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بسم الله الرحمن الرحيم

Submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Food Sciences & Nutrition at the College of Food Sciences & Agriculture of King Saud University

By

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1432/2011

Supervision

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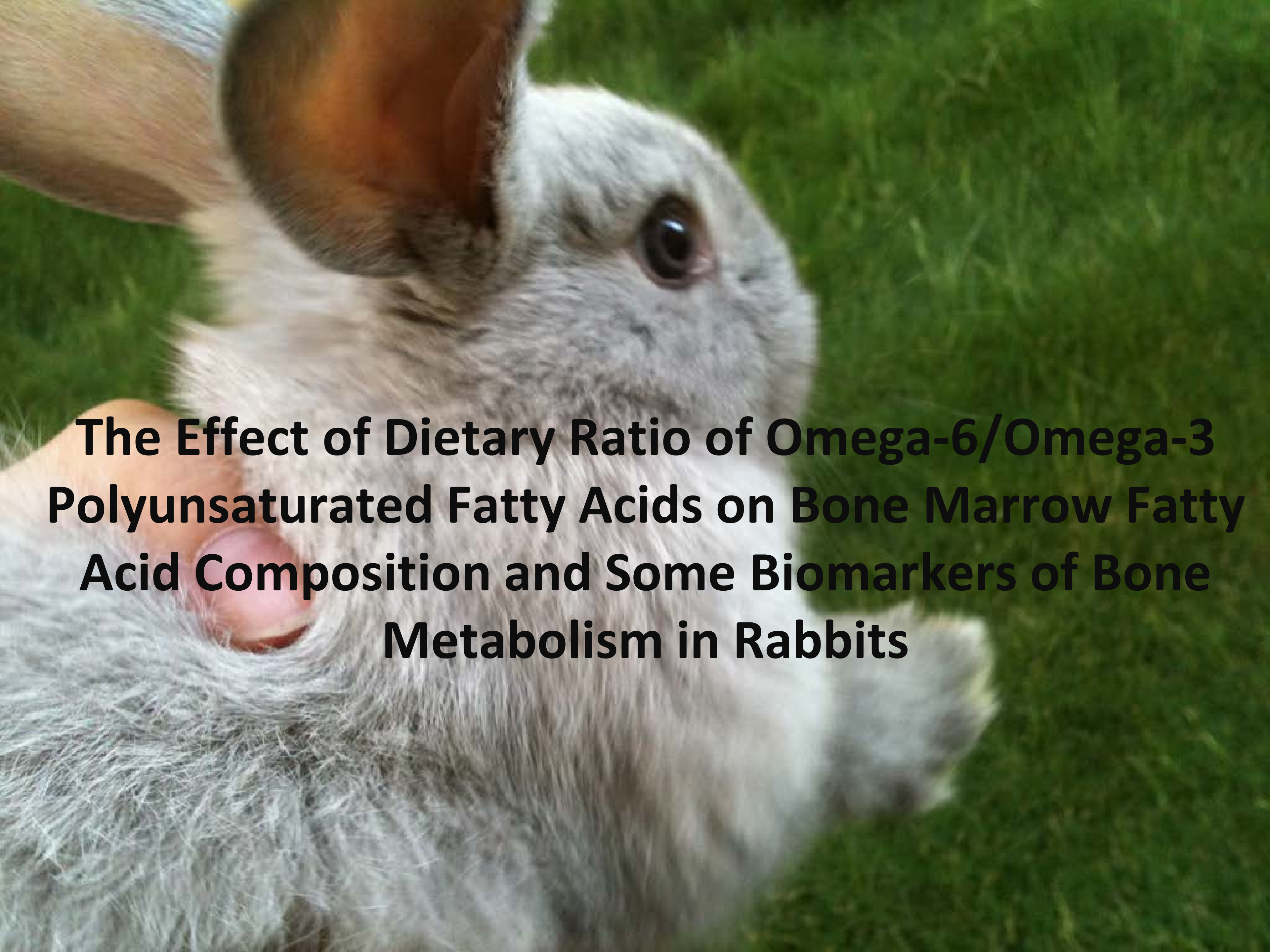
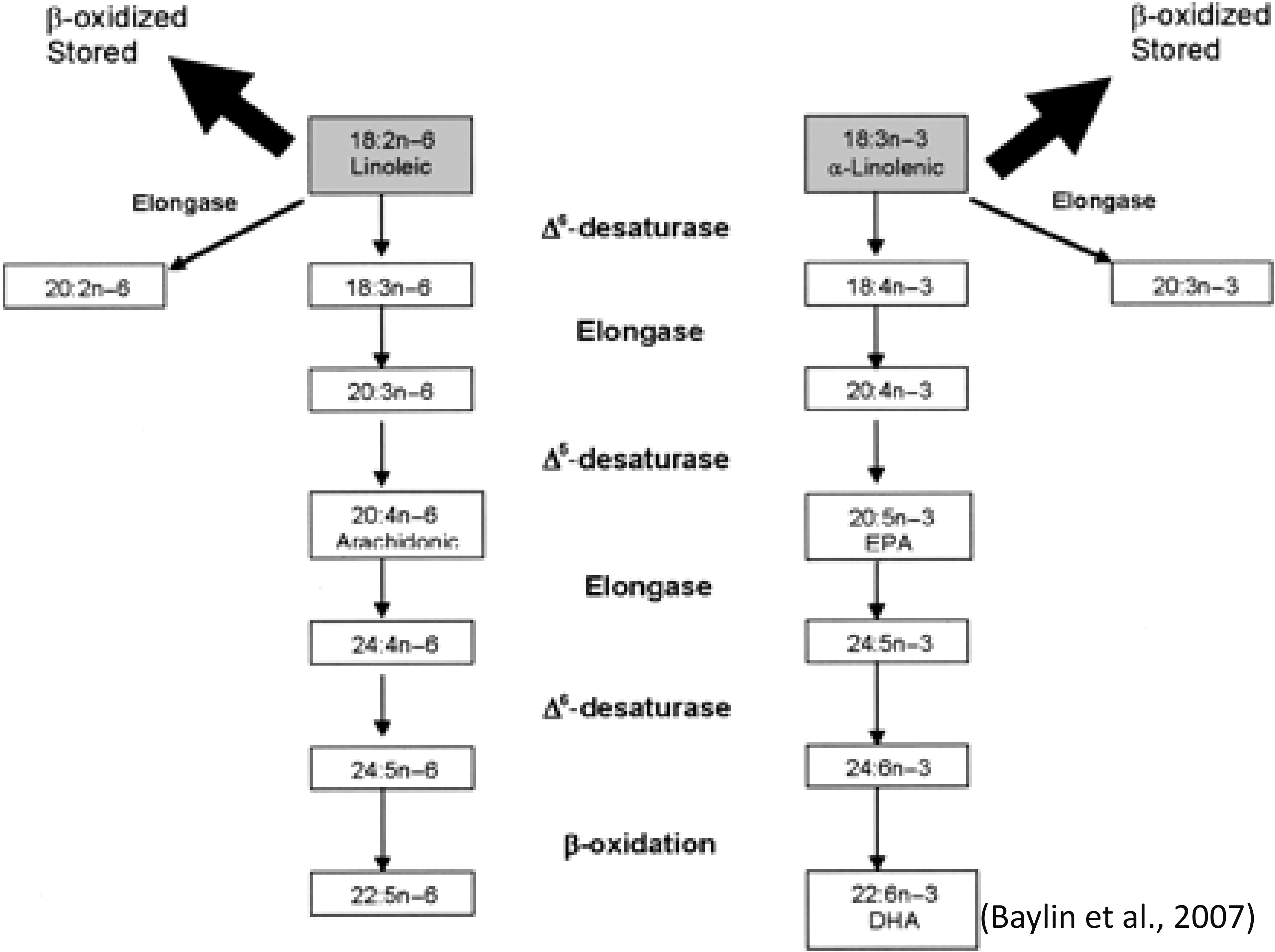


The Effect of Dietary Ratio of Omega-6/Omega-3 Polyunsaturated Fatty Acids on Bone Marrow Fatty Acid Composition and Some Biomarkers of Bone Metabolism in Rabbits

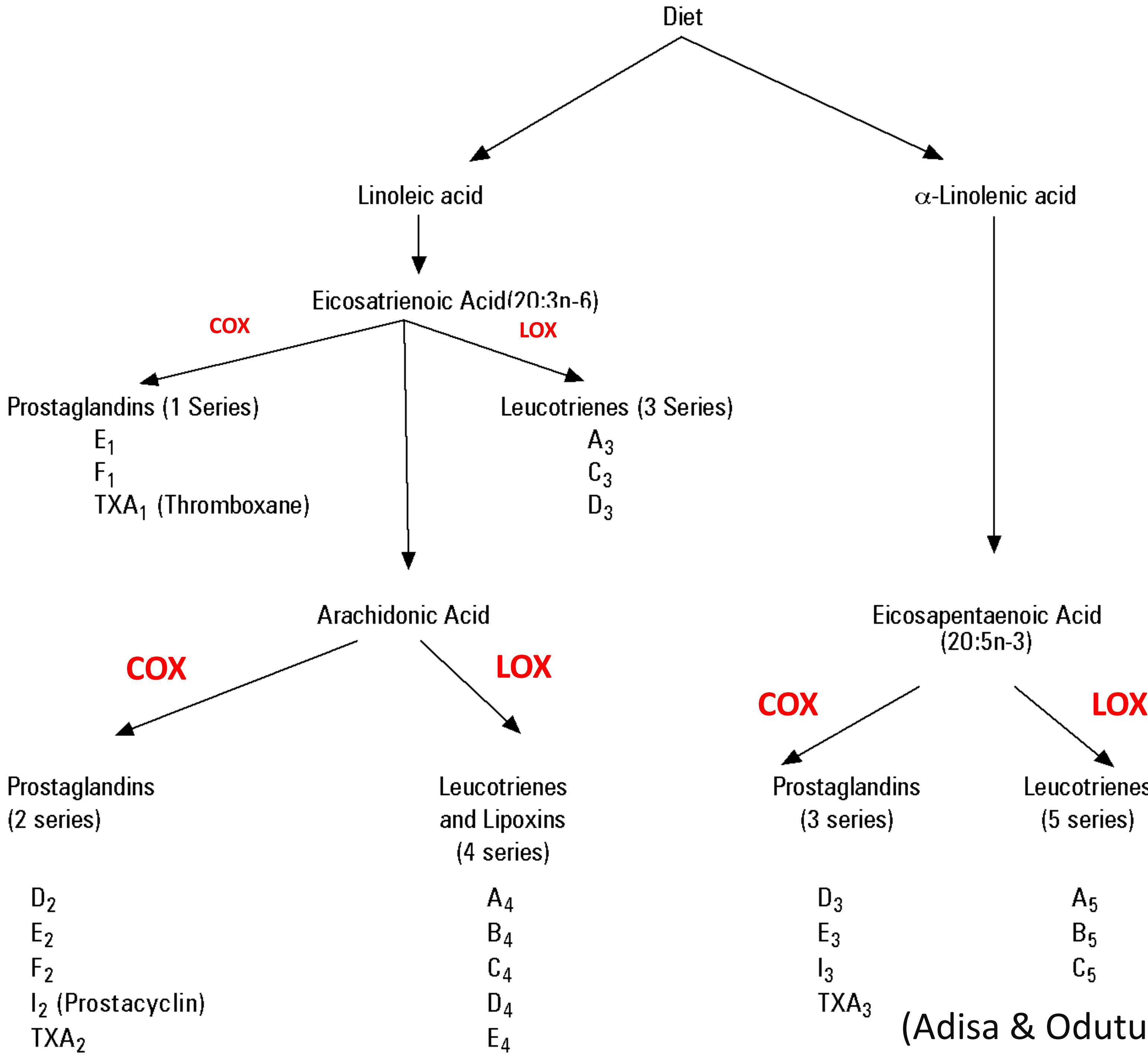
Outlines

- Introduction
- Objectives
- Materials & Methods
- Results & Discussion
- Conclusion
- Future Research

Introduction



(Baylin et al., 2007)



(Adisa & Odutuga, 1999)

Bone Composition

- Cells
  - Osteoblasts
  - Osteoclasts
- Organic Matrix (35%)
- Inorganic (65%)
- Cartilage
- Connective tissue

(Boskey, 1999; Anderson, 2000)

Osteoblasts-bone forming cells

- Mononucleated cells
- Rich in ALP (mineralization)
- Produce organic components for bone formation
- Produce regulatory factors
    - Growth factors
    - Eicosanoids
    - Cytokines

(Baron, 1999; Anderson, 2000)

Osteoclasts-bone resorping cells

- Multinucleated cells
- Rich in ACP
- Synthesize lysosomal enzymes
- Secrete metalloproteinases for bone resorption

(Baron, 1999; Anderson, 2000)

Both osteoblasts & osteoclasts originate from Bone Marrow

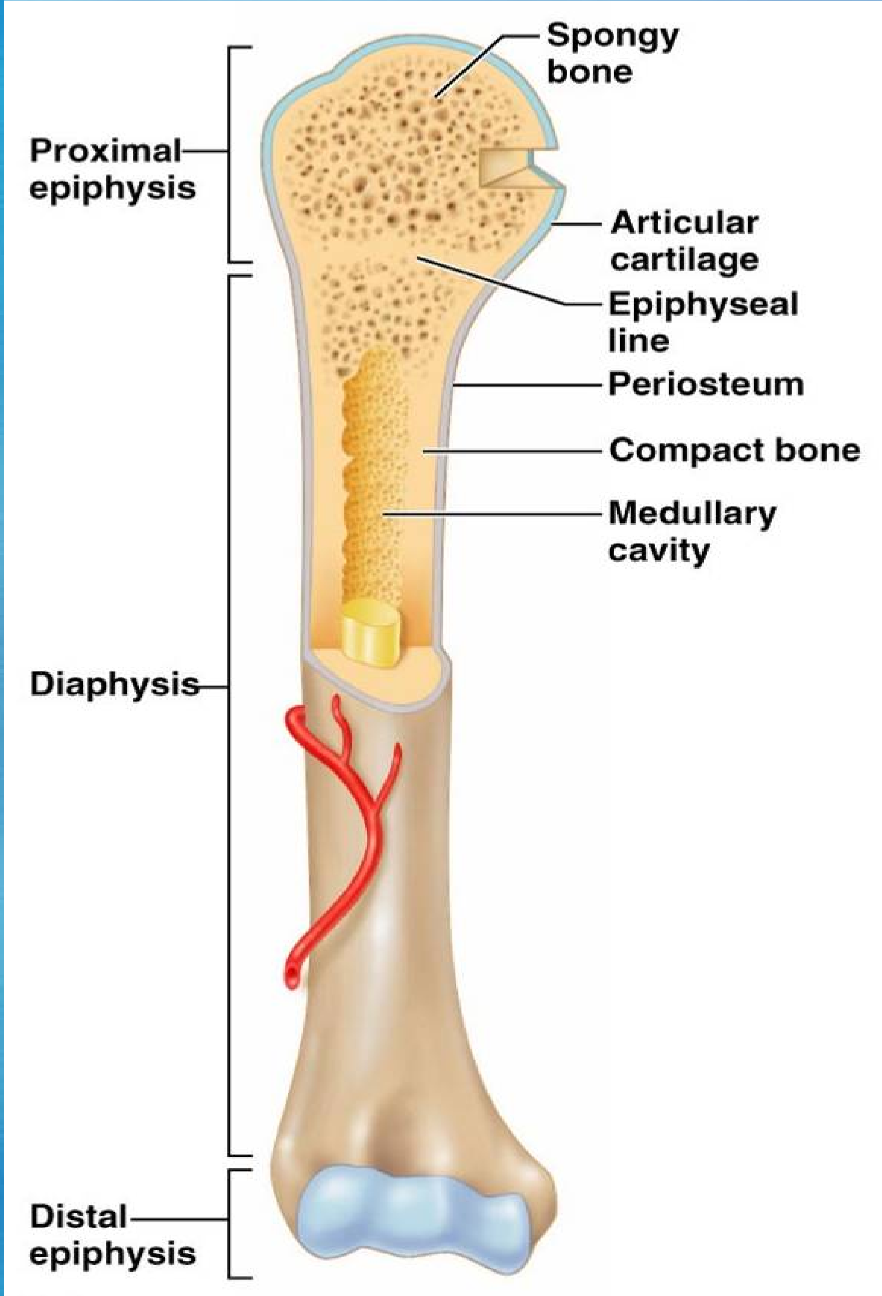
Inorganic

- Calcium (Ca) 99%
- Phosphorous (P) 85%
- Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$
- Magnesium (Mg) 67%
- Zinc (Zn) 29%

(Anderson, 2000; Sherwood, 2004)

Bone Components

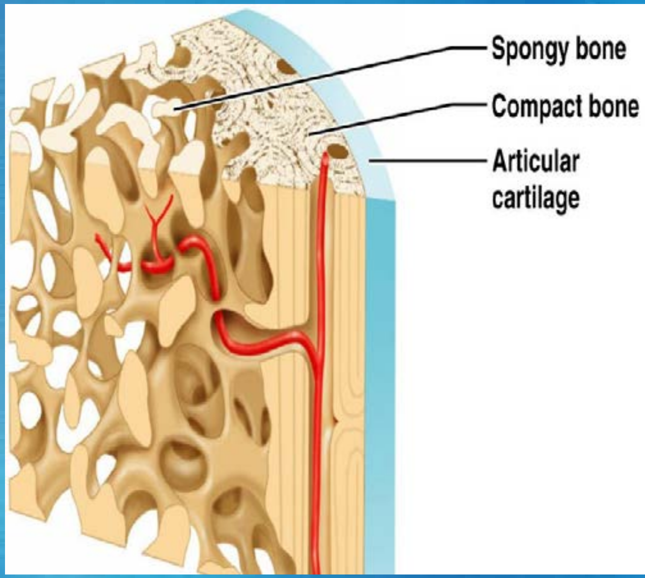
- Diaphysis = Shaft
- Epiphysis = Ends



(Boskey, 1999; Anderson, 2000)

Bone Types

- Cortical (compact/dense)
  - External
  - 80-90% calcified
  - Function: mechanical & protective
-
- Trabecular (spongy/cancellous)
  - Internal
  - 15-25% calcified
  - Function: metabolic



(Boskey, 1999; Anderson, 2000)

Characteristics of Bone

Stiff

Flexible

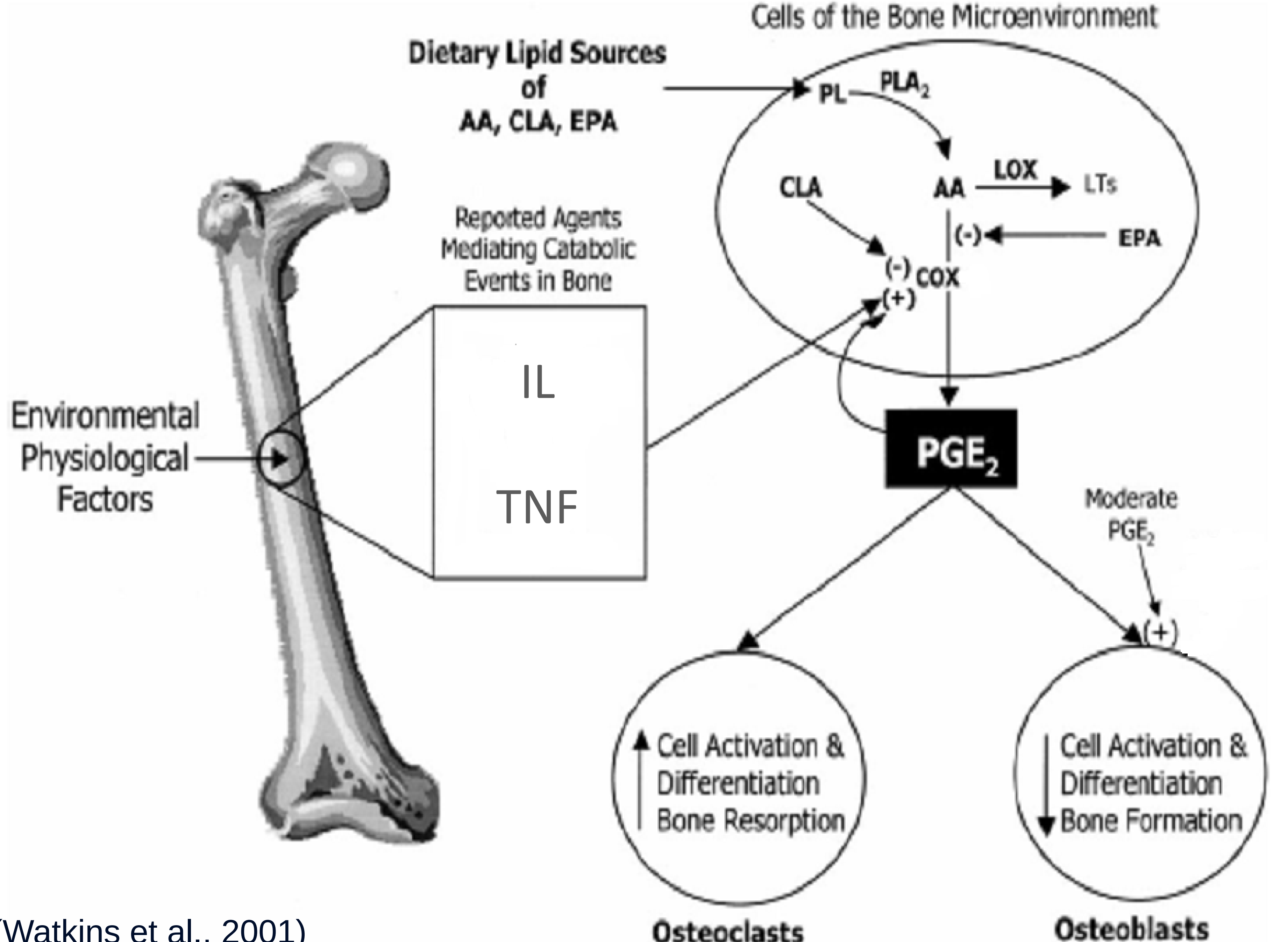
Light

(Currey, 2002; Seeman, 2008)

Bone Metabolism

- Remodeling
  - Resorption
  - Formation
- Modeling
  - Formation
  - Resorption

(Watkins & Seifert, 2000; Sherwood, 2004)

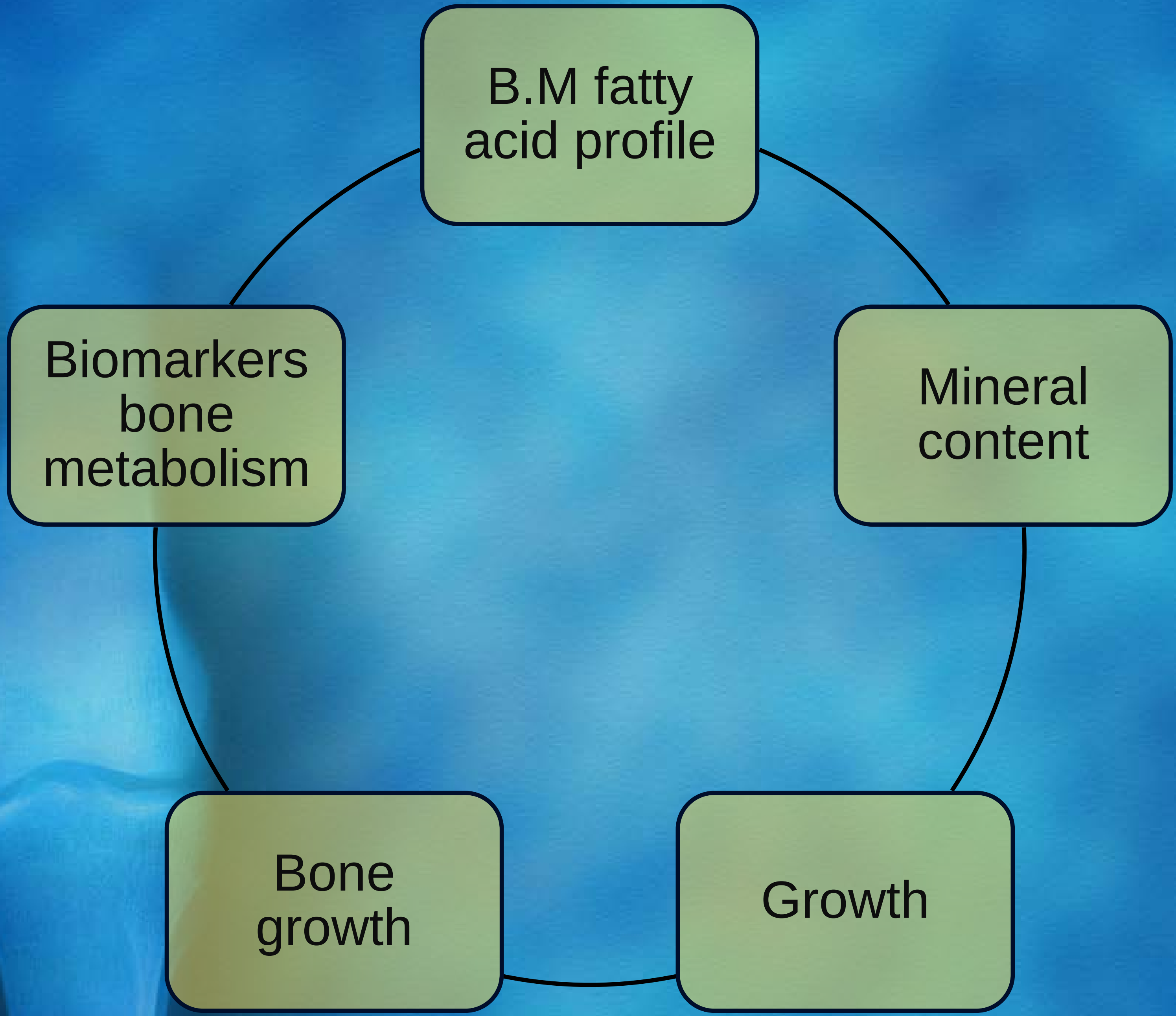


(Watkins et al., 2001)

