



**UNIVERSITÀ POLITECNICA DELLE MARCHE**  
**DIPARTIMENTO DI SCIENZE DELLA VITA E  
DELL'AMBIENTE**

Corso di Laurea in Scienze Biologiche

***Effect of dietary microalgal supplementation on fatty acid composition of egg yolk in laying hens***

***Effetto dell'integrazione alimentare di microalghe sulla composizione di acidi grassi del tuorlo d'uovo nelle galline ovaiole***

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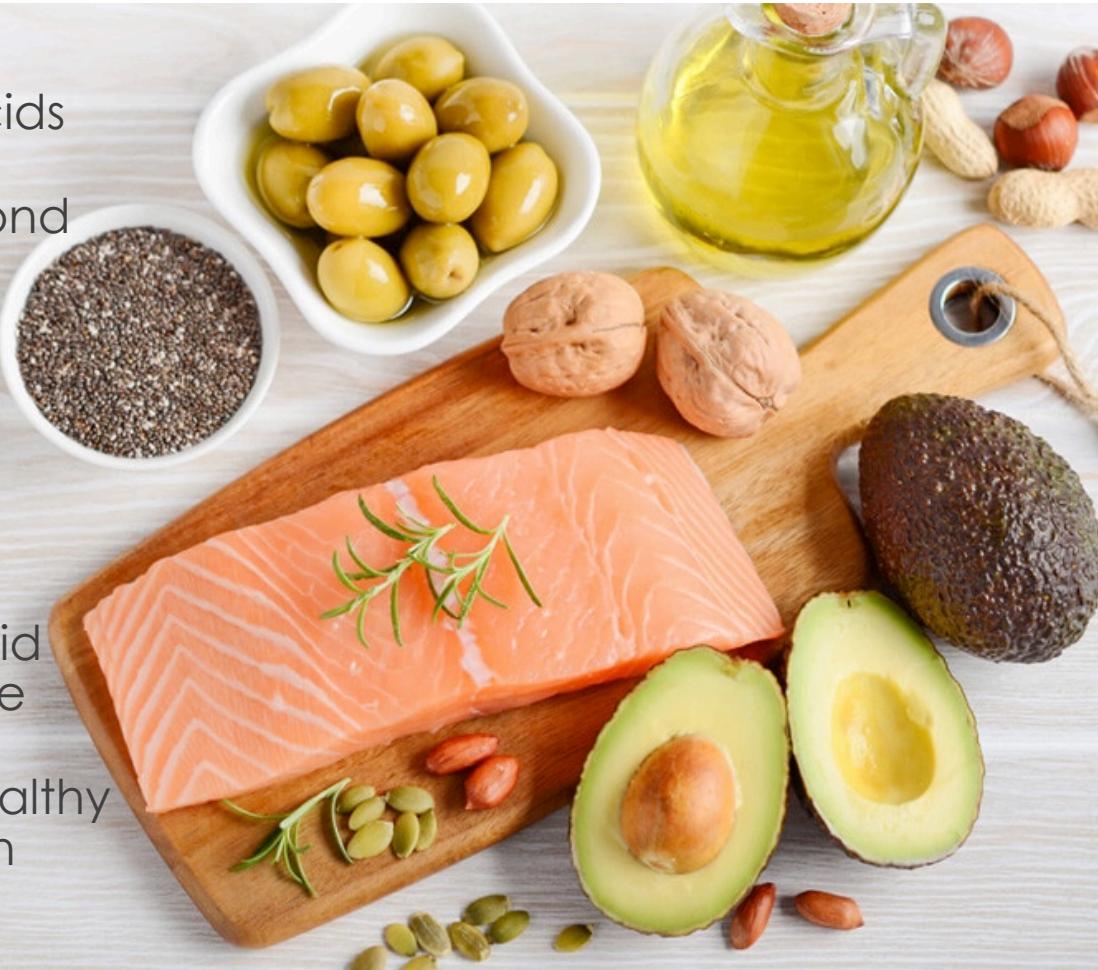
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# INTRODUCTION

Omega-3 are polyunsaturated fatty acids characterized by the presence of a double bond located three carbons away from the terminal methyl group.

