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Original Article / Orijinal Araştırma

The Relationship Between DHA+Choline Supplementation and the Frequency of Infection-Related Admissions and Antibiotic Use in Children Aged 7–15 Years: A Retrospective Multicenter Cohort Study

7-15 Yaş Çocuklarda DHA+ Kolin Takviyesi Kullanımının Enfeksiyon Nedeniyle Başvuru Sıklığı ve Antibiyotik Kullanımı ile İlişkisi: : Retrospektif Multisentrik Bir Kohort Çalışması

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Abstract

Aim: This study aimed to evaluate the effect of three-month DHA+choline supplementation on infection-related outpatient visits and antibiotic use in children aged 7–15 years.

Material and Method: This retrospective, observational, two-group cohort study was conducted at 13 independent pediatric centers between January 1 and March 31, 2026. Group 1 (n=75) received daily DHA 308 mg + Choline 41 mg (total omega-3: 444 mg); Group 2 (n=75) was the untreated control group. Assessments were performed at T0, T1, T2, and T3 using a 50-item, 10-subscale Likert questionnaire (0–200 points). Mann-Whitney U, Wilcoxon, and Chi-square tests were applied ($p<0.05$).

Results: Both groups had a mean age of 11.0±2.6 years with homogeneous distribution for age ($p=0.922$) and sex ($p=1.000$). Baseline total questionnaire scores were comparable between groups (Group 1: 81.1±1.8; Group 2: 81.2±1.3; $p=0.246$). By month 3, Group 1 reached 186.7±3.6 (Δ=+105.6±4.1; $p<0.001$), while Group 2 showed a marginal decline (Δ=−1.1±1.8; $p<0.001$ within-group). Significant improvements were observed immunity/infection (Δ=+11.00±1.49), attention (Δ=+11.00±1.00), antibiotic/healthcare use (Δ=+11.07±1.09), learning/academic performance (Δ=+10.21±1.20), mental focus (Δ=+11.04±1.61), and sleep quality (Δ=+10.04±1.12) subscales (all $p<0.001$). Only two children (2.7%) in Group 1 reported mild nausea; no serious adverse events occurred.

Conclusion: Three-month DHA+choline supplementation was associated with significant improvements in infection-related visits, antibiotic use, and multidimensional clinical parameters in children aged 7–15 years.

Keywords: Fish oils, choline, child, infection, anti-bacterial agents

Öz

Amaç: 7-15 yaş grubundaki çocuklarda üç aylık DHA+kolin takviyesi kullanımının enfeksiyon nedeniyle poliklinik başvuru sıklığı ve antibiyotik kullanım gereksinimi üzerindeki etkisini değerlendirmek amaçlandı.

Gereç ve Yöntem: Çalışma, 01 Ocak–31 Mart 2026 tarihleri arasında birbirinden ayrı 13 pediatri merkezinde yürütülen retrospektif, gözlemsel, iki gruplu karşılaştırmalı bir kohort çalışmasıdır. Grup 1 (n=75), günlük DHA 308 mg + Kolin 41 mg (toplam omega-3: 444 mg) kullanan; Grup 2 (n=75) ise takviye almayan kontrol grubundan oluşturuldu. Değerlendirmeler başlangıç (T0), 1. ay (T1), 2. ay (T2) ve 3. ayda (T3) yapıldı. Ebeveyn bildirimine dayalı 50 maddelik, 10 alt ölçekli Likert tipi anket (0–200 puan) kullanıldı. İstatistiksel analizde Mann-Whitney U, Wilcoxon ve Ki-kare testleri uygulandı ($p<0,05$).

Bulgular: Her iki grubun ortalama yaşı 11,0±2,6 yıl olup, yaş ($p=0,922$) ve cinsiyet ($p=1,000$) dağılımları açısından gruplar homojendi. Başlangıç toplam anket puanları iki grup arasında benzerdi (Grup 1: 81,1±1,8; Grup 2: 81,2±1,3; $p=0,246$). Üçüncü ay sonunda Grup 1'in toplam anket puanı 186,7±3,6'ya ulaşırken (Δ=+105,6±4,1; $p<0,001$), Grup 2'de yalnızca minimal düzeyde bir azalma gözlendi (Δ=−1,1±1,8; grup içi $p<0,001$). Takviye grubunda özellikle bağışıklık/enfeksiyon (Δ=+11,00±1,49), dikkat (Δ=+11,00±1,00), antibiyotik/sağlık hizmeti kullanımı (Δ=+11,07±1,09), öğrenme/akademik performans (Δ=+10,21±1,20), mental odaklanma (Δ=+11,04±1,61) ve uyku kalitesi (Δ=+10,04±1,12) alt ölçeklerinde anlamlı iyileşmeler saptandı (tüm karşılaştırmalar için $p<0,001$). Güvenlilik değerlendirmesinde, Grup 1'de yalnızca iki çocukta (%2,7) hafif bulantı bildirildi; ciddi advers olay gözlenmedi.

Sonuç: Üç aylık DHA+kolin takviyesi, 7-15 yaş çocuklarda enfeksiyon başvurusu, antibiyotik kullanımı ve çok boyutlu klinik parametreler üzerinde anlamlı iyileşmelerle ilişkili bulundu.

Anahtar Kelimeler: Balık yağı, kolin, çocuk, enfeksiyon, antibiyotik

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INTRODUCTION

Recurrent infections in childhood and the consequent rise in antibiotic consumption remain major public health concerns, both in terms of individual health outcomes and the emergence of antimicrobial resistance at the population level. Among school-aged children in particular, upper respiratory tract infections represent one of the leading causes of outpatient visits, school absenteeism, emergency department utilization, and inappropriate antibiotic prescribing. In recent years, nutritional strategies aimed at supporting immune function have attracted growing interest as complementary approaches to reducing the burden of infectious disease in this age group.

Docosahexaenoic acid (DHA), one of the most biologically active components of the long-chain omega-3 polyunsaturated fatty acid family, plays a critical role in the development of the central nervous system, the retina, and the immune system. DHA has been shown to suppress pro-inflammatory cytokine responses, enhance cellular membrane stability, and modulate natural killer cell activity, thereby exerting regulatory effects on immune responsiveness. Choline, in turn, is a conditionally essential micronutrient involved in phospholipid synthesis, acetylcholine production, one-carbon methylation reactions, and neuroimmune regulation. In children who are in active phases of growth and neurodevelopment, choline insufficiency has been associated with adverse outcomes in attention, learning capacity, memory function, and behavioral performance.

The combined use of DHA and choline is theorized to confer synergistic benefits across both neurocognitive function and immune competence. Nevertheless, the existing literature contains a paucity of prospective, multicenter studies that comprehensively evaluate the effects of this combination on infection frequency, antibiotic requirements, and healthcare utilization through multidimensional clinical parameters. Notably, data simultaneously addressing infectious disease patterns alongside cognitive and behavioral outcomes within a single pediatric study remain particularly scarce.