Objectives

To determine:

1. The effect of different dietary ω-6/ω-3 ratios from different dietary oil sources on:



B.M fatty acid profile
Mineral content
Growth
Bone growth
Biomarkers bone metabolism

To determine:

2. The correlation between bone marrow specific LCPUFA concentrations, their ratios &

   Mineral content

   ALP activity

   PGE_2 level

3. Whether male & female rabbits respond differently to dietary ω-6/ω-3 ratios


Materials & Methods

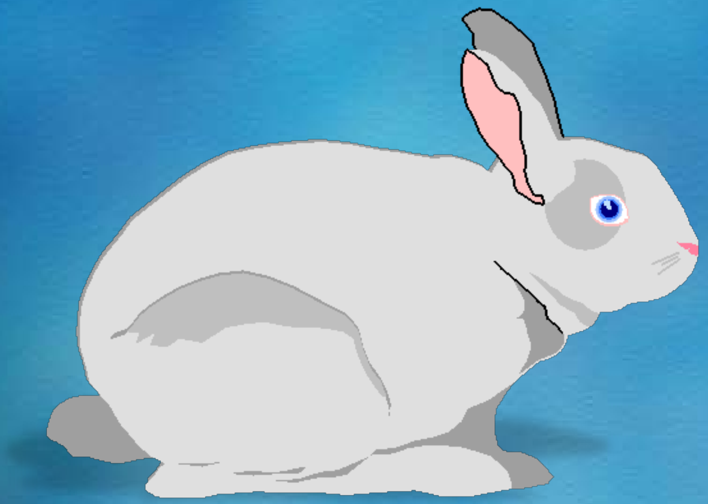
Diets

- Basal diet (47152- Rabbit 18/14 Pellet, without fat) (ARASCO)
- Soy bean oil (SBO)
- Sesame oil (SO)
- Fish oil (FO) (DHA 40% + EPA 30%)
- Two types of marine brown microalgae oils of the genus *Crypthecodinium cohnii*
  - DHA 40% (40 g/100 g of fatty acids)
  - ARA 40% (40 g/100 g of fatty acids

Amounts of oils added (70 g/Kg diet) to each of the 5 experimental diets

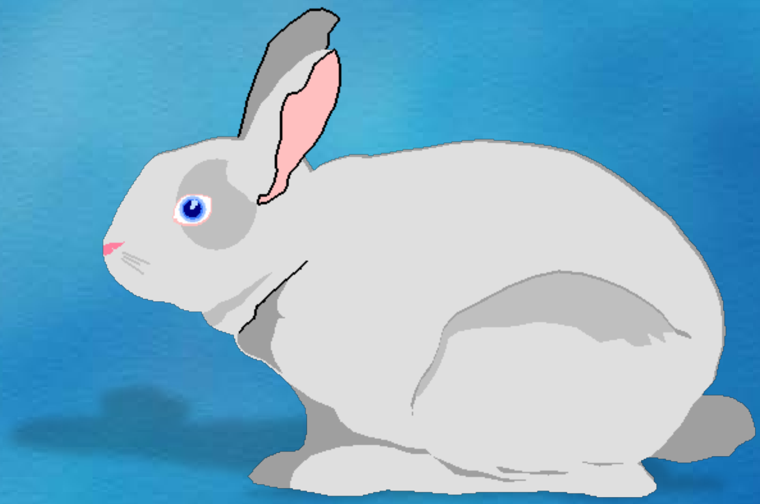
	SBO oil	SO oil	FO oil	DHA oil	ARA oil
SBO diet (Control)	70	-	-	-	-
SO diet	20	50	-	-	-
FO diet	20	-	50	-	-
DHA diet	20	-	-	50	-
DHA/ARA diet	20	-	-	25	25

Animals



25 ♂

20 ♀



Experimental Design

Growth Indicators

- Weight gain (Wt gain) (g)
  =(g final body weight – g initial body weight)

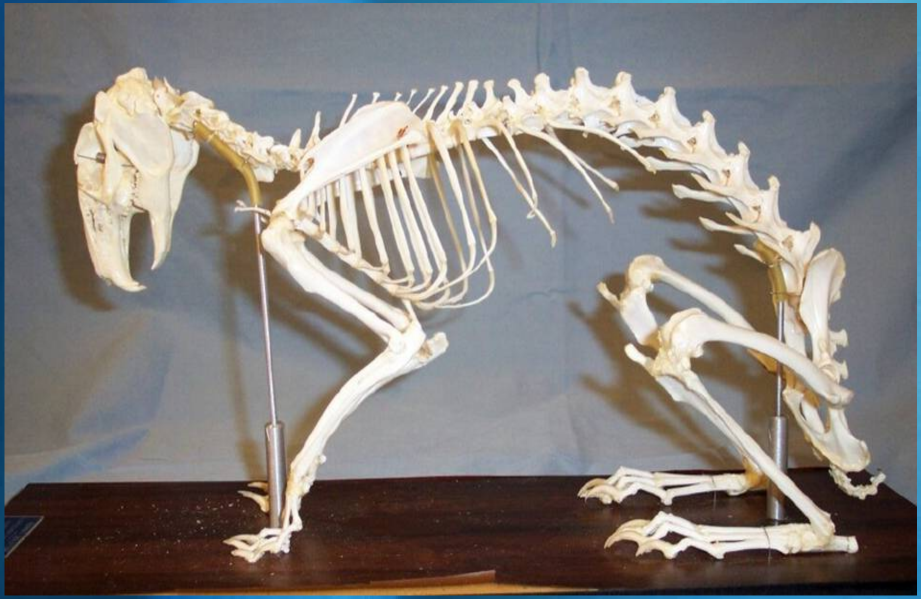
- Food efficiency (FE)
  =(total g weight gain/total g food consumed)

- Growth rate (GR) (g/day)
  =(total g weight gain/100 days study period)

Samples Collection

Bones

Blood



Blood

- 10 ml of blood was collected via cardiac puncture

- In a vacutainer heparinized tube

Bones

- Excised & carefully freed of soft tissue by gentle scraping with a scalpel
- Rinsed with normal saline (0.9% NaCl) & dried using a lint-free paper towel
- Stored in a plastic container at –20°C until elicitation of bone marrow

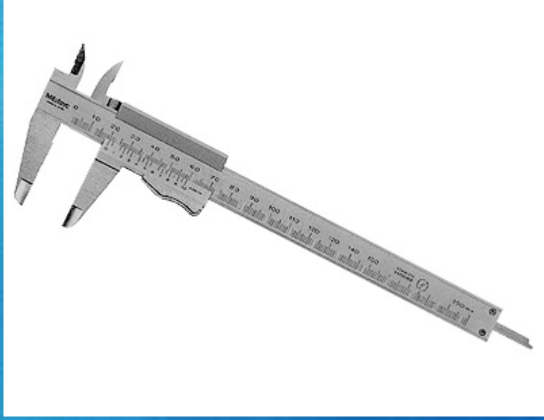
(Dekel et al., 1981)


SBO
1M
Femur
الفخذ
Tibia
الساق
SBO
2M
R
L
1M
SBO
SBO
1M
Humerus
العضد
Radius
الساعد
L
R
L
1M
SBO

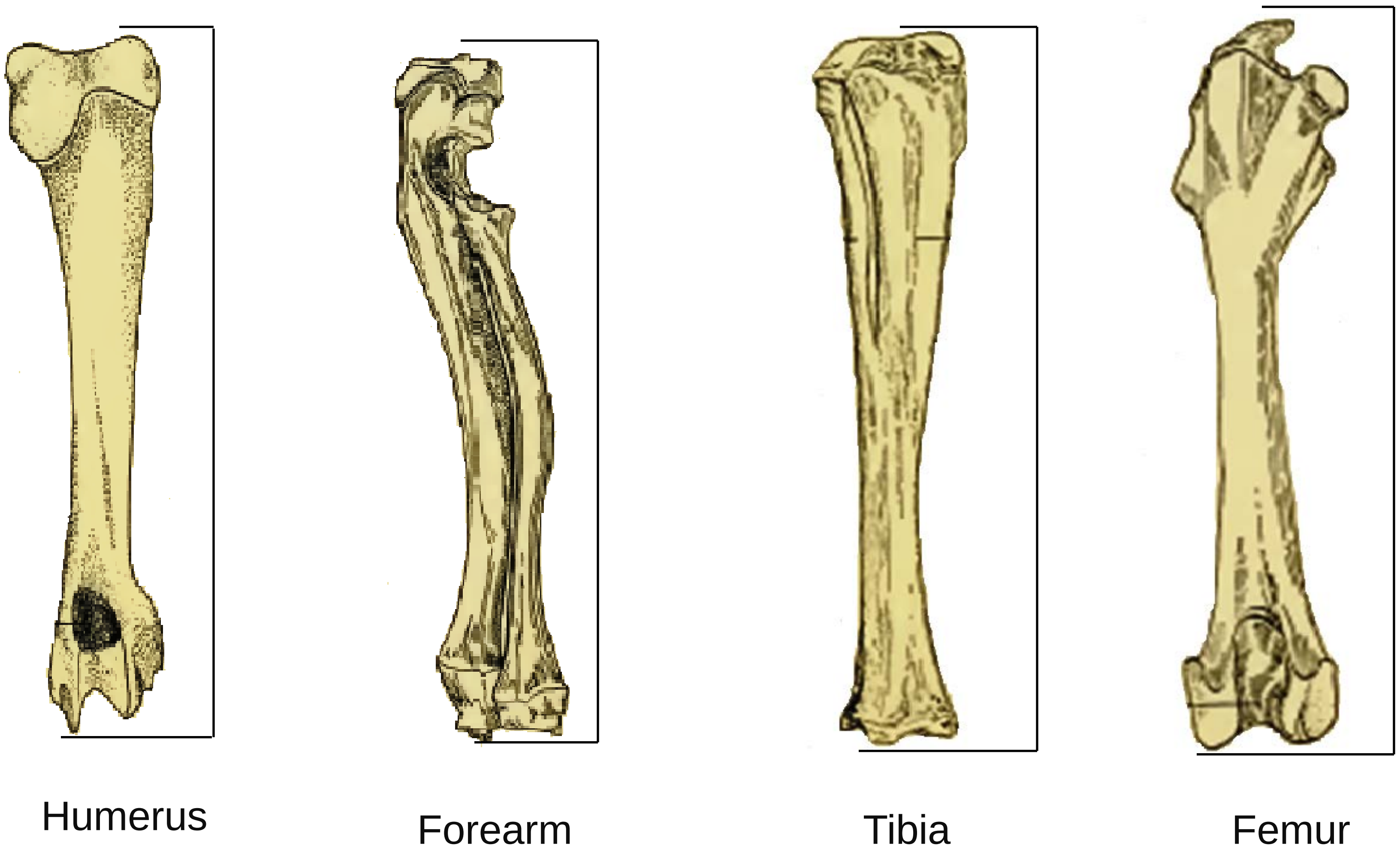
Bone Growth Indicators

- Weight (Wt)

- Length (Lt)
- Width (Wd)

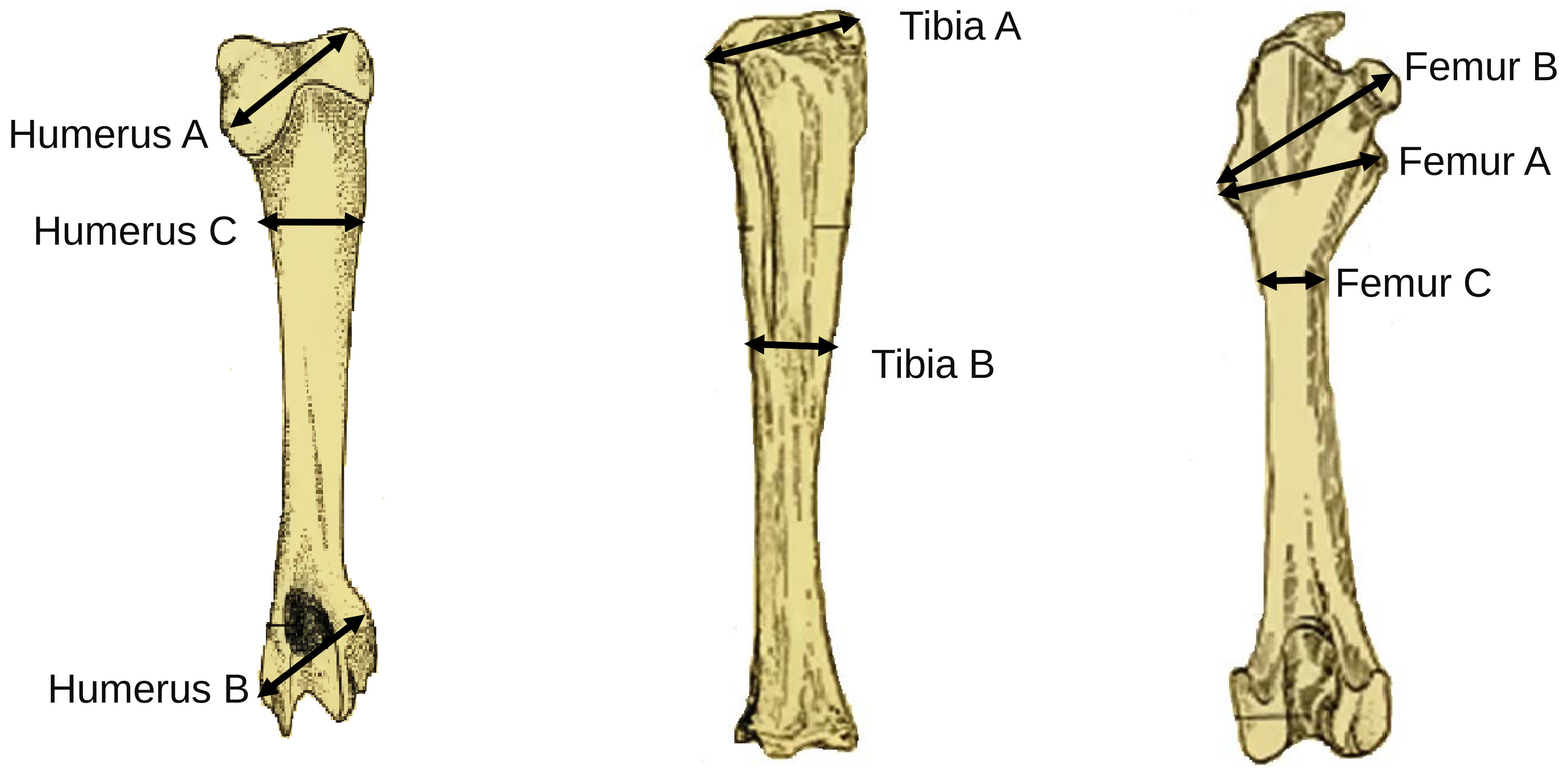


Method of taking bone length measurements



(Reichling & German, 2000)

Method of taking bone width measurements



(Reichling & German, 2000)

Samples Preparation

- Blood was centrifuged to obtain plasma at 4000 rpm (1753 x g) for 8 min at 4°C

- Plasma samples were stored refrigerated at 4°C for one week

Samples Preparation

- Bone epiphysis was removed using a fine saw

- Incision was made along the bone using a sharp non-serrated knife

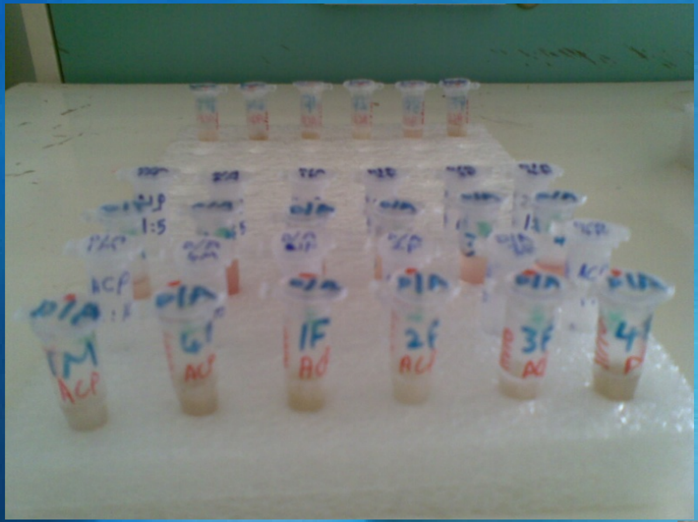
- Bone marrow was removed & weighed to the nearest 0.0001 g

- Stored in a plastic tube at –20°C until used for lipid extraction & fatty acid analysis

Samples Preparation

- Bone samples were rinsed again with normal saline
- Dried using a lint-free paper towel
- Stored in a plastic tube at –20°C until used for PGE_2 & mineral content analysis

Analysis



Enzymes

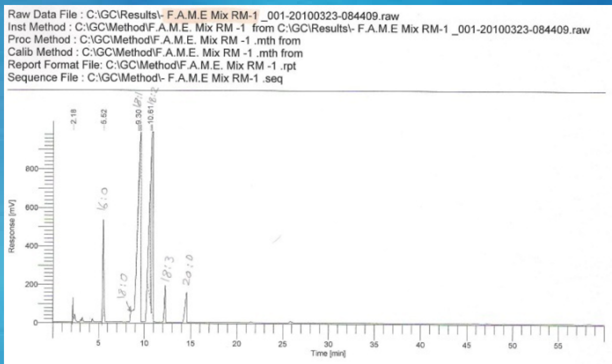
- ALP activity according to Bowers & McComb (1966)
- ACP activity according to Ellis et al. (1971)
- Using a commercial colorimetric/kinetic method kits
- The optical density measured at 405 nm using a spectrophotometer
- Enzyme activity was expressed as (U/L)

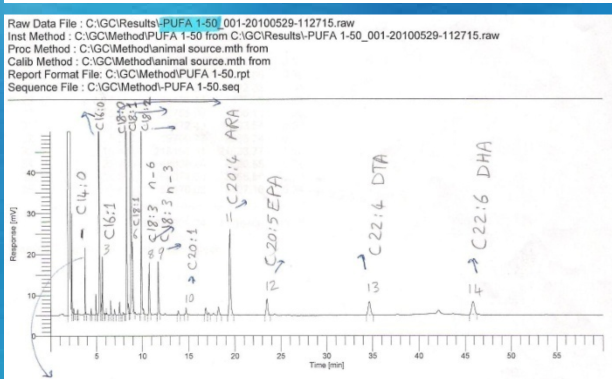
FA analysis

- Bone marrow samples homogenized at 4°C using bench top Homogenizer
- Total lipids extracted according to Folch et al. (1957)
- Transmethylated to fatty acid methyl esters (FAMEs) according to Bligh & Dyer (1959)

FA analysis

- FAMEs separated by gas chromatography (GC)
- FAMEs (C14-C22) identified by comparison with standard fatty acids PUFA-2 animal source & F.A.M.E Mix RM-1 Oil Reference
- FA concentration expressed as g/100 g of total fatty acids





Bone culture & PGE_2

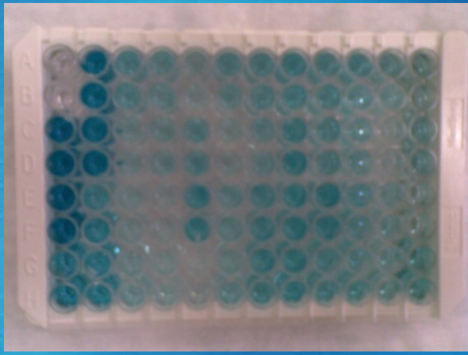
- Bone organ culture performed as previously described (Blanaru et al., 2004; Mollard et al., 2005)

- Tibia samples (1 g) incubated in 10 ml of Hank's balanced salt solution for 2 h at 37°C in a shaking water bath, followed by the removal of bone & rapid freezing of solution at –20°C

Bone culture & PGE_2

- PGE_2 analyzed by enzyme-linked immunosorbent assay (ELISA) technique using a rabbit polyclonal antibody PGE_2 kit

- PGE_2 levels expressed as ng/g bone



Mineral content

- Femur samples (300 mg) digested in 6 ml of concentrated nitric acid (HNO_3) & 2 ml hydrogen peroxide (H_2O_2) for 30 min in a microwave digestion system

- Ca, Mg & Zn concentrations measured using an inductively coupled plasma mass spectrometer (ICP-MS)

Mineral content

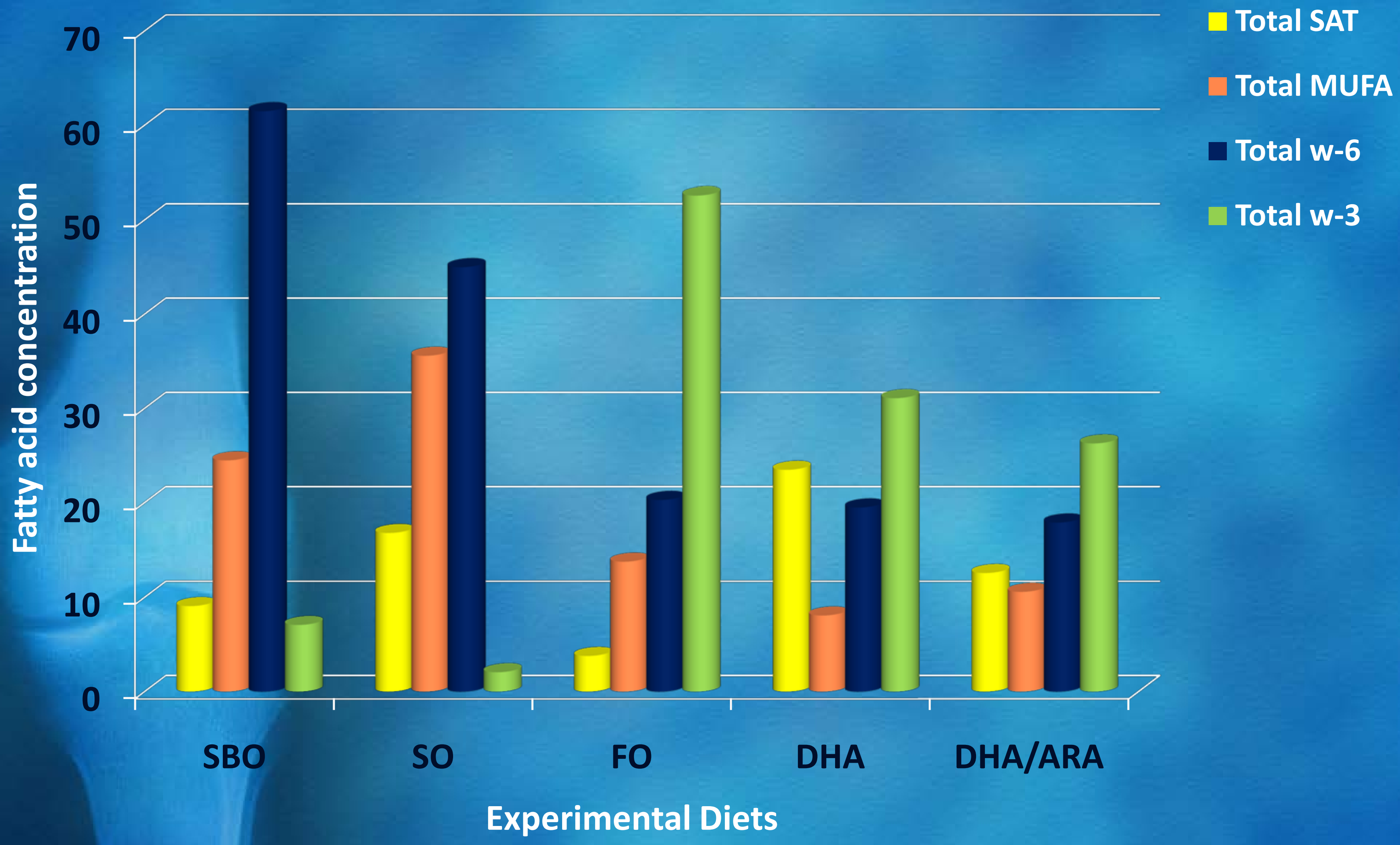
- P concentration measured using molybdo-vanadate colorimetric method according to the AOAC (1962)

