

Effect of lactoferrin in oral

by Nur Aisyah Widjaja

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RESEARCH ARTICLE

Effect of lactoferrin in oral nutrition supplement (ONS) towards IL-6 and IL-10 in failure to thrive children with infection [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Growth failure due to infection in children is a major health problem throughout the world. It provokes a systemic immune response, with increased interleukin (IL)-6 and reduced IL-10. Lactoferrin (Lf) is a multifunctional iron-binding protein that can be found in whey protein inside formula milk such as oral nutrition supplement (ONS), which can upregulate anti-inflammatory cytokines (IL-10) and modulate pro-inflammatory cytokines. This study investigates the effect of Lf supplementation in ONS on IL-6 and IL-10 levels in children with failure to thrive and infection.

Methods: We performed a quasi-experimental pre- and post-study in children aged 12–60 months old with failure to thrive due to infectious illness. The subjects received 400 ml of oral nutritional supplements (ONS, 1 ml equivalent to 1 kcal) each day for 90 days, and their parents received dietary advice and medication based on the underlying illness. Blood was drawn to measure IL-6 and IL-10 before and after the intervention.

Results: There were 75 subjects recruited and divided into group-1 and group-2 based on age. The incidence of undernutrition was 37.33%. Lf in ONS intervention improved body weight and body length. Lf also reduced IL-6, although there was not a significant difference before and after the intervention. However, the IL-6 reduction was significantly higher in subjects with undernutrition compared with subjects with weight faltering. Pre-intervention IL-6 levels were higher in children with stunting than in children with normal stature. There was a greater change in IL-6 in children with severe stunting than in children with normal stature or stunting. IL-10 was significantly reduced after the intervention.

Conclusions: In addition to improving body weight and length, Lf supplementation in ONS improved immune response homeostasis by balancing IL-6 and IL-10 levels and by improving the IL-6/IL-10 ratio.

Open Peer Review


Approval Status ? ?


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
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Keywords

IL-6, IL-10, lactoferrin, growth failure



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Introduction

Growth failure is still an important health problem, with ⁵⁵ weight-for-age (WAZ) and length-for-age (LAZ) declining during the golden period or *golden 1,000 days* (period during pregnancy until second years of life),¹ and insignificant growth thereafter.² Nutritional intervention during this period will impact a child's growth, development and ability to thrive.¹ Infection in children causes growth failure by provoking a systemic immune response which affects the nutritional status,³ especially as a result of a reduction of insulin-like growth factor 1 (IGF-1).⁴

⁹¹ Undernutrition refers to children who are underweight, stunted or wasted, or have nutrient deficiency which makes the children vulnerable to infection.⁵ The induction of the acute phase response and proinflammatory cytokine production caused by infection directly affect bone remodeling which is important for long bone growth,³ and also inhibits chondrogenesis.⁶ Pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor-alpha (TNF- α) were found to have increased in stunted children, which also increases leptin levels leading to a limited appetite.⁴ These proinflammatory cytokines also cause bone breakdown.⁶ IL-6 has anti- and pro-inflammatory functions. After it binds with IL-6 receptors in the liver, it stimulates hepatocytes to produce acute-phase proteins and cytokines via multiple signaling pathways.⁷

⁴² Oral nutritional supplements (ONS), also known as food for special medical purposes, contain both macro- and micronutrients that are sufficient to meet daily nutritional needs for those at risk of malnutrition.⁸ ONS is prescribed to increase nutritional intake due to insufficiency in diets to meet daily nutritional requirements,⁹ particularly protein and calories.¹⁰ ONS not only provides some benefits for hospital admission patients such as a reduced length of hospital stay (LOS), reduced inpatient cost, complication rates, readmission rates and improved lean body mass recovery,¹¹ but also improves energy intake and nutritional status such as body weight, length and mid-arm circumference.¹² For children, ONS is a dairy milk-based product, which is designed to provide an energy density of 1–1.5 kcal/ml, and it is expected to be effective in improving growth.¹³

Lactoferrin (Lf) concentration within the whey protein that is contained in the modified cow's milk formula is only 0.1 mg/ml.¹⁴ ONS contains 10.8 g of protein per 100 g, which is 46% whey and 54% casein. The effect of Lf supplementation (dose 0.6 g/L and 1.00) compared to standard infant formula on body weight showed no significant difference in children until 12 months old.¹⁵ Lf acts as an innate immune regulator and defense due to its antimicrobial properties.¹⁶ It can interact with the immune system, such as influencing cytokine activity by upregulating anti-inflammatory cytokines (IL-4 and IL-10) or modulating proinflammatory cytokines.¹⁷ An *in vitro* study showed that Lf (10 mg/mouse *i.v.*) before thymectomy reduced IL-6 by 70%, and TNF- α by 30% 4 hours after an operation.¹⁸ A study in adults showed that Lf reduced systemic inflammatory biomarkers by 61%, improved immune function by 75%, changed immune cell activity by 40% and reduced respiratory tract infection outcomes by 60%. In adults, lactoferrin has been shown to reduce IL-6 by 24.9 pg/mL.¹⁹

Here we investigate the effect of lactoferrin in ONS towards IL-6 and IL-10 in failure to thrive children with infection for 90 days of intervention.

