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CONTENTS

CHAPTER 1

Body Condition, Sexual Dimorphism and Shell Injuries in Thrace Populations of *Testudo hermanni* (Northwestern Türkiye)

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Introducion

Most vertebrate species exhibit marked morphological and physiological differences depending on the geographical region and environmental conditions in which they live. This geographical variation, particularly observed among distant populations, can enhance a species' adaptive capacity through the influence of environmental factors (DeWitt and Scheiner, 2004). Reptiles represent one of the vertebrate groups that exhibit a high degree of phenotypic plasticity in response to variable environmental conditions. This flexibility, supported by physiological, behavioral, and morphological adaptation mechanisms, strengthens the survival and environmental resilience of species (Bonnet et al., 2001a; Aubret et al., 2007). Thus, the morphological diversity observed in reptiles plays a critical role in understanding ecosystem dynamics (Djordjević et al., 2011).

Tortoises, as a reptilian group, are distinguished by their diverse shell morphology. The structure of the shell plays a critical role in biological processes such as reproduction, movement, and competition, while body size and shape are directly related to ecological and sexual selection processes (Djordjević et al., 2013). In females, shell structure supports reproductive output, whereas in males it serves as an advantageous trait in sexual selection (Kaddour et al., 2008; Djordjević et al., 2011; Baycan and Tosunoğlu, 2023). These differences form the basis of sexual dimorphism, creating distinct variations between males and females in terms of body size, resource use, and reproductive strategies (Fairbairn, 1997; Djordjević et al., 2011). Such divergences, which support intersexual niche differentiation, directly affect the ecological roles and reproductive success of individuals (Shine, 1989; Fairbairn, 1997; Djordjević et al., 2011). Additionally, body condition, calculated as the ratio of body mass to body size, provides essential information about the nutritional status and physiological condition of tortoises (Hennen, 1991, 1997; Lagarde et al., 2002; Baycan and Tosunoğlu, 2023).

Injuries to the carapace and plastron may result from both natural and anthropogenic factors (Dodd, 2001; Meek, 2007; Saumure et al., 2007; Biaggini and Corti, 2018). Natural causes include intraspecific competition, predatory attacks, and accidental falls, whereas anthropogenic sources encompass agricultural machinery, vehicle collisions, and artificial barriers (Meek and Inskeep, 1981; Saumure and Bider, 1998; Hailey, 2000a; Gibbons et al., 2001; Dodd, 2001; Draud et al., 2004; Biaggini and Corti, 2018). Shell injuries on tortoises serve as indicators of environmental and human-induced impacts and provide valuable insights into population dynamics. Such injuries, in particular, can be used as biological indicators to assess the intensity of human activities and their effects on ecosystems (Duro et al., 2021; Buica et al., 2014). The spread of intensive agricultural activities accelerates habitat loss, making *Testudo* populations distributed throughout the Mediterranean region more vulnerable to both natural and anthropogenic shell injuries (Biaggini and Corti, 2018).

Testudo hermanni (Gmelin, 1789) is widely distributed across Europe and the Mediterranean Basin, inhabiting agricultural lands, rugged grasslands, areas with sparse vegetation, and habitats near forests. However, the species is listed among those under threat due to habitat loss, pollution (Mingo et al., 2016), habitat degradation caused by agricultural practices (Matache et al., 2006; Rozylowicz and Dobre, 2009), forest fires (Hailey, 2000), and the pet trade (Cheylan, 2001; Fernández-Chacón et al., 2011). According to the International Union for Conservation of Nature (IUCN), the species is globally classified as "Vulnerable (VU)" (Luiselli, 2024). The overall distribution range of the species encompasses regions in Europe with Mediterranean and sub-Mediterranean climates (Cheylan, 2001; Karaman, 2017). In Türkiye, however, its distribution is limited to the Thrace region (Baran et al., 2021).

Various studies have been conducted on the morphometry, sexual dimorphism, and shell injuries of *T. hermanni* (Jackson, 1980; Amiranashvili, 2000; Djordjević et al., 2011; Djordjević et al., 2013; Kicaj et al., 2016; Labus et al., 2016; Biaggini and Corti, 2018; Makridou et al., 2019; Djordjević et al., 2019; Duro et al., 2021). Although research on the morphometry and body condition index of *T. hermanni* populations in the Thrace region is limited, no study has previously focused on shell injuries in this species. This study investigates morphometric measurements, ratios, sexual dimorphism, body condition indices, and shell injuries of *T. hermanni* populations from two different stations in the Thrace region, with the aim of comparing morphological differences both within and between populations. Through the comparison of these two populations, the effects of different habitat types on the morphology and body condition of the species were determined.

Materials and Methods

Study Area

The Thrace Region is a transitional area located in northwestern Türkiye, connecting Asia and Europe, and covers an area of 23,764 km^2. It is bordered by Bulgaria to the north, the Black Sea to the northeast, Greece to the west, and the Aegean and Marmara Seas to the south. The region is separated from Anatolia by the Bosphorus and the Dardanelles Straits (Yılmaz, 1981; Erdoğan, 2010).

Testudo hermanni populations in the Thrace Region were collected from two stations with distinct habitat characteristics. The first station, Kırklareli/Karakoç (41°47'5.62"N, 27°12'33.07"E), represents an open habitat featuring small shrubs (382 m a.s.l.) and wetlands and livestock grazing and agricultural activity were also observed in the area. The second station, Edirne/Keşan (40°45'16.29"N, 26°42'53.24"E), is characterized by a more closed habitat with dense tree cover (78 m a.s.l.). The distance between the two stations is approximately 121.42 km (Figure 1).

All analyses involving animal specimens were conducted with the necessary ethical approvals obtained from the Local Ethics Committee for Animal Experiments of Çanakkale Onsekiz Mart University (decission no 2023/01-06).

Figure 1. Study sites [A: Map view of the study areas, B: Kırklareli/Karakoç, C:Edirne/Keşan(source:https://maps.google.com)].

Data Collection

Testudo hermanni specimens were hand-collected from the study stations between April and September 2024. Individuals were marked on the marginal scutes following the method described by Stubbs et al. (1984). The sex of each individual was determined using external morphological characteristics such as the concavity of the plastron (Willemsen and Hailey, 2003). Juvenile individuals with undetermined sex (carapace length <10 cm) were excluded from the analyses (Willemsen & Hailey, 2003).

Body measurements were taken using a digital caliper (accuracy: 0.01 mm) and a measuring tape, while weights were recorded using a digital scale (accuracy: 1 g) and an analog scale. Body weight was measured before the animals urinated or defecated. After conducting morphometric analyses, photographs of each specimen were taken, and injuries on the carapace and plastron were documented. After analyses, the individuals were released back into their original habitats.

Morphometric Measurements and Indices

The following morphological parameters were recorded:

Body Weight (BW): Total body mass. Straight Carapace Length (SCL): Measured along the dorsal midline from the anterior edge of the nuchal scute to the posterior edge of the supracaudal scute. Carapace Width (CW): Taken at the level of the 6th marginal scutes. Plastron Length (PL): Distance from the tip of the gular scutes to the tip of the anal scutes. Plastron Width (PW): Measured at the level of the 6th marginal scutes. Shell Height (SH): Maximum vertical distance from the ground (contact point of the plastron) to the midpoint of the suture between the 2nd and 3rd vertebral scutes. Anal Notch Width (ANW): Linear width of the suture between the two anal scutes. Claw Length (CL): Length of the claw at the tip of the tail.

To compare males and females both within and between populations, five morphometric ratios (SCL/PL, SCL/PW, SCL/CW, PL/PW, SCL/SH) were examined. The Body Condition Index (BCI) was calculated as the ratio of body weight (g) to the estimated shell volume (cm^3), obtained by multiplying carapace length (SCL), height (SH), and width (CW) (Nagy et al., 2002). Shell volume was calculated in cubic centimeters using the formula SCL × SH × CW. BCI value greater than 0 indicates better health due to favorable environmental conditions, while negative values suggest poor health conditions caused by malnutrition or stress factors (Stevenson and Woods, 2006; Mollov et al., 2021). Additionally, BCI values were compared by considering elevation differences between the localities to evaluate the potential effects of environmental conditions on individual condition. The Sexual Dimorphism Index (SDI) was calculated according to the method of Lovich and Gibbons (1992).

