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REVIEW ARTICLE

Association of Protein Intake with Anemia among Adolescent Girls in Indonesia: A Systematic Review

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ABSTRACT

Introduction: Anemia remains highly prevalent among adolescent girls. Protein intake influences haemoglobin synthesis, iron transport, and overall diet quality. However, evidence regarding the association between protein intake and anemia remains inconsistent. This systematic review examined the association between protein intake and anemia among adolescent girls in Indonesia.

Methods: A systematic review was conducted in accordance with PRISMA 2020. PubMed, Embase, Scopus, Web of Science, and Google Scholar were searched from database inception to 24 August 2025 using terms related to anemia, protein intake, and adolescents. Eligible studies involved Indonesian adolescent girls aged 10-19 years, assessed dietary protein intake, and reported anemia-related outcomes, including haemoglobin concentration or iron-status indicators such as ferritin or haematocrit. Risk of bias was appraised using design-appropriate Joanna Briggs Institute (JBI) tools.

Results: Eighteen studies published between 2016 and 2025 met the inclusion criteria. Findings were inconsistent. Inadequate protein intake generally tended to be associated with lower haemoglobin levels or higher anemia risk; however, only two studies reported multivariable-adjusted estimates specifically for the protein-anemia association, and adjusted estimates were attenuated after accounting for important confounders.

Conclusion: Protein intake may be associated with anemia among adolescent girls in Indonesia, but its independent contribution remains uncertain because of substantial heterogeneity and confounding by iron intake, overall dietary quality, menstrual factors, and other determinants. Longitudinal and intervention studies are needed to clarify protein-specific effects.

Keywords: Anemia; Protein Intake; Haemoglobin; Adolescent Girls; Systematic Review.

INTRODUCTION

Anemia remains one of the most prevalent nutritional disorders affecting adolescent girls worldwide and continues to pose a substantial public health challenge despite ongoing nutritional interventions. Adolescence is characterised by rapid growth, expansion of blood volume, and profound hormonal changes. The onset of menstruation further increases the physiological demand for iron and other nutrients required for erythropoiesis, thereby increasing susceptibility to anemia (1,2). Anemia in this age group is associated with impaired physical growth, reduced cognitive performance, decreased school achievement, lower immunity, and adverse maternal outcomes in later life (3,4). For these reasons, adolescence represents a critical window for interventions aimed at preventing anemia and improving health across the life course.

Although iron deficiency has traditionally been regarded as the principal cause of anemia, contemporary evidence indicates that the condition arises from a far more complex interplay of nutritional, physiological, infectious, and socioeconomic determinants. In addition to iron intake, other factors including vitamin C, folate, vitamin B12, total energy intake, menstrual blood loss, infection, inflammation, and socioeconomic conditions may substantially influence haemoglobin status (5,6). These observations indicate that anemia cannot be adequately explained by a single nutrient deficiency alone and requires a broader nutritional and epidemiological perspective.

Among nutritional factors, protein intake has attracted growing attention because of its biological relevance to haemoglobin synthesis and iron metabolism. Proteins provide amino acids required for globin chain formation and support iron transport, storage, and utilization (7). However, protein intake should be interpreted carefully because total protein intake, animal-source protein, plant-source protein, and protein-rich food consumption represent distinct nutritional constructs. Animal-source foods may support haemoglobin synthesis not only through protein content but also through coexisting nutrients such as highly bioavailable heme iron, vitamin B12, zinc and other micronutrients essential for erythropoiesis (8,9). Consequently, the association observed between protein intake and anemia may reflect broader dietary adequacy rather than an independent biological effect attributable solely to protein. Distinguishing these competing explanations remains an important challenge for nutritional epidemiology.

Evidence from previous studies has produced inconsistent conclusions regarding the relationship between dietary protein intake and anemia among adolescent girls. Several investigations reported that adequate protein intake was associated with higher haemoglobin concentrations or lower odds of anemia, whereas others observed that these associations became weaker or disappeared after adjustment for iron intake, menstrual characteristics, or additional confounding variables (10–13). These inconsistencies may partly reflect methodological differences in dietary assessment, anemia definitions, sample size, and statistical adjustment across studies. More importantly, it remains unclear whether protein intake acts as an independent determinant of anemia or merely serves as a proxy for broader dietary quality and greater consumption of animal-source foods. Despite increasing research on adolescent anemia in Indonesia, no previous systematic review has critically synthesized the evidence with explicit consideration of methodological quality, confounding, and protein source differentiation.

