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The Effects of a Diet Containing Yoghurt with Krill Oil Consumed by Rats During Their Pregnancy on Long Bones of Their Offspring

Ratların Gebelik Döneminde Tükettikleri Krill Yağlı Yoğurt İçeren Diyetin Yavrularının Uzun Kemikleri Üzerindeki Etkisi

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ABSTRACT

The use of calcium, vitamins, minerals, and omega 3 fatty acids during pregnancy are recommended to support the bone development of infants. The aim of this study is to feed pregnant rats with the probiotic yoghurt mixed with krill oil, which is rich in these features, and examine the morphometric development of long bones in their offspring. For this purpose, a total of twelve 2-month-old offspring including 6 in the experimental group (offspring of pregnant rats fed with yoghurt mixed with krill oil) and 6 in the control group (offspring of pregnant rats fed a standard feed), were used in the study. When they became 2 months old, their biometric measurements were taken. After euthanasia, long bones of the offspring (Humerus, antebrachium, femur, ossa cruris) were cleaned by maceration. These bones were photographed. Morphometric measurements of the length and width of these bones were made using the Image J program. In the SPSS (20.0 Version) program, the parameters were compared between the right and left leg bones and between the control and experimental groups by running the Independent Samples T test. Additionally, Pearson's correlation test was applied between the parameters. The results of the study indicated that this diet with krill oil and yogurt consumed by pregnant rats had positive effects on the length parameters of the bones and biometric parameters of their offspring.

Keywords: Krill oil, morphometry, rat, yoghurt.

ÖZ

Gebelikte yavruların kemik gelişimini desteklemek için kalsiyum, vitamin, mineral ve omega 3 yağ asitlerinin kullanımı önerilmektedir. Bu çalışmanın amacı, bu özelliklerden zengin olan krill yağı ile karıştırılmış probiyotik yoğurt ile gebe ratları beslemek ve yavrularında uzun kemiklerin morfometrik gelişimini incelemektir. Bu amaçla deney grubunda 6 adet (krill yağı ile karıştırılmış yoğurtla beslenen gebe ratların yavruları) ve kontrol grubunda 6 adet (standart yemle beslenen gebe ratların yavruları) olmak üzere toplam 12 adet 2 aylık yavru rat çalışmada kullanıldı. Yavru ratlar 2 aylık olduklarında biyometrik ölçümleri alındı. Ötanazi sonrasında yavruların uzun kemikleri (Humerus, antebrachium, femur, ossa cruris) maserasyonla temizlendi. Bu kemikler fotoğraflandı. Bu kemiklerin uzunluk ve genişliklerinin morfometrik ölçümleri Image J programı kullanılarak yapıldı. SPSS (20.0 Versiyonu) programında sağ ve sol bacak kemikleri arasında ve kontrol ve deney grupları arasında Independent Samples T testi ile parametreler karşılaştırıldı. Ayrıca parametreler arasında Pearson korelasyon testi uygulandı. Çalışmanın sonuçları, gebe ratların tükettiği krill yağı ilaveli yoğurt diyetinin kemik uzunluk parametreleri ve yavrularının biyometrik parametreleri üzerinde olumlu etkileri olduğunu gösterdi.

Anahtar Kelimeler: Krill yağı, morfometri, rat, yoğurt.

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INTRODUCTION

Krill oil is a substance obtained from a sea creature called "*Euphausia Superba*" living in the oceans. It contains high rates of omega 3 fatty acids in the form of phospholipids. It is also a nutritional supplement that contains astaxanthin, vitamin A and vitamin E. Astaxanthin has a strong antioxidant activity. In recent years, Krill oil has become more and more important than fish oil. It is safe to use it during pregnancy. However, the high content of docosahexaenoic acid and eicosapentaenoic acid has increased the importance of krill oil.[1, 2] Furthermore, this oil has recently become the focal point of the researchers in terms of high absorption.[3-7]

Omega 3 fatty acid supplements are known to be important in mental development, hyperlipidemia, premenstrual syndromes, and inflammatory[8] and cardiological diseases.[9] However, multiple unsaturated fatty acids are effective in stimulation of growth. In fact, Omega-6 fatty acids elevate prostaglandin E2 levels and this hormone also suppresses bone development. For this reason, it is suggested that feeding with a ration enriched by omega-3 fatty acids suppresses the release of prostaglandin E2 and promotes bone development.[10-12]

