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Biochemical blood parameters and hormone levels of foraging, nesting, and injured loggerhead sea turtles (*Caretta caretta*) in Turkey

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Abstract: The biochemical blood parameters of 22 (7 foraging, 7 nesting, and 8 injured) loggerhead sea turtles (*Caretta caretta)* from the southwest coast of Turkey were investigated. A total of 27 blood variables were analyzed in three groups (plasma enzymes, plasma metabolites, and plasma ions), and 4 steroid hormones: testosterone (T), estradiol (E_2), progesterone (Pro), and corticosterone (B). Triglyceride, cholesterol, low-density lipoprotein, very low-density lipoprotein, magnesium, and phosphorus were higher in nesting females; urea was high in foraging individuals; and sodium, potassium, and chloride were lower while estradiol levels were higher in injured individuals. In addition, plasma steroid hormone levels T, Pro, and B were assessed in healthy and stranded individuals. The results of the present study provide valuable blood parameters for the medical care of the species in rescue centers and help us to understand the physiological response of loggerhead sea turtles to different conditions.

Key words: Loggerhead sea turtle, *Caretta caretta*, plasma biochemistry, steroid hormone, health, rehabilitation

1. Introduction

Extensive conservation efforts have been made to protect sea turtles in the last three decades in the Mediterranean, but most of the threats such as coastal development, incidental catch, collision with marine vehicles, and intentional killings remain (Casale and Margaritoulis 2010, Başkale et al., 2018). In addition, natural diseases are also a threat to sea turtles (George, 1997; Norton et al., 2005; Jacobson et al., 2006). The loggerhead sea turtle (*Caretta caretta*) is the most abundant sea turtle species in the Mediterranean (Broderick et al., 2002); its main nesting areas are Greece, Turkey, Cyprus, and Libya (Kasparek et al., 2001; Margaritoulis et al., 2003; Canbolat, 2004; Casale and Margaritoulis, 2010), and nesting in other Mediterranean countries is rare. However, loggerhead sea turtles are encountered in the entire Mediterranean (Casale and Margaritoulis, 2010). To reduce the negative anthropogenic effects and increase sea turtle conservation efficiency, a total of 34 confirmed sea turtle rescue centers, 8 first-aid stations, and 7 informal rescue centers are in operation in the Mediterranean (Ullmann and Stachowitsch, 2015).

Biochemical blood parameters are an essential tool that might be used for diagnosing the health status of sea turtles (George, 1997; Aguirre and Balazs, 2000; Whiting et al., 2007; Flint et al., 2009), and are widely used at rescue centers. Most of the biochemical blood parameters of sea turtles were well documented by different researchers (Stamper et al., 2005; Deem et al., 2006, 2009; Whiting et al., 2007; Casal et al., 2009; Flint et al., 2009, 2010; Fong et al., 2010; Camacho et al., 2015; Kelly et al., 2015). However, very few studies on blood biochemistry were reported from the Mediterranean region (Gelli et al., 2009; Basile et al., 2012). Previous reports suggested that biochemical blood parameter values may vary among geographic locations, habitats, and genetics (Herbst and Jacobson, 2003), breeding status (Deem et al., 2006) and migratory status (Stamper et al., 2005). Plasma steroid hormones are also an important biochemical blood parameter to understand the physiological condition of sea turtles and for diagnosing their health status. Plasma testosterone (T), estradiol (E_2), progesterone (P), and corticosterone (B) levels were reported for different sea turtle species in different physiological condition such as reproductive cycles and endocrinology (Owens, 1976; Licht et al., 1979; Morris, 1982; Owens and Morris, 1985; Wibbels et al., 1987a, 1990; Wibbels, 1988; Owens, 1997; Al-Habsi et al., 2006). Plasma T and E_2 levels were also used for sex

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determination in sea turtles (Wibbels et al., 1987b; Casale et al., 1998, 2002; Wibbels, 1999, 2003). Plasma B levels were also studied as the primary glucocorticoid hormone in response to stress (Gregory et al., 1996; Jessop et al., 1999a, 1999b; Jessop and Hamann, 2004; Hunt et al., 2012; Flower et al., 2015). However, very few studies on plasma hormone levels of Mediterranean loggerhead sea turtles were reported and only T levels were investigated as a sea turtle sexing technique (Casale et al., 1998, 2002).

