcinus depurator*, *Rapana venosa*, *Mytilus galloprovincialis*, South Black Sea of Turkey, Heavy metals, Seafood, EWI.

Introduction

Heavy metals occur naturally; on the other hand their levels have increased with domestic, industrial, mining and agricultural activities. Especially discharge of heavy metals into the marine environment may cause extensive ecological differences, due to their toxicity, persistence and accumulative behaviour in the organisms (Dural *et al.* 2006, Bat *et al.* 2009).

The Black Sea had been exposed to pollution pressure that derives both from human activities and natural sources. Samsun Province is situated in the middle of the Black Sea in Turkey which extends along the coast between two major rivers, Kızılırmak and Yeşilırmak (Figure 1). The city also encounters rapid population growth, urbanization without conforming to city plans and intense agricultural activity involving making use of fertilizers, insecticides, and herbicides etc. (Pınarlı *et al.* 1991). Fertilizers and pesticides are seriously used and increased in agricultural fields in this region and all of them create local problems of heavy metal pollution along the Samsun coasts (Kurt and Özkoç 2004, Bakan *et al.* 2010). Besides small or full scale industrial activities such as food, cement, resin, plastic and textile are carried out in this region (Altaş and Büyükgüngör 2007, Bakan *et al.* 2010). Moreover, the province has the largest Black Sea commercial port which covers an area of 9579 km^2.

The decapod crustacean, *Liocarcinus depurator*, called harbour crab, is a species of crab found in the Black Sea and also North Sea, Atlantic Ocean and Mediterranean Sea. There is no available data on heavy metal levels of *L. depurator* in the Black Sea, while some studies are available on taxonomy, length-weight relationship and condition factor of this species (Ateş 1999, Aydın *et al.* 2013). It is not commercially important species for human consumption in Turkey, but it is a common prey and predator of many marine commercial and non-commercial species that is the main food source for crustaceans, molluscs, polychaetes and fishes (Freire 1996). Gastropods are useful and generally used in the monitoring of metal pollution (Bat *et al.* 2000). Veined Rapa whelk, *Rapana venosa*, is a species of large predatory gastropod whose main diet consists of mollusc species and mussels. Filter feeding organisms, bivalves have a sedentary life, so they are directly influenced by environmental conditions. *Mytilus galloprovincialis*, the Mediterranean mussel, is a species of bivalve have a broad geographical range. It is consumed by humans, thus it is used in monitoring metal pollution (Bat *et al.* 1999; Özden *et al.* 2010). These demersal species are important for transferring energy between trophic levels and to represent coastal environmental conditions.

The aim of this study was aim to determine the concentrations of essential (Cu, Co, Fe, Zn, Mn, Ni) and non-essential (Pb, Cd) heavy metals in the edible tissues of *L. depurator*, *R. venosa* and *M. galloprovincilais* collected from Samsun coasts, Turkey in 2010 and to compare the international and national permissible limits set for heavy metals by FAO/WHO.

Materials and Methods

Study area

The study area, Samsun, is located in the middle of the Southern Black Sea of Turkey (Figure 1) that was selected as sampling location because it is in the way of various industrial and domestic effluent discharges, harbour and agricultural activities.

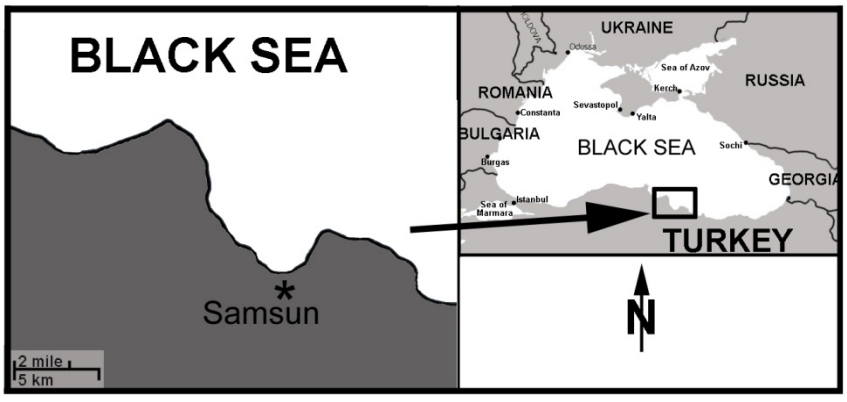


Figure 1. Sampling area from Samsun coasts of the Black Sea, Turkey

Materials

Samples of the harbour crab or sandy swimming crab (*Liocarcinus depurator*), veined Rapa whelk (*Rapana venosa*) and the Mediterranean mussel (*Mytilus galloprovincialis*) were collected by scuba divers from the upper-infra littoral zone of Samsun coasts of the Black Sea, Turkey, at a water depth of 0-5 m, between July and September in 2010.

The size of mussels for marketing changes between 50-80 mm in Europe while the minimum size of the mussels for consumption is approximately 70 mm in length in Turkey (Aral 1999). In this study the size of mussels (6-8 cm) (Bat et al. 2012), snails (8-12 cm) (Bat et al. 2000) and the carapace length (2.5-3 cm) and width (3-3.5 cm) of crabs were chosen for metal analysis.

Methods

The samples were brought to the laboratory and washed thoroughly with double distilled water then were rinsed with clean seawater to remove sand particles and then placed in about 20 litres of constantly aerated clean seawater for 24 hours to allow depuration (Bat *et al*. 1999). Following elimination of the gut contents the shell of the samples were removed and discarded. Subsequently, whole edible bodies of 25 individuals for each species blotted, homogenized and stored at -21°C until required for analysis. Only edible parts of all the samples were analysed. Upon thawing, the whole soft parts were removed using stainless steel instruments. The samples were analysed with 3 replicates for each measurements. Aliquots of 10 g fresh weight were digested with 20 ml HNO_3 at 30-40°C. After a clear yellow solution was formed and this was diluted with double distilled water and filtered before heavy metal (Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe) analysis (modified from Bernhard 1976, UNEP 1984 and 1985).

The heavy metal concentrations were determined by the Flame Atomic Absorption Spectrophotometer (UNICAM 929). All reagents used in the analysis were of reagent grade. Double-deionized water was used for all dilutions. Chemicals were of high pure quality (Merck). The element standard solutions from (Merck) that were used for the calibrations were prepared by diluting stock solutions of 1000 mg L^{-1}.The working standard values were used to plot a standard curve. The standards and blank were treated in the same way as the real samples to minimize matrix interferences during analysis. Laboratory glassware was kept overnight in a 10% v/v HNO_3 solution and then rinsed with deionised double distilled water (UNEP 1984 and 1985). The measured concentrations are expressed in mg/kg wet wt. with the mean ± standard deviation.

Intake Levels Calculation

The average heavy metal weekly intake was calculated according to the following formula:

Heavy metals intake level = average heavy metal content X consumption of seafood per person/ body weight

Statistical analysis

The data collected from heavy metals analysis was statistical analysed by analysis of variance (ANOVA) and with the multiple comparisons Tukey's test to find out significant level among organisms using Statistica 7.0 statistical package program. In all cases, the estimation was carried out at significant level of 0.05 (Zar 1984).

Results and Discussion

The concentrations of the metals in edible tissues of the samples are given in Figure 2 (A, B, C, D, E, F, G and H) along with the statistical parameters. Statistical analysis of the data showed significant differences among all of the samples. The mean heavy metal levels varied among considerably depending on the type of species.

The present study was performed for assessment of eight heavy metals (Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe) in the edible tissues of *L. depurator, R. venosa* and *M. galloprovincilais* collected from Samsun coasts of the Black Sea, Turkey in 2010. These heavy metals were chosen in this study. They are not only toxic to marine organisms, but transfer through the food chain means that they are also potentially harmful to humans (Underwood 1977, Förstner and Wittmann 1983). The bioaccumulation degree depends on the type of food chain and also bioavailability and persistence of the heavy metals in the water.

Cu, Co, Zn, Mn, Ni and Fe are essential elements for organisms whereas Pb and Cd are non-essential metals and have no role in biological processes in living organisms and are toxic at low concentrations. Legal thresholds are not available for essential metals in European Commission Regulation. However, in the edible tissues of the average Zn and Cu concentrations were on average below the maximum tolerance levels for

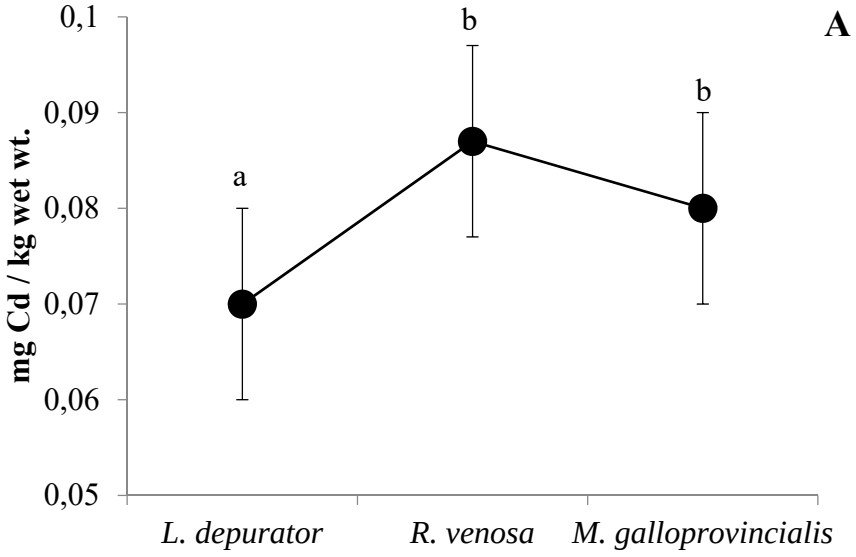
human consumption established by the Turkish and international legislations (Anonymous 1995, MAFF 1995). The highest concentration of Zn (19 ±3 mg/kg wet wt.) found in *L. depurator* is less than 50 mg/kg wet wt. in Crustaceans, well below the guideline level (Anonymous 1995, MAFF 1995). The highest Cu concentration (9 ±3 mg/kg wet wt.) was found in *M. galloprovincialis* and this level is also quite below the guideline level of 20 mg/kg wet wt. for Mollusca (Anonymous 1995; MAFF 1995).

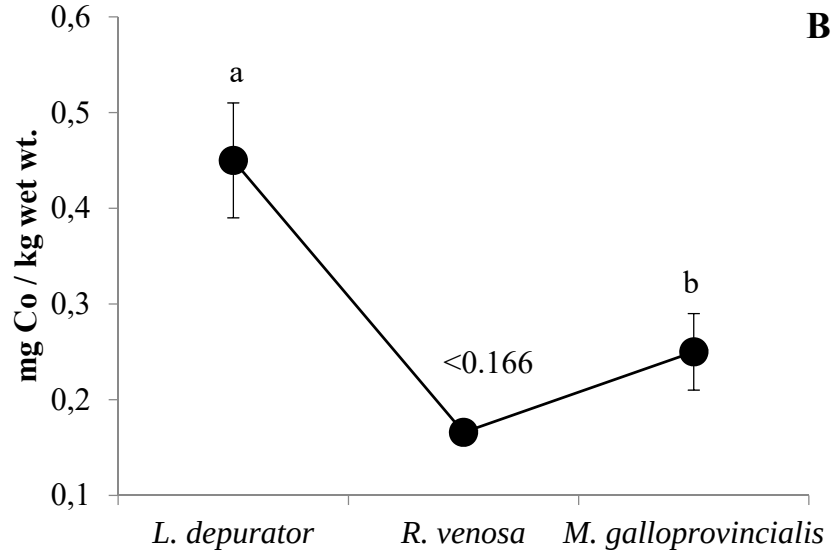
The Cu level (9±3 mg/kg wet wt.) in the mussels of this study higher than those (0.850-3.473 mg/kg wet wt.) in the Sea of Marmara (Mol and Üçok Alakavuk 2011), it is probably related to discharge form urban areas and small industries flowing into the coast of Samsun. It might be said that examined mussels are safe for human consumption regarding Cu and Zn. Cd concentrations in *M. galloprovincialis* (0.008±0.001 mg/kg wet wt.) in this study, were lower than those indicated by Mol and Üçok Alakavuk (2011) for the Sea of Marmara while they were between 0.296 and 0.740 mg/kg wet wt. where Pb levels were similar.

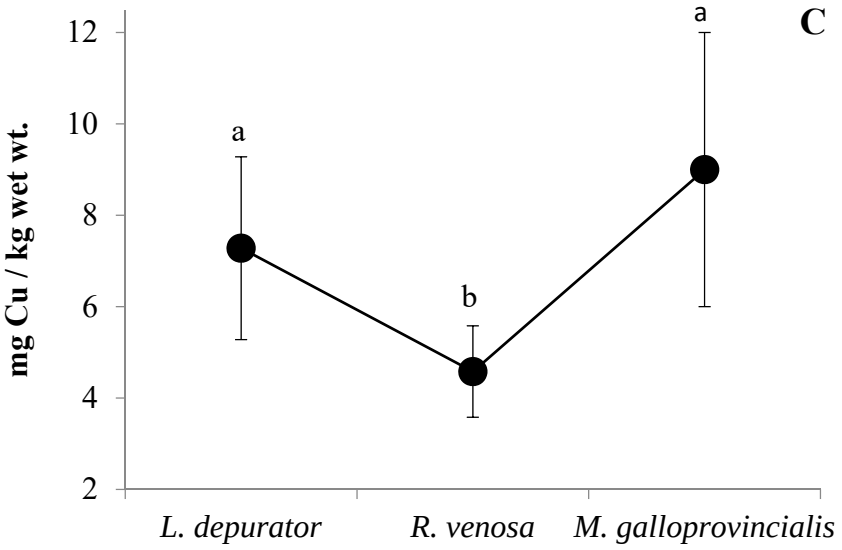
Cd and Pb are toxic and bio accumulative these metals with no known biological function and its absorption may constitute a serious risk to public health. By comparing the data from different the Southern West Black Sea regions (Mülayim and Balkıs 2015) it is possible to note that Cd concentrations (0.087 ±0.01 mg/kg) in *R. venosa* observed in this study in the Samsun coast of the Black Sea were lower than those observed (0.1-1.6 mg/kg) by Mülayim and Balkıs (2015). However Pb levels were similar in both studies.

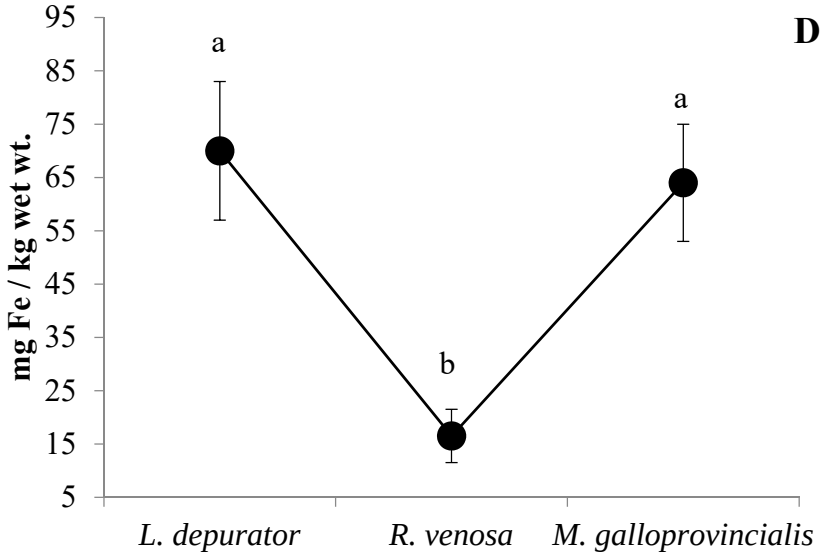
However comparing data obtained in this study, Cd concentrations in crabs were much lower than those reported in other studies (Ayas 2013; Mülayim and Balkıs 2015), where Pb values were similar in all studies. Moreover Cu and Zn levels in *E. verrucosa* were lower than those in blue swimmer crabs (*Portunus pelagicus*) from the North-eastern Mediterranean Sea (Ayas 2013).

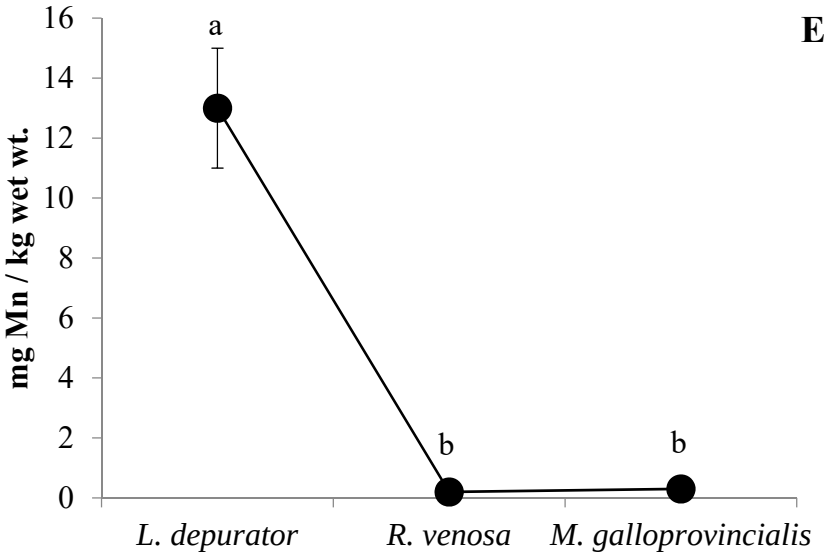
Pb and Cd are extremely toxic to humans through food chains. Commission Regulation (EC) (2006) and Turkish Food Codex (TFC) (2008) indicate that maximum level of Pb in Crustacea and Mollusca are 0.5 and 1.5 mg/kg wet wt., respectively. In case of the maximum level of Cd in Crustacea and Mollusca are 0.5 and 1.0 mg/kg wet wt., respectively.

