An egg intervention improves dietary intakes but does not fill intake gaps for multiple micronutrients among infants in rural Bangladesh

Monica M Pasqualino[1], Saijuddin Shaikh[2], John McGready[3], Md Tanvir Islam[2], Hasmot Ali[2], Tahmeed Ahmed[4], Keith P West Jr[1], Munirul Alam[4], Md Iqbal Hossain[4], Alain B Labrique[1], Amanda C Palmer[1]

[1]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States of America

[2]The JiVitA Project, Gaibandha, Bangladesh

[1]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States of America

[4]icddr,b, Dhaka, Bangladesh

This work was supported, in whole or in part, by the Bill and Melinda Gates Foundation, Grant Number OPP1163259. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission. Researchers at the Johns Hopkins Bloomberg School of Public Health and icddr,b were responsible for the design of the study. The funder contributed to the study design but was not involved in the data collection, analysis, or preparation of the manuscript.

Author disclosures: MMP, SS, JM, MTI, HA, TA, KPW, MA, MIH, ABL, ACP, no conflicts of interest.

Address correspondence to MMP. Mailing address: 615 N Wolfe St, Suite W2505, Baltimore MD 21205. Telephone: 516-661-0745. Email: mpasqua2@jhmi.edu

Word count: 5000
Number of figures: 1
Number of tables: 5
Supplemental Tables 1-3 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents available on the Journal homepage.

Running title: Egg intervention improves infant dietary intakes

Abbreviations used and definitions: AI, Adequate Intake; ASF, animal source foods; HFI, household food insecurity index; IOM, Institute of Medicine; LAZ, length-for-age z-score; LMIC, low- and middle-income country; NCI, National Cancer Institute; USDA, United States Department of Agriculture; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score

Abstract

Background: Eggs are nutrient-rich. Strengthening evidence on the impact of egg consumption on dietary quality can inform complementary feeding guidance.

Objective: To assess the effect of an egg intervention on dietary intakes among six- to 12-months-olds in rural Bangladesh.

Methods: We conducted a cluster-randomized controlled trial allocating clusters (n=566) to enteric pathogen control or placebo treatment, and daily provision of a protein-rich meal, isocaloric meal, egg, or control. Nutrition education was provided to all arms. Our focus here is on the egg and control arms. Infants were enrolled at three months. From six months, we visited households weekly to distribute eggs and measure compliance. A semi-structured feeding questionnaire assessed 24-hour intake at six-, nine-, and 12-months. Assessments were repeated in ~10% of subjects 2-29 days later. Using National Cancer Institute SAS macros, we estimated usual intake distributions for energy, protein, fat, and 18 micronutrients and the proportion meeting intake recommendations. We compared outcomes between arms using clustered bootstrapping.

Results: Data were available from 757 infants (137 clusters) and 943 infants (141 clusters) in the egg and control arms, respectively. In the egg versus control arms, mean usual intakes were higher for energy (610 vs. 602 kcal/d, nine months; 669 vs. 658 kcal/d, 12 months), crude protein (2.2 vs. 1.7 g/(kg·d), nine months; 2.4 vs. 1.9 g/(kg·d), 12 months), available protein (2.0 vs 1.6 g/(kg·d), nine months; 2.1 vs. 1.8 g/(kg·d), 12 months), and for 13 and 14 micronutrients at nine and 12 months, respectively. The proportion meeting intake recommendations for most micronutrients was higher in the egg arm but remained <50% for 15 and 13 micronutrients at nine and 12 months, respectively.

Conclusions: Daily egg consumption improved dietary intakes among Bangladeshi infants, but was insufficient to meet multiple micronutrient intake recommendations, demonstrating the need to be coupled with other strategies.

Keywords: infants, dietary intakes, eggs, animal source foods, South Asia

Introduction

Meeting nutrient intake requirements during the complementary feeding period remains a challenge in low- and middle-income countries (LMICs). Infants and young children six to 23 months of age are typically breastfed and consume relatively small amounts of non-breast milk foods that are usually cereal-based and high in phytate that inhibits mineral absorption (1). In Bangladesh, where 30.8% of children under five are stunted (length-for-age z-scores (LAZ) <-2 below the WHO growth standard median), complementary foods are low in nutrient density, leading to inadequate intakes of micronutrients like B-vitamins, vitamin D, vitamin E, calcium, iron, and zinc (2-8). Deficiencies of these nutrients contribute to negative short- and long-term health outcomes (9). Identifying strategies to improve dietary quality during the complementary feeding period is therefore a public health priority.

Including animal source foods (ASF) in the diets of infants and children is important as they are rich sources of essential amino acids, fatty acids, and bioavailable micronutrients (10, 11). Eggs contain all nine essential amino acids and essential fatty acids, which are critical for early brain and retina development (12). They also contain multiple micronutrients, including vitamin A, B-vitamins, choline, iron, phosphorus, and zinc, that play key roles in early growth and development (13). In Ecuador, a trial providing a daily egg for six months in the complementary feeding period found a positive treatment effect on mean LAZ, whereas a similarly designed trial in Malawi found no effect on linear growth (14, 15). In Malawi, the intervention increased total intakes of nutrients present in high levels in eggs. However, the prevalence of inadequate intake was >80% for several micronutrients, including B-vitamins, calcium, iron, and zinc (16). As diets differ by context, it is important to examine the impact of egg consumption on dietary quality across populations.

In Bangladesh, studies have shown that eggs are included early in the diet (2, 17). According to the 2018 Demographic and Health Survey, 41.0% of breastfeeding children and 51.7% of non-breastfeeding children six to 23 months of age consumed eggs in the 24 hours prior to the survey (7). Eggs also hold practical benefits as they are typically more affordable than other types of ASF (13). Bangladesh has a history of small-scale poultry and egg production programs implemented for nutritional and economic benefits (18, 19). Scaling up production, coupled with efforts encouraging households to include eggs in the diet, has potential to improve access to and utilization of a nutrient-dense food during the complementary feeding period.

We conducted an analysis of secondary dietary outcomes of a cluster-randomized trial that allocated clusters to an enteric pathogen control or placebo treatment of infants, and delivery of a protein-rich meal, isocaloric meal, egg, or control, with all arms receiving nutrition education. Our focus here is on the egg and control arms, irrespective of the enteric pathogen control intervention. We aimed to assess the effect of providing a daily egg for six months to infants six to 12 months on: 1) usual energy and nutrient intakes, and 2) the proportion of subjects with intakes at or above recommendations.

Methods

Setting and study population

The cluster-randomized controlled trial was conducted at the JiVitA Research Site in Gaibandha District, Rangpur Division in northwestern Bangladesh, where various maternal and child health studies have been implemented (20-22). The site, which has a population of ~630,000, has been divided into 566 clusters. The setting is representative of national rural infrastructure, maternal and child nutritional status, and health services (23).

An active pregnancy surveillance system was in place across the full study area under the mCARE-II trial, which tested an mHealth intervention to improve coverage of antenatal and postnatal care (mCARE-II, clinical registration No. NCT02909179). Households were surveyed to identify all married women of reproductive age, who were approached to obtain consent for pregnancy surveillance. Women who became pregnant were recruited for the mCARE-II trial. At enrollment into mCARE-II, data were collected on household and maternal characteristics. Women were followed throughout pregnancy and pregnancy outcomes were registered. Infants born to women enrolled in the mCARE-II trial who survived to three months of age during a one-year enrollment period (September 2018 – September 2019) were considered eligible for the present trial. Enrollment was stopped after nine months (July 2019) under ethical review board guidance because accrual of infants into the cohort exceeded projections by ~30%.