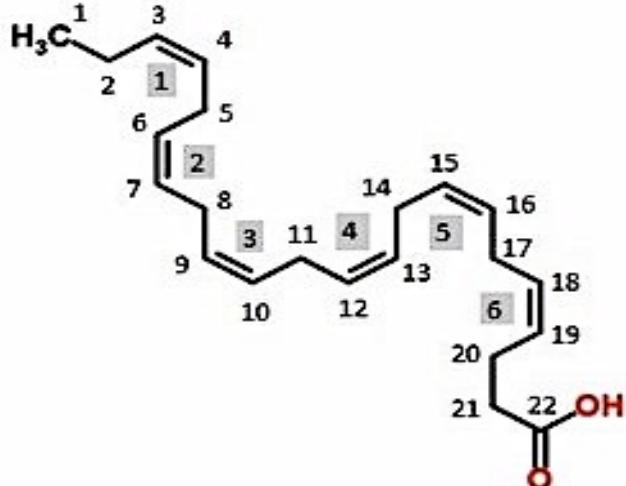
They are also known as **N-3 PUFA**.

They are important constituents of animal lipid metabolism, and they are involved in lowering inflammation levels in healthy adults and in people with metabolic syndrome.

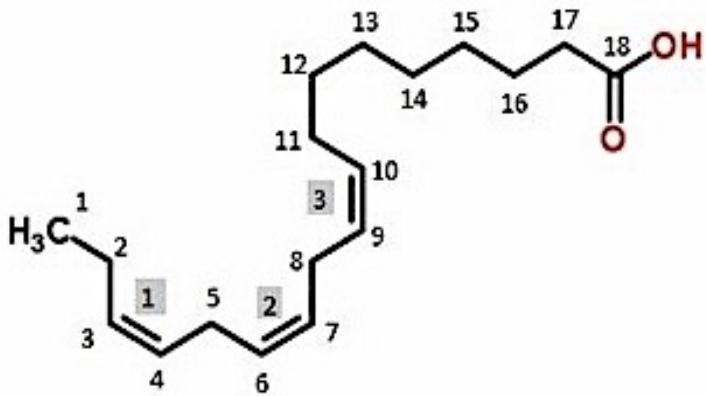


## N-3 PUFA involved

**Docosahexaenoic acid (DHA)** is an Omega-3 long chain PUFA (**LCPUFA**) with 22 carbons and 6 double bonds. It is an essential component of cell membranes, especially in the nervous system, in which it constitutes 85% of membrane phospholipids.



**Docosahexaenoic acid (DHA)**  
22:6n-3



**Alpha-linolenic acid (ALA)**  
18:3n-3

In terrestrial animals it is a semi-essential PUFA. In fact, mammals and birds can produce it from  **$\alpha$ -linolenic acid (ALA)**, another N-3 PUFA typically found in plant seeds, like Flax Seeds. Yet, this conversion is rather inefficient, so supplementation through diet is very important.

The main purpose of the study conducted by Neijat et al. (2016) at the University of Manitoba in collaboration with the Canadian Center for Agri-Food Research in Health and Medicine[1] is to determine the effect of microalgal DHA supplementation on the enrichment of N-3 fatty acids in egg yolk.



## Experimental birds and diets

This study involved 70, 34-weeks-old, Lohmann LSL-Classic laying hens.

All diets were cereal based and formulated to meet minimum nutrient specifications according to published NRC (National Research Council) recommendations and according to management guide for Lohmann hens (Lohmann LSL-Lite, North American Edition).

The hens were first subjected to a 2 weeks adaptation period in which they were fed a commercial layer diet without supplemental omega-3 PUFA or the control diet containing almond oil.



## Experimental birds and diets

Almond oil has been used in the control diet because it is very low in ALA and DHA free. This removes the potential confounding effect on the results of the experimental diets.



