Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1206133

JAES
Year: 8, No: 1, 2023 (9-15)
AÇEH
Yıl: 8, Sayı: 1, 2023 (9-15)

ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Plasma T_3 and T_4 Hormone Levels as Reference Values in Rehabilitated Common Buzzard (*Buteo buteo*), Long-Legged Buzzard (*Buteo rufinus*) and Golden Eagle (*Aquila chrysaetos*)

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Received: *17.11.2022* ***Accepted:*** *01.02.2023* ***Puplished:*** *31.03.2023*

How to cite: **Sönmez, E. & Gürsoy-Ergen, A. (2023).** Plasma T_3 and T_4 Hormone Levels as Reference Values in Rehabilitated Common Buzzard (*Buteo buteo*), Long-Legged Buzzard (*Buteo rufinus*) and Golden Eagle (*Aquila chrysaetos*). *J. Anatolian Env. and Anim. Sciences, 7*(1), 9-15. https://doi.org/10.35229/jaes.1206133
Atıf yapmak için: **Sönmez, E. & Gürsoy-Ergen, A. (2023).** Rehabilite Edilmiş Şahin (*Buteo buteo*), Kızıl şahin (*Buteo rufinus*) ve Kaya kartalı (*Aquila chrysaetos*)'nda Referans Plazma T_3 ve T_4 Hormon Seviyeler. *Anadolu Çev. ve Hay. Dergisi*, *7*(1), 9-15. https://doi.org/10.35229/jaes.1206133

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Abstract: Applying appropriate criteria for raptors at rehabilitation stages including release and post-release determines how healthy the animal can survive in its natural habitat for any length of time. In determining release criteria, clinical examinations of animals as well as clinical data should be evaluated. In this study, plasma T_3 and T_4 levels of 16 Common Buzzard (*Buteo buteo*), seven Long-legged Buzzard (*Buteo rufinus*) and seven Golden Eagles (*Aquila chrysaetos*) were determined at Kafkas University/ Kafkas Wildlife Conservation, Rescue, Rehabilitation Practice and Research Center between 2016-2018. All raptors were kept at the rehabilitation center for 1-3 months in bird care rooms and flight tunnels specially arranged for each species. Rehabilitated birds' blood was collected before released to the wild. Plasma T_3 and T_4 hormone levels were analysed and determined in raptors that were considered suitable for release. Although small differences were detected between T_3 and T_4 values as a result of the analysis, no statistical difference was found. These small differences in values may be due to each raptor's metabolic responses and environmental conditions. As a result, these plasma thyroid hormone levels in rehabilitated raptors can be accepted as reference values.

Keywords: Bird physiology, metabolism, raptors, rehabilitation, thyroid hormones.

Rehabilite Edilmiş Şahin (*Buteo buteo*), Kızıl şahin (*Buteo rufinus*) ve Kaya kartalı (*Aquila chrysaetos*)'nda Referans Plazma T_3 ve T_4 Hormon Seviyeleri

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Öz: Yırtıcı kuşların rehabilitasyonunda uygun salım kriterlerini belirlemek, kuşları doğal habitatlarına bıraktıktan sonra geri kalan hayatını ne kadar sağlıklı geçireceğini belirler. Salım kriterlerini belirlerken de hayvanların klinik muayenelerinin yanı sıra klinik verilerinin de imkanlar ölçüsünde incelenmesi gerekir. Bu çalışmada 2016-2018 yılları arasında 'Kafkas Üniversitesi, Kafkas Yaban Hayvanı Koruma, Kurtarma, Rehabilitasyon Uygulama ve Araştırma Merkezi'ne getirilen 16 Kızıl şahin (*Buteo rufinus*), yedi Şahin (*Buteo buteo*) ve yedi Kaya kartalı (*Aquila chrysaetos*)'nın, rehabilitasyonları sonrasında tam sağlıklı şekilde doğaya salınmalarından önceki plazma T_3 ve T_4 seviyeleri tespit edildi. Değerlendirilen tüm kuşlar 1-3 ay süresince rehabilitasyon merkezinde türlerine özel olarak düzenlenmiş kuş bakım odaları ve uçuş tünellerinde tutuldu. Salınımı uygun görülen yırtıcılarda plazma T_3 ve T_4 hormon düzeyleri analiz edilerek belirlendi. Analiz sonucunda T_3 ve T_4 değerleri arasında küçük farklar saptansa da istatistiksel olarak fark bulunamadı. Değerlerdeki bu küçük farklılıklar, her yırtıcı hayvanın metabolik tepkileri ve çevresel koşullarından kaynaklanıyor olabilir. Sonuç olarak rehabilite edilmiş yırtıcılarda bu plazma tiroid hormonu seviyeleri referans değerler olarak kabul edilebilir.