Randomization

The trial had a 2x4 factorial, cluster-randomized controlled design. The primary aim was to test the independent and combined effects of a protein intervention and enteric pathogen control intervention on linear growth. The first randomly allocated factor, which was masked, was enteric pathogen control or placebo treatment. The second randomly allocated factor was unmasked and consisted of a protein-rich blended food, isocaloric blended food, egg, and control. We expanded these factors out to eight groups for randomization. To obtain an equal number of clusters and geographic balance across arms, we used block randomization; blocks consisted of administrative areas of ~15 clusters each. An analyst on the study team used random number seeds to run randomization sequences, one of which was randomly selected among a subset of sequences considered balanced based on anthropometric and socioeconomic measures from previous trials.

Sample Size

The trial was designed based on the primary outcome of linear growth and an initial one-year enrollment period. Data from a previous trial at the site (2012 – 2014) that evaluated the effect of complementary food supplements on growth informed the anticipated cohort yield, expected mean LAZ change from six to 12 months, and within cluster variance and between-cluster coefficient of variation (20). Under these assumptions and α=0.05, a cohort of 3180 infants would enable the detection of a mean difference of 0.165 in LAZ between groups at 12 months with 80% power. However, accrual into the cohort was ~30% greater than expected due to an inaccurate assumption that fertility would decline based on pregnancy data last registered at the site (2008 – 2012). Based on actual births, enrollment at three months, and estimated 10% loss to follow up, we shortened the enrollment period from one year to nine months. This yielded an anticipated ~5400 outcome measures.

Intervention

Infants received presumptive treatment of azithromycin or a placebo at ~six and nine months and one of three nutrition interventions or the control (i.e.., no nutrition intervention). The use of azithromycin was informed by evidence of high exposure to enteric pathogens among infants in Bangladesh, and consistency in biomarkers of environmental enteric dysfunction between our study site and that of a study in Mirpur, Bangladesh assessing gut function and environmental exposures (24, 25). The nutrition interventions included provision of a daily protein-rich blended food, isocaloric blended food, or egg for six months starting at ~six months of age. Nutrition education was provided to all arms.

Eggs were obtained from Kazi Farms Group, an established commercial egg producer in Bangladesh that meets European Union production standards (26). Eggs were procured at a standard weight of 60 g. Field distributors visited households weekly, delivering eight eggs at each visit (one extra egg provided in case of breakage or spoilage). The first visit was scheduled for the week following an infant's six-month birthday. If the family was unavailable, distributors visited up to three additional times, until the infant reached seven months. At each visit, distributors provided standard cooking and feeding instructions, which included to prepare the egg how the infant preferred; feed the whole egg in addition to regular food and breast milk; not share the eggs with family members; and, if the infant became sick, follow medical guidance on feeding. Distributors provided nutrition education once a month. This consisted of standardized age-specific audio recordings and pamphlets developed from Alive and Thrive modules that covered appropriate breastfeeding, complementary feeding, and hygiene practices (27, 28).

Data collection

Household consent visits were scheduled when infants reached three months and began in September 2018. Six-month household visits and intervention delivery began in January 2019. Twelve-month household visits were scheduled through April 2020 but were suspended in March 2020 due to the onset of COVID-19. Remaining cases were reached over telephone. Field interviewers administered questionnaires with primary caregivers when infants reached ~six, nine, and 12 months. At each visit, infant weight and length were measured using standard protocols (29). Breastfeeding status was assessed at each visit by asking the respondent if the infant was currently being breastfed. If yes, she was asked how many times in the last 24 hours the infant had been breastfed, with response options of 1-10, 11-20, or ≥ 21 times. Household food insecurity index (HFI) was estimated at the six-month visit using a nine-item questionnaire (30).

Intake of non-breast milk food and beverages was assessed at each visit using a semi-structured infant feeding questionnaire. The questionnaire, which had been previously evaluated for validity, included a pre-specified list of individual foods and mixed dishes selected based on the most frequently consumed foods reported in the study area (4, 31, 32). Nineteen items were included for six-month-olds and 31 items for nine- and 12-month-olds. For each item, the interviewer asked, “From yesterday morning to today morning, has the infant been fed [the item]?” If the caregiver responded yes, the interviewer asked how much was offered using a set of standard measures (i.e., spoons, bowls, glasses). The interviewer then asked how much the infant ate, recording this amount as a portion of the quantity offered (i.e., half, one quarter, three quarters, one third, two thirds, whole units). After completing the list, the interviewer asked if the infant had eaten any additional items. Up to eight additional items were recorded. Respondents were instructed to not report the eggs provided by the trial because trial egg intake was recorded when assessing intervention compliance. Caregivers of infants residing in a subset of 28 clusters, which were selected based on proximity to the field office, were approached for consent to participate in repeated dietary intake assessments conducted by substudy team members. This repeated assessment was planned for two to six days after each initial assessment in ~10% of subjects.

Compliance with the intervention was assessed weekly. At each visit, respondents were asked to recall, for each day of the previous week, the amount of egg offered to the infant, consumed, left over, or shared. Reported consumption was categorized as none, whole egg, greater than half, half, or less than half.

Ethical approval

Protocols were approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health (Baltimore, MD) and Research and Ethics Review Committees of the International Center for Diarrhoeal Disease Research, Bangladesh (Dhaka, Bangladesh). Written parental consent was obtained at enrollment. The trial was registered as NCT03683667 at clinicaltrials.gov.

Statistical analysis

Data on household and maternal characteristics of infants, collected when their mothers were enrolled into the mCARE-II trial, and infant anthropometry and diet assessed at ~six months of age (baseline) were summarized by arm as mean ± SD for continuous variables, and number of subjects and percentages for binary and categorical variables. To characterize socioeconomic status, we calculated a Living Standards Index using principal components analysis based on household assets and dwelling characteristics (33). We categorized household food insecurity as none (HFI = 9), mild (HFI >9 to <16), and severe (HFI ≥16) based on an examination of the distribution (30). We calculated LAZ, weight-for-length z-scores (WLZ), and weight-for-age z-scores (WAZ) using WHO Child Growth Standards and classified infants as stunted (LAZ <-2), wasted (WLZ <-2), or underweight (WAZ <-2) (8). We assessed differences in baseline characteristics using linear and logistic regression models with generalized estimating equations or multinomial regression models with robust standard error estimation to adjust for clustering.

Baseline characteristics of subjects included analyses were compared with those excluded due to loss-to-follow up or missingness. Analyses were performed using Stata version 14.2 (Stata Corp LP, College Station, TX) and SAS version 9.4 (Cary, NC). Analyses were completed on an intention-to-treat basis using a complete case approach. *P*-values were adjusted for multiple comparisons accounting for 22 dietary components such that $P < 0.05/22 = 0.002$ was considered statistically significant.

Observed dietary intakes and protein quality adjustment

Outcome data were primarily missing on compliance, or trial egg intake. This occurred because intervention delivery and data collection were carried out by two different cadres of staff (distributors and interviewers, respectively). It was therefore possible for the six-month intervention period to begin before the six-month interview and to end before the 12-month interview, resulting in no available compliance data at the 12-month timepoint. After excluding subjects with missing data, we compared the age distributions between arms at 12 months. We applied the age distribution of the egg arm (12.0 ± 0.1 months) to the control arm by comparing the percentage of subjects falling into sub-groups (e.g., 11.9, 12.0, 12.1 months). For baseline diet, we excluded subjects for whom the six-month interview occurred after the first visit from the field distributor and likewise compared the age distributions between arms.

After exploring variability in trial egg intake, we extracted the compliance data from the day preceding each dietary assessment and added these as an additional food item to the dataset of food and beverage intakes for that day. Although repeated dietary intake assessments were planned for two to six days after the first assessment, they occurred up to 29 days after. We explored within-subject variation in intakes by number of days between the initial and repeated assessments and excluded repeated assessments conducted 21 days after the first.

