

Isocaloric Substitution of Animal-Protein with Plant-Protein and Academic Performance in Spanish Adolescents: EHDLA Study

José Francisco López-Gil* (josefranciscolopezgil@gmail.com), Carlos Cristi-Montero (carlos.cristi.montero@gmail.com), Jacinto Muñoz-Pardeza (mpjacinto27@gmail.com), Camila Miño (camilamino501@gmail.com), Fiorella Quiroz-Cárdenas (fiorella.quiroz@yahoo.com), Rodrigo Yáñez-Sepúlveda (rodrigo.yanez.s@unab.cl), Doris Quiroz-Cárdenas⁹ (dorislqc@hotmail.com), Lucille Ridgell (lucilleridgell@gmail.com), Dong Keon Yon (yonkkang@gmail.com), Miguel López-Moreno (miguel.lopez@ufv.es).

Corresponding author

* José Francisco López-Gil, PhD.

✉ School of Medicine, Universidad Espíritu Santo, Samborondón, Ecuador.

📧 josefranciscolopezgil@gmail.com

Table S1. Classification of food frequency questionnaire items.

ID	Food item	Protein source	Food group	Protein (g/serving)	Notes
1	Milk	Animal	Dairy	3.0	Dairy product, high protein
2	Yogurt	Animal	Dairy	2.0	Dairy product
3	Chocolate	Other	Other	0.5	Low protein
4	Puffed cereals	Plant	Cereals	1.0	Cereal product
5	Maria biscuits	Other	Cereals	0.8	Cereal product, bakery
6	Chocolate biscuits	Other	Cereals	0.8	Cereal product, bakery
7	Muffins, cake	Other	Cereals	1.0	Cereal product, bakery
8	Croissant, donut, Danish pastry	Other	Cereals	1.2	Cereal product, bakery
9	Salad	Plant	Vegetables	0.5	Fresh vegetables
10	Green beans, chard, spinach	Plant	Vegetables	0.8	Fresh vegetables
11	Side vegetables	Plant	Vegetables	0.8	Fresh vegetables
12	Potatoes	Plant	Root vegetables	1.5	Starchy vegetable
13	Legumes	Plant	Legumes	4.0	High plant protein (>3 g/serving)
14	White rice	Plant	Cereals	1.5	Cereal grain
15	Pasta	Plant	Cereals	2.0	Cereal grain
16	Soups and creams	Other	Other	0.8	Soup/base
17	Eggs	Animal	Eggs	3.0	Animal protein source
18	Chicken or turkey	Animal	Poultry	8.0	Animal protein source (>6 g/serving)
19	Beef, pork, lamb	Animal	Red meat	7.0	Animal protein source (>6 g/serving)
20	Ground meat, sausage, hamburger	Animal	Processed meat	6.0	Processed animal protein (>6 g/serving)
21	White fish	Animal	White fish	7.0	Fish - lean (>6 g/serving)
22	Oily fish	Animal	Oily fish	8.0	Fish - fatty (>6 g/serving)
23	Seafood	Animal	Seafood	6.0	Shellfish/seafood (>6 g/serving)
24	Croquettes, empanadas, pizza	Other	Other	3.0	Mixed processed, mainly animal
25	Bread	Plant	Cereals	1.5	Cereal product
26	Ham, cured meats, deli meats	Animal	Processed meat	5.0	Processed meat (>6 g/serving)
27	Fresh cheese	Animal	Dairy	3.5	Dairy product
28	Other cheeses	Animal	Dairy	5.0	Dairy product (>3 g/serving)
29	Citrus fruits	Plant	Fruits	0.5	Fruit
30	Other fruits	Plant	Fruits	0.5	Fruit
31	Canned fruits	Plant	Fruits	0.3	Processed fruit
32	Natural fruit juice	Plant	Fruits	0.3	Fruit juice
33	Commercial fruit juice	Plant	Processed fruits	0.2	Fruit juice
34	Nuts	Plant	Nuts	2.5	High plant protein (>2 g/serving)
35	Dairy desserts	Other	Dairy	1.5	Dairy product
36	Cream or chocolate cakes	Other	Other	0.8	Processed baked goods, cereal-based
37	Candy	Other	Other	1.0	Candy/confectionery
38	Snack bags	Other	Other	1.0	Salty snacks/chips
39	Ice cream	Other	Other	1.0	Frozen dairy dessert
40	Sugar-sweetened beverages	Other	Other	0.0	Sugar-sweetened beverage
41	Low-calorie beverages	Other	Other	0.0	Non-caloric beverage
42	Wine, sangria	Other	Other	0.0	Alcoholic beverage
43	Beer	Other	Other	0.2	Alcoholic beverage
44	Non-alcoholic beer	Other	Other	0.1	Non-alcoholic beverage
45	Distilled beverages	Other	Other	0.0	Alcoholic beverage