- The optical density measured at 410 nm using a spectrophotometer

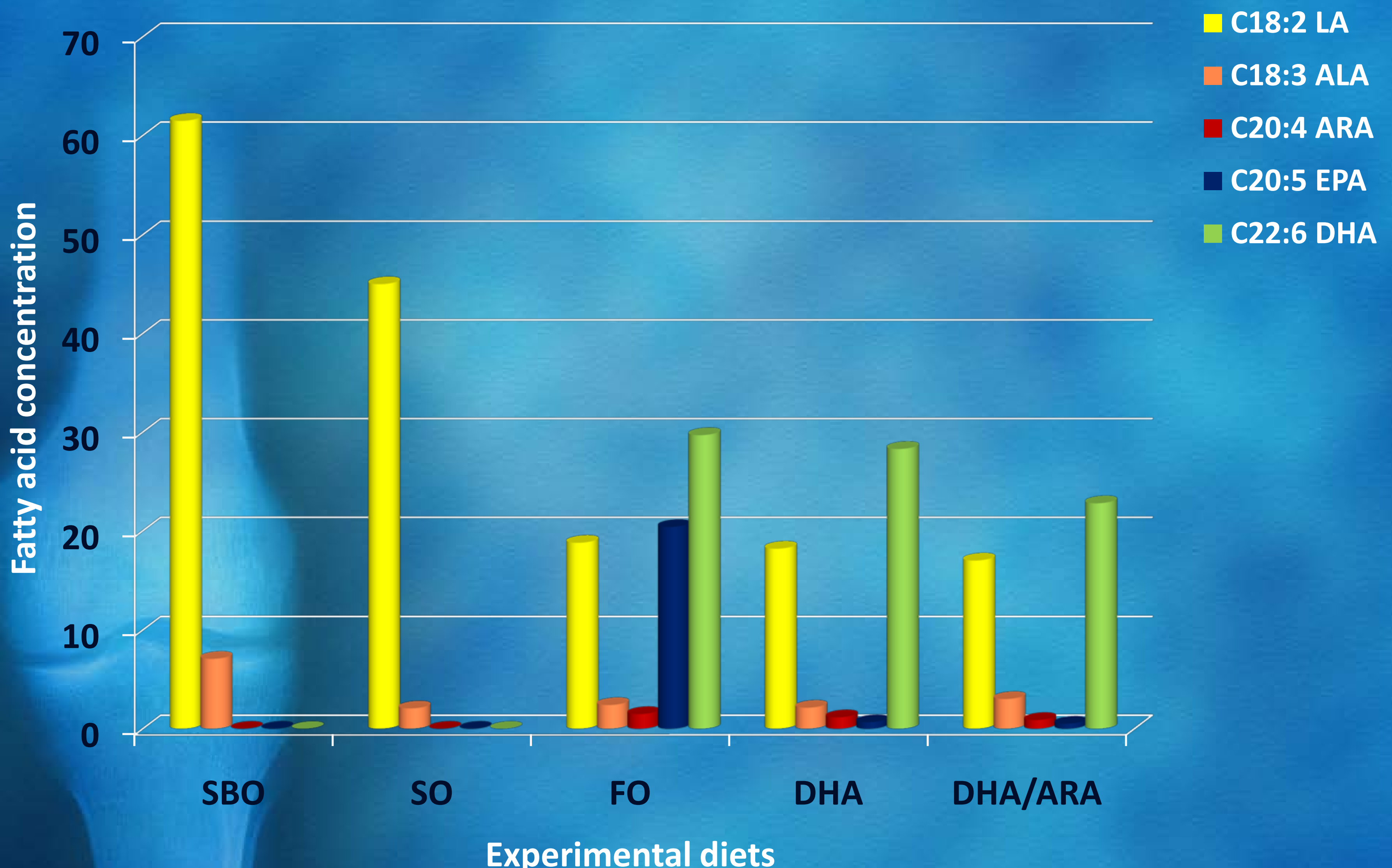
- Minerals' concentration expressed as mg/g bone

Results & Discussion

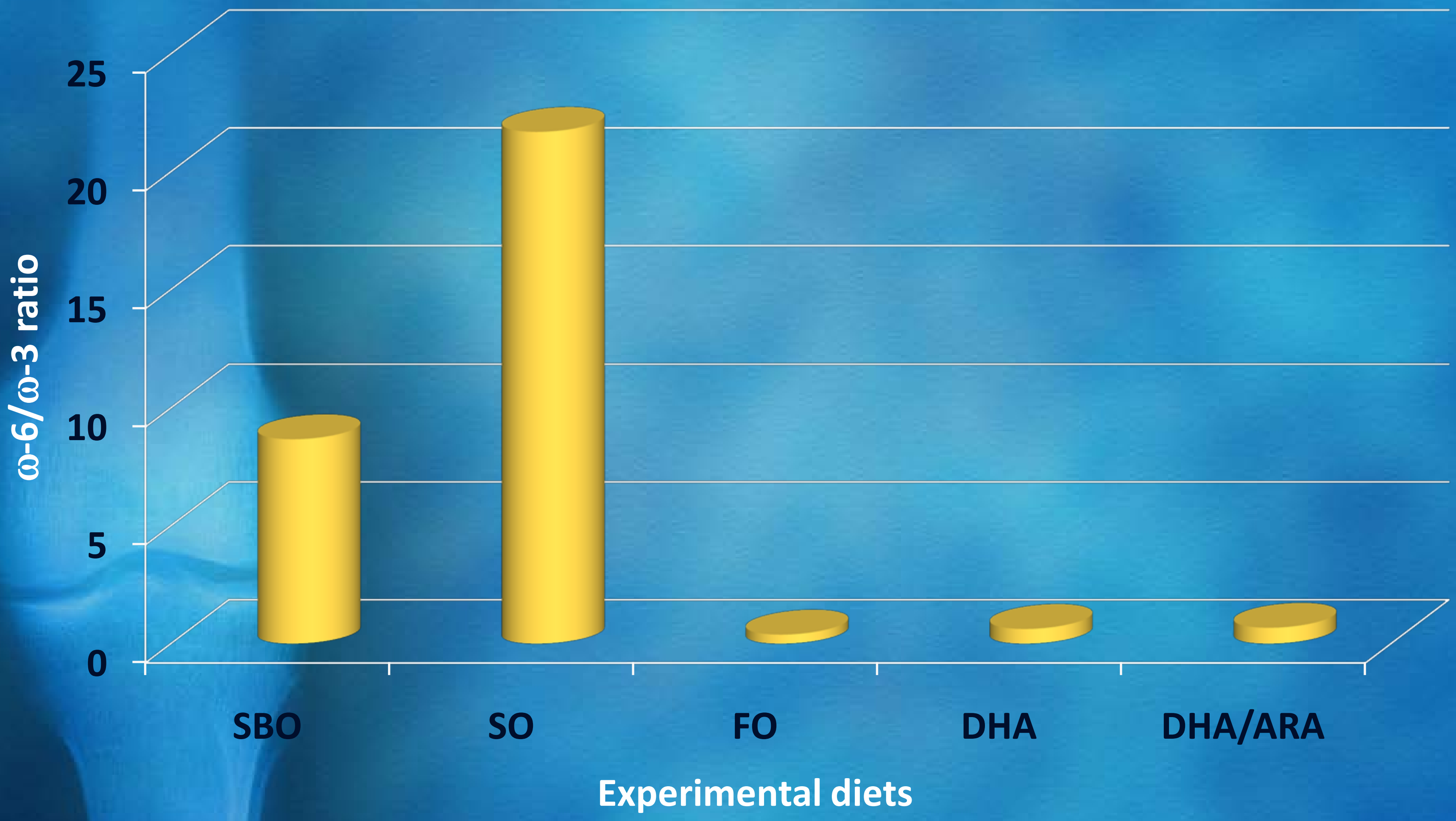
Fatty acid composition (g/100 g total fatty acids) of the experimental diets



Fatty acid composition (g/100 g total fatty acids) of the experimental diets



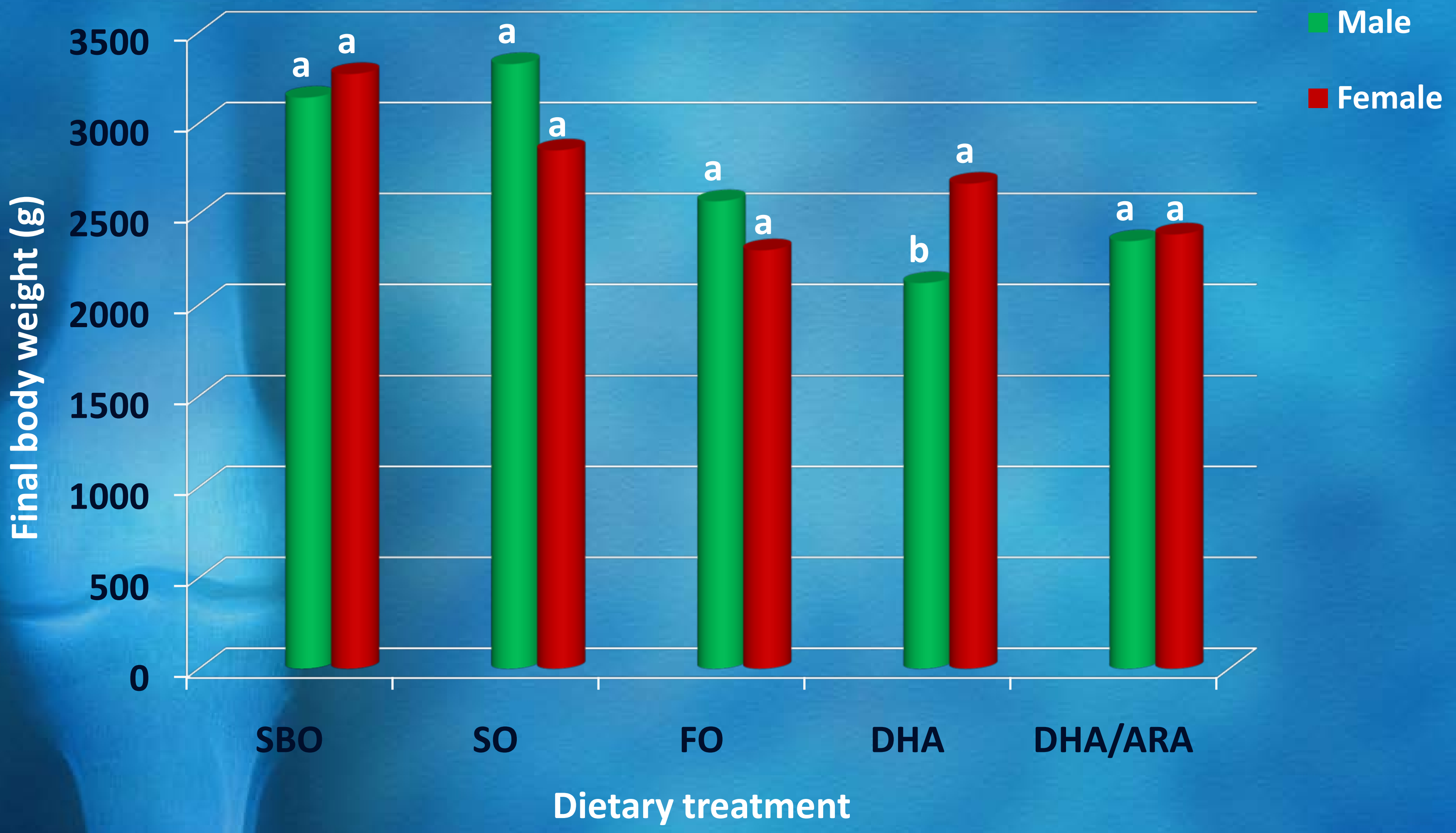
ω-6/ω-3 ratio of the experimental diets



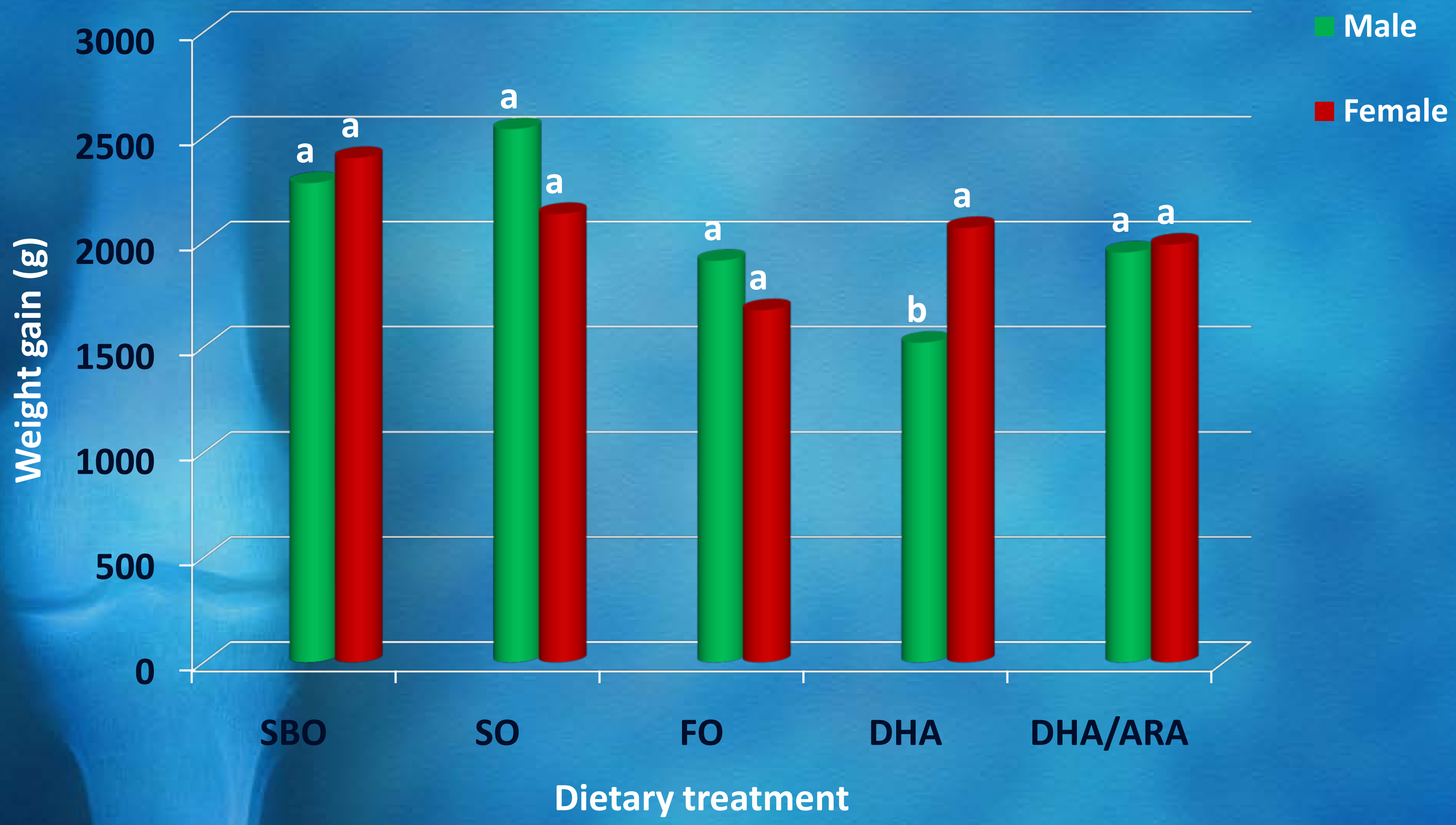
Growth indicators of male & female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratio for 100 days

Growth indicator	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
FB Wt (g)	**3140 Aa ±91.38**	3270 Aa ±165.53	**3325 Aa ±119.90**	2850 Aab ±332.92	2570 Ab ±152.15	2300 Ab ±200	**2120 Bc ±37.42**	2666.67 Aab ±109.29	2350 Abc ±50	2387.50 Ab ±132.88
Wt gain (g)	**2280 Aab ±101.98**	2400 Aa ±178.19	**2537.50 Aa ±131.30**	2133.33 Aa ±290.59	1910 Ab ±151.16	1675 Aa ±225	**1520 Bc ±33.91**	2066.67 Aa ±60.09	1950 Ab ±50	1987.50 Aa ±135.98
GR (g/day)	**22.80 Aab ±1.02**	24.20 Aa ±1.69	**25.50 Aa ±1.20**	21.33 Aa ±2.91	19.40 Ab ±1.60	17 Aa ±2	**15.40 Bc ±0.40**	21 Aa ±0.58	19.50 Ab ±0.50	20 Aa ±1.41
FE	0.41 Aa ±0.01	0.42 Aa ±0.01	0.42 Aa ±0.01	0.39 Aa ±0.03	0.43 Aa ±0.01	0.41 Aa ±0.07	**0.37 Bb ±0.01**	0.46 Aa ±0.01	0.44 Aa ±0.01	0.48 Aa ±0.02

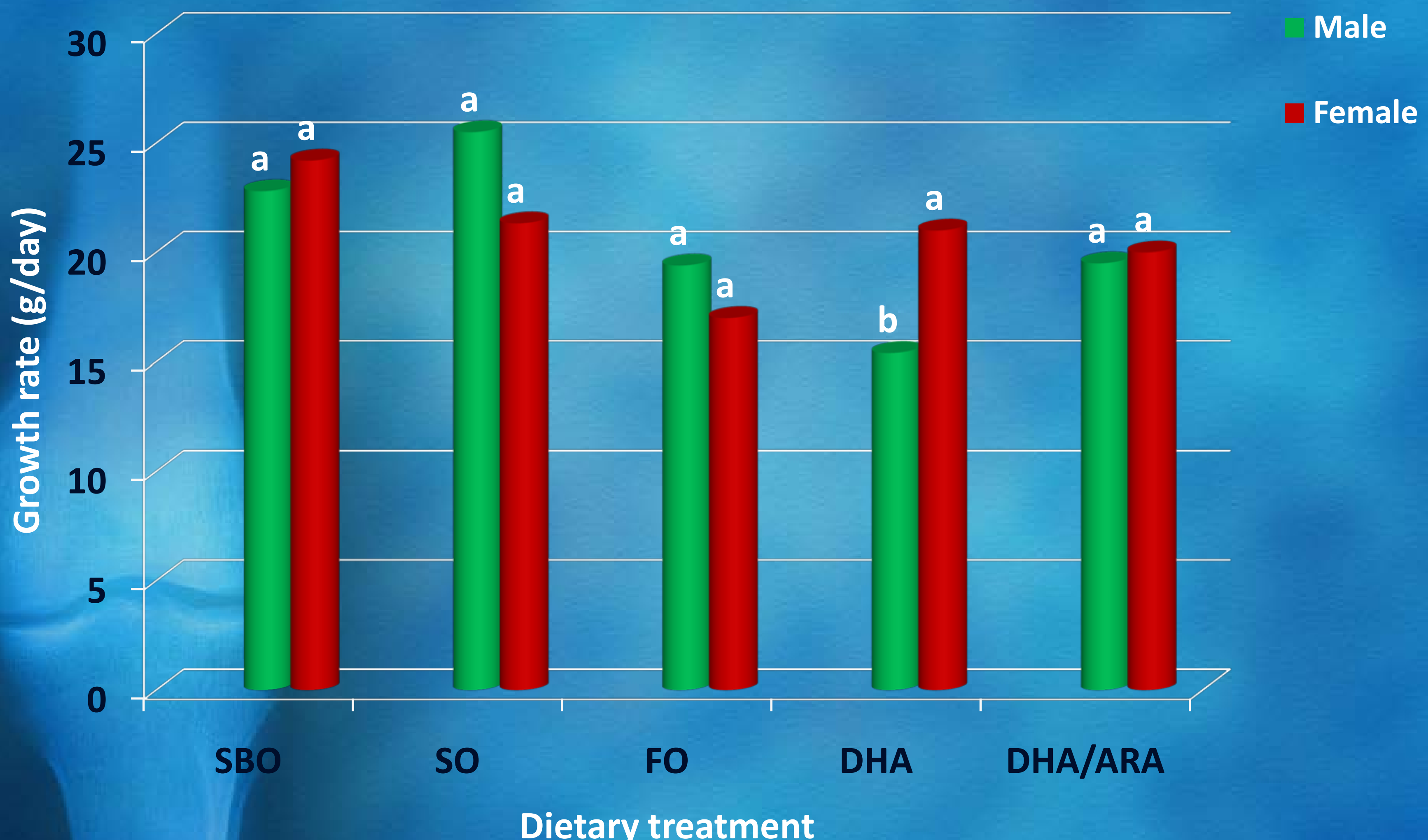
Comparison of final body weight between male & female rabbits



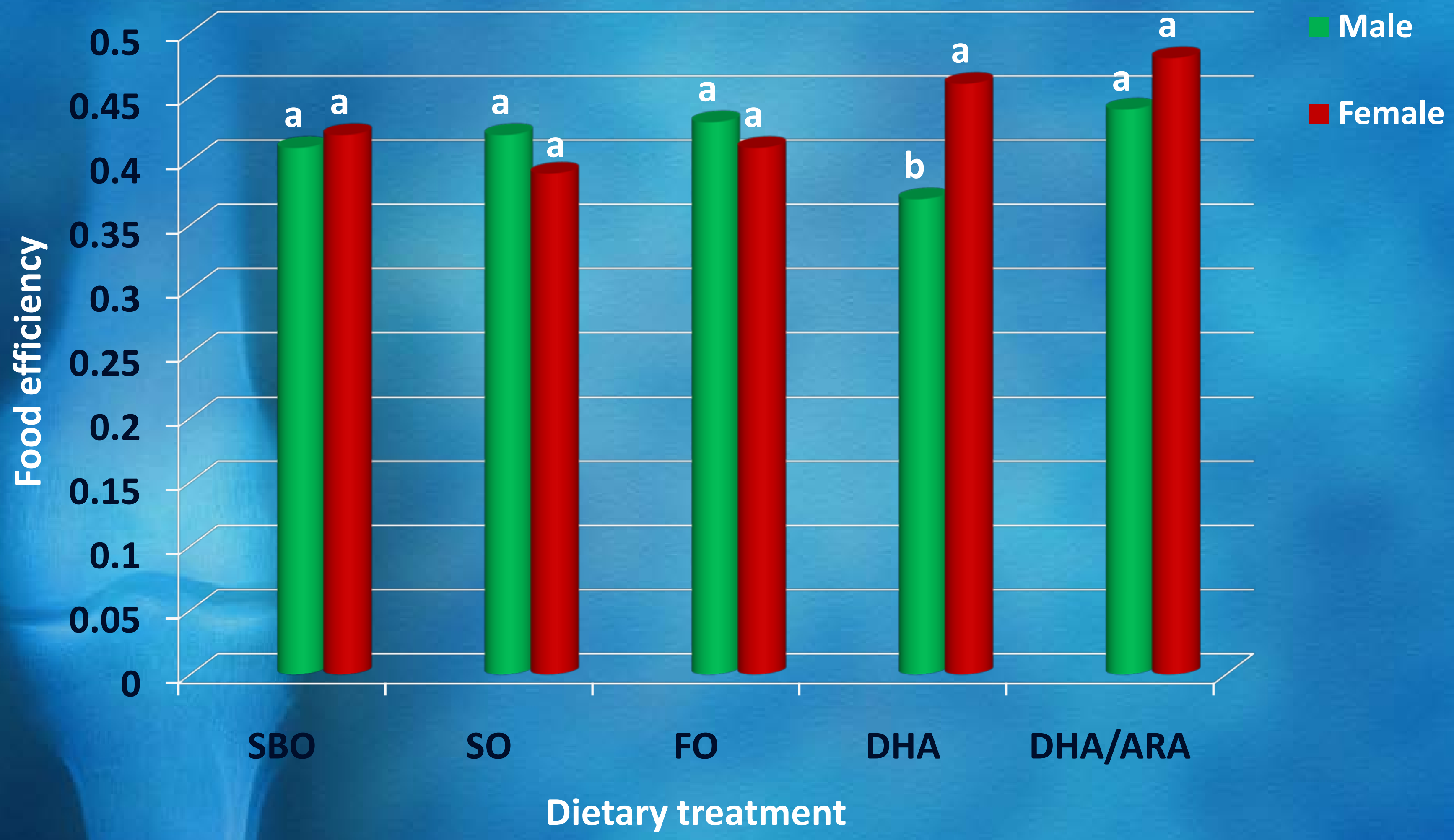
Comparison of weight gain between male & female rabbits



Comparison of growth rate between male & female rabbits



Comparison of food efficiency between male & female rabbits

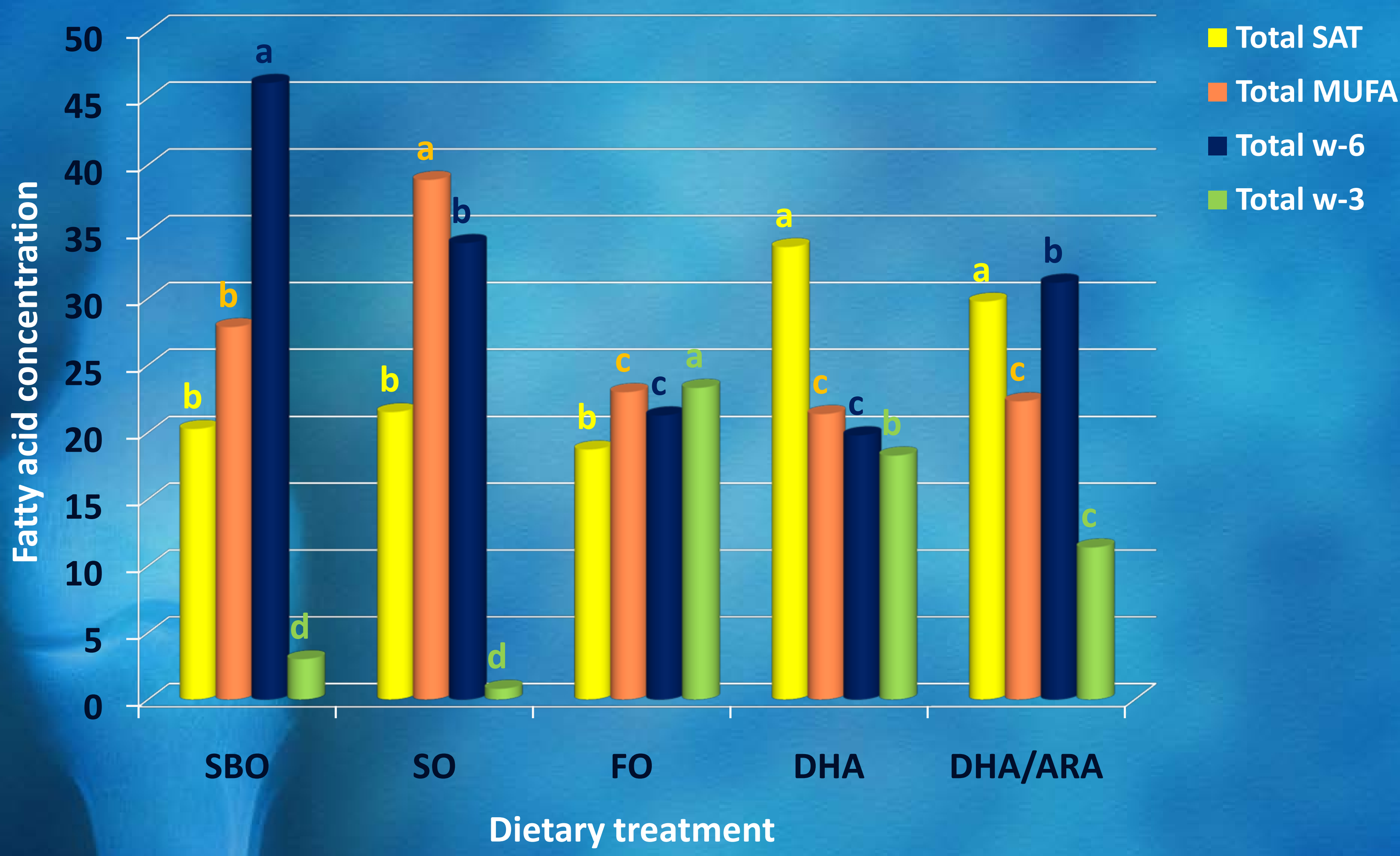


Long-term Supplementation with Different Dietary ω-6/ω-3 Ratios Alters Bone Marrow Fatty Acid Profile and Biomarkers of Bone Formation and Resorption in Growing Rabbits

