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Nutrigenomic evaluation of omega-3 fatty acid enriched feed on egg quality parameters in commercial layer hens

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Abstract

The fatty acid profile of a table egg is almost entirely a mirror of what the hen eats, yet the molecular machinery linking dietary omega-3 intake to yolk lipid remodelling has only begun to be mapped in production-scale flocks. This feeding trial coupled classical egg quality assessment with hepatic gene expression profiling to evaluate graded levels of flaxseed-derived omega-3 fatty acids in 120 Hy-Line W-36 laying hens over a 12-week production cycle at the São Paulo Research Institute of Animal Sciences (January-April 2023). Birds were randomly allocated to four isocaloric, isonitrogenous diets differing only in ground flaxseed inclusion: 0% (control), 1.5%, 3.0%, and 4.5% (n=30 per group). Eggs collected weekly were analysed for weight, shell thickness, Haugh unit, yolk colour index, and yolk fatty acid composition by gas chromatography. Liver biopsies taken at weeks 6 and 12 were assayed by RT-qPCR for expression of FADS2, ELOVL5, PPAR α , and SREBP-1c. Yolk omega-3 content rose in a dose-dependent fashion from 3.2 mg/g in controls to 18.9 mg/g at 4.5% flaxseed ($p<0.001$), driven mainly by increases in α -linolenic acid (ALA) and docosahexaenoic acid (DHA). The n-6:n-3 ratio dropped from 14.8:1 to 3.1:1 in the highest inclusion group. Shell thickness, egg weight, and Haugh unit remained unaffected across treatments. Hepatic FADS2 and ELOVL5 mRNA levels were upregulated 2.4-fold and 1.9-fold, respectively, at the 4.5% level, while PPAR α expression rose 1.7-fold, pointing to coordinated transcriptional activation of the desaturase-elongase cascade. These data confirm that flaxseed inclusion up to 4.5% enriches egg yolk omega-3 content without compromising shell or albumen quality, and they identify FADS2 upregulation as the rate-limiting genomic response driving long-chain PUFA deposition in the yolk.

Keywords: Nutrigenomics, omega-3 fatty acids, flaxseed, egg quality, layer hens, FADS2, ELOVL5, yolk fatty acid profile, gene expression, poultry nutrition

1. Introduction

What a hen deposits into her egg yolk is dictated, to a remarkable degree, by the lipids she absorbs from the gut. This nutritional plasticity makes the table egg one of the simplest vehicles for delivering health-promoting omega-3 polyunsaturated fatty acids (n-3 PUFA) to human consumers ^[1]. Flaxseed, fish oil, and marine algae have all been tested as dietary omega-3 sources in layer rations, with flaxseed being the most cost-effective and widely available option in tropical poultry systems ^[2].

Conventional egg yolks contain omega-6 and omega-3 fatty acids at a ratio near 15:1, a value far above the 4:1 ceiling recommended by nutritional guidelines for cardiovascular protection ^[3]. Feeding ground flaxseed at levels between 2% and 10% of the diet shifts this ratio downward by flooding hepatic lipid metabolism with α -linolenic acid (ALA, 18:3n-3), the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ^[4]. But the efficiency of ALA-to-DHA conversion varies across bird genotypes and is regulated by a chain of desaturase and elongase enzymes encoded by the FADS2 and ELOVL5 genes, among others ^[5].

Nutrigenomics the field examining how dietary components alter gene expression offers a tool to move beyond simple input-output feeding trials and ask *why* some inclusion levels yield disproportionate jumps in yolk DHA while others plateau. If FADS2 expression saturates above a certain ALA threshold, there is little point in pushing flaxseed inclusion higher;

conversely, if the gene continues to respond linearly, higher levels may be economically justified [6]. Only a handful of published trials have paired egg fatty acid data with hepatic transcriptomics in commercial layer genotypes, and none have done so under Brazilian production conditions where ambient temperatures and feed ingredient profiles differ from temperate-zone experiments [7].

Shell and albumen quality form the second axis of concern. Producers will not adopt omega-3 enrichment strategies if egg weight drops, shells weaken, or Haugh units decline, because these traits govern market grade and consumer acceptance [8]. Reports on this front are mixed: some trials noted marginally thinner shells at high flaxseed levels, while others found no effect up to 15% inclusion [2, 9]. Reconciling these discrepancies requires controlled dose-response designs that measure both external quality traits and internal lipid composition simultaneously. This research was designed to fill that gap by feeding four graded levels of flaxseed to commercial Hy-Line W-36 hens, measuring weekly egg quality parameters over 12 weeks, and profiling hepatic expression of key lipid metabolism genes at the midpoint and endpoint of the trial.