Yoghurt is a fermented dairy product that is rich in protein, calcium, phosphorus, and especially B-group vitamins.[13, 14] Probiotics in yoghurt positively affect the absorption of minerals such as calcium, phosphorus, and zinc. By means of minerals it contains, yoghurt has significant effects on bone development.[15] However, some components are added to increase the nutritional value of yoghurt. Yoghurt enriched with omega-3 fatty acids has a positive effect on consumption in terms of food quality.[16] Probiotic yoghurt added with krill oil has been reported to have very good physical, chemical, microbiological, and sensory properties and a high nutritional value.[17]

It is recommended to consume calcium-rich foods, vitamins, mineral supplements and omega-3 foods to support the bone development of infants during pregnancy.[18] Yoghurt with krill oil used in the study is rich in these features. For this reason, this study aims to investigate morphometric development of the long bones in the offspring of rats fed yoghurt with krill oil during their pregnancy.

MATERIALS AND METHODS

Animals

In the study, a total of 24 rats including 12 pregnant rats and their 12 offspring were used. The pregnant rats were divided into two groups as experimental and control groups including 6 rats in each.

Experimental Group

This study was approved by the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (No: 1046, Date: 07.03.2023).

The experimental group consisted of male offspring (2 months old, n=6) of rats that fed probiotic yoghurt with 2 %krill oil during their pregnancy. These pregnant rats were given standard feeding for 21 days and during this period, yoghurt with krill oil (daily dose: 1 ml) was given them via gavage once a day.

It is known that gender has an effect on bone morfometria. In the study, the gender factor was taken into account in case the morphometric difference that would affect the results could be gender-related. For this reason, only male rats were used as an experimental material in the study.

Control Group

The control group consisted of male offspring (2 months old, n=6) of rats that fed the standard feed during their pregnancy.

The offspring, which were included the experimental and control groups, were reared in similar care units for 2 months. They were fed the standard feed in this process. They were euthanized under the anesthesia with xylazine-ketamine at the end of 2 months.

First of all, the weight, head-tail length and tail length parameters of these rats were determined. Then, their long bones (Humerus, Antebrachium, Femur, Ossa Cruris) were cleaned from rough meats via maceration. Cleaned bones were photographed. In the Image J program, morphometric measurements were carried out over the photographs (Figures 1, 2, 3, and 4) and the results were recorded. Morphometric parameters were determined upon the literature review. [19]

Statistical Analyses

The parameters were analyzed using the SPSS (20.0 version) packaged software. These parameters were compared in terms of groups (control group- experimental group) and direction (right-left) by independent samples t test. Moreover, Pearson's correlation test was performed between these parameters.

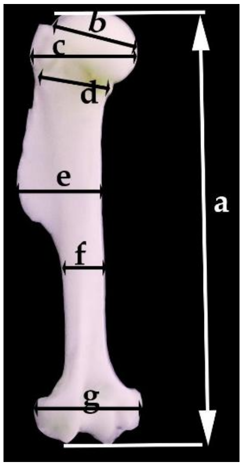

Figure 1. Morphometric measurements determined on humerus (Caudal view of the left humerus): a. Humerus length, b. caput humeri width, c. proximal width, d. collum humeri width, e. largest width of the corpus, f. smallest width of the corpus, g. distal width

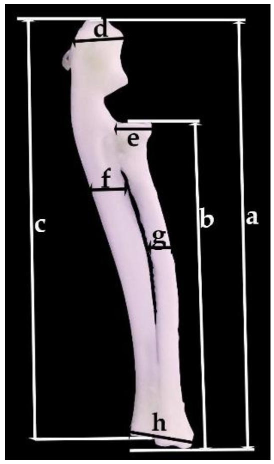

Figure 2. Morphometric measurements determined on antebrachium (lateral view of the left antebrachium (radius and ulna)) a. Antebrachium length, b. radius length, c. ulna length, d. proximal width of ulna, e. proximal width of radius, f. largest corpus width of the ulna, g. largest corpus width of the radius, h. distal width