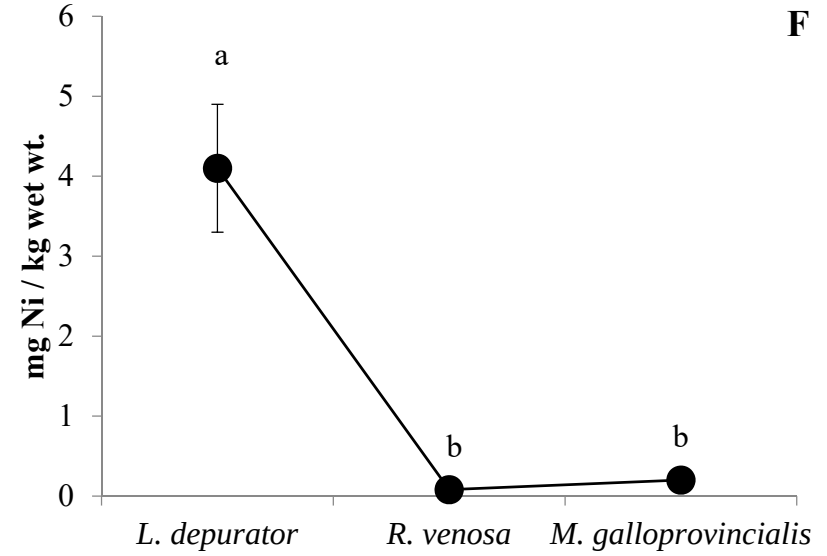


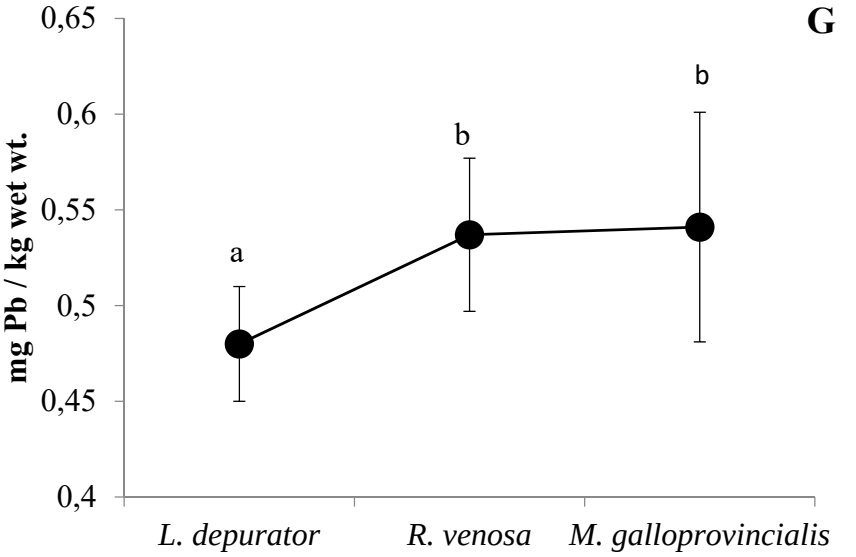

B
mg Co / kg wet wt.
0,6
0,5
0,4
0,3
0,2
0,1
a
b
<0.166
L. depurator
R. venosa
M. galloprovincialis


C
mg Cu / kg wet wt.
12
10
8
6
4
2
a
b
a
L. depurator
R. venosa
M. galloprovincialis


D
mg Fe / kg wet wt.
95
85
75
65
55
45
35
25
15
5
a
b
a
L. depurator
R. venosa
M. galloprovincialis


E
mg Mn / kg wet wt.
16
14
12
10
8
6
4
2
0
a
b
b
L. depurator
R. venosa
M. galloprovincialis


F
mg Ni / kg wet wt.
6
5
4
3
2
1
0
a
b
b
L. depurator
R. venosa
M. galloprovincialis


G
mg Pb / kg wet wt.
0,65
0,6
0,55
0,5
0,45
0,4
a
b
b
L. depurator
R. venosa
M. galloprovincialis

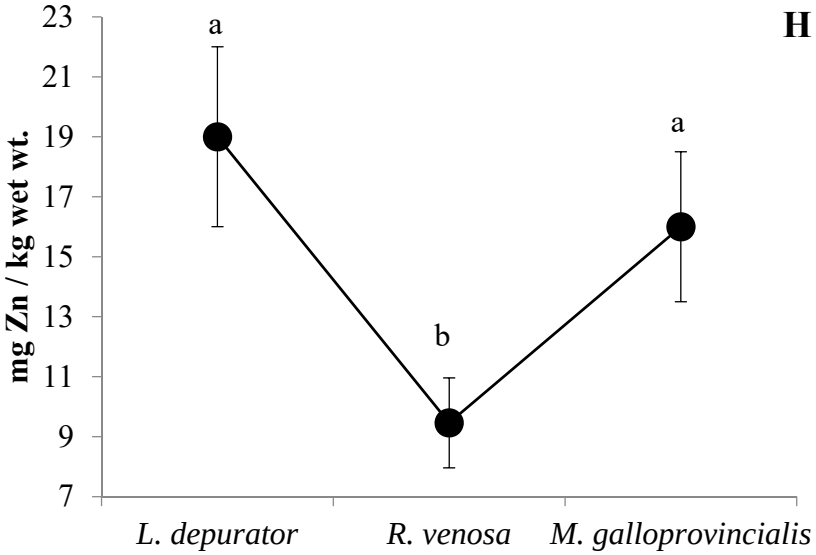


Figure 2 (A,B,C,D,E,F,G,H). The means with standard deviations (vertical line) of Cd (A), Co (B), Cu (C), Fe (D), Mn (E), Ni (F),Pb (G) and Zn (H) concentrations (mg/kg wet wt.) in the edible tissues of *Liocarcinus depurator* (decapoda), *Rapana venosa* (gastropoda) and *Mytilus galloprovincilais* (mollusca) from Samsun coasts of the Black Sea Turkey in 2010. a, b = The same letters beside the vertical bars in each graph indicate the values are not significantly different (P>0.05).

Overall, the findings from the present study revealed that Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe concentrations in the edible tissues of *L. depurator*, *R. venosa* and *M. galloprovincilais* were lower than the maximum permissible limit as recommended by the Commission Regulation (EC, 2006) and Turkish Food Codex (TFC, 2008). Aquatic organisms having toxic metals could present a toxic threat for the consumer which is dependent on the metal concentration and amount of their consumed. Therefore, the tolerable weekly intakes were estimated by means of references for edible tissues of aquatic organisms consumed by people. According to FAO estimates of Molluscs and other (except fish) daily consumption in Turkey is 1 g per person (FAO 2010). This is also equivalent to 7 g/week. However, the annual quantity of seafood (including fish) consumed is 6,918 kg / person in 2010 (TUIK 2014), which is equivalent to 18.95 g/day for Turkey. The tolerable weekly intake of heavy metals as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). PTWI is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects. The estimated daily intake (EDI) and estimated weekly intake (EWI) in this study were calculated and presented in Table 1. Intake estimates were expressed as per unit body weight (mg/kg body wt. /weekly and daily).

Although the estimated Co intake is 0.2 to 1.8 mg/day (Codex, 1995), JECFA has not evaluated cobalt. There is no evidence that the intake of cobalt is ever limiting in the human diet, and no Recommended Dietary Allowances (RDA) is necessary (National Academy of Sciences 1989). It seems that the use of cobalt in general causes no problems (Council of Europe 2001). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Cu, Pb, Zn, Cd, Mn, Ni and Fe of 3.5, 0.025, 7, 0.007, 2-5, 0.035 and 5.6 mg/kg body weight/week which was equivalent to 245, 1.75, 490, 0.49, 140-350, 2.45 and 392 mg/week for a 70 kg adult person, respectively (National Academy of Sciences, 1989; FAO/WHO, 1996; Council of Europe, 2001; WHO, 2011). In the present study the EWIs and EDIs for Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe from consumption of seafood from Samsun coasts of the Blacks Sea were estimated (see Table 1).

Table 1. Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of heavy metals in edible tissues of seafood from Samsun Coastal waters of the Black Sea, Turkey.

Metals	PTWI[a]	PTDI[b]	EWI[c]		EDI[d]	
			Minimum	Maximum	Minimum	Maximum
Cu	245	35	0.608	1.194	0.087	0.171
Co	-	-	<0.022	0.060	<0.003	0.008
Pb	1.75	0.25	0.064	0.072	0.009	0.010
Zn	490	70	1.255	2.520	0.179	0.360
Cd	0.49	0.07	0.009	0.012	0.001	0.002
Mn	140-350	20-50	0.022	1.724	0.003	0.246
Ni	2.45	0.35	0.010	0.544	0.001	0.078
Fe	392	56	2.191	9.286	0.313	1.327

[a]PTWI (Provisional Tolerable Weekly Intake) (mg/week/70 kg body wt.)

[b]PTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body wt.)

[c]EWI (Estimated Weekly Intake) (mg/week/ kg body wt.)

[d]EDI (Estimated Daily Intake) (mg/day/ kg body wt.)

Conclusion

The obtained data with guidelines showed that the metal levels in *L. depurator*, *R. venosa* and *M. galloprovincilais* are below the permissible level defined by Commission Regulation (EC), MAFF and Turkish Food Codex. On the other hand these data provided that the heavy metal concentrations in these organisms do not present any danger to human health. Consequently, adverse human health effects may occur if contaminated seafood is consumed too much. However it can be seen that the estimated EWIs and EDIs of heavy metals in this study are far below the recommended PTWIs and/or PTDIs and indicated no adverse effects to the consumers. It may be suggested that continuous care must be taken to biomonitor the heavy metal levels in the harbour crab or sandy swimming crab, veined Rapa whelk and the Mediterranean mussel especially if they exceed international and local permissible limits for human consumption.

It was concluded that benthic organisms from the Samsun coast of the Black Sea, Turkey is safe as regards the heavy metals studied. Thus, heavy metal levels in seafood must be monitored periodically with respect to consumer health. On the other hand, these data could allow people to make informed decisions about which product to eat in order to reduce the risks of contaminants.

Acknowledgments

This study was presented as a poster presentation with Turkish abstract only in the 18th National Fisheries Symposium, 1-4 of September 2015, Izmir, Turkey.

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FULL PAPER TAM MAKALE

ANTIBACTERIAL ACTIVITY OF ROYAL JELLY AND RAPE HONEY AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus* STRAINS

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Abstract:

Antimicrobial resistance of pathogenic bacteria is a growing public health problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a significant burden on healthcare services because it is involved in severe difficult to treat infections in humans. Several non-antibiotic approaches regarding the treatment of infections caused by MRSA as well as of other resistant bacteria have been studied. Since ancient times royal jelly and honey have been considered both as foods and medicines, and recently have been identified as potential alternative medicines to treat bacterial infections, especially of the skin and soft tissue. The aim of the present study was to investigate the antibacterial effect of honey, royal jelly and their mixtures against MRSA *in vitro*. At least 3 decimal reductions of MRSA count were observed in Tryptone Soy broth with concentrations of 40% rape honey (RP), 20% and 30% royal jelly (RJ), 30% RJ:RH (1:100). In general, honey and royal jelly, individually or in combination, appeared to have a potential as alternative therapeutic agents against MRSA infections, but clinical studies are needed for confirmation.

Keywords: Honey, Royal jelly, Antibacterial activity, MRSA

Introduction

Although the therapeutic effect of honey in treating of infected wounds is known since the ancient times, more recently has been reported its inhibitory effect on a great number of bacteria including both aerobes and anaerobes, as well as Gram-positive and Gram-negative (Cooper et al., 2002) and also fungi and yeasts (Brady et al., 1996).

The high antibacterial effect of RJ has also been reported (Blum et al., 1959; Melliou & Chinou, 2005).

The antibacterial activity of royal jelly, rape honey, individually and in combination has been reported against resistant strain of *E. coli* (Dinkov et al., 2014) and *A. hydrophila* (ATCC 7965) (Stratev et al., 2015).

Antibiotic-resistant bacteria represent a critical problem in modern medicine world-wide (WHO, 2014) and consequently, scientific efforts have been developed to control bacterial infections with alternative medicines beyond conventional antibiotic therapy. Honey (Molan, 1997), propolis (Kujumgiev et al., 1999) and royal jelly (Fontana et al., 2004) are among these alternative therapeutic agents.

The aim of this study was to determine the antimicrobial effect of rape honey and royal jelly, individually or in combination against MRSA.

Materials and Methods

Test substances

Test substances were Bulgarian rape honey, royal jelly and mix of rape honey and royal jelly. The rape bee honey (RH) and royal jelly (RJ) were obtained from beekeepers, immediately after the flowering of rape from the region of Stara Zagora, Bulgaria. During the honey collection period bees were not treated with carbohydrate syrups or antimicrobial drugs. Until the analysis, RH and RJ samples were stored in sterilized jars and sterile plasic tubes at 0-4 °C, respectively. Water content, pH, free acidity, electrical conductivity, diastase and invertase activity, specific optical activity and hydroxymethylfurfurol (HMF) content were assayed as per the harmonized methods of the European honey commission (Bogdanov et al., 1997). The botanical origin of the samples was established by their melissopalynological, organoleptic, physical and chemical characteristics (Oddo et al., 2004; von der Ohe et al., 2004). All data referring to physical and chemical parameters of rape honey were statistically processed by the Student's t-test and presented as mean and standard deviation (SD) (Table 1).

Royal jelly was pipetted directly from queen's cells. The following parameters of samples were determined: sugars (fructose, glucose, sucrose) by HPLC according to Sesta (2006); proteins by Folin-Ciocalteu reagent; water content by refractometry; dry matter of the sample by subtracting the water content from 100; pH values -potentiometrically by pH meter Mi 150 (1% water solution of royal jelly); total acidity by titration with 0.1 N NaOH according to ON 2576693-84 about fresh and lyophilized royal jelly; electrical conductivity of 1 % water solution of royal jelly by conductometer (11, 15) (Table 2). All royal jelly samples were kept refrigerated at -20 °C in dark bottles. Solutions containing 10, 20, 30 and 40% (v/v) of each test substances were prepared in sterile Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany). To prevent photodegradation of glucose oxidase which is associated with antimicrobial activity in honey (Balkanska et al., 2012), all test substances were stored in the dark and dilutions were prepared immediately prior to testing (Sherlock et al., 2010).

Table 1. Physicochemical parameters of rape honey

Parameters	Mean	SD	Maximum	Minimum
Water content (%)	16.8	0.2108	17	16.6
Free acidity (meq.kg^{-1})	36.3	1.1595	38	35
pH	3.232	0.01032	3.25	3.22
Conductivity (mS.cm^{-1})	0.128	0.00105	0.13	0.127
Diastase activity (Ghote), (DN)	12.9	0.1051	13.1	12.8
Hydroxymethilfurfurol (HMF), (mg.kg $^{-1}$)	14.89	0.3528	15.36	14.4
Invertase activity (IN)	10.643	0.0241	10.69	10.62
Specific optical rotation, $[\alpha]_D^{20}$	(–) 12	0.8164	(–) 13	(–) 11

Table 2. Physicochemical characteristics of royal jelly

Parameters	Mean	SD	Maximum	Minimum
Water content (%)	62.7	1.43452	63.7	60.2
pH	3.97	0.07776	4.06	3.78
Total acidity (mL 0.1n NaOH/g)	4.08	0.38084	4.51	3.31
Electrical conductivity (μS/cm)	197	14.0791	224	180
Proteins (%)	16.94	1.37065	19.36	14.81
Fructose (%)	4.83	0.75832	6.19	3.59
Glucose (%)	3.85	0.99522	5.65	2.7
Sucrose (%)	1.70	0.86652	4.25	0.64

Bacterial strains and preparation of inoculum

Three MRSA isolates belonging to Prof. D. Sergelidis collection were used in our study. These isolates belonged to spa types t127 (isolated from goat carcass), t4038 (isolated from unpasteurized goat's milk) and t548 (isolated from marinated anchovies). They were stored in cryo-tubes containing Tryptone Soy broth (Merck, Darmstadt, Germany) supplemented with 15% glycerol at -80 °C. Prior to experiments the MRSA strains were incubated for 35 °C in TSB (Merck, Darmstadt, Germany) for 24 h and then a loopfull was streaked onto Blood agar and incubated for 24 h at 35 °C. Three to four colonies were taken from the Blood agar and suspended in 5 mL sterile physiological solution for preparation of bacterial suspension adjusted to the 0.5 McFarland standard ($1.5x10^8$ CFU/mL). Decimal dilutions to 10^{-4} in 9 mL sterile TSB were prepared from the initial suspension.

Experimental design

Prior to the experiment, 50% (w/v) dilutions of RH, RJ and RH and RJ mix in TSB (Merck, Darmstadt, Germany) were prepared. Thereafter, serial dilutions of the 50% stock solutions were prepared in order to obtain 40%, 30%, 20% and 10% (v/v) RH, 30%, 20% and 10% (v/v) RJ, and 40%, 30%, 20% and 10% RH and RJ mix, respectively. TSB (Merck, Darmstadt, Germany) was used as a control.

The tubes were inoculated with the bacterial cultures from each MRSA isolate according to the method described by Patton et al. (2006). The inoculated tubes were incubated at 35 °C for 48 h. In order to the determination of survived staphylococci after 24 and 48 h, serial 10-fold dilutions in 0.1% peptone water supplemented with 2.5% NaCl were prepared. Thereafter, 0.1 mL from each tube was spread plated onto Baird Parker agar (Merck, Darmstadt, Germany) containing 0.0025% w/v potassium tellurite and rabbit plasma fibrinogen. Typical *S. aureus* colonies were counted after incubation at 35 °C for 24 h. For the detection of survivors at populations lower than 10 CFU/g, the first dilution was incubated for enrichment at 35 °C for 24 h and then 0.1 mL was spread plated onto Baird Parker agar.