From the third week the hens were assigned randomly 1 of 6 experimental diets (10 hens/treatment) containing graded levels of total N-3 PUFA (0.20%, 0.40% and 0.60%, by wt of diet) derived from either:

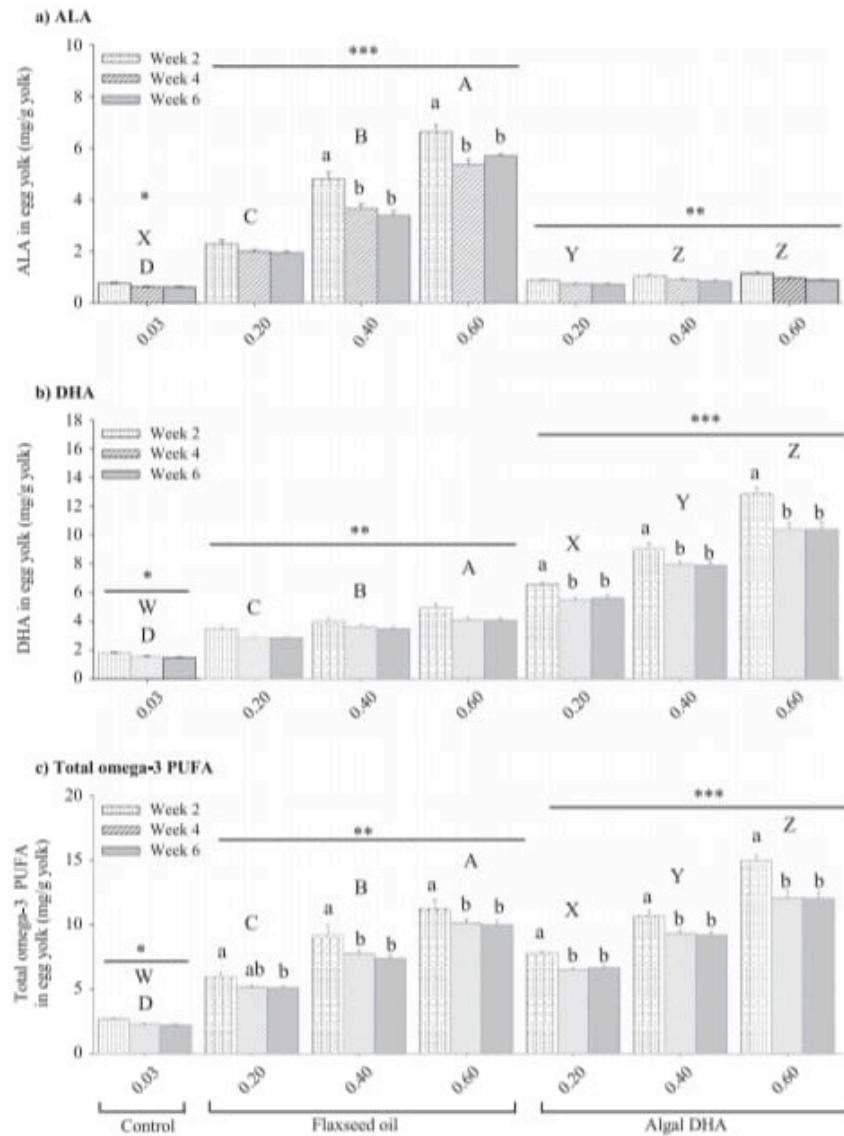
- **Algal DHA** (DHAgold™ S17-B, dried whole-cell algae product by DSM)
- **Flaxseed oil** (provider of precursor **ALA**)

## Photoperiod

A lighting regime of 16 h of light and 8 h of dark was used for the entire experiment.

# Results and discussion

Yolks derived from either precursor ALA or preformed DHA decreased the Omega6/Omega3 ratio to similar levels (with the range of 3.3 to 1 ratio), depositing 3-fold more DHA in the egg yolk when laying hen diets contained 0.60% of total omega-3 fatty acids with algal DHA (3.36 g/100 g diet) over flaxseed oil (1.19 g/100 g diet), achieving a steady state of accumulation within 4 weeks of feeding.