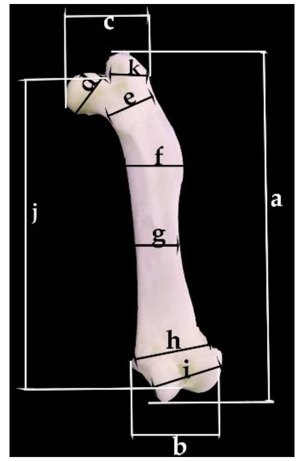

Figure 3. Morphometric measurements determined on femur (Caudal view of the right femur) a. Femur length, b. distal width, c. proximal width, d. caput femoris width, e. collum femoris width, f. largest width of the corpus, g. smallest width of the corpus, h. largest distal width (above the condylus level), i. condylus width, j. smallest length of corpus, k. trochanter major width

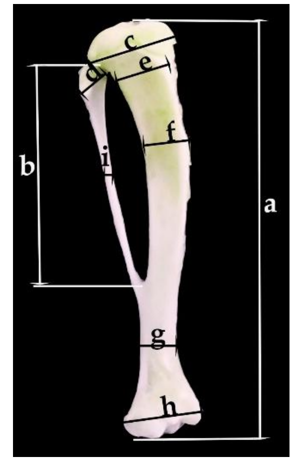

Figure 4. Morphometric measurements determined on ossa cruris (Caudal view of the left ossa cruris (tibia and fibula): a. tibia length, b. fibula length, c. tibia's proximal width, d. fibula's proximal width, e. collum tibia width, f. largest width of the corpus, g. smallest width of corpus, h. distal width, i. corpus fibula width

Probiotic Yoghurt Mixed With 2% Krill Oil

Krill oil is rich in omega 3 fatty acids, astaxanthin, vitamin A and vitamin E.[20] Tables 1, 2, and 3 show the nutritional values of the probiotic yoghurt with 2% krill oil used in the study.

RESULTS

Table 4 shows biometric parameters of the rats. In the comparison made in terms of length (Head - tail length and tail length), the rats in the experimental group had statistically significantly longer values ($P < .05$). The parameter of weight was greater in the experimental group, but it was not statistically significant ($P > .05$).

Table 1. Nutritional values of probiotic yoghurt mixed with 2% Krill oil.

Main components		Minerals	
Serum	31.08%	Ca (Calsium)	3894.40 ppm
Protein	4.30%	Mg (magnesium)	1323.15 ppm
Oil	4.18 %	P (phosphor)	1245.30 ppm
dry matter	14.45%	K (potassium)	1932.68 ppm
Ash	0.89 %	Na (sodium)	2169.45 ppm

Table 2. Amounts of volatile aroma compounds contained in probiotic yoghurt mixed with 2% Krill oil.

Matter	Amount (ppm)
Acetaldehyde	27.06
Ethanol	2039.12
Diacetyl	22.03
Acetoin	5.81

Table 3. Microbiology of probiotic yoghurt mixed with 2% Krill oil.

Microbiological analysis	Amount (log10 KOB/g)
Lactobacillus delbrueckii subsp. bulgaricus	5.68
Aerobic mesophilic bacteria count	8.52
Lactobacillus acidophilus	7.02
yeast numbers	3.85
Mold	(<1 log10 KOB/g
Coliform	(<1 log10 KOB/g)
Streptococcus salivarius subsp. thermophilus	9.24

Table 4. Biometric parameters of the experimental group and control group in offspring.

Biometric value	Control group	Experimental group
Weight (gr)	201.83 ±18.91	220.16±10.18
Head-tail length (mm)	193.53± 6.92*	211.83 ±13.20*
Tail length (mm)	158.00± 14.11*	181.46 ±17.68*

*: Comparison between control and experimental groups, $P < .05$.

Table 5 shows morphometric parameters of humerus. Accordingly, the humerus was statistically significantly longer in the experimental group; whereas, the proximal and distal width of humerus was statistically significantly greater in the control group ($P < .05$). Except for width of the collum humeri, the other parameters related to width were larger in the control group, but they were not statistically significant ($P > .05$).