Methods

Ethical statement

⁷² The study passed ethical exemption and was declared to be ethically appropriate by the Health Research Ethics Committee, Airlangga University, Surabaya, Indonesia, number 226/EC/KEPK/FKUA/2021 on October 4th 2021 and registered on [ClinicalTrials.gov](https://clinicaltrials.gov) number ID: NCT05289674, initial released on May 3rd, 2022.

Participants

A quasi experimental pre- and post- study design was performed from October 2021 until July 2022 recruiting children aged between 1 years (12-months-old) and 5 years (60-months-old) with failure to thrive due to infectious illness (mainly urinary tract infection and tuberculosis (TB)) diagnosed by a paediatrician (the researcher) based on clinical and laboratory findings at Husada Utama Hospital outpatient unit, Surabaya, Indonesia. The subjects included in the study were excluded if they had fluid retention, organomegaly, a tumor mass, congenital abnormalities, cerebral palsy or hormonal disorders and syndromes. A written informed consent was signed by the parents as approval to participate in the study after the researcher explained the importance, the risks and the benefits of this study.

¹⁴ Sample size

The sample size was determined using the formula below:

$$n1 = n2 = \frac{(Z\alpha\sqrt{2PQ} + Z\beta\sqrt{P1Q1 + P2Q2})^2}{(P1 - P2)^2}$$

Note:

N = sample size

$Z\alpha$ = standard deviation (α) 5% (1.96)

$Z\beta$ = power, the researchers determined 90% (0.842)

$P1$ = clinical judgement 15% = 0.1

$P2$ = standard effect $P2 = 25.5\% = 0.25$.²⁰

$P = \frac{1}{2}(P1+P2) = 0.175$

$Q = 1-P = 0.825$

$Q1 = 1-P1 = 0.9$

$Q2 = 1-P2 = 0.75$

The sample size was 80 subjects; pre- and post-design needed for the study was 160 samples.

Interventions

The subjects were given an oral nutritional supplement or ONS with Lf (1 ml ~1 kcal, 400 ml/day), SGM Eksplor Gain Optigrow[®] prescribed by the researchers for 15 days consumption (equal to 4 boxes of 400 g) for initial intervention to detect any adverse reaction. The authors used this formula due to its relatively cheaper cost compared to other high calorie formula (ONS) available in Indonesia. The parents also had dietary counseling, animal protein was provided and a medication plan was given according to the underlying disease. Parents were asked to report any side effects to the researcher's team by phone for further medical treatment. The parents were asked to visit the doctor after 14 days of ONS consumption for anthropometric measurements, compliance and side effect monitoring at day 15. While visiting, parents also received ONS for the next 15 days consumption (day 16 to 30), and were asked to visit the doctor again on day 30, 60 and 90. At the 30-day visit, parents received ONS for two months' consumption (day 31 to day 60, and day 61 until day 90) (8 boxes of 400 g) and anthropometric measurements.

Blood was withdrawn via vena cubiti by a laboratory employee at Husada Utama Hospital to measure IL-6 (human IL-6 ELISA kit, code E0090Hu, BT Lab) and IL-10 (human IL-10 ELISA kit, code E0102Hu, BT Lab) before (day 0, when the parents agreed to participate in this study) and after the intervention (day 90). After the blood samples were collected, they were placed in a non-EDTA containing tube for micro-centrifugation to separate blood plasma from blood serum at 3000 rpm for 10 minutes. The supernatant was removed and placed in a PCR tube of 1.5 mL, then kept in a freezer at -4°C.

An indirect sandwich ELISA was performed to analyse IL-6 and IL-10 levels before- and after nutritional intervention using blood serum. For the sandwich ELISA, all reagents (standard solution, wash buffer, substrate solution A, substrate solution B and stop solution) were brought to room temperature before use (27°C).²¹

Preparation of standard solution

A total of 120 μ L standard solution (640 ng/ml) was diluted with 12 μ L standard diluent to produce a 320 ng/L standard stock solution, and it was then allowed to rest for 15 minutes. Standard duplication points were made using a serial dilution of standard stock solution to produce 160 ng/L, 80 ng/L, 40 ng/L and 20 ng/L solutions.

Preparation for wash buffer solution

Then 20 ml of wash buffer concentrate 25 \times was added to distilled water to yield 500 mL of 1 \times wash buffer. The wash buffer was mixed gently if crystals formed in the concentrate until the crystals had completely dissolved.

Assay procedure

The assay procedure was performed at room temperature after we determined the number of strips required for the assay, and then we inserted the strips in the frames for use.

1. 50 μ L of the standard solution was added into all the sample wells.
2. Then 50 μ L standard solution was added into the standard wells.
3. 40 μ L of sample was added to the sample wells and then 10 μ L of human IL-6 or IL-10 antibody was added. Then 50 μ L streptavidin-HRP was added to sample wells and standard wells, but not the blank control well. Each of them were mixed before the wells were placed on the plate and then sealed for incubation at 37°C for 60 minutes.
4. After 60 minutes of incubation, the seals were removed, and the plates were washed 5 times with wash buffer; the wells were soaked in 300 μ L of wash buffer for 30 seconds to 1 minute for each wash.
5. 50 μ L of substrate solution A and 50 μ L of substrate solution B were added to each well and the plate was covered and incubated for 10 minutes at 37°C in the dark.
6. 50 μ L of stop solution was added to each well, so that the blue colour changed to yellow immediately.