This systematic review critically synthesized the available evidence concerning the association between protein intake and anemia among adolescent girls in Indonesia. Specifically, the review evaluated the consistency and strength of the evidence, examined the role of methodological quality and confounding in shaping observed associations, and explored whether protein intake is better interpreted as an independent nutritional exposure or as a marker of overall dietary quality. By identifying key evidence gaps, the review sought to inform health-promotion strategies, including adolescent nutrition education and school-based anemia prevention, and to support the development of integrated nutrition policies and future research.

METHODS

Study design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020). The methodology was designed to support transparent and reproducible reporting throughout the review (14). The review protocol was not prospectively registered in PROSPERO or another systematic-review registry before study commencement. This lack of prospective registration may reduce methodological transparency and increase the possibility of selective reporting and is therefore considered a limitation of the review.

Literature search strategy

A systematic literature search was conducted in PubMed, Embase, Web of Science, Scopus, and Google Scholar. The final database search was conducted on 24 August 2025. Controlled vocabulary (e.g., MeSH and Emtree terms) and free-text keywords related to anemia, protein intake, and adolescents were used. Boolean operators (AND and OR) and proximity operators (NEAR/W) were applied as appropriate. Geographic terms such as "Indonesia" and "Indonesian" were intentionally omitted to improve identification of studies not indexed with location-specific keywords. Geographic eligibility was instead applied during screening, and only studies conducted in Indonesia or reporting data specific to Indonesian adolescent girls were retained. The verbatim search strategies are presented in Table 1. Searches were limited to human studies but were not restricted by language or publication year.

Table 1. Search Strategies Used for Online Databases

Database	Search strategies	Records
PubMed	("Anemia, Iron-Deficiency"[Mesh] OR "Anemia"[Mesh] OR anemia[tiab] OR anemia[tiab] OR "iron deficiency anemia"[tiab] OR "iron-deficiency anemia"[tiab] OR hemoglobin[tiab] OR haemoglobin[tiab]) AND ("Dietary Proteins"[Mesh] OR "Protein Intake"[tiab] OR "dietary protein"[tiab] OR "protein consumption"[tiab] OR protein[tiab] AND intake[tiab]) AND ("Adolescent"[Mesh] OR adolescent*[tiab] OR teen*[tiab] OR youth[tiab]) AND ("Female"[Mesh] OR girl*[tiab] OR female*[tiab])	95
Embase	('iron deficiency anemia'/exp OR anemia:ti,ab,kw OR anemia:ti,ab,kw OR 'iron deficiency anemia':ti,ab,kw OR hemoglobin:ti,ab,kw OR haemoglobin:ti,ab,kw) AND ('dietary protein intake'/exp OR 'dietary protein'/exp OR 'protein intake':ti,ab,kw OR 'dietary protein':ti,ab,kw OR 'protein consumption':ti,ab,kw OR (protein NEAR/3 intake):ti,ab,kw) AND (adolescent/exp OR adolescent*:ti,ab,kw OR teen*:ti,ab,kw OR youth:ti,ab,kw) AND (female/exp OR girl*:ti,ab,kw OR female*:ti,ab,kw)	76
Web of Science	TS=((anemia OR anemia OR "iron deficiency anemia" OR "iron-deficiency anemia" OR hemoglobin OR haemoglobin) AND ("protein intake" OR "dietary protein" OR "protein consumption" OR (protein NEAR/3 intake)) AND (adolescent* OR teen* OR youth) AND (girl* OR female*)	34
Scopus	TITLE-ABS-KEY((anemia OR anemia OR "iron deficiency anemia" OR "iron deficiency anemia" OR hemoglobin OR haemoglobin) AND ("protein intake" OR "dietary protein" OR "protein consumption" OR (protein W/3 intake)) AND (adolescent* OR teen* OR youth) AND (girl* OR female*)	285
Google Scholar	(anemia OR anemia OR "iron deficiency anemia" OR "iron deficiency anemia" OR hemoglobin OR haemoglobin) AND ("protein intake" OR "dietary protein" OR "protein consumption" OR protein) AND (adolescent* OR teen* OR youth*) AND (girl* OR female*)	1435

Eligibility criteria
Research focusing on adolescent girls aged 10–19 years and presented dietary protein intake whether total, animal-source, or plant-based measured through dietary recalls, food frequency questionnaires, dietary records, or intervention protocols. Eligible studies also needed to report outcomes related to anemia, haemoglobin concentration, or iron status indicators such as ferritin or haematocrit. Articles were considered ineligible if they examined adult populations or heterogeneous samples where adolescent-specific findings could not be extracted separately. Studies were also excluded if protein intake was not assessed or reported, or if they were categorized as case reports, narrative reviews, editorials, conference proceedings without complete manuscripts, or experimental studies involving animals or laboratory models.