Table 5. Morphometric parameters of humerus in offspring (Average value ± standard deviation) (mm).						
Humerus	Control – right leg	Control- left leg	Control	Experimental right leg	Experimental- left leg	Experimental
Humerus length	23.99 ± 0.29	24.18 ± 0.25	24.08 ± 0.27*	24.90 ± 0.87	24.70 ± 1.04	24.80 ± 0.92*
Caput humeri width	4.18 ± 0.14	4.06 ± 0.25	4.12 ± 0.20	4.05 ± 0.18	3.79 ± 0.43	3.92 ± 0.34
Proximal width	6.06 ± 0.25*	6.12 ± 0.40	6.09 ± 0.32*	5.76 ± 0.15*	5.68 ± 0.24	5.72 ± 0.19*
Distal width	6.65 ± 0.36^{b}	6.03 ± 0.52^{b*}	6.34 ± 0.54*	6.05 ± 0.55^{a}	5.30 ± 0.32^{a*}	5.67 ± 0.58*
Largest width of the corpus	4.04 ± 0.29^{b}	4.58 ± 0.31^{b}	4.31 ± 0.40	3.76 ± 0.27^{a}	4.31 ± 0.24^{a}	4.04 ± 0.37
Smallest width of the corpus	2.15 ± 0.16	2.20 ± 0.18	2.18 ± 0.16	2.00 ± 0.17	2.17 ± 0.23	2.09 ± 0.21
Collum humeri width	3.11 ± 0.12^{b}	3.67 ± 0.39^{b}	3.39 ± 0.40	3.25 ± 0.33	3.60 ± 0.22	3.43 ± 0.32
Condylus humeri width	3.53 ± 0.14^{b}	2.79 ± 0.51^{b}	3.16 ± 0.52	3.36 ± 0.28	2.88 ± 0.50	3.12 ± 0.46

* : shows the difference between control and experimental groups ($P < .05$). ^{a},: shows the difference between right and left humerus in the experimental group ($P < .05$). ^{b} : shows the difference between right and left humerus in the control group ($P < .05$).

Table 6 shows the correlation values between parameters of humerus. Based on this table, no significant correlation was observed between humerus length and the other parameters. In both groups, there was a significant negative correlation between the largest width of corpus and the distal width and between the width of condylus humeri and the collum humeri ($P < .001$)

Table 6. The correlation values between morphometric parameters of humerus in offspring (c: control group, e: experimental group).											
TOP-c DOWN e	HL	CHW	PW	DW	LWC	SWC	Collum	Condylus	W	HTL	TL
HL		-.522	.229	-.215	.024	.441	.391	-.421	.245	.416	.162
CHW	.210		-.329	.197	-.444	-.146	-.634*	.261	-.449	.451	.487
PW	-.086	.220		-.142	.283	.291	.480	-.462	-.541	-.042	-.162
DW	.419	.182	.007		-.817**	-.437	-.793**	.666*	.426	-.201	-.114
LWC	.023	-.382	-.096	-.745**		.221	.889**	-.584*	-.111	-.237	-.126
SWC	-.164	-.839**	.169	-.182	.369		.366	-.568	.103	.771	.200
Collum	.223	-.274	-.490	-.267	.634*	.093		-.752**	-.041	-.566	-.654
Condylus	-.050	.443	.276	.386	-.592*	-.271	-.577*		.687	-.329	-.486
W	-.032	.828*	.022	-.166	-.483	-.877*	-.025	-.117		-.077	.180
HTL	-.637	.534	-.041	-.780	-.020	-.672	.036	.-487	.642		.642
TL	-.583	.452	-.141	-.819*	.187	-.661	.244	-.555	.631	.959**	

HL: Humerus length, CHW: caput humeri width, PW: Proximal width, DW: Distal width, LWC: Largest width of the corpus, SWC: Smallest width of the corpus, Collum: Collum humeri width, Condylus: Condylus humeri width, W: Weight of offspring, HTL: Head-tail length, TL: Tail length.*: $P < .05$, **: $P < .01$

Table 7 shows morphometric parameters of antebrachium. The length values of radius, ulna and total antebrachium were greater in the experimental group. However, the length of the radius was statistically significantly greater in the experimental group ($P < .05$). Distal width of the left radius was statistically significantly larger in the experimental group ($P < .05$). The parameters of ulna and radius were greater in the control group, while the corpus width was larger in the experimental group. However, these differences were not statistically significant ($P > .05$).

Table 7. The morphometric parameters of antebrachium in offspring (mm).