Table S2. Comparison of participants included in the analytical sample versus those excluded.

Variable	Excluded from analytical sample N = 609¹	Included (analytical sample) N = 769¹	p-value²
Age (years)	15.0 (13.0, 17.0)	14.00 (13.0, 15.0)	<0.001
Sex			<0.001
Male	338 (56%)	342 (44%)	
Female	271 (44%)	427 (56%)	<0.001
Body mass index (kg/m ²)	21.9 (19.5, 25.9)	21.7 (19.3, 25.3)	0.571
Missing	117	0	
FAS-III (score)	8.0 (6.0, 9.0)	8.0 (7.0, 10.0)	<0.001
Missing	267	0	
GPA (score)	4.7 (3.0, 6.3)	6.3 (4.7, 8.0)	<0.001
Missing	115	0	
Language (score)	5.0 (3.0, 7.0)	7.0 (5.0, 8.0)	<0.001
Missing	44	0	
Maths (score)	4.0 (2.0, 6.0)	6.0 (4.0, 8.0)	<0.001
Missing	91	0	
Foreign language [English] (score)	5.0 (3.0, 7.0)	6.0 (5.0, 8.0)	<0.001
Missing	43	0	
YAP-S Physical activity (score)	2.7 (2.2, 3.1)	2.6 (2.2, 3.0)	0.665
Missing	296	0	
YAP-S Sedentary behaviors (score)	2.6 (2.2, 3.2)	2.6 (2.2, 3.0)	0.004
Missing	296	0	

¹Data expressed as median (first quartile, third quartile) or numbers (percentage). ²Wilcoxon rank sum test (for continuous variables); Pearson's chi-squared test (for categorical variables). FAS-III, Family Affluence Scale-III; GPA, grade point average.

Table S3. Sensitivity analysis examining the effect of a 5% isocaloric energy substitution of plant protein for animal protein on academic performance outcomes using a partition model approach (adjusted for father's educational level).

Outcome	B (95% CI)	β (95% CI)	P-value
GPA	1.19 (0.68, 1.70)	0.55 (0.32, 0.79)	<0.001
Language	1.13 (0.66, 1.61)	0.49 (0.28, 0.69)	<0.001
Maths	1.18 (0.66, 1.70)	0.47 (0.26, 0.68)	<0.001
Foreign language	1.40 (0.81, 1.98)	0.62 (0.36, 0.88)	<0.001

B = unstandardized coefficient; β = standardized coefficient (in SD units); CI = confidence interval. Substitution effect calculated using partition model approach. Represents the change in academic performance per 5% energy substitution from animal-based protein to plant-based protein sources. Models adjusted for energy intake, carbohydrates, fat, other protein, age, sex, educational level (father), sleep duration, physical activity, sedentary behaviors, and body mass index. Socioeconomic status was not included in the models. Coefficients and 95% CIs from robust regression models ('lmrob' function, 'KS2014' setting). Primary or less education (n = 257; 34.6%), secondary education (n = 314; 42.3%), university education (n = 172; 23.1%)

Table S4. Sensitivity analysis examining the effect of a 5% isocaloric energy substitution of plant protein for animal protein on academic performance outcomes using a partition model approach (adjusted for mother's educational level).