Study selection
Citations retrieved from all databases were compiled in reference-management software, and duplicate records were identified and removed before screening. For Google Scholar, the search was conducted directly using the specified search strategy and yielded 1,435 records. The retrieved records were exported in RIS format and imported into Mendeley. Records retrieved from all databases were subsequently combined. Two reviewers independently screened the titles and abstracts and subsequently assessed potentially eligible full-text articles. Studies were excluded if they did not involve adolescent girls, did not assess protein intake, or were conducted outside Indonesia. Disagreements were resolved by consensus with a third reviewer.

Data extraction
Data were extracted using a standardised form that captured study characteristics, including age, region, study design, sample size, dietary-assessment method, and definition of anaemia. The extracted findings included the protein exposure, adjusted confounders, effect estimates, and interpretation of the strength of evidence. Data were initially extracted by one reviewer and independently checked by two other reviewers. Any discrepancies identified during the checking process were discussed and resolved by consensus among the reviewers.

Data synthesis
The quality of the included studies was critically evaluated using the Joanna Briggs Institute (JBI) appraisal tools. The specific checklist applied was determined based on the methodological design of each study. Two reviewers independently conducted the appraisal, and any discrepancies were resolved through consensus. Due to heterogeneity in study designs, dietary assessment tools, protein intake definitions, and outcome measures, a meta-analysis was not feasible. Instead, a qualitative synthesis was conducted, summarising the direction, strength, and consistency of associations between protein intake and anemia or haemoglobin outcomes. For descriptive classification of appraisal results, the review team used thresholds of >=80% for low risk of bias, 60-79% for low-to-moderate risk, and <60% for moderate risk. These thresholds were applied consistently and are not official JBI cut-off values.

Ethical approval
Ethical approval was not required for this review, as it utilized only secondary data extracted from previously published studies that were publicly accessible. The study did not include participation by human subjects or the collection of any primary data.

RESULTS

The literature search identified 1925 records. Following the screening process, 18 studies met all inclusion criteria and were included in this systematic review. The study identification process is presented in Figure 1.