Shell Injuries

To assess the condition and severity of shell injuries, the Carapace Index and Plastron Index developed by Saumure et al. (2007) and Biaggini and Corti (2018) were used. Following this methodology, the carapace and plastron in the photographs of the specimens were each divided into four equal regions (Figure 2). Each region was scored between 0 and 3 to evaluate injury severity. The scoring was as follows: 0: Shell intact, no damage or only superficial abrasions present. 1: Small and limited-depth abrasions or indentations less than 0.5 cm on the marginal scutes. 2: Moderate abrasions larger than 1 cm and/or indentations deeper than 0.5 cm. 3: Severe damage, presence of deep indentations or fractures in the inner shell regions. For each tortoise, the scores of the four regions were summed, and the total score was divided by the maximum score of 12, resulting in an index value ranging between 0 and 1. This method allowed for a quantitative assessment of shell injuries.

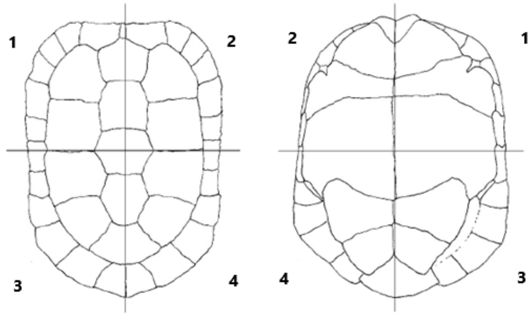

Figure 2. Shell regions for the carapace index and plastron index (Biaggini and Corti, 2018).

In order to evaluate the causes of shell injuries, a visual analysis was conducted on the carapace and plastron photographs of the specimens. In this analysis, the carapace was divided into five regions and the plastron into four regions for examination (Figure 3). Injuries were classified under two main categories based on their origin: anthropogenic and natural. Anthropogenic injuries included those resulting from human-related factors such as collisions with vehicles, accidents occurring during agricultural activities, and direct physical impacts (e.g., blows from an axe or blunt objects). Natural injuries referred to damage clearly caused by environmental factors, such as falls, abrasions during digging, lesions, or fire. This category also included claw or bite marks attributed to predators.

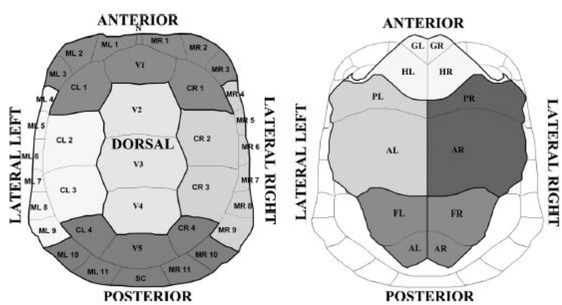

Figure 3. Zoning model of the carapace and plastron for injury assessment (Buica et al., 2014).

Statistical Analyses

Data obtained from the morphometric measurements and shell injuries of the specimens were analyzed using SPSS (version 26) and R Project for Statistical Computing software packages. The Kolmogorov-Smirnov Normality Test was used to assess the distribution of the data. Comparisons between sexes and populations were conducted using the Mann-Whitney U Test for non-parametric data and the Student's t-test for parametric data. The Chi-square test (χ^2 test) was used to examine whether there were differences between categorical variables in shell injuries. Correlations between SCL and BW, BCI, and carapace and plastron indices within and between populations were investigated using the Spearman and Pearson Correlation Tests. In all cases, a p-value ≤ 0.05 was considered statistically significant.

Results

Morphometric Measurements and Indices

As part of the morphological analyses, measurements were examined for a total of 78 specimens (38♂♂, 40♀♀), including 34 individuals from the Kırklareli population (13♂♂, 21♀♀) and 44 individuals from the Edirne population (25♂♂, 19♀♀) (Table 1).

In the SDI calculations, the values for the Kırklareli (0.540) and Edirne (0.900) populations were found to be positive. These results indicate that both populations exhibit female-biased sexual dimorphism. In addition, in most of the morphological measurements, female individuals were found to be larger than males in both populations.

Table 1. Descriptive statistics of morphological measurements for all populations.

KIRKLARELİ												
Morphometric Measurements and Indices	Male						Female					
	N	Min	Max	Mean	SE	SD	N	Min	Max	Mean	SE	SD
BW	13	550	1400	898,08	69,921	252,104	21	560	1840	1220,24	83,864	384,313
SCL	13	14,00	17,70	15,49	0,366	1,320	21	13,5	20,80	17,75	0,468	2,148
CW	12	11,2	22,5	13,36	0,970	3,361	18	12,3	24,0	14,91	0,631	2,679
PL	12	13,0	18,5	13,94	0,429	1,489	20	10,7	19,0	16,27	0,495	2,216
PW	11	9,60	15,10	10,86	0,459	1,523	11	11,00	13,80	12,19	0,268	0,890
SH	7	7,20	9,10	8,04	0,257	0,681	7	8,10	10,60	9,70	0,344	0,911
ANW	7	3,8	5,6	4,81	0,236	0,625	7	3,2	5,9	4,50	0,434	1,150
CL	7	0,3	1,5	0,97	0,179	0,475	7	0,5	1,4	0,85	0,121	0,320
BC(g/cm^3)	7	0,54	0,67	0,60	0,020	0,053	7	0,51	0,68	0,56	0,022	0,059
SCL/PL	12	0,95	1,25	1,10	0,024	0,084	20	0,92	1,34	1,09	0,023	0,105
SCL/PW	11	1,17	1,55	1,43	0,032	0,108	11	1,31	1,65	1,50	0,028	0,095
KCL/CW	12	0,78	1,47	1,18	0,059	0,204	18	,85	1,48	1,20	0,035	0,151
PL/PW	10	1,22	1,42	1,30	0,020	0,064	10	1,23	1,54	1,40	0,026	0,083
SCL/SH	7	1,69	2,06	1,89	0,056	0,148	7	1,66	2,15	1,92	0,061	0,162
EDİRNE												
Morphometric Measurements and Indices	Male						Female					
	N	Min	Max	Mean	SE	SD	N	Min	Max	Mean	SE	SD
BW	25	320	1300	742,60	45,277	226,385	18	460	1520	1043,89	70,526	299,217
SCL	25	10,1	19,0	14,90	0,429	2,145	19	12,8	19,2	16,33	0,395	1,723
CW	19	8,3	135,0	18,78	6,471	28,209	17	10,1	21,0	13,18	0,555	2,289
PL	21	9,20	15,00	12,82	0,294	1,350	17	11,61	18,50	14,89	0,493	2,035
PW	21	7,0	12,0	9,82	0,301	1,379	16	8,4	14,9	10,95	0,437	1,750
SH	16	5,5	10,5	7,61	0,302	1,210	14	6,3	11,1	8,45	0,333	1,247
ANW	16	2,5	5,4	4,40	0,218	0,872	14	2,2	11,2	4,53	0,571	2,137
CL	16	0,4	1,6	1,11	0,088	0,354	13	0,3	3,3	0,83	0,214	0,772
BC(g/cm^3)	16	0,04	0,69	0,503	0,036	0,147	13	0,47	0,64	0,57	0,011	0,041
SCL/PL	21	1,00	1,35	1,16	0,017	0,079	17	0,91	1,33	1,108	0,020	0,085
SCL/PW	21	1,20	2,28	1,49	0,046	0,213	16	1,10	1,66	1,48	0,038	0,153
KCL/CW	19	0,11	1,43	1,16	0,061	0,267	17	0,80	1,40	1,25	0,031	0,131
PL/PW	19	1,16	2,14	1,32	0,048	0,211	15	0,83	1,46	1,34	0,039	0,152
SCL/SH	16	1,72	2,22	1,95	0,038	0,152	14	1,48	2,19	1,92	0,049	0,185

The SCL measurements in male individuals ranged between 14.00 cm and 17.70 cm in the Kırklareli population, and between 10.10 cm and 19.00 cm in the Edirne population. The highest SCL values in males were observed in the Kırklareli population. In female individuals, SCL ranged from 13.50 cm to 20.80 cm in the Kırklareli population, and from 12.80 cm to 19.20 cm in the Edirne population. Based on the results, female individuals from the Kırklareli population were larger than those from the Edirne population. However, no statistically significant differences were detected between male and female individuals across populations ($p > 0.05$) (Figure 4).