We converted portion sizes into weight equivalents using a study-specific standard database, and food weights into observed energy and nutrient intakes using a food composition table previously developed for the site (4). We added data on choline and vitamin B12 to the table from the United States Department of Agriculture (USDA) Standard Reference Database, and data on amino acids from the USDA database and the Bangladesh Food Composition Table (34, 35). We adjusted amino acid values depending on the protein value of the original source (36). Energy and nutrient intakes were summed across all items for each subject to estimate observed intakes from complementary foods.

We assumed that energy intake from breast milk was inversely related to energy intake from complementary foods (6). For each subject, we estimated total energy requirements based on FAO sex-, weight-, and age-specific guidelines (37). We subtracted energy consumed from complementary foods from energy requirements, resulting in the assumed energy from breast milk. This was divided by the energy density of breast milk in LMICs (0.63 kcal/g) to estimate the amount of breast milk consumed (38). Breast milk consumption in liters was multiplied by the amount of each nutrient in mature breast milk. The breast milk content of nutrients affected by maternal status were obtained from the literature: vitamin A (227 μg/L), thiamin (0.16 mg/L), riboflavin (0.22 mg/L), vitamin B6 (0.10 mg/L), choline (90 mg/L), and vitamin B12 (0.28 μg/L) (39-41). For amino acids, values were obtained from WHO/FAO/United Nations University, and for remaining nutrients from WHO (38, 42). Observed energy and nutrient intakes from breast milk were added to those from complementary foods to estimate total observed intakes.

We adjusted observed protein intakes using the Digestible Indispensable Amino Acid Score method to estimate available protein. Because ileal digestibility factors are not available for most foods, fecal digestibility factors were assigned to each food item, as recommended by FAO (43). We obtained fecal digestibility factors from FAO and the literature (36, 44, 45).

Usual intake distributions

Using SAS macros from the National Cancer Institute (NCI), we estimated usual total intake distributions for energy, crude and available protein, fat, vitamin A, thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, choline, vitamin C, vitamin D, vitamin E, calcium, copper, iron, magnesium, phosphorous, potassium, and zinc (46). We ran the MIXTRAN macro to transform the observed intake data to approximate a normal distribution using a Box-Cox transformation procedure. For energy and nutrients consumed daily, we fit a one-part non-linear mixed model using the NLMIXED procedure. For components consumed episodically, we fit a correlated two-part non-linear mixed model.

At baseline, we pooled subjects and included a treatment covariate due to the small sample size and observed lack of difference in food group consumption between arms (47). At nine and 12 months, we ran models separately by arm to allow regression coefficients and variance terms to differ by treatment assignment. Each model included a random term for individual subjects and covariates for sex and weekend day (Friday or Saturday). Assessment sequence was specified in each model. We used the DISTRIB macro to run a Monte Carlo simulation, with weekend days assigned a weight of two out of seven days per week. To estimate percent energy from protein and fat, we used SAS macros that estimate the distribution of the ratio of two dietary components (48). We used clustered bootstrapping with n=1000 replicates to test differences in mean total usual intakes by arm at each visit.

For each nutrient, we used the usual intake distributions to estimate the proportion of infants with intakes at or above recommendations by age and arm (49). For fat, vitamin A, vitamin C, vitamin E, B-vitamins, choline, calcium, copper, magnesium, phosphorus, and potassium, we used the Adequate Intake (AI) values of the Institute of Medicine (IOM). Although it is not possible to estimate the prevalence of intake adequacy/inadequacy using the AI, groups with mean intakes at or above the AI can be assumed to have a low prevalence of inadequate intakes (50-52). For vitamin D, we used the Recommended Daily Allowance value of the IOM (53). For crude and available protein, we assessed intake adequacy relative to requirements on a per-kilogram body weight basis (1.12 g/(kg·d) for six- and nine-month-olds, 0.95 g/(kg·d) for 12-months-olds) (43). We evaluated the prevalence of zinc intake adequacy relative to the Estimated Average Requirement (4 mg/d) from the International Zinc Nutrition Consultative Group for an unrefined diet with low zinc bioavailability (54). For iron, we used the probability approach based on the distribution of usual iron intake and distribution of iron requirements. The IOM provides an iron requirement distribution for six to 12-month-olds, which reflects the 10% iron bioavailability typical of a high phytate diet (55, 56). Differences in proportions between groups for each nutrient were estimated at each visit using clustered bootstrapping with n=1000 replicates.

Results

There were 3398 eligible infants from 283 clusters (**Figure 1**). Among these, parental consent was obtained for 3051 infants (89.8%). Twelve-month interviews were completed for 2640 infants (86.5% of those consented). After excluding subjects missing compliance data and comparing the age distributions between arms at 12 months, 757 infants (137 clusters) in the egg arm and 943 infants (141 clusters) in the control arm were retained for analyses. Baseline characteristics of those included and excluded were comparable; observed differences in maternal age and education and infant age and stunting were of small magnitude (**Supplemental Table 1**).

Baseline characteristics were balanced between arms, aside from wasting (**Table 1**). Across arms, most infants lived in food secure households (67.6%) and had mothers who had completed primary education (71.9%). Infants were a mean age of 6.3 ± 0.3 months. Prevalence of stunting, wasting, and underweight were 19.6%, 6.1%, and 16.4%, respectively. The intervention began for most subjects in the egg arm (77.1%) before the six-month interview. Subjects for whom baseline dietary intake data were assessed were younger than those excluded (**Supplemental Table 2**).

Among those for whom baseline dietary intake were analyzed, breastfeeding status, frequency, and estimated breast milk intake did not differ between arms (**Table 2)**. At the nine- and 12-month visits, ~98% of infants in each arm continued to be breastfed; estimated breast milk intake was lower in the egg versus control arms. At each visit, food group consumption was generally comparable between arms (**Table 3**). At ~12 months, dairy and liver consumption were higher in the egg arm (p-value <0.05). Almost all caregivers in the egg arm and about one-third in the control reported infants had consumed an egg in the 24 hours prior to the nine- and 12-month interviews. Other than trial eggs, fish was the most commonly consumed ASF in each arm (~50% at 12 months). Across arms, most ~12-month-olds had consumed cereals (94.2%), tubers (63.4%), or biscuits (59.9%) in the last 24 hours; 30.0% and 10.0% had consumed fruits or vegetables rich in vitamin A and vitamin C, respectively.

Estimated mean total usual intakes at the six-month visit were comparable between groups; despite significant p-values for various nutrients, the magnitude of difference in mean intakes was small (**Table 4**). Among nutrients for which we compared six-month intakes to the AI, estimated mean total intakes were equal to or greater than the AI for fat and copper. Estimated mean usual energy and nutrient intakes increased with age in both groups. At ~nine- and 12-months, estimated mean total usual intakes were higher for energy and most nutrients in the egg versus control arms, particularly for those present in eggs (e.g., protein, vitamin A, B-vitamins, choline, phosphorus, zinc) (**Supplemental Table 3**). Estimated mean total usual intakes of vitamin C and calcium were higher in the control arm at ~nine- and 12-months, and of potassium at ~12-months.