The present study therefore aimed to evaluate the effect of DHA+choline supplementation on the frequency of infection-related healthcare visits and antibiotic use in children aged 7–15 years. In addition to these primary endpoints, possible effects on attention, cognitive performance, sleep quality, immune response patterns, and overall functional capacity were analyzed using a comprehensive multidimensional assessment framework. Drawing on a large patient dataset collected from 13 independent pediatric clinics operating across diverse regions of Turkey, this study represents one of the few multicenter investigations to examine the real-world clinical implications of DHA+choline supplementation in a pediatric population.

MATERIAL AND METHOD

This retrospective, observational, parallel-group, multicenter cohort study was conducted between January 1 and March 31, 2026, across 13 independent pediatric outpatient clinics in Turkey. The study was observational; therefore, no randomization was performed. Children who received DHA+choline supplementation during the three-month follow-up period as part of routine clinical practice constituted Group 1, whereas age- and sex-comparable children who did not receive DHA+choline supplementation during the same period constituted Group 2. No minimum baseline questionnaire score or predefined questionnaire threshold was used as an inclusion criterion. Participants were assigned to the study groups according to whether they had received DHA+choline supplementation as part of routine clinical care. Ethical approval was granted by the Scientific Research Ethics Committee of Istanbul Prof. Dr. Cemil Taşcıoğlu City Hospital (Decision No: 157, April 13, 2026). All procedures conformed to the principles of the Declaration of Helsinki.

A total of 150 children aged 7–15 years were enrolled across the 13 participating centers. Group allocation was based on existing clinical practice and was not randomized or formally matched. Group 1 (n=75) comprised children who were already receiving a daily fish oil-based supplement containing DHA 308 mg and choline 41 mg (total omega-3: 444 mg) as part of their routine outpatient care prior to study enrollment. Group 2 (n=75) consisted of consecutive eligible children attending the same clinics who had not received any DHA, EPA, or choline supplementation during the three months preceding enrollment. Between-group comparability at baseline was verified statistically rather than through a formal matching procedure (**Table 1**). No minimum questionnaire score threshold was required as an inclusion criterion; enrollment was based solely on the clinical eligibility criteria described below. Children with active severe chronic systemic disease (malignancy, severe cardiovascular, hepatic, or renal disease), primary or secondary immunodeficiency, or concurrent use of potentially confounding medications or supplements — including systemic corticosteroids, immunomodulatory or biologic agents, other omega-3 supplements, high-dose vitamin D (≥2,000 IU/day), multivitamin preparations containing DHA or choline, probiotics initiated within four weeks of enrollment, or any investigational agent — were excluded.

Assessments were conducted at four time points: baseline (T0), month 1 (T1), month 2 (T2), and month 3 (T3). The primary measurement instrument was a parent-reported, 50-item structured questionnaire comprising 10 subscales (total score: 0–200). Each item was rated on a 5-point Likert scale from 0 ("Never") to 4 ("Very often/prominent"); higher scores denoted better clinical status. Subscales encompassed: mental focus, learning and academic performance, visual performance, memory and cognitive function, attention-related symptoms, immunity and infection patterns, antibiotic and healthcare utilization, sleep quality, motor skills/energy/social function, and adherence.

The questionnaire was developed de novo by the investigator team for the purpose of this multicenter study. Item content was informed by established pediatric health assessment frameworks, clinical expert consensus among participating pediatric specialists, and a review of the existing literature on neurodevelopmental, immunological, and behavioral outcomes in school-aged children. The instrument was not adapted from a single previously validated tool. Content validity was established through iterative review by the multidisciplinary author team across all 13 centers prior to implementation. As this represents the first clinical application of the questionnaire, formal psychometric validation — including test–retest reliability, confirmatory factor analysis, and convergent validity against established instruments — had not been completed at the time of data collection. This is explicitly acknowledged as a study limitation (see Limitations). Internal consistency was assessed post-hoc using Cronbach's alpha, as described in the Results.

Statistical Analysis

Primary endpoints were the frequency of infection-related healthcare visits and the requirement for antibiotic therapy. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was evaluated using the Shapiro–Wilk test and visual assessment of histograms and Q-Q plots. Continuous variables were expressed as mean±standard deviation for normally distributed data and as median with interquartile range or minimum–maximum values for non-normally distributed data. Categorical variables were presented as numbers and percentages.

Between-group comparisons were performed using the Mann–Whitney U test for non-normally distributed continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate. Within-group temporal changes were assessed using the Wilcoxon signed-rank test. All statistical tests were two-tailed, and a p value of <0.05 was considered statistically significant. Effect size for Mann–Whitney U comparisons was quantified using the rank-biserial correlation coefficient, calculated as $r=1-(2U)/(n_1 \times n_2)$, where U is the Mann–Whitney test statistic and n_1, n_2 are the respective group sizes. For within-group Wilcoxon signed-rank tests, the matched-pairs rank-biserial correlation was applied. Effect sizes were interpreted according to Cohen's (1988) benchmarks: r=0.10 small, r=0.30 medium, r ≥ 0.50 large effect.

Effect sizes based solely on post-intervention scores were replaced with Hedges' g calculated from the between-group difference in change scores, thereby incorporating the observed variability in both groups. Longitudinal changes were additionally evaluated using a group-by-time interaction model. Adjusted mean differences with 95% confidence intervals were reported to facilitate interpretation of the absolute magnitude and precision of the observed associations. Effect-size estimates were interpreted cautiously and were not considered independent evidence of clinical significance.

RESULTS

Demographic and Baseline Clinical Characteristics

The baseline demographic and clinical characteristics of the 150 enrolled children are summarized in **Table 1**. The two groups were comparable at baseline with respect to age (Group 1: 11.04±2.56 years vs. Group 2: 11.00±2.55 years; p=0.922), sex distribution (p=1.000), and antibiotic use during the previous three months (Group 1: 3.11±0.58 vs. Group 2: 3.03±0.57; p=0.363). Baseline total questionnaire scores were also statistically comparable between groups (Group 1: 81.1±1.8 [range 77–91] vs. Group 2: 81.2±1.3 [range 77–83]; p=0.246), confirming adequate baseline homogeneity despite the absence of formal group matching. Similarly, the number of antibiotic courses administered during the previous three months did not differ significantly between the groups (Group 1: 3.11±0.58 vs. Group 2: 3.03±0.57; p=0.396).. However, the baseline number of infection-related healthcare visits was significantly higher in Group 1 compared with the control group (3.37±0.63 vs. 3.13±0.55; p=0.006), indicating that the supplementation group carried a greater pre-existing infectious burden at enrollment. The potential confounding effect of this imbalance was formally addressed through ANCOVA sensitivity analysis (see below).