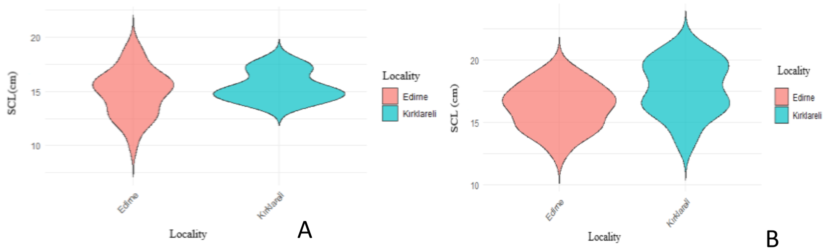

Figure 4. Comparison of SCL values between the two populations (A: Male, B: Female).

The BW (Body Weight) of male individuals in the Kırklareli population ranged between 550 g and 1440 g, while that of males in the Edirne population ranged between 320 g and 1300 g. According to the data obtained, the BW of male individuals in the Kırklareli population was found to be higher than that of males in the Edirne population. Similarly, the BW of female individuals in the Kırklareli population ranged between 560 g and 1840 g, whereas in the Edirne population it ranged between 460 g and 1520 g (Table 1). Female individuals from the Kırklareli population were also found to be heavier than those from the Edirne population (Figure 5).

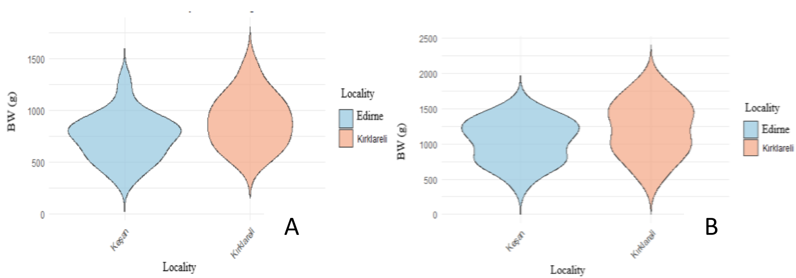

Figure 5. Comparison of BW values between populations (A: Male, B: Female).

Comparison of Males and Females Within Populations

In the Kırklareli population, statistically significant differences were found between male and female specimens in five morphological measurements (BW, SCL, PL, PW, SH) ($p < 0.05$), while no significant differences were found in the morphological ratios ($p > 0.05$). In the Edirne population, significant differences were observed between male and female specimens in six morphological measurements (BW, SCL, PL, PW, SH, CL), as well as in the ratios SCL/PL, SCL/CW, and PL/PW ($p <0.05$) (Table 2).

Table 2. Evaluation of morphological measurements and indices between male and female individuals within populations using Student's t-test and Mann-Whitney U tests (t: t-test value, df: degrees of freedom, p: significance level, U: Mann-Whitney U value, * Measurements with significant differences between male and female individuals).

Morphometric Measurements and Indices	KIRKLARELİ				Morphometric Measurements and Indices	EDİRNE			
	t	df	p	U		t	df	p	U
BW*	**2,679**	**32**	**0,012**		**BW***	**3,595**	**30,260**	**0,001**	
SCL*	**3,412**	**32**	**0,002**		**SCL***	**2,449**	**41,839**	**0,019**	
CW	1,334	19,976	0,197		CW			0,476	139,000
PL*	**3,219**	**30**	**0,003**		**PL***	**3,602**	**26,722**	**0,001**	
PW*	**2,500**	**16,120**	**0,024**		**PW***	**2,112**	**27,874**	**0,044**	
SH*	**3,850**	**11,113**	**0,003**		SH*	1,876	27,231	0,071	
ANW	-,635	12	0,537		ANW			0,466	94,500
CL	-,528	10,528	0,609		CL			**0,007**	**43,000**
BC(g/cm^3)	-1,533	11,833	0,151		BC(g/cm^3)			0,114	68,000
SCL/PL	-,277	27,463	0,784		**SCL/PL**			**0,028**	**103,500**
SCL/PW	1,657	19,694	0,113		SCL/PW			0,304	134,500
SCL/CW	0,311	18,902	0,759		**SCL/CW**			**0,023**	**90,000**
PL/PW	0,789	19,744	0,439		**PL/PW**			**0,017**	**90,000**
SCL/SH	0,251	11,903	0,806		SCL/SH	-,413	25,277	0,683	

Comparison of Male and Female Individuals Between Populations

When male and female individuals across populations were compared, a significant difference was found only in body condition among males, whereas in females, differences were observed in CW, PL, and PW measurements ($p < 0.05$) (Table 3). Regarding the morphometric measurements with significant differences, female individuals from the Kırklareli population were found to be larger than those from the Edirne population.

Table 3. Comparison of morphological measurements and ratios of male and female individuals across all populations (p: significance level, U: Mann-Whitney U value, * Measurements with significant differences between male and female individuals).

Morphometric Measurements and Indices	MALE		Morphometric Measurements and Indices	FEMALE	
	p	U		p	U
BW	0,071	104,000	BW	0,121	134,000
SCL	0,579	144,500	SCL	0,054	128,500
CW	0,903	111,000	**CW***	**0,005**	**67,000**
PL	0,123	85,000	**PL***	**0,044**	**104,000**
PW	0,131	77,500	**PW***	**0,014**	**38,000**
SH	0,192	36,500	SH	0,040	21,500
ANW	0,345	42,000	ANW	0,654	43,000
CL	0,500	46,000	CL	0,213	30,000
BC(g/cm^3) *	**0,038**	25,000	BC(g/cm^3)	0,272	29,000
SCL/PL	0,075	78,500	SCL/PL	0,411	143,000
SCL/PW	0,539	100,000	SCL/PW	0,844	84,000
SCL/CW	0,530	98,500	SCL/CW	0,210	115,000
PL/PW	0,416	95,000	PL/PW	0,537	75,500
SCL/SH	0,640	49,000	SCL/SH	0,881	47,000

The correlation and distribution graph of SCL and BW values, which indicate body size, is presented in Figure 6. A statistically significant positive correlation was found between SCL ($r = 0.866$, $p = 0.000$) and BW ($r = 0.866$, $p = 0.000$) measurements among males from the Kırklareli and Edirne populations. Similarly, a statistically significant positive correlation was also found between SCL ($r = 0.929$, $p = 0.000$) and BW ($r = 0.929$, $p = 0.000$) measurements among females.

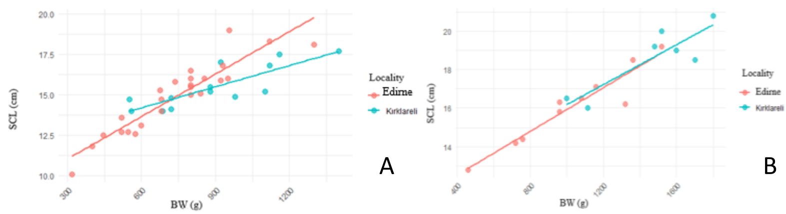

Figure 6. Correlation graph of SCL and BW values in male and female specimens between populations (A: Male, B: Female).

The BC value in male individuals ranged from 0.54 to 0.67 cm³ in the Kırklareli population and from 0.004 to 0.69 cm³ in the Edirne population. In female individuals, it ranged from 0.51 to 0.68 cm³ in the Kırklareli population and from 0.47 to 0.69 cm³ in the Edirne population (Figure 7).

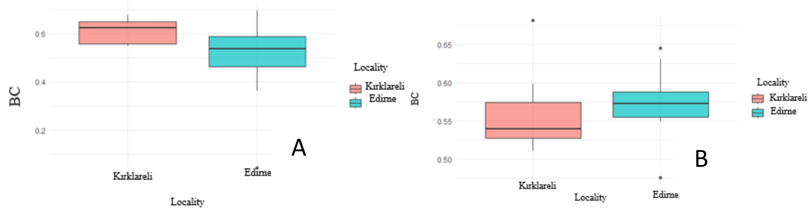

Figure 7. Comparison of Body Condition (BC) between male (A) and female (B) individuals from two different populations.