Material and Methods

Field Experimental Design: The experiment followed a completely randomised design with four treatments and 30 replicates (individual hens) per treatment. Hy-Line W-36 pullets (n=120) at 22 weeks of age were sourced from a single commercial hatchery and housed in individual wire cages (40 × 45 × 42 cm) in a naturally ventilated, curtain-sided poultry house at the São Paulo Research Institute of Animal Sciences, São Paulo, Brazil. A two-week pre-experimental adaptation period on a standard corn-soybean meal basal diet preceded the 12-week data collection phase (January 8 - April 2, 2023). During adaptation, baseline eggs from all birds were sampled for initial fatty acid profiling. Treatments consisted of the basal diet plus ground flaxseed at 0% (T₁-Control), 1.5% (T₂), 3.0% (T₃), and 4.5% (T₄), formulated to be isocaloric (2,800 kcal ME/kg) and isonitrogenous (16.5% crude protein). Feed and water were offered *ad libitum*. The photoperiod was maintained at 16L:8D throughout. Ambient temperature inside the house ranged from 24°C to 33°C, and relative humidity from 55% to 82%, typical of the São Paulo summer-autumn season.

Material: Ground golden flaxseed (*Linum usitatissimum*) was obtained from a certified supplier (ALA content: 22.3 g/100 g dry matter, verified by gas chromatography). The basal diet comprised yellow corn (58.2%), soybean meal (24.6%), limestone (9.1%), dicalcium phosphate (1.7%), soybean oil (3.0%), vitamin-mineral premix (0.5%), DL-methionine (0.15%), salt (0.35%), and choline chloride (0.05%). For flaxseed-containing diets, soybean oil and a portion of the corn fraction were replaced by ground flaxseed to maintain energy and protein equivalence. Diet samples were collected monthly and analysed for proximate composition and fatty acid content at the Institute's Nutrition Laboratory. The

Institutional Animal Ethics Committee approved the protocol (Approval No. SPRIAS-CEUA/2022-089, dated 14 November 2022).

Methods

Eggs were collected daily, but formal quality assessments were conducted once per week on three consecutive eggs per hen. Egg weight was recorded to 0.01 g. Shell thickness was measured at the equator, blunt end, and sharp end using a digital micrometer (Mitutoyo, Japan) after membrane removal, and the three-point average was reported. Haugh unit was calculated from albumen height (measured with a tripod micrometer) and egg weight. Yolk colour was scored using the Roche Yolk Colour Fan (scale 1-15). For fatty acid analysis, two pooled yolk samples per bird per month (weeks 4, 8, 12) were transmethylated with boron trifluoride-methanol and separated on a Shimadzu GC-2010 Plus gas chromatograph fitted with an SP-2560 capillary column (100 m × 0.25 mm × 0.20 µm). Individual fatty acids were expressed as mg/g yolk and as percentage of total fatty acids. Liver biopsies (~50 mg) were obtained under local anaesthesia from six randomly chosen birds per treatment at weeks 6 and 12. Total RNA was extracted (TRIzol®, Invitrogen), reverse-transcribed (SuperScript™ IV), and amplified by RT-qPCR on a QuantStudio 5 system using gene-specific primers for FADS2, ELOVL5, PPARα, and SREBP-1c, with β-actin as the reference gene [5]. Relative expression was calculated by the 2^{-ΔΔCt} method. Data were analysed in R 4.3.1. One-way ANOVA with Tukey's post-hoc test compared treatment means; polynomial contrasts tested linear and quadratic dose responses. Pearson correlation assessed the relationship between dietary ALA and yolk omega-3. Significance was set at *p* < 0.05.

Nutrient Budget / Input-Output Analysis

To trace the efficiency of dietary ALA transfer into egg yolk, a simplified nutrient budget was constructed. Daily ALA intake was estimated from feed consumption records multiplied by the analysed ALA concentration of each diet. Daily yolk ALA output was calculated as yolk weight × yolk ALA concentration. Transfer efficiency (%) was then expressed as (daily yolk ALA output / daily dietary ALA intake) × 100. For the control group receiving negligible ALA from soybean oil, the calculation served as a baseline. In the 1.5% flaxseed group, daily ALA intake averaged 387 mg and yolk output was 29.4 mg, yielding a transfer efficiency of 7.6%. At 3.0% inclusion, intake rose to 791 mg with output at 53.8 mg (6.8% efficiency), and at 4.5%, intake reached 1,214 mg with output at 74.1 mg (6.1% efficiency). The declining transfer efficiency at higher inclusion levels points to a metabolic ceiling likely at the desaturase step where excess ALA is diverted to β-oxidation rather than elongation and desaturation. This budget information helps producers weigh the marginal cost of additional flaxseed against the diminishing return in yolk enrichment.