Antebrachium	Control – right leg	Control – left leg	Control	Experimental – right leg	Experimental – left leg	Experimental
Antebrachium Length	28.03 ± 0.22	28.34 ± 0.58	28.18 ± 0.45	28.36 ± 0.72	28.18 ± 1.74	28.27 ± 0.97
Ulna length	27.00 ± 0.20	27.43 ± 0.64	27.21 ± 0.51	27.10 ± 0.84	26.96 ± 1.24	27.03 ± 1.01
Radius length	20.84 ± 0.25*	21.38 ± 0.54	21.11 ± 0.49*	21.78 ± 0.53*	21.92 ± 0.76	21.85 ± 0.63*
Proximal width of ulna	3.25 ± 0.44	3.27 ± 0.44	3.26 ± 0.42	3.29 ± 0.36	3.04 ± 0.25	3.16 ± 0.32
Proximal width of radius	2.17 ± 0.17	2.19 ± 0.25	2.18 ± 0.20	2.27 ± 0.41	2.03 ± 0.20	2.15 ± 0.33
Distal width	3.85 ± 0.47	4.14 ± 0.60*	4.00 ± 0.54	3.98 ± 0.51[a]	4.83 ± 0.31[a*]	4.40 ± 0.60
Largest corpus width of the ulna	1.87 ± 0.27	2.07 ± 0.11	1.97 ± 0.22	2.02 ± 0.30	2.18 ± 0.22	2.10 ± 0.26
Largest corpus width of the radius	1.23 ± 0.12	1.36 ± 0.20	1.30 ± 0.17	1.39 ± 0.09	1.41 ± 0.18	1.38 ± 0.04

Statistical analysis between control and experimental groups, [a]: shows the difference between right and left antebrachium in the experimental group * : $P < .05$.

Table 8 shows correlation values between the morphometric parameters of antebrachium. Based on this table, a significant positive correlation was observed between total length and length of ulna and radius in both groups ($P < .01$). Moreover, there was a significant positive correlation between the head-tail length and the length of the antebrachium in the experimental group ($P < .05$).

Table 8. The correlation values between morphometric parameters of antebrachium in offspring (c: control group, e: experimental group).

TOP-c DOWN -e	AL	UL	RL	PWU	PWR	DW	LCWU	LCWR	W	HTL	TL
AL		.780**	.789**	.408	.082	-.234	.289	.785**	-.849*	.173	-.324
UL	.956**		.703*	.275	-.262	-.008	.457	.769**	-.552	-.098	.203
RL	.946**	.884**		.526	.192	-.283	.379	.646*	-.097	.059	.324
PWU	.547	.555	.453		-.199	-.393	-.194	.084	-.041	.662	.395
PWR	.351	.286	.298	.670*		-.124	.098	-.106	-.490	-.362	-.541
DW	.131	.225	.254	-.119	-.092		.583*	-.111	-.305	.012	.433
LCWU	.487	.472	.526	.246	.495	.624*		.477	-.363	-.307	.344
LCWR	-.150	-.225	-.229	-.099	-.361	-.125	-.235		-.196	-.125	.221
W	.722	.808	.493	.344	.132	.645	.258	.073		-.077	.180
HTL	.831*	.841	.794	.458	.115	.002	-.048	.045	.642		.642
TL	.683	.717	.630	.295	.162	-.015	.051	.052	.631	.959**	

AL: Antebrachium length, UL: Ulna length, RL: Radius length, PWU: Proximal width of ulna, PWR: Proximal width of radius, DW: Distal width of antebrachium, LCWU: Largest corpus width of the ulna, LCWR: Largest corpus width of the radius, W: Weight of offspring, HTL: Head-tail length, TL: Tail length *: $P < .05$, **: $P < .01$.

Table 9 shows morphometric parameters of femur. Accordingly, the femoral length was greater in experimental group, but the difference between groups was not statistically significant ($P > .05$). Width of caput femoris and the largest width of corpus were larger in the control group and this difference between the groups was statistically significant ($P < .05$). The parameters related to the width of distal femur (Condylus width, distal width) were larger in the experimental group, but this difference between groups was not statistically significant ($P > .05$)

Table 10 shows correlation values between morphometric parameters of femur. While a significant negative correlation was observed between the head - tail length and length of the femur ($P < .05$) in the control group, there was a weak positive correlation (not significant) between them in the experimental group ($P > .05$).

Table 9. Morphometric parameters of femur in offspring (mm).