Outcome	B (95% CI)	β (95% CI)	P-value
GPA	1.07 (0.64, 1.50)	0.50 (0.30, 0.70)	<0.001
Language	0.96 (0.58, 1.34)	0.41 (0.25, 0.57)	<0.001
Maths	1.08 (0.63, 1.54)	0.43 (0.25, 0.61)	<0.001
Foreign language	1.25 (0.75, 1.75)	0.56 (0.33, 0.78)	<0.001

B = unstandardized coefficient; β = standardized coefficient (in SD units); CI = confidence interval. Substitution effect calculated using partition model approach. Represents the change in academic performance per 5% energy substitution from animal-based protein to plant-based protein sources. Models adjusted for energy intake, carbohydrates, fat, other protein, age, sex, educational level (mother), sleep duration, physical activity, sedentary behaviors, and body mass index. Socioeconomic status was not included in the models. Coefficients and 95% CIs from robust regression models ('lmrob' function, 'KS2014' setting). Primary or less education (n = 225; 29.4%), secondary education (n = 305; 39.9%), university education (n = 235; 30.7%)

Table S5. Distribution of protein energy density from fish, nuts, and reference plant food groups in the analytical sample (n = 769).

Food group	Median % energy (Q1, Q3)	Mean % energy (SD)	% with zero intake
White fish	1.44 (0.55, 2.47)	1.69 (1.54)	24.1
Oily fish	1.88 (0.91, 3.03)	2.15 (1.99)	21.6
Seafood/shellfish	0.85 (0.00, 1.52)	1.01 (1.11)	35.1
Nuts	0.78 (0.29, 1.34)	0.91 (0.84)	22.5
Legumes (reference plant staple)	1.50 (0.93, 2.20)	1.66 (1.19)	7.4
Cereals (reference plant staple)	0.11 (0.00, 0.41)	0.27 (0.39)	48.9

Q1, first quartile; Q3, third quartile; SD, standard deviation.