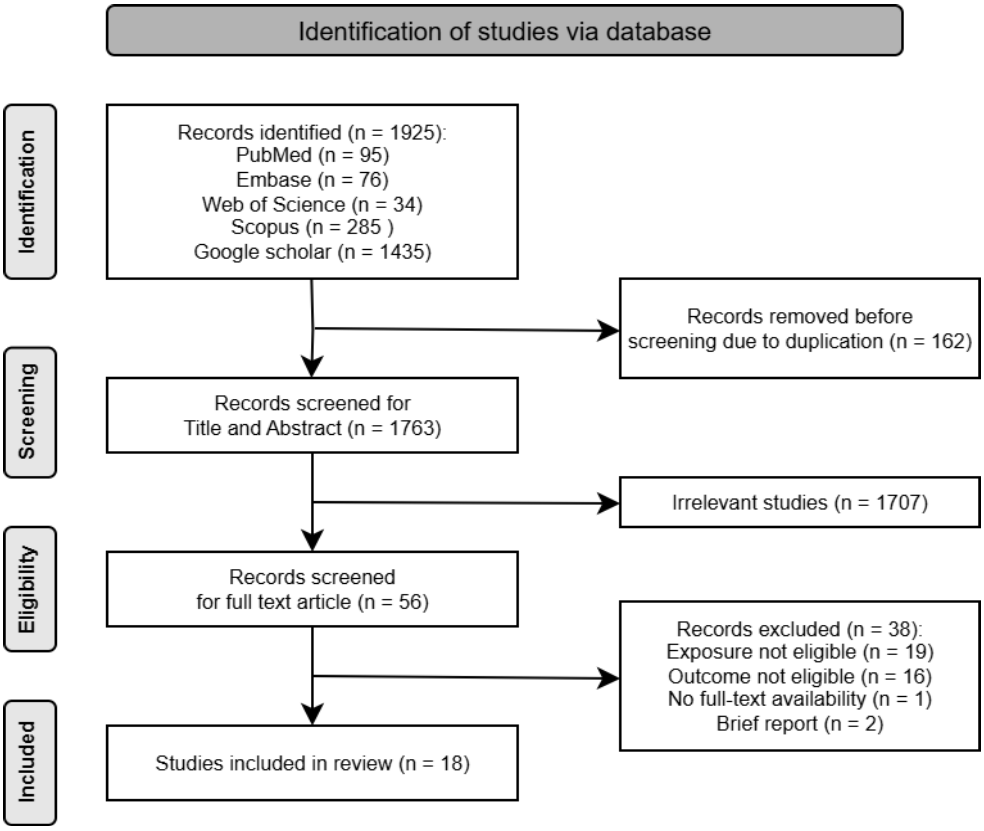

Figure 1. PRISMA Flow Chart Diagram of the Systematic Literature Review Strategy

Study characteristics

A total of 18 studies published between 2016 and 2025 were included in this review. The characteristics of the included studies are summarized in Table 2.

Table 2. Characteristics of Included Studies

Authors/years	Age (years old)	Region	Study design	Sample Size	Dietary Assessment	Anemia Definition
Hidayanti, et al. (2025) (15)	12-18	Tasikmalaya	Cross sectional	323	24-hour dietary recall	Hb <12 g/dL
Widiasta, et al. (2025) (16)	18-19	Bandung	Cross sectional	13	24-hour dietary recall	Normal, mild, moderate, severe
Fauziyah, et al. (2024) (17)	15-18	Tasikmalaya	Cross sectional	150	SQ-FFQ	Hb <12 g/dL
Abby, et al. (2023) (18)	12-15	Serang	Cross sectional	61	SQ-FFQ	Hb <12 g/dL
Handayani, et al. (2023) (19)	13-15	Bengkulu	Cross sectional	44	24-hour dietary recall	Hb <12 g/dL
Jumiyati, et al. (2023) (20)	13-14	Rejang Lebong	Cross sectional	112	SQ-FFQ	Hb <12 g/dL
Lestari, et al. (2022) (21)	15-21	Cianjur	Cross sectional	122	Questionnaire	Hb <12 g/dL
Sari, et al. (2022) (22)	10-19	Sumedang	Cross sectional	95	24-hour dietary recall	Hb <12 g/dL
Baha, et al. (2021) (23)	12-13	Majene	Cross sectional	126	FFQ	Hb <12 g/dL
Fithria, et al. (2021) (24)	15-17	Muna	Cross sectional	57	24-hour dietary recall	Hb <12 g/dL
Ekasanti, et al. (2020) (25)	10-15	Kendari	Cross sectional	97	24-hour dietary recall	Hb <12 g/dL

Authors/years	Age (years old)	Region	Study design	Sample Size	Dietary Assessment	Anemia Definition
Pratama, et al. (2020) (26)	12-15	Banjarmasin	Cross sectional	88	24-hour dietary recall	Hb <12 g/dL
Sholicha, et al. (2019) (27)	15-18	Gresik	Cross sectional	62	SQ-FFQ	Hb <12 g/dL
Sholihah, et al. (2019) (28)	15-16	Surabaya	Case control	44	24-hour dietary recall	Hb <12 g/dL*
Silvia, et al. (2019) (29)	16-18	Semarang	Cross sectional	70	FFQ	Anemia (8–10.9 g/dL), mild anemia (11–11.9 g/dL), and normal (≥12 g/dL)
Khasanah, et al. (2017) (30)	10-19	Ungaran	Cross sectional	68	FFQ	Hb <12 g/dL*
Lewa, (2016) (31)	16-18	Palu	Cross sectional	75	24-hour dietary recall	Hb <12 g/dL
Restuti, et al. (2016) (32)	16-18	Jember	Cross sectional	71	24-hour dietary recall	Hb <12 g/dL*

Note: * Abbreviations: Hb=haemoglobin; FFQ=Food Frequency Questionnaire; SQ-FFQ=Semi-Quantitative Food Frequency Questionnaire. * For Sholihah et al., Khasanah et al., and Restuti et al., the anemia definition was not explicitly reported in the original study and was therefore identified in this review using the WHO criterion for adolescent girls (haemoglobin <12 g/dL). These entries are marked with an asterisk.