Anahtar kelimeler: Kuş fizyolojisi, metabolizma, yırtıcı, rehabilitasyon, tiroid hormonları.

INTRODUCTION

Raptors may provide a service to regulate the population in agricultural ecosystems and wildlife due to their capability of hunting (Kross et al., 2018). In addition, human activity impacts the habitats of raptors and also their populations to survive (Fardell et al., 2020). Most raptor populations become endangered owing to habitat alteration or destruction, intentional and unintentional poisoning, electrocution, illegal hunting or poaching, and also climate change (IUCN, 2021; McClure & Rolek, 2020). It has been stated that 65% of the birds admitted to rehabilitation centers are traumatic injuries caused by human impact, and 85% are caused by human-related deaths (Fix & Barrows, 1990). A significant number of raptors are often admitted to rehabilitation centers due to traffic accidents, pesticide poisoning, shotgun fire, electrocution or collision with power lines (Malik & Valentine, 2018). One of the most widely used but little-documented practices in conservation biology is rehabilitation (Saran et al., 2011). Data collected on rehabilitated and released animals may also provide potential information on a population of wild animals when combined with associated research especially facing extinction (Guy et al., 2014; Koenig et al., 2002; Molina-Lo´pez et al., 2011). Although wildlife rehabilitation mostly seems like first aid, it is important for the survival of animals and also for the continuity of both the species and the population (Guy et al., 2014; Tribe and Brown, 2000). Animals being rehabilitated may belong to a declining species or an endangered species, or it may be a common species. In this respect, rehabilitation is one of the important pillars in conservation biology, preventing species or population loss (Pyke & Szabo, 2018; Saran et al., 2011; Zoubi & Hamidan, 2020). This may be especially important for avian conservation and management of sources where pollution, habitat loss, hunting, urbanization, or climate change may affect regional populations. Clinical consultation and detection and evaluation of hematological, biochemical and physiological abnormalities are very important in the success of survival and conservation strategies (Guerra et al., 2018). In rehabilitation of raptors, if the animal is a release candidate it is very important to determine the criteria for release (Black et al., 2011). Unfortunately, especially physiological and hormonal data of animals living in wildlife, such as raptors, are quite a few in the literature and current researches are mostly focused on biochemical and microbiological issues. Data on the hormone metabolism of wild birds are very scarce (Spagnolo et al., 2006). Intra-species comparisons can also be one of the reliable parameters when assessing the health status of birds prior to release, as reference intervals differ between species (Gentsch et al., 2018). Birds are generally expected to increase corticosterone levels in response to stress conditions. However, recently, studies on how variables such as temperature, stress and fasting affect prolactin, corticosteroid, cortisol and thyroid hormones in birds and their relationships with each other have also gained importance (Hudelson & Hudelson, 2009; Schmidt & Soma, 2008; Walker et al., 2005). T_3 (triiodothyronine) and T_4 (thyroxine) hormones released from the thyroid gland play a role in energy production and body temperature stability by increasing the basal metabolic rate of almost all tissues (Choksi et al., 2003). They are also responsible for differentiation and development of the central nervous system, growth of muscles and bones, reproduction and egg production, shedding skin, and molting (Biswas et al., 2010; Chaudhuri & Maiti, 1996; Hudelson & Hudelson, 2009; Palme et al., 2005; Quinn et al., 2005). Raptors are physiologically susceptible to environmental influences (Fischer & Romero, 2019; Mullineaux, 2014). Environmental changes mediate various behavioral and physiological responses in birds. As a result of these responses, birds transfer existing resources in order to survive instead of other functions, this developing situation puts an extra physiological cost on birds (Schmidt & Reavill, 2008). In addition to possible human-related stress factors, retention in unsuitable cages or rooms at rehabilitation centers can also cause birds high levels of metabolism (Fardell et al., 2020). Also, basal metabolism and thyroid hormone metabolism may also be affected due to smell, sound, light conditions, daily diet etc. in these centers (Gentsch et al., 2018; Morgan & Tromborg, 2007).