When body condition values were examined according to elevation, no significant difference was found between the Kırklareli and Edirne populations. The Kırklareli station was classified as an open area due to the presence of sparse shrubs, while the Edirne station was considered a closed area due to the presence of dense trees. No significant difference was detected between these two habitat types in terms of BC values ($p > 0.05$) (Figure 8).

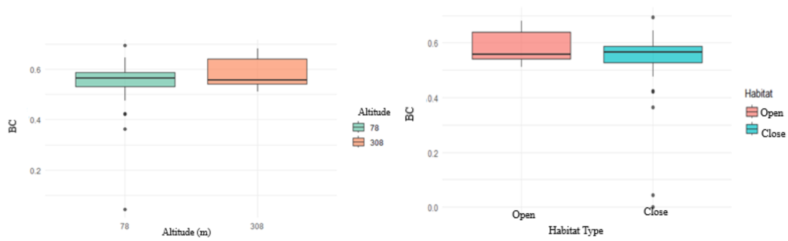

Figure 8. Comparison of BC values of populations with habitat parameters.

Shell Injuries

In the context of shell injuries, the carapace and plastron injuries of 26 specimens (8♂♂, 18♀♀) from the Kırklareli population and 36 specimens (22♂♂, 14♀♀) from the Edirne population were examined.

Comparison of Shell Injuries Between Males and Females Within Populations

When comparing shell injuries and injury indices between male and female specimens within the populations, injuries were detected in 100% of males and 88.88% of females from the Kırklareli population. Carapace index values for males ranged from 1.00 to 0.44, and plastron index values ranged from 0.16 to 0.66; for females, the carapace index ranged from 0.33 to 0.66, and the plastron index ranged from 0.16 to 0.66. No significant difference was found between male and female specimens in terms of shell injuries on the carapace and plastron ($\chi^2 = 0.963$, df = 1, $p = 0.326$), or in their carapace and plastron index values ($p > 0.05$).

In the Edirne population, shell injuries on the carapace and plastron were found in 81% of male specimens and 92% of female specimens. Carapace and plastron index values for both male and female specimens ranged from 0.16 to 1.00. No significant difference was observed between male and female specimens of the Edirne population in terms of carapace and plastron injuries ($\chi^2 = 0.872$, df = 1, $p = 0.350$), or in their carapace and plastron index values ($p > 0.05$) (Table 4).

Table 4. Comparison of carapace and plastron index values between male and female specimens from the Kırklareli and Edirne population.

KIRKLARELİ														
	Male						Female						Mann Whitney-U test	
Shell Injury Index	N	Min	Max	Mean	SE	SD	N	Min	Max	Mean	SE	SD	U	p
Carapace Index	8	0,16	1,00	0,44	0,930	0,263	15	0,33	0,66	0,43	0,031	0,122	53,00	0,623
Plastron Index	8	0,16	0,66	0,41	0,054	0,154	14	0,16	0,66	0,37	0,046	0,175	45,500	0,448
EDİRNE														
	Male						Female							
Shell Injury Index	N	Min	Max	Mean	SE	SD	N	Min	Max	Mean	SE	SD	U	p

Carapace Index	18	0,16	1,00	0,42	0,059	0,250	13	0,16	0,66	0,35	0,035	0,129	107,0	0,676
Plastron Index	14	0,16	1,00	0,52	0,069	0,260	8	0,16	0,66	0,39	0,076	0,217	40,500	0,275

Shell injuries on the carapace and plastron were detected in 92% (24 specimens) of the Kırklareli population and in 86% (31 specimens) of the Edirne population. No statistically significant difference was found between the two populations in terms of carapace and plastron injuries ($\chi^2 = 0.579$, df = 1, $p = 0.447$). Based on the carapace and plastron index results, the carapace index ranged from 0.16 to 1.00 and the plastron index ranged from 0.16 to 0.66, with no significant difference observed between the two populations ($p > 0.05$) (Table 5).

Table 5. Comparison of carapace and plastron indices between Kırklareli and Edirne populations.

	KIRKLARELİ						EDİRNE						Mann Whitney-U test	
Shell Injury Index	**N**	**Min**	**Max**	**Mean**	**SE**	**SD**	**N**	**Min**	**Max**	**Mean**	**SE**	**SD**	**U**	**p**
Carapace Index	23	0,16	1,00	0,43	0,037	0,178	31	0,16	1,00	0,39	0,037	0,208	288,500	0,208
Plastron Index	22	0,16	0,66	0,39	0,035	0,165	22	0,16	1,00	0,47	0,052	0,248	199,500	0,299

Comparison of Shell Injuries Between Male and Female Individuals Across Populations

When comparing carapace and plastron injuries, as well as carapace and plastron indices, between male and female individuals across populations, no statistically significant differences were found in terms of shell injuries in males ($\chi^2 = 1.678$, df = 1, $p = 0.195$) or females ($\chi^2 = 0.146$, df = 1, $p = 0.702$) between the two populations. Similarly, no statistically significant differences were observed in carapace and plastron indices ($p > 0.005$) (Table 6).

Table 6. Comparison of male and female individuals between populations.

	Male						Mann Whitney-U test		Female						Mann Whitney-U test	
Shell Injury Index	N	Min	Max	Mean	SE	SD	U	p	N	Min	Max	Mean	SE	SD	U	p
Carapace Index	26	0,16	1,00	0,43	0,048	0,249	66,50	0,765	28	0,16	0,66	0,39	0,024	0,129	63,00	0,118
Plastron Index	22	0,16	1,00	0,48	0,048	0,229	45,00	0,482	22	0,16	0,66	0,38	0,039	0,186	54,00	0,920

Differences in Localization of Injuries

When the locations of shell injuries were compared, injuries were detected on the carapace in 95% and on the plastron in 91% of the individuals from the Kırklareli population. Among males from the Kırklareli population, 100% had injuries on both the carapace and the plastron, while among females, injuries were observed on the carapace in 83% and on the plastron in 77%.

In the Edirne population, 86% of the individuals had injuries on the carapace and 58% on the plastron. Among males, 81% had injuries on the carapace and 40% on the plastron, whereas in females, injuries were detected on the carapace in 92% and on the plastron in 64%. No statistically significant differences were found between male and female individuals within both populations.

Description of Injuries

In tortoise shells, the dorsal and anterior regions are primarily affected by anthropogenic injuries, while the posterior surface of the shell is more influenced by natural injuries. Direct anthropogenic injuries (e.g., axe strikes, blunt force trauma) damage multiple scutes regardless of their position on the carapace or plastron, whereas agricultural machinery causes deeper injuries affecting a greater number of scutes. Natural injuries, resulting from events such as falls, abrasion, or fire, may be observed over larger surface areas. The injuries on the carapace and plastron of specimens from the Kırklareli population were determined to be of natural origin, while in the Edirne population, the carapace injuries were of natural origin and the plastron injuries were identified as anthropogenic (Figure 9).

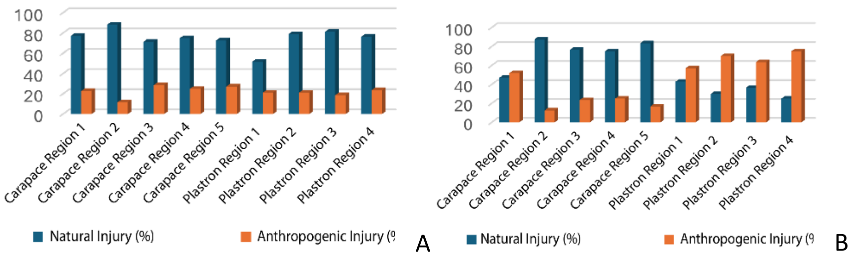

Figure 9. Distribution of shell injuries in Kırklareli and Edirne populations (A: Kırklareli, B: Edirne).

When examining the injuries in the carapace and plastron regions of male and female individuals from the Kırklareli population, it was found that the rate of natural injuries was significantly higher than that of anthropogenic injuries (Figure 10).