Femur	Control – right	Control - left	Control	Experimental - right	Experimental - left	Experimental
Femur length	33.05 ± 0.42	32.60 ± 0.74	32.82 ± 0.63	33.23 ± 1.31	33.73 ± 0.96	33.48 ± 0.32
Caput femoris width	4.00 ± 0.13*	3.81 ± 0.16	3.90 ± 0.17*	3.69 ± 0.14*	3.58 ± 0.24	3.63 ± 0.19*
Trochanter major width	3.00 ± 0.19	3.15 ± 0.07	3.07 ± 0.15	3.29 ± 0.42	3.30 ± 0.33	3.29 ± 0.36
Smallest width of the corpus	4.09 ± 0.27[b]	3.76 ± 0.19[b]	3.93 ± 0.28	3.83 ± 0.17	3.63 ± 0.16	3.73 ± 0.14
Largest width of the corpus	5.30 ± 0.29*	5.00 ± 0.38	5.15 ± 0.36*	4.94 ± 0.14*	4.82 ± 0.08	4.88 ± 0.12*
Proximal width	7.68 ± 0.11	7.71 ± 01.7	7.69 ± 0.14	7.69 ± 0.43	7.76 ± 0.41	7.72 ± 0.40
Largest distal width (above the condylus level)	6.33 ± 0.21[b]	6.64 ± 0.17[b]	6.49 ± 0.24	6.51 ± 0.24	6.57 ± 0.29	6.54 ± 0.25
Condylus width	6.79 ± 0.23	6.61 ± 0.27	6.70 ± 0.25	6.77 ± 0.10	6.91 ± 0.20	6.84 ± 0.50
Smallest length of corpus	29.66 ± 0.23	29.19 ± 0.73	29.42 ± 0.57	29.41 ± 0.70	29.41 ± 0.96	29.41 ± 0.80
Collum femoris width	4.38 ± 0.13	4.68 ± 0.47	4.53 ± 0.36	4.32 ± 0.17[a]	4.73 ± 0.12[a]	4.53 ± 0.25

* : shows the difference between control and experimental groups (*P* < .05). [a] : shows the difference between right and left femur in the experimental group (*P* < .05). [b]: shows the difference between right and left femur in the control group (*P* < .05).

Table 10. The correlation values between morphometric parameters of femur in offspring (c: control group, e: experimental group).

TOP-c DOWN -e	FL	Caput	TMW	SWC	LWC	PW	LDW	condylus	SLC	collum	W	HTL	TL
FL		.332	.051	.100	.331	-.204	-.050	-.163	.832**	.248	.358	-.345	-.839*
Caput	.430		.223	.467	.465	.079	-.392	.160	.427	-.132	.278	-.249	-.771
TMW	-.119	.372		-.329	-.282	-.189	.175	-.377	-.048	.003	.230	-.362	-.866*
SWC	-.407	-.129	-.493		.852**	.516	-.195	.837**	.333	.021	-.503	-.350	-.314
LWC	.272	.394	-.444	.429		.615*	-.002	.743**	.536	.443	-.095	-.344	-.386
PW	.622*	-.015	-.320	-.225	.368		.315	.594*	.185	.567	-.664	-.071	.375
LDW	.385	.228	.436	-.439	-.177	.526		-.244	-.029	.622*	-.480	-.138	.028
Condylus	.450	-.105	-.127	-.197	-.285	.479	.341		-.007	.170	-.486	-.307	-.142
SLC	.799**	.371	-.221	-.221	.250	.356	-.044	.434		.232	.507	.056	-.571
Collum	.192	-.298	.030	-.276	-.590*	-.042	.090	.463	-.024		-.419	-.733	-.205
W	.290	.488	-.325	.341	.405	.200	.148	.582	.245	-.199		.180	-.077
HTL	.173	.115	.093	.206	-.149	-.303	.308	.177	.145	.026	.631		.642
TL	.275	.240	.187	-.049	-.056	-.072	.554	.224	.175	-.240	.642	.959**	

FL: Femur length, Caput: Caput femoris width, TMW: Trochanter major width, SWC: Smallest width of the corpus, LWC: Largest width of the corpus, PW: Proximal width, LDW: Largest distal width (above the condylus level), Condylus: Condylus width, SLC: Smallest length of corpus, Collum: Collum femoris width, W: Weight of offspring, HTL: Head-tail length, TL: Tail length. *: *P* < .05, **: *P* < .01.