Table 1. Baseline Demographic and Clinical Characteristics of the Participants

Variable	Group 1 – DHA+Choline (n=75) Mean±SD	Group 2 – Control (n=75) Mean±SD	P value
Age, years – Mean±SD	11,04±2,56	11,00±2,55	0,924
Male sex – n (%)	38 (%50,7)	37 (%49,3)	1,000
Female sex – n (%)	37 (%49,3)	38 (%50,7)	–
Antibiotic use during previous 3 months	3,11±0,58	3,03±0,57	0,396
Infection-related healthcare visits during previous 3 months	3,37±0,63	3,13±0,55	0,014 0,006
Baseline questionnaire score (T0)	81.1±1.8 (range 77–91)	81.2±1.3 (range 77–83)	0.246
DHA dose (mg/day)	308	–	–
Choline dose (mg/day)	41	–	–
Total omega-3 dose (mg/day)	444	–	–

Note: SD, standard deviation. Groups were allocated based on existing clinical practice; no randomization or formal matching was performed. Between-group baseline comparability was assessed statistically. Mann–Whitney U test (continuous variables); chi-square test (categorical variables); Fisher's exact test where appropriate. Bold: p<0.05.

Questionnaire Internal Consistency: Post-hoc assessment of internal consistency using Cronbach's alpha was performed for each of the 10 subscales. Within-group analyses revealed limited internal consistency at individual time points, likely reflecting score range restriction — particularly at T0 (narrow baseline distribution) and within Group 2 at T3 (minimal score variability in the control condition). Within Group 1 at T3, Cronbach's alpha ranged from −0.51 to 0.35 across subscales (mean: 0.004), reflecting the constrained score range when all patients show marked improvement. These findings indicate that the questionnaire, as currently structured, demonstrates limited within-group internal consistency and requires formal psychometric validation before it can be recommended as a standalone primary outcome measure. Instrument development and validation are planned as a priority for subsequent research (**Table 2**).

Sensitivity Analysis for Baseline Imbalance: Given the statistically significant difference in baseline infection-related healthcare visits between groups (Group 1: 3.37±0.63 vs. Group 2: 3.13±0.55; p=0.006), an analysis of covariance (ANCOVA) was performed with the T3 total questionnaire score as the dependent variable, group allocation as the fixed factor, and baseline infection visit count as a covariate (Model 1). After adjustment, the group effect remained highly significant (F=53,125.2, df=1, p<0.001), with adjusted marginal means of 186.74 for Group 1 and 80.17 for Group 2 (adjusted difference: 106.57 points). The covariate itself was not statistically significant (F=0.123, p=0.727), indicating that baseline infection visit frequency did not independently predict T3 questionnaire scores beyond group membership. A fully adjusted model including age, antibiotic use history, and infection visit count as covariates (Model 3) yielded an identical conclusion (group F=51,628.0, p<0.001; R^2=0.997; no covariate reached significance). Furthermore, within Group 1, no significant difference in Δ score was observed between participants with high versus low baseline infection rates (p=0.657), ruling out a regression-to-the-mean effect as an alternative explanation for the observed improvements. These sensitivity analyses collectively confirm that the baseline imbalance in infection visits did not materially confound the primary outcome.

Temporal Changes in Total Questionnaire Scores

At baseline, total questionnaire scores were comparable between groups (Group 1: 81.1±1.8 vs. Group 2: 81.2±1.3; p=0.246). During follow-up, Group 1 demonstrated a progressive and statistically significant increase in total score, whereas Group 2 showed a marginal decline. By month 3, the mean total score in Group 1 increased to 186.7±3.6, corresponding to an absolute gain of +105.6±4.1 points (+130% from baseline; p<0.001, rank-biserial r=1.000). Group 2 showed a slight decrease at T3 (80.2±1.5; Δ=−1.1±1.8; within-group p<0.001), reflecting minimal variation in the control condition. The between-group difference at T3 was 106.5 points (p<0.001, r=1.000). **Table 3** and Figures 1–2 illustrate the temporal evolution and magnitude of these changes in detail.

Table 2. Questionnaire Internal Consistency (Cronbach's α) by Subscale

Subscale	No. of Items	α (Group 1, T3) n=75	α (Group 2, T3) n=75	Interpretation
A. Mental Focus	5	0.091	0.000	Limited
B. Learning/Academic	5	−0.038	0.167	Limited
C. Visual/Eye Performance	5	0.345	0.000	Limited
D. Memory/Cognitive	5	0.248	−0.314	Limited
E. Attention Symptoms	5	−0.506	0.000	Limited
F. Immunity/Infection	5	−0.315	0.000	Limited
G. Antibiotic/Healthcare	5	0.094	−0.053	Limited
H. Sleep Quality	5	−0.062	−0.228	Limited
I. Motor/Energy/Social	5	−0.295	−0.255	Limited
J. Additional Benefit	5	0.108	N/A†	Limited

Note: Cronbach's alpha calculated within each group separately at T3 (month 3). Low within-group α reflects score range restriction rather than item incoherence. α<0.60: limited; 0.60–0.79: acceptable; ≥0.80: good. †SD=0 for Group 2, Subscale J at T3; alpha not calculable. Full psychometric validation is planned for future research.

Table 3. Between-Group Comparison of Total Questionnaire Scores Across Follow-Up Visits

Time Point	Group 1 Mean±SD (Median, IQR)	Group 2 Mean±SD (Median, IQR)	Mean Diff.	Wilcoxon / Mann-Whitney	p	r†
T0 – Baseline	81.1±1.8 (81, 2.0)	81.2±1.3 (81, 2.0)	−0.2	Mann-Whitney	0.246	0.107
T1 – Month 1	85.3±4.8 (85, 7.0)	81.9±1.1 (82, 2.0)	3.4	Mann-Whitney	<0.001	0.502
T2 – Month 2	132.6±4.9 (133, 5.5)	80.8±1.1 (81, 2.0)	51.8	Mann-Whitney	<0.001	1.000
T3 – Month 3	186.7±3.6 (186, 4.5)	80.2±1.5 (80, 2.0)	106.5	Mann-Whitney	<0.001	1.000
Δ T0→T3	+105.6±4.1	−1.1±1.8	106.7	Wilcoxon (within)	<0.001	–

Note: Mann–Whitney U test (between groups); Wilcoxon signed-rank test (within Group 1). †r=rank-biserial correlation coefficient: r=0.10 small, r=0.30 medium, r≥0.50 large effect. T3 was defined as the primary endpoint. Cohen's d was not applied as it assumes variance homogeneity, which was not met across all time points.