The experiment was performed twice and the results are presented as mean values.

Results and Discussion

There were not survived cells of MRSA t127 (3.34 $\log_{10}$ reduction) after 24 h incubation in TSB with 40% RH, with 20 and 30% RJ and with 30 and 40% mix of RJ:RH (1:100). A reduction of 1.95 $\log_{10}$ and 1.23 $\log_{10}$ was observed in TSB with 30% RH and 20% RJ:RH after 48 h incubation (1:100) (Table 3). The counts in the other concentrations of all substances were more than 8 $\log_{10}$ after 48 h.

A reduction of 3.17 $\log_{10}$ of MRSA t548 was observed after 24 h incubation in TSA with 10, 20 and 30% RJ and with 40% RJ:RH (1:100) (Table 4). Although a reduction almost 3.17 $\log_{10}$ was observed after 24 h in TSA with 40% RJ:RH (1:100), staphylococcal cell count reached 3.54 $\log_{10}$ at 48 h. The counts in the other concentrations of all substances were more than 7 $\log_{10}$ after 48 h.

The population of MRSA t4038 was reduced by 3.2 $\log_{10}$ after 24 h in TSB with 40% RH, 10, 20 and 30% RJ, 20-40% RJ:RH (1:100), and after 48 h in TSB with 30% RH and again with 20, 30 and 40% RJ:RH (1:100) (Table 5). The population reached 7 $\log_{10}$ after 24 h incubation in TSB with 10 and 20% RH and then after 48 h incubation it declined to 3.47 and 3.26 $\log_{10}$, respectively. In all other cases the population was grown by at least 7 $\log_{10}$. In general, there are not many references in the international literature on the antimicrobial activity of honey and royal jelly, and particularly for the *S. aureus* and MRSA.

Table 3. Antibacterial activity of Rape Honey (RP), Royal Jelly and mix RJ:RH (1:100) at several concentration in Tryptone Soy broth (TSB) against MRSA t127

Substance	Concentration	Initial inoculum	Counts after 24h	Counts after 48h
RH	10%	3.34 CFU/mL	>8 log CFU/mL	>8 log CFU/mL
	20%		>8 log CFU/mL	>8 log CFU/mL
	30%		3.53 CFU/mL	1.39 CFU/mL
	40%		0	0
RJ	10%	3.34 CFU/mL	>8 log CFU/mL	>8 log CFU/mL
	20%		0	0
	30%		0	0
RJ:RH (1:100)	10%	3.34 CFU/mL	>8 log CFU/mL	>8 log CFU/mL
	20%		3.58 log CFU/mL	2.11 log CFU/mL
	30%		0	0
	40%		0	0

Table 4. Antibacterial activity of Rape Honey (RP), Royal Jelly and mix RJ:RH (1:100) at several concentration in Tryptone Soy broth (TSB) against MRSA t548

Substance	Concentration	Initial inoculum	Counts after 24h	Counts after 48h
RH	10%	3.17 CFU/mL	>7 log CFU/mL	>7 log CFU/mL
	20%		>7 log CFU/mL	>7 log CFU/mL
	30%		>7 log CFU/mL	>7 log CFU/mL
	40%		3.54 log CFU/mL	>7 log CFU/mL
RJ	10%	3.17 CFU/mL	0	0
	20%		0	0
	30%		0	0
RJ:RH (1:100)	10%	3.17 CFU/mL	>7 log CFU/mL	>7 log CFU/mL
	20%		>7 log CFU/mL	>7 log CFU/mL
	30%		>7 log CFU/mL	>7 log CFU/mL
	40%		0	3.47 log CFU/mL

Table 5. Antibacterial activity of Rape Honey (RP), Royal Jelly and mix RJ:RH (1:100) at several concentration in Tryptone Soy broth (TSB) against MRSA t4038

Substance	Concentration	Initial inoculum	Counts after 24h	Counts after 48h
RH	10%	3.2 CFU/mL	>7 log CFU/mL	3.47 log CFU/mL
	20%		>7 log CFU/mL	3.26 log CFU/mL
	30%		3.29 log CFU/mL	0
	40%		0	0
RJ	10%	3.2 CFU/mL	0	0
	20%		0	0
	30%		0	0
RJ:RH (1:100)	10%	3.2 CFU/mL	>7 log CFU/mL	>7 log CFU/mL
	20%		0	0
	30%		0	0
	40%		0	0

The MIC of four varieties of honey from Algeria for *S. aureus* ranged between 20% and 21% (v/v), while the MIC of RJ was 2% (v/v). When honey and RJ were used jointly, all honey varieties had a more than 50% decrease in MIC with 1% (v/v) RJ (Boukraa et al., 2008). In another study in Algeria, the MIC of RJ was 1.7% (vol /vol) against *S. aureus* and 2% against *Escherichia coli* (Boukraa et al., 2009). When starch was added in RJ, a MIC decrease of 61% and 30% against *S. aureus* and *E. coli*, respectively. Manuka honey showed a MIC of 6% and 7% against methicillin-resistant and methicillin-sensitive *S. aureus* (Alzahrani et al., 2012).

The broad spectrum of antibacterial activity of honey is mainly against Gram-positive bacteria (Marcucci et al., 2001) and is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys. The antimicrobial action of the hydrogen peroxide in honey that is produced by glucose oxidase (Dustmann, 1979; Taormina et al., 2001), the high osmolarity (honey consists of 80% w/v of sugars) (Dustmann, 1979), the presence of lysozyme and its high antimicrobial potential (Bogdanov, 1997) are well characterized (Molan, 1992). Recently, methylglyoxal (MGO) in manuka honey and the antimicrobial peptide bee defensin-1 in revamil honey have been identified as important antibacterial compounds (Sesta, 2006; Adams et al., 2008; Mavric et al., 2008).

RJ has shown antimicrobial effects against a wide range of bacteria, viruses, yeast, and fungi (Alreshoodi & Sultanbawa, 2015). It has been reported that RJ has antibacterial activity against both Gram-positive and Gram-negative bacteria due mainly to fatty acids present in RJ, such as trans-10-hydroxydec-2-enoic acid, 3-hydroxydodecanoic acid, 11-oxododecanoic acid, and 11-S-hydroxydodecanoic acid (Melliou & Chinou, 2005; Alreshoodi & Sultanbawa, 2015). Furthermore, a series of short peptides (jelleines, royalisin) present in RJ have also been shown to possess strong antibacterial properties against Gram-positive and Gram-negative bacteria and yeasts (Fujiwara et al., 1990; Fontana et al., 2004; Alreshoodi & Sultanbawa, 2015; Bilikova et al., 2015).

Conclusion

As the development of antibiotic-resistant bacteria spreads and an increasing interest in the alternative use to antibiotics therapies has been developed, bee products may receive renewed recognition as healing agents. In general, honey and royal jelly, alone or in combination, appear to have a potential as alternative therapeutic agents against MRSA infections, but clinical studies are needed for confirmation.

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FULL PAPER TAM MAKALE

THE MATRIX EFFECT OF TUNA AND MAHI-MAHI ON BIOGENIC AMINE DETECTION

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Abstract:

Method of standard addition (MSA) is used to account for and quantify matrix effect. In this study, MSA was used to analyze the matrix effect of mahi-mahi and tuna in respect to biogenic amine detection. Two different detection methods were used in conjunction with the MSA procedure: a colorimetric strip that detects volatile biogenic amines and a histamine-specific ELISA (enzyme-linked immunosorbant assay), which detects histamine in the liquid phase. Trained sensory experts were utilized to grade mahi-mahi and tuna. The grading system acted as a measure of fish quality. A biogenic amine cocktail was created to act as the standard spike for the MSA procedure. It contained histamine and other biogenic amine compounds of varying volatility that are present in nature. The ELISA-MSA showed a clear correlation between increase in histamine and a lower quality sample (higher numerical grade). The colorimetric strips did not have a clear correlation between biogenic amine content and grade. Comparing the two methods demonstrates that the matrix effect of the tuna and mahi-mahi is likely more prominent with gas phase compounds than liquid phase compounds.

Keywords: Biogenic amines, ELISA, Histamine, Mahi-Mahi, Tuna

Introduction

Histamine and other biogenic amines are found in food products and can have toxicological implications. These compounds are known to affect various biological systems including the respiratory, gastrointestinal, cardiovascular, hematological, and immunological systems (Prester, 2011). Other biogenic amines of toxicological interest include tyramine and cadaverine. These compounds compete with histamine for oxidation by histamine metabolizing enzymes, thus increasing the toxicity of histamine itself (Halász, 1994). Histamine is a major food safety concern as the compound is heat-stable and thus not affected by any cooking process (Ahmed, 1991). The primary illness associated with the presence of histamine in fish is scombroid poisoning.

Scombroid poisoning has a number of symptoms ranging from nausea, vomiting, GI distress, and many signs of an anaphylactic attack like swelling and itching. Although rare, these symptoms can be severe enough to result in death. The symptoms appear quickly, normally within an hour of consuming the spoiled fish (CDC, 2013). Scombroid poisoning was initially associated with *Scombroidae* fish (including tuna and mackerel) but other vectors have been identified outside of this classification including mahi-mahi. Tuna and mahi-mahi are the two most implicated causes of scombroid poisoning in the US (CDC, 2013).

The principle seafood product attributed to scombroid poisoning is spoiled fish, mainly tuna species that naturally contain high levels of histidine (a precursor of histamine). The conversion of this amino acid to the toxic biogenic amines is carried out by certain bacterial species including *Morganella morganii*, *Klebsiella pneumonia*, *Hafnia alvei*, *Pseudomonas putrefaciens*, and *Clostridium perfringens* that contain the histidine decarboxylase enzyme (Ashie, 1996; Hidalgo and others, 2013). Moderate temperature and low pH favor the formation of histamine (Hidalgo and others, 2013). Histamine does not exist in even distribution within the fish muscle. Instead, it is found in highest concentration in areas around the dorsal and interior muscles (Yamanaka, 1982).

Imported fish (particularly mahi-mahi) have been the source of many scombroid poisoning outbreaks in the United States. The higher ambient water temperature of the tropics and poor handling conditions likely contribute to the formation of histamine (Ahmed, 1991). This helps to explain how non-scombroid species can be vectors for histamine poisoning.

A matrix effect occurs when the components of the sample other than the analyte interfere with the determination of the target analyte's concentration. To account for the matrix effect and produce an accurate quantification of the analyte, the method of standard additions (MSA) is often used. This method consists of measuring the analyte within the sample before and after the addition of known amounts of analyte standard (or "spikes") are added. The responses of the spiked and unspiked samples are plotted and regressions are used to determine the analyte concentration in the original sample (Saxberg, 1979). The FDA often recommends this method for measuring furan content in food samples (Altaki, 2007). However, MSA has mostly been used for liquid samples or liquid extracts. The MSA method should be able to be used for gas phase detection methods, given that gas phase quantitation has been extensively studied. Pinho and others (2002) have shown how volatile measurement of solid-liquid matrices can be done using solid-phase microextraction (SPME) to measure volatile free fatty acids in cheeses.

The aim of this study was to determine the concentration of biogenic amines in tuna and mahi-mahi using a MSA procedure via volatile measurement of biogenic amines and histamine. The hypothesis was that the MSA procedure would allow for the determination of matrix effect of the fish tissue along with the quantification of biogenic amines and histamine in both mahi-mahi and tuna samples.

Materials and Methods

Samples and Sensory Grading System

Frozen mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) filets were obtained from waters in the South Pacific near Indonesia, and were evaluated by up to five FDA and National Marine Fisheries Service (NMFS) sensory experts during a grading session according to the standard sensory grading system of the FDA ORA Laboratory Manual (FDA, 2013). The fish filets were received from the FDA/NMFS frozen, individually vacuum packed, and shipped overnight on dry ice. Each filet had been evaluated

by sensory experts and graded on a 1 through 7 scale, representing the quality of the fish. This grading system is based on olfaction. Each filet was rated on a 100-point scale and ranked into one of seven grades. The cutoff between a pass and fail sample is a score of 50, with all scores above 50 being a "pass" (grades 1-4) and all scores below 50 being a "fail" (grades 5-7). The same grading scale was used in the work of Pivarnik et al. (2001).

Storage and Preparation of Fish Samples

Upon arrival, the fish filets were stored at -20˚C. For each grade of fish, seven samples were defrosted overnight at room temperature (~20˚C). The defrosted filets were cut into smaller pieces by hand, and then homogenized twice using a blender (Total Blend Classic WildSide, Blendtec, Orem, UT). In order to assure uniformity of sample, food grade dye was added to the sample to confirm visually that the sample was homogenized adequately. The individual homogenized filets were combined and homogenized again to form a single batch (a "composite" sample) of pureed fish of one grade. Six 50 g samples of the homogenized fish of each grade were used for the method of standard addition experiment. The samples were placed in sealed mason jars allowing for headspace accumulation.

Preparation of Bromophenol Blue (BPB) Strips

A solution was prepared by dissolving 1% bromophenol blue (BPB) (Acros Organics, Geel, Belgium) in 70% aqueous ethanol (Fischer Scientific, Pittsburgh, PA). One-inch squares of filter paper were soaked in this solution for one minute before being allowed to dry for an hour. These strips were used to measure the volatile amine content of the fish samples.

Colorimetric Evaluation – BPB Strips

The sample exposed BPB indicator strips were measured using a colorimeter (Chroma Meter CR-400/410, Konica Minolta, Tokyo, Japan) on the L*a*b* scale. Unexposed indicator strips were also measured using the colorimeter as the control to the exposed strips. The difference in b* value between the unexposed (control) and exposed strips was calculated. Using the biogenic amine standard curve (Figure 1), the ppm of volatile biogenic amines in the samples were detected and calculated.

Biogenic Amine Standard Curve – BPB Strips

A biogenic amine standard curve was created using the biogenic amine standard cocktail (histamine, cadaverine, tyramine, dimethylamine, trimethylamine) of four different concentrations (0, 10, 15, 20 and 50 ppm) in Milli-Q water (EMD Millipore Synergy Ultrapure Water Systems, Darmstadt, Germany). The BPB strips were exposed to the solutions for 60 minutes in a sealed jar. The b* value difference between the unexposed BPB indicator strips and the exposed BPB strips was calculated to produce a standard curve to confirm that the response of the aqueous biogenic amine solutions increased with concentration (Figure 1).

BPB Method of Standard Addition (MSA) Procedure

A biogenic amine standard cocktail was made with equal concentrations of histamine (Sigma-Aldrich, St Louis, MO), cadaverine (Fluka, Switzerland), tyramine (Acros Organics, Geel, Belgium), dimethylamine (Acros Organics, Geel, Belgium), and

trimethylamine (Acros Organics, Geel, Belgium). Three different concentrations of this cocktail were used: 10 ppm, 20 ppm, and 50 ppm. The cocktail was created and diluted with Milli-Q water. Duplicate samples were run for each concentration of standard cocktail. The spike cocktail was mixed with the sample fish in a 1:1 ratio (50 g fish to 50 mL spike solution). The unspiked sample (initial reading) was 50 g of homogenized fish diluted with 50 mLs of Milli-Q water. Next, the BPB indicator strips were added to the jars. The samples were heated in 45˚C water bath (Isotemp 220, Fischer Scientific, Pittsburgh, PA) for 60 minutes. The calculated b* value was graphed against the ppm of standard cocktail spike for each grade. A standard addition plot was used to determine the concentration of biogenic amines in each unspiked sample by extrapolation of the equation of the line to the x-axis.

Histamine ELISA Method of Standard Addition (MSA) Procedure

The AOAC (No. 070703) validated Neogen Veratox ® test kit (Neogen Corp, Lansing, MI) is a histamine-specific ELISA test that was utilized in this study. In accordance with the kit instructions, 10 g samples of tuna or mahi-mahi were diluted with a 10 mL spike of each standard cocktail concentration and 80 mL of Milli-Q water. The procedure was performed in duplicate for each spike

concentration. The standard solutions provided with the Veratox ® kit and standard biogenic amines were used to construct separate standard curves. The concentration of histamine was calculated both by using the software provided by the manufacturer (Standard ELISA), and graphically using the equation of the line. The ELISA-MSA procedure using the equation of the line established the point where the line intersected the x-axis, thus giving the concentration of histamine in the unspiked sample. Unspiked samples (5 replications per grade) were also analyzed using the ELISA assay. The results of the ELISA assay given by the manufacturer software are reported to the third decimal place. Therefore, calculated histamine concentrations have four significant figures (Ebbing and Gammon, 1998).

Results and Discussion

Two standard curves were created for the biogenic amine cocktail. Figure 1 shows the standard curve for the cocktail using the BPB strip method. The b* score is the difference between the b* values of the unexposed and exposed strips. The b* value of the colorimeter represents a scale from yellow to blue; the more yellow hue present, the more positive the b* value, alternatively the more blue hue present, the more negative the b* value. With higher amine concentration, the BPB strips become more blue and as a result the b* difference score (difference between the unexposed and exposed strips) increases as the concentration of volatile biogenic amines increases. Figure 2 shows the standard curve for the cocktail using the ELISA kit. Table 1 shows a comparison between the actual histamine concentration as part of the biogenic amine cocktail and the experimentally determined histamine values by the ELISA kit assay. This was done to observe if there was any cross-reactivity with the other biogenic amines present in the cocktail. It should be noted that the actual histamine ppm represents the one-fifth portion of the overall cocktail concentration that is histamine. Multiple studies have shown that there are no significant cross-reactivity issues with the Neogen kit (Lupo and Mozola, 2011; Hungerford and Hu, 2012). The results of this study are consistent with these previous conclusions.