In the present study, we aimed to examine the biochemical blood parameters of loggerhead turtles from the eastern Mediterranean basin and compare these parameters among healthy nesting, foraging, and injured loggerhead sea turtles from the Turkish coastline.

2. Materials and methods

This study was approved by the Pamukkale University Animal Care and Use Committee (protocol number 17.02.2009 – PAUHDEK – 2009/006). Sampling from marine habitats during this study was approved by the Republic of Turkey Ministry of Food, Agriculture, and Livestock (protocol number 05.02.2010/004069).

2.1. Study site, turtle capture, and sampling

This study was conducted on healthy loggerhead sea turtles captured from foraging individuals in Köyceğiz-Dalyan Specially Protected Area (SPA), from nesting females at Dalyan Beach at the boundary of Köyceğiz-Dalyan SPA, and from injured individuals admitted to the Sea Turtle Research, Rescue and Rehabilitation Center (DEKAMER), Dalyan, Turkey. These individuals were grouped and named *foraging*, *nesting*, and *injured*, respectively.

We obtained blood samples from loggerhead sea turtles in 2009. The turtles were captured by hand by a swimmer from the foraging ground in September. The turtles were then taken on board. Nesting females were spotted at night on the nesting beach in June and the turtles captured following the nesting process. Within 5 min of capture, blood samples were obtained from the dorsal cervical sinus (Owens and Ruiz, 1980) using a 21-gauge needle attached to a 10-mL syringe, and then were transferred into lithium heparin blood tubes. The needles were removed prior to blood transfer to prevent possible hemolysis. The blood samples then were immediately placed on ice. The blood samples were transferred to DEKAMER laboratory and centrifuged at 3000 rpm for 5 min within 3 h. Blood samples from injured turtles were obtained following admission to DEKAMER by the same procedure. The plasma was drawn off and stored at –20 °C until analysis.

The common carapace length measurement method described by Bolten (1999) was used. Curved carapace length maximum (CCL_{max}) from tip to the tip of the carapace and curved carapace width (CCW) were measured in centimeters, using a soft millimetric tape measure. Straight carapace length maximum (SCL_{max}) from tip to the tip of the carapace and straight carapace width (SCW) were measured in centimeters using calipers after blood sampling. Adult loggerhead sea turtles in the Mediterranean are smaller than those in other populations (Margaritoulis et al., 2003), and individuals larger than 70 cm CCL were considered to be adults in Turkey (Türkozan et al., 2013). However, quite a large number of nesting females were observed between 65 and 70 cm CCL in the study area (Kaska et al., 2016). Therefore, individuals with CCL_{max} > 65 cm were considered to be adults.

2.2. Analysis of biochemical blood parameters

Plasma biochemistry was analyzed with a COBAS 6000 analyzer (Roche Instruments) and commercial kits were used for the following 25 parameters, and the data were then grouped by plasma enzymes (alanine transaminase: ALT, aspartate transaminase: AST, alkaline phosphatase: ALP, lactate dehydrogenase: LDH, gamma-glutamyl transferase: GGT, creatine kinase: CK, and CK-MB, amylase: Amy), metabolites (glucose: Glu, creatinine: CR, total protein: TP, albumin: ALB, globulin: Glob, cholesterol: CHOL, high-density lipoprotein: HDL, urea, uric acid: UA, triglyceride: TG), and ions (sodium: Na, potassium: K, chloride: Cl, calcium: Ca, magnesium: Mg, inorganic phosphor: P, iron: Fe). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels were calculated after analysis.