**Table 4**  
Fatty acid composition of egg yolk (mg/yolk) as a function of increasing levels of total omega-3s using flaxseed oil in diets of hens fed from 36 to 42 weeks of age<sup>1</sup>.

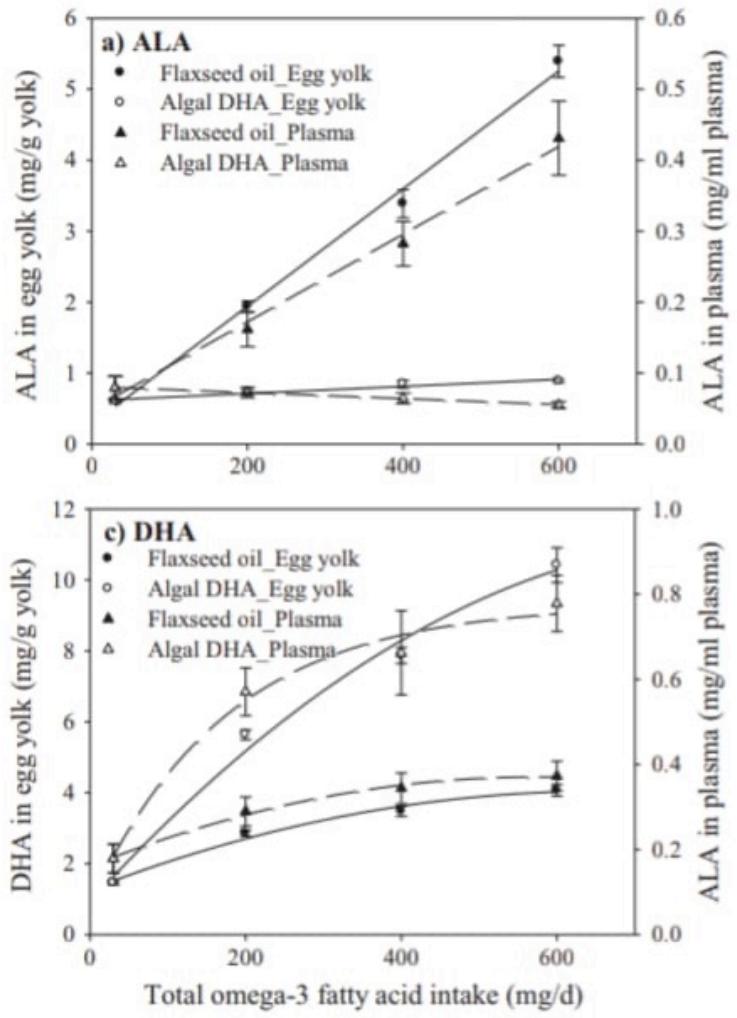
	Week	Diet effect				Week effect				P-value			
		Total omega-3 in diet (%)				SE	wk 2	wk 4	wk 6	SE	diet	wk	diet x wk
		(wk)	0.03	0.20	0.40								
TSFA <sup>2</sup>	2	1623	1544	1527	1616								
	4	1406	1381	1325	1332								
	6	1377	1377	1325	1359								
	Overall	1469	1434	1415	1436	49.0	157 <sup>a</sup>	137 <sup>b</sup>	1359 <sup>b</sup>	34.0	0.90	<0.0001	0.88
TMUFA <sup>2</sup>	2	2027	1953	1849	1900								
	4	1729	1767	1729	1519								
	6	1705	1703	1543	1631								
	Overall	1820	1807	1707	1683	62.3	193 <sup>a</sup>	1686 <sup>b</sup>	1645 <sup>b</sup>	43.1	0.31	<0.0001	0.48
LA	2	564	555	544	584								
	4	494	491	508	473								
	6	483	493	477	497								
	Overall	514	513	510	518	19.2	562 <sup>a</sup>	492 <sup>b</sup>	488 <sup>b</sup>	13.1	0.99	<0.0001	0.75
ARA	2	88.6	74.4	62.9	65.1								
	4	61.4	65.3	57.9	49.7								
	6	79.5	68.4	56.2	51.4								
	Overall	83.2 <sup>a</sup>	69.4 <sup>b</sup>	59.0 <sup>c</sup>	55.4 <sup>c</sup>	1.81	72.8 <sup>a</sup>	63.6 <sup>b</sup>	63.8 <sup>b</sup>	1.28	<0.0001	<0.0001	0.29
ALA	2	12.7	37.2	71.8 <sup>a</sup>	114 <sup>a</sup>								
	4	10.4	33.3	61.5 <sup>a</sup>	88.7 <sup>c</sup>								
	6	10.3	33.7	58.2 <sup>a</sup>	97.2 <sup>b</sup>								
	Overall	11.2 <sup>d</sup>	34.7 <sup>c</sup>	63.8 <sup>b</sup>	99.9 <sup>a</sup>	2.33	58.9 <sup>a</sup>	48.5 <sup>b</sup>	49.9 <sup>b</sup>	1.58	<0.0001	<0.0001	<0.05
EPA	2	0.28	0.89	2.56	3.90								
	4	0.19	0.85	2.12	3.67								
	6	0.24	0.97	2.23	3.40								