When comparing Figures 1 and 2, the shapes of the two curves differ. Figure 2 shows a direct linear relationship between concentration of the cocktail and concentration of detected histamine. The graph in Figure 1 lacks this distinctive shape; and the response is possibly an exponential relationship. This could be due to a number of reasons. First is the quantitative limit for the colorimeter and the strips themselves (the ability to differentiate between two sets of values). As the concentration of the cocktail approaches 50 ppm, one of two situations is likely occurring: the strips have reacted completely and can no longer change to a more intense blue color or the colorimeter has reached its detector saturation limit for the b* value (the ability to measure a very negative b* value). Therefore, it may have been possible the colorimeter could not accurately measure the pronounced blue color produced by a higher volatile amine concentration. Dye impregnated membranes have previously been used to indicate freshness in other products (Kuswandi, 2013). This type of technology is established, but quantitation issues many have limited its application in this particular situation.

Table 1. Expected vs. Actual Results of ELISA Cocktail Standard Curve.

Cocktail Concentration (ppm)	Actual Histamine Concentration (ppm)	Experimentally Determined Histamine – Standard ELISA (ppm)
10	2	3.894
15	3	5.496
20	4	7.358
50	10	15.91

Table 2. Mahi-Mahi Volatile Biogenic Amine and Histamine Results.

Grade	Volatile Biogenic Amines (ppm) – BPB-MSA	Histamine (ppm) – ELISA-MSA	Histamine (ppm) – Standard ELISA
M-1	35.17	3.389	8.399 ± 2.9
M-2	34.67	0.4086	0.65 ± 0.20
M-3	81.49	0.7989	3.690 ± 1.5
M-4	41.83	0.3830	1.261 ± 0.23
M-5	33.53	0.9583	1.438 ± 0.32
M-7	99.05	24.21	155.3 ± 20

* ± Standard error (SE) is reported only for the Standard ELISA procedure since an MSA procedure does not permit the computation of SE.

Table 3. Tuna Volatile Biogenic Amine and Histamine Results.

Grade	Volatile Biogenic Amines (ppm) – BPB-MSA	Histamine (ppm) – ELISA-MSA	Histamine (ppm) – Standard ELISA
T-1	25.37	0.0867	0.2362 ± 0.072
T-2	256.0	0.0130	0.1329 ± 0.13
T-3	58.60	0.0663	0.789 ± 0.27
T-4	111.0	0.0753	0 ± 0.0
T-5	194.6	5.239	81.99 ± 28
T-6	44.50	5.090	197.7 ± 71
T-7	27.10	0.5320	36.09 ± 6.8

* ± Standard error (SE) is reported only for the Standard ELISA procedure since an MSA procedure does not permit the computation of SE.

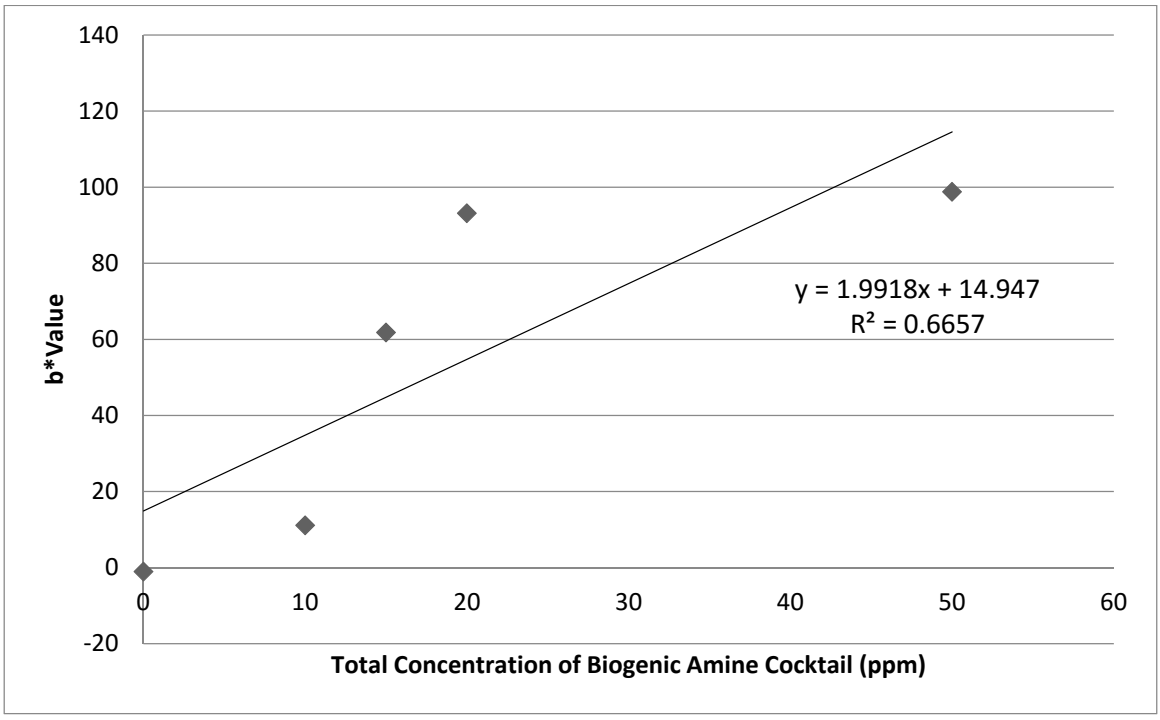

Figure 1. Biogenic Amine Cocktail Standard Curve – BPB Strips.

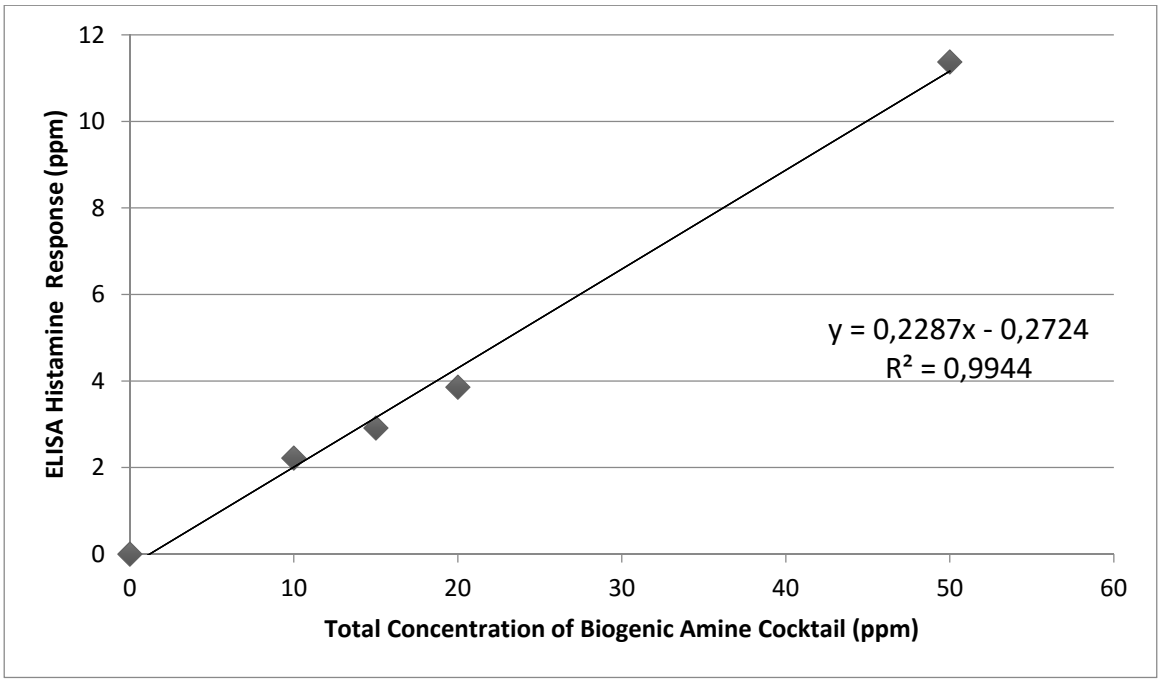

Figure 2. Biogenic Amine Cocktail Standard Curve – ELISA Assay.

The class and phase of analyte being measured can account for these differences. The BPB strips measure gas phase biogenic amines while the ELISA kit measures liquid phase histamine. Ashie (1996) showed that a number of compounds, including trimethylamine, make up the volatile aroma mixture given off by fish. This is part of the reason why the standard cocktail used in this study contains more than just histamine and why the strips can be considered a non-specific assay. Because the grading of the fish is performed via olfactory detection, multiple volatile compounds were needed to best formulate a legitimate spiking solution. The ELISA assay, however, measures a specific compound, rather than a class of compounds. The phase of detection of this assay also could impact its effectiveness in this situation. Rather than the analyte being driven off by heat, as is the case with the BPB strip method, the histamine is directly extracted in the liquid phase from the fish, yielding a more direct means of detection.

The differences in the target analyte and detection method are likely responsible for the difference in the two standard curves when comparing the two assays. Given the nature of the MSA methodology, linearity of the graph is important for providing an accurate calculation of given analyte. When comparing the two assays, BPB strips and ELISA, the ELISA assay produced a curve with better linearity and better curve fit (Figures 1 & 2). This is likely due to the nature of the assay as discussed above and how it more accurately measures a specific compound (histamine) versus a class of compounds.

For the majority of the ELISA-MSA graphs used to calculate the histamine content in the unspiked samples, the linearity was ideal. Any poor linearity for the ELISA graphs may be due to the limit of quantitation with the Neogen assay. As the grades of samples approached the "fail" range, extra dilution steps were necessary to bring the sample back within the ideal detection range for histamine (2-40 ppm). The kit is more reliable within the manufacturer specified detection range; any samples analyzed that exceed this range (despite further dilution) are more likely to be inaccurately measured.

Graph linearity and the limit of quantitation of the assays need to be taken into account when analyzing the calculated ELISA-MSA values in the right hand columns of Tables 2 and 3. The more accurately calculated concentrations are for Grades 1-5 in Table 2 given the linearity of the graphs that were produced. Similarly, the calculated values in Table 3 are also likely more accurate for the "pass" grades. This trend for both mahi-mahi and tuna samples demonstrates the limit for quantitation for the ELISA assay.

In Table 3, we see an anomaly with the Grade 7 tuna: it has a much lower calculated histamine

concentration than the other two "fail" grades (Grades 5 and 6). This could be caused by an initial concentration that exceeded the detection range of the assay. The Grade 7 tuna samples underwent 3 extra dilutions to put it in the range of quantitation, more than any other sample grade. The limit of quantitation may explain the trend shown in Table 3. As for Table 2, Grade 1 of the mahi-mahi has higher histamine concentration than any other grades excluding Grade 7.

Both Tables 2 and 3 show the results of the ELISA-MSA and standard ELISA procedures. When comparing the standard ELISA values to the ELISA-MSA values, most of the standard ELISA values are higher than those found in the ELISA-MSA procedure. This may be attributed to the methodology of how the ELISA-MSA (spiked) concentrations were calculated and demonstrates why linearity is a major factor in producing accurate results with the MSA assay.

Overall, with both ELISA assays, greater histamine concentrations are seen in lower quality samples (e.g. Grades 5, 6 and 7) as expected. In comparison, there is no true trend with the BPB-MSA data. Lack of a linear increase of analyte as spoilage increases may be attributed to the poor linearity of the graphs used to calculate the volatile biogenic amine contents, which relates back to the overall matrix effect. Others have reported the less linear the MSA graph is, the stronger the effect of the matrix or other interfering compounds that inhibit the selectivity of the matrix (Saxberg, 1979). When comparing the different natures of the two assays and the results derived from them, it can be deduced that the matrix effect is much more prominent for gaseous phase (volatile) compounds than liquid phase compounds.

The BPB-MSA results may be explained due to the nature of volatile compounds: volatile compounds tend to be lipophilic (lack hydrophobicity) and therefore more likely to be bound in the flesh than present in the gas phase (Druaux and Voilley, 1997). Comparing the MSA and ELISA methodologies demonstrates this principle. The data for the ELISA (liquid-phase) assay followed more of an expected pattern than for the BPB strips (gaseous-phase assay) likely due to the fact that there was more of the histamine present in the flesh (histamine is non-volatile), not bound by protein (histamine is formed from unbound histidine) and present mostly in the lipid portion of the fish. The results reported by Lerke (1978) demonstrated that histamine is found in highest concentrations in the lipid portion near the gut cavity. Both free histidine and histamine is found in greater concentration in the fattier red muscle tissue (Hultin, 1984; Lukton and Olcott, 1958; Takagi et al., 1969). A major concern as fish spoil is protease breakdown, which leads to muscle softening (Hultin, 1984). Bacterial spoilage both directly causes formation of histamine (Yamanaka, 1982) while also indirectly breaking down protein through the formation of trimethylamine oxide (Hultin, 1984). The bacterial breakdown of the protein portion of the fish by the protein creates a positive feedback loop as it provides an ideal environment for further microbiological growth (Lerke, 1967). The breakdown of the flesh through spoilage, and release of biogenic amines that results, likely overwhelms the BPB assay and leads to poor results for lower quality (higher grade) samples.

Conclusion

The ELISA-MSA results demonstrated an increase in histamine content as the quality of either tuna or mahi-mahi decreased. A comparison of the two assays (BPB strips and ELISA) showed that the matrix effect was much stronger for volatile compounds than aqueous compounds. The lipophilic nature of aromatic compounds helps partially account for the discrepancy between the results of the two MSA assays. The ELISA assay seemed to be better adept at extracting and detecting the desired compounds, it provided a more accurate representation of the trend in the fish samples; that is, histamine increases as quality decreases. Given these conclusions, the sensory grading system does represent what it intends to: a higher quality fish being safer to consume. However, the fact that these grades are based upon an orthonasal panel draws into question how accurate orthonasal measurement is for fish when there seems to be such a strong matrix effect with the volatile compounds. Further research should determine what proportion of volatile spoilage indicators (biogenic amines in this case) are present in the liquid and gaseous phase at each grade to assure that grading accurately reflects the safety of the product.

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FULL PAPER TAM MAKALE

ISOLATION OF *Salmonella* spp. AND OTHER MEMBERS OF *Enterobacteriaceae* FROM HORSE MACKEREL (*Trachurus trachurus*), SOLD IN PUBLIC MARKETS OF ISTANBUL, TURKEY

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Abstract:

Aim of this investigation is to provide the general information about prevalence of members of *Enterobacteriaceae* in horse mackerel (*Trachurus trachurus*) sold in open public markets in Istanbul, Turkey. Horse mackerel were randomly selected and collected in warm and cold seasons of the year. Purchased samples were kept in sterile insulated bags with ice and transported to the laboratory for microbiological analyses. *Citrobacter* spp. showed highest prevalence (45.56%), followed by *Proteus mirabilis* (22.62%), *Proteus vulgaris* (9.17%) and *Escherichia coli* (7.64%). The other isolates were *Shigella sonnei* (4.28%), *Shigella dysenteria* (3.36%), *Salmonella* Typhimurium (1.83%), *Salmonella* Paratyphi A (1.52%), *Klebsiella pneumoniae* (1.22%), *Klebsiella oxytoca* (1.22%), *Enterobacter aerogenes* (0.91%) and *Enterobacter cloacae* (0.61%). The percentages of the isolates, obtained in spring and autumn, were also compared. The *Citrobacter* spp., *P. mirabilis* and *P. vulgaris* were the dominantly isolated species, during spring season. *P. mirabilis* and *Citrobacter* spp. were also dominant in autumn. However, numbers and percentages of isolated pathogens (*E. coli, S. sonnei, S. Dysenteriae, S.* Typhimurium *and S.* Paratyphi A*)* were much more in autumn than that of spring. Since samples are sold without any chilling treatment in public markets, air temperature might be the reason of higher pathogen isolation in autumn. It is essential to implement cold chain as well as to prevent secondary contamination and to improve quality control.

Keywords: *Salmonella, Enterobacteriaceae*, Pathogen, Fish, Contamination, Public market

Introduction

The members of *Enterobacteriaceae* family are widespread in the environment and their natural habitat is gastrointestinal tract of warm-blooded animals. They may play an important role in the fish spoilage and some bacterial species of this family are very pathogenic (Guiraud, 2003; Shabarinath et al., 2007; Lopez Da Silva et al., 2010). Pathogens such as *Salmonella* spp., *Shigella spp*. and certain *E. coli* can cause severe diarrhea (Lindberg et al., 1998). Especially *Salmonella* is dominating intestinal tract of animals (birds, reptiles, and farm animals) and humans. *Salmonella* species are extensively distributed in nature (water reservoirs, coastal water contaminated with human or animal feces) and causing outbreaks worldwide. According to Centers for Disease Control and Prevention (CDCP), almost 400 persons die every year with acute Salmonellosis. This bacterium has been isolated from fish and other seafood (Jay, 2000a; Huss et al., 2004; CDCP, 2010). It may be transferred to the seafood due to the poor hygienic conditions during transportation and marketing (Temiz, 1998). Prevalence of *Salmonella* and some bacterial species, belonging to the *Enterobacteriaceae* family, have been studied in different regions of the world and health risks were evaluated. Microbiological quality of seafood in Croatia (Topic Popovic et al., 2010), fish in Khartoum, Sudan (Yagoub, 2009), fish and crustaceans in Coimbatore, India (Hatha and Lakshmanaperumalsamy 1997), shrimp in India (Hatha et al., 1998; Jonnalagadda and Bhat, 2004), seafood in Greece (Papadopoulou et al., 2007), cooked shellfish in UK (Sagoo et al., 2007), hygiene conditions of wholesale fishmarket in Istanbul (Ucok, 2003), microbiological quality of stuffed mussel in Istanbul (Bingol et. al., 2008), determinetion of pathogen microorganisms in seafood in Istanbul (Ucok Alakavuk, 2009) and quality of fish from retail markets in Istanbul (Mol and Tosun 2011), were studied.