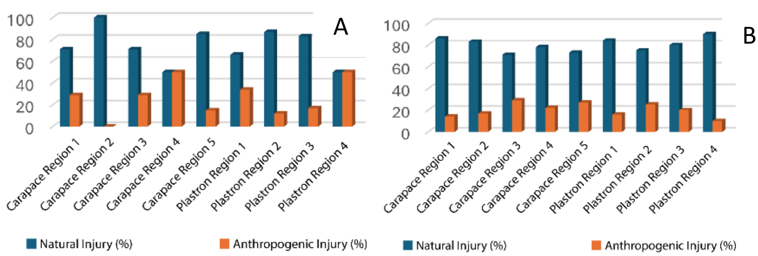

Figure 10. Distribution of shell injuries in male and female individuals from the Kırklareli population (A: Male, B: Female).

When the injuries in the carapace and plastron regions of male and female individuals from the Edirne population were examined, it was found that natural injuries were more common on the carapace, while anthropogenic injuries were more prevalent on the plastron (Figure 11).

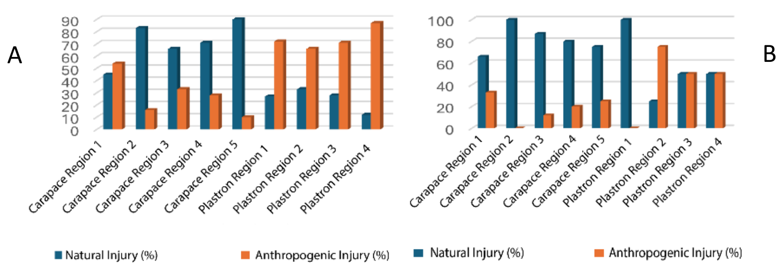

Figure 11. Distribution of shell injuries in male and female specimens from the Edirne population (A: Male, B: Female).

The natural and anthropogenic shell injuries identified in the Kırklareli and Edirne populations are presented in Figures 12–15.

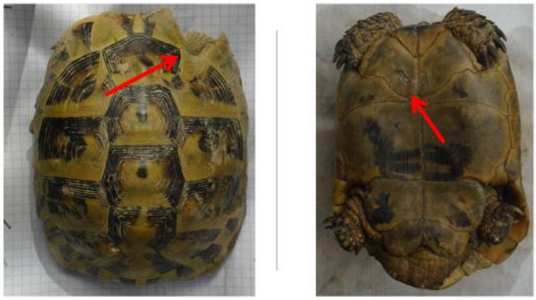

Figure 12. Anthropogenic shell injuries in the Kırklareli population.

Figure 13. Natural shell injuries in the Kırklareli population.

Figure 14. Anthropogenic shell injuries in the Edirne population.

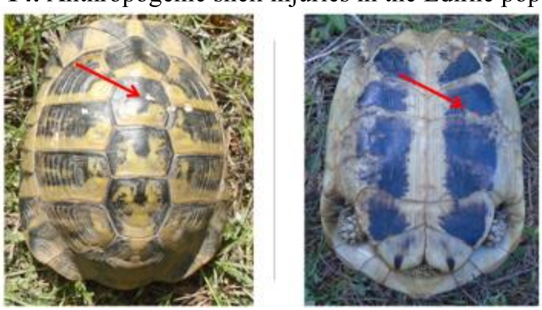

Figure 15. Natural shell injuries in the Edirne population.

Discussion

In tortoises, SDI is the most commonly used and accepted formula for calculating sexual size dimorphism. Positive values indicate female-biased sexual size dimorphism, while negative values represent male-biased sexual size dimorphism (Lovich and Gibbons, 1992; Baycan and Tosunoğlu, 2023). Previous studies have attributed these sex-based differences to variations in maturation timing between sexes (Lovich and Gibbons, 1990), differences in mortality rates (Hailey and Willemsen, 2000), or unmeasured environmental influences such as high ambient temperatures or abnormal rainfall patterns (Duro et al., 2021). Studies on sexual size dimorphism in *T. hermanni* populations from different localities have shown male-biased dimorphism in some populations (Hailey et al., 1988) and female-biased in others (Ljubisavljević et al., 2012; Djordjević et al., 2013; Duro et al., 2021). According to the positive SDI values obtained in the present study, both populations showed female-biased sexual size dimorphism.

In tortoises, total body size is an important morphological trait influenced by sexual selection within populations (Berry and Shine, 1980; Stubbs et al., 1984). Thus, many morphometric studies on tortoises have found that females are larger than males (Hailey and Willemsen, 2000; Ljubisavljević et al., 2012; Djordjević et al., 2013; Duro et al., 2021). Because males need to move faster to find mates, their body sizes are smaller (Berry and Shine, 1980), whereas females tend to be larger due to fecundity selection (Bonnet et al., 2001b; Bulté et al., 2008a, b; Djordjević et al., 2011). Furthermore, under suitable environmental conditions (availability of food, ambient temperatures), individuals may grow quickly, while unfavorable conditions can limit body size and halt growth; additionally, males and females do not face the same reproductive selective pressures (Willemsen and Hailey, 2003; Vetter, 2006). As a result, there may be size differences between male and female individuals (Djordjević et al., 2011). In the present study, significant morphological differences were found between males and females within both stations. The habitat of the Kırklareli population is open, with an abundance of water and food resources, while the habitat of the Edirne population is a closed area with more limited food and resource availability. These environmental differences seem to have led to sex-specific effects on body size, with females found to be larger than males.

Willemsen and Hailey (1999) stated that body size in tortoises across populations is influenced by latitude, with individuals living at higher latitudes being larger, and those at lower latitudes being smaller. Djordjević et al. (2011), in their study in Eastern Serbia, emphasized that the general morphological traits and ratios of *T. hermanni* may vary according to environmental factors. Accordingly, significant changes in certain morphometric measurements were observed depending on the habitat characteristics of different populations. The

Kırklareli population of *T. hermanni* inhabits an open shrubland near large wetland areas, while the Edirne population lives in a more closed, forested area. In our study, when female individuals of both populations were compared, significant differences were observed in CW, PL, and PW measurements, with the Kırklareli population being larger. Nikolić et al. (2020) reported that the preferred habitats of *T. hermanni* species are open shrublands, grasslands, and forested areas, emphasizing that their interaction with vegetation plays a key role in feeding and protection. These findings suggest that morphological differences in *T. hermanni* populations are largely influenced by the physical and environmental characteristics of their habitats.

Body condition is generally considered an important metric for assessing an animal's overall health and physical state (Peig and Green, 2009, 2010). Therefore, it is used as an important indicator to understand how tortoises respond to seasonal and environmental changes (Bonnet et al., 2001; Frankerberger et al., 2024). Body condition may vary depending on environmental factors such as habitat conditions and the availability of food and water in each region (Duro et al., 2021). In this study, the body condition indices (BC) of individuals from both populations were found to be positive, and a significant difference was observed between male individuals across populations. The BC values of male individuals from the Kırklareli population were higher compared to those from the Edirne population. This finding may be associated with the fact that the Kırklareli population lives in open habitats with easier access to food and water resources. In contrast, the closed habitat with dense vegetation in the Edirne population may limit access to resources and negatively affect the body condition of individuals. These results are consistent with previous studies that emphasize the sensitivity of body condition to environmental factors (Bonnet et al., 2001; Duro et al., 2021; Lecq et al., 2014; Frankerberger et al., 2024). The positive BC values indicate that the tortoises are heavier than their predicted body mass and in good general physical condition. This suggests that environmental conditions in the study areas may enhance feeding opportunities or indicate high habitat quality. Therefore, the findings of this study are in line with the current literature and reaffirm that habitat characteristics have a determining effect on body condition.

Shell injuries in tortoises are directly related to habitat structure and the level of human impact; these factors play a significant role in the type and frequency of injuries (Meek, 2007). Carapace and plastron index values can be used to determine the severity of shell injuries (Saumure et al., 2007; Biaggini and Corti, 2018). In this study, shell injuries were observed in *Testudo hermanni* individuals from the Kırklareli and Edirne populations; the carapace index values of individuals from the Kırklareli population were found to be higher than those from the Edirne population. The Kırklareli population inhabits an area with intensive agricultural and livestock activities, which is considered the primary reason for these high injury index values. Similarly, previous studies have shown that individuals located within 1 km of agricultural fields experienced more widespread and severe shell damage due to anthropogenic activities (Biaggini and Corti, 2018).