Testosterone (T), estradiol (E_2), progesterone (Pro), and corticosterone (B) levels were determined by radioimmunoassay (RIA), electrochemiluminescence immunoassay (ECLIA), and ELISA, respectively. Plasma samples were extracted two times prior to analysis; 3 mL of diethyl ether were added to 200 µL of plasma sample. After addition of diethyl ether, samples were vortexed for 1 min and centrifuged for 5 min at 2500 rpm. The ether phase was taken and the same procedure was repeated. The samples were then evaporated in a water bath at 37 °C and 1.5 mL of Tris buffer saline was added, followed by analysis. T and Pro levels were measured with RIA kits (DIA Source TESTO–RIA–CT KIP 1709 for T, and Immunotech IM1188 for P). Extraction was performed before analysis and ECLIA was performed for E_2 analysis (Cobas Estradiol II 03000079 122 kits). B levels were measured with EIA by using Cayman Chemical kits (Cat. No. 500651 and Cat. No. 10005590).

2.3. Data analysis

We used nonparametric Kruskal–Wallis and Mann–Whitney U tests for comparing analysis results between the study groups by computer software (Minitab Ver. 16.2). Standard ANOVA could not be conducted because of a failed normality test (Kolmogorov–Smirnov test). Results significant at $P \leq 0.05$ were used for all analyses, and test results were given separately.

3. Results

Blood samples were collected from 22 loggerhead sea turtles and CCL_{max} was measured for all individuals. Of these sampled turtles, 14 individuals were female with a mean CCL_{max} of 73.6 cm ± 4.08, while 7 individuals were male with a mean of 71.6 ± 2.20 cm and an individual was a subadult (60.0 cm).

Plasma TG, Chol, LDL, VLDL, urea, Na, K, Cl, P, Mg, and E_2 levels were significantly different between the groups, and statistical analysis results are given in the related tables. In addition, the mean values of ALP, ALT, AST, GGT, LDH, Amy, CK, CK-MB, UA, HDL, Fe, CR, T, Pro, and B levels were different between the groups, but the differences were not statistically significant.

Plasma biochemistry levels and descriptive statistics of plasma enzymes are presented in Table 1. Nesting females had higher level of plasma ALT, AST, LDH, CK-MB but lower GGT and Amy levels. Plasma enzymes showed similar patterns in foraging and injured turtles. Plasma metabolites are presented in Table 2. The mean plasma cholesterol levels were higher in nesting than in foraging and injured turtles, but only nesting and foraging turtles showed significantly different levels. As with cholesterol, triglyceride levels were higher in nesting females, but only nesting and injured turtles showed significantly different levels. Injured turtles had lower mean HDL levels, but the groups did not significantly differ. Plasma LDL and VLDL levels showed similar patterns as cholesterol. Plasma urea levels were highest in foraging turtles while the lowest levels were seen in nesting females. Plasma glucose levels were higher in injured turtles, but the groups did not significantly differ. However, we excluded a glucose

Table 1. Descriptive statistics of plasma enzymes.

	Group	N	Min	Max	Mean	SD
ALP (U/L)[a]	Foraging	7	5.0	15.0	8.7	4.03
	Nesting	7	6.0	15.0	8.9	3.63
	Injured	8	4.0	23.0	10.3	5.75
ALT (U/L)[b]	Foraging	7	1.1	8.0	3.6	2.70
	Nesting	7	3.6	23.2	7.7	7.13
	Injured	8	0.0	8.2	3.1	2.66
AST (U/L)[c]	Foraging	7	81.7	286.2	183.6	66.70
	Nesting	7	127.5	457.0	221.5	111.20
	Injured	8	106.3	294.8	186.6	65.10
GGT (U/L)[d]	Foraging	7	0.0	16.0	3.0	5.77
	Nesting	7	0.0	2.0	0.9	0.90
	Injured	8	0.0	15.0	4.1	5.17
LDH (U/L)[e]	Foraging	7	0.0	52.0	12.7	18.34
	Nesting	7	7.0	248.0	58.3	85.00
	Injured	8	0.0	57.0	20.6	19.90
Amy (U/L)[f]	Foraging	7	289.0	2080.0	1130.0	575.00
	Nesting	7	475.0	833.0	677.1	107.70
	Injured	8	509.0	1473.0	1149.0	351.00
CK (U/L)[g]	Foraging	7	140.0	2191.0	825.0	873.00
	Nesting	7	110.0	677.0	337.3	213.50
	Injured	8	233.0	4505.0	1164.0	1411.00
CK-MB (U/L)[h]	Foraging	7	56.9	142.7	110.2	31.00
	Nesting	7	72.0	328.1	188.4	89.20
	Injured	8	21.1	174.4	93.0	60.30