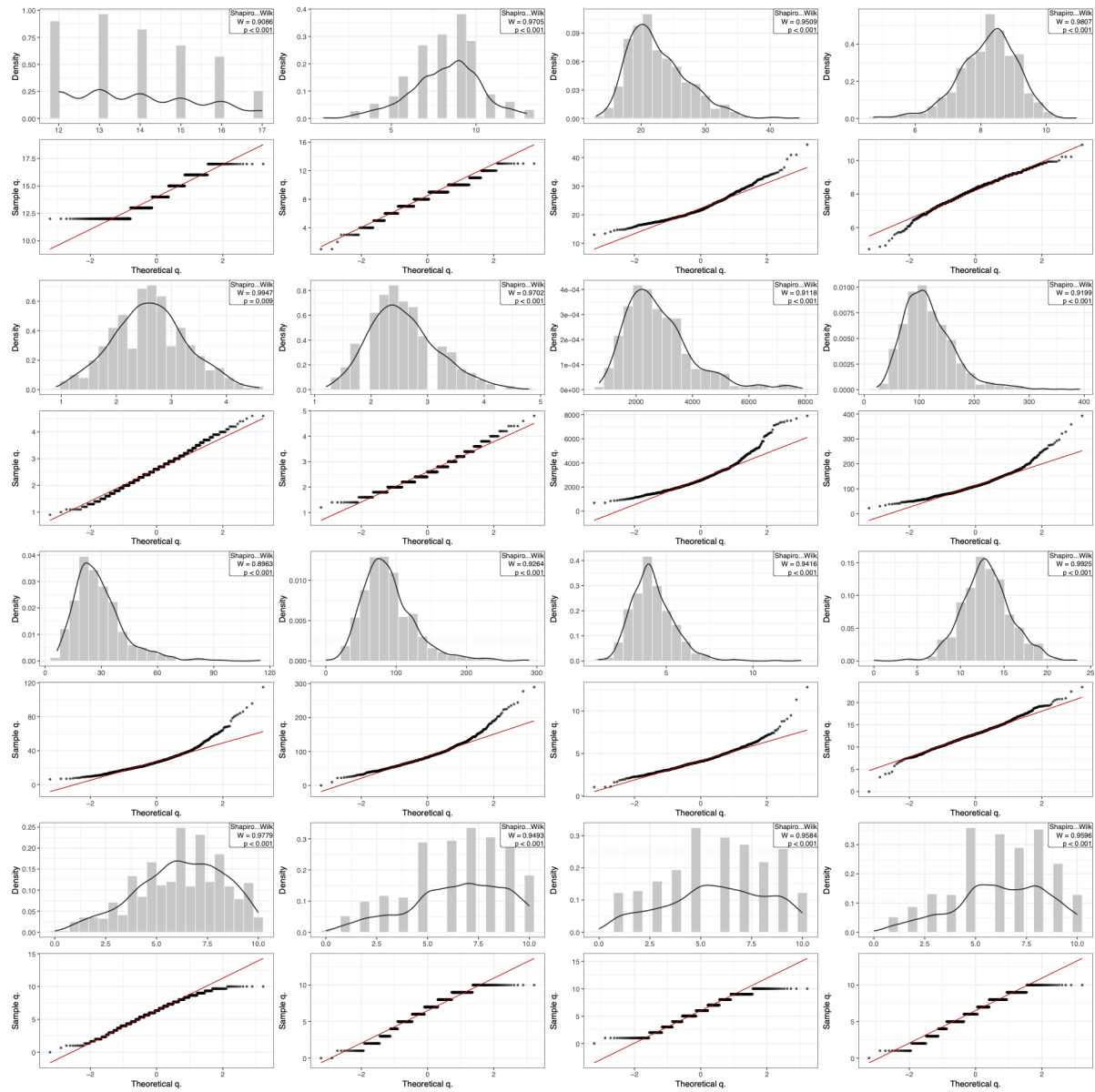


Figure S1. Normality diagnostics for continuous variables in the analytical sample (n = 769). Histogram + density (top), quantile–quantile (Q–Q) plot (bottom). Shapiro-Wilk statistic (W) and p–value are shown on the density plots.

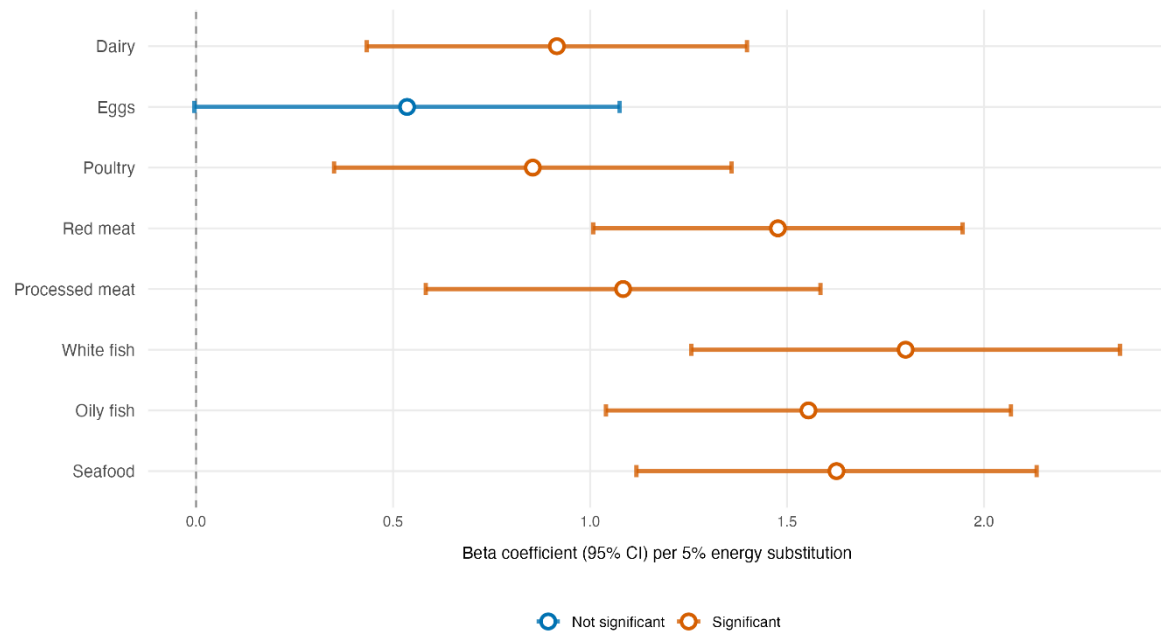


Figure S2. Supplementary forest plot showing unstandardized associations between isocaloric substitution of protein derived from specific animal-based food sources with plant-based protein and Grade Point Average (GPA). Beta coefficients (95% CI) per 5% energy substitution are shown in original units (points).