No infants met intake recommendations for micronutrients at baseline, except for riboflavin, folate, choline, and copper (**Table 5**). At ~12-months, almost all infants in both arms had adequate protein intakes, even after adjusting for protein availability. In both arms, estimated mean total fat intake was greater than the AI and >60% of infants met the recommendation (**Tables 4 & 5).** Estimated mean total intakes of thiamin, riboflavin, niacin, vitamin B6, choline, and phosphorus were at or above the respective AI in the egg arm, but not in the control. The proportion of infants meeting the AI for B-vitamins, choline, magnesium, and phosphorus were particularly higher in the egg versus control arms, but remained <50% for thiamin, riboflavin, vitamin B6, folate, and magnesium in the egg arm. Estimated mean total calcium intake was greater than the AI only in the control arm and the proportion meeting the recommendation was higher in the control versus egg arms. Less than 5% of infants in each arm met recommendations for vitamin A, vitamin C, iron, potassium, and zinc, and none met those for vitamin D or vitamin E. Similar trends were found at the nine-month visit.

Discussion

The provision of a daily egg during the early complementary feeding period increased estimated usual intakes of energy and protein among infants at ~nine and 12 months of age. For most micronutrients, the estimated mean total usual intakes and the proportion of infants meeting intake recommendations were higher in the egg arm compared to the control. However, among those receiving the intervention, <50% met the recommended intakes at ~12 months for thiamin, riboflavin, vitamin B6, folate, calcium, and magnesium; <5% for vitamin A, vitamin C, iron, potassium, and zinc; and none for vitamins D or E.

Few studies have examined the contribution of increasing ASF consumption on dietary intakes during the complementary feeding period in LMICs. Our finding of a small difference in estimated mean total usual energy intake between arms was not surprising, given the method used to estimate breast milk intake. Our assumption of an inverse relationship between energy intake from breast milk and that from complementary foods, based on a study in Bangladesh that measured breast milk intake using test weighing, has been used by other studies estimating energy intake (6, 50, 57). However, the method has limitations. It is possible that subjects' total energy intake was greater or less than their energy requirements, and their energy and nutrient intakes from breast milk differed from our estimations. Considering the observed breastfeeding practices and variation in energy intake from complementary foods, this method was preferable to using WHO-published average breast milk intake amounts (38, 57).

The higher estimated usual intakes of crude and available protein in the egg arm were expected given the protein and amino acid content of eggs. The egg trial in Malawi, a meat and milk supplement trial in Kenya, and fortified food supplement trials in LMICs yielded similar results on protein intake (16, 58-60). After adjusting for protein quality, almost all subjects in our study had adequate protein intakes. Other studies in LMICs adjusting protein intake similarly reported adequate protein intake among infants and children (16, 36, 61). The estimated total usual intakes of fat were comparable between groups in our study due to the high fat content of breast milk (38).

Although we estimated a greater mean intake of vitamin A in the egg versus control arms, almost all subjects across arms did not meet the AI. Overall, consumption of vitamin A-rich foods like liver, meat, fruits, and vegetables was low. Additionally, more than half of the total estimated energy consumed at each visit was from breast milk, the vitamin A content of which is affected by maternal status (38). This suggests that daily egg consumption would be insufficient to boost infants' vitamin A intakes to recommended amounts without intervening on other parts of the diet, particularly when maternal intake of the vitamin is low. It is possible that we underestimated the breast milk content of vitamin A. We used a value estimated from a study in Bangladesh that assessed maternal and infant serum retinol concentrations and breast milk vitamin A concentration (41). A more recent publication from our study area reported a lower prevalence of vitamin A deficiency based on maternal serum retinol concentration (62). Further research is needed on maternal dietary intake and breast milk nutrient composition in this setting.

Our findings indicate that consuming an egg a day could significantly improve intakes of B-vitamins and choline, but the benefits of doing so would be strengthened if integrated within efforts to increase diversity in the diet. B-vitamins are found in a variety of foods, including flesh and organ meat, dairy, dark leafy vegetables, and legumes, yet these foods were consumed by <30% of subjects at ~12 months. Similar conclusions can be made regarding vitamin C, which is not found in eggs, and vitamins D and E, which are present but not in high amounts. Across arms, most subjects did not meet recommended intakes for these nutrients, reflecting low quality and diversity in the background diet. For example, only ~10% consumed vitamin C-rich foods (e.g., citrus fruits, berries, mango) at ~12 months. Like our study, the egg trial in Malawi observed higher intakes for several micronutrients in the egg arm, but that the prevalence of inadequacy was high for many micronutrients (16). Across contexts, when the typical diet is lacking in multiple micronutrients, encouraging caregivers to regularly feed infants and children eggs should be part of a comprehensive dietary strategy addressing the specific nutrient gaps of the population. Quality dietary assessments across populations are needed to identify these gaps.

Although meeting nutrient needs has been challenging in LMICs, lessons have been learned from interventions promoting recommended infant and young child feeding practices (28). An assessment of programs in Bangladesh, Malawi, Peru, and Zambia concluded that complementary feeding practices, including dietary diversity, can be improved if interventions focus on the constraints to food access and use behavior change approaches that enable households to prepare and feed appropriate foods for infants and children (63). The Alive & Thrive program in Bangladesh, for example, delivered intensified interpersonal counseling, mass media, and community mobilization through an existing nationwide health program, achieving improvements in complementary feeding practices and demonstrating the beneficial impact of a large-scale behavior change intervention on diet (28). Encouraging inclusion of different types of ASF in the diet is of particular important. Iron and zinc are found in low concentrations in breast milk, and complementary foods must be rich in these minerals for infants to meet requirements (50). Our egg intervention contributed to higher estimated mean iron and zinc intakes, but it was not enough to meet gaps, particularly as consumption of other ASF like meat was low. Cost and cultural factors may limit access to ASF. Several programs in LMICs have intervened on household animal production, successfully increasing consumption of targeted foods when designed to address the population's specific needs and constraints (64). Evaluating the contextual factors influencing access to nutrient-dense foods like ASF can also reveal if other strategies should be considered, such as micronutrient supplementation or food fortification.

There were some limitations to our study. We did not directly observe whether infants consumed trial eggs or provide messaging on not selling trial eggs. However, field workers visited households weekly to identify challenges in compliance. Our trial was partially blinded. This could have resulted in biased outcome assessments, although our interviewers were highly trained and had experience objectively assessing infant dietary intake. Our dietary intake assessment was not open-ended, which may have led to an underestimation of intakes. However, the pre-specified list was developed based on data collected in the study area and respondents had the opportunity to report additional foods. We did not directly measure breast milk intake and used previously published values of breast milk nutrient content, which can differ by setting (65). We did not analyze the nutrient profile of the eggs provided by the trial, which can vary (66, 67). A main strength of our study was collection of multiple days of intakes to estimate within-subject variance, which increased the precision of estimated usual intakes. We also used a study-specific food composition table expanded to estimate vitamin B12 and choline intakes, and amino acid intakes to adjust for protein quality.

Consumption of nutrient-dense foods like ASF, legumes, fruits, and vegetables was low in this rural area of Bangladesh, leading to relatively low intakes of numerous micronutrients. The provision of a daily egg starting at ~six months of age increased estimated usual intakes of energy and most nutrients, as well as the proportion meeting intake recommendations for nutrients important for early growth and development. Our findings indicate that promoting egg consumption should be coupled with other strategies designed to the specific needs of the population to ensure infants reach the recommended intakes for the nutrients typically lacking in their diet.

Acknowledgments

The authors express special gratitude to Laura Caulfield and Jessica Fanzo at Johns Hopkins Bloomberg School of Public Health and to the study participants, without whom this research would not have been possible. The authors' responsibilities were as follows – MMP, SS, HA, TA, KPW, MA, MIH, ABL, ACP designed research; MMP, SS, MTI, HA conducted research; MMP analyzed data, advised by JM; MMP wrote the paper; MMP and ACP had primary responsibility for the final content. All authors have read and approved the final manuscript.

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

References

1. Dewey KG. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: an evolutionary perspective. J Nutr 2013;143(12):2050-4.