Public markets are the main suppliers of fish and other seafood to the public. However, fish and other seafood are sold in Turkish public markets without chilling. Since high environmental temperature is the most important reason to encourage bacterial growth (Jay, 2000b) warmer seasons are very risky for the microbial safety of fish, displaying on the counter without any chilling treatment. On the other hand, air temperature in Istanbul City is between -2 to 5.7°C in winter, 5.5 to 17.06°C in spring, and 7.2 to 19.36°C in autumn (Turkish State Meteorological Service (TSMS), 2013). Then, the warmer seasons (spring and autumn) are the most risky periods, regarding the growth of *Salmonella* and other members of *Enterobacteriaceae* on fish, sold in public markets without chilling. Horse mackerel (*Trachurus trachurus*), a common fish in Turkish waters, having an annual catching value of 12213.2 ton (TUIK, 2015), and this catch has been offered for domestic market as well as export market. Since it is very popular for Turkish consumers, horse mackerel is one of the top selling fish in public markets. The aim of this study, is the determination of *Salmonella* and members of *Enterobacteriaceae* on horse mackerel, sold in public markets in Istanbul.

Materials and Methods

Sample collection

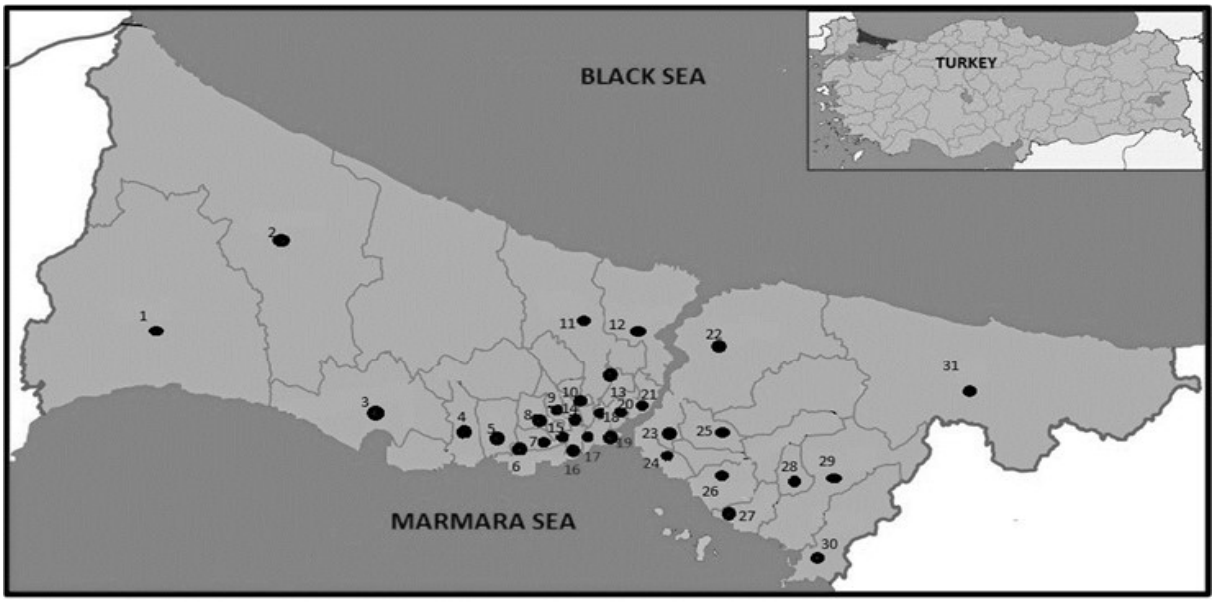
The average air temperatures were 13.7°C in spring and 15.75°C in autumn, during this study. The highest air temperatures in spring and in autumn were 22.2°C, and 26.0°C, respectively. Horse mackerel samples (16.03 ±5.91g, 11.76 ±1.24cm) were randomly purchased from the public markets in 31 districts of Istanbul (Figure 1) twice in spring and twice in autumn. Therefore, sampling was performed 124 times. Thirty individuals of horse mackerels were purchased from each public market (31 markets). Therefore, a total of 3720 fish samples (30 individual's x 124 times) were used in this investigation. Samples were collected from public markets between the hours of 09:00-12:00, they were kept in sterile insulated bags, iced and transported to the laboratory in less than 3 hours.

Microbiological examination

The samples, purchased from each of 31 public markets were homogenized separately. Then, 25 g of homogenate was added to the enrichment broth (225 mL Lactose Broth, Merck, 1.07661.0500), and incubated at 37°C for 24-48 hours. 0.1 mL homogenate from enrichment broth was transferred into the 10 mL of Rappaport-Vassiliadis Broth (Merck 1.07700.500) and then incubated at 42°C for 24 hours for the selective enrichment. At the same time 1 mL homogenate from lactose broth was transferred in to 10 mL Tetrathionate Broth (Merck 1.05285.0500) and then incubated for 24 hours at 43°C. After the incubation, a loopful of broth cultures were streaked onto XLT4 Agar (Merck 1.13919.0500) and Bismut Sulfite Agar

(Merck 1.05418.0500). Plates were incubated at 35°C for 48 h. After icubation, black zone all colonies (XLT4) and black center with metallic sheen colonies (BSA) streaked on TSI (Triple Sugar Iron) Agar (Merck 1.03915.0500) and LI (Lysine Iron) Agar (Merck 1.11640.0500). TSI and LI slant agar were incubated at 35°C for 48 h. and at 35°C for 16-24 h. respectively. All slant agar tubes were tested biochemically (motility, urea, H_2S, MR-VP, indole, ONPG, lactose, sucrose, mannitol, malonate, sitrat) after incubation. All salmonella cultures were confirmed by using Salmonella Latex Kid (OXOID FT 0203,0204, 0205) after biochemical tests. (Andrews et al., 2007). *Enterobacteriaceae* colonies were identified by using a scheme 'Caracteristique des principales *Enterobacteries'* (Guiraud, 2003).

Figure1. Public markets in 31 districts of Istanbul



Results and Discussion

Some members of *Enterobacteriaceae* such as *Salmonella sp., E. coli, Proteus sp.* and *Klebsiella sp.* may cause serious infections. Therefore, monitoring of *Enterobacteriaceae* in seafood is important for public health (Al-Mutairi, 2011). Out of 124 sampling 77 (62.10%) showed a positive growth for *Enterobacteriaceae* (Table 1). Percentages of isolates, from *Enterobacteriaceae* positive samples were 69.72% in spring and 30.27% in autumn (Table 2). The isolated species were *S.* Typhimurium, *S.* Paratyphy A, *E. coli*, *S. dysenteriae*, *S. sonnei, Citrobacter* spp., *P. mirabilis*, *P. vulgaris, K. pneumonia*, *K. oxytoca, E. aerogenes* and *E. cloacae.* The numbers and percentages of bacterial species, isolated from horse mackerels during in spring and autumn were shown in Table 3. These organisms were isolated from shrimp in India (Jonnalagadda and Bhat, 2004), seafoods in Greece (Papadopoulou et al., 2007), fresh and frozen seafoods in Croatia (Topic Popovic et al., 2010), fresh fish in Sudan (Goja, 2013), similarly. Some of the potential pathogenic microorganisms (*Citrobacter* spp., *P. mirabilis* and *P. vulgaris)* were isolated during spring season. *Citrobacter spp.* (42.81%), *P. mirabilis* (14.67%) and *P. vulgaris* (8.25%) were mostly determined in spring. *P. mirabilis* (7.95%) *and Citrobacter spp.* (2.85%) were also determined in autumn. However, pathogenic species such as *S.* Typhimurium (%1.83), *S.* Paratyphy A (1.52%), *E. coli* (7.33%), *S. dysenteriae* (3.05%), *S. sonnei* (3.66%) were dominant in autumn (Table 3). Therefore, even the percentage of isolates was higher in spring; autumn was considered as a more risky season. The high percentage of pathogenic bacteria in autumn might be attributed to the suitability of air temperature for their survival and multiplication. Absence of

chilling treatment in public markets allows bacterial growth as well. Yagoub, (2009) reported the highest presence (66%) of *Enterobacteriaceae* from raw fish from a market in autumn, similarly. They have isolated some highly pathogenic agents such as *Salmonella* spp., *Shigella* spp., and the pathogenic *E. coli*. They have mentioned about the possible public health risks, due to this microbial activity in the autumn. Maintenance of sanitation and temperature control are very important for microbial quality, especially in warmer seasons due to the high ambient temperature (Mol and Tosun, 2011).

Salmonella spp. are one of the most important reasons of gastrointestinal diseases. Maintenance of personal hygiene is needed to prevent transmitting of these bacteria, since gastrointestinal tract is the main reservoir of them (Huss et al., 2004). According to European Union (EU) regulations on 25 g of seafood must not contain *Salmonella sp*. (Forsythe, 2010a). In this study, samples were free of *S.* Typhimurium and *S.* Paratyphi A in spring season (Table 3). However, 1.83% of the samples contained *S.* Typhimurium and 1.52% of them were *S.* Paratyphi A positive in autumn. Likewise, 2.8% of raw seafood were *Salmonella* positive, according to Heinitz et al., (2000). Brands et al., (2005) reported a higher percentage of isolated *Salmonella* from oysters in the summer (13.4%) than winter (1.6%), similarly. The prevalence of *Salmonella* in shrimp have been reported as 11% and 53%, from Hyderabad, India (Jonnalagadda and Bhat, 2004) and Thailand (Minami et al., 2010). Likewise, 14.25% of the fish, from retail markets in Coimbatore, India and the 15% shellfish from markets in Ho Chi Minh City, Vietnam have been reported as positive for *Salmonella* (Hatha and Lakshmanaperumalsamy, 1997; Hao Van et al., 2007). It may be concluded that, higher air temperature encourages the growth of *Salmonella*. In this study, the presence of a higher amount of *S.* Typhimurium and *S.* Paratyphi A in autumn season, might be the result of higher ambient temperature.

Table 1. Numbers and percentages of samplings, showing a positive growth of *Enterobacteriaceae*

		Positive growth of *Enterobacteriaceae*	**Percentage of occurrence (%)**
Sampling season	**Autumn (n=62)**	29	46.77
	Spring (n=62)	48	77.42
TOTAL (n=124)		77	62.10

Table 2. Numbers and percentages of isolates, from *Enterobacteriaceae* positive samples

Isolated organism	**Total**	**Autumn**		**Spring**	
	Number of isolates	**Number of isolates**	**Percentage (%)**	**Number of isolates**	**Percentage (%)**
Enterobacteriaceae	327	99	30.27	228	69.72

Table 3. Numbers and percentages of bacterial species, isolated from horse mackerel samples

Isolated bacterial species	Autumn		Spring		Total	
	Number of isolates	Percentage (%)	Number of isolates	Percentage (%)	Number of isolates	Percentage (%)
Escherichia coli	24	7.33	1	0.3	25	7.64
***Salmonella* typhimurium**	6	1.83	ND	-	6	1.83
***Salmonella* paratyphi A**	5	1.52	ND	-	5	1.52
Shigella dysenteriae	10	3.05	1	0.3	11	3.36
Shigella sonnei	12	3.66	2	0.61	14	4.28
Proteus mirabilis	26	7.95	48	14.67	74	22.62
Proteus vulgaris	3	0.91	27	8.25	30	9.17
Enterobacter cloacae	2	0.61	ND	-	2	0.61
Enterobacter aerogenes	1	0.3	2	0.61	3	0.91
Klebsiella oxytoca	1	0.3	3	0.91	4	1.22
Klebsiella pneumoniae	ND	-	4	1.22	4	1.22
***Citrobacter* spp.**	9	2.85	140	42.81	149	45.56

ND: Not detected

E. coli has been found in the intestinal flora of humans and warm-blooded animals. This microorganism may transfer to the foods due to the poor hygienic conditions, cross contamination or contaminated water (Huss et al., 2004). In the present study, *E. coli* was one of the dominant bacterial species in autumn; and the percentage of isolated *E. coli* was higher (7.33%) in autumn than that of the samples collected in spring (0.3%) (Table 3). High prevalence of *E. coli* in fish, from local retailers in Greece has been reported by Papadopoulou et al., (2007). Similarly, 6.7% of the fish and seafood samples, from wholesale and retail markets in Seoul, Korea; have been reported as positive (Ryu et al., 2012). Lopez Da Silva et al., (2010) isolated *E. coli* from 10% of fish, from street markets in Sao Paulo, Brazil. On the other hand, the dominant isolate in the fish, from public market in Khartoum, Sudan (Yagoub, 2009) and in the catfish, from different markets in Nigeria (Adebayo-Tayo et al., 2012) has been reported as *E. coli*. Likewise, 48.95% of fish and fish products from Punjab, India (Gupta et al., 2013); and 38.8% of fish and shellfish Mangalore, India (Kumar et al. 2005) have been reported as *E. coli* positive. Ananchaipattana et al., (2012) also reported the highest presence (70%) of *E. coli* from seafood from retail markets (Open and supermarket) in Thailand. It was seen that; a higher isolation rates of *E. coli* have been reported in warmer climate countries.

Shigella species are the natural inhabitants of the intestinal tract of humans (Ray and Bhunia 2008). Poor personal hygiene is one of the main reasons of *Shigella* contamination, and generally the largest number of *Shigella* outbreaks have been seen in the warmer months (Huss et al., 2004). In this study, *S. dysenteriae* (3.05%) and *S. sonnei* (3.66%) were mostly seen in autumn (Table 3). Out of 150 collected samples from a fish market in Sudan, 2.2% showed positive isolation of *Shigella spp.* (Yagoub, 2009). Likewise; David et al., (2009) reported a high prevalence (39.7%) of *Shigella spp*. in fish, harvested from Kenya. Regarding the higher occurrence of *Shigella* spp. in middle climate countries; our results were associated with the higher ambient temperature in autumn.

Proteus species have a high proteolytic activity and they may easily spoil seafood, stored above refrigeration temperatures. *Proteus vulgaris* and *Proteus mirabilis* may cause diarrhea when they infect foods. Poor hygiene rules are the main cause of contamination (Ayhan, 2000). In this study, the percentages of *Proteus mirabilis*, isolated from horse mackerel were 14.67% in spring, and 7.95% in autumn (Table 3). As to *Proteus vulgaris*, the percentages of isolation in spring and autumn were 8.25% and 0.91%, respectively. The isolation of *Proteus* spp. from fish has also been reported in former studies (David et al., 2009; Lopez- Sabater et al., 1996). Yagoub, (2009), isolated *Proteus spp*. in fish with an incidence of 10.2%. The prevalence

of *Proteus* spp. in shrimp from wholesale markets has been reported as 25% by Jonnalagadda and Bhat, (2004).

Escherichia, Klebsiella, Enterobacter and *Citrobater* are the "coliforms". Coliforms are naturally present in the gastrointestinal tract of man and animals. Isolation of these microorganism groups indicates fecal contamination (Forsythe, 2010b). In our study, samples were free of *Klebsiella pneumoniae* in autumn season. *Klebsiella pneumoniae* was isolated 1.22% in spring season. *Klebsiella oxytoca* was isolated 0.3% in autumn and 0.91% in spring (Table 3). The prevalence of *Klebsiella* spp. in shrimps, imported from Thailand has been reported by Navaz et al. (2012). *Klebsiella pneumoniae* was isolated from blue crab (Reinhard et. al. 1996) and tropical marine fish (Singh et al., 2012), in former studies. *Enterobacter cloacae* was isolated 0.61 % in autumn. The samples were free of *Enterobacter cloacae* in spring season. *Enterobacter aerogenes* isolated from horse mackerel samples were 0.61% in spring, and 0.3 % in autumn (Table 3). The presences of *Klebsiella* spp. and *Enterobacter* spp. in fish have been reported as 2.4%, and 10.8% respectively (Yagoub, 2009). Adededji and İbrahim, 2011, and David et al., 2009 have also reported contamination of shrimp and fish with *E. aerogenes*. The present study, the percentage of isolated *Citrobacter* spp. was higher (42.81%) in spring than that of the samples collected in autumn (2.8%) (Table 3). Contamination of seafood with *Citrobacter freundii* (Papadopoulou et al., 2007) and fish with *Citrobacter* spp. (Yagoub, 2009) have reported in former studies. *Citrobacter* spp. are widely distributed in the environment (soil, water, plants). These bacteria can be found in the intestinal flora of humans (Baylis et al., 2011). High prevalence reason for *Citrobacter* spp. may be poor personal hygiene and cross contamination.

Conclusion

It was concluded that horse mackerel, selling without any chilling treatment in the public markets of Istanbul might be a source of primary pathogens and opportunistic pathogens. The opportunistic pathogens were dominantly isolated during spring, while primary pathogens were dominant in autumn. Air temperature was regarded as an important reason of pathogen growth. Microbial contamination of fish selling in public markets can be prevent by good hygiene practice. Results of this study may be helpful to realize inadequacies and may conduce to improve selling conditions.