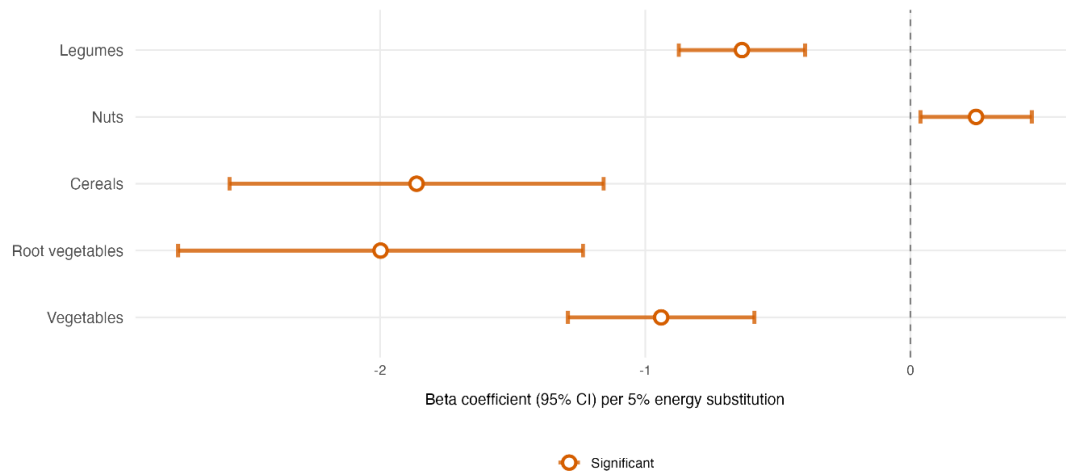


Figure S3. Supplementary forest plot showing unstandardized associations between isocaloric substitution of protein derived from specific animal-based sources with plant-based protein and Grade Point Average (GPA). Beta coefficients (95% CI) per 5% energy substitution are shown in original units (points).

Table S6. Sensitivity analysis of standardized associations (β) between replacing fish-derived protein with plant protein and grade point average (GPA) under alternative subsamples.

Fish subgroup	Subsample	N	β (95% CI)	P-value
White fish	Full analytical sample	769	0.84 (0.59, 1.09)	<0.001
White fish	Participants with fish intake > 0	584	1.17 (0.84, 1.50)	<0.001
White fish	Participants with intake $\geq$ median among consumers (1.89% energy)	292	1.19 (0.85, 1.52)	<0.001
Oily fish	Full analytical sample	769	0.73 (0.49, 0.96)	<0.001
Oily fish	Participants with fish intake > 0	603	0.99 (0.69, 1.28)	<0.001
Oily fish	Participants with intake $\geq$ median among consumers (2.36% energy)	302	0.90 (0.62, 1.19)	<0.001
Seafood/shellfish	Full analytical sample	769	0.76 (0.52, 1.00)	<0.001
Seafood/shellfish	Participants with fish intake > 0	499	0.99 (0.69, 1.28)	<0.001
Seafood/shellfish	Participants with intake $\geq$ median among consumers (1.28% energy)	250	0.64 (0.44, 0.83)	<0.001

Models used the partition approach with robust regression (lmrob, KS2014), adjusted for total energy intake, carbohydrate and fat density, residual protein, other animal protein sources (held constant), age, sex, FAS-III, sleep duration, physical activity, sedentary behavior, and BMI. Substitution effect = $\beta_{\text{plant}} - \beta_{\text{fish}}$. Estimates are standardized (SD units of GPA) per 5% energy substitution.