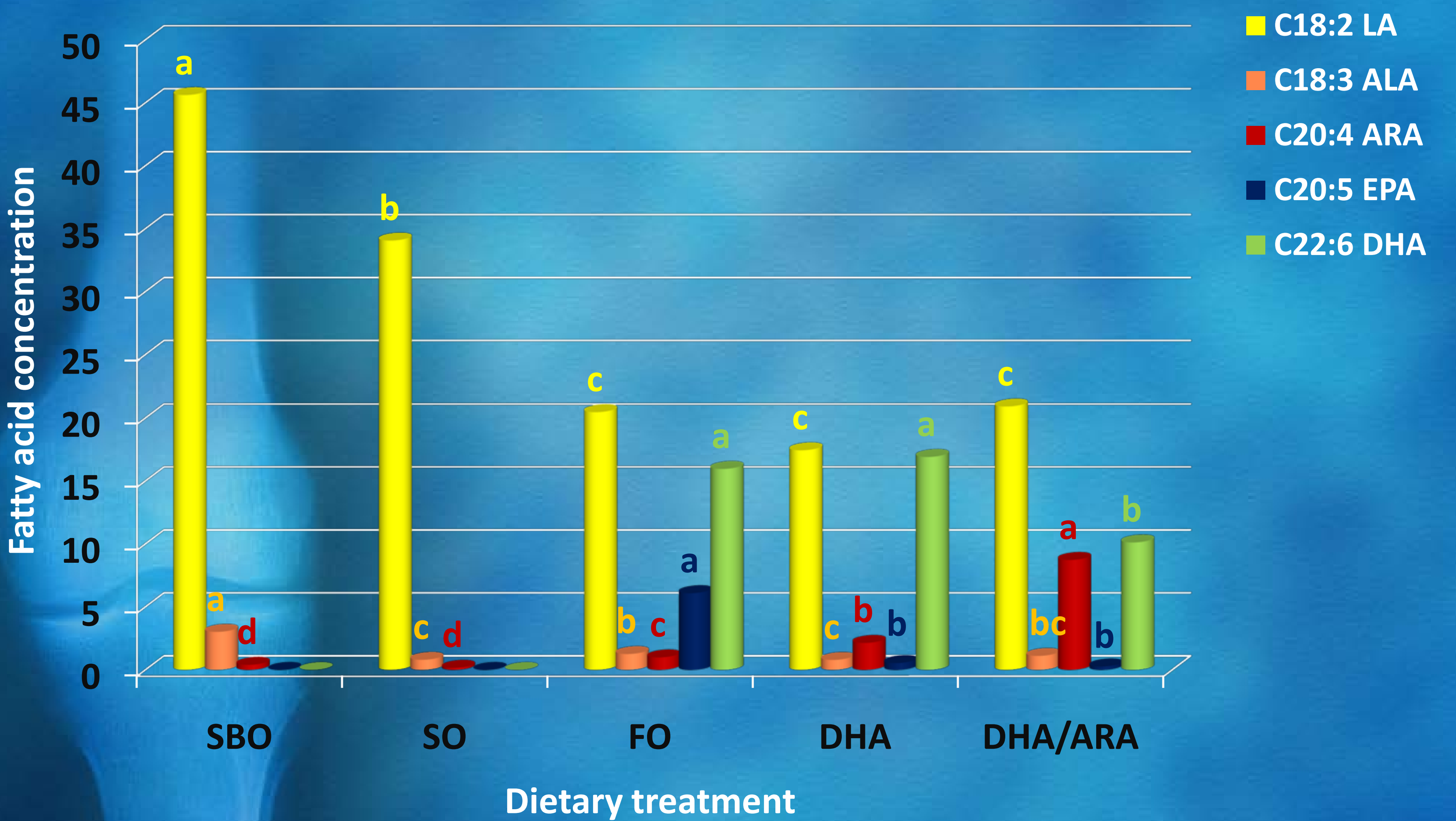
Al-Nouri, D. M., Al-Khalifa, A. S., and Shahidi, F.

Prepared in manuscript format

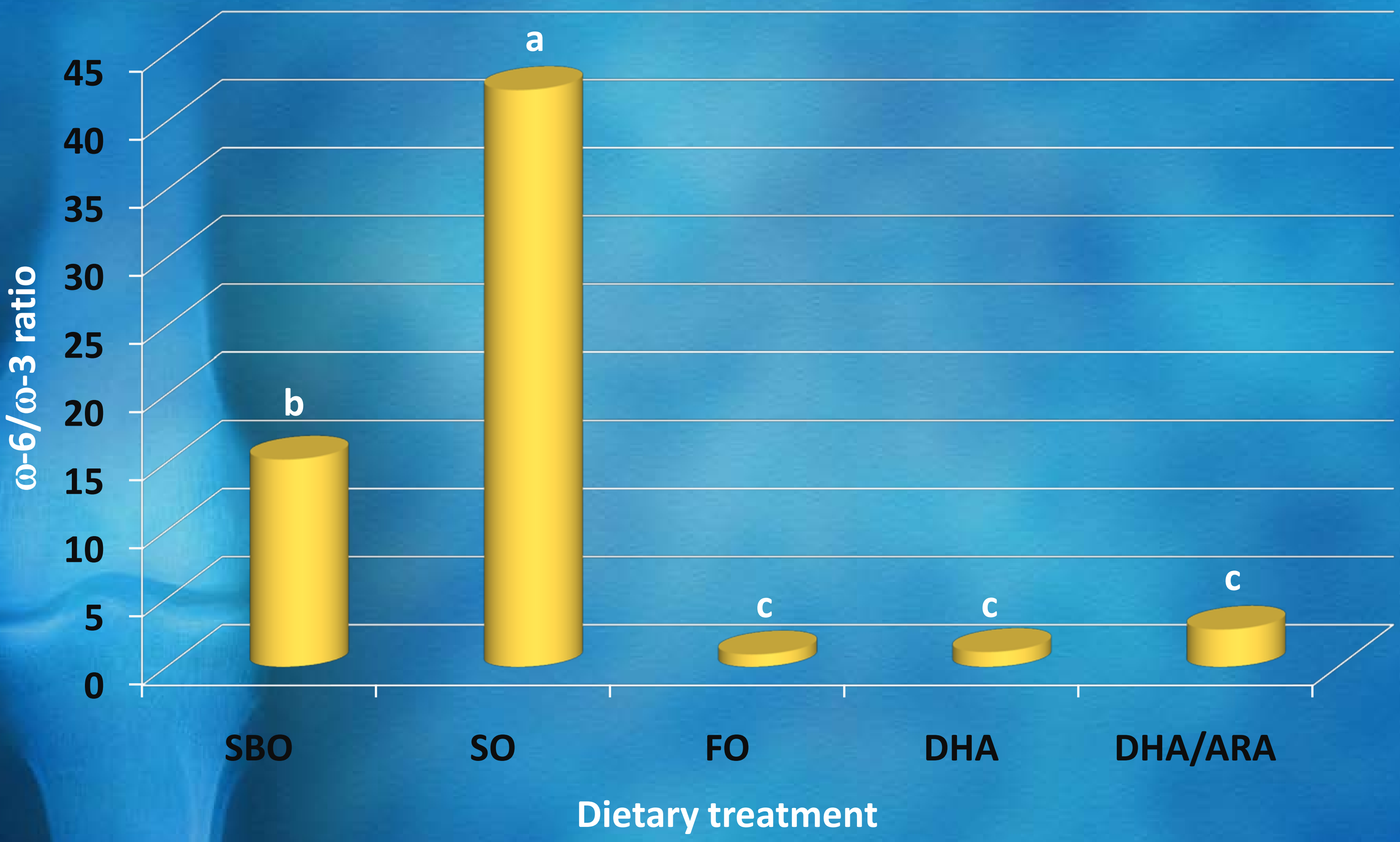
Bone marrow fatty acid profile of male rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days



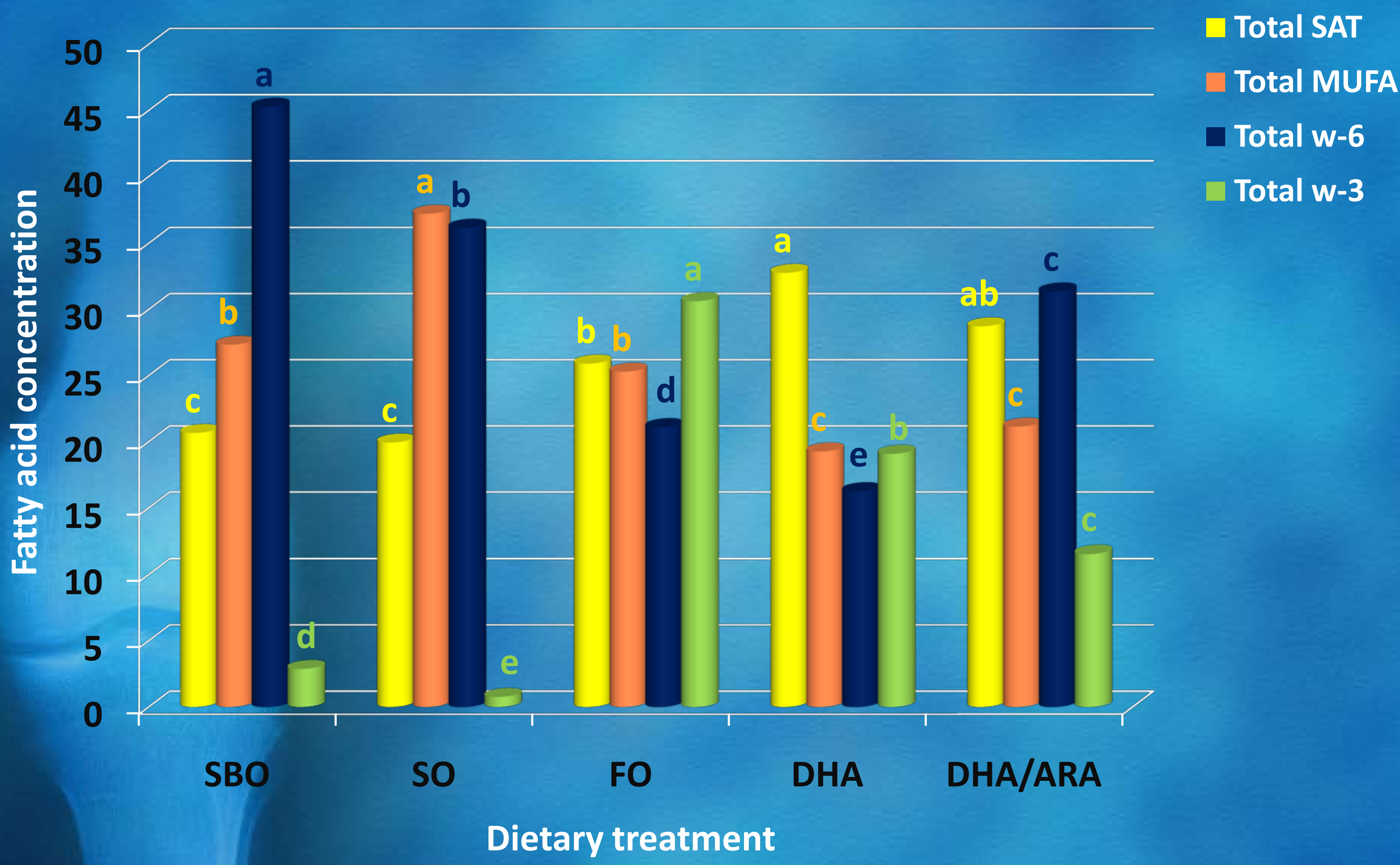
Bone marrow fatty acid profile of male rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days



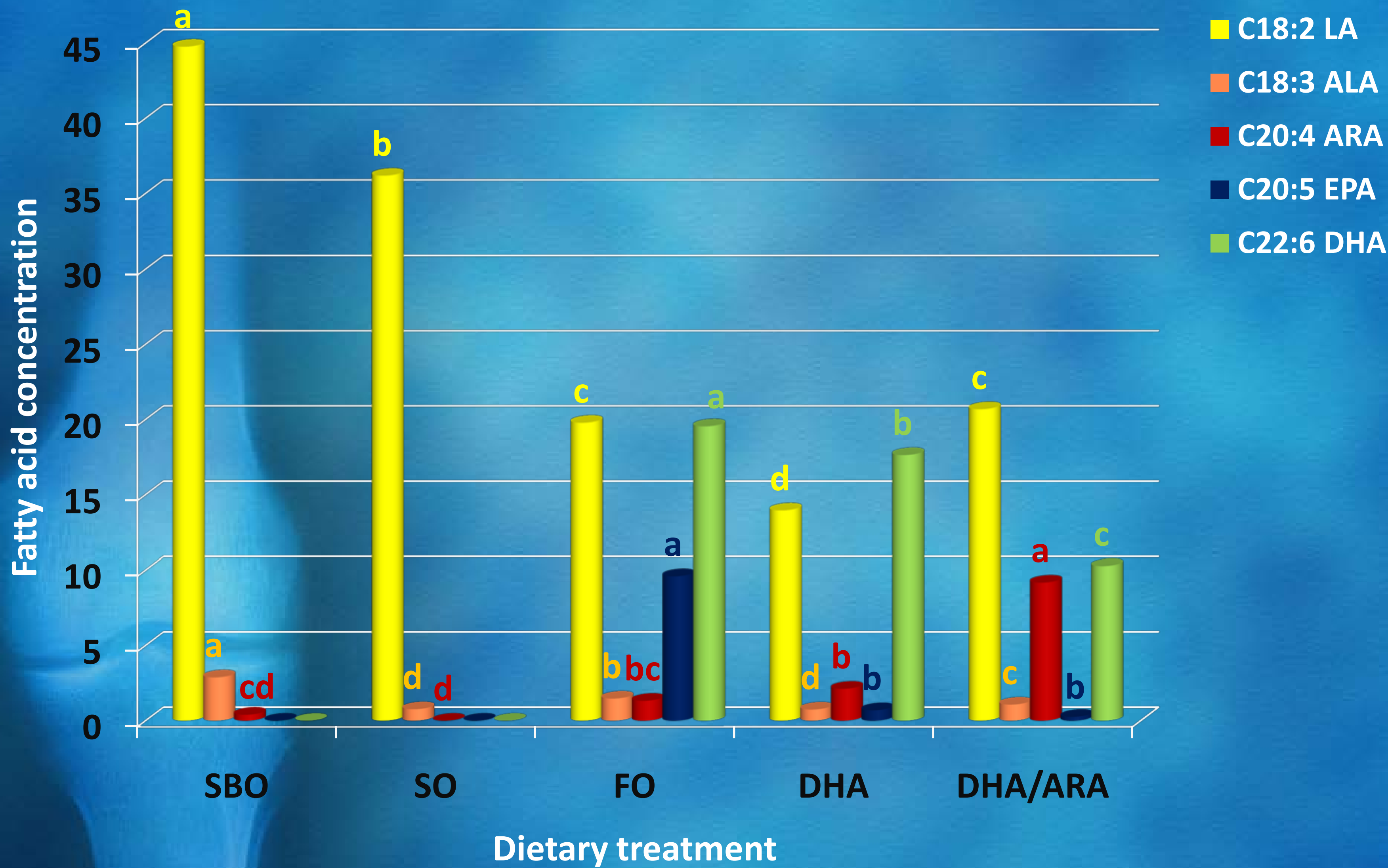
Bone marrow ω-6/ω-3 ratio of male rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days



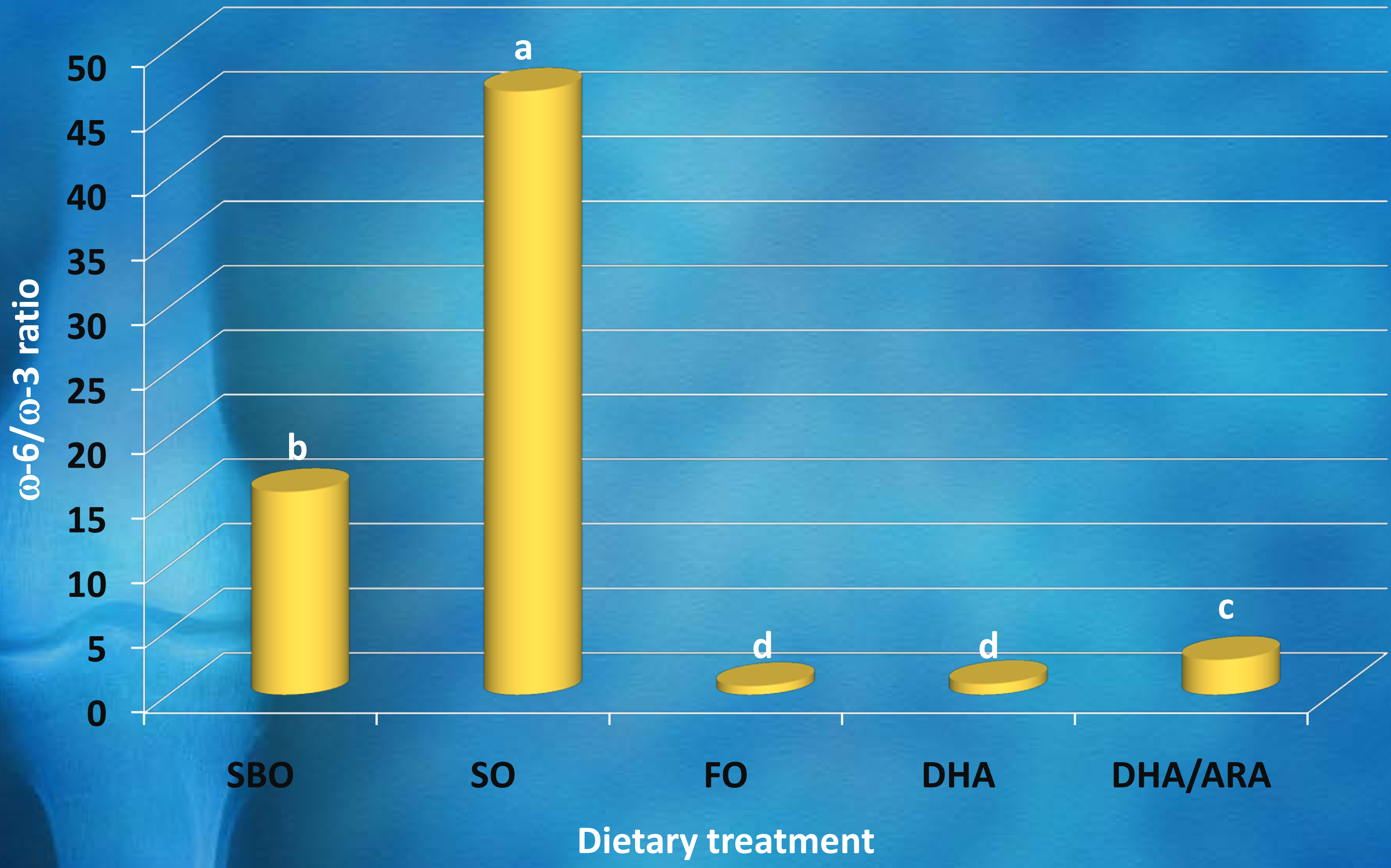
Bone marrow fatty acid profile of female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days



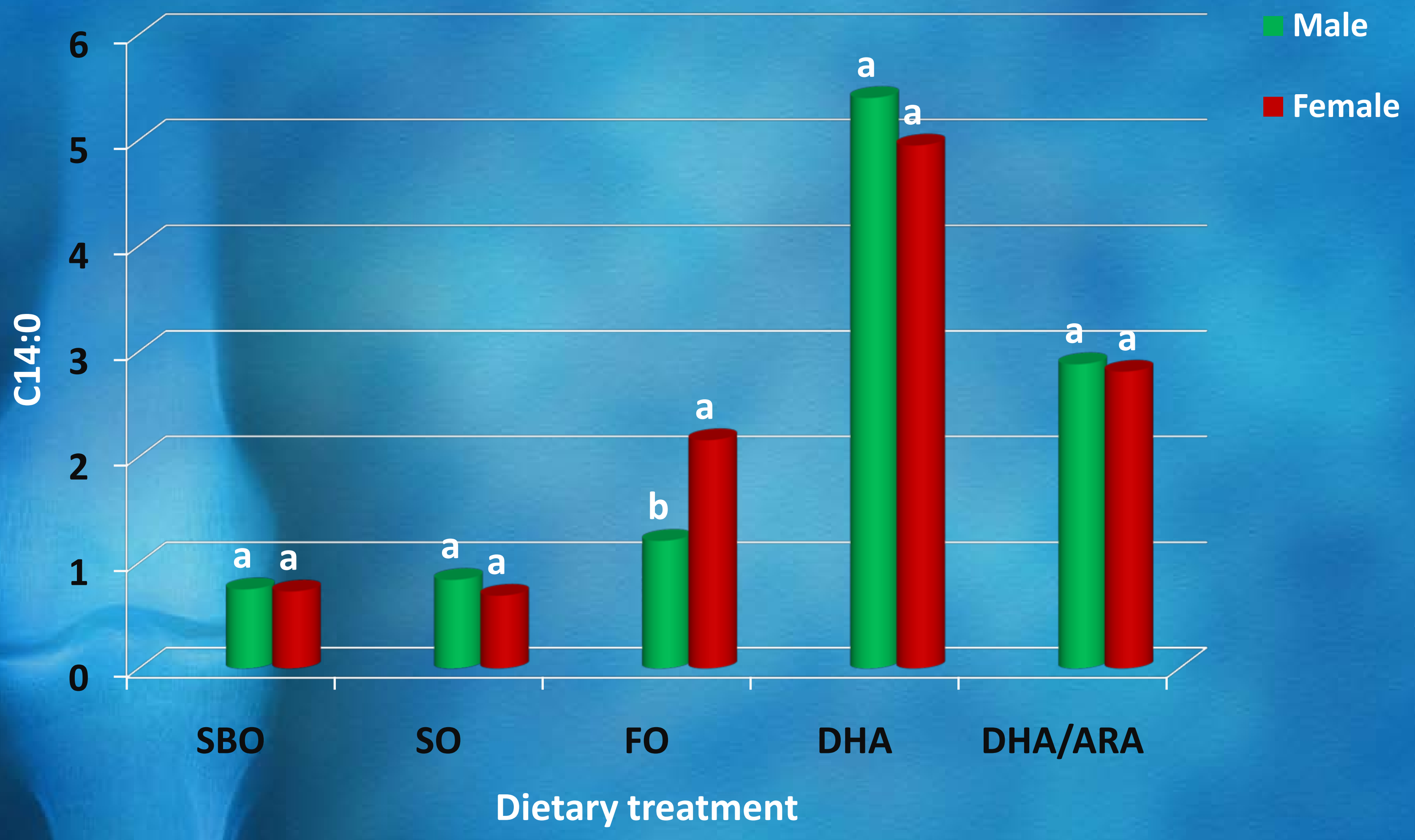
Bone marrow fatty acid profile of female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days