Most studies were conducted on Java Island (n = 11). Participants in all included data were adolescent girls aged 10-19 years, with study sample sizes ranging from 13 to 323 participants. Almost all included studies used a cross-sectional design, indicating that the available evidence was primarily observational and limiting the ability to establish temporal relationships or causal inference. Dietary intake was commonly assessed using a 24-hour dietary recall. Anemia was primarily defined as a haemoglobin concentration <12 g/dL for adolescent girls.

Evidence on Association between Protein Intake and Anemia

The included studies reported associations between protein intake and anemia among adolescent girls in Indonesia. A synthesis of the main findings is presented in Table 3.

Table 3. Main Findings of Studies Included

Authors/years	Protein Exposure	Adjusted Confounders	Effect Size	Interpretation
Hidayanti, et al. (2025) (15)	<80% RDA vs ≥80% RDA	Not adjusted	cOR=2.677 (95% CI: 1.086–6.598); p=0.045	Weak evidence
Widiasta, et al. (2025) (16)	Severe deficiency (<70% RDA), moderate deficiency (70–79% RDA), mild deficiency (80–89% RDA), adequate (90–119% RDA), and excess (>120% RDA)	Not adjusted	r=0.489; p=0.90	Weak evidence
Fauziyah, et al. (2024) (17)	Insufficient (<80% RDA) vs Sufficient (≥80% RDA)	Protein intake, iron intake, and vitamin C intake	AOR=8.51 (95% CI 1.065–68.06); p=0.008	Moderate–strong evidence
Abby, et al. (2023) (18)	Adequate vs inadequate (<80% RDA)	Not adjusted	PR=3.310; p=0.034	Moderate evidence
Handayani, et al. (2023) (19)	Insufficient (<80% RDA) vs Sufficient (≥80% RDA)	Not adjusted	r=-0.128; p=0.407	Weak evidence
Jumiyati, et al. (2023) (20)	Protein (g/day)	Not adjusted	r=0.362; p=0.000	Moderate evidence
Lestari, et al. (2022) (21)	Severe deficiency (<70% RDA), moderate deficiency (70–79% RDA), mild deficiency (80–89% RDA), adequate (90–119% RDA), and excess (>120% RDA)	Not adjusted	r=0.171; p=0.059	Weak evidence
Sari, et al. (2022) (22)	Good vs poor(<80% RDA)*	Not adjusted	p=0.394	Weak evidence
Baha, et al. (2021) (23)	Never, rarely, sometimes, often	Not adjusted	p=0.674	Weak evidence
Fithria, et al. (2021) (24)	Adequate vs inadequate (<80% RDA)*	Not adjusted	p=0.466	Weak evidence
Ekasanti, et al. (2020) (25)	Low (<80%) vs enough (≥80% RDA)	Protein nutrient intake, iron nutrient intake, nutritional intake of vitamin C, menstrual status	cOR=8.267 (95% CI: 2.591–26.437); AOR=2.012; p=0.007	Strong evidence
Pratama, et al. (2020) (26)	Adequate vs inadequate (<80% RDA)*	Not adjusted	p=0,149	Weak evidence
Sholicha, et al. (2019) (27)	Adequate vs inadequate (<80% RDA)	Not adjusted	r=0.663; p=0.000	Moderate–strong evidence

Authors/years	Protein Exposure	Adjusted Confounders	Effect Size	Interpretation
Sholihah, et al. (2019) (28)	Adequate vs inadequate (<80% RDA)	Not adjusted	p=<0.001; cOR=30.33	Strong evidence
Silvia, et al. (2019) (29)	Low (<80% RDA), good (80–110% RDA), and high (>110% RDA)	Not adjusted	p=0.047	Weak–moderate evidence
Khasanah, et al. (2017) (30)	Severe deficiency, moderate deficiency, mild deficiency, and normal	Not adjusted	r=0.308; p=0.006	Weak–moderate evidence
Lewa, (2016) (31)	Adequate vs inadequate (<80% RDA)	Not adjusted	p=1.000	Weak–moderate evidence
Restuti, et al. (2016) (32)	deficiency, inadequate, moderate, and good	Not adjusted	r=0.271; p=0.280	Weak evidence

Note: * Abbreviations: RDA= Recommended Dietary Allowances; cOR= Crude Odds Ratio; AOR= Adjusted Odds Ratio; CI=Confidence Interval.
*Where an original study did not explicitly report a protein-adequacy category, the extracted exposure was identified using the review criterion of adequate protein intake >=80% of the RDA. Inferred entries are marked with an asterisk.