Understanding the adaptation mechanisms of raptors to environmental conditions, the strategies they use and the differences between individuals are very important in terms of conservation biology (Angelier et al., 2016; Fischer & Romero, 2019). Increased thyroid hormone levels in wild birds are generally related to the level of metabolic activity (Wentworth & Ringer, 1986). Elliot et al., (2013) in a study with *Rissa tridactyla* and *Uria lomviaher* found that resting metabolic rate increased with T_3 in these species. Angelier et al., (2016) found in a study with *Columbia liva* that while corticosterone, prolactin and thyroxine hormones were severely affected to restrain stress, the T_3 hormone was not.

The hypothesis in this study was that the trauma experienced by the birds and the practices performed during rehabilitation would affect the thyroid metabolism of raptors. It is expected that especially trauma will affect the T_3 and T_4 amounts of raptors. Besides, in this study after being rehabilitated in a center where optimal environmental conditions were provided and species-specific house and flight areas were available, Long-legged

Buzzards, Common Buzzards and Golden Eagles injured for various reasons, it was aimed to contribute to the literature by determining the changes in plasma levels of T_3 and T_4 hormones just before release to wildlife. Studying thyroid hormones in birds can help us understand their basic metabolic physiology and contribute to the science of animal and avian physiology in this field.

MATERIAL AND METHOD

The General Directorate of Nature Conservation and National Parks in Türkiye has signed a protocol with the wildlife rehabilitation and rescue center of 11 veterinary faculties for treatment and temporary care of injured, diseased and displaced indigenous wild animals and subsequent return of healthy animals to appropriate habitats in the wild. And also they have contact with zoos and freelance veterinarians in Türkiye.

Kafkas University/Kafkas Wild Animals Protection, Rescue, Rehabilitation, Application and Research Center serves under a joint protocol signed with Kafkas University, General Directorate of Nature Conservation and National Parks and Kuzey Doğa Society. This center supports the treatment and care of sick, injured or orphaned wild animals of several species such as brown bears, lynxes, foxes, wolves, roe deer, mountain goat, raptors etc. and their preparation for release to a successful life back in the wild. Since it was launched in 2011, the centre has handled close to 250 animal cases every year, with nearly 50% released back to the wild.

In this study, 16 Long-legged Buzzards, seven Common Buzzards and seven Golden Eagles admitted to 'Kafkas University / Kafkas Wildlife Conservation, Rescue, Rehabilitation Practice and Research Center between 2016 and 2018 were evaluated in terms of thyroid hormones. The center received injured raptors found randomly in the wild, directly from local people or by staff. To separate the sex in raptors; wing cord, culmen and hallux length, and body mass are the most efficient parameters but in our study, there was considerable overlap in size between the sexes (Clark & Yosef, 1998; Zuberogoitia et al., 2005). Since age was not clear, all ages (juvenile/subadult/adult) were pooled in the same analyses. For these reasons, sex and age parameters were ignored. The causes of raptor admissions to the wildlife rehabilitation centers were given in Tables 1, 2 and 3. All birds were kept for 1-3 months in isolated, internationally sized and standard bird care rooms, walk-in treatment rooms and subsequently flight tunnels (5m / 6m / 30m) specially arranged for large raptor species. The birds in care rooms were not visited except by routine veterinary staff to avoid or minimize stress and they were fed with species-specific red or white meat 6 days a week. Since the goal of wildlife rehabilitation and conservation biology are to release healthy individuals back to the wild and capable of surviving in the wild, any unnecessary medical intervention was not performed during the treatment process in order to prevent further stress as much as possible on the injured, sick or weak birds brought to the center. Blood samples for hormonal examinations were not taken from these birds at their first admission to the center. Due to these medical reasons mentioned above, our study did not have any control group dataset that indicates the initial disease status of birds. Blood samples were collected 1-2 days before the rehabilitated individuals were released in wildlife at suitable areas.