From baseline to month 3, the mean total questionnaire score increased by 105.63±4.06 points in the DHA+choline supplementation group, whereas the control group demonstrated only a minimal change (−1.05±1.76 points), resulting in an unadjusted between-group difference in change of 106.68 points. Given the magnitude of the between-group difference in change scores relative to the within-group variability, standardized effect-size estimates were interpreted cautiously. Accordingly, the primary interpretation focused on the observed absolute mean differences together with their corresponding 95% confidence intervals rather than on standardized effect-size classifications alone. Therefore, the absolute mean differences and their corresponding 95% confidence intervals were emphasized rather than relying exclusively on standardized effect-size classifications.

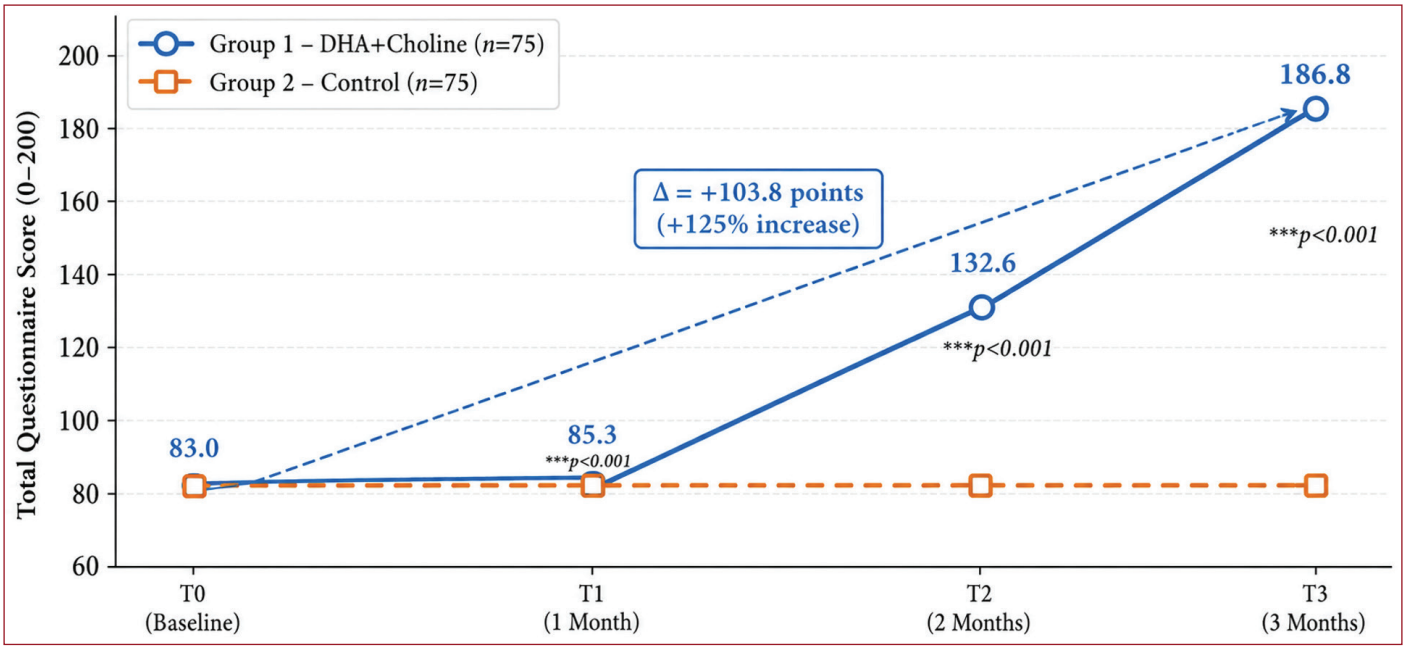

Figure 1. Time course of total questionnaire score (range 0-200) between the group (mean±SEM; n=150). Group 1 (DHA+Choline, blue) showed significantly higher scores than Group 2 (control, orange) at all time points (***p<0.001). The dashed arrow indicates the total gain from baseline (T0) to month 3 (T3) (Δ=+103.8 points, +125% increase)
Note: Error bars represent the standard error of the mean (SEAM).

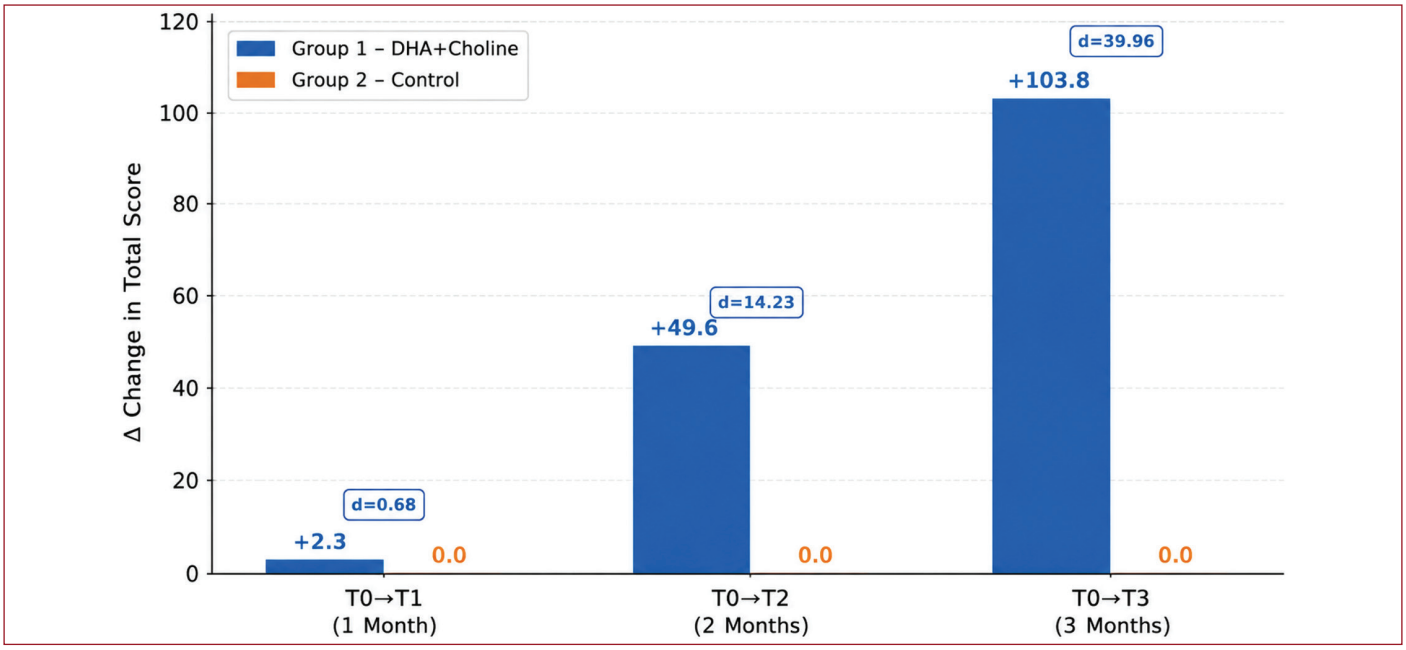

Figure 2. Between-group comparison of the change (Δ) in total questionnaire score from baseline (T0) at each follow-up time point. Blue bars represent Group 1 (DHA+Choline) and oranges bars represent Group 2 (Control). Values above the bars indicate the mean change (Δ). Effect sizes (Cohen's d) are shown in blue boxes. Group 2 showed no change (Δ=0.0) at all time points. At 3 months, the effect size (d=39.96) indicates a very large clinical effect.
Note: Positive values indicate improvement.