Meek (2007), in a study on *T. hermanni* individuals in Croatia and Montenegro, found that males had lower injury rates compared to females. However, in our study, evaluations of individuals from the Kırklareli and Edirne populations showed that male specimens had higher carapace and plastron injury index values and thus experienced more injuries.

Biaggini and Corti (2018) reported that the location, number, and type of shell injuries in tortoises could serve as indicators of the source and intensity of stress experienced by individuals. The same study emphasized that shell injuries could be photographed to distinguish between anthropogenic and natural trauma; natural injuries were generally characterized by irregular edges and surface abrasions along the marginal scutes and plastron. In contrast, anthropogenic injuries were described as more localized, deep, and sharply defined, often resulting from impacts with cutting or blunt objects, and crushing injuries from agricultural machinery or vehicles. Similarly, Moraru et al. (2018) reported that some lesions observed in tortoises were natural injuries resulting from contact with rough surfaces and keratin layer damage caused by dog feces.

In this study, the lesions identified in the carapace and plastron regions of individuals from the Kırklareli population were largely determined to be of natural origin. The observation of many stray dogs in this region during fieldwork may have contributed to the formation of these natural injuries, consistent with the findings of Moraru et al. (2018). In contrast, while superficial injuries were detected on the carapace of individuals from the Edirne population, deeper marks and shell fractures were observed on the plastron; the nature of these injuries is consistent with anthropogenic causes. This indicates that anthropogenic pressure in the Edirne area may have a negative impact on the shell integrity of individuals.

Conclusion

In this study, the morphometric characteristics, body conditions, and shell injuries of two different *Testudo hermanni* populations from the Thrace Region were comparatively evaluated. The findings revealed that both populations exhibit female-biased sexual size dimorphism, and that the body condition index showed significant variation only among males between populations. Additionally, statistically significant morphological differences were observed between sexes and stations. However, no statistically significant differences were found within or between populations in terms of the carapace and plastron indices calculated to assess the severity of shell injuries. Regarding the sources of injuries, it was determined that injuries to the carapace and plastron in the Kırklareli population were caused by natural factors, while in the Edirne population, carapace injuries were natural, and plastron injuries were anthropogenic.

In conclusion, this study is the first comprehensive examination of shell injuries in a vulnerable tortoise species, *Testudo hermanni,* in Türkiye, providing a detailed evaluation of carapace and plastron indices. The findings provide an important scientific basis for ecological monitoring, habitat management, and the development of sustainable conservation strategies for the protection of the species.

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CHAPTER 2

Innovative Approaches in The Dairy Industry: Alternative Products and Sustainability

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1. INTRODUCTION

Adequate and balanced nutrition plays a vital role in protecting and improving individual health, thereby contributing to a higher quality of life. It refers to the daily intake of the necessary amounts of energy and nutrients required by the body. These essential nutrients and energy sources are obtained through the foods we consume. For the purpose of ensuring adequate and balanced nutrition, foods are categorized into four main groups: meat and meat products, milk and dairy products, fruits and vegetables, and bread and cereals. The milk and dairy products group includes foods derived from milk, such as yogurt, cheese, and powdered milk. These products are significant sources of various essential nutrients, including protein, calcium, phosphorus, vitamin B2 (riboflavin), and vitamin B12 (Ünal & Besler, 2008; Örmeci Kart & Demircan, 2014).

Milk and dairy products, which are among the sources of animal protein, are not at the desired level in terms of either production or consumption in Türkiye. In European Union countries, the average per capita consumption of milk and dairy products is approximately 331 kg per year in milk equivalent. In contrast, this figure is 171 kg per year in Türkiye (Şahin & Yurdakul, 1996; Şahin et al., 2001). According to studies on milk consumption, the daily intake of 1 liter of milk can meet the entire calcium and phosphorus requirements of adults and nearly all of the needs of children aged 10 to 12 (Karakaya & Akbay, 2013).

Milk, which is essential at every stage of human life, is also a good source of both macro- and micronutrients, excluding vitamin C and iron. It is particularly beneficial for bone health during childhood, pregnancy and lactation, and old age (Ünal & Besler, 2008). Clinical and biochemical studies on the health effects of milk have shown that the consumption of low-fat milk, in particular, plays a significant role in reducing the risk of hypertension, dental diseases, colon cancer, and cardiovascular diseases (Terin et al., 2015).

Dairy products are secondary products derived from milk, including cheese, yogurt, butter, and ayran (Gündüz et al., 2013). Among these, cheese holds a significant place due to its richness in protein, fat, minerals, and vitamins. Milk fat is commonly consumed in the form of butter in Türkiye and is favored by consumers for its pleasant taste. In addition to its palatability, milk fat plays an important role in nutrition due to its content of physiologically valuable fatty acids, its ease of digestion, and the presence of fat-soluble vitamins such as A, D, E, and K (Uzunöz & Gülşen, 2007).

Research has shown that consumers are generally aware of the positive effects of milk on human health, primarily due to its role as a significant source of calcium. However, some consumers still hold certain biases regarding milk consumption. For example, there is a widespread belief that milk causes weight gain and that packaged milk contains preservatives (Akbay & Tiryaki, 2007). In this context, national policies should be developed to eliminate consumer misconceptions and to raise awareness about the importance of consuming high-quality food products (Gündüz et al., 2013).

2. Milk and Dairy Product Consumption

Milk is a vital component of the human diet, offering essential nutrients that support growth, development, and overall health across all age groups. The annual per capita consumption of various dairy products in Türkiye includes 41.50 kg of milk, 18.40 kg of cheese, 30.60 kg of yogurt, 18.40 kg of ayran, and 1.78 kg of butter. In contrast, the average milk consumption per person in the European Union stands at 65.00 kg (ZMO, 2018). The level of milk and dairy product consumption can be influenced by multiple factors, such as product quality, pricing, hygienic conditions, as well as individual preferences and taste. Furthermore, socio-economic and demographic factors-particularly income level, educational attainment, age, gender, household size, maternal employment, and the presence of children in the household-are reported to play a significant role in shaping milk consumption patterns (Şengül, 2004; Akbay & Tiryaki, 2007). According to Şimşek and Açıkgöz (2011), 81% of students believe that milk is a necessary component of the diet at every stage of life. Yogurt is a highly valuable fermented dairy product known for enhancing the bioavailability of nutrients and serving as an easily digestible source of protein (Marette & Picard-Deland, 2014). However, its final nutritional profile can vary significantly depending on factors such as the type of milk used and the inclusion of sweeteners or other additives (Aryana & Olson, 2017). Yogurt is particularly important in early life nutrition, as it is commonly consumed and recommended for children in many countries, serving as a key dietary source of glucose (Williams et al., 2015; Devenish et al., 2019). In the United States, yogurt consumption is notable, with 64% of men and 41% of women consuming it at least once a week (Wang et al., 2013). In response to public health efforts to address obesity, the total sugar content in fruit-flavored yogurts has been reduced in the UK in recent years (Moore et al., 2020). Additionally, yogurt's fat content is a point of consideration in dietary planning, and there is now a greater preference for semi-skimmed milk over whole milk in the context of healthy eating (Wechsler & Wernick, 1992).

3. Innovative Approaches

3.1 Advanced Biotechnological Approaches

Recent advancements in biotechnology have proven to be highly valuable for enhancing the quality characteristics of livestock-derived products, particularly within the dairy sector (Güner, 2025). In many developing countries, microbial inoculants have been widely utilized to optimize dairy product attributes, such as taste, aroma, texture, shelf stability, and nutritional value. Probiotic-based foods, forming a key segment of the functional food market, are experiencing significant growth due to increasing consumer interest. Although dairy remains the dominant medium for probiotic delivery, the food industry is actively developing alternative probiotic-rich products that offer comparable health advantages (Sundarraj et al., 2018). Additionally, contemporary biotechnological innovations have introduced transformative opportunities for the dairy industry, facilitating broader access to milk and its derivatives, particularly for economically disadvantaged populations. Given the industry's central objective of supplying affordable, nutritious, and high-quality dairy products, the integration of biotechnology throughout various stages of milk production and processing has become essential. Continued investment in research and development is expected to amplify the influence of biotechnology on food quality and diversity, especially with the aim of producing healthier and more palatable options. The ongoing evolution of technological capabilities suggests that biotechnology will maintain a significant role in food safety and processing innovation. In particular, its application in areas such as bio-preservation of dairy products, the engineering and production of probiotics, enzyme synthesis, bioactive peptides derived from milk, functional ingredient development, and starter culture enhancement through genetic modification is of paramount importance (Nurye Gebeyehu, 2023).