[a] Kruskal–Wallis H = 0.51; DF = 2; P > 0.05
[b] Kruskal–Wallis H = 4.05; DF = 2; P > 0.05
[c] Kruskal–Wallis H = 0.29; DF = 2; P > 0.05
[d] Kruskal–Wallis H = 1.43; DF = 2; P > 0.05
[e] Kruskal–Wallis H = 3.91; DF = 2; P > 0.05
[f] Kruskal–Wallis H = 6.00; DF = 2; P < 0.05
[g] Kruskal–Wallis H = 4.06; DF = 2; P > 0.05
[h] Kruskal–Wallis H = 5.39; DF = 2; P > 0.05

Table 2. Descriptive statistics of plasma metabolites.

Variable	Group	N	Min	Max	Mean	SD
TG (mg/dL)[a]	Foraging	7	32.1	178.2	91.3	60.70
	Nesting	7	161.8	544.8	322.4	119.50
	Injured	8	7.6	311.1	95.1	103.0
CHOL (mg/dL)[b]	Foraging	7	75.1	198.1	147.1	41.70
	Nesting	7	177.6	394.9	288.9	74.00
	Injured	8	60.4	438.7	174.8	147.10
HDL (mg/dL)[c]	Foraging	7	17.9	56.8	31.4	13.63
	Nesting	7	17.3	93.6	35.3	26.26
	Injured	8	16.9	33.6	26.6	5.42
LDL (mg/dL)[d]	Foraging	7	49.0	141.0	97.4	29.40
	Nesting	7	113.0	292.0	201.0	60.20
	Injured	8	21.0	351.0	127.3	132.90
VLDL (mg/dL)[e]	Foraging	7	6.0	36.0	18.3	12.19
	Nesting	7	32.0	109.0	64.4	23.96
	Injured	8	2.0	62.0	19.1	20.36
CR (mg/dL)[f]	Foraging	7	0.00	0.08	0.03	0.034
	Nesting	7	0.00	0.11	0.06	0.039
	Injured	8	0.00	0.27	0.07	0.091
Glu (mg/dL)[g]	Foraging	7	75.3	103.0	88.2	9.50
	Nesting	7	49.9	115.9	84.1	19.77
	Injured	7	16.9	99.6	78.5	28.70
TP (g/dL)[h]	Foraging	7	2.44	5.56	4.36	1.238
	Nesting	7	3.44	4.75	4.20	0.449
	Injured	8	3.72	7.20	5.05	1.173
Alb (g/dL)[i]	Foraging	7	0.69	1.54	1.20	0.326
	Nesting	7	0.92	1.46	1.17	0.183
	Injured	8	0.88	1.75	1.31	0.282
Glob (g/dL)[j]	Foraging	7	2.0	4.0	3.14	0.900
	Nesting	7	2.0	3.0	2.86	0.378
	Injured	8	3.0	6.0	3.75	1.165
Urea (mg/dL)[k]	Foraging	7	102.2	299.4	213.5	71.00
	Nesting	7	10.9	52.1	29.8	15.11
	Injured	8	32.5	203.3	111.1	54.70
UA (mg/dL)[l]	Foraging	7	0.4	1.4	0.76	0.355
	Nesting	7	0.4	0.9	0.73	0.180
	Injured	8	0.4	1.1	0.58	0.225
Fe (ug/dL)[m]	Foraging	7	33.9	93.3	59.8	20.81
	Nesting	7	29.3	85.6	56.5	19.18
	Injured	8	19.6	58.7	39.5	13.78

[a] Kruskal–Wallis H = 11.69; DF = 2; P < 0.003; Mann–Whitney Foraging/Nesting W = 68.0; P > 0.05; Foraging/Injured W = 52.0; P > 0.05; Nesting/Injured W = 31.0; P < 0.005

[b] Kruskal–Wallis H = 7.09; DF = 2; P < 0.03; Mann–Whitney Foraging/Nesting W = 30.0; P < 0.005; Foraging/Injured W = 65.0; P > 0.05; Nesting/Injured W = 71.0; P > 0.05