Table 11 shows morphometric parameters of ossa cruris. As shown in this table, tibia and fibula lengths were greater in the experimental group, but this difference was not statistically significant (*P* > .05). Tibia's proximal width and the narrowest diameter of the corpus were larger in the control group (*P* < .05). The parameters related to width, except for the largest diameter of corpus tibia and the corpus fibula, were larger in the control group, but this difference was not statistically significant (*P* > .05).

Table 11. Morphometric parameters of ossa cruris in offspring (mm).

Ossa cruris	Control – right leg	Control – left leg	Control	Experimental – right leg	Experimental – left leg	Experimental
Tibia length	36.93 ± 1.15	36.55 ± 0.45	36.74 ± 0.86	37.45 ± 0.86	37.04 ± 0.93	37.25 ± 0.88
Fibula length	20.85 ± 0.85	20.64 ± 0.83	20.74 ± 0.81	21.48 ± 0.42	20.76 ± 0.82	21.12 ± 0.72
Tibia's proximal width	7.65 ± 0.45	8.02 ± 0.30	7.83 ± 0.41*	7.44 ± 0.28	7.48 ± 0.57	7.46 ± 0.43*
Fibula's proximal width	2.12 ± 0.37[b]	2.62 ± 0.35[b]	2.37 ± 0.43	1.79 ± 0.19[a]	2.39 ± 0.26[a]	2.09 ± 0.38
Distal width	6.38 ± 0.94	6.60 ± 0.42	6.49 ± 0.71	5.92 ± 0.27[a]	6.34 ± 0.26[a]	6.13 ± 0.33
Largest width of the corpus	3.83 ± 0.31	3.45 ± 0.35	3.64 ± 0.37	4.09 ± 0.23[a]	3.46 ± 0.23[a]	3.77 ± 0.40
Smallest width of corpus	2.93 ± 0.17*	2.89 ± 0.24	2.91 ± 0.20*	2.58 ± 0.17*	2.59 ± 0.24	2.58 ± 0.20*
Collum tibia width	6.04 ± 0.30	5.69 ± 0.33	5.86 ± 0.35	5.95 ± 0.40	5.68 ± 0.49	5.81 ± 0.45
Corpus fibula width	0.98 ± 0.04	0.91 ± 0.06	0.95 ± 0.06	1.08 ± 0.24	0.92 ± 0.01	1.00 ± 0.18

* : shows the difference between control and experimental groups ($P < .05$). [a] : shows the difference between right and left crus in the experimental group ($P < .05$). [b]: shows the difference between right and left crus in the control group ($P < .05$).

Table 12 shows the correlation values between morphometric parameters of ossa cruris. A significant positive correlation was observed between both groups in terms of fibula and tibia length values ($P > .05$). A significant positive correlation was determined between tibia length and largest width of the corpus tibia and between tibia length and collum tibia width in the experimental group ($P < .05$).

Table 12. The correlation values between morphometric parameters of ossa cruris in offspring (c: control group, e: experimental group)

TOP-c DOWN -e	TL	FL	TPW	FPW	DW	LWC	SWC	CTW	CFW	W	HTL	TL
TL		.749**	.236	.319	.276	.380	.488	.533	.361	-.012	.748	.648
FL	.612*		.149	.247	.280	.235	.578*	.502	-.027	-.564	.637	.498
TPW	.453	-.037		.567	.656*	-.222	.399	.236	-.108	-.228	.061	.423
FPW	-.045	-.333	.001		.374	-.627*	-.158	-.346	.036	-.475	.531	.311
DW	.506	-.062	.456	.536		-.223	.465	.299	.213	-.535	-.021	-.051
LWC	.589*	.534	.211	-.537	-.256		.591*	.700*	-.044	.877*	.338	.372
SWC	-.124	-.216	-.070	-.136	.143	.025		.690*	-.159	-.153	.257	.379
CTW	.709**	.669*	-.093	-.129	.155	.534	-.303		.104	-.039	.413	.674
CFW	.569	.416	.316	-.559	.161	.478	-.114	.337		-.112	.004	.162
W	-.747	-.716	-.614	.809	-.792	-.289	-.194	-.786	-.441		-.077	.180
HTL	-.419	-.027	-.110	.248	-.594	-.383	-.832*	-.172	-.245	.642		.642
TL	-.316	.049	.003	.142	-.446	-.336	-.860*	-.193	.003	.631	.959**	

TL: Tibia length, FL: Fibula length, TPW: Tibia's proximal width, FPW: Fibula's proximal width, DW: Distal width, LWC: Largest width of the corpus, SWC: Smallest width of
CTW: Collum tibia width, CWF: Corpus fibula width, W: Weight of offspring, HTL: Head-tail length, TL: Tail length,*: $P < .05$, **: $P < .01$.