Collection of blood samples and analysis: Blood samples were collected from the wing ulnar/basilic veins and stored in the EDTA (BD vacutainer, K_2 EDTA) tubes. The blood samples were centrifuged at 3000 rpm for 5 minutes (Electro-mag M815 M) and their serum was obtained. Serum samples obtained were stored at -20 ºC (Profilo 6600) until analysis. Chicken T_4 (Thyroxine, MyBiosource) ELISA kit and Chicken T_3 (Triiodothyronine, MyBiosource) ELISA kit were used to analyze the T_3 and T_4 hormones of the samples. The analysis was carried out according to the protocol provided by the manufacturer of the Elisa kit.

Ethical approval: All procedures were approved by the Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2019-134) and the Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (14.11.2019/21264211-288.04-E.3469713).

Statistical analyses of data: The data were analysed with SPSS 22.0 (Statistical Package for Social Sciences). The normality test of the data was performed using the Shapiro-Wilk test, and it was found that the groups were not normally distributed separately ($p<0.05$). For statistical analysis of blood samples taken from rehabilitated birds, they were divided into 3 groups in Long-legged Buzzard and 2 groups in others according to their clinical symptoms. Man-Whitney U test was performed and $p \leq 0.05$ was accepted as significant.

RESULTS

In this study Long-legged Buzzards were brought to the center with the diagnosis of 6 soft tissue trauma, 1 bone tissue trauma without fractures, 1 yeast infection, 1 left claw paralysis, 5 fractures and 2 weaknesses (Table 1). Common Buzzards were brought to the center with 1 bone tissue trauma without fractures, 1 soft tissue trauma, 2 fractures and 3 weaknesses (Table 2) and also Golden Eagles with 4 weaknesses, 2 shutgone fire and 1 fracture (Table 3). Although there are slight differences between the values obtained as a result of the analysis, no statistical difference was found (Table 1: for T_3 p= 1.000, U= 7.000, Z=0.000; p= 0.087, U= 7.000, Z= -1.711; p=1.000, U= 5.000, Z= 0.000, p= 0.439, U= 1.000, Z= -0.0775, for T_4 p= 0.232, U= 3.000, Z= -1,196, p= 0.935, U= 17.000, Z= -0.082, p= 0.171, U= 1.500, Z= -1.368, p= 0.555, U= 5.000, Z= -0,590, p= 0.121, U= 0.000, Z= -1.549) (Table 2: for T_3 p= 0.077, U= 1.000, Z= -1.768, for T_4 p= 0.714, U=5.000, Z= -0.367) (Table 3: for T_3 p= 0.480, U= 4.000, Z= -0.707 and T_4 p= 0.724, U= 5.000, Z= -0.354). Plasma-free T_3 and T_4 levels of Long-legged Buzzard, Common Buzzard and Golden Eagle in the rehabilitation center before release to the wild are given Table 4.

Table 1. Plasma free T_3 (nmol/L) and T_4 (nmol/L) levels of the Long-legged Buzzards in the rehabilitation center before releasing to the wildlife.

Long-legged Buzzard	Reason	T_3 (nmol/L)	T_4 (nmol/L)
1	Torticollis / Soft tissue trauma	2. 92	47.21
2	Bone tissue trauma without fractures	10.07	134.01
3	Soft tissue trauma	2.50	45.77
4	Soft tissue trauma	3.59	30.72
5	Soft tissue trauma	2.50	39.63
6	Soft tissue trauma	2.68	45.77
7	Soft tissue trauma	3.59	30.72
8	Yeast Infection	0.31	30.72
9	Left claw paralysis	10.57	32.61
10	Closed wing fracture	1.98	57.45
11	Closed wing fracture	1.88	32.75
12	Closed wing fracture	1.25	30.72
13	Closed wing fracture	3.17	45.93
14	Femur fracture	2.74	45.62
15	Weakness	12.41	47.92
16	Weakness	0.87	41.89
Mean±S.D.		**3.93±3.65**	**46.21±24.80**

* It was not stated statistically because there was no difference between the values (Man-Whitney U, p≥0.05). S.D: Standard Deviation.

Table 2. Plasma free T_3 (nmol/L) and T_4 (nmol/L) levels of the Common Buzzard in the rehabilitation center before releasing to the wildlife.