[c] Kruskal–Wallis H = 0.37; DF = 2; P > 0.05

[d] Kruskal–Wallis H = 6.84; DF = 2; P < 0.03; Mann–Whitney Foraging/Nesting W = 29.5; P < 0.004; Foraging/Injured W = 64.0; P > 0.05; Nesting/Injured W = 70.0; P > 0.05

[e] Kruskal–Wallis H = 11.71; DF = 2; P < 0.003; Mann–Whitney Foraging/Nesting W = 29.0; P < 0.003; Foraging/Injured W = 56.5; P > 0.05; Nesting/Injured W = 81.0; P < 0.005

[f] Kruskal–Wallis H = 1.53; DF = 2; P > 0.05

[g] Kruskal–Wallis H = 0.71; DF = 2; P > 0.05

[h] Kruskal–Wallis H = 2.00; DF = 2; P > 0.05

[i] Kruskal–Wallis H = 1.73; DF = 2; P > 0.05

[j] Kruskal–Wallis H = 2.91 DF = 2; P > 0.05

[k] Kruskal–Wallis H = 15.65; DF = 2; P < 0.001; Mann–Whitney Foraging/Nesting W=77.0; P < 0.002; Foraging/Injured W = 78.0; P < 0.002; Nesting/Injured W = 31.0; P < 0.005

[l] Kruskal–Wallis H = 2.63; DF = 2; P > 0.05

[m] Kruskal–Wallis H = 4.33; DF = 2; P > 0.05

analysis result from the injured group as it showed an extremely outlier plasma glucose level (493.0 mg/dL). The mean plasma Fe levels were lower in injured turtles, but the groups did not significantly differ. The mean values of Na, K, and Cl were lower in injured turtles. Plasma K levels were also significantly different between foraging and nesting turtles. Plasma P and Mg levels were significantly higher in nesting females than in foraging and injured turtles. Plasma Ca levels did not show differences between the groups (Table 3). Plasma lipids were regrouped for detailed analysis considering different physiological conditions and the results are given in Table 4.

Measurements of plasma steroid hormone levels failed for an injured turtle and plasma B level of a nesting female was below the detection range; those samples were subsequently excluded from the statistical analyses. Analysis results of plasma steroid hormone levels are given in Table 5. In addition, plasma steroid hormone levels were regrouped for detailed analysis considering sex and health status and the results are given in Table 6.

Plasma steroid hormone levels showed variation between the study groups but only E_2 levels were significantly different between the groups. In addition, plasma T and B levels were higher in healthy individuals, while E_2 and Pro levels were higher in injured individuals, but only E_2 levels showed significant differences.

4. Discussion

The present study provides the first biochemical blood parameters for loggerhead sea turtles from the Mediterranean coast of Turkey. Blood parameters were analyzed and compared in three groups: foraging, nesting, and injured turtles. Plasma enzymes did not show significant differences between the groups and plasma enzyme levels were lower than in previous studies or at the lower limit (Stamper et al., 2005; Jacobson et al., 2007; Casal et al., 2009; Deem et al., 2009; Gelli et al., 2009; Flint et al., 2010; Delgado et al., 2011; Fazio et al., 2012a, 2012b; Rousselet et al., 2013). Conversely, Amy levels were higher than in previous studies. Plasma CK levels were

Table 3. Descriptive statistics of plasma ions.

	Group	N	Min	Max	Mean	SD
Na (mmol/L)[a]	Foraging	7	160.0	179.0	164.4	6.58
	Nesting	7	160.0	174.0	162.9	5.05
	Injured	8	146.0	172.0	152.9	8.34
K (mmol/L)[b]	Foraging	7	3.46	4.78	4.20	0.525
	Nesting	7	4.79	5.47	5.05	0.287
	Injured	8	2.76	4.26	3.38	0.512
Cl (mmol/L)[c]	Foraging	7	110.5	129.0	119.8	5.38
	Nesting	7	112.0	128.2	116.5	5.68
	Injured	8	99.8	112.6	106.5	4.66
P (mmol/L)[d]	Foraging	7	4.14	6.17	5.27	0.816
	Nesting	7	7.96	13.02	9.47	1.980
	Injured	8	2.49	11.68	6.04	2.557
Ca (mg/dL)[e]	Foraging	7	7.66	9.37	8.58	0.614
	Nesting	7	3.99	11.63	8.56	2.524
	Injured	8	6.29	10.97	8.49	1.648
Mg (mg/dL)[f]	Foraging	7	2.98	4.20	3.80	0.410
	Nesting	7	5.08	7.42	5.84	0.774
	Injured	8	2.89	4.62	3.77	0.634