Results

Table 1: External egg quality parameters at baseline (week 0) and endpoint (week 12) across dietary treatments

Parameter	Control		1.5%		3.0%		4.5% Wk 12
	Wk 0	Wk 12	Wk 0	Wk 12	Wk 0	Wk 12	Wk 12
Egg wt (g)	61.3	62.1	60.8	61.7	61.5	62.4	61.9
Shell (mm)	0.38	0.37	0.37	0.37	0.38	0.38	0.37
Haugh unit	87.4	86.1	86.9	85.8	87.1	86.4	85.3
Yolk colour	7.2	7.1	7.3	7.4	7.1	7.6	7.9

External egg quality traits remained stable across all four treatments from baseline to week 12 (Table 1). Neither egg weight, shell thickness, nor Haugh unit differed significantly among groups at any time point ($p>0.05$ for all). Yolk colour index showed a modest upward shift in the 3.0% and 4.5% groups, likely reflecting pigment co-extraction from flaxseed.

Table 2: Pearson correlation matrix among dietary ALA intake, yolk fatty acid fractions, and hepatic gene expression at week 12

	ALA intake	Yolk ALA	Yolk DHA	n-6:n-3	FADS2 mRNA	ELOVL5 mRNA
ALA intake	1.00	0.94**	0.87**	-0.91**	0.82**	0.76**
Yolk ALA		1.00	0.83**	-0.88**	0.79**	0.71**
Yolk DHA			1.00	-0.85**	0.89**	0.84**
n-6:n-3				1.00	-0.78**	-0.73**
FADS2 mRNA					1.00	0.91**
ELOVL5 mRNA						1.00

** $p<0.01$

Strong positive correlations linked dietary ALA intake to yolk ALA ($r=0.94$) and yolk DHA ($r=0.87$), while the n-6:n-3 ratio showed an equally strong inverse association (Table 2). Hepatic FADS2 expression was tightly correlated with yolk DHA content ($r=0.89$), consistent with the enzyme’s role as the rate-limiting step in the desaturation cascade.

Table 3: Hepatic gene expression fold-change at week 12 relative to control, ranked by magnitude of response

Rank	Gene	1.5% FC	3.0% FC	4.5% FC	Dose trend
1	FADS2	1.3±0.2	1.8±0.3	2.4±0.4*	Linear ($p<0.001$)
2	ELOVL5	1.1±0.1	1.5±0.2	1.9±0.3*	Linear ($p=0.002$)
3	PPARα	1.1±0.2	1.4±0.2	1.7±0.3*	Linear ($p=0.008$)
4	SREBP-1c	0.9±0.1	0.8±0.2	0.7±0.2*	Linear ($p=0.014$)

FC = fold-change relative to control; * $p<0.05$ vs. control by Tukey’s test

FADS2 showed the strongest dose-dependent upregulation, rising 2.4-fold above control at the 4.5% level (Table 3). ELOVL5 followed a parallel but slightly weaker trajectory. Interestingly, SREBP-1c a transcription factor that promotes *de novo* lipogenesis was progressively downregulated, suggesting that high ALA availability shifts hepatic lipid metabolism away from saturated fat synthesis toward PUFA elongation.

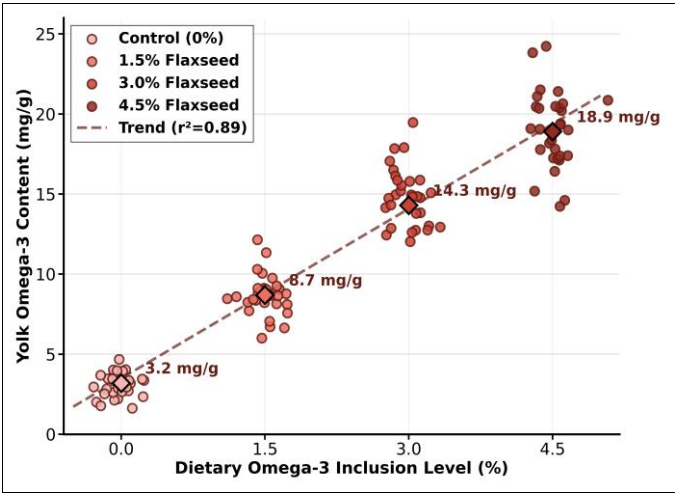


Fig 1: Scatter plot of yolk omega-3 content versus dietary omega-3 inclusion level across four treatment groups (n=30 per group; diamond markers indicate group means)