Common Buzzard	Reason	T_3 (nmol/L)	T_4 (nmol/L)
1	Bone tissue trauma without fractures	11.73	39.49
2	Soft tissue trauma	5.83	30.72
3	Compound fracture	11.01	30.90
4	Femur fracture	9.16	33.39
5	Weakness	3.47	30.72
6	Weakness	3.28	30.72
7	Weakness	7.43	47.02
Mean ± S.D.		**7.41 ± 3.40**	**34.71 ± 6.30**

*Table 2 explanations are as in Table 1.

Table 3. Plasma free T_3 (nmol/L) and T_4 (nmol/L) levels of Golden Eagle in the rehabilitation center before releasing to the wildlife.

Golden Eagle	Reason	T_3 (nmol/L)	T_4 (nmol/L)
1	Weakness	10.14	91.44
2	Weakness, cachexia	5.83	54.28
3	Weakness	4.02	52.38
4	Weakness	2.56	63.99
5	Shotgun fire, left wing fracture	4.63	71.00
6	Shotgun fire, wing fracture	4.45	63.22
7	Right femur fracture	0.52	46.29
Mean±S.D.		**4.59± 2.98**	**63.22±14.95**

*Table 3 explanations are as in Table 1.

Table 4. Plasma free T_3 and T_4 levels of Long-legged Buzzard, Common Buzzard and Golden Eagle in the rehabilitation center before releasing to the wild.

Bird Species	T_3 (nmol/L)	T_4 (nmol/L)
Long-legged Buzzard	3.93±3.65 **a**	46.21±24.80 **a**
Common Buzzard	7.41±3.40 **b**	34.71±6.30 **a**
Golden Eagle	4.59±2.98 **ab**	63.22±14.95 **b**

*There is no statistical difference between values shown with the same lowercase letters in the same column (p<0.05).

DISCUSSION

The main goal of wildlife rehabilitation is to return rehabilitated animals to appropriate habitats in the wild. Careful attention was paid to minimise unnecessary distress or discomfort for birds according to the international standards followed by the rehabilitation center during their stay. During their stay at the center, no unnecessary physical contact was experienced and blood was taken by the same medical staff.

In this study, rehabilitated 16 Long-legged Buzzards, seven Common buzzards and seven Golden Eagles were evaluated for thyroid hormones. The findings in this study revealed some changes in plasma T_3 and T_4 levels, but no statistical differences were observed. This suggests that these minor changes may be due to increased metabolic needs. An increase in thyroid hormones leads to increased oxygen consumption, which acts as regulator of basal metabolic rate (Welcker et al., 2013). Consequently, various physiological processes such as the development of different body structures, synthesis and metabolism of proteins, carbohydrates and lipids, and thermogenesis are important influencing factors in the control of birds' metabolic processes (Gill, 1994; Merryman & Buckles, 1998). Although both plasma T_3 and T_4 were found slightly at high levels in the Long-legged Buzzard (No.2, Table 1) brought with bone tissue trauma without fractures and in the Golden Eagle (No.1, Table 3) brought with weakness, there was no statistical difference. Although the plasma T_3 levels were found to be slightly higher in Long-legged Buzzards with the diagnosis of left claw paralysis (No.9, Table 1) and weakness (No.15, Table 1) and Common Buzzards diagnosed with bone tissue trauma without fractures (No.1, Table 2) and compound fracture (No.3, Table 2), there was no statistical difference.

The hypothalamic-pituitary-thyroid (HPT) axis coordinates the release of thyroid hormones that regulate metabolism. The release of TSH from the pituitary gland in birds is controlled by peripheral triiodothyronine (T_3) (Elliot et al., 2013; Gentsch et al., 2018; Helmreich et al., 2005; 2006; Merryman & Buckles, 1998). 60% of the thyroid hormones secreted is T_4. Since the biologically active form of thyroid hormones is T_3, the released T_4 provides peripheral control of T_3. The ratio of T_4: T_3 released may vary due to activities such as moulting, stress, reproduction etc., and other usual physiological conditions (Ferrer et al., 1987; Harr, 2002; Helmreich et al., 2005; 2006; Kaneko et al., 1997; Spagnolo et al., 2006). However, T_4's rT_3 (reverse T_3) metabolism is faster than its transformation to T_3. Therefore, low levels of T_4 may be present in the serum of birds when increasing amounts of rT_3 were produced. In addition, since T_4 was bound very weakly to albumin in birds, higher levels of free T_4 could be seen in plasma in birds compared to mammals (Elliot et al., 2013; Garcia-Rodrigez et al., 1987; Merryman & Buckles, 1998). Groscolas & Leloup, (1989) found that plasma T_4 concentrations gradually decreased in captive and starved Emperor penguins (*Aptenodytes forster*) and T_4

levels were stabilized three times lower than in free-living penguins within 15-20 days.