[a] Kruskal–Wallis H = 9.45; DF = 2; P < 0.008; Mann–Whitney Foraging/Nesting W = 60.5; P > 0.05; Foraging/Injured W = 78.0; P < 0.02; Nesting/Injured W = 78.0; P < 0.02

[b] Kruskal–Wallis H = 16.24; DF = 2; P < 0.001; Mann–Whitney Foraging/Nesting W = 28.0; P < 0.002; Foraging/Injured W = 76.0; P < 0.03; Nesting/Injured W = 84.0; P < 0.002

[c] Kruskal–Wallis H = 13.34; DF = 2; P < 0.001; Mann–Whitney Foraging/Nesting W = 63.0; P > 0.05; Foraging/Injured W = 82.0; P < 0.003; Nesting/Injured P = 0.003

[d] Kruskal–Wallis H = 11.04; DF = 2; P < 0.004; Mann–Whitney Foraging/Nesting W = 28.0; P = 0.0022; Foraging/Injured W = 52.0; P > 0.05; Nesting/Injured W = 78.0; P < 0.02

[e] Kruskal–Wallis H = 0.26; DF = 2; P > 0.05

[f] Kruskal–Wallis H = 13.75; DF = 2; P < 0.001; Mann–Whitney Foraging/Nesting W = 28.0; P < 0.003; Foraging/Injured W = 59.0; P > 0.05; Nesting/Injured W = 84.0; P < 0.002

Table 4. Descriptive statistics of plasma TG, and cholesterol levels of healthy individuals according to sex.

Variable	Groups	N	Min	Max	Mean	SD
TG (mg/dL)	Nesting females	7	161.8	544.8	322.4	119.50
	Foraging females	2	32.1	141.8	87.0	77.60
	Foraging males	4	35.7	178.2	108.2	61.90
	Foraging subadult	1	32.6	32.6	32.6	-
CHOL (mg/dL)	Nesting females	7	177.6	394.9	288.9	74.00
	Foraging females	2	121.0	184.3	152.7	44.80
	Foraging males	4	135.0	198.1	162.4	27.20
	Foraging subadult	1	75.1	75.1	75.1	-
HDL (mg/dL)	Nesting females	7	17.3	93.6	35.3	26.26
	Foraging females	2	17.9	56.8	37.3	27.50
	Foraging males	4	21.4	37.4	31.4	6.96
	Foraging subadult	1	19.5	19.5	19.5	-
LDL (mg/dL)	Nesting females	7	113.0	292.0	201.0	60.20
	Foraging females	2	97.0	99.0	98.0	1.41
	Foraging males	4	74.0	141.0	109.3	27.50
	Foraging subadult	1	49.0	49.0	49.0	-
VLDL (mg/dL)	Nesting females	7	32.0	109.0	64.4	23.96
	Foraging females	2	6.0	28.0	17.0	15.60
	Foraging males	4	7.0	36.0	21.8	12.53
	Foraging subadult	1	7	7	7.0	-

Table 5. Descriptive statistics of plasma steroid hormones.

Variable	Group	N	Min	Max	Mean	SD
Testosterone (ng/mL)[a]	Foraging	7	0.17	11.5	3.26	3.950
	Nesting	7	0.12	7.84	2.02	2.850
	Injured	7	0.15	5.73	1.06	2.060
Estradiol (pg/mL)[b]	Foraging	7	23.2	428.7	207.1	144.10
	Nesting	7	10.2	176.8	63.7	72.00
	Injured	7	71.0	2540.0	1039.0	865.00
Progesterone (ng/mL)[c]	Foraging	7	0.01	0.09	0.05	0.029
	Nesting	7	0.01	4.92	0.99	1.795
	Injured	7	0.01	8.85	1.44	3.290
Corticosterone (ng/mL)[d]	Foraging	7	0.010	5.230	1.474	2.3120
	Nesting	6	0.008	0.230	0.096	0.0915
	Injured	7	0.006	0.140	0.057	0.0471