We then determined the optical density (OD value) of each well immediately using a microplate reader set at 450 nm of wavelength within 30 minutes after the stop solution was added, and then the standard curve was made.²¹

Body weight was measured using a Seca 354 digital baby scale or a Seca 813 electronic flat scale) and body length/height was measured using a Seca 415 infantometer or Seca 213 stadiometer). Both measurements were taken twice by a trained nurse in the outpatient department of Husada Utama Hospital. The weight and length/height were the average value of the two measurements. When the subjects were weighed and measured, they wore light clothes without footwear or hair accessories. Anthropometry measurement for weight-for-age z-score (WAZ), length-for-age or height-for-age z-score or height-for-age z-score (LAZ/HAZ) and weight-for-length or weight-for-height z-score or weight-for-height z-score (WLZ/WHZ) were determined using WHO Anthro offline version 3.2.2. All the data are summarized in the underlying data²² and extended data.²³

Statistical analysis

Statistical analysis conducted in this study was a test of normality and homogeneity, independent sample T-test or Mann-Whitney U test, Fischer exact test, Pearson chi-square, paired sample T-test or Wilcoxon, two-way ANOVA and one-way ANOVA using IBM SPSS Statistics version 21.

Results

Seventy-five subjects were involved in the study and divided into two groups based on the age of the participant: group-1 (age 1–2 years, n = 39) and group-2 (age 2–5 years old, n = 36), as summarized in Figure 1.

Table 1 summarizes the characteristics of the subjects who participated in the study. The ratio of male/female was 12/13 and there was no significant difference in gender distribution in both groups ($p = 0.108$). There was no significant difference in the main complaint ($p = 0.229$), duration of complaints ($p = 0.580$), WAZ ($p = 0.482$) and WLZ/WHZ ($p = 0.499$). Age, ideal body weight and height age were lower in group-1 compared to group-2 ($p < 0.05$). LAZ was lower in group-1 compared to group-2 (-1.95 ± 1.17 vs. -1.19 ± 0.86 , $p = 0.002$).

The incidence of underweight and severely underweight children in group-1 and group-2 were 33.33% and 5.33% respectively, and there was no significant difference in WAZ categories in both groups ($p = 0.874$). While stunted and severely stunted children in group-1 and group-2 were 25.33% and 13.33% respectively, with a higher incidence of stunted/severely stunted children in group-1 compared to group-2 (56.41% vs. 19.45%, $p = 0.004$). However, the incidence of stunted/severely stunted children in group-1 was predominantly boys (6 boys vs. 1 girl). The incidence of wasted and severely wasted children in group-1 and group-2 were 12% and 2.67% ($p = 0.486$).