2. Campbell RK, Hurley KM, Shamim AA, Shaikh S, Chowdhury ZT, Mehra S, de Pee S, Ahmed T, West KP, Jr., Christian P. Effect of complementary food supplementation on breastfeeding and home diet in rural Bangladeshi children. Am J Clin Nutr 2016;104(5):1450-8.

3. Rah JH, Akhter N, Semba RD, de Pee S, Bloem MW, Campbell AA, Moench-Pfanner R, Sun K, Badham J, Kraemer K. Low dietary diversity is a predictor of child stunting in rural Bangladesh. Eur J Clin Nutr 2010;64(12):1393-8.

4. Campbell RK, Hurley KM, Shamim AA, Shaikh S, Chowdhury ZT, Mehra S, Wu L, Christian P. Complementary food supplements affect dietary nutrient adequacy and home food consumption in children 6-18 mo old in a randomized controlled trial in rural Bangladesh. J Nutr 2018;148:1-8.

5. Arsenault JE, Yakes EA, Islam MM, Hossain MB, Ahmed T, Hotz C, Lewis B, Rahman AS, Jamil KM, Brown KH. Very low adequacy of micronutrient intakes by young children and women in rural Bangladesh is primarily explained by low food intake and limited diversity. J Nutr 2013;143(2):197-203.

6. Kimmons JE, Dewey KG, Haque E, Chakraborty J, Osendarp SJM, Brown KH. Low nutrient intakes among infants in rural Bangladesh are attributable to low intake and micronutrient density of complementary foods. J Nutr 2005;135(3):444-51.

7. National Institute of Population Research and Training (NIPORT) and ICF. Bangladesh Demographic and Health Survey 2017-18. Dhaka, Bangladesh and Rockvlle, Maryland, USA: NIPORT and ICF, 2020.

8. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76-85.

9. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham-McGregor S, Katz J, Martorell R, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013;382(9890):427-51.

10. Dror DK, Allen LH. The importance of milk and other animal-source foods for children in low-income countries. Food Nutr Bull 2011;32(3):227-43.

11. Neumann C, Harris DM, Rogers LM. Contribution of animal source foods in improving diet quality and function in children in the developing world. Nutr Res 2001;22:193-220.

12. Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. J Am Diet Assoc 2009;109(4):668-79.

13. Iannotti LL, Lutter CK, Bunn DA, Stewart CP. Eggs: the uncracked potential for improving maternal and young child nutrition among the world's poor. Nutr Rev 2014;72(6):355-68.

14. Iannotti LL, Lutter CK, Stewart CP, Gallegos Riofrio CA, Malo C, Reinhart G, Palacios A, Karp C, Chapnick M, Cox K, et al. Eggs in early complementary feeding and child growth: a randomized controlled trial. Pediatrics 2017;140(1).

15. Stewart CP, Caswell B, Iannotti L, Lutter C, Arnold CD, Chipatala R, Prado EL, Maleta K. The effect of eggs on early child growth in rural Malawi: the Mazira Project randomized controlled trial. Am J Clin Nutr 2019.

16. Caswell BL, Arnold CD, Lutter CK, Iannotti LL, Chipatala R, Werner ER, Maleta KM, Stewart CP. Impacts of an egg intervention on nutrient adequacy among young Malawian children. Matern Child Nutr 2021;17(3):e13196.

17. Thorne-Lyman AL, Valpiani N, Akter R, Baten MA, Genschick S, Karim M, Thilsted SH. Fish and meat are often withheld from the diets of infants 6 to 12 months in fish-farming households in rural Bangladesh. Food Nutr Bull 2017;38(3):354-68.

18. Shanta IS, Hasnat MA, Zeidner N, Gurley ES, Azziz-Baumgartner E, Sharker MAY, Hossain K, Khan SU, Haider N, Bhuyan AA, et al. Raising backyard poultry in rural Bangladesh: financial and nutritional benefits, but persistent risky practices. Transbound Emerg Dis 2017;65(5):1454-64.

19. Nielsen H, Roos N, Thilsted SH. The impact of semi-scavenging poultry production on the consumption of animal source foods by women and girls in Bangladesh. J Nutr 2003;133(11):4027S-30S.

20. Christian P, Shaikh S, Shamim AA, Mehra S, Wu L, Mitra M, Ali H, Merrill RD, Choudhury N, Parveen M, et al. Effect of fortified complementary food supplementation on child growth in rural Bangladesh: a cluster-randomized trial. Int J Epidemiol 2015;44(6):1862-76.

21. West KP, Jr., Christian P, Labrique AB, Rashid M, Shamim AA, Klemm RD, Massie AB, Mehra S, Schulze KJ, Ali H, et al. Effects of vitamin A or beta carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh: a cluster randomized trial. Jama 2011;305(19):1986-95.

22. Merrill RD, Shamim AA, Ali H, Jahan N, Labrique AB, Schulze K, Christian P, West KP, Jr. Iron status of women is associated with the iron concentration of potable groundwater in rural Bangladesh. J Nutr 2011;141(5):944-9.

23. Labrique AB, Christian P, Klemm RD, Rashid M, Shamim AA, Massie A, Schulze K, Hackman A, West KP, Jr. A cluster-randomized, placebo-controlled, maternal vitamin A or beta-carotene supplementation trial in Bangladesh: design and methods. Trials 2011;12:102.

24. McCormick BJJ, Lee GO, Seidman JC, Haque R, Mondal D, Quetz J, Lima AAM, Babji S, Kang G, Shrestha SK, et al. Dynamics and Trends in Fecal Biomarkers of Gut Function in Children from 1-24 Months in the MAL-ED Study. Am J Trop Med Hyg 2017;96(2):465-72.

25. Campbell RK, Schulze KJ, Shaikh S, Mehra S, Ali H, Wu L, Raqib R, Baker S, Labrique A, West KP, Jr., et al. Biomarkers of Environmental Enteric Dysfunction Among Children in Rural Bangladesh. J Pediatr Gastroenterol Nutr 2017;65(1):40-6.

26. European Commission. Agriculture and rural development: Eggs. Internet: https://agriculture.ec.europa.eu/farming/animal-products/eggs_en (accessed September 4, 2022).

27. Alive and Thrive. Internet: www.aliveandthrive.org (accessed August 9, 2020).

28. Menon P, Nguyen PH, Saha KK, Khaled A, Sanghvi T, Baker J, Afsana K, Haque R, Frongillo EA, Ruel MT, et al. Combining intensive counseling by frontline workers with a nationwide mass media campaign has large differential impacts on complementary feeding practices but not on child growth: results of a cluster-randomized program evaluation in Bangladesh. J Nutr 2016;146(10):2075-84.

29. de Onis M, Onyango AW, Van den Broeck J, Chumlea WC, Martorell R. Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. Food Nutr Bull 2004;25:S27-S36.

30. Na M, Mehra S, Christian P, Ali H, Shaikh S, Shamim AA, Labrique AB, Klemm RD, Wu LS, West KP, Jr. Maternal detary diversity decreases with household food insecurity in rural Bangladesh: a longitudinal analysis. J Nutr 2016;146(10):2109-16.

31. Chowdhury ZT, Hurley KM, Campbell RK, Shaikh S, Shamim AA, Mehra S, Christian P. Novel method for estimating nutrient intakes using a semistructured 24-hour diet recall for infants and young children in rural Bangladesh. Curr Dev Nutr 2020;4(9):nzaa123.

32. Christian P, Kim J, Mehra S, Shaikh S, Ali H, Shamim AA, Wu L, Klemm R, Labrique AB, West KP, Jr. Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 Trial. Am J Clin Nutr 2016;104(4):1175-82.