[a] Kruskal–Wallis H = 2.28; DF = 2; P > 0.05
[b] Kruskal–Wallis H = 11.58; DF = 2; P < 0.003; Mann–Whitney Foraging/Nesting W = 69.0; P < 0.05; Foraging/Injured W = 35.0; P < 0.03; Nesting/Injured W = 75.0; P < 0.005
[c] Kruskal–Wallis H = 3.26; DF = 2; P > 0.05
[d] Kruskal–Wallis H = 2.78; DF = 2; P > 0.05

Table 6. Descriptive statistics of plasma steroid hormone levels of healthy and injured turtles according to sex.

Health status	Variable	Sex	N	Min	Max	Mean	SD
Healthy	Testosterone (ng/mL)	Female	9	0.12	7.84	1.93	2.476
		Male	4	0.29	11.50	4.86	4.780
		Subadult	1	0.17	0.17	0.17	-
	Estradiol (pg/mL)	Female	9	10.2	176.8	69.1	63.30
		Male	4	263.7	428.7	312.4	77.80
		Subadult	1	23.2	23.2	23.2	-
	Progesterone (ng/mL)	Female	9	0.01	4.92	0.78	1.608
		Male	4	0.02	0.06	0.05	0.017
		Subadult	1	0.08	0.08	0.08	-
	Corticosterone (ng/mL)	Female	8	0.01	0.23	0.09	0.085
		Male	4	0.24	5.23	2.54	2.670
		Subadult	1	0.01	0.01	0.01	-
Injured	Testosterone (ng/mL)	Female	4	0.15	5.73	1.64	2.730
		Male	3	0.27	0.32	0.29	0.029
	Estradiol (pg/mL)	Female	4	71.0	2540.0	908.0	1113.00
		Male	3	607.0	1689.0	1214.0	553.00
	Progesterone (ng/mL)	Female	4	0.01	8.85	2.22	4.420
		Male	3	0.04	1.09	0.39	0.603
	Corticosterone (ng/mL)	Female	4	0.02	0.14	0.08	0.055
		Male	3	0.01	0.05	0.03	0.023

between the previously reported ranges (Jacobson et al., 2007; Deem et al., 2009; Gelli et al., 2009; Flint et al., 2010; Delgado et al., 2011; Rousselet et al., 2013). In general, the mean plasma ALT, AST, LDH, and CK-MB levels were highest in nesting females, while the mean plasma GGT, Amy, and CK levels were lowest. Foraging and injured groups showed similar results for the plasma enzymes.

Plasma lipid levels were reported to be increased during vitellogenesis in leatherback sea turtles (Deem et al., 2006) and loggerhead sea turtles (Deem et al., 2009). TG and Chol levels in the present study were significantly higher in nesting females. Gelli et al. (2008) reported lower TG and Chol levels from Italy. This area is known as a foraging area and loggerhead sea turtle nesting is not observed in the area (Bentivegna, 2002; Casale and Margaritoulis, 2010). In the present study, the mean Chol values of foraging turtles were similar to the reported values from Italy, and our results were in agreement with previous studies' increased Chol levels during vitellogenesis.

Glucose levels in the present study were within the limits of previous reports on loggerhead sea turtles (Bolten et al., 1992; Stamper et al., 2005; Jacobson et al., 2007; Casal et al., 2009; Deem et al., 2009; Gelli et al., 2009; Flint et al., 2010; Delgado et al., 2011; Rousselet et al., 2013). Injured individuals' plasma glucose levels were lower than those of foraging and nesting females, but the groups were not significantly different. Similar plasma glucose levels were reported for foraging, nesting, and injured loggerhead sea turtles (Deem et al., 2009). Plasma CR levels in the present study were much lower than those in previous studies for the same species (Casal et al., 2009; Deem et al., 2009; Gelli et al., 2009; Flint et al., 2010; Fazio et al., 2012a, 2012b; Rousselet et al., 2013).