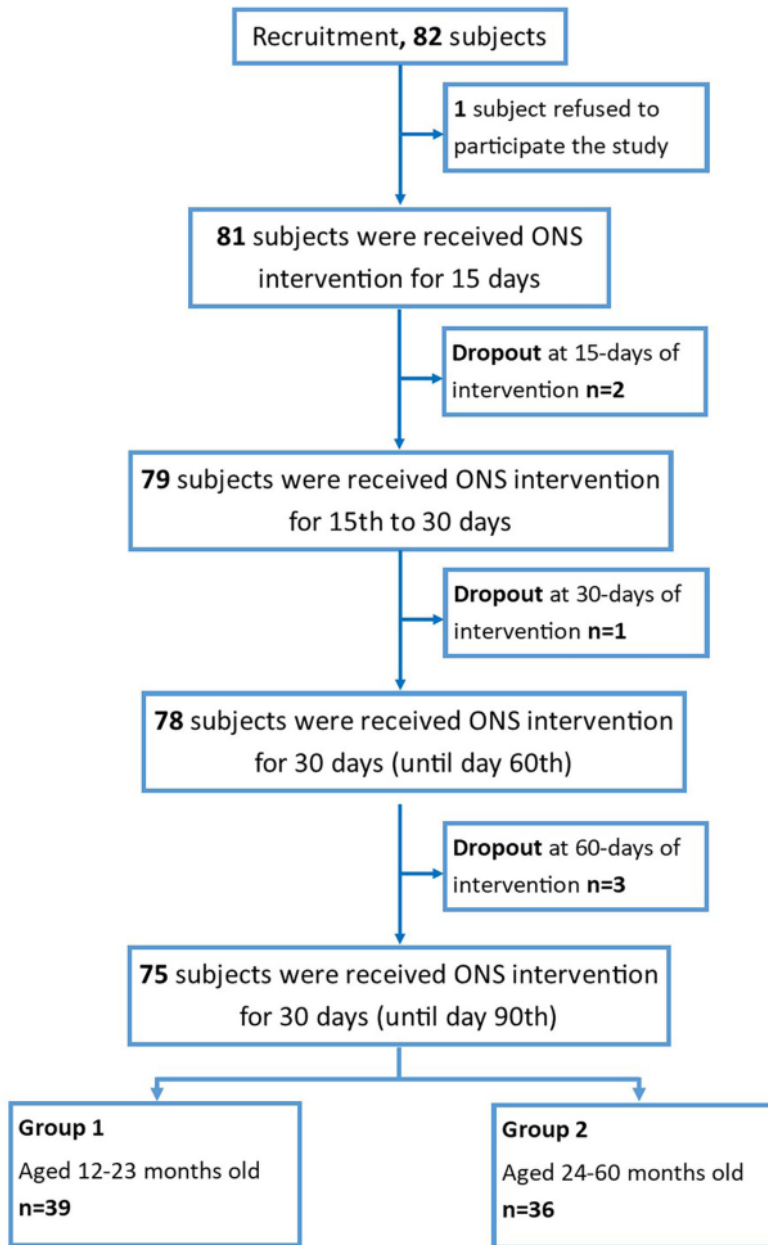


Figure 1. Flowchart of the subject's recruitment.

The effect of ONS on body weight and body length/height change is summarized in Table 2. Initial body weight before treatment was lower in group-1 compared to group-2 ($p = 0.000$). post intervention body weight was lower in group-1 than in those of group-2 ($p = 0.000$) but the weight change (Δ body weight) in both groups showed no significant difference (922.56 ± 671.28 vs. 855.55 ± 577.16 g, $p > 0.05$). The initial body length/height was shorter in group-1 compared to group-2 ($p = 0.000$), so the late body length/height was shorter in group-1 compared to group-2 ($p = 0.000$). Body length/height change was greater in group-1 compared to group-2 (3.49 ± 1.43 vs. 2.08 ± 1.04 cm, $p = 0.000$).

Table 1. Subject's characteristic during the study.

| Subject's characteristics | Group-1 (mean ± SD) | Group-2 (mean ± SD) | p |
|---|------------------------|------------------------|--------------------|
| Age, months | 16.74 ± 3.63 | 35.44 ± 7.70 | 0.000 ¹ |
| Gender, n (%) | | | 0.108 ² |
| - Male | 15 (36.59) | 21 (58.33) | |
| - Female | 24 (58.53) | 15 (41.67) | |
| Main complains: | | | 0.229 ³ |
| - Feeding difficulties with stagnant body weight gain | 10 (25.64) | 16 (44.44) | |
| - Stagnant body weight gain and length gain | 1 (2.56) | 0 | |
| - Body weight gain | 27 (69.29) | 20 (55.56) | |
| - Fever with liquid pups | 1 (2.56) | 0 | |
| Duration of complaint, months | 7.84 ± 4.29 | 12.23 ± 11.15 | 0.580 ¹ |
| WAZ | -1.52 ± 1.08 | -1.35 ± 0.99 | 0.482 ⁴ |
| WAZ categories, n (%) | | | 0.874 ³ |
| - Weight faltering (normo-weight) | 25 (64.10) | 21 (58.33) | |
| - Underweight | 12 (30.77) | 13 (36.11) | |
| - Severely underweight | 2 (5.13) | 2 (5.56) | |
| LAZ/HAZ | -1.95 ± 1.17 | -1.19 ± 0.86 | 0.002 ⁴ |
| LAZ/HAZ categories, n (%) | | | 0.004 ³ |
| - Normo-stature | 17 (43.59) | 29 (80.56) | |
| - Stunted | 14 (35.90) | 5 (13.89) | |
| - Severely stunted | 8 (20.51) | 2 (5.56) | |
| WLZ/WHZ | -0.85 ± 0.92 | -1.01 ± 1.12 | 0.499 ⁴ |
| WLZ/WHZ categories, n (%) | | | 0.486 ³ |
| - Good nutrition | 35 (89.74) | 29 (80.56) | |
| - Wasted | 3 (7.69) | 6 (16.67) | |
| - Severely wasted | 1 (2.56) | 1 (2.78) | |

Note: WAZ = weight-for-age z-score; LAZ = length-for-age z-score; HAZ = height-for-age z-score; WLZ = weight-for-length z-score; WHZ = weight-for-height z-score.

¹Mann-Whitney U Test.

²Fischer's Exact Test.

³Pearson Chi Square.

⁴Independent Sample T-Test.

Table 2. Body weight (in g) and body length/height (in cm) changes after intervention.

| Parameters | Group-1 (mean ± SD) | Group-2 (mean ± SD) | p |
|---|------------------------|------------------------|--------------------|
| Initial body weight | 8,735.38 ± 1,318.64 | 12,164.17 ± 1,688.89 | 0.000 ¹ |
| Late body weight | 9,657.95 ± 1,607.48 | 13,019.72 ± 1,768.91 | 0.000 ¹ |
| Body weight change (Δ Body weight) | 922.56 ± 671.28 | 855.55 ± 577.16 | 0.646 ¹ |
| Initial body length/height | 75.14 ± 5.26 | 91.39 ± 4.80 | 0.000 ¹ |
| Late body length/height | 78.63 ± 5.02 | 93.47 ± 4.84 | 0.000 ¹ |
| Body length/height change (Δ length/height) | 3.49 ± 1.43 | 2.08 ± 1.04 | 0.000 ¹ |

¹Independent sample T-test.