3.2 Bio-preservation Strategies

Despite significant advancements in modern food processing technologies and the implementation of stricter microbiological safety standards, the risk of foodborne illnesses and spoilage has not been entirely eradicated. Consequently, the food industry remains committed to identifying innovative strategies for producing minimally processed, ready-to-eat products that retain their sensory and nutritional attributes. Among these approaches, bio-preservation-particularly through the use of bacteriocins-has emerged as a promising solution for extending the shelf life of such foods without compromising their chemical integrity or nutritional value. Bacteriocins are antimicrobial peptides considered safe due to their susceptibility to degradation by proteolytic enzymes within the mammalian gastrointestinal tract. These peptides are predominantly synthesized by lactic acid bacteria (LAB), which are commonly employed in fermented food systems. The application of bacteriocins, whether in purified form or secreted naturally by LAB strains during fermentation, presents a viable and health-conscious alternative to traditional chemical preservatives in dairy processing. In practice, bacteriocins may be incorporated into dairy matrices either as isolated compounds, through the inclusion of bacteriocinogenic LAB during fermentation, or as adjunct cultures. Their effectiveness in inhibiting pathogenic microorganisms in milk, yogurt, and cheese has been well-documented. A notable recent advancement in this field is the integration of bacteriocins into bioactive packaging systems either in purified or semi-purified form, or via bacteriocin-producing LAB-enabling their direct application to food surfaces for enhanced microbial control (Silva et al., 2018).

3.3 Probiotics

The term "probiotic," derived from the meaning "for life," refers to live microorganisms that, when administered in adequate amounts, confer health benefits to the host. The most commonly utilized probiotic strains belong to the genera Lactobacillus and Bifidobacterium; however, other microorganisms such as Bacillus, Pediococcus, and various yeast species have also demonstrated potential as effective probiotics. Probiotics are incorporated into a wide range of dairy-based products including fermented milk, yogurt, cheese, butter, ice cream, and infant formulas. These microorganisms may be employed either independently as starter cultures or in combination with conventional starters. Alternatively, they may be added post-fermentation. In either case, probiotics contribute significantly to the sensory profile of the product-enhancing aroma, flavor, and texture-while also imparting numerous health-promoting properties (Gao et al., 2021).

Fermented dairy products, in particular, serve as highly effective vehicles for delivering viable probiotic strains, optimizing their biological functionality within the human gastrointestinal system. These microorganisms can be concentrated and added in relatively small quantities to milk-based matrices where they can actively proliferate. Yogurt, especially bio-yogurt, is a widely recognized probiotic-rich functional food, characterized by the presence of viable bacterial cultures at the time of consumption.

Probiotics have traditionally been used as dietary supplements and therapeutic agents for gastrointestinal health. Recently, their value has been further acknowledged due to their strain-specific functional benefits, which include immune modulation, restoration of disrupted intestinal microbiota, reinforcement of mucosal barrier function, and alleviation of lactose intolerance.

Ongoing research is increasingly focusing on the potential of probiotics as biotherapeutic agents in the management of various chronic metabolic and inflammatory diseases. These include type 2 diabetes, cardiovascular diseases (CVD), obesity, irritable bowel syndrome (IBS), inflammatory bowel diseases such as ulcerative colitis (UC) and Crohn's disease (CD), as well as acute gastrointestinal infections, hypercholesterolemia, respiratory tract infections, hypertension, colorectal cancer, and urinary tract infections (UTIs) (Nurye Gebeyehu, 2023).

3.4 Industrial Enzyme Production

The biotechnological production of enzymes has emerged as a vital component of modern food processing, offering a cost-effective solution that significantly reduces investment and operational expenditures. Enzymes, as bio-catalytic agents, play a crucial role in transforming food components and enhancing product quality through controlled biochemical reactions. In this context, biotechnology serves as a powerful tool for tailoring enzymatic activity to meet the specific functional requirements of the food matrix.

Within the food and dairy industries, several key enzymes—including microbial protease, lipase, and galactosidase—are derived from beneficial microorganisms. These enzymes are particularly valued for their thermotolerance, stability under heat and acidic conditions, and overall robustness, which make them highly suitable for industrial-scale applications (Al-Manhel, 2018).

The industrial use of enzymes in food processing has historical roots dating back to 1874, when Danish scientist Christian Hansen successfully extracted rennin (also known as chymosin) from the stomachs of calves for use in cheese production. This milestone marked the beginning of enzymatic innovation in food technology. Subsequently, bovine chymosin became the first enzyme to be produced biotechnologically via *Escherichia coli* using recombinant DNA techniques. This advancement paved the way for the development of engineered enzymes tailored to specific functional and consumer demands.

Today, advances in genetic engineering enable the large-scale synthesis of custom-designed enzymes using recombinant DNA technology. These enzymes have found widespread application in the food industry, where they enhance efficiency, improve sensory qualities, and ensure consistent product performance (Al-Manhel, 2018).

3.5 Digital Dairying: Smart Farm Management with the Internet of Things (IoT)

Internet of Things (IoT); It is also called a system where artificial intelligence-based integrated sensors, objects and smart devices can communicate and move with each other without the need for human control (Ambrosin et al., 2016; Conti et al., 2018).

The utilisation of the Internet of Things (IoT) in the domain of animal farming constitutes a subfield of precision agriculture, wherein analytical measurements are employed to optimise business decisions (Abdullahi et al., 2020). The Internet of Things (IoT) has been utilised for the purpose of tracking animals, particularly in rural areas where livestock are accustomed to grazing in flocks, as this method is likely to prevent the loss of animals (Dlodlo and Kalezhi 2015). In summary, the performance of data collection, analysis and decision-making processes for farm animals is now possible through the use of IoT-based devices.

Electronic ear tags are the most commonly used application method. It enables the tracking and management of individual animals within the herd, and facilitates the rapid and uninterrupted acquisition of data such as estrus, pregnancy, productivity and disease (Anonymous, 2022).

In the context of animal husbandry, sensors are devices that facilitate the instantaneous wireless transmission of essential information, including body temperature, step count, and the location of animals, to the primary machinery. The utilisation of these devices has the capacity to facilitate the tracking and management of both the herd as a whole and its constituent elements. These devices facilitate the easy, unmanned, and instantaneous performance of tasks that require measurement. In recent times, there has been an increasing prevalence of their utilisation across a wide range of disciplines, including but not limited to meteorology, geology and animal husbandry (Özcan, 2022).

Virtual fences, In animal husbandry, fences are barriers created to prevent animals from crossing the determined boundaries. These barriers can be fixed, temporary or virtual thanks to the advantages provided by today's technologies. Thanks to the audible and shock system integrated into the collar, it warns when the animal crosses the virtually created fence boundary and causes the animal to change direction. This collar, which has a two-stage warning system, gives an audible warning as soon as the animal crosses the fence nerve. If the animal ignores the audible warning and tries to cross the fence, it applies an electric shock at a level that will not harm the animal and causes the animal to change direction (Lomax, et al., 2019; McSweeney et al., 2020).

Computer vision, the creation of decision-making mechanisms by tracking animals individually and instantly is possible thanks to developing computer technologies with increasing storage capacity. Today, computer vision has begun to be widely used in data collection, analysis and decision-support mechanisms. The use of computer vision in the livestock sector is also rapidly increasing. It is used for purposes such as image classification, object detection, image segmentation, pose estimation and tracking in cattle, sheep/goats, pigs and poultry (Li et al., 2021).

3.6 Precision Fermentation in Dairy Innovation

Conventional dairy production relies heavily on animal husbandry, which raises concerns regarding environmental sustainability, resource efficiency, and animal welfare. In response to these challenges, precision fermentation has emerged as a transformative technology within the dairy industry, offering a novel approach to producing milk proteins without the need for cows (Ranjbar, 2025).