Plasma urea levels were dramatically lower in nesting females, while plasma urea levels were highest in foraging turtles. These results were similar to those of a previous report by Deem et al. (2009), which may suggest fasting during the nesting period. The plasma level of UA was lowest in injured turtles but did not differ from nesting and foraging turtles. Increased UA levels could be associated with dehydration and injuries, but we have a reverse situation in this study.

Nesting females had higher plasma Ca levels, but the groups did not significantly differ. Plasma P levels were within the limits of previously reported results. Ca:P ratio was < 1 in nesting females and > 1 in foraging and injured individuals. Plasma P levels are likely to be increased during egg production (Deem et al., 2006). Therefore, a

low Ca:P ratio in nesting females could be expected. The mean plasma Fe levels were considerably low in injured turtles, but the groups did not differ. Plasma Fe results of the present study were higher than previously reported Fe levels (Bolten et al., 1992). Plasma Mg levels of nesting females were significantly higher than those of foraging and injured turtles. Plasma Na, K, and Cl levels were within the known limits. Injured turtles had the lowest level of plasma ions. Deem et al. (2009) suggested that decreased food intake could lead to low plasma K levels, which may explain the low K levels of injured turtles. The same situation could be expected for nesting females due to fasting during the nesting period, but conversely nesting females had the highest K levels. Our results did not indicate fasting for nesting females except for urea.

We analyzed plasma steroid hormone levels of T, E_2, Pro, and B under the same three groups. Among the four studied hormones, only E_2 was significantly different among the groups. Injured loggerhead sea turtles had the highest plasma E_2 levels, while nesting females had the lowest plasma E_2 levels. Plasma E_2 levels are at the highest levels during vitellogenesis and then gradually decrease during the mating and nesting period in green sea turtles (Al-Habsi et al., 2006), in leatherback sea turtles (Rostal et al., 2001), and in loggerhead sea turtles (Wibbels, 1988). Plasma T levels are known to be increased prior to mating in both sexes and then eventually decrease after the mating period in loggerhead sea turtles (Wibbels, 1988; Whittier et al., 1997), Kemp's ridley sea turtles (Rostal et al., 1998), and leatherback sea turtles (Rostal et al., 2001). The same pattern was reported for plasma Pro levels of female sea turtles, but the decrease in plasma Pro levels occurs more slowly (Whitter et al., 1997; Rostal et al., 1998, 2001). In addition, no data are available for male sea turtles for Pro levels in the previous studies. Plasma B levels are known to be altered in response to stress and previous studies reported changes in plasma B levels in different conditions for sea turtle species (Gregory et al., 1996; Whitter et al., 1997; Jessop et al., 1999a, 1999b, 2000, 2002; Jessop and Hamman, 2004; Blanvillain et al., 2008; Flower et al., 2015).

In the present study, healthy female turtles were sampled during the nesting period or postnesting period. Moreover, all but one individual were adults. When the health status of sampled individuals was considered, plasma T and E_2 and B levels of males were higher than in females in the healthy group, while the Pro levels were lower. However, plasma T, E_2, Pro, and B levels of males were lower than those of females in the injured group. Plasma E_2 and Pro levels were dramatically increased in injured turtles for both sexes. No significant changes were observed for T and B levels of females between healthy and injured turtles. When healthy and injured groups were compared together, E_2 and Pro levels were higher in the injured group, while T and B levels were lower. The role of steroid hormones, particularly E_2, in proliferation and tissue damage is well known. Therefore, altered plasma steroid hormone levels may be associated with hormonal response to injuries in the present study, but we avoid drawing a precise conclusion due to the small sample size.

In summary, here we presented the first blood biochemistry parameters for loggerhead sea turtles of different health and reproductive status from Turkey. We avoided the calculation of reference intervals as we had a relatively small sample size (n = 22); the minimum sample size for calculating the reference intervals was considered 120 individuals (Campbell, 1995; Geffre et al., 2009). Nonetheless, we provided valuable blood parameters for the medical care of the species at rescue centers in the Mediterranean. Our findings may also provide information to understand the physiological response of loggerhead sea turtles to different conditions.

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