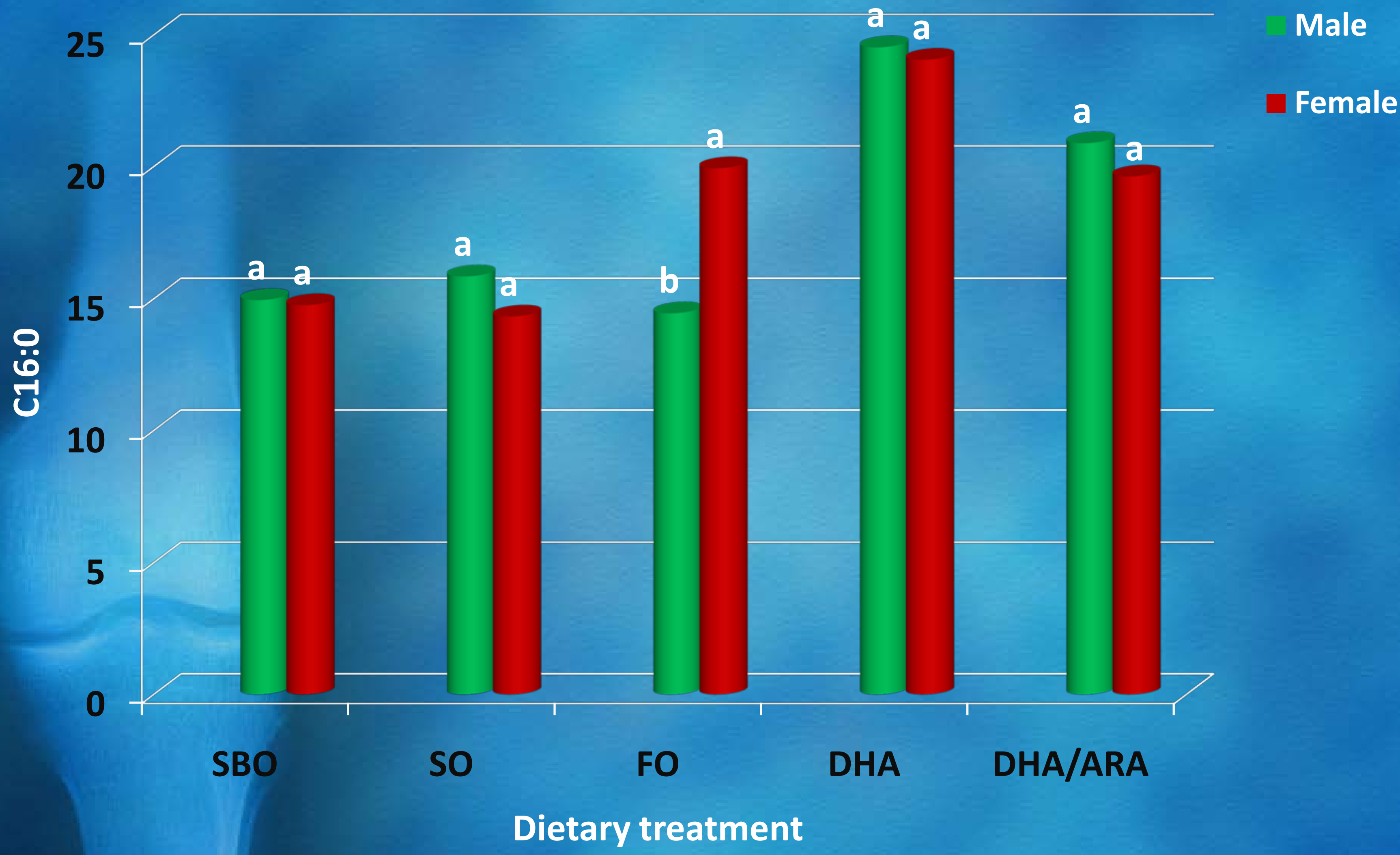
Bone marrow ω-6/ω-3 ratio of female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days



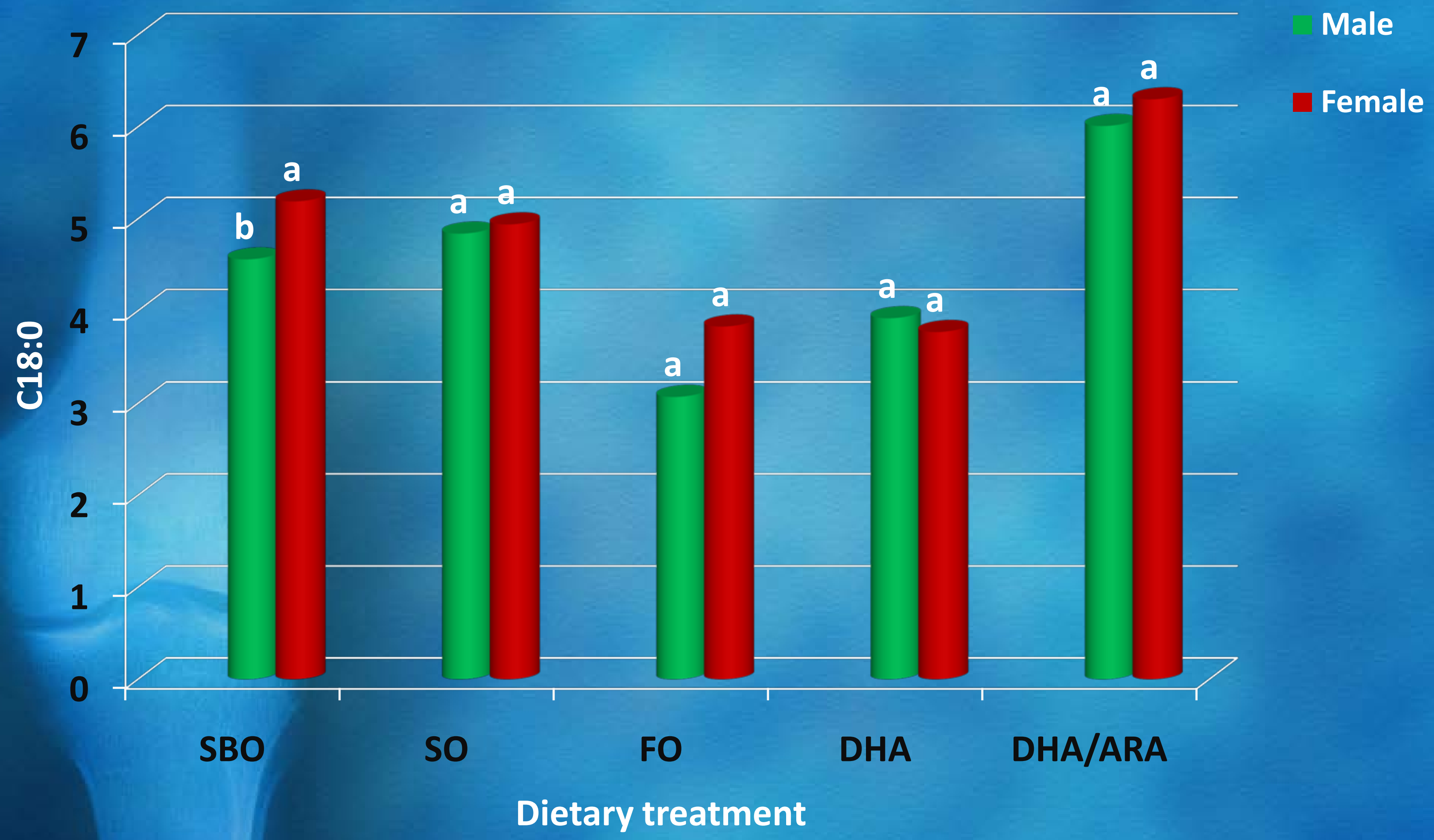
Comparison of C14:0 concentration between male & female rabbits



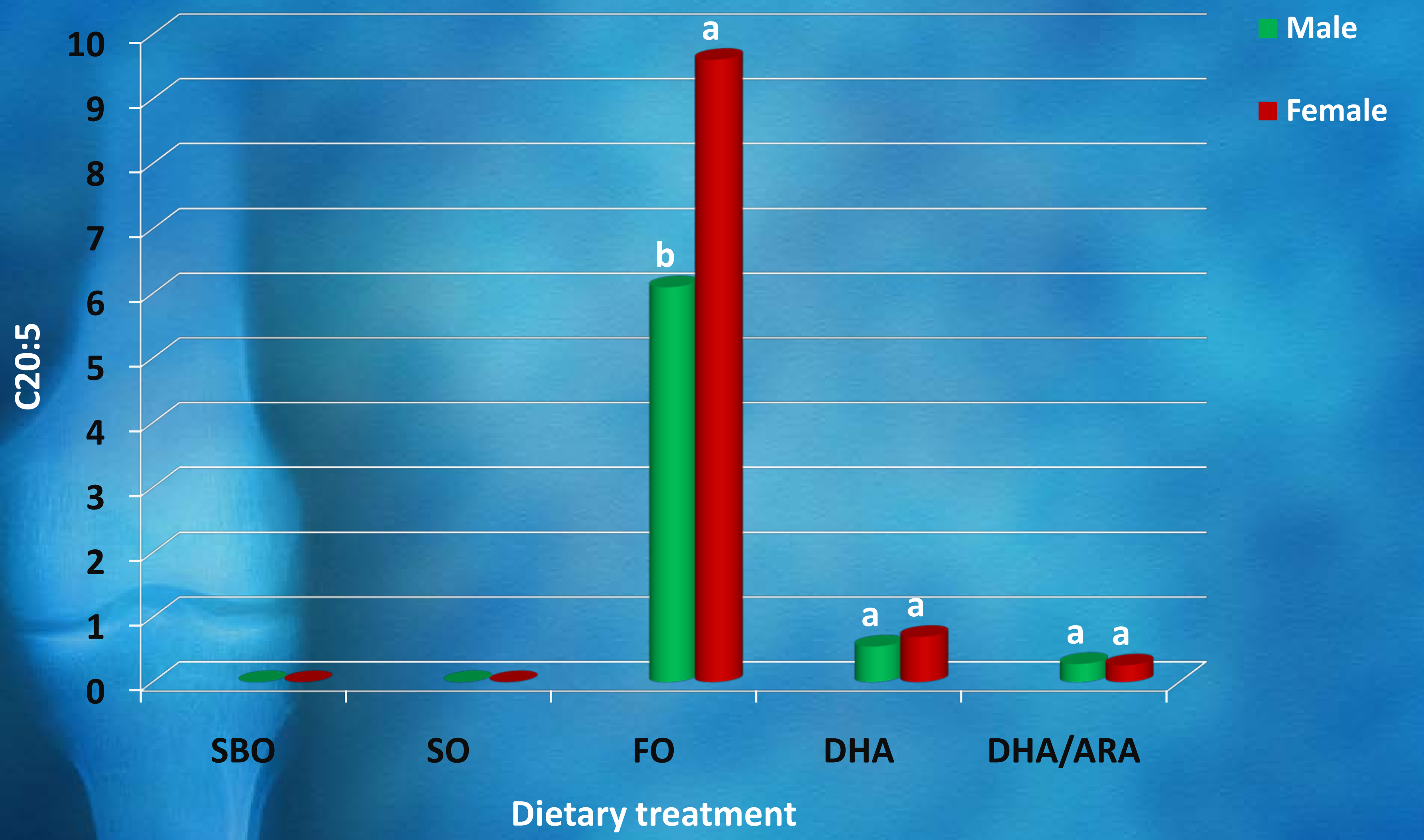
Comparison of C16:0 concentration between male & female rabbits



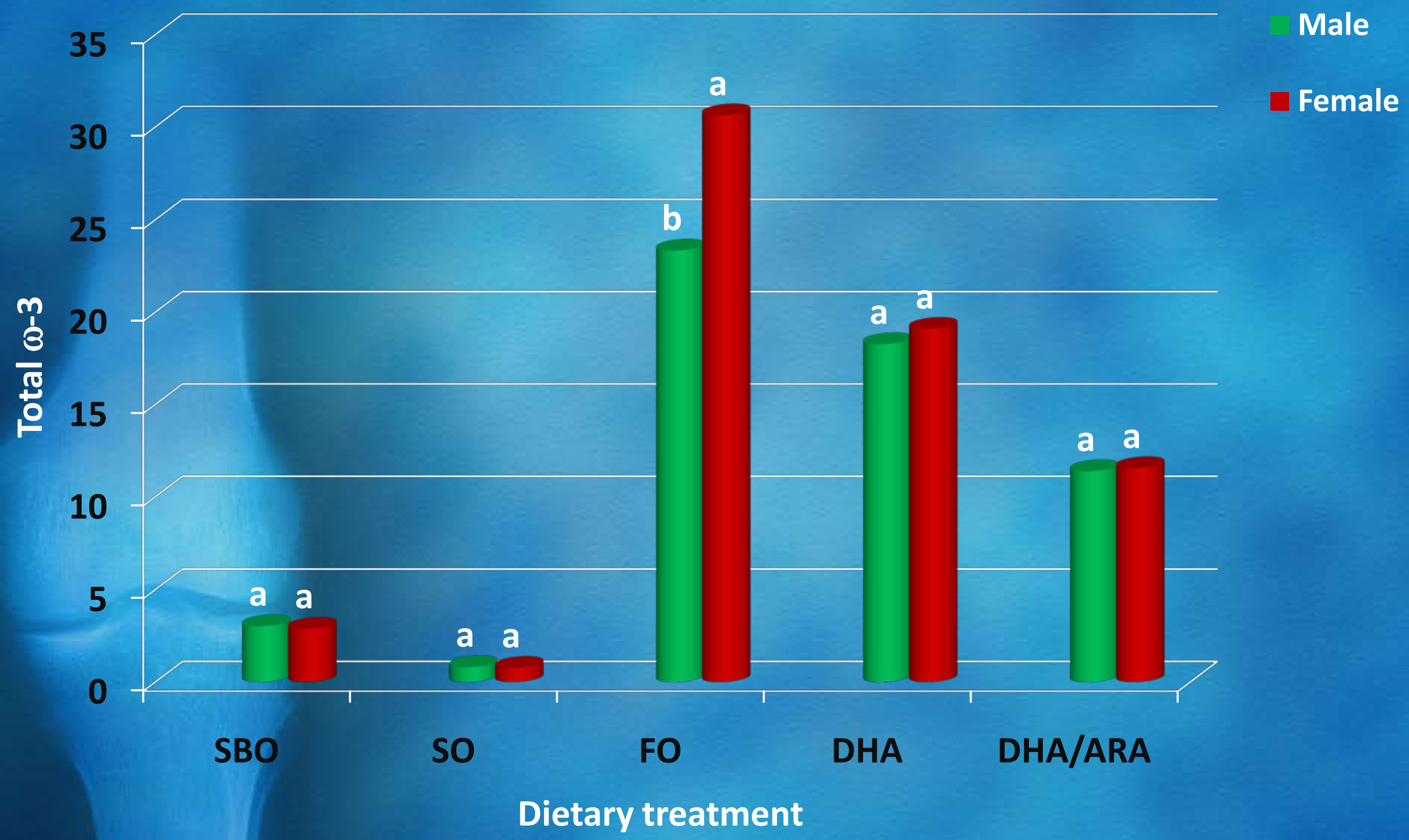
Comparison of C18:0 concentration between male & female rabbits



Comparison of C20:5 concentration between male & female rabbits



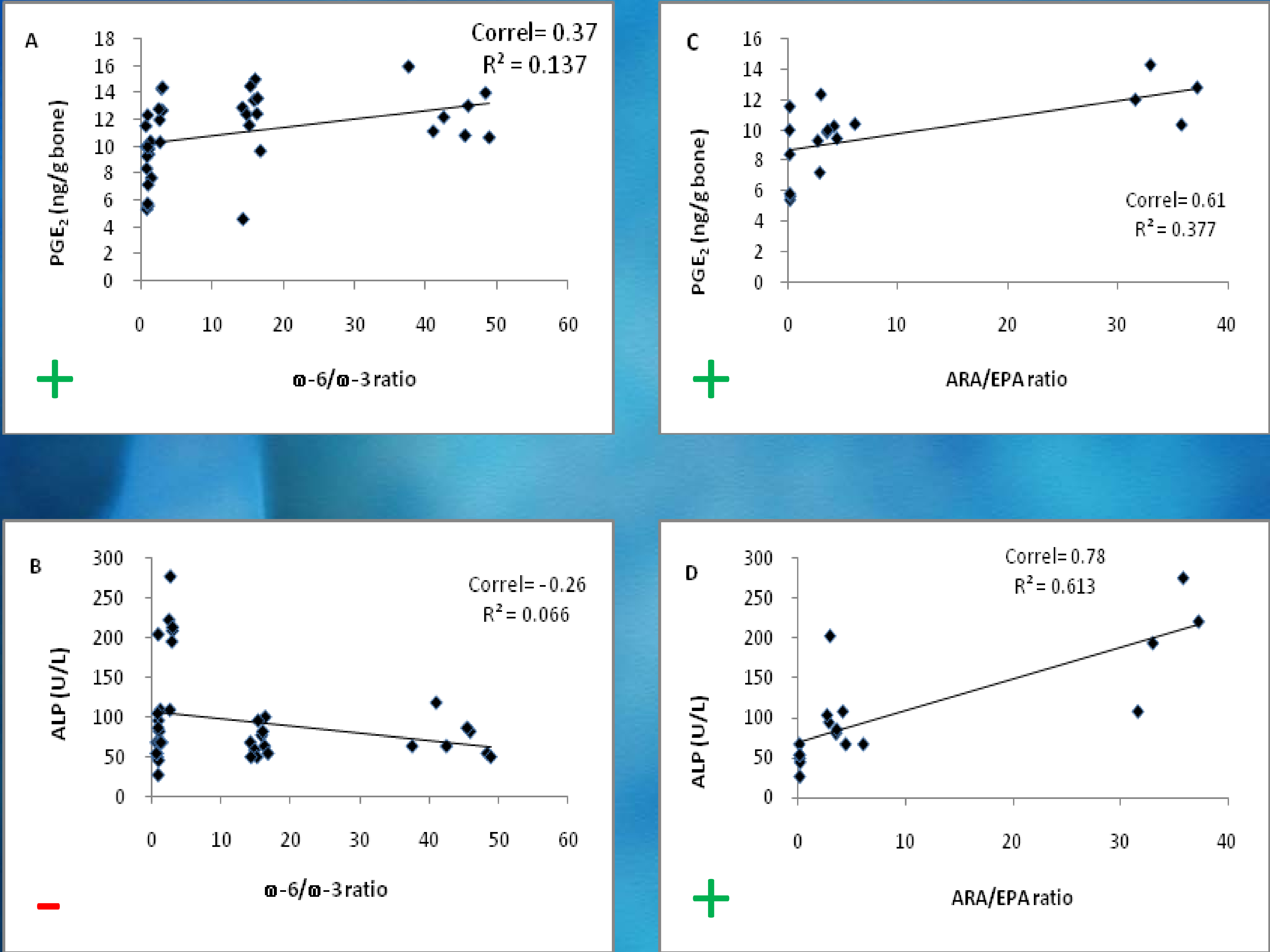
Comparison of total ω-3 concentration between male & female rabbits

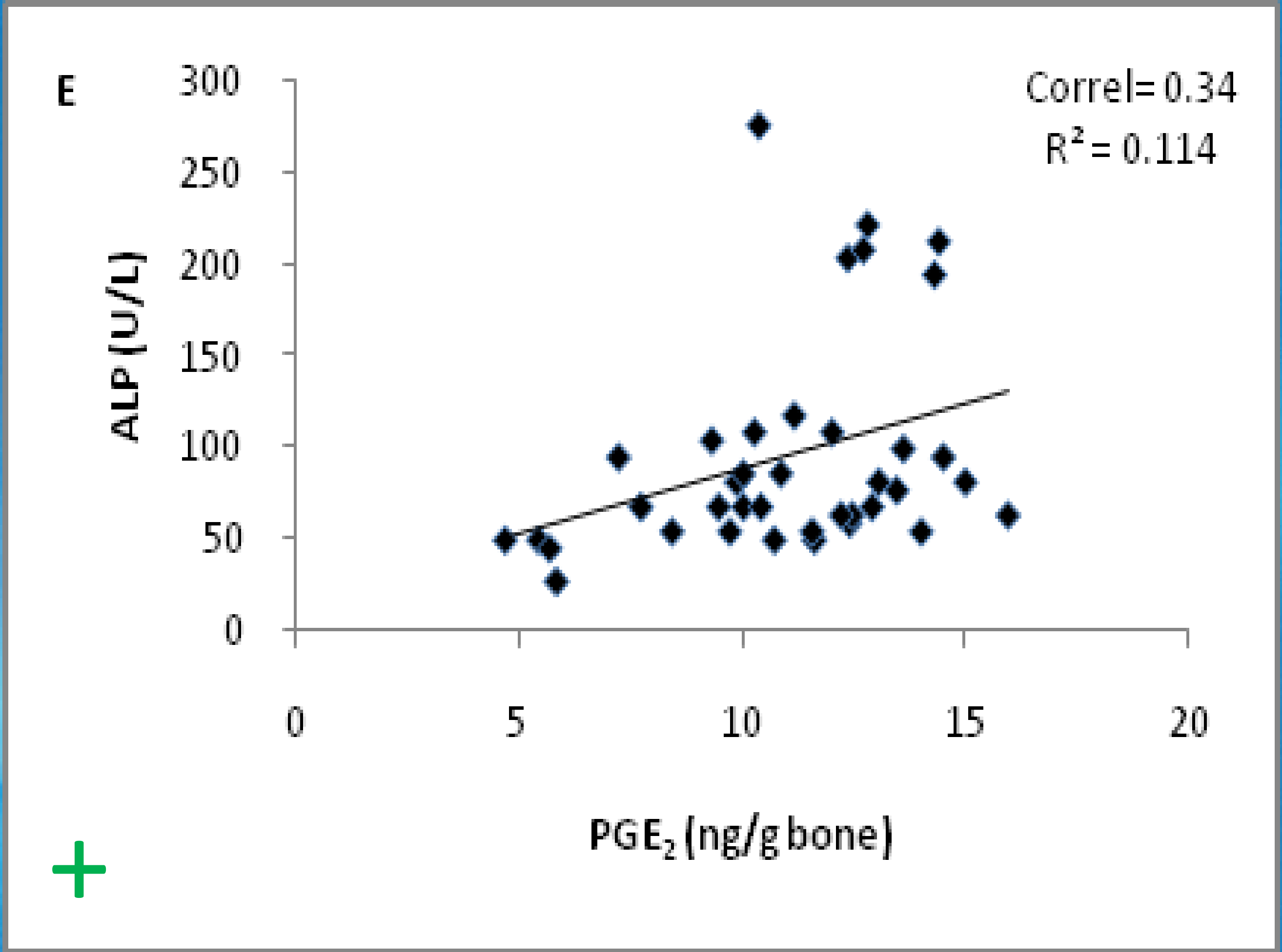


Plasma enzymes level & *ex vivo* PGE_2 release from tibia of male & female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days

	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
ALP (U/L)	63.47 ^{Ab} ±4.53	76.16 ^{Abc} ±10.28	74.80 ^{Ab} ±14.51	72.53 ^{Abc} ±11.41	51.68 ^{Ab} ±7.67	54.40 ^{Ac} ±0.00	84.32 ^{Ab} ±7.93	131.47 ^{Ab} ±36.64	**158.67 ^{Aa} ±49.87**	**226.67 ^{Aa} ±17.56**
ACP (U/L)	25.25 ^{Aa} ±4.23	26.27 ^{Aa} ±1.39	32.42 ^{Aa} ±2.70	22.18 ^{Aa} ±3.55	29.00 ^{Aa} ±3.28	33.27 ^{Aa} ±0.86	30.71 ^{Aa} ±5.09	26.73 ^{Aa} ±3.17	38.39 ^{Aa} ±0.86	33.27 ^{Aa} ±6.13
PGE_2 (ng/g)	12.56 ^{Aa} ±0.31	11.49 ^{Aa} ±1.95	13.33 ^{Aa} ±1.05	11.53 ^{Aa} ±0.76	**6.91 ^{Ac} ±0.87**	9.98 ^{Aa} ±1.58	**9.43 ^{Ab} ±0.58**	10.55 ^{Aa} ±0.92	12.35 ^{Aa} ±0.35	12.96 ^{Aa} ±0.94

No differences between male & female rabbits in either ALP, ACP or PGE_2


A
Correl= 0.37
R^2 = 0.137
PGE_2 (ng/g bone)
ω-6/ω-3 ratio
C
Correl= 0.61
R^2 = 0.377
PGE_2 (ng/g bone)
ARA/EPA ratio
B
Correl= -0.26
R^2 = 0.066
ALP (U/L)
ω-6/ω-3 ratio
D
Correl= 0.78
R^2 = 0.613
ALP (U/L)
ARA/EPA ratio


E
Correl= 0.34
R² = 0.114
ALP (U/L)
300
250
200
150
100
50
0
0
5
10
15
20
PGE2 (ng/g bone)

Conclusion

- Growth indicators of male rabbits were affected by the dietary treatments

- Different dietary oil sources with varying ω-6/ω-3 ratios significantly altered the fatty acid profile of bone marrow

- As the concentration of EPA & DHA elevated, the concentration of ARA declined in bone marrow

Conclusion

- There was no effect of diet on ACP activity in both males & females
- There was a favorable effect of ARA/EPA ratio in ALP activity than the overall ω-6/ω-3 ratio
- The result of this study revealed a stimulating effect of PGE_2 on ALP activity

The Effect of Long-term Supplementation with Different Dietary ω-6/ω-3 Ratios on Minerals Content and *ex vivo* PGE_2 Release in Bone of Growing Rabbits

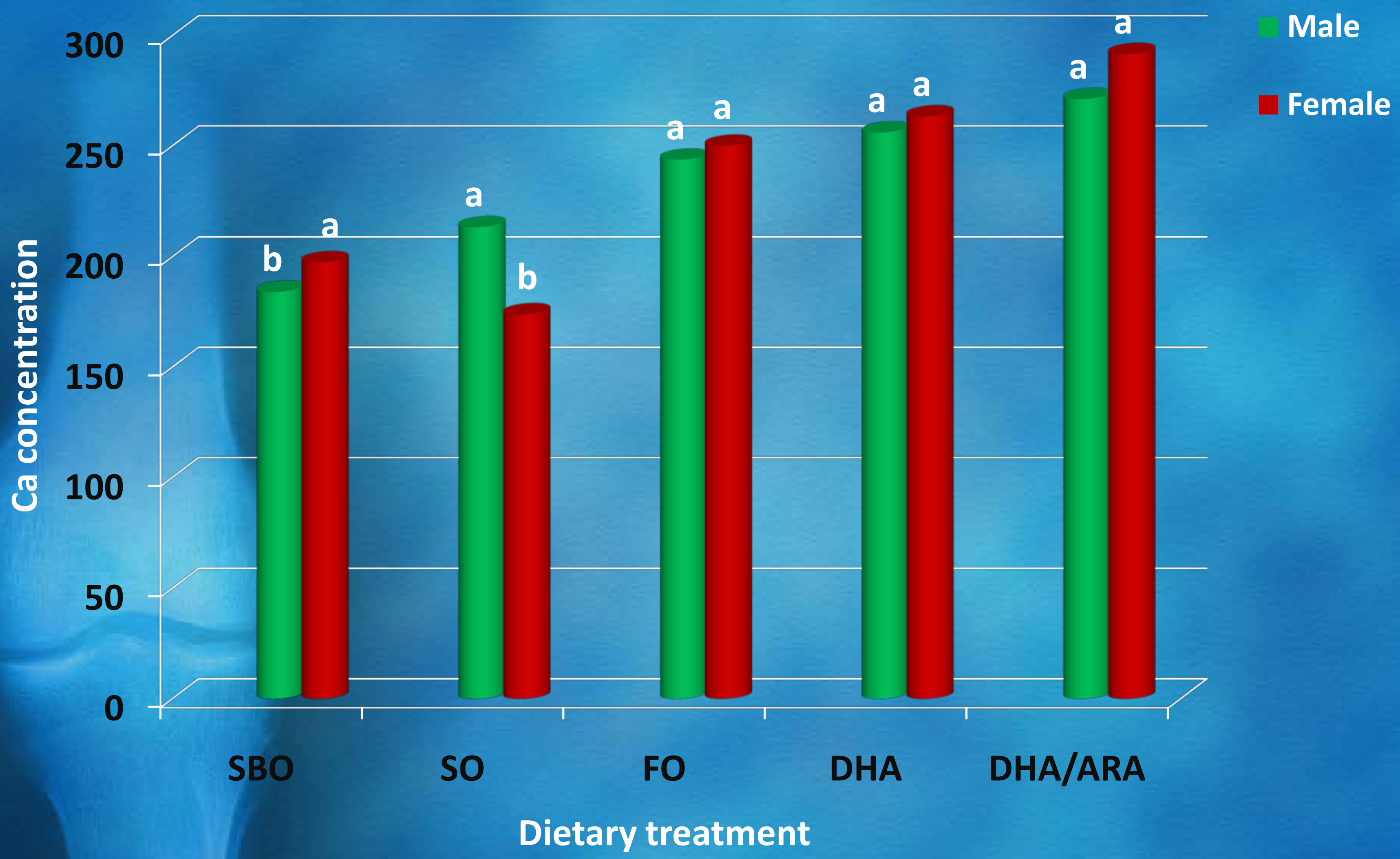
Al-Nouri, D. M., Al-Khalifa, A. S., and Shahidi, F.