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GÖRME ENGELLİ ÇOCUKLARDA BESLENME DURUMU, FİZİKSEL AKTİVİTE VE DİYET KALİTESİNİN DEĞERLENDİRİLMESİ

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Öz:

Bu araştırma, yetersiz fiziksel aktivite ve şişmanlık açısından risk altında bulunan görme engelli çocukların beslenme durumlarını, diyet kalitelerini ve fiziksel aktivite düzeylerini değerlendirmek amacıyla planlanmıştır. Araştırmaya Ankara'daki bir görme engelliler ilköğretim okulunda öğrenim gören ve farklı düzeylerde görme kusuru bulunan 6-11 yaş arası 59 çocuk dahil edilmiştir. Aile ve okul öğretmenleri ile görüşülerek anket formu doldurulmuş ve Akdeniz Diyeti Kalite İndeksi (KIDMED) uygulanmıştır. Çocukların boy uzunlukları ve vücut ağırlıkları ölçülerek, beden kütle indeksleri (BKİ) hesaplanmıştır. BKİ sonuçları, Dünya Sağlık Örgütü (WHO) 2007/5-19 yaş referans değerlerine göre değerlendirilmiştir. Çocukların fiziksel aktivite süreleri saat bazında kaydedilmiştir. Günlük uyku süresinin erkek çocuklarda 9.4 ±1.1, kız çocuklarda 9,6 ±1.1 saat olduğu ve çocukların günde yaklaşık 2.8 saatlerini bilgisayar/TV başında ve ders çalışarak geçirdikleri belirlenmiştir. Erkek çocuklarda günlük kalsiyum, posa ve niasin, kız çocuklarda posa, kalsiyum ve enerji gereksinimlerini karşılama oranlarının düşük olduğu saptanmıştır. KIDMED skorlarına göre çocukların %27.1'inin diyet kalitelerinin çok düşük olduğu, %55.9'ünün diyetlerinin iyileştirilmesi gerektiği belirlenmiştir. Tüm çocukların %44'ünün hafif şişman/şişman olduğu bulunmuştur. Görme engelli çocuklarda özellikle açık alanda yapılan fiziksel aktivitelerin sınırlı olduğu, diyet kalitelerinin düşük olduğu ve aynı yaş grubu sağlıklı çocuklara göre hafif şişmanlık/şişmanlık görülme sıklığının bu çocuklarda daha yaygın olduğu gösterilmiştir.

Anahtar Kelimeler: Görme engeli, Beslenme durumu, Aktivite, Diyet kalitesi

Abstract:

Assessment of Nutritional Status, Physical Activity and Diet Quality of Visually Disabled Children

This research was designed to evaluate nutritional status, dietary quality and physical activity of visually impaired children, who have been shown to have ahigh risk for insufficient physical activity and obesity. The research was conducted in a primary school for visually impaired children in Ankara, Turkey. 59 children aged 6-11 years were included, with different degrees of visual impairment. Parents and school teachers helped to complete a questionnaire, from which the Mediterranean Diet Quality Index (KIDMED) was calculated. Height and body weight of the children were measured and body mass index (BMI) calculated. BMI values were evaluated in comparison to the World Health Organization's (WHO) 2007 reference values for children aged 5-19 years. The children's' physical activity time was recorded on an hourly basis. Average sleep duration during the day was 9.4 ±1.1 hours for boys and 9.6 ±1.1 hours for girls. The children spent a mean 2.8 hours per day sitting to play computer games or watch TV. Daily calcium, fiber and niacin intake were low in boys, and daily fiber, calcium and energy intake low in girls. According to the KIDMED score, 27.1% of children had a diet identified as "very low quality diet" and 55.9% were categorized as "needs to be improved". 44% of all children were overweight/obese. Physical activity of visually impaired children is limited, especially outdoor activity. Visually impaired children also have a low quality diet compared with healthy children of the same age group. Furthermore, they have a tangentially higher risk for overweight/obesity.

Keywords: Visual impaired, Nutritional status, Activity, Diet quality

Giriş

Çocukluk çağı, büyüme ve gelişme sürecine özgü besin ögesi gereksinimlerinde meydana gelen değişimlerin yanında, yaşam boyu sürecek beslenme alışkanlıklarının kazanılması açısından büyük önem taşımaktadır (Montero, 2005). Bu dönemde sağlıklı beslenme ile bedensel, duygusal-sosyal gelişim ve okul başarısı/devamlılığı olumlu yönde etkilenmekte, aynı zamanda gerek çocukluk döneminde gerekse yetişkinlik dönemine temel oluşturan doğru beslenme alışkanlıklarının kazanılması ile bazı kronik hastalıklardan korunabilmek mümkün olabilmektedir (Datar ve ark. 2004; Kiess ve ark. 2004; Lobstein & Baur, 2005; Mikkila ve ark. 2003; O'Toole ve ark. 2007; Organization, 2000). Yaşamın erken dönemlerinde yapılan eksik ya da hatalı beslenme uygulamaları ile yetersiz fiziksel aktivitenin yol açtığı şişmanlığın yetişkin dönemde de kalıcı olması ve pek çok kronik hastalık açısından risk oluşturması nedeniyle, sorunun ciddiyetine olan ilgi her geçen yıl artmaktadır (Haynos & O'Donohue, 2012).

Tüm dünyada, çocuk ve adolesanlarda şişmanlık prevalansı son 20 yılda ciddi boyutlarda artış göstermektedir. Amerika Birleşik Devletleri'nde 2007-2008 yılı verilerine göre 6-11 yaş arası okul çağı çocuklarının %20'si ve 12-19 yaş arası adolesanların %18'inin şişman olduğu, şişmanlığın 1980 yılından itibaren 3 kat arttığı belirtilmektedir (DeMattia & Denney, 2008; Ogden ve ark. 2010). Ülkemizde ise 2011 yılında yayınlanan "Okul Çağı Çocuklarında Büyümenin İzlenmesi Projesi" raporunda, 6-10 yaş arasındaki çocukların %6.5'inin şişman, %14.3'ünün hafif şişman/kilolu, %7,9'unun zayıf ve %1.3'ünün ise çok zayıf olduğu saptanmıştır. Yine aynı raporda Türkiye'de yapılmış olan çalışmalarda çocuk ve gençlerde şişmanlık sıklığının %1.1-16.0; hafif şişmanlık sıklığının %7.5-17.8; zayıflık sıklığının %4.0-17.1 ve bodurluk sıklığının %7.5-24.7 olduğu belirtilmiş ve Türk çocuklarında zayıflık ve şişmanlığın her ikisinin de iki uçlu sorunlar yumağı olduğuna dikkat çekilmiştir (Bakanlığı, 2011).

Şişmanlığın ortaya çıkmasında sağlıksız beslenme alışkanlıkları ve fiziksel aktivitenin yetersiz olması en önemli etkenlerdir (Adachi-Mejia ve ark., 2007; Brown ve ark. 2008; Giammattei ve ark. 2003; Jahns ve ark. 2001; Ludwig & Gortmaker, 2004; Sherry, 2005; Stettlerve ark. 2004; Veugelers & Fitzgerald, 2005). Toplumun sosyal ve ekonomik yapısı beslenme alışkanlıklarının temel belirleyicisidir. Sağlıksız beslenme alışkanlıkları gibi fizik aktivite yapmaya ilişkin davranışların da sosyal ve çevresel etmenlerden etkilendiği bilinmektedir. Kişilerin davranışlarının belirlenmesinde bireyin fiziksel yapısının da önemli rolü vardır. Özellikle fiziksel aktiviteyi kısıtlayan bazı durumlarda şişman olma olasılığında artma söz konusudur. Bu gruplar arasında yer alan görme engeli de fizik aktiviteyi kısıtlayan önemli bir sağlık sorunu olarak ortaya çıkmakta, görme engelli çocuklar da şişmanlık açısından toplumdaki önemli risk gruplarından birini oluşturmaktadır.

Pek çok çalışmada görme engelli çocukların, fiziksel aktivitelere katılımlarının sınırlı olması nedeniyle, aynı yaştaki sağlıklı çocuklara göre daha sedanter bir yaşam tarzı sürdürdükleri ve fiziksel aktivitelerinin daha az olduğu saptanmıştır (Armbruster ve ark. 2005; Aslan ve ark. 2012; Chen & Lin, 2011; Houwen ve ark. 2009; Kozub & Oh, 2004; L. Lieberman & McHugh, 2001; L. J. Lieberman ve ark. 2006; Longmuir & Bar-Or, 1994; Longrnuir & Bar-Or, 2000; Ponchillia ve ark. 2002; Sit ve ark. 2002). Aslan ve ark. (2012) tarafından yapılan bir çalışmada, görme engelli çocukların genellikle hafif-orta düzeyde fiziksel aktivitelere katıldıkları, şiddetli/yoğun fiziksel aktivite katılımının ise çok düşük olduğu (görme kapasitesi düşük olanlar için 0.03 ±0.12 saat/hafta, hiç görmeyen çocuklarda 0.00 ±0.00 saat/hafta) gösterilmiştir. Benzer şekilde Houwen ve arkadaşları (Houwen ve ark. 2009) tarafından yapılan bir araştırmada, ilköğretim çağındaki sağlıklı ve görme engelli çocukların orta ve yoğun/şiddetli fiziksel aktivite katılımları karşılaştırıldığında, görme engelli çocuk ve adolesanların aktivite düzeylerinin sağlıklı yaşıtlarına göre daha düşük olduğu tespit edilmiştir.

Görme engelli çocuklardaki beslenme durumunu değerlendiren sınırlı sayıda çalışma bulunmaktadır. İspanya'da 8-18 yaş arasındaki görme engelli çocuklarda yapılan bir araştırmada, sağlıklı nüfus için belirlenen beslenme hedefleri ile karşılaştırıldığında, görme engelli çocukların diyetinin dengesiz olduğu (karbonhidrat alımı düşük, yağ alımı çok yüksek) ve çocukların yalnızca %11,9'unun diyet kalitesinin iyi düzeyde olduğu belirlenmiştir (Montero, 2005). Aynı çalışmada, sağlıklı çocuklar ile karşılaştırıldığında, görme engelli çocuklarda kilolu (%25,8) ve şişman çocuk (%11,8) prevalansının daha yüksek olduğu saptanmıştır.

Sağlıklı çocukların olduğu gibi görme engelli çocukların da ideal vücut ağırlıklarının korunabilmesi ve ileriye dönük hastalık riskinin azaltılabilmesi için bu çocukların sağlıklı beslenme ve aktivite konusunda durumlarının tanımlanması önemlidir. Bu araştırma, 6-11 yaş arası görme engelli okul çağı çocuklarının enerji ve besin ögesi alımları, antropometrik ölçümleri ve fiziksel aktivite düzeylerini değerlendirmek amacıyla planlanmıştır.

Materyal ve Metot

Araştırmanın Genel Planı

Bu araştırma, Nisan -Mayıs ayları arasında, Ankara İlinde bulunan bir görme engelliler ilköğretim okulunda öğrenim gören, farklı düzeyde görme kusuru bulunan ve araştırmaya katılmaya istekli okul çağındaki (6-11 yaş grubu) çocuklar üzerinde yürütülmüştür. Beden kütle indeksini ve fiziksel aktivite durumunu etkileyebilecek; diyabet, kemik, böbrek, karaciğer, kalp, akciğer rahatsızlığı gibi kronik hastalıkları olan, nörolojik, psikiyatrik ve ortopedik rahatsızlıkları olan ve son bir yıl içerisinde düzenli ilaç ya da besin desteği kullanmış olan/kullanmaya devam eden çocuklar araştırma kapsamına dâhil edilmemiştir.

Çalışmanın Etik Kurul izni Hacettepe Üniversitesi Girişimsel Olmayan Araştırmalar Etik Kurulu'ndan alınmıştır. Araştırma öncesinde ayrıca İlçe Kaymakamlığı ile İlçe Milli Eğitim Müdürlüğü'nden gerekli izinler alınmıştır. Araştırmaya katılan tüm çocuklara ve ailelerine araştırmacı diyetisyen tarafından araştırmanın içeriği ve amacı ile ilgili genel bir bilgi verilmiş, araştırmaya katılmayı kabul eden her aileye onam formu okutulup imzalatılmıştır.

Araştırmacı tarafından hazırlanan anket formu (sosyo-demografik özellikler, beslenme alışkanlıkları, fiziksel aktivite alışkanlıkları) çocuğun bakımından sorumlu kişi ile görüşülerek tamamlanmış, yine aynı kişilerden 24 saatlik geriye dönük hatırlatma yöntemi ile çocukların besin tüketim kayıtları alınmıştır. Ayrıca çocukların boy uzunlukları ve vücut ağırlıkları ölçülerek kayıt edilmiştir.

Antropometrik Ölçümler

Araştırmaya katılan tüm çocukların vücut ağırlıkları ve boy uzunlukları ölçülerek, beden kütle indeksleri (BKİ) hesaplanmıştır. Antropometrik ölçümler bireyler iç giyimleriyle ve ayakkabısız iken SECA marka elektronik boy ölçerli terazi ile ve Frankfort düzlemde yapılmıştır (Bakanlığı, 2014; Pekcan, 2008). Ölçümlerin tamamı aynı araştırmacı uzman diyetisyen tarafından, okul tarafından tahsis edilen bölümde yapılmıştır.

Çocukların beden kütle indeksleri [Vücut ağırlığı (kg)/boy uzunluğu $(m)^2$] denkleminden hesaplanmıştır. BKİ sonuçları, WHO 2007/5-19 yaş referans değerlerine ve WHO'nun önerdiği kesişim değerlerine göre sınıflandırılmıştır (WHO, 2012). Yaşa göre beden kütle indeksi z-skoru değerlendirmesinde 2 standart sapma altında olanlar "çok zayıf", 1-2 standart sapma altında olanlar "zayıf", bir standart sapma altında ve üstünde olanlar arasında olanlar "normal", 1-2 standart sapma ve üstünde olanlar "hafif şişman" ve 2 standart sapmanın üzerinde olanlar ise "şişman" olarak değerlendirilmektedir.

Besin Tüketim Durumu ve Diyet Örüntüsünün Saptanması

Günlük diyet ile alınan enerji ve besin öğeleri miktarlarının saptanması amacı ile araştırmaya katılan çocukların ailelerinden geriye dönük 24 saatlik hatırlatma yöntemi ile besin tüketim kayıtları alınmıştır (Lee & Nieman, 2003). Besin tüketim kaydı alınırken besin miktarlarının saptanmasında 'Yemek ve Besin Fotoğraf Kataloğu-Ölçü ve Miktarlar' kitabından faydalanılmıştır (Rakıcıoğlu ve ark. 2012). Besin tüketim kayıtlarında yer alan yemeklerin besin bazında içeriklerini saptamak için standart tarifelerden faydalanılmıştır (Baysal, 2009; Kutluay, 1994). Tüketilen besinlerin miktarları saptandıktan sonra, Beslenme Bilgi Sistemleri Paket Programı (BEBİS) 6.1 versiyonu kullanılarak; çocukların günlük enerji, makro ve mikro besin ögeleri alımları hesaplanmıştır. Enerji ve besin ögelerinin günlük alım miktarları ortalama, standart sapma, alt ve üst değerler olarak değerlendirilmiştir. Günlük enerji ve besin ögelerinin yeterlilik durumlarının değerlendirilmesinde "Türkiye'ye Özgü Beslenme Rehberi" verileri kullanılmıştır (Hizmetleri & Müdürlüğü, 2004).

Diyet Kalitesi (KIDMED İndeksi)

Katılımcıların ailelerine, çocuklarının beslenme alışkanlıklarına yönelik Serra-Majem ve arkadaşları (Serra-Majem ve ark. 2004) tarafından geliştirilen "Akdeniz Diyeti Kalite İndeksi" (Mediterranean Diet Quality Index-KIDMED) uygulanmıştır. Bu indeks 0-12 arasında puanlanan ve Akdeniz Tipi besin tüketimi modelinin ilkelerini baz alan 16 maddelik kısa bir anketten oluşmaktadır.

KIDMED indeksinin içerdiği sorulardan 12'si olumlu, 4'ü olumsuz sorular olup, olumlu sorulara evet cevabı verenler "+1", olumsuz sorulara evet

cevabı verenler ise "-1" puan almakta ve bu puanların toplanması ile değerlendirme sonunda 0-12 arasında değişen puanlar elde edilmektedir. İndeks sonucu elde edilen toplam puana göre diyet kalitesi 3 grup altında incelenmektedir:

≥8 : Optimal Akdeniz tipi diyet

4-7 : Akdeniz tipi diyet ile uyumlu olabilmesi için diyet kalitesi iyileştirilmeli

≤3 : Diyet kalitesi çok düşük

Fiziksel Aktivitenin Saptanması

Sedanter aktivite (uyku, bilgisayar ve televizyon ile ders çalışma) için harcanan süre ile çocukların aktif olarak (açık alanda oyun oynama, spor kulübünde yapılan planlı fiziksel aktiviteler ve okuldaki beden eğitimi ders saatleri) geçirdikleri süreler anket formu ile saat bazında sorgulanmış ve kaydedilmiştir. Fiziksel aktivite için harcanan sürelerin ortalama, standart sapma, alt ve üst değerleri hesaplanmıştır.

İstatistiksel Değerlendirme

Verilerin istatistiksel değerlendirmesi, SPSS 15.0 istatistik paket programı kullanılarak yapılmıştır. Anket formunda yer alan sorulara ilişkin nitel veriler, sayı ve yüzde olarak değerlendirilmiş, nicel verilerin aritmetik ortalama ($\bar{X}$), standart sapma (S), alt ve üst değerleri hesaplanmıştır.

Cinsiyete göre çocukların enerji ve bazı besin ögelerinin referans alım düzeylerini karşılama oranları arasındaki farklılığın değerlendirilmesinde parametrik koşulları sağlayan veriler için 'Student t testi', parametrik koşulları sağlayamayan veriler için 'Mann Whitney U testi' kullanılmıştır. Tüm istatistiksel analizler sonucunda $p<0,05$ olan değerler istatistiksel olarak anlamlı derecede farklı olarak kabul edilmiştir.

Bulgular ve Tartışma

Çocukların Demografik Özellikleri

Bu araştırma, Nisan 2013-Mayıs 2013 tarihleri arasında, ilköğretim okulunda öğrenim gören, farklı düzeyde görme kusuru bulunan ve araştırmaya katılmaya istekli 6-11 yaş arasındaki 38 erkek (%64.4) ve 21 (%35.6) kız çocuk olmak üzere toplam 59 çocuk ile yapılmıştır. Erkek çocukların yaş ortalaması 9.6 ±1.4 yıl ve kız çocukların yaş ortalaması 8.7 ±1.4 yıl olarak saptanmıştır.