Precision fermentation involves the use of genetically engineered microorganisms—such as yeasts, fungi, or bacteria—to produce specific functional proteins, including casein and whey proteins. These microorganisms are cultivated in bioreactors where they synthesize the desired proteins, which are then isolated and combined with plant-based components to create dairy alternatives that mimic the taste, texture, and nutritional profile of conventional milk (Cho et al., 2025).

Precision fermentation offers several advantages over traditional methods of food production, particularly in the context of dairy alternatives. One of the primary benefits is its environmental impact. Compared to conventional dairy farming, precision fermentation significantly reduces greenhouse gas emissions, land use, and water consumption. This makes it a more sustainable and environmentally friendly option for producing dairy-like products.

Additionally, products generated through precision fermentation are naturally lactose-free, which makes them suitable for individuals with lactose intolerance, a growing concern in many populations. Furthermore, the process is animal-free, which aligns with ethical considerations and the increasing demand for vegan and animal-friendly food choices. By eliminating the need for animal-derived ingredients, precision fermentation offers a solution that appeals to those seeking plant-based alternatives or those concerned with animal welfare (Ranjbar, 2025).

From a quality and safety standpoint, precision fermentation allows the production of proteins in highly controlled environments, ensuring consistent quality, traceability, and safety. These controlled conditions reduce the risk of contaminants and ensure that the final product is pure and reliable.

One of the most notable commercial applications of precision fermentation is the production of non-animal whey protein (β-lactoglobulin) by Perfect Day, a biotechnology company based in the United States. This protein is structurally identical to the one found in cow's milk and exhibits the same functional properties, such as foaming, emulsification, and gelling. The ability to produce proteins that mirror the characteristics of traditional animal-derived proteins without the environmental and ethical costs of animal farming is a key advantage of this technology (Cankorur-Cetinkaya et al., 2018).

4. Alternative Products

4.1 Plant-Based Dairy Alternatives

In recent years, consumer expectations for beverages have evolved beyond basic hydration. There is a growing interest in products that align with diverse lifestyles and offer functional health benefits such as enhanced energy intake, delay of age-related decline, and the prevention of chronic diseases and stress-related conditions. In response to these demands, the beverage industry has increasingly focused on developing nutritionally enriched and functionally enhanced food products to meet the evolving needs of health-conscious consumers (Makinen et al., 2016).

Functional food development commonly involves the direct consumption of products exhibiting nutraceutical properties or the isolation and incorporation of bioactive compounds as functional ingredients. Within this context, milk remains one of the most widely consumed beverage categories and is regarded as a complete food. It provides a balanced profile of macronutrients—proteins, lipids, and carbohydrates-as well as essential micronutrients such as calcium, selenium, riboflavin, vitamin B12, and pantothenic acid (Janssen et al., 2016; Sethi et al.,2016).

However, several challenges have emerged in recent years. In some regions of the world, access to dairy products remains limited. Additionally, certain micronutrients (e.g., iron, folate) and bioactive compounds (e.g., phenolic compounds) are present in milk only in trace amounts. Furthermore, health issues such as milk protein allergies and lactose intolerance have contributed to a growing demand for plant-based milk alternatives, which are increasingly being developed to mimic the sensory, nutritional, and functional properties of traditional dairy milk (Bridges, 2018; Park, 2018).

The production of plant-based milk alternatives initially began with soy-based beverages and has since diversified into a wide range of products derived from ingredients such as oats, almonds, coconuts, hemp seeds, and cocoa. These beverages offer consumers a variety of alternatives to dairy milk, driven by both nutritional and ethical considerations. Compared to animal-based milk, the production of plant-based beverages generally requires significantly lower energy input per unit of product and allows for compositional flexibility depending on consumer demand, making them advantageous in terms of both production efficiency and market adaptability (Park, 2018).

The growing popularity of dietary lifestyles such as vegetarianism, lacto-vegetarianism, and ovo-vegetarianism, along with increased attention to animal welfare and environmental sustainability often amplified through food blogs and social media has contributed to the rapid expansion of the plant-based beverage market. While these products serve as an affordable nutritional alternative for populations in developing regions with limited access to cow's milk, in countries such as Turkey, they are predominantly preferred by individuals in higher income brackets (Sebastiani et al., 2019).

5. Sustainability

5.1 Zero-Waste

The concept of a zero-waste dairy industry has gained increasing attention in recent years as part of broader efforts to enhance sustainability in food production systems. In this context, zero-waste refers to the efficient utilization of all raw materials, by-products, and waste streams generated during milk processing. Whey, one of the major by-products of cheese and casein production, was traditionally considered waste but is now widely valorized for its nutritional and functional properties in the production of protein concentrates, bioactive peptides, and fermented beverages (Smithers, 2008). Moreover, dairy sludge and wastewater are being increasingly explored for biogas generation through anaerobic digestion, contributing to renewable energy production and reducing environmental burden (Usmani et al., 2022). Innovations in membrane filtration, enzymatic hydrolysis, and microbial fermentation have further enabled the recovery of valuable compounds such as lactose, minerals, and organic acids from dairy effluents (Álvarez-Castillo et al., 2021). The integration of circular economy principles in dairy processing not only minimizes waste but also creates economic value from secondary streams, promoting a more sustainable and resource-efficient dairy industry.

5.2 Sustainable Packaging Solutions

Packaging has become an integral part of daily life, and with increasing consumption, the use of packaging materials continues to rise steadily. It is reported that millions of tons of plastic waste are generated annually, yet only approximately 9% of this waste is successfully recycled. The remaining portion is disposed of in landfills or accumulates in marine environments, including seas and oceans (Pan et al.,2016).

The widespread use of plastic materials in the packaging industry raises significant environmental concerns. These materials are primarily derived from finite petroleum resources, and most conventional plastics are non-biodegradable, persisting in the environment for extended periods. As a result, they contribute substantially to the growing problem of solid waste accumulation and have become a major factor in the global climate crisis (Caferoğlu, 2021).

In recent years, increasing attention has been directed toward the development of bio-based and biodegradable packaging materials as alternatives to petroleum-derived plastics. These materials, sourced from renewable resources and capable of decomposing naturally after use, have become a major focus in sustainable packaging design. As an alternative to petroleum-based plastic packaging, research has increasingly focused on the design and development of biodegradable packaging materials derived from bioplastics. These bioplastics are obtained from a variety of renewable sources, including starch, proteins, cellulose, and various microorganisms such as bacteria, fungi, and algae. Their ability to decompose naturally after use makes them promising candidates for sustainable packaging solutions (Caferoğlu, 2021; Lanjekar et al., 2024).

6. Conclusion

Over the past three decades, global dairy production and consumption have experienced substantial growth, and current projections suggest that this upward trend will persist in the coming years. Despite this global increase, certain regions—particularly within the European Union—have observed a decline in cattle populations, largely due to growing concerns over environmental sustainability, animal welfare, and regulatory pressures.

In response to these challenges, various technological innovations have emerged to support the intensification and modernization of dairy farming systems. Recent advancements, including automated milking systems, precision sensors, blockchain-based traceability tools, and automated feeding mechanisms, have demonstrated potential in enhancing milk yield, improving resource efficiency, and promoting better animal welfare standards. Concurrently, innovations in milk processing technologies have contributed significantly to the production of safer, higher-quality dairy products that meet modern health and safety standards. These advanced processing techniques also offer promising avenues for minimizing greenhouse gas (GHG) emissions throughout the production, processing, and storage phases of the dairy supply chain.

In parallel, the integration of biotechnology into the dairy sector has expanded rapidly. Biotechnological applications now encompass a wide range of areas, including animal nutrition, food processing, biopreservation, probiotic development, enzyme production, the synthesis of milk-derived bioactive peptides, functional ingredient manufacturing, starter culture optimization, and genetic improvement strategies. These innovations collectively contribute to enhanced productivity, food safety, and sustainability.

However, access to such advanced technologies remains largely concentrated in developed countries, limiting their widespread adoption in low- and middle-income regions. To address global food security challenges and reduce the environmental footprint of dairy production, it is imperative to facilitate broader access to these technologies, particularly among smallholder and resource-constrained farmers in developing nations. Enhancing global accessibility will be essential for increasing milk yield per animal, lowering GHG emissions, and meeting the nutritional demands of a growing global population.

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