Prepared in manuscript format

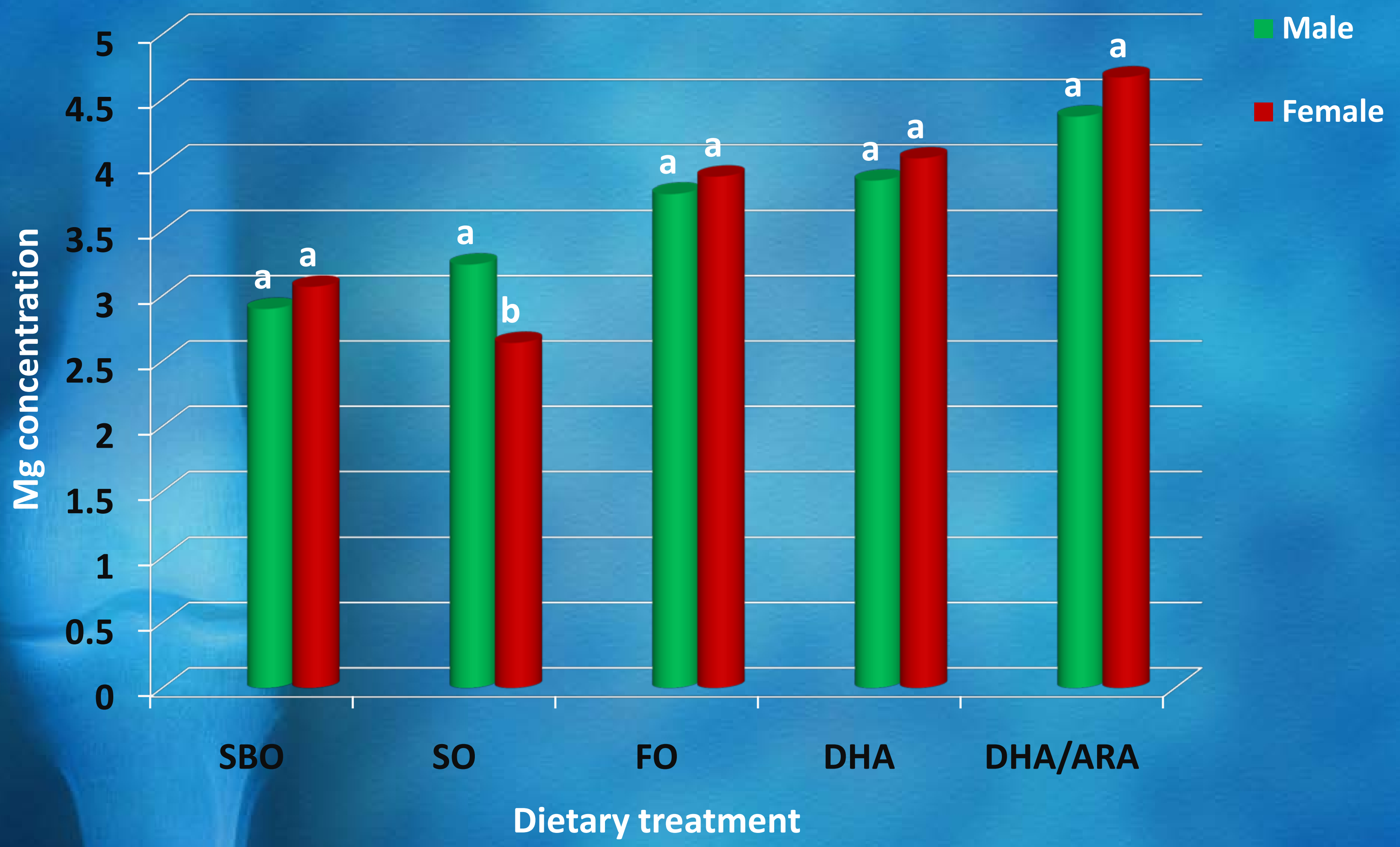
Minerals content & *ex vivo* PGE_2 level in bone of male & female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days

	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
Ca	184.15 Bb ±2.57	197.56 Ac ±2.18	213.45 Ab ±3.74	174.00 Bd ±4.50	243.79 Aa ±15.49	250.11 Ab ±1.01	256.14 Aa ±6.13	263.44 Ab ±2.52	271.27 Aa ±7.69	291.70 Aa ±6.55
P	114.79 Ab ±5.08	122.83 Ab ±1.11	132.16 Aa ±2.72	135.52 Aa ±3.40	136.32 Aa ±2.00	136.44 Aa ±0.12	131.94 Aa ±2.44	130.15 Aa ±1.29	134.21 Aa ±1.75	134.48 Aa ±2.66
Mg	2.90 Ac ±0.12	3.07 Ac ±0.04	3.24 Ac ±0.10	2.64 Bd ±0.04	3.78 Ab ±0.23	3.91 Ab ±0.19	3.88 Aab ±0.14	4.05 Ab ±0.04	4.37 Aa ±0.16	4.67 Aa ±0.11
Zn	0.08 Ab ±0.004	0.09 Aab ±0.004	0.10 Aa ±0.008	0.08 Bbc ±0.003	0.07 Ab ±0.005	0.07 Ac ±0.003	0.07 Ab ±0.004	0.08 Aabc ±0.01	0.10 Aa ±0.009	0.10 Aa ±0.002
PGE_2 (ng/g)	12.56 Aa ±0.31	11.49 Aa ±1.95	13.33 Aa ±1.05	11.53 Aa ±0.76	6.91 Ac ±0.87	9.98 Aa ±1.58	9.43 Ab ±0.58	10.55 Aa ±0.92	12.35 Aa ±0.35	12.96 Aa ±0.94

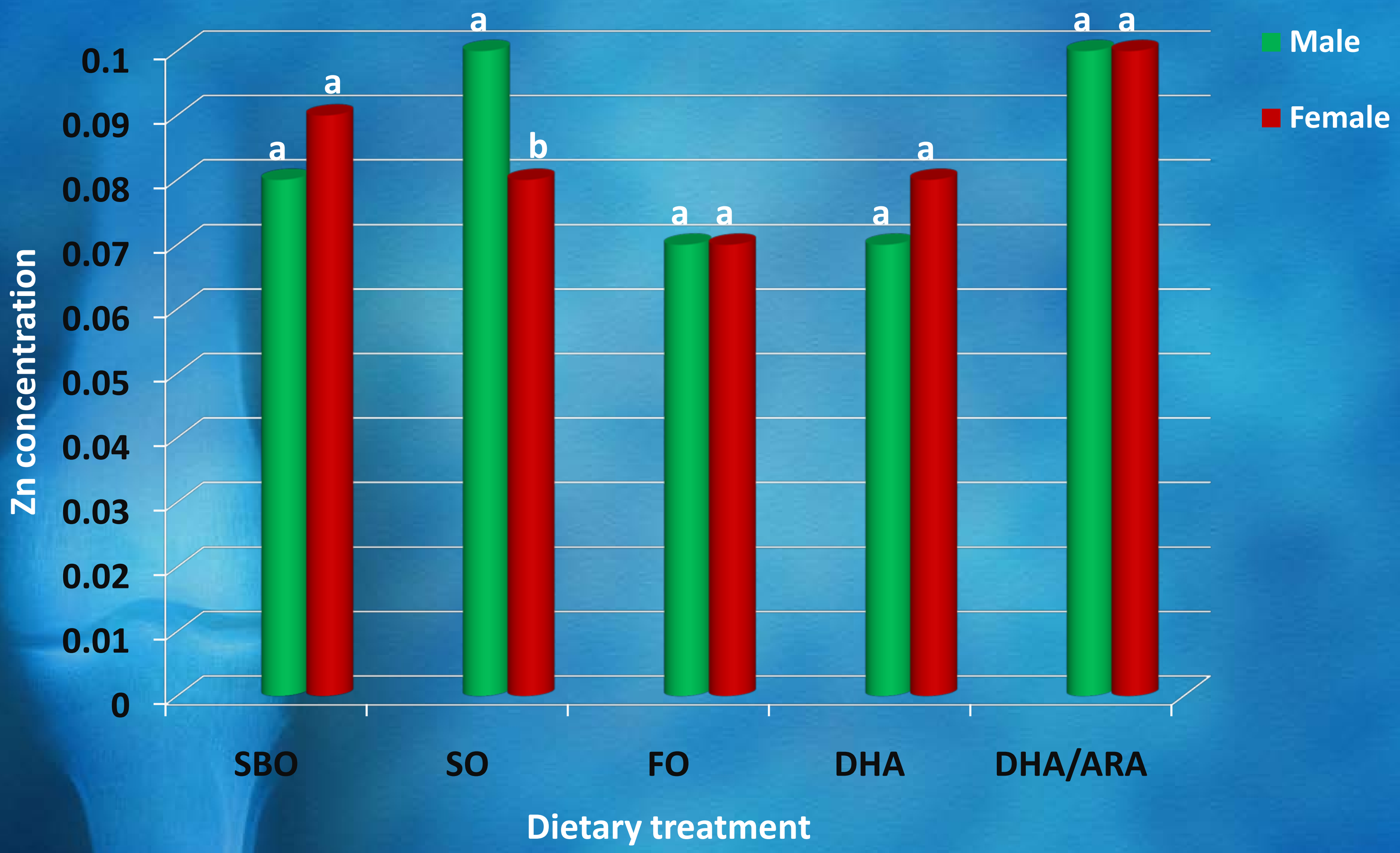
Comparison of Ca concentration between male & female rabbits



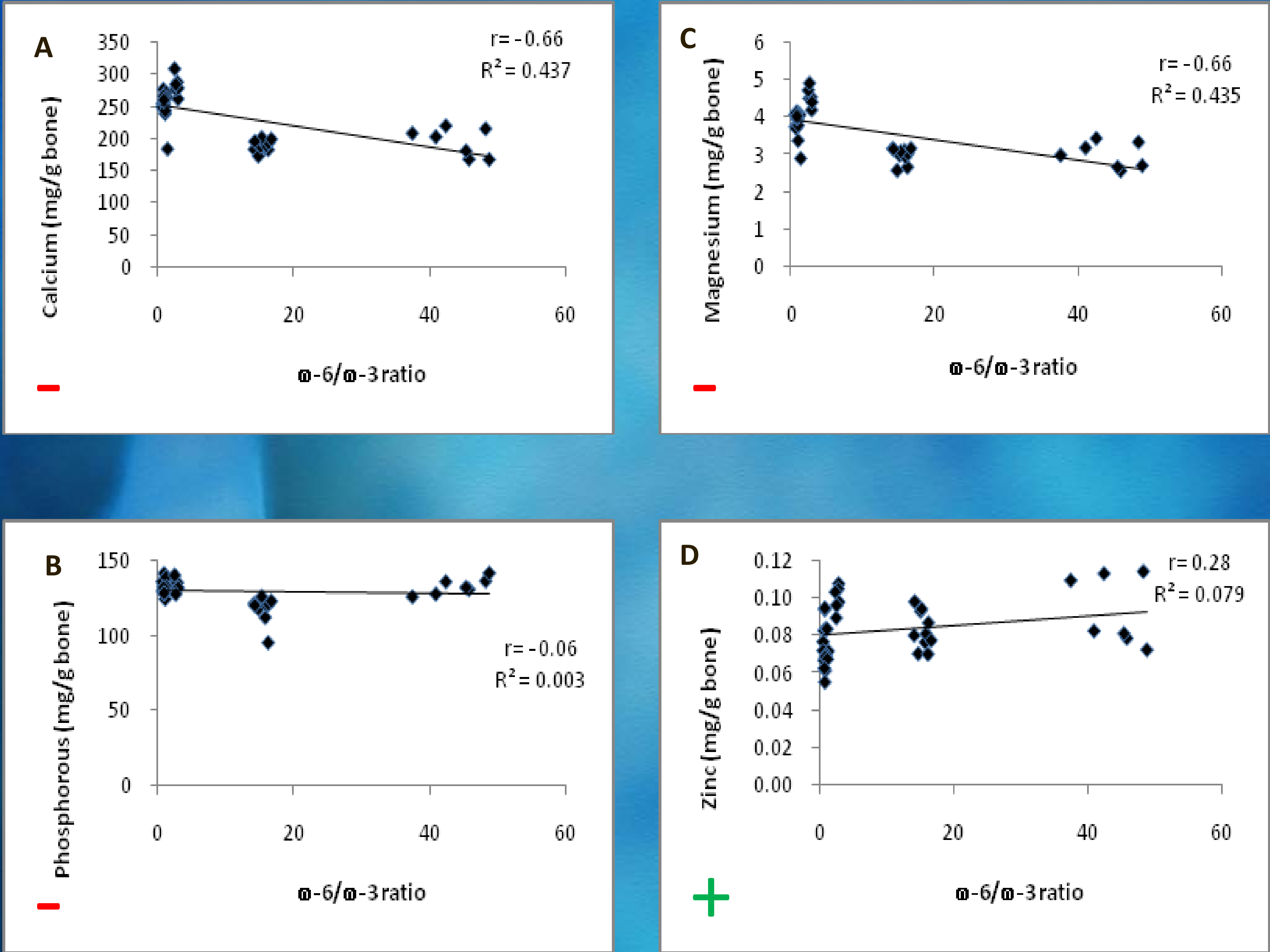
Comparison of Mg concentration between male & female rabbits

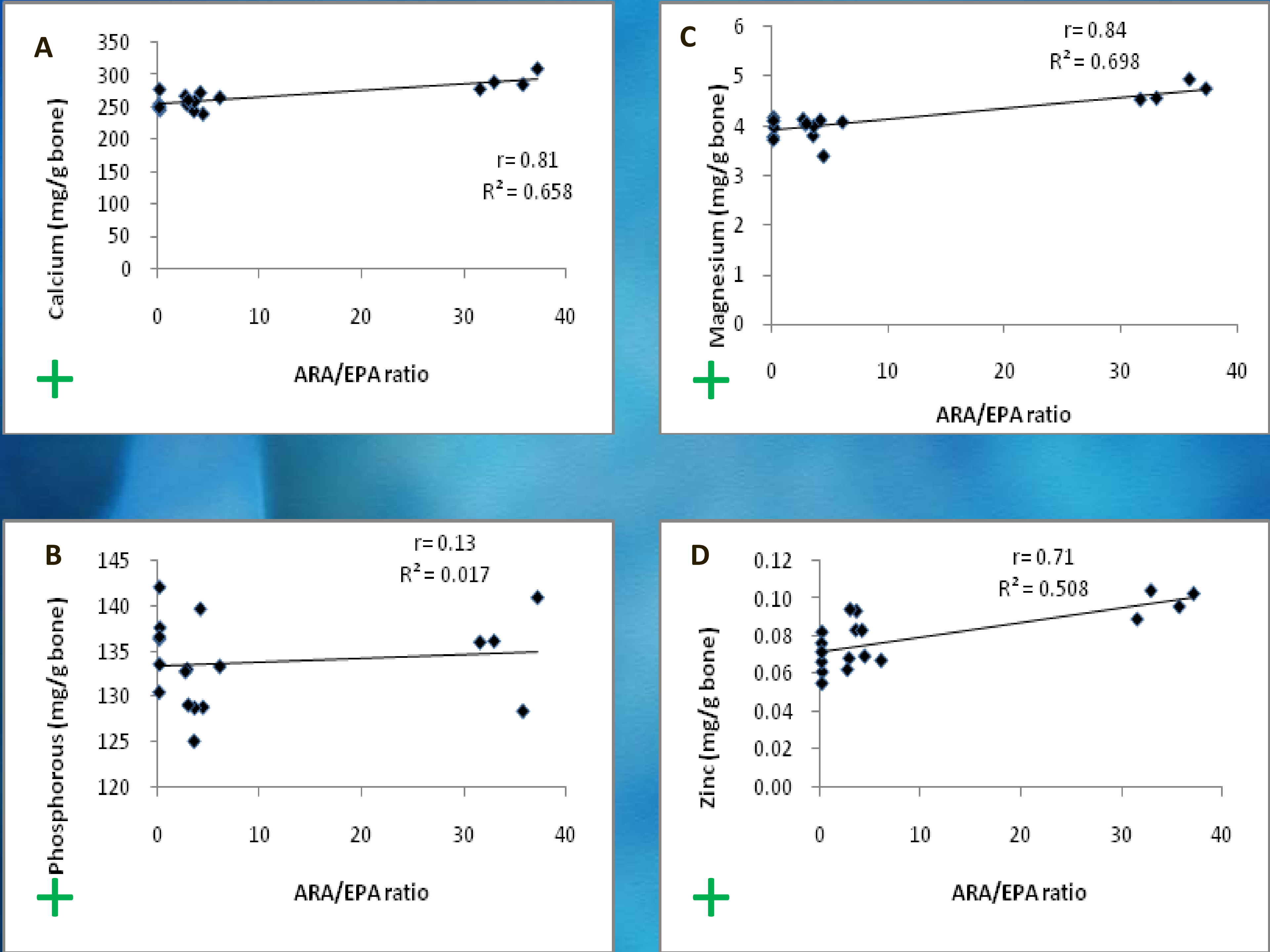


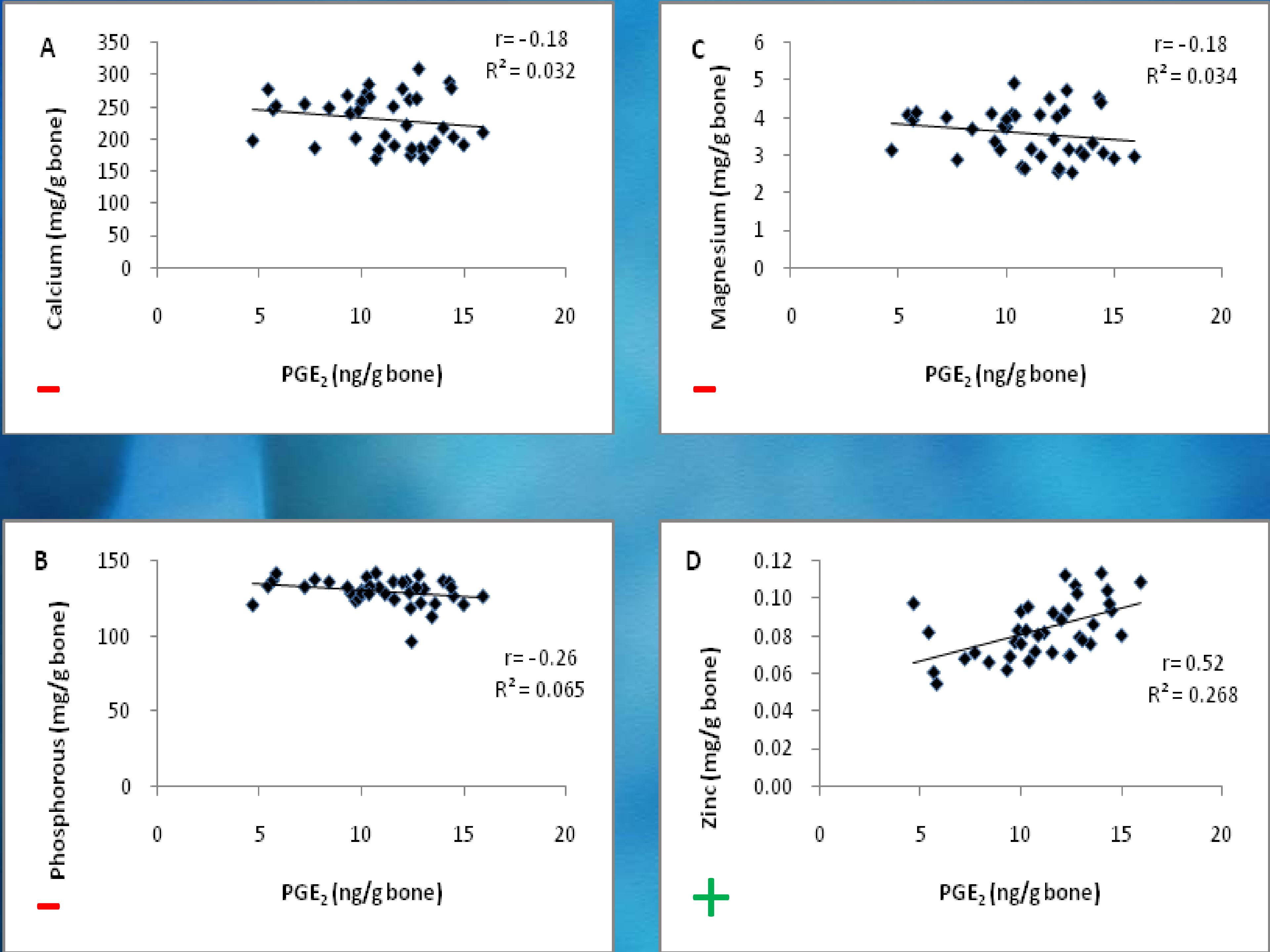
Comparison of Zn concentration between male & female rabbits



No differences between male & female rabbits in P


A
r= -0.66
R² = 0.437
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0
20
40
60
ω-6/ω-3 ratio
C
r= -0.66
R² = 0.435
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0
20
40
60
ω-6/ω-3 ratio
B
r= -0.06
R² = 0.003
Phosphorous (mg/g bone)
150
100
50
0
0
20
40
60
ω-6/ω-3 ratio
D
r= 0.28
R² = 0.079
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
ω-6/ω-3 ratio


A
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0
10
20
30
40
ARA/EPA ratio
r= 0.81
R² = 0.658
C
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0
10
20
30
40
ARA/EPA ratio
r= 0.84
R² = 0.698
B
Phosphorous (mg/g bone)
145
140
135
130
125
120
0
10
20
30
40
ARA/EPA ratio
r= 0.13
R² = 0.017
D
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
ARA/EPA ratio
r= 0.71
R² = 0.508


A
r= -0.18
R^2 = 0.032
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0
5
10
15
20
PGE_2 (ng/g bone)
C
r= -0.18
R^2 = 0.034
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0
5
10
15
20
PGE_2 (ng/g bone)
B
r= -0.26
R^2 = 0.065
Phosphorous (mg/g bone)
150
100
50
0
0
5
10
15
20
PGE_2 (ng/g bone)
D
r= 0.52
R^2 = 0.268
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
5
10
15
20
PGE_2 (ng/g bone)

Conclusion

- The higher concentration of ω-3 PUFA found in bone marrow was associated with reduced *ex vivo* PGE_2 release in bone

- The reduction in ω-6/ω-3 ratio through the inclusion of ω-3 LCPUFA in the diet from either fish oil or marine algae oils has been shown to increase bone Ca, P & Mg contents

Conclusion

- The reduction in ω-6/ω-3 ratio through the inclusion of ω-3 LCPUFA in the diet from marine algae oils has been shown to maintain optimal Ca/P ratio in the bone

- There was a favorable effect of ARA/EPA ratio in bone Ca, P, & Mg contents than the overall ω-6/ω-3 ratio

The Effect of Bone Marrow ARA, EPA, and DHA on Femur Minerals Content and Biomarkers of Bone Metabolism in Growing Rabbits

Al-Nouri, D. M., Al-Khalifa, A. S., and Shahidi, F.

