Supplementary Material

^{1}H NMR Spectroscopy and MVA to Evaluate the Effects of Caulerpin-Based Diet on *Diplodus Sargus* Lipid Profiles

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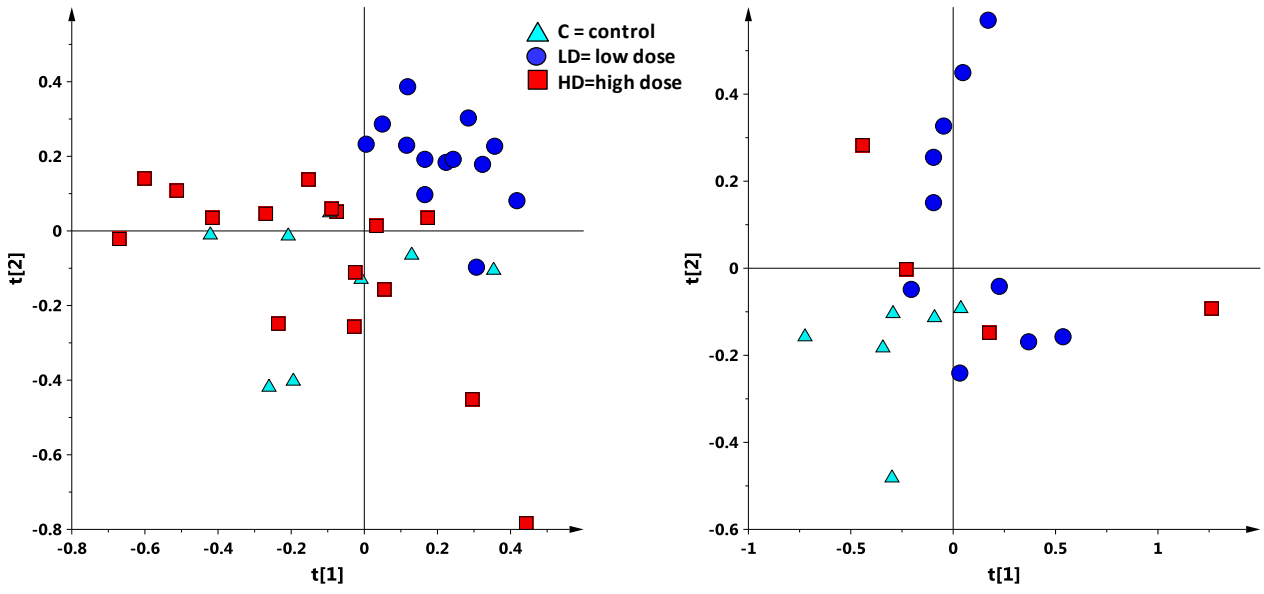

(**a**) (**b**)

Figure S1. (**a**) PCA (4 components, R^2X=0.80, Q^2=0.70) score plot obtained from ^{1}H NMR lipid extracts for experiment 1 (3 days treatment); (**b**) PCA (4 components, R^2X=0.89, Q^2=0.74) score plot for experiment 2 (10 days treatment).

FOLD CHANGE EXP-1 (37 samples)

Table S1. Fatty acid percentage calculated by integration of unbiased signals in the ^{1}H NMR spectra of lipid extracts for EXP-1 samples.

	%PUFA	%EPA	%DHA	%DUFA	%MUFA	%UFA	%SFA
C	23.23	3.75	12.25	6.32	22.37	48.08	51.92
HD	22.31	3.57	11.29	7.47	19.20	51.02	48.98
LD	24.13	3.46	10.11	8.01	14.00	53.86	46.14

Table S2. Ratio calculated from percentage of FA values.

	%PUFA	%EPA	%DHA	%DUFA	%MUFA	%UFA	%SFA
C/LD	0.963	1.083	1.212	0.789	1.598	0.893	1.125
C/HD	1.041	1.050	1.085	0.845	1.165	0.942	1.060

Table S3. Fold change (FC) values.

FC	%PUFA	%EPA	%DHA	%DUFA	%MUFA	%UFA	%SFA
C/LD	0.054	-0.115	-0.277	0.342	-0.676	0.164	-0.170
C/HD	-0.058	-0.070	-0.117	0.243	-0.221	0.085	-0.084

FOLD CHANGE EXP-2 (20 samples)

Table S4. Fatty acid percentage calculated by integration of unbiased signals in the ^{1}H NMR spectra of lipid extracts for EXP-2 samples.