Çalışmaya katılan çocukların bazı demografik özellikleri Tablo 1'de gösterilmiştir. Buna göre çocukların çoğunluğunun (%28.3) ilköğretim üçüncü sınıfa kayıtlı olduğu gözlenmiştir. Genel olarak incelendiğinde, çocukların annelerinin %61.1'inin ilkokul düzeyi ve altı, babalarının ise %55.9'unun ortaokul ve üzeri eğitim düzeyine sahip olduğu belirlenmiştir. Araştırmaya katılan çocukların ailelerinin gelir düzeyleri değerlendirildiğinde, ailelerin %61'inin ayda 1.000 TL'den daha az gelire sahip olduğu, %35.6'sının ise asgari ücret ile geçindiği saptanmıştır.

Çocukların Enerji ve Besin Ögeleri Alımlarının Değerlendirilmesi

Çocukların aileleri ve öğretmenleri ile görüşülerek geriye dönük hatırlatma yöntemi ile alınan 24 saatlik besin tüketim kayıtlarından yola çıkılarak, günlük enerji ve bazı besin ögelerini ortalama alım düzeyleri hesaplanmıştır (Tablo 2). İki çocuğun ailesine ulaşılamaması nedeniyle besin tüketim kayıtları alınamamış ve değerlendirme toplam 57 çocuk üzerinden yapılmıştır.

Tablo 2'de görüldüğü gibi erkek çocukların enerji alımları 1636.0 ±584.4 kkal/gün, kız çocukların enerji alımları ise 1396.2 ±403.6 kkal/gün'dür. Enerjinin makro besin ögeleri arasındaki dağılıma bakıldığında, enerjinin karbonhidrat ve yağdan gelen oranlarının (%) erkek ve kız çocuklar arasında benzer olduğu (erkek çocuklar için sırasıyla %51.0 ±9.0 ve %37.0 ±7.7; kız çocuklar için sırasıyla %46.6 ±10.0 ve %39.6 ±8.8; $p>0.05$), enerjinin proteinden gelen oranının ise kız çocuklarda, erkek çocuklara göre anlamlı olarak yüksek olduğu saptanmıştır (erkek ve kız çocuklar için sırasıyla %12.0 ±3.0 ve %13.8 ±3.1; $p<0.05$).

Araştırma kapsamına alınan çocukların günlük enerji ve besin ögeleri alımları, Türkiye'ye Özgü Beslenme Rehberi'nde yer alan aynı yaş ve cinsiyete sahip çocuklar için önerilen referans alım düzeyleri ile karşılaştırılmış ve önerileri karşılama yüzdeleri hesaplanmıştır (Tablo 3). Buna göre görme engelli erkek çocuklarda sırasıyla günlük kalsiyum (%49.6), posa (%56.8) ve niasin (%73.7) alımlarının, kız çocuklarda ise sırasıyla günlük posa (%54.0), kalsiyum (%57.6) ve enerji (%72.0) alımlarının referans alım düzeylerini karşılama oranlarının düşük olduğu saptanmıştır. Erkek ve kız çocukların günlük diyetle besin ögesi alım düzeylerinin Türkiye'ye özgü önerileri karşılama oranları arasında istatistiksel olarak anlamlı bir farkın olmadığı belirlenmiştir.

Fiziksel Aktivite Durumunun Değerlendirilmesi

Tablo 4'te araştırma kapsamına alınan görme engelli çocukların bazı günlük aktivite sürelerinin ortalama değerleri verilmiştir. Buna göre sedanter aktiviteler arasında yer alan günlük uyku süresi erkek çocuklarda 9.4 ±1.1; kız çocuklarda 9.6 ±1.1 saattir. Çocukların bilgisayar/TV başında ve ders çalışarak geçirdikleri toplam sürenin hem erkek hem de kız çocuklarda günde yaklaşık 2.8 saat olduğu saptanmıştır.

Çocukların aktif olarak geçirdikleri süreler incelendiğinde, erkek ve kız çocukların günde ortalama 1.3 saatlerini açık alanda oynayarak geçirdikleri gösterilmiştir. Bir spor kulübüne kayıtlı olan çocukların (n=5) haftada ortalama 0.1–0.2 saatlerini spor kulübünde aktivite yaparak geçirdikleri belirlenmiştir. Çocukların okuldaki haftalık beden eğitimi derslerinde aktivite yaparak geçirdikleri sürelerin ortalaması ise 1.0–1.2 saat olarak saptanmıştır (Tablo 4).

Tablo 1. Çocukların demografik özelliklerine göre dağılımı

	Sayı	**%**
Cinsiyet		
Erkek	38	64.4
Kız	21	35.6
Sınıfı		
1. sınıf	12	20.3
2. sınıf	11	18.6
3. sınıf	17	28.8
4. sınıf	12	20.3
5. sınıf	7	11.9
Annenin öğrenim durumu		
Okur-yazar değil	6	10.2
Okur yazar	5	8.5
İlkokul mezunu	25	42.4
Ortaokul/İlköğretim mezunu	8	13.6
Lise mezunu	11	18.6
Üniversite mezunu	4	6.8
Babanın öğrenim durumu		
Okur-yazar değil	4	6.8
Okur yazar	3	5.1
İlkokul/İlköğretim mezunu	19	32.2
Ortaokul mezunu	9	15.3
Lise mezunu	14	23.7
Üniversite mezunu	10	16.9
Gelir düzeyi		
Asgari ücret	21	35.6
800 – 1.000 TL	15	25.4
1.000 – 3.000 TL	23	39.0

Tablo 2. Çocukların günlük enerji (kkal) ve bazı besin ögelerini alım düzeylerinin ortalama ($\bar{X}$), standart sapma (S), alt ve üst değerleri

	Erkek (n=36)			Kız (n=21)		
	$\bar{X} \pm S$	Alt	Üst	$\bar{X} \pm S$	Alt	Üst
Enerji ve Makro Besin Ögeleri						
Enerji (kkal)	1636.0 ± 584.4	632.0	2759.0	1396.2 ± 403.6	737.0	2076.0
Protein (g)	48.5 ± 19.2	19.3	88.1	48.3 ± 16.3	18.7	78.3
Protein (E%)[a]	12.0 ± 3.0♣	6.0	20.6	13.8 ± 3.1♣	8.8	19.3
Karbonhidrat (g)	210.9 ± 87.6	62.2	364.9	159.3 ± 50.6	80.3	260.8
Karbonhidrat (E%) [a]	51.0 ± 9.0	37.5	72.6	46.6 ± 10.0	25.7	62.8
Yağ (g)	66.6 ± 25.0	19.9	117.1	62.9 ± 26.2	23.3	114.6
Yağ (E%) [a]	37.0 ± 7.7	21.5	50.8	39.6 ± 8.8	26.4	62.3
Kolesterol (mg)	177.3 ± 133.9	30.4	770.5	165.4 ± 92.6	36.2	342.6
Diyet posası (g)	15.2 ± 8.0	3.1	39.4	13.7 ± 6.0	6.4	27.3
Mineraller						
Kalsiyum (mg)	505.4 ± 280.2	85.3	1361.6	542.7 ± 289.7	47.7	1029.3
Magnezyum (mg)	206.3 ± 96.6	52.5	542.4	179.3 ± 73.8	44.9	309.6
Fosfor (mg)	830.5 ± 325	287.0	1672.8	806.8 ± 280.7	228.3	1168.2
Demir (mg)	9.3 ± 4.9	2.1	23.6	7.4 ± 2.6	3.2	14.0
Çinko (mg)	7.0 ± 2.9	2.7	13.6	6.8 ± 2.4	2.6	10.9
Vitaminler						
A vitamini (mcg)	646.2 ± 821.4	81.2	4500.6	994.3 ± 1359.8	121.6	5277.6
E vitamini (mg)	20.9 ± 10.7	4.6	47.7	16.1 ± 10.0	2.8	39.3
C vitamini (mg)	57.9 ± 46.2	2.2	200.3	61.4 ± 45.5	4.9	175.1
Tiamin (mg)	0.7 ± 0.3	0.2	1.6	0.6 ± 0.2	0.2	0.9
Riboflavin (mg)	1.0 ± 0.5	0.4	2.4	1.0 ± 0.4	0.3	1.8
Niasin (mg)	7.1 ± 3.6	1.5	14.9	6.7 ± 2.7	2.5	14.4
B_6 vitamini (mg)	1.0 ± 0.5	0.2	2.1	0.9 ± 0.3	0.3	1.6
B_{12} vitamini (mcg)	1.7 ± 1.2	0.0	4.8	1.7 ± 1.4	0.1	5.4
Folik asit (mcg)	228.2 ± 109.5	80.7	578.3	203.5 ± 80.9	72.2	349.7

[a] Student t test, ♣ İstatistiksel fark: $p<0.05$, E%: Makro besin ögelerinin enerjiden gelen oranı (%)

Tablo 3. Çocukların günlük enerji ve bazı besin ögesi alımlarının Türkiye'ye Özgü Beslenme Rehberi önerilerini karşılama oranlarının (%) ortalama ($\bar{X}$), standart sapma (S), alt ve üst değerleri

	Erkek (n=36)	Kız (n=21)	
	$\bar{X} \pm S$	$\bar{X} \pm S$	p [a]
Enerji (kkal)	76.9 ± 27.9	72.0 ± 21.1	0.488
Protein (g)	126.5 ± 57.1	145.0 ± 60.4	0.252
Posa (g)	56.8 ± 31.2	54.0 ± 23.8	0.731
Kalsiyum (mg)	49.6 ± 27.6	57.6 ± 31.3	0.317
Fosfor (mg)	112.0 ± 67.7	128.1 ± 60.8	0.372
Magnezyum (mg)	120.6 ± 73.2	118.4 ± 57.1	0.905
Demir (mg)	93.1 ± 49.1	73.7 ± 26.0	0.186[b]
Çinko (mg)	99.3 ± 61.3	113.0 ± 47.1	0.384
A vitamini (mcg)	116.2 ± 138.5	192.5 ± 271.7	0.248
E vitamini (mg)	245.4 ± 142.8	207.9 ± 141.9	0.343
B1 vitamini (mg)	94.8 ± 48.0	93.1 ± 38.7	0.892
B2 vitamini (mg)	142.2 ± 71.9	156.6 ± 67.5	0.461
Niasin (mg)	73.7 ± 41.6	75.7 ± 35.9	0.851
B6 vitamini (mcg)	129.2 ± 72.5	139.6 ± 64.0	0.591
B12 vitamini (mcg)	115.7 ± 83.2	126.0 ± 95.6	0.924
Toplam folik asit (mcg)	94.0 ± 51.9	83.7 ± 36.4	0.428
C vitamini (mg)	86.2 ± 68.3	94.9 ± 68.1	0.648

[a] Student t testi [b] Mann Whitney U testi

Tablo 4. Çocukların bazı günlük aktivite (uyku, bilgisayar, TV, ders çalışma) sürelerinin (saat/gün) ortalama ($\bar{X}$), standart sapma (S), alt ve üst değerleri

	Erkek (n=38)			Kız (n=21)		
	$\bar{X} \pm S$	Alt	Üst	$\bar{X} \pm S$	Alt	Üst
Uyku süresi	9.4 ± 1.1	6.0	11.0	9.6 ± 1.1	7.0	11.0
Bilgisayar, TV süresi	1.5 ± 1.3	-	4.0	1.1 ± 1.2	-	4.0
Ders çalışma süresi	1.3 ± 0.7	-	3.0	1.7 ± 0.7	0.5	3.0
Açık alanda oynama süresi (saat/gün)	1.3 ± 0.9	-	3.0	1.3 ± 1.0	-	3.0
Spor kulübünde aktivite süresi (saat/hafta)	0.1 ± 0.4	-	2.0	0.2 ± 0.4	-	1.5
Beden eğitimi (saat/hafta)	1.2 ± 0.7	-	2.0	1.0 ± 0.6	-	2.0

Diyet Kalitesi

Görme engelli çocukların diyet kalitelerini belirlemek amacıyla kullanılan KIDMED indeksi skorlarının cinsiyete göre dağılımı Tablo 5'de gösterilmiştir. Buna göre, araştırma kapsamına alınan çocukların %27.1'inin diyet kalitelerinin çok düşük olduğu, %55.9'ünün diyetlerinin iyileştirilmesi gerektiği ve %17'sinin Akdeniz tipi diyet ile uyum gösteren bir diyet tükettikleri belirlenmiştir. Kız çocuklarının ortalama KIDMED skoru, erkek çocukların ortalama KIDMED skorundan yüksek olmasına rağmen bu farkın istatistiksel açıdan anlamlı olmadığı saptanmıştır (KIDMED skorları erkek ve kız çocuklar için sırasıyla: 4.76 ±2.11, 5.62 ±2.99; t= -1.282, p= 0.205).

Antropometrik Ölçümler

Çalışmaya alınan kız çocukların vücut ağırlıkları ortalaması 32.7 ± 13.4 kg ve boy uzunlukları ortalaması 129.6 ± 12.1 cm olarak bulunmuştur. Erkek çocuklar için ağırlık ve boy uzunluğu ortalamaları ise sırasıyla 31.8 ± 8.0 kg ve 130.9 ± 7.8 cm'dir. Tablo 6'da çocukların vücut ağırlığı ve boy uzunluğu değerlerinden hesaplanarak bulunan BKİ değerlerinin DSÖ-MGRS 2007 5-19 yaş grubu sağlıklı çocukların Z-skor referans verilerine göre dağılımı gösterilmiştir. Buna göre, araştırma kapsamına alınan çocukların %40.7'sinin (erkek: %44.7; kız: %33.3) yaşa göre normal beden kütle indeksi değerleri (≥-1SD - 1SD) arasında olduğu bulunmuştur. Hafif şişmanlık (≥ 1SD – < 2SD) ve şişmanlığın (≥ 2SD) görülme oranlarına bakıldığında, hafif şişmanlığın kız çocuklarda (erkek: %18.4; kız: %28.6), şişmanlığın ise erkek çocuklarda (erkek: %23.7; kız: %19.0) daha yaygın olduğu gözlenmiştir. Genel olarak incelendiğinde ise tüm çocukların %44.0'ının hafif şişman veya şişmanlık sınıfı içerisinde olduğu saptanmıştır. Çocuklarda zayıf ve çok zayıf olma durumları değerlendirildiğinde, çocukların %1.7'sinin çok zayıf, %13.6'sının ise zayıf olduğu bulunmuştur. Kız çocuklar arasında yalnızca 1 çocuk (%4.8) çok zayıf sınıfında yer alırken, erkek çocuklar arasında çok zayıf çocuk bulunmamaktadır. Kız çocukların %14.3'ü, erkek çocukların ise %13.2'si BKİ Z-skorlarına göre zayıf olduğu belirlenmiştir (Tablo 6).

Çocukluk çağı, büyüme ve gelişmenin hızlı olduğu, yaşam boyu sürebilecek davranışların büyük ölçüde oluştuğu bir dönemdir. Bu dönemde görülen şişmanlığın genellikle yetişkin dönemde de kalıcı olması ve pek çok kronik hastalık açısından risk oluşturması nedeniyle, sorunun ciddiyetine olan ilgi her geçen yıl artmaktadır (Haynos & O'Donohue, 2012; Magarey ve ark. 2003).

Şişmanlığın ortaya çıkmasında sağlıksız beslenme alışkanlıkları ve fiziksel aktivitenin yetersiz olması en önemli etkenlerdir (Adachi-Mejia ve ark. 2007; Brown ve ark. 2008; Giammattei ve ark. 2003; Jahns ve ark. 2001; Ludwig & Gortmaker, 2004; Sherry, 2005; Stettler ve ark., 2004; Veugelers & Fitzgerald, 2005). Ailelerin oyun alanlarını güvenli bulmaması (DeMattia & Denney, 2008), televizyon, bilgisayar ve video başında geçirilen sürenin giderek uzaması (Adachi-Mejia ve ark. 2007; Bakanlığı, 2011; Stettler ve ark. 2004) ve uyku sürelerinin azalması (Ludwig & Gortmaker, 2004; Veugelers & Fitzgerald, 2005) çocukluk çağı şişmanlığı riskini artırmaktadır. Diğer taraftan özellikle aktiviteleri kısıtlı olan özel gruplar arasında yer alan görme engelli çocuklar da şişmanlık açısından çocuklar arasında önemli risk gruplarından birini oluşturmaktadır (Montero, 2005).

Bu araştırmada, Ankara'daki bir görme engelliler okulunda öğrenim gören 6-11 yaş arası okul çağı

çocuklarında beslenme durumu, diyet kalitesi ve fiziksel aktivite durumu incelenmiştir. Araştırmanın sonucunda DSÖ'nün BKİ Z-skor referans verilerine göre çocukların %44'ünün hafif şişman (%22) ya da şişman (%22); %15.3'ünün ise zayıf/çok zayıf olduğu bulunmuştur. Türkiye'de aynı yaş grubu çocuklarda yapılan TOÇBİ Projesi'nde ise BKİ Z-skor referans verilerine göre hafif şişman/şişman olan çocukların sıklığı %20.8 iken, çocukların %9.2'sinin ise zayıf/çok zayıf olduğu saptanmıştır (Bakanlığı, 2011). Engelsiz çocuklar ile karşılaştırıldığında bu araştırmaya alınan görme engelli çocuklarda başta şişmanlık olmak üzere kötü beslenmiş (malnütrisyonlu) çocukların sıklığının daha yüksek olduğu belirlenmiştir. İspanya'da Montero ve arkadaşları (Montero, 2005) tarafından benzer bir çalışmada da, 8-18 yaş grubundaki görme engelli çocukların %37.6'sının şişman (%11.8) veya hafif şişman (%25.8) olduğu ve bu değerlerin aynı toplumdaki engelsiz çocuklarda görülen şişmanlık sıklığından yüksek olduğu bildirilmiştir.