	Overall	0.24 <sup>d</sup>	0.91 <sup>c</sup>	2.30 <sup>b</sup>	3.66 <sup>a</sup>	0.11	1.91	1.71	1.71	0.093	<0.0001	0.21	0.57
DPA	2	2.29	4.78	6.49	8.05								
	4	1.96	3.83	5.40	6.36								
	6	2.16	3.75	5.63	6.40								
	Overall	2.14 <sup>d</sup>	4.12 <sup>c</sup>	5.84 <sup>b</sup>	6.94 <sup>a</sup>	0.24	5.40 <sup>a</sup>	4.39 <sup>b</sup>	4.48 <sup>b</sup>	0.17	<0.0001	<0.0001	0.26
DHA	2	29.6	54.0	68.0	74.7								
	4	25.9	47.5	62.8	64.2								
	6	24.7	49.4	59.9	66.9								
	Overall	26.7 <sup>c</sup>	50.3 <sup>b</sup>	63.6 <sup>a</sup>	68.6 <sup>a</sup>	1.61	56.6 <sup>a</sup>	50.1 <sup>b</sup>	50.2 <sup>b</sup>	1.22	<0.0001	<0.001	0.74
Total n-6	2	659	635	611	654								
	4	582	566	561	527								
	6	568	566	538	541								
	Overall	603	587	570	574	21.2	640 <sup>a</sup>	558 <sup>b</sup>	553 <sup>b</sup>	14.5	0.71	<0.0001	0.80
Total n-3	2	44.2	96.5	148 <sup>a</sup>	214 <sup>a</sup>								
	4	38.3	85.4	130 <sup>a</sup>	167 <sup>b</sup>								
	6	37.6	88.2	121 <sup>a</sup>	174 <sup>b</sup>								
	Overall	40.1 <sup>d</sup>	90.0 <sup>c</sup>	133 <sup>b</sup>	185 <sup>a</sup>	3.90	126 <sup>a</sup>	105 <sup>b</sup>	105 <sup>b</sup>	2.57	<0.0001	<0.0001	<0.001
n-6/n-3 ratio	2	15.1	6.50	4.35	3.36								
	4	15.6	6.57	4.42	3.16								
	6	15.2	6.41	4.27	3.20								
	Overall	15.3 <sup>d</sup>	6.49 <sup>b</sup>	4.35 <sup>c</sup>	3.24 <sup>d</sup>	0.074	7.33	7.46	7.28	0.056	<0.0001	0.063	0.078

<sup>a-d</sup>Different superscripts between treatments (effect of diet) or periods (effect of week), within a row, are significantly different at  $P < 0.05$ .

<sup>a,b</sup>Different superscripts within a column for each parameter, are significantly different ( $P < 0.05$ ), represents a diet by week interaction.