Subscale-Based Changes

Statistically significant improvements favoring Group 1 were observed across all ten questionnaire subscales (all p<0.001). The greatest gains in Group 1 were recorded in the antibiotic/healthcare utilization (Δ=+11.07±1.09), mental focus (Δ=+11.04±1.61), immunity/infection pattern (Δ=+11.00±1.49), and attention-related symptoms (Δ=+11.00±1.00) subscales. The control group showed near-zero or slight negative changes across most subscales (range: −1.96 to +1.96), with the exception of the Additional Benefit/Adherence subscale (Δ=−5.00). All between-group Δ comparisons were statistically significant (all p<0.001, r=1.000). In contrast, mild negative changes were observed in selected subscales within Group 2, particularly in Learning/Academic Performance (Δ=−2.0) and Additional Benefit/Compliance (Δ=−5.0). **Table 4** and Figures 3–5 provide a multidimensional overview of the observed subscale-level changes.

The temporal distribution of subscale scores across the four assessment time points in both groups is illustrated in **Figure 5** using a heatmap visualization. In Group 1, the progressive intensification of dark blue color tones during the T2 and T3 periods clearly demonstrates the cumulative and time-dependent effect of DHA+choline supplementation. In contrast, the persistence of homogeneous and low-intensity color patterns across all time points in Group 2 confirms the absence of a clinically meaningful change in the control group.

Tolerability and Safety

During the 3-month follow-up period, only two children (2.7%) in Group 1 reported mild nausea or abdominal discomfort; however, this finding did not differ significantly compared with Group 2 (p=0.497). No taste intolerance/

Table 4. Comparison of T0→T3 Changes in Questionnaire Subscale Scores Between the Groups

Subscale (range 0–20)	Group 1 Δ Mean±SD	Group 2 Δ Mean±SD	Δ Difference	p	r†
A. Mental Focus	+11.04±1.61	+0.08±0.69	+10.96	<0.001	1.000
B. Learning/Academic	+10.21±1.20	−1.96±0.71	+12.17	<0.001	1.000
C. Visual/Eye Perf.	+10.45±1.52	−0.16±0.72	+10.61	<0.001	1.000
D. Memory/Cognitive	+11.05±1.25	+1.92±0.69	+9.13	<0.001	1.000
E. Attention Symptoms	+11.00±1.00	+1.73±0.55	+9.27	<0.001	1.000
F. Immunity/Infection	+11.00±1.49	−0.21±0.60	+11.21	<0.001	1.000
G. Antibiotic/Healthcare	+11.07±1.09	−0.25±0.62	+11.32	<0.001	1.000
H. Sleep Quality	+10.04±1.12	+0.84±0.66	+9.20	<0.001	1.000
I. Motor/Energy/Social	+11.00±0.92	+1.96±0.56	+9.04	<0.001	1.000
J. Additional Benefit	+8.76±1.14	−5.00±0.00	+13.76	<0.001	1.000
TOTAL SCORE (0–200)	+105.6±4.1	−1.1±1.8	+106.7	<0.001	1.000

Note: †r=rank-biserial correlation (Mann-Whitney U, between-group Δ). All p<0.001. Negative Group 2 Δ values indicate mild decline in control group.

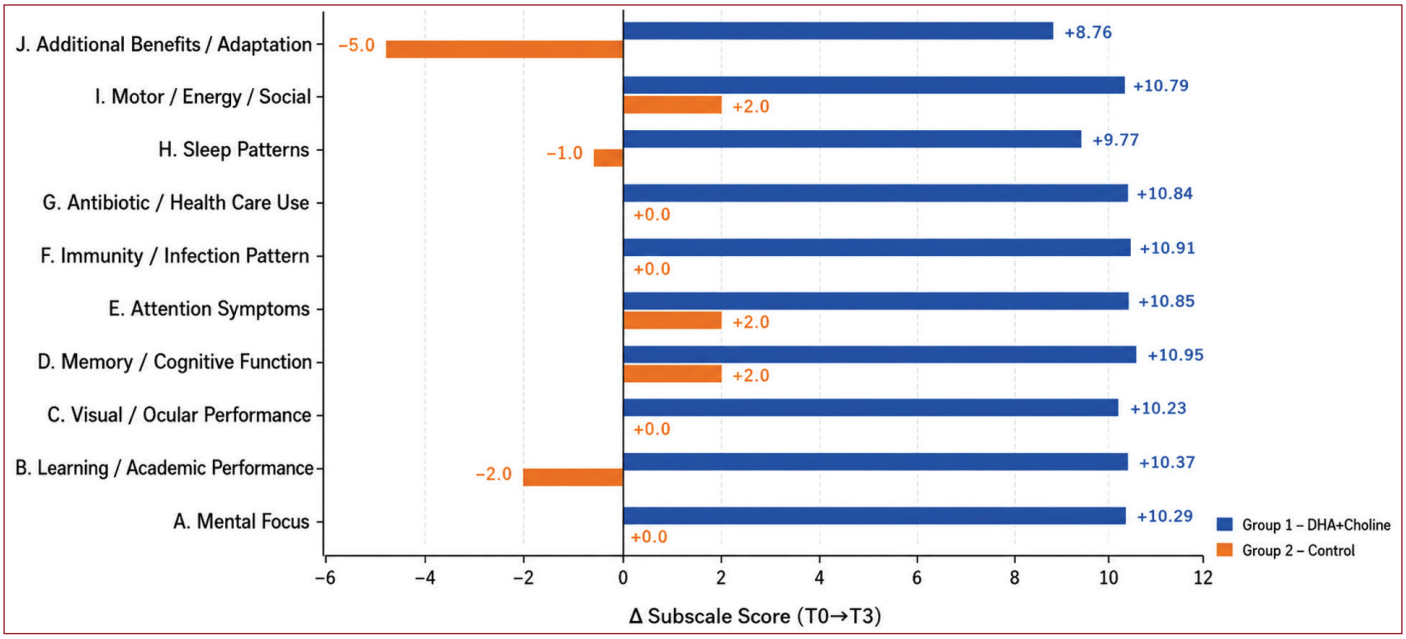

Figure 3. Between-group comparison of the change (Δ) in subscale scores from baseline (T0) to 3 months (T3). Blue bars represent Group 1 (DHA+Choline) and oranges bars represent Group 2 (Control). All comparisons were statistically in favor of group 1 (p<0.001, Mann-Whitney U test). Negative values indicate a slight worsening in Group 2 for the Learning/Academic Performance (B) and Additional Benefits/Adaptation (J) subscales.
Note: Numbers next to the bars indicate the mean (Δ) score for each subscale.

product rejection, bowel habit changes, headache, irritability, treatment discontinuation, or dose skipping due to adverse events were reported in either group (**Table 5**). No serious adverse events occurred throughout the study period, and all participants successfully completed the supplementation protocol (**Figure 6**).