	%PUFA	%EPA	%DHA	%DUFA	%MUFA	%UFA	%SFA
C	16.88	1.77	9.22	12.44	14.70	55.99	44.01
HD	16.52	2.48	8.89	11.78	19.88	51.82	48.18
LD	22.28	3.37	10.08	9.07	17.31	51.33	48.67

Table S5. Ratio calculated from percentage of FA values.

	%PUFA	%EPA	%DHA	%DUFA	%MUFA	%UFA	%SFA
C/LD	1.320	1.902	1.094	0.729	1.178	0.917	1.106
C/HD	1.349	1.359	1.134	0.770	0.871	0.991	1.010

Table S6. Fold change (FC) values.

FC	%PUFA	%EPA	%DHA	%DUFA	%MUFA	%UFA	%SFA
C/LD	-0.401	-0.9278	-0.130	0.455	-0.236	0.125	-0.145
C/HD	-0.432	-0.442	-0.181	0.377	0.199	0.014	-0.014

Table S7. One-way Anova results for EXP-1 samples.

```
> AnovaModel.2 <- aov(X.PUFA ~ trattamento, data=dsE7_TOT)

> summary(AnovaModel.2)
             Df Sum Sq Mean Sq F value Pr(>F)
trattamento   2  23.69  11.847   2.127  0.135
Residuals    34 189.33   5.568
```

```
> with(dsE7_TOT, numSummary(X.PUFA, groups=trattamento, statistics=c("mean",
+    "sd")))
      mean       sd data:n
C  23.23309 2.519209      8
HD 22.31423 2.485671     16
LD 24.12908 2.086125     13

Fit: aov(formula = X.PUFA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
HD - C == 0   -0.9189     1.0218  -0.899    0.643
LD - C == 0    0.8960     1.0604   0.845    0.677
LD - HD == 0   1.8148     0.8811   2.060    0.113
(Adjusted p values reported -- single-step method)

> AnovaModel.5 <- aov(X.EPA ~ trattamento, data=dsE7_TOT)

> summary(AnovaModel.5)
            Df Sum Sq Mean Sq F value Pr(>F)
trattamento  2  0.410  0.2051   0.967   0.39
Residuals   34  7.211  0.2121

> with(dsE7_TOT, numSummary(X.EPA, groups=trattamento, statistics=c("mean", "sd")))
      mean       sd data:n
C  3.751333 0.3737482      8
HD 3.572989 0.4962436     16
LD 3.463528 0.4600527     13

Fit: aov(formula = X.EPA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
HD - C == 0   -0.1783     0.1994  -0.894    0.646
LD - C == 0   -0.2878     0.2069  -1.391    0.355
LD - HD == 0  -0.1095     0.1720  -0.637    0.800
(Adjusted p values reported -- single-step method)



> AnovaModel.6 <- aov(X.DHA ~ trattamento, data=dsE7_TOT)

> summary(AnovaModel.6)
            Df Sum Sq Mean Sq F value Pr(>F)
trattamento  2  23.92  11.961   5.034 0.0122 *
Residuals   34  80.78   2.376
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> with(dsE7_TOT, numSummary(X.DHA, groups=trattamento, statistics=c("mean", "sd")))
      mean       sd data:n
C  12.24687 1.093859      8
HD 11.28827 1.842136     16
LD 10.10589 1.338741     13
```