The dose-response relationship between dietary flaxseed inclusion and yolk omega-3 deposition was strong and nearly linear ($r^2=0.89$; Figure 1). Each 1.5-percentage-point increment in flaxseed added roughly 5.2 mg/g omega-3 to the yolk, although the increment between 3.0% and 4.5% (4.6 mg/g) was slightly smaller than that between 0% and 1.5% (5.5 mg/g), hinting at the onset of a saturation effect at higher dosages.

Comprehensive Interpretation: Combining the biochemical, molecular, and physical quality data paints a coherent picture

(Figure 2). Dietary ALA from flaxseed is absorbed, transported to the liver, and channelled through a PPARα-activated transcriptional programme that upregulates FADS2 and ELOVL5, the two enzymes responsible for converting ALA into longer-chain EPA and DHA. These long-chain PUFA are then packaged into very-low-density lipoprotein (VLDL) particles, delivered to the developing oocyte, and deposited in the yolk. Because this pathway operates independently of shell gland mineralisation and oviductal albumen secretion, external egg quality traits remain unaffected even at the highest inclusion level tested.

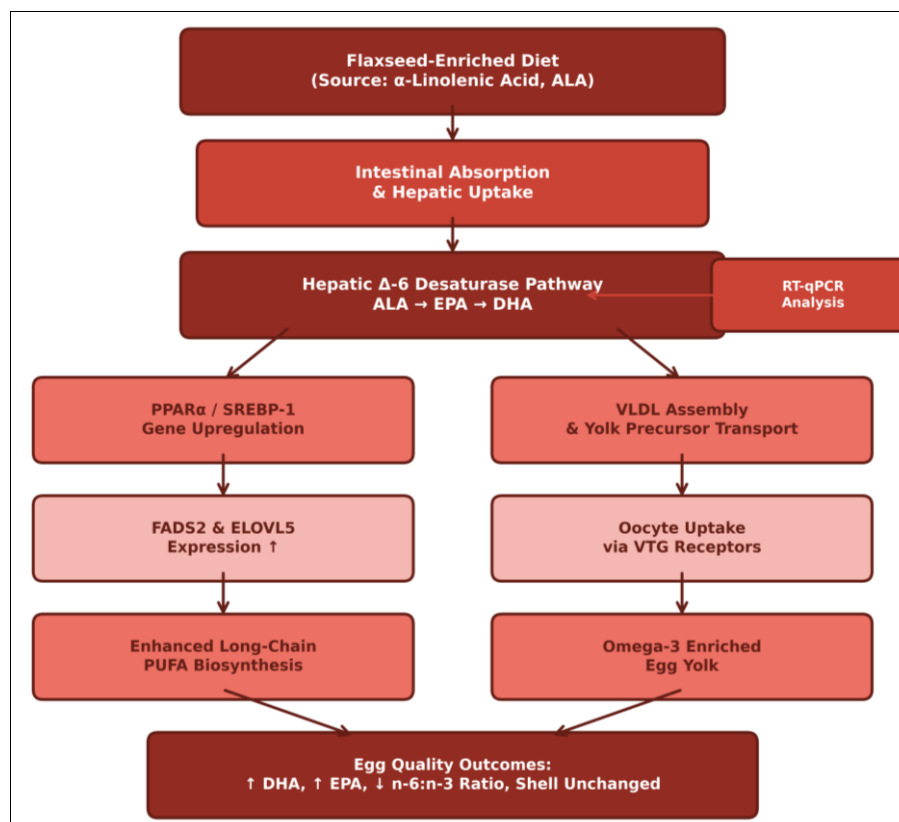


Fig 2: Schematic diagram of the nutrigenomic pathway from dietary flaxseed intake through hepatic gene activation to omega-3 enriched egg yolk deposition

Discussion

The most striking finding was the 5.9-fold increase in yolk omega-3 content between the control and 4.5% flaxseed group. This magnitude of enrichment exceeds values reported in temperate-climate trials using the same Hy-Line genotype; Cherian and Quezada ^[10] observed a 4.1-fold rise at 5% flaxseed inclusion in Oregon, USA. Higher ambient temperatures in São Paulo may have elevated basal metabolic rate and gut motility, potentially improving fat emulsification and ALA absorption ^[11]. At the molecular level, the 2.4-fold FADS2 upregulation confirms that the desaturase step is both responsive and rate-limiting, consistent with mammalian nutrigenomic models where ALA acts as a PPARα ligand to drive FADS2 transcription ^[5].