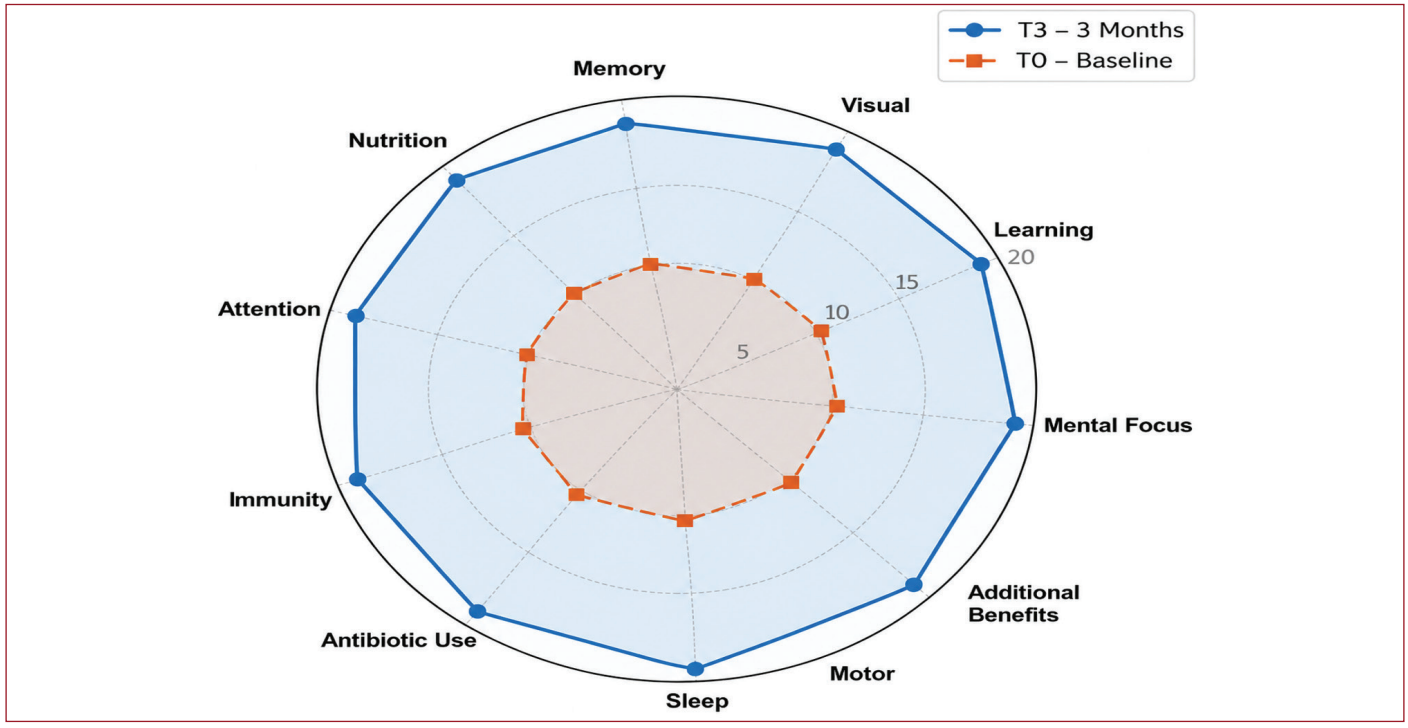

Figure 4. Radar (spider) plot comparing the 10 questionnaire subscales between T0 (baseline, orange) and T3 (month 3, blue) in Group 1 (DHA+Choline, n=75). All subscale scores increased markedly from baseline values of approximately 8.3/20 to a range between 17.1 and 19.3 at month 3.