IL-6 and IL-10 levels during the intervention are summarized in Table 3. The levels of IL-6 post-intervention (day 90) were not significantly different from pre-intervention (128.45 ± 109.92 vs. 111.76 ± 78.10 pg/mL, $p = 0.554$), although there was a decline (-16.68 ± 91.09 pg/mL) in both groups (-13.42 ± 97.80 vs. -20.23 ± 84.46 pg/mL, $p = 0.749$). There was no significant difference in IL-6 levels before the treatment in both groups ($p < 0.232$) and after treatment ($p <$

Table 3. IL-6 and IL-10 in both group-1 and group-2 (in pg/mL).

| IL-6 markers | Group-1 (mean \pm SD) | Group-2 (mean \pm SD) | p |
|---|----------------------------|----------------------------|--------------------|
| Initial levels of IL-6 | 113.80 \pm 109.24 | 144.31 \pm 109.96 | 0.232 ¹ |
| Initial levels of IL-6 based on nutritional status | | | 0.669 ² |
| - Weight faltering | 94.12 \pm 85.49 | 126.13 \pm 93.17 | |
| - Undernutrition | 136.76 \pm 130.54 | 191.58 \pm 139.54 | |
| Late levels of IL-6 | 100.38 \pm 73.95 | 124.09 \pm 81.59 | 0.191 ¹ |
| Late levels of IL-6 based on nutritional status | | | 0.121 ² |
| - Weight faltering | 95.31 \pm 70.06 | 115.52 \pm 76.85 | |
| - Undernutrition | 108.51 \pm 81.65 | 166.89 \pm 98.51 | |
| Δ IL-6 | -13.42 \pm 97.80 | -20.23 \pm 84.46 | 0.749 ¹ |
| Δ IL-6 levels based on nutritional status | | | 0.001 ² |
| - Weight faltering | -9.00 \pm 83.72 | -19.86 \pm 64.76 | |
| - Undernutrition | -18.56 \pm 114.38 | -21.18 \pm 126.85 | |
| Initial levels of IL-10 | 423.66 \pm 385.66 | 501.88 \pm 400.43 | 0.392 ¹ |
| Initial levels of IL-10 based on nutritional status | | | 0.131 ² |
| - Weight faltering | 407.09 \pm 367.31 | 411.74 \pm 318.22 | |
| - Undernutrition | 442.99 \pm 415.93 | 736.23 \pm 507.72 | |
| Late levels of IL-10 | 240.41 \pm 165.75 | 283.89 \pm 161.27 | 0.254 ¹ |
| Late levels of IL-10 based on nutritional status | | | 0.650 ² |
| - Weight faltering | 211.15 \pm 100.40 | 256.25 \pm 137.08 | |
| - Undernutrition | 274.56 \pm 217.39 | 355.76 \pm 202.48 | |
| Δ IL-10 | -183.24 \pm 378.50 | -217.99 \pm 294.61 | 0.661 ¹ |
| Δ IL-10 levels based on nutritional status | | | 0.113 ² |
| - Weight faltering | -195.94 \pm 345.44 | -155.49 \pm 234.96 | |
| - Undernutrition | -168.43 \pm 423.58 | -380.47 \pm 378.91 | |
| Initial value of IL-6/IL-10 ratio | 0.33 \pm 0.28 | 0.33 \pm 0.14 | 0.991 ¹ |
| Initial value of IL-6/IL-10 ratio based on nutritional status | | | 0.179 ² |
| - Weight faltering | 0.28 \pm 0.17 | 0.34 \pm 0.16 | |
| - Undernutrition | 0.38 \pm 0.37 | 0.29 \pm 0.16 | |
| Late value of IL-6/IL-10 ratio | 0.43 \pm 0.12 | 0.45 \pm 0.12 | 0.616 ¹ |
| Late value of IL-6/IL-10 ratio based on nutritional status | | | 0.560 ² |
| - Weight faltering | 0.43 \pm 0.12 | 0.44 \pm 0.11 | |
| - Undernutrition | 0.44 \pm 0.11 | 0.47 \pm 0.12 | |
| Δ IL-6/IL-10 ratio | 0.12 \pm 0.29 | 0.12 \pm 0.17 | 0.262 ¹ |
| Δ IL-6/IL-10 ratio based on nutritional status | | | 0.125 ² |
| - Weight faltering | 0.15 \pm 0.19 | 1.00 \pm 0.16 | |
| - Undernutrition | 0.06 \pm 0.37 | 0.18 \pm 0.21 | |

¹Independent sample T-test.

²Two-way ANOVA.

0.191). IL-10 level was significantly reduced after the intervention (461.20 ± 392.12 became 261.28 ± 163.97 pg/mL, $\Delta = 199.92 \pm 339.01$ pg/mL, $p=0.000$). The reduction in IL-10 showed no significant difference in either group (-183.24 ± 378.50 vs. -217.99 ± 294.61 pg/mL, $p = 0.518$).