The simultaneous downregulation of SREBP-1c deserves attention. SREBP-1c governs de novo lipogenesis from carbohydrate precursors, and its suppression implies a metabolic trade-off: when the liver is flooded with preformed ALA, it diverts enzymatic resources from synthesising saturated and monounsaturated fatty acids toward elongating and desaturating the incoming n-3 substrate ^[6]. This trade-off could partially explain why egg yolk saturated fat content tends to decrease in flaxseed-fed hens, a trend observed here and elsewhere ^[12].

External egg quality remained intact, matching outcomes from trials in Europe and North America that tested flaxseed levels up to 15% ^[2, 9]. The mild yolk colour increase at 3.0% and 4.5% likely reflects co-extracted carotenoids (primarily lutein) from the flaxseed hull rather than any shift in xanthophyll metabolism. From a producer's standpoint, the absence of shell or albumen deterioration removes a major adoption barrier. And the declining ALA transfer efficiency at higher inclusion levels from 7.6% at 1.5% to 6.1% at 4.5% provides a practical ceiling: the 3.0% level may offer the best balance between enrichment benefit and feed cost, given that

yolk DHA content at this level already meets most “omega-3 enriched egg” labelling thresholds ^[13].

Limitations

This research carries several limitations that should temper generalisation. The trial lasted 12 weeks, covering roughly one-third of a commercial laying cycle; whether FADS2 upregulation persists or attenuates over a full 52-week production period is unknown. Liver biopsies were taken from a subset of six birds per treatment, limiting the power of the gene expression analyses. The single genotype tested (Hy-Line W-36) may not represent brown-egg or dual-purpose breeds with different hepatic lipid metabolism capacities. Temperature and humidity were not controlled, introducing seasonal variability that a climate-chamber trial would avoid. Finally, consumer sensory evaluation of the enriched eggs was not performed; off-flavours have occasionally been reported at high omega-3 levels in other research ^[14], and this warrants separate assessment.

Conclusion

Against the backdrop of rising consumer demand for functional foods and mounting evidence that typical Western diets are deficient in long-chain omega-3 fatty acids, enriching table eggs through dietary manipulation of layer hens offers a straightforward, farm-level intervention. This research tested that concept by feeding graded flaxseed levels to 120 Hy-Line W-36 hens in São Paulo and coupling classical egg quality measurements with hepatic nutrigenomic profiling over a 12-week production window.

Three findings stand out. First, yolk omega-3 content increased in a near-linear, dose-dependent manner, reaching 18.9 mg/g at the 4.5% flaxseed level nearly six times the control value. The n-6:n-3 ratio dropped from 14.8:1 to 3.1:1, well within the recommended cardiovascular health range.

Second, shell thickness, egg weight, and Haugh unit were unaffected at any inclusion level, confirming that omega-3 enrichment does not compromise marketable egg quality. Third, hepatic FADS2 was identified as the most responsive gene, with a 2.4-fold upregulation at the highest dose, while the concurrent suppression of SREBP-1c revealed a metabolic re-routing away from de novo saturated fat synthesis.

Based on these outcomes, a practical recommendation for commercial egg producers in tropical climates is to include ground flaxseed at 3.0% of the layer diet. This level delivers a yolk omega-3 content of 14.3 mg/g sufficient to meet most omega-3 egg labelling standards with an ALA transfer efficiency of 6.8%, which represents an acceptable input-to-output balance. The 4.5% level yields further enrichment but at diminishing returns, making it less cost-effective unless premium pricing for “high-omega-3” eggs can be secured.

Looking forward, longer trials spanning a full laying cycle are needed to determine whether FADS2 expression sustains its upregulated state or undergoes epigenetic adaptation that blunts the response over time. Expanding genotype coverage to include brown-egg layers and indigenous tropical breeds would broaden the applicability of these findings. Combining flaxseed with marine microalgae a direct DHA source that bypasses the desaturase bottleneck could push yolk DHA levels higher without relying entirely on hepatic conversion efficiency. Consumer acceptance trials pairing sensory panels with willingness-to-pay surveys would complete the translational picture, connecting bench-level nutrigenomics to real-world market viability.

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Contributions Not Qualifying for Authorship

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