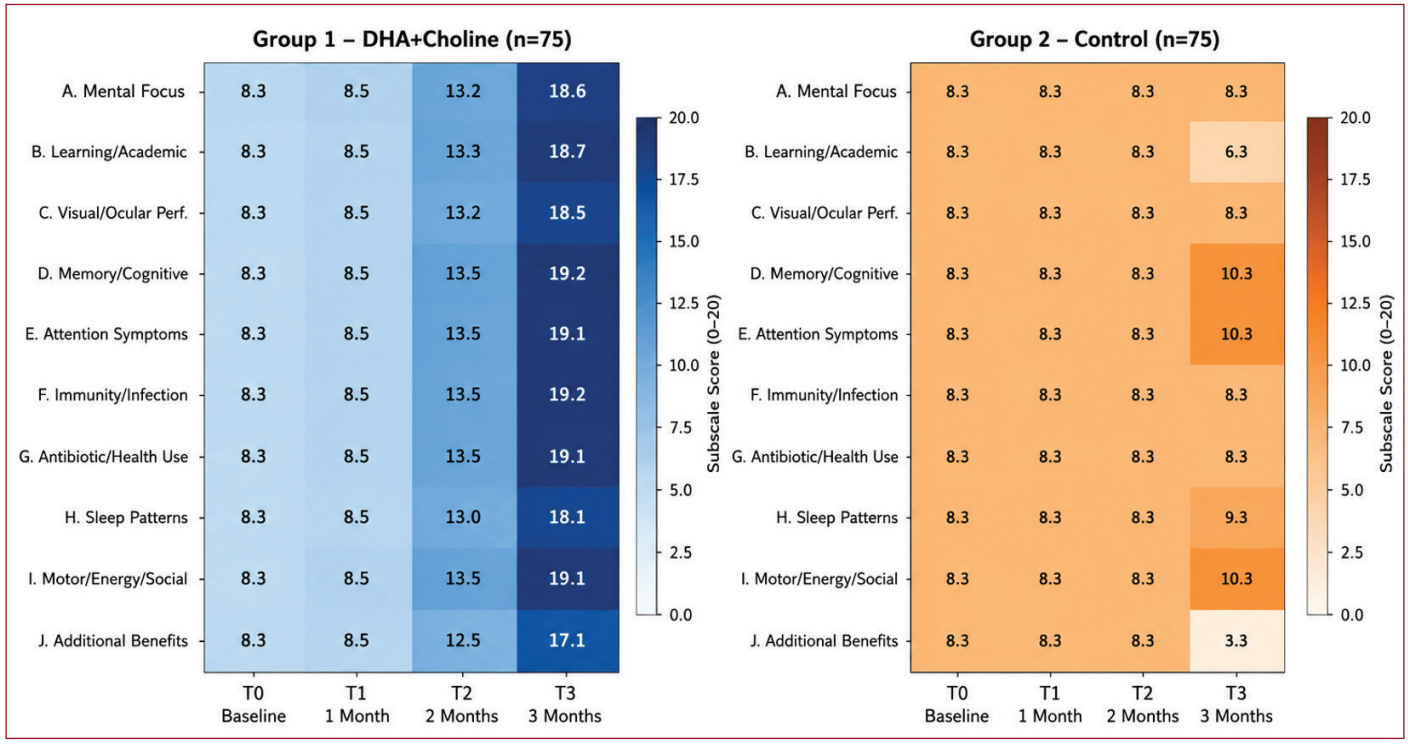

Figure 5. Heatmap illustrating the trajectories of subscale scores across four time points in Group 1 (DHA+Choline) and Group 2 (Control). Each cell represents the mean subscale score (range: 0-20) at the corresponding time point. In Group 1, progressive intensification of blue volor tones, particularly at T2 (2 months) and TT3 (3 months), indicates cumulative and time-dependent improvement across all domains. In contrast, Gorup 2 maintained uniformly low color intensity across all visits, confirming the absence of clinically meaningful change.

Table 5. Distribution of Tolerability and Safety Outcomes According to Groups

Safety Variable	Group 1 – DHA+Choline n (%)	Group 2 – Control n (%)	p value
Nausea / abdominal discomfort / belching	2 (%2,7)	0 (%0,0)	0,497
Taste intolerance / product rejection	0 (%0,0)	0 (%0,0)	1,000
Diarrhea / constipation / bowel habit changes	0 (%0,0)	0 (%0,0)	1,000
Headache / irritability	0 (%0,0)	0 (%0,0)	1,000
Treatment discontinuation / dose skipping due to adverse events	0 (%0,0)	0 (%0,0)	1,000
Any serious adverse event	0 (%0,0)	0 (%0,0)	–

Note: Fisher's exact test was applied. No participant discontinued supplementation during follow-up. Serious adverse events were defined as life-threatening events or those requiring hospitalization.

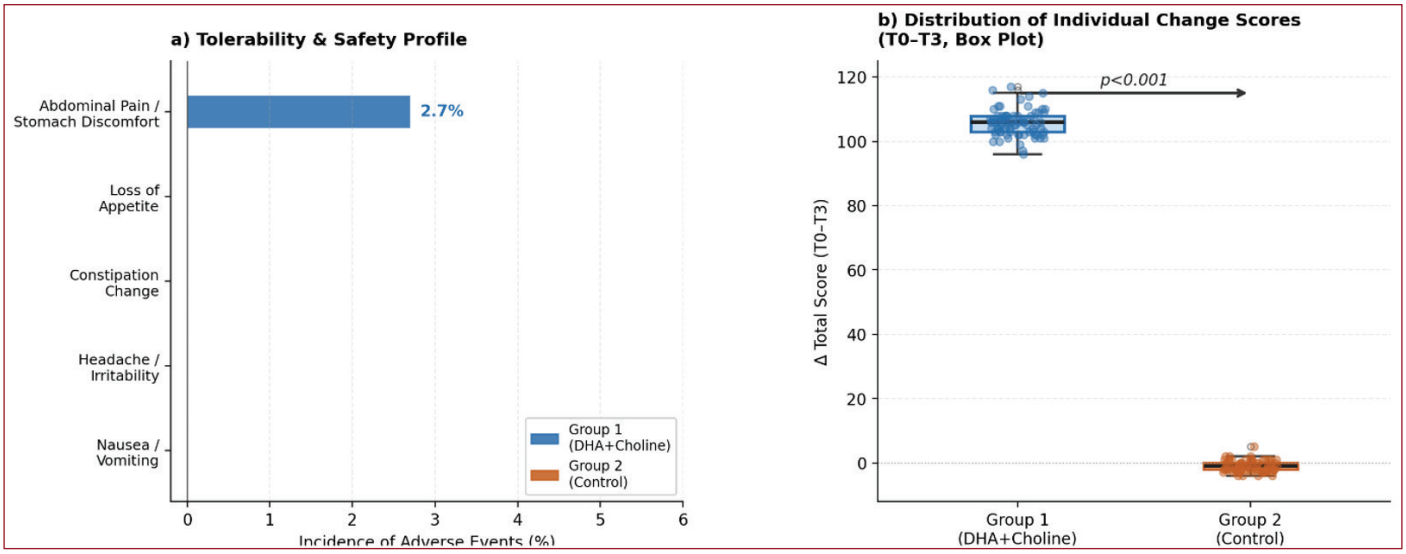

Figure 6. a) Tolerability and safety profile: During the 3-month intervention period, Group 1 (DHA+Choline) reported a single adverse event (abdominal pain/ stomach discomfort) with an incidence of 2.7%. No adverse events were reported in Group 2 (Control). All events were mild and did not require discontinuation. **b)** Distribution of individual change scores (Δ total score, T0-T3): Group 1 demonstrated substantial improvements with scores concentrated above 90 points, whereas Group 2 showed no meaningful change with scores clustered around zero. The between-group diffirence was hihly significant (p<0.001, Mann-Whitney U test).

DISCUSSION

In this retrospective multicenter cohort study, three months of DHA+choline supplementation in children aged 7–15 years was associated with significant improvements in infection-related healthcare visits, antibiotic use, and multiple clinical parameters. Particularly, the marked improvements observed in the subscales of immunity/ infection pattern, attention-related symptoms, learning-academic performance, sleep regulation, and antibiotic/ healthcare utilization suggest that the DHA+choline combination may provide substantial clinical benefits not only in neurocognitive domains but also in immunological and behavioral functions (1–3).

DHA is one of the principal structural components of phospholipid membranes within the central nervous system and plays a critical role in synaptic plasticity, neurotransmitter transmission, and neuronal membrane fluidity (4,5). Choline, on the other hand, is an essential micronutrient involved in acetylcholine synthesis, membrane integrity, myelination, and methylation pathways (6). In recent years, combined DHA and choline supplementation has been proposed to generate stronger neurocognitive outcomes compared with omega-3 supplementation alone. Simultaneous support of cholinergic neurotransmission and membrane stabilization may exert synergistic effects on attention, learning, and memory functions (7,8). In the present study, the remarkably high Δ scores observed in attention-related symptoms, mental focus, and learning/academic performance strongly support these biological mechanisms.