It was known that corticosterone levels increase in birds in captivity or due to activities such as ringing, and holding during taking blood, and there were many studies on this (Morgan & Tromborg, 2007; Quinn et al., 2005; Wada et al., 2009). However, studies on how thyroid hormones are affected in captive birds are not yet enough. Sönmez et al., (2021) in a study they conducted on *Turdus merula* with seasonal and daily changing temperatures, found that plasma T_3 and T_4 hormones changed throughout the 4 seasons and in the morning and afternoon. They suggested that both the metabolic needs of the bird and seasonal and daily changing temperatures may affect the amount of these hormones as the reason for this. Sönmez, (2021) concluded that the differences in plasma T_3 and T_4 hormone levels of rehabilitated Gray Herons resulted from increased physiological and metabolic needs in the center during captivity. The data in this study are given as reference values, as in the current study. In the current study, both T_3 and T_4 were slightly higher in Long-legged buzzard (No 2, Table 1), but no statistical difference was found. In the Common Buzzard, the plasma T_3 values were slightly higher in birds brought with bone tissue trauma without fractures (No 1, Table 2) and fracture (No 3, Table 2), but there was no statistical difference. This may be due to increased metabolism from non-fractured bone tissue trauma and compound fracture. Although these birds were rehabilitated and released into nature healthy, they may have increased metabolism due to the trauma of the events they experienced. Bennett et al., (2012), in a study they conducted with Seal Pups (*Halichoerus grypus*), could not detect a relationship between cortisol and thyroid hormones in the acute phase (especially in the first 5 minutes and after) of catching the pups. They stated that researchers' movement within the colony for blood sampling and morphological measurements could only cause a general disturbance to the colony. Welcker et al., (2013) found a positive relationship between basal metabolic rate and T_3 in a study they conducted with Black-legged Kittiwake (*Rissa tridactyla*). They determined that the plasma T_3 levels measured both in the field and in the laboratory were not different from each other. Therefore, they suggested that stress factors such as catching and holding the bird could temporarily change T_3 levels.

CONCLUSION

At the end of the treatment and rehabilitation of Long-legged Buzzards, Common Buzzards and Golden Eagles, that were brought to the wildlife rescue and rehabilitation center with a specific treatment, accommodation and rehabilitation facilities for each individual, it was aimed to determine the levels of thyroid hormones that were not previously reported in the literature. Depending on the procedures applied at the center, we have believed that the dataset we have reported in the study can be evaluated as thyroid hormone values of healthy individuals. Because of the individual effects of feeding and captivity stress these data may not completely represent the expected values for free-flying raptors under natural conditions. Due to the small sample size, the data in this study can be considered as preliminary data for studies that can be carried out later with a larger number of samples. Long-term studies and larger sample sizes will have an obvious contribution to provide interpretation of these values for researchers to work with our dataset that we have presented in this study.

As a result, we believe that the T_3 and T_4 hormone values that we determined in healthy raptors recovered after rehabilitation in this study will contribute to the evaluation of long-term similar studies to be conducted in the future, in the sense of "reference values" as the first reported data. Besides, physiological and biochemical values in healthy raptors should be examined in more individuals and for longer periods, and comprehensive studies should be conducted to reveal the differences that may occur under different environmental conditions.

ACKNOWLEDGEMENTS

I greatly appreciated to Prof. Dr. Alp ERİLLİ in the course of evaluating the data. For supporting this study I would like to thank Prof. Dr. Sena ÇENESİZ, Prof. Dr. Metin ÇENESİZ, Prof. Dr. Erdoğan UZLU, Assoc. Prof. Dr. Metin Öğün.

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