There was significantly improvement in the IL-6/IL-10 ratio after the intervention (0.33 ± 0.22 vs. 0.44 ± 0.12 , $\Delta = 0.11 \pm 0.23$, $p = 0.000$). The reduction in the IL-6/IL-10 ratio showed no significant difference in either group (0.11 ± 0.29 vs. 0.12 ± 0.17 , $p = 0.991$).

The levels of IL-6 based on anthropometric categories are summarized in Table 4. Based on the LAZ categories, there was a significant difference in IL-6 levels pre-intervention ($p = 0.045$), in which the stunted group had higher levels of IL-6 compared to those with a normal stature (212.06 ± 146.05 vs. 115.81 ± 93.84 pg/mL, $p = 0.037$). Although there was no significant difference, IL-6 was higher in the stunted group compared to those who were severely stunted (212.06 ± 146.05 vs. 85.45 ± 89.06 cm, $p=0.057$). There was no significant difference in post-intervention levels of IL-6 ($p = 0.083$); however, IL-6 was lower in normal stature children compared to stunted and severely stunted children. Although IL-6 levels were higher in the stunted group compared to the severely stunted group, there was no significant difference between both groups (212.06 ± 146.05 vs. 85.45 ± 89.06 pg/mL, $p = 0.057$). Changes in IL-6 (Δ IL-6) based on the LAZ/HAZ categories showed no significant difference ($p = 0.055$), but the changes in severely stunted children were higher compared to the stunted group (47.33 ± 93.48 vs. -41.66 ± 108.69 pg/mL, $p = 0.036$) and the normal stature group (47.33 ± 93.48 vs. -20.28 ± 77.36 pg/mL, $p = 0.031$). This was due to increased IL-6 in severely stunted children, but reduced IL-6 in stunted children and those of normal stature.

The levels of IL-6 based on the WAZ categories showed no significant difference pre-intervention ($p = 0.903$) or post-intervention ($p = 0.173$), but the change in IL-6 (Δ IL-6) showed a significant difference ($p = 0.014$), where the WAZ in severely underweight children increased, while the underweight and weight faltering decreased. Therefore, severely underweight children had higher changes of IL-6 compared to stunted children (78.14 ± 32.06 vs. -44.19 ± 137.03 pg/mL, $p = 0.012$) and weight faltering/normal weight children (78.14 ± 32.06 vs. -16.09 ± 73.95 pg/mL, $p = 0.001$).

The initial and late changes of IL-6 (Δ IL-6) based on WLZ/WHZ categories showed no significant difference ($p > 0.05$). The initial level of IL-6 was higher in good nutritional status subjects compared to wasted and severely wasted. But after

Table 4. IL-6 levels based on anthropometry categories.

| Markers | Pre-intervention (mean \pm SD) | Post-intervention (mean \pm SD) | Δ IL-6 (mean \pm SD) |
|----------------------------------|----------------------------------|-----------------------------------|-------------------------------|
| IL-6 based on LAZ/HAZ categories | | | |
| - Normo-stature | 115.81 \pm 93.84 | 104.81 \pm 48.67 | -20.28 \pm 77.36 |
| - Stunted | 212.06 \pm 146.05 | 117.51 \pm 115.94 | -41.66 \pm 108.69 |
| - Severely stunted | 85.45 \pm 89.06 | 132.78 \pm 103.03 | 47.33 \pm 93.48 |
| p value | 0.045 ² | 0.083 ¹ | 0.055 ² |
| IL-6 based on WAZ categories | | | |
| - Normo-weight | 125.59 \pm 99.41 | 109.49 \pm 75.87 | -16.09 \pm 73.95 |
| - Underweight | 145.73 \pm 151.51 | 101.54 \pm 73.55 | -44.19 \pm 137.03 |
| - severely underweight | 103.70 \pm 85.38 | 181.84 \pm 111.85 | 78.14 \pm 32.06 |
| p value | 0.903 ² | 0.173 ¹ | 0.014 ² |
| IL-6 based on WLZ/WHZ categories | | | |
| - Good nutritional status | 131.54 \pm 110.91 | 109.49 \pm 75.98 | -22.05 \pm 92.83 |
| - Wasted | 129.02 \pm 110.60 | 138.75 \pm 97.46 | 9.73 \pm 84.73 |
| - Severely wasted | 26.83 \pm 5.26 | 63.04 \pm 8.72 | 36.22 \pm 3.46 |
| p value | 0.235 ² | 0.391 ¹ | 0.148 ² |

Note: WAZ = weight-for-age z-score; LAZ = length-for-age z-score; HAZ = height-for-age z-score; WLZ = weight-for-length z-score; WHZ = weight-for-height z-score.

¹One-way ANOVA.

²Kruskal-Wallis.