Although numerous studies have investigated the effects of omega-3 fatty acids on attention, behavior, and cognitive performance in children, studies simultaneously evaluating DHA+choline supplementation together with multidimensional clinical parameters remain extremely limited (9–11). In particular, large-scale pediatric datasets evaluating infection patterns, antibiotic utilization, sleep regulation, attention symptoms, and cognitive performance within the same study framework are scarce in the literature. From this perspective, the present study represents one of the rare multidimensional real-world analyses assessing both neurocognitive and immunological outcomes in school-aged children.

One of the most remarkable findings of the present study was the significant improvement observed in the

immunity/infection pattern and antibiotic/healthcare utilization subscales. Omega-3 fatty acids are known to suppress proinflammatory cytokine production, modulate T-cell responses, and enhance natural killer cell activity (12–14). Furthermore, DHA contributes to the synthesis of resolvins and protectins, which are specialized pro-resolving mediators involved in the resolution phase of inflammation (15). Considering these biological mechanisms, the reduction in infection-related healthcare visits and antibiotic use observed in our study appears biologically and pathophysiologically plausible.

The observed baseline imbalance in infection-related visits between groups (Group 1: 3.37±0.63 vs. Group 2: 3.13±0.55; p=0.006) warranted formal statistical evaluation. ANCOVA analyses revealed that the baseline infection visit frequency was not a significant predictor of T3 total questionnaire scores (F=0.123; p=0.727) after accounting for group allocation, and the adjusted marginal means remained virtually identical to the unadjusted values (Group 1: 186.74 vs. Group 2: 80.17; adjusted difference: 106.57 points). Importantly, within Group 1, participants with higher baseline infection frequencies did not demonstrate greater improvements than those with lower frequencies (p=0.657), effectively ruling out regression-to-the-mean as an alternative explanation for the observed gains. These findings support the conclusion that the baseline imbalance in infection burden did not materially confound the primary outcome of this study. Indeed, the fact that Group 1 achieved substantial improvements despite entering the study with a higher infectious burden may further underscore the clinical relevance of the DHA+choline supplementation effect.

Globally, school-aged children are reported to experience approximately 6–8 healthcare visits annually due to upper respiratory tract infections, while antibiotic prescription rates remain considerably high (16,17). In Türkiye, unnecessary antibiotic use among school-aged children continues to exceed the European average and remains a major public health concern because of its contribution to antimicrobial resistance (18). Therefore, the substantial improvement observed in infection patterns and antibiotic utilization among children receiving DHA+choline supplementation may be clinically meaningful not only at the individual level but also from a broader public health perspective.

The improvements observed in sleep regulation, motor/energy/social functioning, and overall behavioral performance are also clinically noteworthy. Sleep quality during childhood has been strongly associated with attention span, learning capacity, and emotional regulation (19). DHA levels have been suggested to influence sleep regulation through melatonin metabolism and neuronal membrane functions (20–29). Accordingly, the significant improvement observed in the sleep-related subscale may be associated with the concurrent enhancement in attention and cognitive performance.

One of the major strengths of the present study is the inclusion of real-world data obtained from 13 independent pediatric clinics located across different geographical regions of Türkiye. The participation of centers representing both eastern and western regions of the country, including İstanbul, Yalova, Muş, Şırnak, and Siirt, enhances the representativeness of the study population and provides a more comprehensive reflection of the Turkish pediatric population. To our knowledge, DHA+choline studies involving such a broad geographical distribution and data collection from 13 separate pediatric centers are extremely limited in the literature. Moreover, multicenter pediatric studies simultaneously evaluating infection patterns and neurocognitive outcomes are virtually nonexistent. Therefore, the present study provides an original and valuable contribution to the real-world pediatric literature originating from Türkiye.

The standardized effect estimates observed in the present study were considerably larger than those generally reported in pediatric nutritional supplementation studies. These estimates should not be interpreted solely according to conventional small, moderate, and large effect-size thresholds. Their magnitude reflects both the substantial difference in longitudinal change between the groups and the relatively narrow within-group distribution of questionnaire scores. Accordingly, the absolute changes, adjusted between-group differences, and confidence intervals may provide a more clinically interpretable representation of the findings. The unusually large estimates also warrant cautious interpretation and independent confirmation in prospective studies using externally validated outcome measures.

This study has several limitations that warrant acknowledgment. First, the retrospective and observational design precludes the establishment of causal relationships and does not allow for randomization or blinding, introducing the possibility of selection bias and unmeasured confounding. Second, the primary outcome instrument is a parent-reported questionnaire developed de novo for this study; formal psychometric validation — including test–retest reliability and construct validity against established instruments — has not yet been performed, and this represents a priority for future research. Third, the narrow score range observed at baseline in both groups (Group 1: range 77–91; Group 2: range 77–83) reflects the relatively homogeneous clinical profile of the enrolled population and partially contributes to the high rank-biserial correlation values (r=1.000) observed at later time points; this should be considered when interpreting the magnitude of between-group differences. Fourth, the baseline imbalance in infection-related healthcare visits (Group 1 vs. Group 2; p=0.006) represents a potential confound that was addressed through ANCOVA sensitivity analysis, though residual confounding cannot be fully excluded. Fifth,

the Additional Benefit/Adherence subscale (J) in the control group showed no intragroup variability at T3 (Δ=−5.00±0.00), which may reflect a floor effect specific to this domain. Finally, the three-month follow-up period limits conclusions regarding long-term sustainability of the observed benefits. Prospective, randomized, placebo-controlled multicenter trials are needed to confirm and extend the findings of the present study.

Third, the primary outcome instrument is a 50-item, parent-reported questionnaire developed de novo for this study. Formal psychometric validation — including test–retest reliability, confirmatory factor analysis, and construct validity against established instruments — had not been completed prior to data collection. Post-hoc within-group Cronbach's alpha analysis revealed limited internal consistency across subscales (range: −0.51 to 0.35), likely reflecting score range restriction at individual time points rather than item incoherence. Nonetheless, this represents a significant limitation, and future studies should prioritize formal validation of this instrument before its adoption as a primary outcome measure.

CONCLUSION

The present study demonstrates that DHA+choline supplementation may be associated with significant and clinically meaningful improvements in infection-related healthcare visits, antibiotic use, attention-related symptoms, cognitive performance, sleep regulation, and overall functioning in school-aged children. Particularly, the combined use of choline with DHA may generate stronger neurocognitive and immunological outcomes by simultaneously supporting neuronal membrane stability and cholinergic neurotransmission. The findings of this study suggest that DHA+choline supplementation may represent a promising and clinically relevant supportive strategy in pediatric practice.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Istanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Scientific Research Ethics Committee (Date: 13.04.2026, Decision No: 157).

Informed Consent: Informed consent was obtained from all the subjects involved in the study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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