Protein intake exposure was measured heterogeneously across studies. Several studies categorized protein intake based on adequacy relative to the Recommended Dietary Allowance (RDA) using a cut-off of 80% of RDA to distinguish adequate and inadequate intake. Other studies classified protein intake into multiple categories or reported as continuous values in grams per day.
Evidence synthesis indicated inconsistent findings regarding the association between protein intake and anemia among adolescent girls. Of the 18 included studies, nine reported statistically significant associations, eight found no statistically significant relationship, and one reported a borderline non-significant result. Studies with significant findings generally reported that insufficient protein intake was associated with lower haemoglobin levels or increased anemia risk.
Methodological heterogeneity was evident among the included studies, with variations in dietary assessment techniques, including 24-hour dietary recalls and food frequency questionnaires, as well as differences in anemia definitions and hemoglobin cut-off values. Protein intake exposure was also measured differently, with most studies assessing total protein intake without distinguishing protein sources. In addition, adjustment for potential confounding factors varied substantially, with only a limited number of studies applying multivariable analysis to control for important covariates such as iron intake, menstrual characteristics, vitamin intake, and socioeconomic factors.
An important pattern emerged when comparing adjusted and unadjusted analyses. Stronger associations were more commonly reported in studies relying on bivariate or minimally adjusted analyses, whereas studies using multivariable adjustment generally reported attenuated effect estimates after controlling for confounding variables. Overall, the findings indicate that higher protein intake tends to be associated with better hemoglobin status and lower anemia risk among adolescent girls. Nevertheless, the magnitude and consistency of the observed association differed considerably among the included studies.

Risk of bias assessment

The methodological rigor of the eighteen included studies was assessed using the Joanna Briggs Institute (JBI) critical appraisal tools, with the appropriate checklist selected according to each study design. Results of quality assessment and risk of bias are presented in Table 4.

Table 4. JBI Critical Appraisal for Cross-Sectional Studies

Study/ Question	Were the criteria for inclusion in the sample clearly defined?	Were the study subjects and the setting described in detail?	Was the exposure measured in a valid and reliable way?	Were objective, standard criteria used for measurement of the condition?	Were confound-ing factors identified?	Were strategies to deal with confound-ing factors stated?	Were the outcomes measured in a valid and reliable way?	Was appro-priate statistical analysis used?	Total
Hidayanti, et al. (2025) (15)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8
Widiasta, et al. (2025) (16)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Fauziyah, et al. (2024) (17)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8
Abby, et al. (2023) (18)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8
Handayani, et al. (2023) (19)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Jumiyati, et al. (2023) (20)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Lestari, et al. (2022) (21)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7

Study/ Question	Were the criteria for inclusion in the sample clearly defined?	Were the study subjects and the setting described in detail?	Was the exposure measured in a valid and reliable way?	Were objective, standard criteria used for measurement of the condition?	Were confound-ing factors identified?	Were strategies to deal with confound-ing factors stated?	Were the outcomes measured in a valid and reliable way?	Was appro-priate statistical analysis used?	Total
Sari, et al. (2022) (22)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7
Baha, et al. (2021) (23)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Fithria, et al. (2021) (24)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Ekasanti, et al. (2020) (25)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8
Pratama, et al. (2020) (26)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Sholicha, et al. (2019) (27)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7
Silvia, et al. (2019) (29)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7
Khasanah, et al. (2017) (30)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	7
Lewa, et al. (2017) (31)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6
Restuti, et al. (2017) (32)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	6

The appraisal showed variability in risk-of-bias profiles. Using the prespecified review-team thresholds, ten studies were classified as having a low risk of bias and eight as having a low-to-moderate risk of bias; no study was classified as having a moderate risk of bias. The design-specific JBI checklist was used for the case-control study by Sholihah et al.

The most frequent methodological concerns involved exposure quantification, reliance on self-reported dietary assessment, limited representativeness of study samples, and inadequate control of confounding variables. Protein intake was predominantly evaluated through single or multiple 24-hour dietary recalls, while some studies employed food frequency questionnaires. Although these dietary assessment instruments are commonly applied in nutritional epidemiological research, their accuracy may be limited by recall bias and challenges in reflecting habitual dietary patterns, potentially resulting in misclassification of protein intake exposure.

Residual confounding was identified as the most important source of bias. Several studies identified major anemia-related confounders such as iron intake, menstrual characteristics, vitamin C intake, and socioeconomic status, but only two studies reported multivariable-adjusted estimates specifically for the protein-anemia association. Most studies relied primarily on bivariate analyses, including chi-square tests, correlations, or Fisher's exact tests, without adequate adjustment for potential confounders. Consequently, observed associations may partly reflect broader nutritional or contextual factors rather than protein intake alone.