Table 5. IL-10 levels based on anthropometry categories.

| Markers | Pre-intervention | Post-intervention | ΔIL-10 |
|---|--------------------|--------------------|--------------------|
| IL-10 based on LAZ/HAZ categories | | | |
| - Normo-stature (n = 52) | 420.51 ± 350.98 | 242.98 ± 133.22 | -177.53 ± 292.88 |
| - Stunted (n = 13) | 666.72 ± 451.21 | 302.92 ± 213.36 | -363.80 ± 413.83 |
| - Severely stunted (n = 10) | 405.61 ± 469.84 | 302.30 ± 213.36 | -103.31 ± 422.42 |
| p value | 0.114 ¹ | 0.535 ¹ | 0.076 ² |
| IL-10 based on WAZ categories | | | |
| - Weight faltering (normo-weight (n = 56) | 437.99 ± 351.68 | 253.73 ± 148.63 | -184.25 ± 297.31 |
| - Underweight (n = 15) | 497.15 ± 477.20 | 260.51 ± 216.90 | -236.64 ± 440.80 |
| - severely underweight (n = 4) | 651.36 ± 630.80 | 369.88 ± 147.97 | -281.48 ± 536.04 |
| p value | 0.538 ¹ | 0.397 ¹ | 0.956 ² |
| IL-10 based on WLZ/WHZ categories | | | |
| - Good nutritional status (n = 64) | 457.82 ± 370.84 | 257.03 ± 159.36 | -200.79 ± 331.63 |
| - Wasted (n = 9) | 571.20 ± 536.68 | 320.00 ± 201.23 | -251.19 ± 421.01 |
| - Severely wasted (n = 2) | 74.55 ± 40.29 | 133.16 ± 25.89 | 58.61 ± 14.41 |
| p value | 0.268 ¹ | 0.302 ¹ | 0.156 ² |

Note: WAZ = weight-for-age z-score; LAZ = length-for-age z-score; HAZ = height-for-age z-score; WLZ = weight-for-length z-score; WHZ = weight-for-height z-score.

¹One-way ANOVA.

²Kruskal-Wallis.

the intervention, IL-6 levels in good nutritional status subjects were reduced (-22.05 ± 92.83 pg/ml), while the wasted and severely wasted group increased (9.73 ± 84.73 and 36.22 ± 3.46 pg/ml respectively).

Initial and late levels of IL-10, and changes of IL-10 based on the anthropometric categories are summarized in Table 5. There was no significant difference in IL-10 before and after the intervention, or in changes of IL-10 ($p < 0.05$) based on the LAZ/HAZ categories. A similar phenomenon was also seen in the WAZ and WLZ/WHZ categories ($p < 0.05$).

The IL-6/IL-10 ratio based on anthropometric measurements is summarized in Table 6. The IL-6/IL-10 ratio based on the LAZ/HAZ categories showed no significant difference pre- and post-intervention. However, ONS supplementation increased the IL-6/IL-10 ratio in all LAZ/HAZ categories. In the WAZ categories, severely underweight children had a lower IL-6/IL-10 ratio compared to underweight children, even though there was no significant difference. The IL-6/IL-10 ratio increased after ONS therapy in all WAZ categories. A higher increment was seen in the severely underweight, but there was no significant difference. The WLZ/WHZ categories also showed no significant difference in the initial and late changes of the IL-6/IL-10 ratio.

Table 6. IL-6/IL-10 ratio based on anthropometric

| Markers | Pre-intervention | Post-intervention | ΔIL-10 |
|---|--------------------|--------------------|--------------------|
| IL-6/IL-10 based on LAZ/HAZ categories | | | |
| - Normo-stature (n = 52) | 0.32 ± 0.16 | 0.43 ± 0.11 | 0.12 ± 0.02 |
| - Stunted (n = 13) | 0.39 ± 0.38 | 0.45 ± 0.13 | 0.07 ± 0.41 |
| - Severely stunted (n = 10) | 0.30 ± 0.27 | 0.46 ± 0.11 | 0.15 ± 0.27 |
| p value | 0.390 ² | 0.764 ¹ | 0.698 ¹ |
| IL-6/IL-10 based on WAZ categories | | | |
| - Weight faltering (normo-weight (n = 56) | 0.34 ± 0.24 | 0.44 ± 0.16 | 0.10 ± 0.26 |
| - Underweight (n = 15) | 0.30 ± 0.16 | 0.43 ± 0.11 | 0.12 ± 0.13 |
| - severely underweight (n = 4) | 0.19 ± 0.04 | 0.47 ± 0.16 | 0.28 ± 0.18 |
| p value | 0.141 ² | 0.809 ¹ | 0.340 ¹ |

Table 6. Continued

| Markers | Pre-intervention | Post-intervention | ΔIL-10 |
|--|--------------------|--------------------|--------------------|
| IL-6/IL-10 based on WLZ/WHZ categories | | | |
| - Good nutritional status (n = 64) | 0.33 ± 0.23 | 0.44 ± 0.11 | 0.11 ± 0.25 |
| - Wasted (n = 9) | 0.28 ± 0.14 | 0.45 ± 0.12 | 0.17 ± 0.18 |
| - Severely wasted (n = 2) | 0.44 ± 0.31 | 0.49 ± 0.16 | 0.05 ± 0.15 |
| p value | 0.675 ² | 0.804 ¹ | 0.739 ¹ |

Note: WAZ = weight-for-age z-score; LAZ = length-for-age z-score; HAZ = height-for-age z-score; WLZ = weight-for-length z-score; WHZ = weight-for-height z-score.