Multiple Comparisons of Means: Tukey Contrasts

Fit: aov(formula = X.DHA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)	
HD - C == 0	-0.9586	0.6675	-1.436	0.3326	
LD - C == 0	-2.1410	0.6927	-3.091	0.0107	*
LD - HD == 0	-1.1824	0.5756	-2.054	0.1141	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

```
> with(dsE7_TOT, numSummary(X.DUFA, groups=trattamento, statistics=c("mean",
+   "sd")))
      mean       sd data:n
C  6.315064 1.908038      8
HD 7.473351 2.257739     16
LD 8.005193 1.440296     13
```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: aov(formula = X.DUFA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
HD - C == 0	1.1583	0.8363	1.385	0.358
LD - C == 0	1.6901	0.8679	1.947	0.140
LD - HD == 0	0.5318	0.7212	0.737	0.742

```
> AnovaModel.8 <- aov(X.MUFA ~ trattamento, data=dsE7_TOT)

> summary(AnovaModel.8)
            Df Sum Sq Mean Sq F value Pr(>F)
trattamento  2  383.5  191.75   3.892 0.0301 *
```

```
Residuals   34 1675.2   49.27
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> with(dsE7_TOT, numSummary(X.MUFA, groups=trattamento, statistics=c("mean",
+   "sd")))
      mean       sd data:n
C  22.37170 8.244414      8
HD 19.19655 8.009060     16
LD 14.00173 4.446400     13

Multiple Comparisons of Means: Tukey Contrasts


Fit: aov(formula = X.MUFA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
HD - C == 0    -3.175      3.039  -1.045   0.5529
LD - C == 0    -8.370      3.154  -2.654   0.0312 *
LD - HD == 0   -5.195      2.621  -1.982   0.1312
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)



> AnovaModel.9 <- aov(X.UFA ~ trattamento, data=dsE7_TOT)

> summary(AnovaModel.9)
            Df Sum Sq Mean Sq F value  Pr(>F)
trattamento  2  169.4   84.72   5.318 0.00978 **
Residuals   34  541.6   15.93
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> with(dsE7_TOT, numSummary(X.UFA, groups=trattamento, statistics=c("mean", "sd")))
      mean       sd data:n
C  48.08015 5.023999      8
HD 51.01588 4.292175     16
LD 53.86400 2.717480     13


Multiple Comparisons of Means: Tukey Contrasts


Fit: aov(formula = X.UFA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:
```

```
            Estimate Std. Error t value Pr(>|t|)
HD - C == 0     2.936      1.728   1.699  0.21910
LD - C == 0     5.784      1.794   3.225  0.00756 **
LD - HD == 0    2.848      1.490   1.911  0.14999
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)


> AnovaModel.10 <- aov(X.SFA ~ trattamento, data=dsE7_TOT)

> summary(AnovaModel.10)
            Df Sum Sq Mean Sq F value  Pr(>F)
trattamento  2  169.4   84.72   5.318 0.00978 **
Residuals   34  541.6   15.93
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


> with(dsE7_TOT, numSummary(X.SFA, groups=trattamento, statistics=c("mean", "sd")))
       mean       sd data:n
C  51.91985 5.023999      8
HD 48.98412 4.292175     16
LD 46.13600 2.717480     13

             Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts


Fit: aov(formula = X.SFA ~ trattamento, data = dsE7_TOT)

Linear Hypotheses:
            Estimate Std. Error t value Pr(>|t|)
HD - C == 0    -2.936      1.728  -1.699  0.21908
LD - C == 0    -5.784      1.794  -3.225  0.00761 **
LD - HD == 0   -2.848      1.490  -1.911  0.15007
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

Table S8. One-way Anova results for EXP-2 samples.

```
             Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

> with(DSL_M25_simca, numSummary(PUFA, groups=trattamento, statistics=c("mean", "sd")))
```

```
     mean       sd data:n
C 22.28495 1.526364      6
HD 16.51727 3.000546     4
LD 16.87872 2.283117     10

Fit: aov(formula = X.PUFA ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
          Estimate Std. Error t value Pr(>|t|)
HD - C == 0  -5.7677     1.4482  -3.983  0.00265 **
LD - C == 0  -5.4062     1.1586  -4.666  < 0.001 ***
LD - HD == 0   0.3615     1.3273   0.272  0.95973
---

> with(DSL_M25_simca, numSummary(EPA, groups=trattamento, statistics=c("mean", "sd")))
     mean       sd data:n
C 3.366663 0.5306937      6
HD 2.478220 0.5833759     4
LD 1.769966 0.2715293     10

Fit: aov(formula = EPA ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
          Estimate Std. Error t value Pr(>|t|)
HD - C == 0  -0.8884     0.2753  -3.227   0.0128 *
LD - C == 0  -1.5967     0.2203  -7.249   <0.001 ***
LD - HD == 0  -0.7083     0.2523  -2.807   0.0307 *


> AnovaModel.4 <- aov(DHA ~ trattamento, data=DSL_M25_simca)
            Df Sum Sq Mean Sq F value Pr(>F)
trattamento  2   4.17   2.083   0.449  0.646
Residuals   17  78.87   4.639