<sup>1</sup> Data are presented as least square means (LSM) ± standard error (SE),  $n=9$  per treatment.

<sup>2</sup> Total saturated fatty acids (TSFA): myristic (14:0), palmitic (16:0), stearic (18:0); total monounsaturated fatty acids (TMUFA): palmitoleic (16:1) and oleic (18:1); Total n-6= sum of LA, GLA and ARA; Total n-3= sum of ALA, EPA, DPA and DHA.



There is a significantly higher nutritional level of enrichment of eggs (providing almost half the daily allowance for omega-3 LCPUFA for the general population i.e. ~400–500 mg/day), achieved by including a source of dietary preformed DHA (such as microalgae) compared to precursor ALA (flaxseed oil) in the laying hen diets.

## Conclusions

Other studies [2-5], showed that marine algae promote efficient yolk DHA deposition with the decrease of the Omega-6/Omega-3 ratio.

However, doubling the amount of the algae in the diet did not result in doubled omega-3 LCPUFA deposition into the eggs.

Taken together, the results indicate that the deposition of DHA reaches a plateau when a higher level of preformed LCPUFA is used in laying hen diets.

However, a significant accumulation of DHA is achieved with preformed sources compared to that endogenously synthesized from precursor ALA.

Further investigation is needed into the mechanism leading to the decline in the efficiency of DHA enrichment with preformed DHA as well as synthesized DHA at higher level of supplementation of their primary sources of omega-3 fatty acid in laying hen diets.

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## *Sunto esteso in lingua Italiana*

### Introduzione

Lo scopo principale dello studio condotto da Neijat et al. (2016) presso l'Università di Manitoba in collaborazione con il Canadian Centre for Agri-Food Research in Health and Medicine [1] è quello di determinare l'effetto della supplementazione di DHA microalgale sull'arricchimento degli acidi grassi N-3 nel tuorlo d'uovo.

Questo studio ha coinvolto 70 galline ovaiole Lohmann LSL-Classic di 34 settimane. Tutte le diete erano a base di cereali e formulate per soddisfare le specifiche nutrizionali minime secondo le raccomandazioni pubblicate dal NRC (National Research Council) e secondo la guida di gestione per le galline Lohmann (Lohmann LSL-Lite, North American Edition).

## Diete

Le galline sono state dapprima sottoposte a un periodo di adattamento di 2 settimane durante il quale sono state alimentate con una dieta commerciale per galline ovaiole senza omega-3 aggiunti o la dieta di controllo contenente olio di mandorle.

Dalla terza settimana alle galline è stata assegnata in modo casuale 1 di 6 diete sperimentali (10 galline/trattamento) contenenti livelli graduati di PUFA N-3 totale (0,20%, 0,40% e 0,60%, in peso della dieta) derivanti da:

- DHAgoldTM S17-B (prodotto di alghe a cellule intere essiccate di DSM)
- Olio di semi di lino (contenente il precursore ALA)

Per l'intero esperimento è stato utilizzato un regime di illuminazione di 16 ore di luce e 8 ore di buio.

## Risultati

E' stato rilevato un livello di arricchimento delle uova significativamente più alto (fornendo quasi la metà del fabbisogno giornaliero di omega-3 per la popolazione generale, cioè ~ 400-500 mg/giorno), utilizzando una fonte di DHA algale rispetto al precursore ALA (dall'olio di semi di lino) nelle diete per galline ovaiole.

Considerando altri studi simili [2-5], tutti i risultati indicano che la deposizione di DHA raggiunge un plateau quando viene utilizzato un livello più alto di LCPUFA nelle diete.

Tuttavia, si ottiene un accumulo più significativo di DHA utilizzando il DHA alglae rispetto a quello ottenuto utilizzando fonti contenenti il precursore ALA.

Sono necessarie ulteriori indagini per fare chiarezza sul meccanismo che porta al declino dell'efficienza dell'arricchimento del tuorlo con omega-3 a un livello più elevato di integrazione.