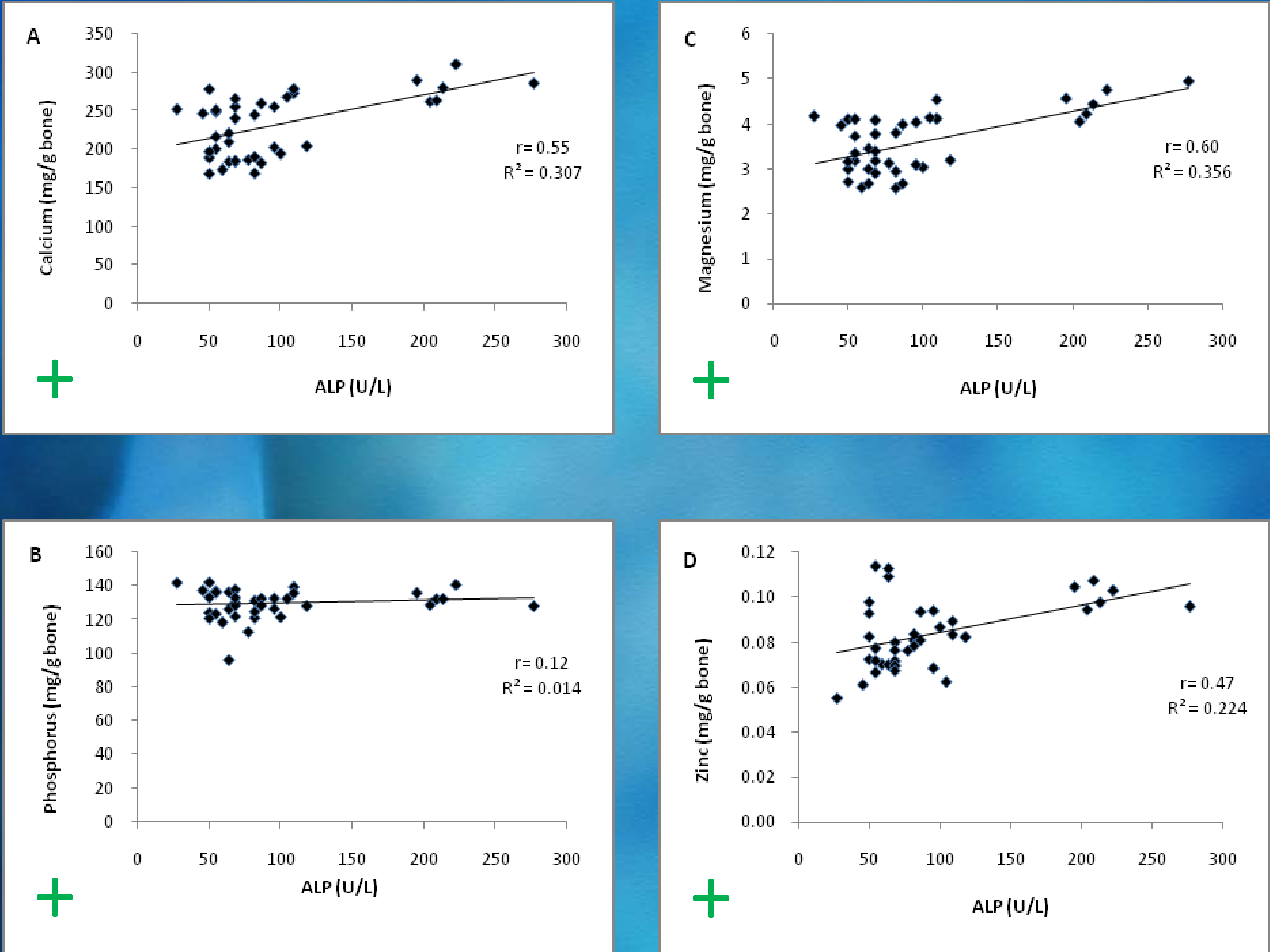
Prepared in manuscript format

Bone marrow fatty acids of male & female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days

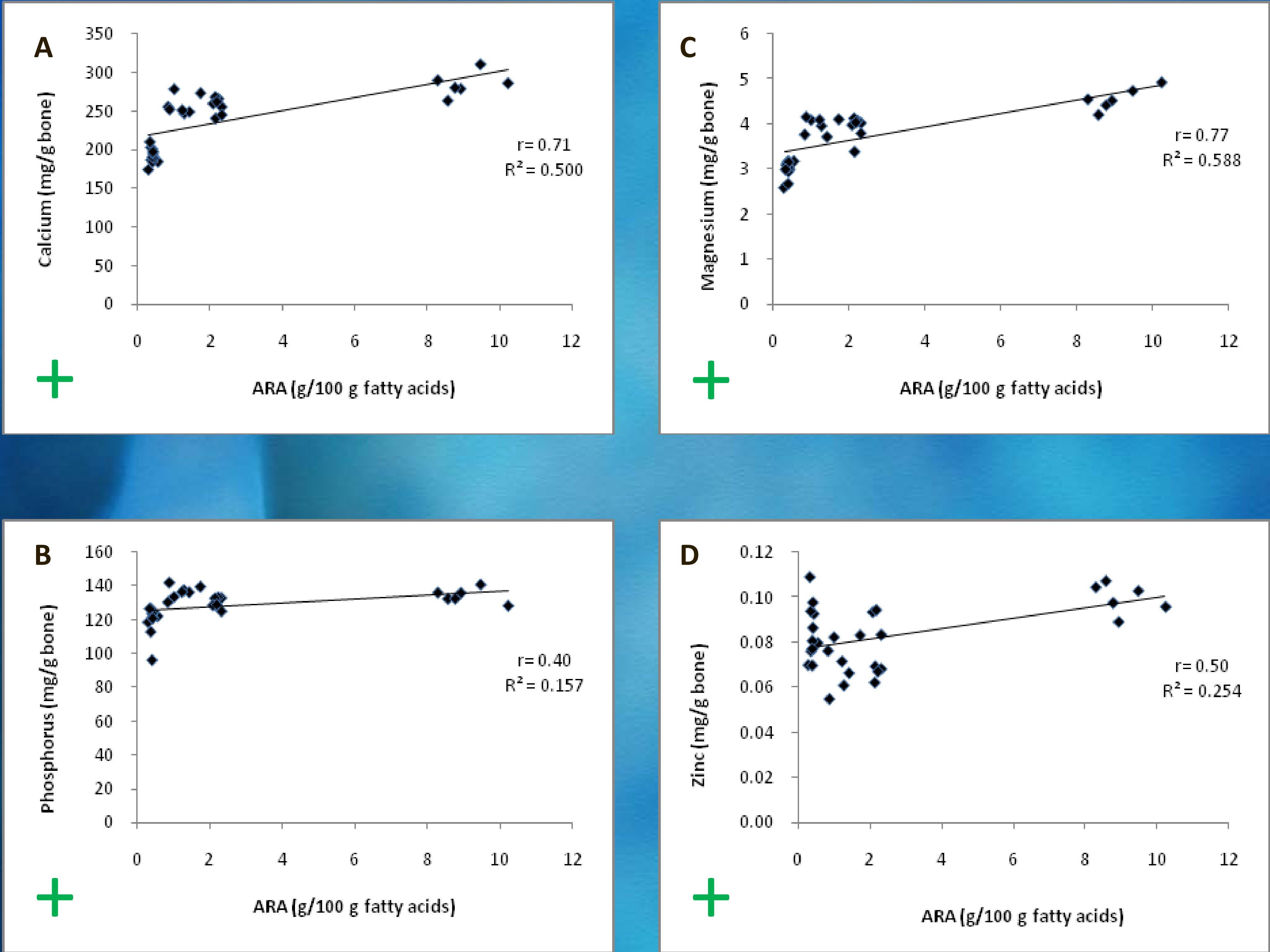
Fatty acid	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
C18:2 ω-6	**45.63^{Aa}**	**44.77^{Aa}**	34.07^{Ab}	36.19^{Ab}	20.48^{Ac}	19.79^{Ac}	17.45^{Ac}	13.96^{Ad}	20.93^{Ac}	20.69^{Ac}
C20:4 ω-6	**0.41^{Ad}**	**0.40^{Acd}**	**0.17^{Ad}**	**Tr^{Ad}**	1.00^{Ac}	1.33^{Abc}	2.15^{Ab}	2.13^{Ab}	**8.74^{Aa}**	**9.19^{Aa}**
C18:3 ω-3	**3.03^{Aa}**	**2.89^{Aa}**	0.81^{Ac}	0.77^{Ad}	1.27^{Ab}	1.48^{Ab}	0.80^{Ac}	0.75^{Ad}	1.12^{Abc}	1.08^{Ac}
C20:5 ω-3	-	-	-	-	**6.10^{Ba}**	**9.61^{Aa}**	0.55^{Ab}	0.70^{Ab}	0.28^{Ab}	0.26^{Ab}
C22:6 ω-3	-	-	-	-	**15.97^{Aa}**	**19.55^{Aa}**	**16.92^{Aa}**	17.64^{Ab}	10.13^{Ab}	10.28^{Ac}
LA/ALA	15.07^{Ac}	15.53^{Ac}	**42.25^{Aa}**	**46.74^{Aa}**	16.00^{Ac}	13.44^{Ad}	22.27^{Ab}	18.62^{Ab}	18.65^{Abc}	19.12^{Ab}
LA+ARA/ ALA+DHA	15.20^{Ab}	15.67^{Ab}	**42.34^{Aa}**	**46.74^{Aa}**	1.24^{Ac}	1.00^{Acd}	1.12^{Ac}	0.87^{Bd}	2.64^{Ac}	2.64^{Ac}
ARA/EPA	-	-	-	-	0.13^{Ac}	0.14^{Ab}	4.21^{Ab}	3.08^{Ab}	**31.62^{Aa}**	35.36^{Aa}
ARA/DHA	-	-	-	-	0.05^{Ac}	0.07^{Ab}	0.13^{Ab}	0.12^{Ab}	**0.86^{Aa}**	0.89^{Aa}
ARA/EPA +DHA	-	-	-	-	0.03^{Ac}	0.05^{Ac}	0.12^{Ab}	0.12^{Ab}	**0.85^{Aa}**	0.88^{Aa}

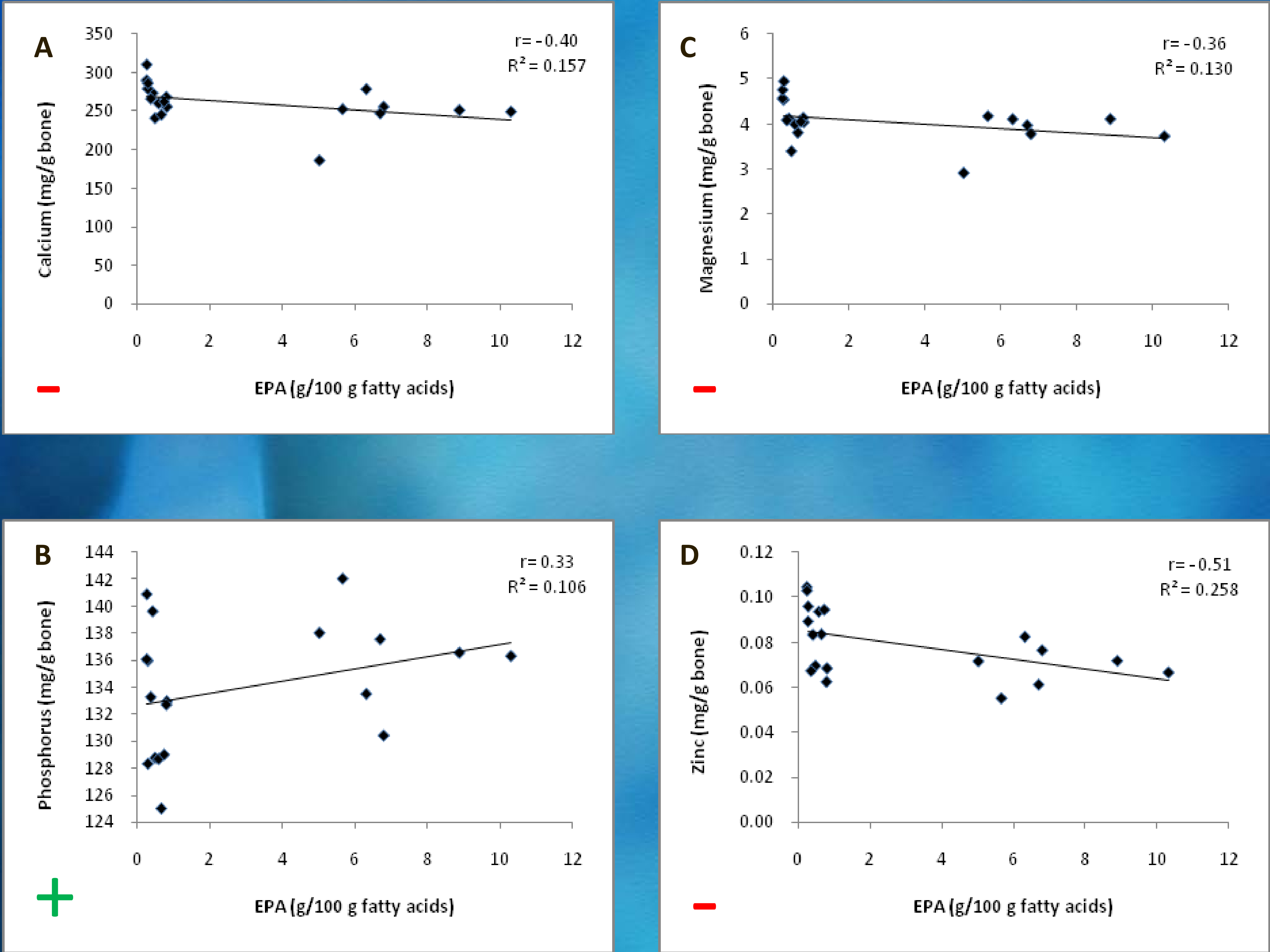
Plasma ALP & ACP activity & femur minerals content of male & female rabbits fed diets with different dietary oil sources & varying ω-6/ω-3 ratios for 100 days

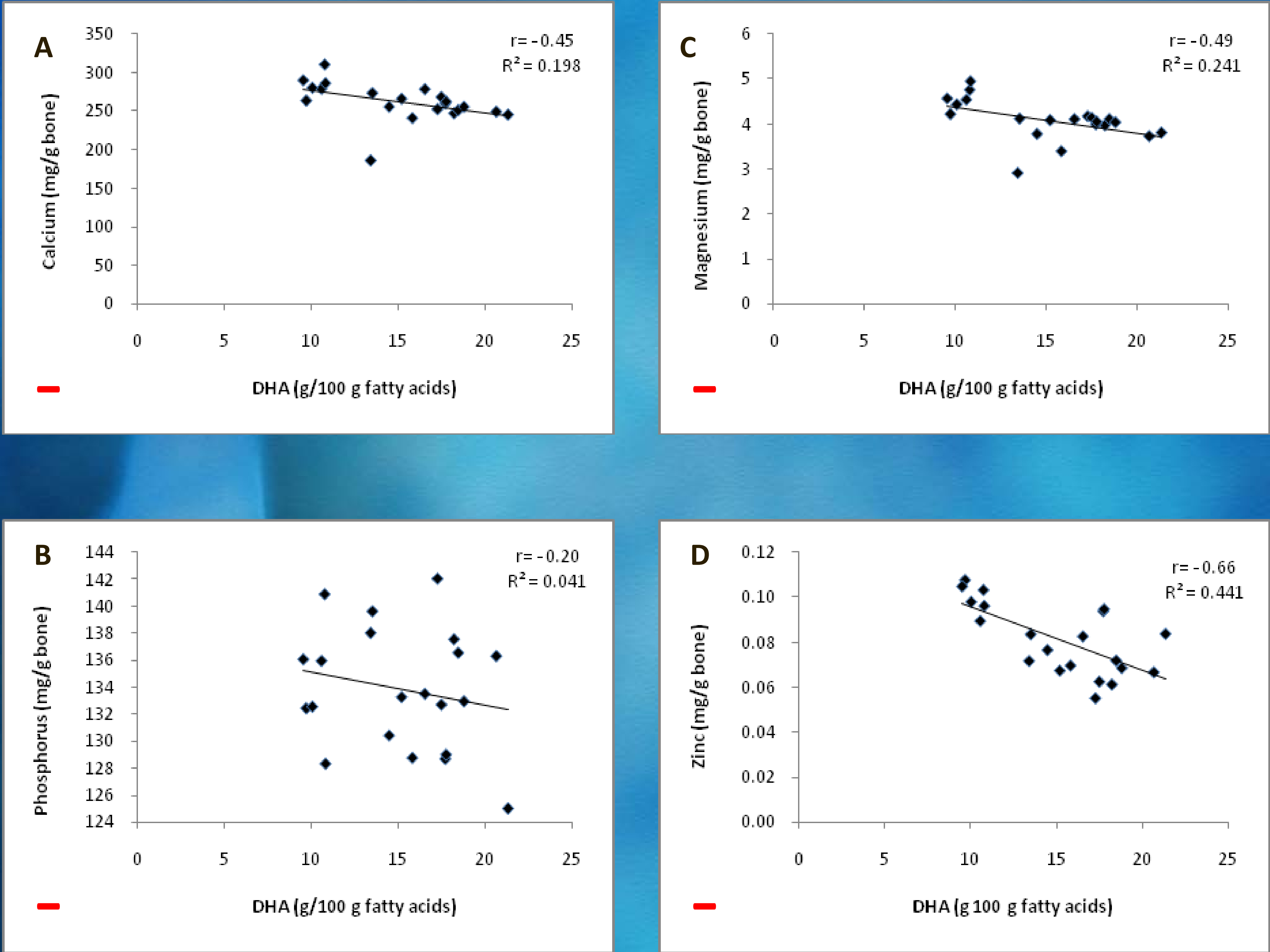
	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
ALP (U/L)	63.47 ^{Ab} ±4.53	76.16 ^{Abc} ±10.28	74.80 ^{Ab} ±14.51	72.53 ^{Abc} ±11.41	51.68 ^{Ab} ±7.67	54.40 ^{Ac} ±0.00	84.32 ^{Ab} ±7.93	131.47 ^{Ab} ±36.64	158.67 ^{Aa} ±49.87	226.67 ^{Aa} ±17.56
ACP (U/L)	25.25 ^{Aa} ±4.23	26.27 ^{Aa} ±1.39	32.42 ^{Aa} ±2.70	22.18 ^{Aa} ±3.55	29.00 ^{Aa} ±3.28	33.27 ^{Aa} ±0.86	30.71 ^{Aa} ±5.09	26.73 ^{Aa} ±3.17	38.39 ^{Aa} ±0.86	33.27 ^{Aa} ±6.13
Ca	184.15 ^{Bb} ±2.57	197.56 ^{Ac} ±2.18	213.45 ^{Ab} ±3.74	174.00 ^{Bd} ±4.50	243.79 ^{Aa} ±15.49	250.11 ^{Ab} ±1.01	256.14 ^{Aa} ±6.13	263.44 ^{Ab} ±2.52	271.27 ^{Aa} ±7.69	291.70 ^{Aa} ±6.55
P	114.79 ^{Ab} ±5.08	122.83 ^{Ab} ±1.11	132.16 ^{Aa} ±2.72	135.52 ^{Aa} ±3.40	136.32 ^{Aa} ±2.00	136.44 ^{Aa} ±0.12	131.94 ^{Aa} ±2.44	130.15 ^{Aa} ±1.29	134.21 ^{Aa} ±1.75	134.48 ^{Aa} ±2.66
Mg	2.90 ^{Ac} ±0.12	3.07 ^{Ac} ±0.04	3.24 ^{Ac} ±0.10	2.64 ^{Bd} ±0.04	3.78 ^{Ab} ±0.23	3.91 ^{Ab} ±0.19	3.88 ^{Aab} ±0.14	4.05 ^{Ab} ±0.04	4.37 ^{Aa} ±0.16	4.67 ^{Aa} ±0.11
Zn	0.08 ^{Ab} ±0.004	0.09 ^{Aab} ±0.004	0.10 ^{Aa} ±0.008	0.08 ^{Bbc} ±0.003	0.07 ^{Ab} ±0.005	0.07 ^{Ac} ±0.003	0.07 ^{Ab} ±0.004	0.08 ^{Aabc} ±0.01	0.10 ^{Aa} ±0.009	0.10 ^{Aa} ±0.002

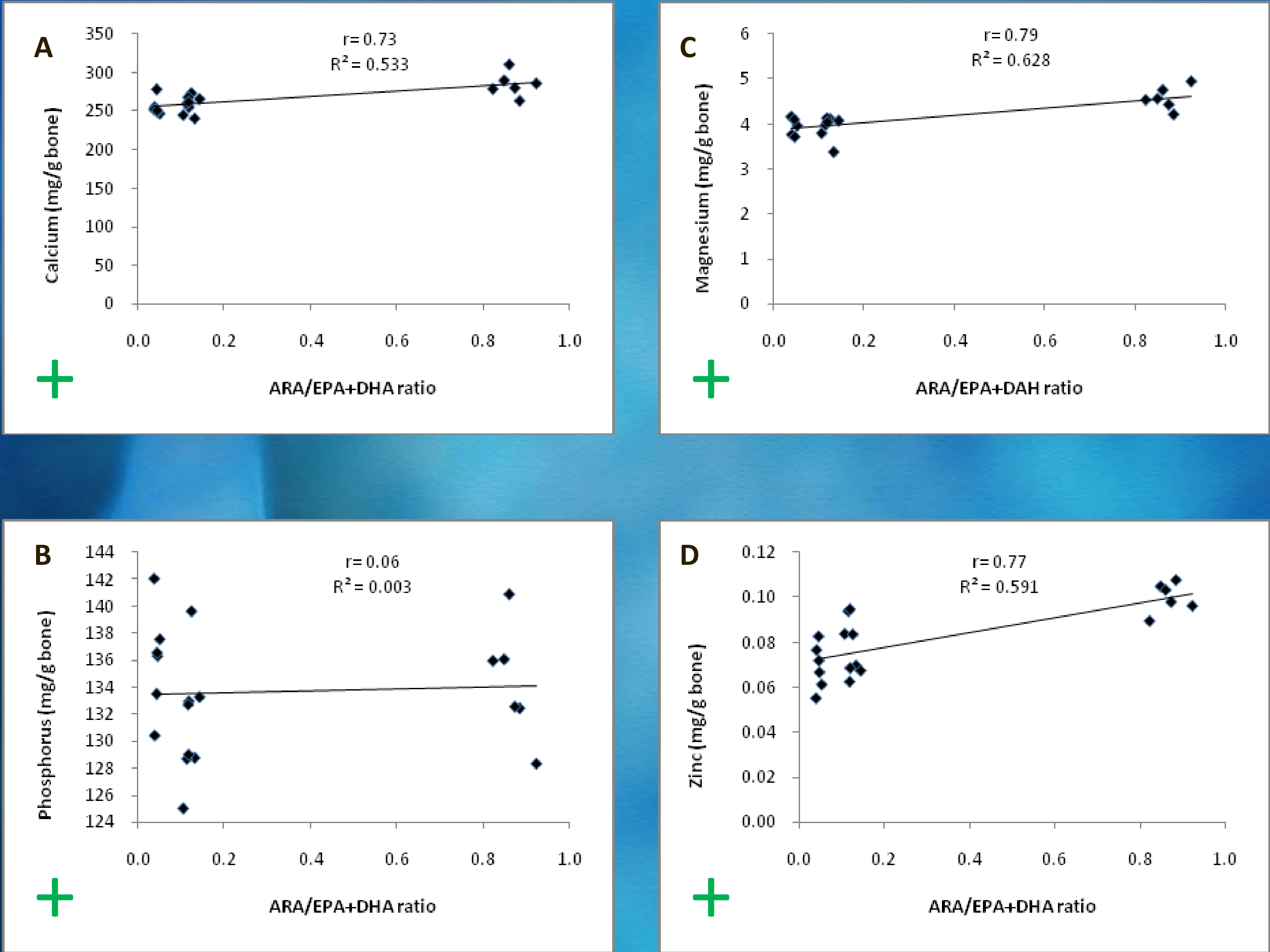


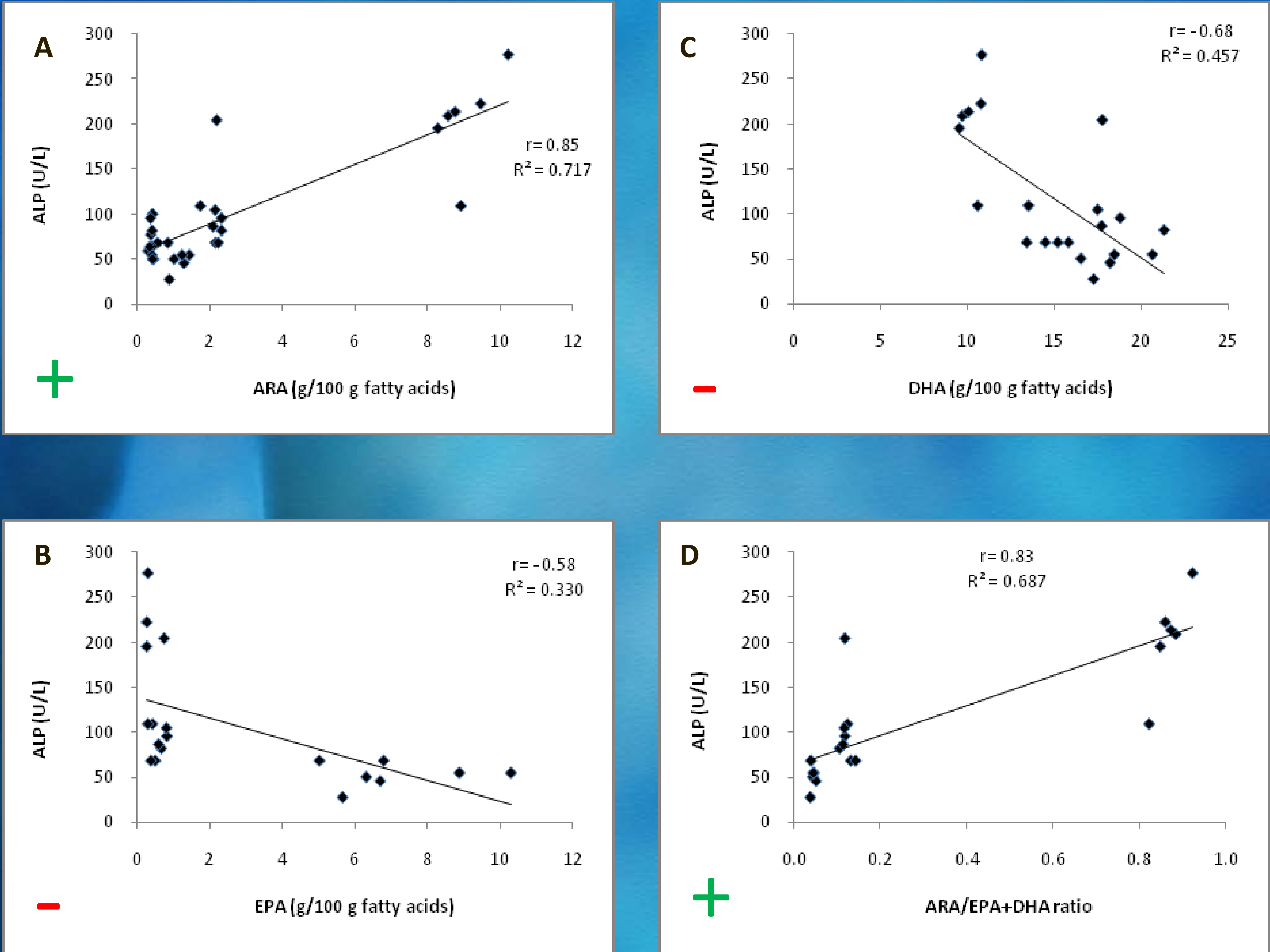
A
Calcium (mg/g bone)
ALP (U/L)
r= 0.55
R² = 0.307
C
Magnesium (mg/g bone)
ALP (U/L)
r= 0.60
R² = 0.356
B
Phosphorus (mg/g bone)
ALP (U/L)
r= 0.12
R² = 0.014
D
Zinc (mg/g bone)
ALP (U/L)
r= 0.47
R² = 0.224