Therefore, high JBI scores were not interpreted as equivalent to high-certainty evidence. Greater interpretive weight was assigned to studies with larger sample sizes, stronger dietary assessment methods, objective hemoglobin measurements, and multivariable-adjusted analyses. Overall, the available evidence was considered to provide moderate confidence in the association between protein intake and anemia among adolescent girls. However, the evidence remains insufficient to establish whether protein intake independently contributes to the development of anemia.

DISCUSSION

Summary of Evidence

Evidence from eighteen eligible studies published from 2016 to 2025 was synthesized in this systematic review. Overall, the findings suggest a general tendency toward an inverse association, in which adequate protein intake may be associated with lower anemia prevalence or reduced odds of anemia. However, the observed associations varied considerably across studies, suggesting that the relationship between protein intake and anemia is complex and may not reflect a simple direct effect. This heterogeneity was further reflected in study quality, particularly in differences in dietary exposure assessment and control of confounding variables (25,33).

An important contribution of this review is the recognition that protein intake may be interpreted through multiple pathways. Protein may contribute directly to haemoglobin synthesis by supplying amino acids required for globin production, but it may also function as a marker of broader dietary quality or as an indirect indicator of animal-source food consumption, which provides additional nutrients relevant to erythropoiesis (34–36). Recent reviews also support that anemia in adolescent girls is multifactorial and cannot be explained by a single nutrient deficiency alone (6).

Sources of Heterogeneity Across Studies

Several methodological, nutritional, and biological factors may explain the heterogeneity of findings across studies. Differences in nutrient interactions, participant characteristics, statistical power, and measurement quality may partly explain why significant associations were observed in some studies but not in others. First, iron intake likely played a major modifying role. Participants reporting higher protein intake also tended to have greater consumption of animal-derived foods, potentially providing increased exposure to heme iron with higher bioavailability. Therefore, observed associations may partly reflect greater intake and better bioavailability of iron, rather than protein alone (18,20,24,33). The role of iron bioavailability especially from animal-source foods has been consistently emphasized in recent literature on adolescent anemia (9).

Second, menstrual characteristics substantially influenced anemia risk. Adolescents experiencing prolonged or heavy menstrual bleeding may develop anemia despite adequate dietary intake because iron loss exceeds dietary replacement (21,22,37). Menstrual blood loss remains one of the strongest non-dietary contributors to iron deficiency anemia in adolescent girls (9).

Third, sample size affected statistical power. The lack of statistically significant findings in some studies may be partly attributable to limited sample sizes, potentially resulting in insufficient statistical power to detect existing associations and increasing the risk of type II error and reducing the ability to detect moderate associations (19,27,28). Fourth, dietary assessment quality varied considerably. Most included studies relied on single 24-hour recalls or self-reported dietary assessment instruments, including food frequency questionnaires, which are vulnerable to recall-related measurement errors and may inadequately represent habitual intake. Misclassification of protein intake may therefore attenuate true associations.

Adjusted Versus Unadjusted Evidence

The strength of the evidence differed according to methodological rigor. Only two of the 18 studies, Fauziyah et al (17). and Ekasanti et al. (24), reported multivariable-adjusted estimates specifically for the protein-anemia association. Hidayanti et al (14). conducted multivariable regression for haemoglobin levels, but the protein-anemia estimate extracted for Table 3 was a crude estimate; Abby et al. (17) used bivariate analyses and did not report a multivariable-adjusted estimate for this association. Stronger inverse associations were generally reported in unadjusted or minimally adjusted analyses.

In the two studies reporting adjusted protein-anemia estimates, effect estimates were interpreted after controlling for key covariates, including iron intake and, where applicable, vitamin C intake and menstrual status (15,17,18,25). The attenuation of associations after adjustment suggests that confounding contributes substantially to the observed relationship between protein intake and anemia. Similar concerns have been reported in systematic reviews of anemia determinants among adolescent girls (6).

Statistically significant findings were not automatically interpreted as strong evidence. Several studies reported significant associations despite substantial methodological limitations, including small sample sizes, single dietary assessments, and inadequate control of confounding, which contributed to moderate risk-of-bias ratings in many studies (25–32). Conversely, some non-significant findings from underpowered studies may reflect insufficient statistical power rather than true absence of association.