33. Gunnsteinsson S, Labrique AB, West KP, Jr., Christian P, Mehra S, Shamim AA, Rashid M, Katz J, Klemm RD. Constructing indices of rural living standards in Northwestern Bangladesh. J Health Popul Nutr 2010;28(5):509-19.

34. USDA Agricultural Research Service. USDA National Nutrient Database for Standard Reference. Washington, DC: USDA, 2020.

35. Institute of Nutrition and Food Science. Food Composition Table for Bangladesh. Dhaka, Bangladesh: University of Dhaka, 2013.

36. Arsenault JE, Brown KH. Dietary protein intake in young children in selected low-income countries is generally adequate in relation to estimated requirements for healthy children, except when complementary food intake is low. J Nutr 2017;147(5):932-9.

37. FAO. Human energy requirements. Report of a joint FAO/WHO/UNU expert consultation. Rome, Italy: FAO 2004.

38. WHO. Complementary feeding of young people in developing countries: a review of current scientific knowledge. Geneva, Switzerland: WHO, 1998.

39. Daniels L, Gibson RS, Diana A, Haszard JJ, Rahmannia S, Luftimas DE, Hampel D, Shahab-Ferdows S, Reid M, Melo L, et al. Micronutrient intakes of lactating mothers and

their association with breast milk concentrations and micronutrient adequacy of exclusively breastfed Indonesian infants. Am J Clin Nutr 2019;110(2):391-400.

40. Allen LH. B vitamins in breast milk: relative importance of maternal status and intake, and effects on infant status and function. Adv Nutr 2012;3(3):362-9.

41. Rice AL, Stoltzfus RJ, de Francisco A, Chakraborty J, Kjolhede CL, Wahed MA. Maternal vitamin A or β-carotene supplementation in lactating Bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. J Nutr 1999;129(2):356-65.

42. WHO/FAO/UNU. Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU expert consultation. WHO technical report series 935. Geneva, Switzerland: WHO, 2007.

43. FAO. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. FAO food and nutrition paper 92. Rome, Italy: FAO, 2013.

44. FAO. Protein quality evaluation: report of joint FAO/WHO expert consultation. Rome, Italy: FAO, 1991.

45. Millward DJ, Jackson AA. Protein/energy ratios of current diets in developed and developing countries compared with a safe protein/energy ratio: implications for recommended protein and amino acid intakes. Public Health Nutrition 2004;7(3):387-405.

46. NCI. SAS macros for the NCI method. Internet: https://epi.grants.cancer.gov/diet/usualintakes/macros.html (Accessed August 9, 2020).

47. Zhang S, Krebs-Smith SM, Midthune D, Perez A, Buckman DW, Kipnis V, Freedman LS, Dodd KW, Carroll RJ. Fitting a bivariate measurement error model for episodically consumed dietary components. Int J Biostat 2011;7(1):1-.

48. Freedman LS, Guenther PM, Dodd KW, Krebs-Smith SM, Midthune D. The population distribution of ratios of usual intakes of dietary components that are consumed every day can be estimated from repeated 24-hour recalls. J Nutr 2010;140(1):111-6.

49. Gibson RS. Principles of nutritional assessment. New York: Oxford University Press, 2005.

50. Dewey KG, Brown KH. Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. Food Nutr Bull 2003;24(1):5-28.

51. Murphy SP, Guenther PM, Kretsch MJ. Using the dietary reference intakes to assess intakes of groups: pitfalls to avoid. J Am Diet Assoc 2006;106(10):1550-3.

52. Institute of Medicine. Dietary Reference Intakes: Applications in Dietary Assessment. Washington, DC: National Academies Press 2000.

53. Institute of Medicine. Dietary Reference Intakes: Applications in Dietary Planning. Washington, DC: National Academies Press, 2003.

54. Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, Ruel MT, Sandtröm B, Wasantwisut E, Hotz C. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004;25(1 Suppl 2):S99-203.

55. WHO. Vitamin and mineral requirements in human nutrition. Geneva, Switzerland: WHO, 2004.

56. Institute of Medicine. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Washington, DC: National Academies Press, 2006.

57. Morseth MS, Torheim LE, Chandyo RK, Ulak M, Shrestha SK, Shrestha B, Pripp AH, Henjum S. Severely inadequate micronutrient intake among children 9-24 months in Nepal-The MAL-ED birth cohort study. Matern Child Nutr 2018;14(2):e12552.

58. Murphy SP, Gewa C, Liang L-J, Grillenberger M, Bwibo NO, Neumann CG. School snacks containing animal source foods improve dietary quality for children in rural Kenya. J Nutr 2003;133(11):3950S-6S.

59. Van Hoan N, Van Phu P, Salvignol B, Berger J, Trèche S. Effect of the consumption of high energy dense and fortified gruels on energy and nutrient intakes of 6–10-month-old Vietnamese infants. Appetite 2009;53(2):233-40.

60. Swanepoel E, Havemann-Nel L, Rothman M, Laubscher R, Matsungo TM, Smuts CM, Faber M. Contribution of commercial infant products and fortified staple foods to

nutrient intake at ages 6, 12, and 18 months in a cohort of children from a low socio-economic community in South Africa. Matern Child Nutr 2019;15(2):e12674-e.

61. Beaton GH, Calloway DH, Murphy SP. Estimated protein intakes of toddlers: predicted prevalence of inadequate intakes in village populations in Egypt, Kenya, and Mexico. Am J Clin Nutr 1992;55(4):902-11.

62. Schulze KJ, Mehra S, Shaikh S, Ali H, Shamim AA, Wu LSF, Mitra M, Arguello MA, Kmush B, Sungpuag P, et al. Antenatal multiple micronutrient supplementation compared to iron-folic acid affects micronutrient status but does not eliminate deficiencies in a randomized controlled trial among pregnant women of rural Bangladesh. J Nutr 2019;149(7):1260-70.

63. Sanghvi T, Seidel R, Baker J, Jimerson A. Using behavior change approaches to improve complementary feeding practices. Matern Child Nutr 2017;13 Suppl 2(Suppl 2).

64. Darrouzet-Nardi AF, Miller LC, Joshi N, Mahato S, Lohani M, Rogers BL. Child dietary quality in rural Nepal: Effectiveness of a community-level development intervention. Food Policy 2016;61:185-97.

65. Dror DK, Allen LH. Overview of nutrients in human milk. Adv Nutr 2018;9(Suppl 1):278S-94S.

66. Karsten HD, Patterson PH, Stout R, Crews G. Vitamins A, E and fatty acid composition of the eggs of caged hens and pastured hens. Renew Agric Food Syst 2010;25(1):45-54.

67. Samman S, Kung FP, Carter LM, Foster MJ, Ahmad ZI, Phuyal JL, Petocz P. Fatty acid composition of certified organic, conventional and omega-3 eggs. Food Chemistry 2009;116(4):911-4.

Figure 1. Participant flow diagram of a trial providing a six-month protein intervention comparing the effect of the provision of a daily egg versus control on energy and nutrient intakes.