A
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
ARA (g/100 g fatty acids)
r= 0.71
R² = 0.500
C
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0
2
4
6
8
10
12
ARA (g/100 g fatty acids)
r= 0.77
R² = 0.588
B
Phosphorus (mg/g bone)
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
ARA (g/100 g fatty acids)
r= 0.40
R² = 0.157
D
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
12
ARA (g/100 g fatty acids)
r= 0.50
R² = 0.254


A
r= -0.40
R² = 0.157
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12
EPA (g/100 g fatty acids)
C
r= -0.36
R² = 0.130
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0 2 4 6 8 10 12
EPA (g/100 g fatty acids)
B
r= 0.33
R² = 0.106
Phosphorus (mg/g bone)
144
142
140
138
136
134
132
130
128
126
124
0 2 4 6 8 10 12
EPA (g/100 g fatty acids)
D
r= -0.51
R² = 0.258
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0 2 4 6 8 10 12
EPA (g/100 g fatty acids)


A
r= -0.45
R² = 0.198
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0
5
10
15
20
25
DHA (g/100 g fatty acids)
C
r= -0.49
R² = 0.241
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0
5
10
15
20
25
DHA (g/100 g fatty acids)
B
r= -0.20
R² = 0.041
Phosphorus (mg/g bone)
144
142
140
138
136
134
132
130
128
126
124
0
5
10
15
20
25
DHA (g/100 g fatty acids)
D
r= -0.66
R² = 0.441
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
5
10
15
20
25
DHA (g 100 g fatty acids)


A
r= 0.73
R² = 0.533
Calcium (mg/g bone)
350
300
250
200
150
100
50
0
0.0
0.2
0.4
0.6
0.8
1.0
ARA/EPA+DHA ratio
C
r= 0.79
R² = 0.628
Magnesium (mg/g bone)
6
5
4
3
2
1
0
0.0
0.2
0.4
0.6
0.8
1.0
ARA/EPA+DAH ratio
B
r= 0.06
R² = 0.003
Phosphorus (mg/g bone)
144
142
140
138
136
134
132
130
128
126
124
0.0
0.2
0.4
0.6
0.8
1.0
ARA/EPA+DHA ratio
D
r= 0.77
R² = 0.591
Zinc (mg/g bone)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.0
0.2
0.4
0.6
0.8
1.0
ARA/EPA+DHA ratio


A
r= 0.85
R² = 0.717
ALP (U/L)
ARA (g/100 g fatty acids)
+
C
r= -0.68
R² = 0.457
ALP (U/L)
DHA (g/100 g fatty acids)
−
B
r= -0.58
R² = 0.330
ALP (U/L)
EPA (g/100 g fatty acids)
−
D
r= 0.83
R² = 0.687
ALP (U/L)
ARA/EPA+DHA ratio
+

Conclusion

- Absence of EPA & DHA & depletion of ARA level in bone marrow of rabbits fed SBO or SO diets

- High dietary ω-6/ω-3 ratio had significantly lower bone Ca, P, & Mg contents

Conclusion

- Bone marrow ARA concentration was positively correlated with ALP activity & bone Ca, P, Mg, & Zn contents

- Bone marrow EPA & DHA concentrations were negatively correlated with ALP activity & bone Ca, Mg, & Zn contents

- ARA/EPA+DHA ratio completely eliminates the undesirable effect of EPA or DHA alone on bone mineralization

Effect of Long-term Dietary Lipids on Femur Mineral Content, *Ex vivo* Prostaglandin E_2 release and Bone Growth in Growing Rabbits

Al-Nouri, D. M., and Al-Khalifa, A. S.

Prepared in manuscript format

Bone growth indicators of male and female rabbits fed diets with different dietary oil sources and varying ω-6/ω-3 ratio for 100 days

Length (cm)	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
Humerus	7.01 [Aa] ±0.04	7.02 [Aa] ±0.07	7.10 [Aa] ±0.09	7.00 [Aa] ±0.12	7.03 [Aa] ±0.10	6.88 [Aa] ±0.03	7.11 [Aa] ±0.09	6.93 [Aa] ±0.14	6.88 [Aa] ±0.08	6.85 [Aa] ±0.15
Forearm	7.97 [Aa] ±0.04	7.92 [Aa] ±0.06	8.06 [Aa] ±0.07	7.92 [Aa] ±0.07	8.03 [Aa] ±0.13	7.75 [Aa] ±0.05	7.92 [Aa] ±0.07	7.90 [Aa] ±0.13	7.85 [Aa] ±0.00	7.85 [Aa] ±0.15
Tibia	10.35 [Aa] ±0.09	10.27 [Aa] ±0.11	10.44 [Aa] ±0.16	10.25 [Aa] ±0.18	10.41 [Aa] ±0.16	10.20 [Aa] ±0.10	10.58 [Aa] ±0.12	10.38 [Aa] ±0.16	10.33 [Aa] ±0.08	10.18 [Aa] ±0.22
Femur	9.68 [Aa] ±0.08	9.66 [Aa] ±0.05	9.68 [Aa] ±0.08	9.57 [Aa] ±0.12	9.72 [Aa] ±0.14	9.40 [Aa] ±0.05	9.81 [Aa] ±0.11	9.68 [Aa] ±0.13	9.58 [Aa] ±0.03	9.40 [Aa] ±0.28

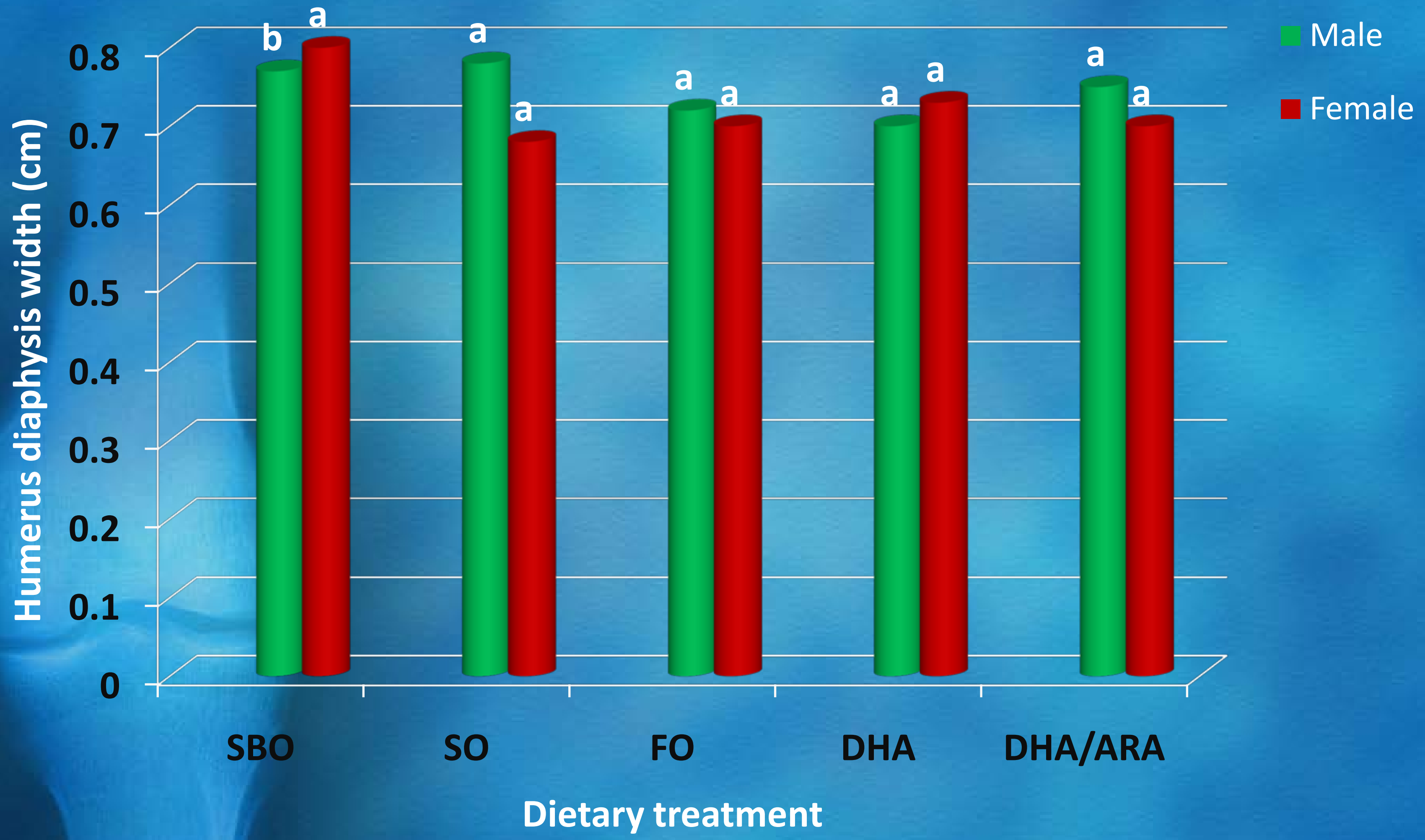
Bone growth indicators of male and female rabbits fed diets with different dietary oil sources and varying ω-6/ω-3 ratio for 100 days

Width (cm)	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
Humerus A	1.23 Aa ±0.06	1.29 Aa ±0.02	1.31 Aa ±0.01	1.27 Aa ±0.03	1.28 Aa ±0.02	1.25 Aa ±0.05	1.26 Aa ±0.02	1.25 Aa ±0.00	1.33 Aa ±0.03	1.29 Aa ±0.03
Humerus B	0.92 Aa ±0.01	0.93 Aa ±0.02	0.96 Aa ±0.01	0.93 Aa ±0.03	0.92 Aa ±0.03	0.88 Aa ±0.03	0.90 Aa ±0.02	0.92 Aa ±0.02	0.93 Aa ±0.03	0.90 Aa ±0.02
Humerus C	0.77 Ba ±0.01	**0.80 Aa ±0.00**	0.78 Aa ±0.01	0.68 Ab ±0.04	0.72 Aa ±0.03	0.70 Ab ±0.00	0.70 Aa ±0.02	0.73 Aab ±0.02	0.75 Aa ±0.05	0.70 Ab ±0.02
Femur A	1.66 Aa ±0.02	1.64 Aa ±0.03	1.60 Aa ±0.04	1.68 Aa ±0.07	1.65 Aa ±0.04	1.55 Aa ±0.05	1.54 Aa ±0.04	1.62 Aa ±0.04	1.63 Aa ±0.03	1.61 Aa ±0.06
Femur B	2.41 Aab ±0.01	2.42 Aab ±0.03	**2.43 Aa ±0.01**	**2.45 Aa ±0.05**	2.36 Aabc ±0.04	2.30 Ac ±0.00	2.29 Ac ±0.02	2.35 Aabc ±0.03	2.33 Abc ±0.08	2.34 Abc ±0.02
Femur C	**0.63 Aa ±0.03**	**0.60 Aa ±0.02**	**0.64 Aa ±0.03**	**0.55 Aa ±0.05**	0.58 Aab ±0.03	0.53 Aa ±0.08	0.49 Ab ±0.01	0.53 Aa ±0.02	0.58 Aab ±0.03	0.51 Aa ±0.03
Tibia A	1.52 Aa ±0.01	1.53 Aab ±0.02	1.55 Aa ±0.02	**1.57 Aa ±0.03**	1.45 Aa ±0.04	1.38 Ad ±0.03	1.46 Aa ±0.02	1.47 Abc ±0.02	1.45 Aa ±0.05	1.44 Acd ±0.02
Tibia B	0.63 Aa ±0.02	**0.62 Aa ±0.03**	0.68 Aa ±0.01	0.60 Bab ±0.00	0.59 Aa ±0.02	0.50 Ac ±0.00	0.57 Aa ±0.03	0.55 Abc ±0.00	0.63 Aa ±0.03	0.56 Aabc ±0.01

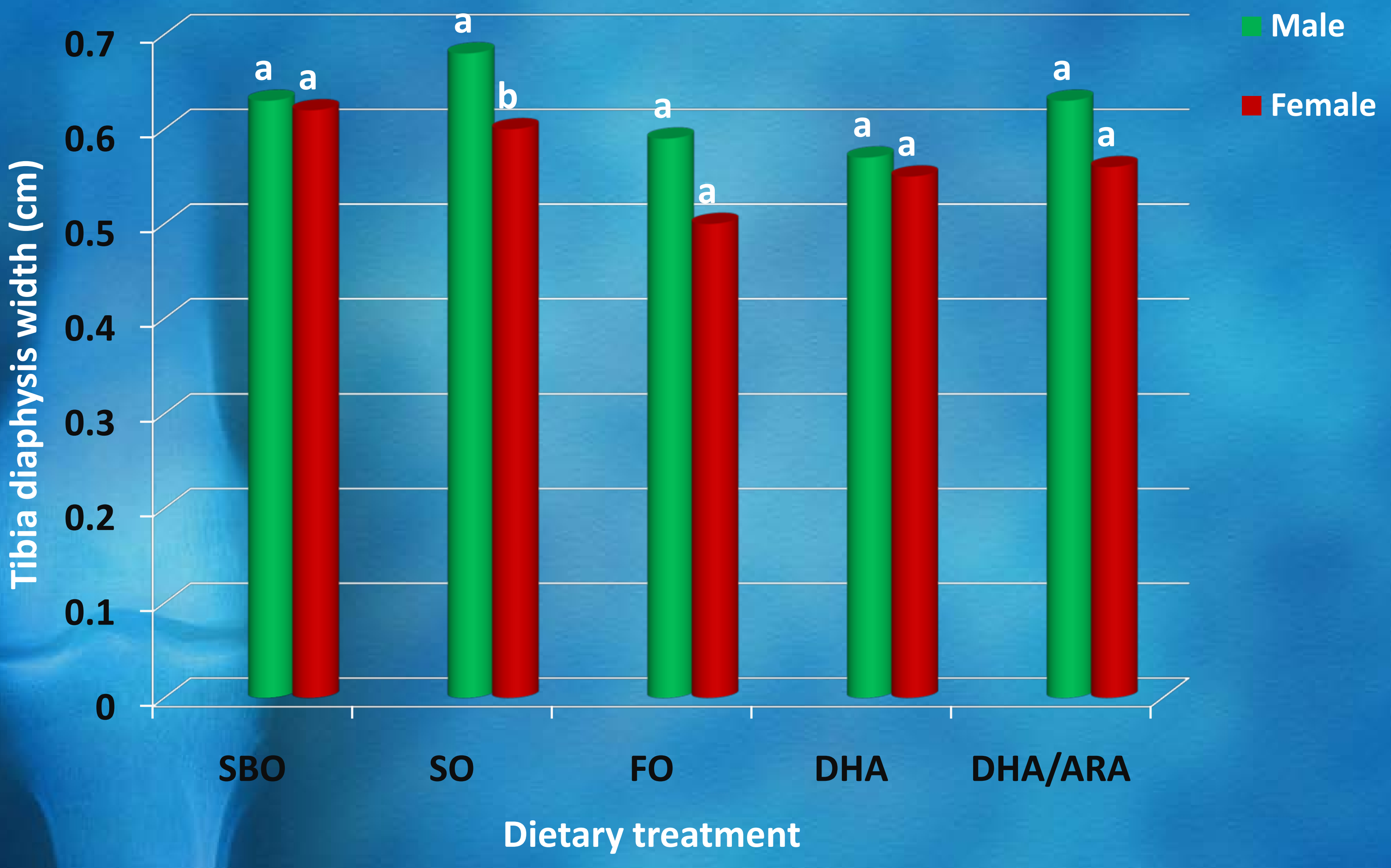
Bone growth indicators of male and female rabbits fed diets with different dietary oil sources and varying ω-6/ω-3 ratio for 100 days

Weight (g)	Dietary treatment									
	SBO		SO		FO		DHA		DHA/ARA	
	M	F	M	F	M	F	M	F	M	F
Humerus	4.51 Aa ±0.14	4.47 Aa ±0.10	4.51 Aa ±0.15	4.02 Ab ±0.15	4.13 Aab ±0.21	3.61 Ab ±0.13	3.80 Ab ±0.07	3.86 Ab ±0.05	3.81 Ab ±0.07	4.01 Ab ±0.16
Forearm	3.81 Aa ±0.11	3.74 Aa ±0.06	3.84 Aa ±0.09	3.42 Aa ±0.19	3.71 Aab ±0.23	3.08 Ab ±0.10	3.24 Ab ±0.09	3.43 Aa ±0.09	3.39 Aab ±0.04	3.63 Aa ±0.08
Tibia	7.83 Aa ±0.28	7.67 Aa ±0.15	7.92 Aa ±0.27	7.14 Aab ±0.30	6.94 Aab ±0.43	5.94 Ac ±0.33	6.34 Ab ±0.18	6.55 Abc ±0.21	6.30 Ab ±0.25	6.02 Ac ±0.29
Femur	9.49 Aa ±0.31	9.18 Aa ±0.18	9.55 Aa ±0.24	8.60 Aab ±0.36	8.54 Aab ±0.45	7.06 Ac ±0.52	7.58 Ab ±0.18	7.85 Abc ±0.23	7.83 Ab ±0.06	7.64 Ac ±0.24

Comparison of humerus diaphysis width between male & female rabbits



Comparison of tibia diaphysis width between male & female rabbits



Conclusion

- Dietary lipid supplementation with various ω-6/ω-3 ratios affects bone width & weight
- & had no effect on bone length

General Conclusion

- The bone marrow fatty acid profile was significantly influenced by & reflected the dietary lipid treatments

- Inclusion the diet with preformed ARA, EPA & DHA is more effective to enrich bone marrow tissue with these essential fatty acids than to supplement with the precursors

- The reduction in the ω-6/ω-3 ratio resulted in significantly

↓ PGE_2 level (FO & DHA)

↑ ALP activity (DHA/ARA)

↑ Ca, P, & Mg contents (FO, DHA & DHA/ARA)

Maintained optimal Ca/P ratio (DHA & DHA/ARA)

- Marine algae oils may be promising dietary sources for promoting bone mineralization during the growing stage
- Alteration in LCPUFA status has not demonstrated tremendous deterioration effects on bone growth

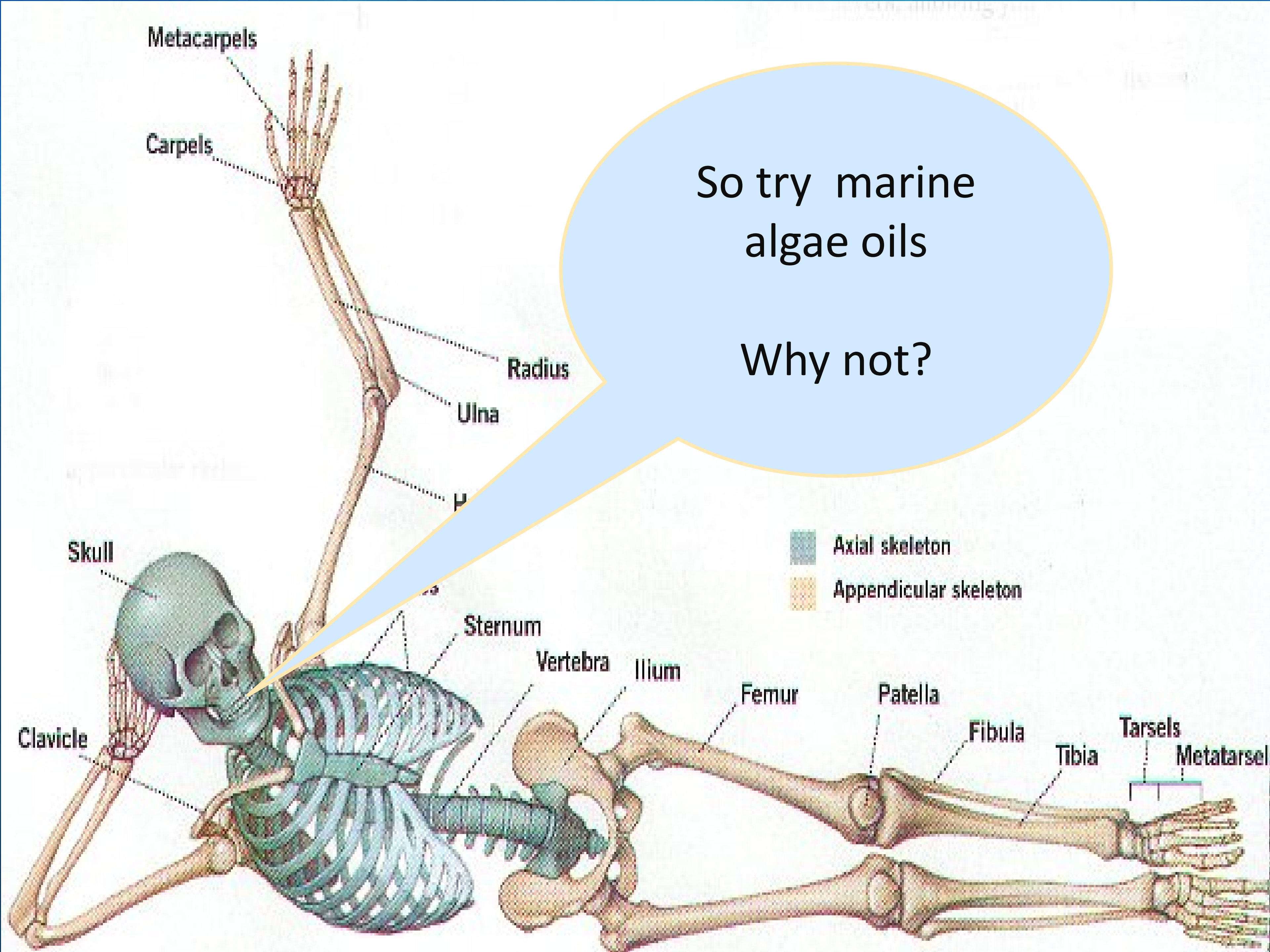
Future Research

- Conduct in adulthood & beyond that peak bone mass is achieved & bone would reflect resorption

- Include other eicosanoids such as PGE_1, PGE_3, leukotrienes & cytokines since they are also involved in bone formation & resorption activities

- Mineral balance studies will explain the change in femur mineral content that was observed in response to dietary LCPUFA supplementation

- Include both genders to clarify the mechanism behind the variation that was existed between males & females


So try marine algae oils
Why not?
Metacarpels
Carpels
Radius
Ulna
Skull
Clavicle
Sternum
Vertebra
Ilium
Femur
Patella
Fibula
Tibia
Tarsels
Metatarsel
Axial skeleton
Appendicular skeleton

First Paper

- International Society for Nutraceuticals & Functional Foods Conference 2011. Sapporo, Japan

- Journal of Functional Food 2012

  IF: 1.308

Second Paper

- The First International Conference of the Saudi Osteoporosis Society 2012. Riyadh, KSA
- Will be publish

Third Paper

- 8th International Symposium on Nutritional Aspects of Osteoporosis 2012. Lausanne, Switzerland

- Will be publish

Fourth Paper

- Journal of Animal & Veterinary Advances 2011

  IF: 0.292

Special Thank

Prof. Al-Khalifa

المختبر المركزي

ابتسام بنت سعود الشريف

غدير بنت منير العتيبي

نوف بنت عبد الله الطوير

المختبر المركزي

عفاف بنت صالح الغامدي

نشوى بنت شفيق عثمان

دانيه بنت عبد الباري العيدروس

المختبر المركزي

أحلام بنت محمد العثمان

شهيره بنت مبارك العسيري

هيفاء بنت صالح البدوي

المختبر المركزي

ندى بنت محمد ميرغني

شروق بنت محمد الحمودي

نوره بنت أحمد الزهراني

وضحى بنت إبراهيم بخاري

مركز البحوث

موضي بنت حسن الزير

شهد بنت علي الراجحي

الدكتورة/ مها بنت حسن داغستاني

الدكتورة/ ناديه بنت عبد العزيز العيسى

الدكتورة/ زبيده بنت عبد النبي بخيت

الدكتورة/ بدريه بنت عمر العبد الكريم


Any
Questions?