When the biometric parameters were examined in the correlation tables, a significant positive correlation was observed between the tail length and head-tail length in the experimental group ($P < .05$), and this correlation was not statistically significant in the control group ($P > .05$).

DISCUSSION

In the study, the long bones of the 2-month-old offspring of rats fed yoghurt with krill oil during their pregnancy were compared morphometrically with those of the control group. Results of the study indicated that the length values of humerus, antebrachium, femur and ossa cruris were greater in the experimental group. However, only length of the radius was statistically significantly greater in the experimental group ($P < .05$). The proximal widths of the bones were higher in the control group. However, the proximal width values of humerus and tibia were statistically significantly larger in the control group ($P < .05$). In the study, it was noteworthy that bone width was larger in the control group and bone length was larger in the experimental group.

A cartilage area called the epiphysis plaque is located between the primary and secondary ossification centers serving the prolongation of the bone. Until the end of the ossification, the cartilage cells in the epiphysis plaques grow by being divided into diaphysis and constantly make cartilage tissue. This cartilage tissue is replaced by bone tissue.[21] When the epiphysial plaque is closed, it can continue to grow transversely while the longitudinal growth of the bone stops.[22] In the mice, epiphysial plaque is not closed exactly due to age and genotype properties and the lack of haversian system. Rats do not have a haversian system as in mice and hamster. In adult rats, especially in some male ones, the growth of the epiphysis is not completed until the age of one year.[21, 23] However, in the literature, it is reported that the degree of elongation and development in each part of the long bones is not the same, and the epiphysis has more elongation than the diaphysis. In the study, the differences in bone length detected between the two groups show the extent to which the growth plaques at different levels are affected.

A study was conducted in which the effects of nutrition on bone development during pregnancy were examined in rat offspring.[24] In the literature,[24] bone elongation rate has been evaluated in offspring of rats fed organic dried apricots during their pregnancy and at different stages of development. Although some elongation was observed in the femur and tibia in the groups fed with organic dried apricots compared to the control group, it was determined that this elongation was not significant ($P > .05$). This is thought to be associated with the fact that hereditary factors have a more significant effect on bone growth than environmental factors.[24] In the study, similar to the literature,[24] the effects of nutrition during pregnancy on bone morphometry were investigated in rat offspring. Unlike the literature,[24] in this study, probiotic yoghurt with krill oil was given to pregnant rats in the experimental group and it was observed that there was no statistical difference between the groups in the length of the femur and tibia ($P > .05$), but there was some growth in these bones in the experimental group, which is compatible with the literature[24]

In the literature,[15] the effect of yoghurt on bone mineralization and the bioavailability of Ca, P and Zn was evaluated in rats. In this study,[15] yoghurt and probiotic yoghurt were given to the rats in the experimental group. As a result of the study, it was revealed that feeding with yoghurt did not have a significant effect on weight. Serum Ca, P, and Zn rates, femur density, and femur calcium rate were also significantly higher in rats fed probiotic yoghurt. Feeding with probiotic yoghurt had beneficial effects in terms of providing significant mineral absorption.[15] In the present study, it was determined that feeding with probiotic yoghurt with krill oil during pregnancy did not have a significant effect on weight, similar to the literature.[15] However, there was a statistically significant difference in radius and humerus length ($P < .05$). On the other hand, a statistically insignificant increase was detected in the lengths of the femur and tibia ($P > .05$).

This study has some limitations in terms of the number of experimental groups. In the study, the possible effects of only krill oil and only yoghurt on bone development could not be evaluated. It may be a hypothesis of another topic.

In conclusion, it was determined that feeding rats with yoghurt with krill oil during their pregnancy had positive effects on the biometric parameters and the length parameters of the bones in their offspring. The average value of bone length was greater in the experimental group. Among morphometric parameters of bone, only radius and humerus length were statistically significant ($P < .05$). Accordingly, it is thought that the increase in length of femur and ossa cruris may be significantly affected when the feeding dose of yoghurt with krill oil is increased.

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