> with(DSL_M25_simca, numSummary(DHA, groups=trattamento,
+   statistics=c("mean", "sd")))
      mean       sd data:n
C 10.081935 1.886349      6
HD  8.891368 1.876738     4
LD  9.215016 2.369036     10

Multiple Comparisons of Means: Tukey Contrasts


Fit: aov(formula = X.DHA ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
          Estimate Std. Error t value Pr(>|t|)
HD - C == 0  -1.1906     1.3903  -0.856    0.672
```

LD - C == 0 -0.8669   1.1123 -0.779   0.719
LD - HD == 0   0.3236   1.2743   0.254   0.965
(Adjusted p values reported -- single-step method)

> AnovaModel.5 <- aov(**DUFA** ~ trattamento, data=DSL_M25_simca)

> summary(AnovaModel.5)
             Df Sum Sq Mean Sq F value Pr(>F)
trattamento 2 43.69 21.844   2.827 0.0871 .
Residuals   17 131.34   7.726

> with(DSL_M25_simca, numSummary(**DUFA**, groups=trattamento,
+   statistics=c("mean", "sd")))
       mean   sd data:n
C 9.069537 1.927444   6
HD 11.781788 5.177614   4
LD 12.436267 1.895603   10

Fit: aov(formula = **DUFA** ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
            Estimate Std. Error t value Pr(>|t|)
HD - C == 0   2.7123   1.7942   1.512   0.3084
LD - C == 0   3.3667   1.4353   2.346   0.0757 .
LD - HD == 0   0.6545   1.6444   0.398   0.9162

> AnovaModel.7 <- aov(**MUFA** ~ trattamento, data=DSL_M25_simca)

> summary(AnovaModel.7)
             Df Sum Sq Mean Sq F value Pr(>F)
trattamento 2   82.1   41.06   0.617 0.551
Residuals   17 1131.9   66.58

> with(DSL_M25_simca, numSummary(**MUFA**, groups=trattamento,
+   statistics=c("mean", "sd")))
      mean   sd data:n
C 17.31323 4.851955   6
HD 19.88096 14.671742   4
LD 14.69904 6.397993   10

Fit: aov(formula = **MUFA** ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
            Estimate Std. Error t value Pr(>|t|)
HD - C == 0   2.568   5.267   0.488   0.877

```
LD - C == 0   -2.614      4.214  -0.620    0.810
LD - HD == 0   -5.182      4.827  -1.073    0.541
```

```
> AnovaModel.12 <- aov(X.UFA ~ trattamento, data=DSL_M25_simca)

> summary(AnovaModel.12)
             Df Sum Sq Mean Sq F value Pr(>F)
trattamento  2  100.0   49.98   2.107  0.152
Residuals   17  403.2   23.72

> with(DSL_M25_simca, numSummary(X.UFA, groups=trattamento,
+   statistics=c("mean", "sd")))
     mean       sd data:n
C 51.33229 2.776968      6
HD 51.81999 7.162467      4
LD 55.98598 4.839500     10

Fit: aov(formula = X.UFA ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
             Estimate Std. Error t value Pr(>|t|)
HD - C == 0   0.4877     3.1438   0.155    0.987
LD - C == 0   4.6537     2.5150   1.850    0.182
LD - HD == 0   4.1660     2.8813   1.446    0.339
```

```
> AnovaModel.10 <- aov(X.SFA ~ trattamento, data=DSL_M25_simca)

> summary(AnovaModel.10)
             Df Sum Sq Mean Sq F value Pr(>F)
trattamento  2  100.0   49.98   2.107  0.152
Residuals   17  403.2   23.72

> with(DSL_M25_simca, numSummary(X.SFA, groups=trattamento,
+   statistics=c("mean", "sd")))
     mean       sd data:n
C 48.66771 2.776968      6
HD 48.18001 7.162467      4
LD 44.01402 4.839500     10

Fit: aov(formula = X.SFA ~ trattamento, data = DSL_M25_simca)

Linear Hypotheses:
             Estimate Std. Error t value Pr(>|t|)
HD - C == 0  -0.4877     3.1438  -0.155    0.987
LD - C == 0  -4.6537     2.5150  -1.850    0.182
LD - HD == 0  -4.1660     2.8813  -1.446    0.338
```