Table 1. Household, maternal, and infant baseline characteristics of infants enrolled in a trial providing a six-month protein intervention comparing the effect of the provision of a daily egg versus control on energy and nutrient intakes, by intervention group[1]

	Control (N = 943)		Egg (N = 757)	
Characteristic	*n*	% or Mean ± SD	*n*	% or Mean ± SD
Household				
Household size	934	4.5 ± 2.0	749	4.4 ± 1.9
LSI quintile				
1st (lowest)	192	20.6	147	19.6
2nd	189	20.2	143	19.1
3rd	194	20.8	164	21.9
4th	187	20.0	149	19.9
5th (highest)	172	18.4	146	19.5
HFI				
None	582	69.1	476	65.9
Mild	243	28.9	224	31.0
Severe	17	2.0	22	3.0
Maternal				
Age, y	922	23.6 ± 5.5	728	23.7 ± 5.4
Education				
No schooling	99	10.6	86	11.5
1-9 y	679	72.8	528	70.9
SSC passed	49	5.3	48	6.4
≥11 y	106	11.4	83	11.1
Infant				
Age, mo	856	6.3 ± 0.3	726	6.3 ± 0.3
Sex, M	943	51.3	757	52.8
Stunting, LAZ <-2	841	20.8	720	18.2
Wasting, WLZ <-2**	832	4.7	718	7.7
Underweight, WAZ <-2	844	17.3	722	15.4

[1] Linear or logistic regression models with generalized estimating equations or multinomial regression models specified with robust standard errors were used to compare characteristics between groups (**$P < 0.05$). HFI, Household Food Insecurity estimated using a nine-item questionnaire collapsed into an index with possible scores ranging from nine to 36 and categorized as none (HFI=9), mild (HFI>9 to HFI<16), and severe (HFI≥16); LAZ, length-for-age z-score; LSI, Living Standards Index calculated based on household assets and dwelling characteristics using principal components analysis; SSC, Secondary School Certificate; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score (30, 33).

Table 2. Estimated breastmilk intake of infants enrolled in a trial providing a six-month protein intervention comparing the effect of the provision of a daily egg versus control on energy and nutrient intakes, by age and intervention group[1]

	6 mo			9 mo			12 mo		
	Control (*n* = 181)	Egg (*n* = 173)	*P*-value	Control (*n* = 867)	Egg (*n* = 720)	*P*-value	Control (*n* = 943)	Egg (*n* = 757)	*P*-value
Breastfed, %[2]	99.4	97.1	0.13	98.6	98.5	0.79	98.4	97.9	0.40
Breastfeeding frequency, %[2]									
1-10 times	10.6	7.7	0.77	15.1	10.9	0.03	20.3	15.8	0.07
11-20 times	66.1	73.2		71.9	72.4		70.6	73.1	
21+ times	23.3	19.0		13.0	16.8		9.2	11.1	
Total energy needs, kcal (Mean ± SD)[3]	544 ± 64	552 ± 75	0.20	594 ± 78	596 ± 77	0.49	641 ± 83	648 ± 84	0.13
Estimated breast milk intake									
Volume, L [Median (25th, 75th pctl)][4]	0.79 (0.69, 0.88)	0.79 (0.68, 0.87)	0.66	0.65 (0.44, 0.80)	0.56 (0.37, 0.71)	<0.001	0.62 (0.37, 0.81)	0.48 (0.21, 0.67)	<0.001
Percent of total energy, % [Median (25th, 75th pctl)]	95 (86, 100)	95 (83, 100)	0.33	70 (48, 85)	62 (41, 75)	<0.001	62 (37, 79)	49 (21, 66)	<0.001

[1] Differences between groups tested using linear or logistic regression models with generalized estimating equations or multinomial regression models with robust standard errors for normally distributed data, and using clustered bootstrapping with n=1000 replicates for non-normally distributed data.

[2] In last 24 hours.

[3] Estimated based on sex-, age-, and weight-specific energy requirements set by FAO (37).

[4] Calculated only if assumed amount of breast milk > 0. Energy density of breast milk based on WHO (38).

Table 3. Consumption of food groups in the last 24 hours among infants enrolled in a trial providing a six-month protein intervention comparing the effect of the provision of a daily egg versus control on energy and nutrient intakes, by age and intervention group[1]

	6 mo			9 mo			12 mo		
Food group, any %	Control (n = 181)	Egg (n = 173)	P-value	Control (n = 867)	Egg (n = 720)	P-value	Control (n = 943)	Egg (n = 757)	P-value
Cereals	40.9	43.4	0.62	92.4	92.1	0.80	93.3	95.2	0.12
Tubers	0.6	2.9	0.12	48.6	47.8	0.65	63.1	63.7	0.93
Legumes	3.3	4.0	0.65	26.1	26.2	0.97	26.1	26.2	0.86
Formula	9.4	13.3	0.25	5.8	6.8	0.39	4.6	4.8	0.86
Dairy	18.2	14.5	0.37	27.2	29.2	0.40	21.2	25.5	0.04
Eggs[2]	11.6	13.3	0.59	30.2	97.6	<0.001	35.0	99.2	<0.001
Fish	8.3	7.5	0.77	40.4	37.9	0.31	47.8	49.1	0.61
Flesh meat	1.1	1.7	0.62	9.5	9.6	0.91	12.9	13.5	0.74
Liver	7.7	4.6	0.24	13.1	11.9	0.50	9.7	13.2	0.02
Vitamin A-rich F/Vs[3]	1.1	5.2	0.05	25.5	25.8	0.89	28.4	32.0	0.12
Vitamin C-rich F/Vs[4]	1.1	3.5	0.11	5.4	5.7	0.75	9.3	10.8	0.40
Biscuits	27.6	28.9	0.80	53.9	53.9	0.96	58.6	61.6	0.22
Other sweet snacks	5.5	5.8	0.94	34.9	38.5	0.22	10.7	13.2	0.13
Savory snacks	0.0	0.0	-	14.1	15.1	0.60	28.5	28.3	0.89

[1] Logistic regression models with generalized estimating equations were used to compare groups. F/Vs, fruits and vegetables.

[2] Includes consumption of eggs provided by the intervention at nine and 12 months.

[3] Includes green leafy vegetables, *khichuri*, mango, pumpkin, papaya, carrot.

[4] Includes mango, papaya, blackberry, orange, *malta*, cauliflower, pomelo, pineapple.

Table 4. Estimated total usual nutrient intakes among infants enrolled in a trial providing a six-month protein intervention comparing the effect of the provision of a daily egg versus control on energy and nutrient intakes, by age and intervention group[1]