Protein-Specific Effect vs Dietary Quality Marker

A key interpretative question is whether protein intake independently influences anemia risk or primarily reflects broader dietary quality. Adolescents with higher protein intake often consume more meat, fish, eggs, and dairy products, which simultaneously provide heme iron, vitamin B12, zinc, and folate—nutrients with well-established roles in erythropoiesis and iron metabolism (18,20,36).

Therefore, higher protein intake may represent healthier dietary patterns and better access to nutrient-dense foods rather than an isolated protein-specific effect. This interpretation aligns with recent evidence suggesting that dietary diversity and overall nutrient adequacy may be more predictive of hemoglobin status than isolated macronutrient intake (38).

An additional complexity is that most included studies assessed total protein intake without differentiating between animal and plant protein sources. This limits interpretation because protein source may substantially modify the observed association. Animal-source foods may improve haemoglobin status through multiple mechanisms, including increased intake of highly bioavailable heme iron and key hematopoietic micronutrients. In contrast, protein obtained from plant sources is often accompanied by non-heme iron, which is less readily absorbed than heme iron, while the presence of phytate compounds may further limit intestinal iron uptake. This distinction between protein sources remains underexplored in Indonesian adolescent populations and represents an important knowledge gap (36).

Collectively, the current evidence remains insufficient to demonstrate that protein intake independently contributes to anemia risk beyond the influence of other dietary and nutritional factors. Instead, protein intake may largely function as a proxy for broader dietary quality, higher consumption of animal-source foods, greater dietary diversity, and more favorable socioeconomic conditions.

Limitations and Cautions

Despite the strength of including studies from diverse geographic and cultural settings, this review has several limitations. First, substantial heterogeneity in study design, dietary-assessment methods, definitions of protein exposure, and anemia outcomes made quantitative synthesis inappropriate. Second, the predominance of cross-sectional studies restricted causal inference because temporality between protein intake and anemia could not be established. Third, variability in adjustment for confounders, including iron intake, vitamin C, folate, menstrual characteristics, and socioeconomic status, may explain inconsistencies across studies. Risk-of-bias assessment further indicated that inadequate control of confounding was a common methodological limitation. Fourth, reliance on self-reported dietary data may introduce recall and reporting bias, particularly among adolescents. Fifth, the lack of prospective protocol registration may increase the risk of selective reporting. Publication bias also remains possible because studies with negative or null findings may be underrepresented.

Recommendations for Future Research

Future research should place greater emphasis on longitudinal cohort designs to provide stronger evidence regarding the temporal relationship and intervention trials to better establish temporality and clarify potential causal pathways between protein intake and anemia. Standardized dietary assessment instruments and consistent definitions of protein intake and anemia indicators are needed to improve comparability across studies. Future research should also ensure adequate control of major confounding factors, including iron and other micronutrient intake, menstrual characteristics, infection or inflammatory status, socioeconomic conditions, and adherence to Iron-Folic Acid Supplementation. More objective dietary assessment methods or biomarker-based approaches are recommended to reduce recall bias, particularly in adolescent populations. Importantly, future studies should aim to disentangle the independent contribution of protein intake from broader dietary quality and animal-source food consumption in order to determine whether observed associations reflect protein-specific effects or overall nutritional adequacy. Recent literature on anemia etiology in Indonesian adolescents similarly identifies this as a major knowledge gap.

Health-promotion implications

The reviewed evidence supports integrated adolescent nutrition education and school-based anemia-prevention strategies that frame adequate protein intake as one component of overall dietary adequacy rather than as a stand-alone intervention. Programs and policies should emphasize balanced nutrient-dense diets, appropriate iron and micronutrient intake, adherence to iron-folic acid supplementation where recommended, and attention to menstrual-health factors. Because the independent effect of protein remains uncertain, policy messages should avoid attributing anemia prevention to protein intake alone.

CONCLUSION

This systematic review suggests that an association between protein intake and anemia was observed in some studies of Indonesian adolescent girls, with adequate protein intake generally linked to lower anemia prevalence or lower odds of anemia. However, the current evidence does not establish a causal relationship, and the independent contribution of protein intake remains uncertain because of the predominance of cross-sectional studies, methodological heterogeneity, and confounding by iron intake, overall dietary quality, animal-source food consumption, menstrual blood loss, and socioeconomic factors. Future longitudinal and intervention studies are needed to disentangle protein-specific effects from other major determinants of anemia.

DECLARATIONS

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