Tablo 5. Çocukların diyet kalitelerinin (KIDMED İndeksi) cinsiyete göre dağılımı

Cinsiyet	Diyet Kalitesi: KIDMED İndeksi değerleri	Sayı	%
Erkek	≤ 3: Diyet kalitesi çok düşük	11	29.0
(n=38)	4-7: Diyet kalitesi iyileştirilmeli	23	60.5
	≥ 8: Optimal Akdeniz tipi diyet	4	10.5
Kız	≤ 3: Diyet kalitesi çok düşük	5	23.8
(n=21)	4-7: Diyet kalitesi iyileştirilmeli	10	47.6
	≥ 8: Optimal Akdeniz tipi diyet	6	28.6
Toplam	≤ 3: Diyet kalitesi çok düşük	16	27.1
(n=59)	4-7: Diyet kalitesi iyileştirilmeli	33	55.9
	≥ 8: Optimal Akdeniz tipi diyet	10	17.0

χ^2=3.133, p=0.209; KIDMED skoru ($\bar{X}$ ± S): erkek = 4.76 ± 2.11, kız= 5.62 ± 2.99; t= -1.282, p= 0.205

Tablo 6. 6-11 yaş arası görme engelli çocukların cinsiyete göre beden kütle indeksi (BKİ) Z skoru dağılımları (%)

	BKİ Z Skoru									
	< -2SD ÇOK ZAYIF		≥ -2SD – < -1SD ZAYIF		≥ -1SD – < 1SD NORMAL		≥ 1SD – < 2SD HAFİF ŞİŞMAN		≥ 2SD ŞİŞMAN	
Cinsiyet	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**
Erkek	-	-	5	13.2	17	44.7	7	18.4	9	23.7
Kız	1	4.8	3	14.3	7	33.3	6	28.6	4	19.0
Toplam	1	1.7	8	13.6	24	40.7	13	22.0	13	22.0

Araştırmaya katılan çocukların demografik özellikleri incelendiğinde, özellikle annelerin yarıdan fazlasının (%61.1) eğitim durumunun ilkokul düzeyi ve altında olduğu ve ailelerin yine yarıdan fazlasının (%61.0) 1000 TL'nin altında gelir düzeyine sahip olduğu gözlenmiştir. Sosyo-ekonomik düzey genellikle çocukluk ve adolesan şişmanlığı (yüksek BKİ) ile negatif olarak ilişkilendirilmektedir (Case ve ark. 2001; Goodman, 1999; Gordon-Larsen ve ark. 2003; Lamerz ve ark., 2005; Wang & Zhang, 2006). Almanya'da yapılan kesitsel bir çalışmada en düşük sosyal statü grubunda yer alan çocuklarda obezite riskinin, en yüksek sosyal statüde yer alan çocuklara göre 3 kat daha yüksek olduğu ve çocuğun şişmanlık durumu ile ilişkili en güçlü göstergenin ailenin eğitim süresi olduğu belirlenmiştir (Lamerz ve ark., 2005). Engelli çocukların ailelerinde yüksek oranda görülen yoksulluk da şişmanlık ile ilişkilendirilecek bir diğer faktördür (Fujiura & Yamaki, 2000). Sınırlı aile bütçesi daha pahalı olan sağlıklı besinlere ulaşımı zorlaştırmakta ve daha ucuz, kalori içeriği daha yüksek olan besinlerin tüketimine yol açmaktadır. Ayrıca engelli olmayan çocuklar, besin tüketimlerini ya da besin seçimlerini kısmen veya tamamen kendileri kontrol edebilseler de, engelli çocuklarda besin tüketimi tercihi genellikle tümüyle aile ya da çocuğun bakımından sorumlu kişiler (öğretmen, bakıcı vb) tarafından kontrol edilmektedir. Bu nedenle ailenin beslenme konusundaki bilgi düzeyinin yetersiz olması, çocuğun vücut ağırlığı üzerinde etkili olabilmektedir (Rimmer ve ark., 2007).

Sağlıklı beden ağırlığı ve kompozisyonunun korunabilmesi için besinlerle alınan enerji ve aktivite ile harcanan enerji arasında bir denge olmalıdır. Araştırmaya alınan çocukların günlük enerji alımları incelendiğinde, hem erkek hem de kız çocukların Türkiye'ye Özgü Beslenme Rehberi'nde önerilen referans enerji alım düzeylerinin tamamını karşılayamadığı görülmüştür (sırasıyla %77 ve %72). Enerjinin makro besin ögelerine göre dağılımına bakıldığında görme engelli erkek ve kız çocuklarda enerjinin proteinden gelen oranının önerilen düzeylerde (%10-20), karbonhidrattan gelen oranının önerilen düzeylerden (enerjinin %50-60'ı) az, yağ oranının ise önerilenden (enerjinin %25-35'i) fazla olduğu saptanmıştır. Montero ve arkadaşları (Montero, 2005) tarafından 8-18 yaş arasındaki görme engelli çocuklarda yapılan bir çalışmada da benzer sonuçlar elde edilmiş ve sağlıklı nüfus için belirlenen beslenme hedefleri ile karşılaştırıldığında, görme engelli çocukların diyetinin dengesiz olduğu (karbonhidrat alımı düşük, yağ alımı çok yüksek) bildirilmiştir. Bu araştırmada ayrıca, görme engelli kız ve erkek çocukların özellikle kalsiyum ve posa için günlük gereksinimlerini karşılama oranlarının oldukça düşük olduğu (<%60) gösterilmiştir. Kemik ve diş yapısının önemli bir kısmını oluşturan kalsiyumun, çocukluk ve adolesan çağında yeterli miktarda alımı, optimal pik kemik kütlesine ulaşılması ve yetişkin dönemde osteoporoz riskinin azaltılması açısından kritik öneme sahiptir ("51. European Food Information Council (2013): Child and Adolescent Nutrition,"). Bu çocuklarda kalsiyum alımının yetersizliği, kalsiyumun zengin kaynakları arasında bulunan süt, yoğurt, peynir gibi süt ürünlerinin yetersiz tüketildiğine işaret etmektedir.

Bu araştırmada KIDMED indeksi kullanılarak çocukların diyet kaliteleri de değerlendirilmiş ve Akdeniz tipi diyet ile uyumuna bakılmıştır. Akdeniz tipi diyet, bitkisel kaynaklı besinlerden zengin (saflaştırılmamış tahıllar, meyve ve sebzeler, kuru baklagiller, yağlı tohumlar), görünür yağ olarak başlıca zeytinyağı içeren, daha fazla balık ve deniz ürünleri ile orta düzeyde yumurta, kümes hayvan etleri ve düşük yağlı süt ürünleri tüketimini sağlayan bir diyet olmakla birlikte, Batı tarzı diyete göre daha az miktarda kırmızı et ve doymuş yağ içermektedir (Bach-Faig ve ark., 2011). Günümüzde Akdeniz tipi diyet ile uyumlu bir beslenme şekline sahip toplumlarda başta kalp hastalıkları ve çeşitli kanser türleri olmak üzere diyabet, astım, Alzheimer gibi çeşitli kronik hastalıkların görülme sıklığının daha düşük olduğu bilinmektedir (Benetou ve ark., 2008; Martínez-González ve ark., 2008). Türkiye'de engelsiz çocuk ve adolesanlarda yapılan araştırmalar incelendiğinde, optimal KIDMED indeksi skoruna sahip olan çocuk ve adolesanların sıklığı Samur ve arkadaşlarının (Samur ve ark. 2008) çalışmasında %76.2 olarak saptanmıştır. Şahingöz ve Şanlıer tarafından (Sahingoz & Sanlier, 2011) 10-14 yaş arası 890 adolesan üzerinde yapılan daha güncel bir çalışmada, KIDMED skoru kullanılarak diyet kalitesi değerlendirilmiş ve çalışmanın sonunda katılımcıların %17.9'unun diyet kalitesinin düşük, %59.2'sinin ise orta düzeyde olduğu ve iyileştirilmesi gerektiği saptanmıştır. Katılımcıların yalnızca %22.9'unun Akdeniz tipi diyete uyumlu bir diyet tükettikleri belirlenmiştir. Ayrıca her üç çalışmada da erkek ve kız çocukların KIDMED indeksi skorları arasında istatistiksel bir farklılığın olmadığı görülmüştür. Avrupa'da İspanya ve Yunanistan gibi Akdeniz tipi diyete yakın diyet tüketen ülkelerde dahi yapılan diğer çalışmalarda da

optimal KIDMED indeksi skorlarına ilişkin benzer sonuçlar elde edilmiş (%8-47), çocuk ve adolesanların yarıdan fazlasının diyet kalitelerinin kötü ya da iyileştirilmesi gerektiği sonucu ortaya çıkmıştır (Kontogianni ve ark., 2008; Mariscal-Arcas ve ark., 2009; Serra-Majem ve ark., 2004). Görme engelli çocuklarda diyet kalitesini değerlendiren fazla sayıda çalışma bulunmamakla birlikte, optimal diyet kalitesine sahip çocuk ve adolesanların sıklığının engelsiz çocuklar ile karşılaştırıldığında oldukça düşük olduğu (%11.9) ve çocukların %34.7'sinin diyet kalitesinin kötü, %53.5'inin ise diyet kalitesinin geliştirilmesi gerektiği sonucuna varılmıştır (Montero, 2005). Bu araştırmada da benzer sonuçlar elde edilmiş ve çocukların yalnızca %17'sinin optimal KIDMED indeksi skoruna sahip olduğu ve yarıdan fazlasında diyet kalitesinin iyileştirilmesi gerektiği saptanmıştır.

Optimal vücut ağırlığının korunabilmesi için enerji alımı kadar fiziksel aktivite ile harcanan enerji de önem taşımaktadır. Vücut ağırlığı denetiminin yanında düzenli fiziksel aktivitenin ayrıca, başta kalp hastalıkları ve diyabet olmak üzere çeşitli kronik hastalıkların riskini azalttığı ve kemik sağlığını geliştirdiği bilinmektedir. Dünya Sağlık Örgütü'nün 2010 yılındaki raporuna göre cinsiyet, ırk, etnik köken ve gelir düzeyi göz önüne alınmadan 5-17 yaş arası çocuk ve adolesanların haftada en az 3 kez ve günde en az 60 dakika orta-ağır şiddette fiziksel aktivite yapmaları önerilmektedir (Bakanlığı, 2011; "Global Recommendations on Physical Activity for Health. Geneva: World Health Organization; 2010. ,"). Ayrıca Amerikan Pediatri Akademisi tarafından çocuklar için belirlenen günlük sedanter aktivite (TV, bilgisayar vb) süresi 2 saat ile sınırlandırılmıştır (Pediatrics, 2001). Ancak yapılan çalışmalarda fiziksel aktivite ve sedanter aktivite için önerilen süreleri karşılama oranlarının düşük (<%20) olduğu (Eaton ve ark., 2010), fiziksel aktivite ve sedanter süre için yapılan her iki öneriyi de karşılayamayan çocuklarda şişmanlık gelişme riskinin 3-4 kat arttığı saptanmıştır (Eisenmann ve ark., 2008).

Engelsiz çocuklar ile karşılaştırıldığında, görme engeli bulunan çocukların daha sedanter bir yaşam tarzı sürdürdüğü, fiziksel aktivitelerinin engelsiz yaşıtlarına göre daha düşük olduğu gösterilmiştir (Aslan ve ark., 2012; Chen & Lin, 2011; Houwen ve ark., 2009; Kozub & Oh, 2004; L. Lieberman & McHugh, 2001; L. J. Lieberman ve ark., 2006; Longmuir & Bar-Or, 1994; Longrnuir & Bar-Or, 2000; Ponchillia ve ark., 2002; Sit ve ark., 2002). Bu duruma yol açan en önemli etmenler arasında görsel, fiziksel ve/veya bilişsel engellerin müsabaka/rekabete dayalı spor aktivitelerine ya da oyunlara katılımı zorlaştırması yer almaktadır (Kasser & Lytle, 2005). Bu araştırmada görme engelli çocukların uyku için günde ortalama 9.5 saat; bilgisayar/TV başında ve ders çalışmak için ortalama 2.8 saat harcadıkları tespit edilmiştir. İspanya'da 8-18 yaş arasındaki görme engelli çocuklarda yapılan çalışmada uyku süresinin ortalama 8.8 saat; toplam sedanter aktivite (TV, ders, bilgisayar) süresinin ortalama 2.6 saat olduğu belirlenmiştir (Montero, 2005). Türkiye'de 2010 yılında yapılan TOÇBİ çalışmasında ise 6-11 yaş grubundaki çocukların uyku ve sedanter aktivite süreleri sırasıyla ortalama 9.6 saat ve 2.8 saattir (Bakanlığı, 2011). Her iki çalışma ile karşılaştırıldığında bu araştırmadaki görme engelli çocukların uyku ve TV-bilgisayar-ders çalışarak geçirdikleri toplam sürelerin görme engelli ve aynı yaş grubu engelsiz çocuklar ile benzer olduğu saptanmıştır. Ayrıca, bu araştırmadaki görme engelli çocukların sedanter olarak geçirdikleri süre engelsiz çocuklar için önerilen süreden (<2 saat) fazladır.

Araştırmaya dâhil edilen çocukların aktif olarak geçirdikleri süreler incelendiğinde ise, erkek ve kız çocukların günde ortalama 1.3 saatlerini açık alanda oynayarak geçirdikleri gösterilmiştir. Türkiye'de aynı yaş engelsiz çocuklarda bu sürenin erkek ve kız çocuklarda dağılımına bakıldığında hafta içi hem erkek hem de kız çocukların ortalama 0.6 saatini, hafta sonu ise erkek çocukların ortalama 2.0; kız çocukların ise ortalama 1.8 saatini dışarıda oyun oynayarak geçirdikleri belirlenmiştir (Bakanlığı, 2011). Ayrıca bu araştırmaya dâhil edilen 58 görme engelli öğrenciden yalnızca %8.5'inin bir spor kulübüne kayıtlı olduğu ve çocukların okulda beden eğitimi dersine harcadıkları sürenin oldukça yetersiz olduğu (<2 saat/hafta) gözlenmiştir. Bu araştırmada, genel olarak değerlendirildiğinde sedanter aktivitelerle ve açık alanda oyun oynanarak geçirilen sürelerin aynı yaş grubundaki sağlıklı çocuklar ile benzerlik göstermesine ve bu çocuklarda günlük enerji alımının gereksinmenin altında olmasına rağmen (<%80) şişmanlık görülme sıklığının daha yüksek olması, görme engelli çocuklarda engelsiz yaşıtlarına oranla aktif olarak geçirilen sürenin orta-yoğun fiziksel aktiviteler yerine hafif düzeyde aktivitelerden oluştuğunu düşündürmektedir. Aslan ve arkadaşları (Aslan ve ark., 2012) tarafından yapılan bir çalışmada, görme engelli çocukların genellikle hafif-orta düzeyde fiziksel aktivitelere katıldıkları, şiddetli/yoğun fiziksel aktivite katılımının ise çok

düşük olduğu gösterilmiştir. Yine benzer araştırmalarda görme kusuru bulunan çocukların okulda beden eğitimi derslerinde yoğun aktivitelere katılımlarının olmadığı bildirilmiştir (Houwen ve ark., 2009; Longrnuir & Bar-Or, 2000; Ponchillia ve ark., 2002). Bu tip spor aktivitelerine katılımın sınırlı ve zor olması, engelli çocukları genellikle yüksek enerji harcatan aktiviteler yerine (futbol, basketbol, yüzme vb) daha çok sedanter aktiviteler ile geçirmek zorunda bırakmakta ve bu durum çocukları şişmanlık riski ile karşı karşıya bırakmaktadır. Ayrıca aktivite yapılabilecek oyun alanların olmaması/yetersiz olması, çocuğun yaşadığı çevrenin güvenli olmaması ve/veya ailenin aşırı korumacı olması, spor için gerekli ekipmana ulaşılamaması, çocukların bakımından sorumlu kişilerin engelli çocuğu aktivitelere adapte etmek konusunda yeterli bilgiye sahip olmaması ve okul bazlı aktivite programlarının eksikliği gibi bazı çevresel etmenler engelli çocukların aktivite yapmalarına engel oluşturmaktadır (Kasser & Lytle, 2005; Rimmer, 2005).

Sonuç

Bu çalışma, antropometrik ölçümlere dayanılarak görme engelli çocuklarda şişmanlık/obezite sıklığının aynı yaş grubundaki engelsiz çocuklara oranla daha yüksek olduğunu göstermiştir. Bu araştırmada, şişmanlığın ortaya çıkışında temel faktörler olan diyetle enerji alımı ve fiziksel aktivite durumunun ele alınmış olmasına rağmen, bir günlük geriye dönük besin tüketim kayıtlarının genel beslenme durumunu yansıtmada yetersiz kalabilmesi ve fiziksel aktivite durumunun, geçerliliği ve güvenilirliği olan akselerometre gibi ölçüm yöntemleri ya da dakika bazında ayrıntılı fiziksel aktivite kayıtları yerine yalnızca beyana dayalı sorular ile değerlendirilmiş olması çalışmanın sınırlılıkları arasında yer almaktadır. Bu araştırmadan elde edilen sonuçların görme engelli tüm çocuklara genellenebilmesi için ileri analizlerde daha büyük bir örneklem sayısı ve kapsamlı, geçerliliği ve güvenilirliği olan ölçüm yöntemleri ile şişmanlık sıklığı, fiziksel aktivite düzeyi, günlük enerji alımı ve harcaması ile beslenme alışkanlıklarının değerlendirilmesi gereklidir.

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