		6 mo			9 mo			12 mo		
		Control (*n*=181)	Egg (*n*=173)	*P*-	Control (*n*=867)	Egg (*n*=720)	*P*-	Control (*n*=943)	Egg (*n*=757)	*P*-
	DRI	Mean ± SD	Mean ± SD	value	Mean ± SD	Mean ± SD	value	Mean ± SD	Mean ± SD	value
Energy, kcal/d	-	545 ± 36	548 ± 36	0.26	602 ± 87	610 ± 93	<0.001	658 ± 111	669 ± 116	<0.001
Protein, g/(kg·d)	0.95/1.12	1.5 ± 0.2	1.5 ± 0.2	0.52	1.7 ± 0.4	2.2 ± 0.4	<0.001	1.9 ± 0.5	2.4 ± 0.6	<0.001
Available protein, g/(kg·d)	0.95/1.12	1.4 ± 0.3	1.3 ± 0.3	0.19	1.6 ± 0.4	2.0 ± 0.4	<0.001	1.8 ± 0.5	2.1 ± 0.6	<0.001
Percent calories from protein	-	7 ± 1	7 ± 0	0.26	9 ± 2	11 ± 1	<0.001	9 ± 1	11 ± 2	<0.001
Total fat, g/d	30*	32 ± 5	32 ± 5	0.35	31 ± 6	31 ± 6	0.02	32 ± 7	32 ± 6	<0.001
Percent calories from fat	-	53 ± 6	53 ± 3	0.38	47 ± 6	46 ± 6	<0.001	45 ± 8	43 ± 7	<0.001
Vitamin A, µg/d	500*	200 ± 5	200 ± 5	0.56	225 ± 39	246 ± 55	<0.001	228 ± 75	270 ± 95	<0.001
Thiamin, mg/d	0.3*	0.2 ± 0.0	0.2 ± 0.0	<0.001	0.2 ± 0.0	0.2 ± 0.0	<0.001	0.2 ± 0.1	0.3 ± 0.1	<0.001
Riboflavin, mg/d	0.4*	0.2 ± 0.1	0.2 ± 0.1	0.01	0.3 ± 0.1	0.4 ± 0.1	<0.001	0.3 ± 0.1	0.4 ± 0.1	<0.001
Niacin, mg/d	4*	1.5 ± 0.4	1.5 ± 0.4	0.24	2.7 ± 0.8	3.6 ± 0.8	<0.001	3.5 ± 1.3	4.5 ± 1.4	<0.001
Vitamin B-6, mg/d	0.3*	0.1 ± 0.0	0.1 ± 0.0	<0.001	0.2 ± 0.1	0.2 ± 0.1	<0.001	0.2 ± 0.1	0.3 ± 0.1	<0.001
Folate, µg/d	80*	70 ± 11	70 ± 11	0.05	71 ± 11	76 ± 17	<0.001	74 ± 17	78 ± 19	<0.001
Vitamin B-12, µg/d	0.5*	0.4 ± 0.0	0.3 ± 0.0	<0.001	0.6 ± 0.3	0.8 ± 0.2	<0.001	0.7 ± 0.4	1.0 ± 0.5	<0.001
Choline, mg/d	150*	80 ± 22	78 ± 22	<0.001	95 ± 24	153 ± 24	<0.001	105 ± 34	168 ± 29	<0.001
Vitamin C, mg/d	50*	31 ± 4	31 ± 4	0.13	30 ± 1	29 ± 11	<0.001	32 ± 9	27 ± 9	<0.001
Vitamin D, µg/d	10	0.5 ± 0.2	0.5 ± 0.2	0.67	0.6 ± 0.2	1.0 ± 0.3	<0.001	0.6 ± 0.3	1.1 ± 0.2	<0.001
Vitamin E, mg/d	5*	1.9 ± 0.3	1.9 ± 0.3	0.24	2.0 ± 0.2	2.0 ± 0.5	<0.001	2.1 ± 0.4	2.1 ± 0.6	0.12
Calcium, mg/d	260*	246 ± 9	246 ± 9	0.94	254 ± 47	242 ± 55	<0.001	270 ± 76	255 ± 46	<0.001
Copper, mg/d	0.22*	0.2 ± 0.0	0.2 ± 0.0	0.04	0.3 ± 0.1	0.4 ± 0.1	<0.001	0.4 ± 0.1	0.5 ± 0.1	<0.001
Iron, mg/d	-	0.5 ± 0.2	0.6 ± 0.2	<0.001	1.5 ± 0.7	2.0 ± 0.4	<0.001	2.0 ± 1.0	2.7 ± 0.8	<0.001
Magnesium, mg/d	75*	32 ± 7	32 ± 7	<0.001	52 ± 16	58 ± 14	<0.001	63 ± 21	71 ± 14	<0.001
Phosphorus, mg/d	275*	140 ± 8	142 ± 8	0.11	193 ± 52	248 ± 48	<0.001	227 ± 63	291 ± 75	<0.001
Potassium, mg/d	860*	449 ± 60	449 ± 60	0.96	504 ± 106	505 ± 98	0.18	552 ± 124	547 ± 140	<0.001
Zinc, mg/d	4	1.2 ± 0.3	1.1 ± 0.3	<0.001	1.7 ± 0.4	2.3 ± 0.4	<0.001	2.0 ± 0.6	2.7 ± 0.7	<0.001

---

[1] Differences in mean usual intakes estimated using clustered bootstrapping with n=1000 replicates. *P*-values were adjusted for multiple comparisons such that $P < 0.05/22 = 0.002$ was considered statistically significant. Available protein intake was estimated using the Digestible Indispensable Amino Acid Score method (43). DRI, Dietary Reference Intake. DRIs listed for infants six to 12 months of age. Adequate Intake values are followed by an asterisk (*). The DRI listed for vitamin D is the Recommended Dietary Allowance value. All other values are Estimated Average Requirements (53).

Table 5. Proportion (%) meeting nutrient intake recommendations among infants enrolled in a trial providing a six-month protein intervention comparing the effect of the provision of a daily egg versus control on energy and nutrient intakes, by age and intervention group[1]

	6 mo			9 mo			12 mo		
	Control (n = 181)	Egg (n = 173)	*P*-value	Control (n = 867)	Egg (n = 720)	*P*-value	Control (n = 943)	Egg (n = 757)	*P*-value
Protein, g/(kg·d)	98.9	98.8	0.97	94.8	99.9	<0.001	99.3	99.9	<0.001
Available protein, g/(kg·d)	84.6	79.8	0.01	90.7	99.6	<0.001	98.0	99.7	<0.001
Total fat, g/d	68.7	69.5	0.33	60.2	60.6	0.35	63.9	62.7	0.01
Vitamin A, µg/d	0.0	0.0	-	0.0	<0.1	<0.001	0.2	2.2	<0.001
Thiamin, mg/d	0.0	0.0	-	1.2	2.6	<0.001	9.7	22.4	<0.001
Riboflavin, mg/d	0.5	0.7	0.13	13.8	29.6	<0.001	21.7	45.9	<0.001
Niacin, mg/d	0.0	0.0	-	6.3	28.2	<0.001	28.2	59.6	<0.001
Vitamin B-6, mg/d	0.0	0.0	-	2.0	2.9	<0.001	11.9	23.1	<0.001
Folate, µg/d	17.4	16.8	0.22	22.0	36.4	<0.001	35.2	43.7	<0.001
Vitamin B-12, µg/d	0.0	0.0	-	60.3	91.2	<0.001	62.4	83.7	<0.001
Choline, mg/d	0.2	0.1	0.22	2.0	52.5	<0.001	10.1	71.7	<0.001
Vitamin C, mg/d	0.0	0.0	-	0.0	4.2	<0.001	3.6	1.5	<0.001
Vitamin D, µg/d	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Vitamin E, mg/d	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-
Calcium, mg/d	0.0	0.0	-	42.8	35.3	<0.001	51.1	42.6	<0.001
Copper, mg/d	61.3	58.8	0.02	94.3	100	<0.001	98.4	99.8	<0.001
Iron, mg/d	0.0	0.0	-	0.3	0.1	<0.001	1.3	1.9	<0.001
Magnesium, mg/d	0.0	0.0	-	8.5	11.1	<0.001	24.5	36.2	<0.001
Phosphorus, mg/d	0.0	0.0	-	7.0	26.2	<0.001	19.9	54.2	<0.001
Potassium, mg/d	0.0	0.0	-	0.3	0.2	0.04	1.7	2.7	<0.001
Zinc, mg/d	0.0	0.0	-	<0.1	0.1	<0.001	0.6	4.8	<0.001

[1] Calculated by comparing usual intake to Adequate Intake values of the Institute of Medicine (IOM), with exception for protein, iron, and zinc (53). Prevalence of adequate crude and available protein intake calculated by comparing usual intake to requirements on a per-kilogram weight basis, or 1.12 g/(kg·d) for six- and nine-month-olds and 0.95 g/(kg·d) for 12-month-olds as described by FAO (44). Prevalence of adequate iron intake was assessed relative to requirement Table I-5 for six- to 12-month-olds from the IOM, and that of zinc relative to the Estimated Average Requirement value (4 mg/d) set by the International Zinc Nutrition Consultative Group (53, 54). Differences between groups tested using clustered bootstrapping with n=1000 replicates. *P*-values were adjusted for multiple comparisons such that $P < 0.05/22 = 0.002$ was considered statistically significant.