¹One-way ANOVA.

²Kruskal-Wallis.

Discussion

The prevalence of stunted and severely stunted children under two-years-old was 33.7%²⁴ and 45.4% in Nigerian children,²⁵ which was higher compared to this study. While the prevalence in two- to five-year-old children in Gaza was 19.6%,²⁶ The prevalence of stunted/severely stunted children was higher in group-1 compared to group-2 in our study, which was similar to the study conducted in Nigeria, accounting for 45.5% vs. 12.2%.²⁵ However, a study in West Sulawesi, Indonesia found that children aged two to five years had a higher incidence of stunted/severely stunted growth compared to children aged one- to two-years-old, 33.64 vs. 23.12%.²⁷

Stunted growth was found to be associated with age, and it was more prevalent in children aged less than 24-months-old.²⁸ Due to the incidence of stunted growth, which was higher in group-1, the LAZ value was significantly lower in group-1 compared to group-2. It was also found that children with stunted growth were significantly shorter in length/height than the control group in another study.²⁹ It was reported that children aged 12–23 months old had an increased risk of stunting by 1.8 times.^{24,30}

In group-2, the incidence of stunted/severely stunted growth was predominant in males, which is in line with Akombi *et al.* (2017), in which male (sex) was one of the stunting risk factors in 0–5-year-olds.²⁵ This is in line with this study, suggesting that males are more vulnerable to health inequalities.³¹ The biological reason is due to the sex difference in the immune and endocrine systems, and testosterone, luteinizing hormone and follicle stimulating hormone are suspected to play a role.³² Feeding practice preferences between boys and girls such as early weaning in boys, and boys tend to consume greater than one meal of complementary feed during 24-hours may also play a part.³³

ONS intervention in undernourished or at nutritional risk children aged nine months to 12-years-old improved body weight by 0.423 kg after six months of intervention and height gain was 0.417 cm compared to the control, with greater gains in weight in the first 7–10 days of intervention (0.089 kg).³⁴ ONS improved growth in underweight children aged five- to 12-years-old after six and 12 months,³⁵ which was in line with this study, where both group-1 and group-2 gained weight. Formula feeding supplemented with lactoferrin is safe for infants under one year old with no difference in growth rate (g/day).¹⁵

Lactoferrin intervention in children with diarrhoea aged 12–36 months old increased the LAZ/HAZ score ($p = 0.03$) compared to the placebo,³⁶ and the children also showed an increment in length/height. A similar result was also found in Vietnamese children aged 24–48 months old in a 12-month intervention. The intervention of 450 kcal of additional ONS during the first three months resulted in an increase in height of 1.62 cm,³⁷ which was lower than our results in a similar group (group-2). A higher calorie density intervention (2.4 kcal/ml vs. 1.5 kcal/ml) for 28 days increased the children's height by 0.87 [0.59–1.16] and 0.55 [0.17–0.93] cm, $p = 0.007$ in children aged greater than one year and less than 12 years old with growth faltering.¹³

Lactoferrin is known to have a bacteriostatic or bactericidal effect and can activate the immune response of an organism, act accordingly and limits tissue damage.³⁸ It can therefore reduce the incidence of acute gastrointestinal symptoms and reduce the duration of respiratory symptoms in children under 12 months old due to viral or bacterial infection.³⁹ Regarding the immunological profile, when comparing an infant who received Lf supplementation vs. non-Lf supplementation vs. standard infant formula, although there was no significant difference between groups, there was an increase in TGF- β 1 (6.5 vs. 4.3 vs. 2.8 ng/mL), TGF- β 2 (0.26 vs. 0.26 vs. 0.22 ng/mL) and IL-2 (0.21 vs. 0.5 vs. 0.4 pg/mL), but a decrease in TNF- α (-2.4 vs. -1.5 vs. -1.7 pg/mL) during a four month intervention.⁴⁰ A study that examined piglets with a 2 ml/day supplementation showed a decrease in bacterial colonies compared to those without Lf supplementation (1.109×10^7 vs. 3.6183×10^8 CFU) via an anal swab after a seven-day intervention.⁴¹ It was stated that Lf induced the

⁴⁶ development of T cell helper type 1 (Th1) immunity, so created the balance of monocytic ⁷⁹ pro- and anti-inflammatory cytokines. In a dose-dependent manner, Lf enhanced pro-inflammatory response *in vitro* (splenocyte and adherent (F4/80⁺) splenocyte populations, bone marrow derived monocytes (BMM), and J774A.1 cultured cells) and induced IL-12 and IL-10 production and increased the ratio IL-12:IL-10 in lipopolysaccharide (LPS) stimulated cells.⁴² A study of *Mycobacterium tuberculosis* infection treated with Bacillus Calmette–Guérin (BCG) and Lf emulsified with Freund's adjuvant in mice showed a decreased mycobacterial load in the lungs and spleen. It also increased the protection against *M. tuberculosis*,^{16,43,44} via downregulation of proinflammatory mediators (TNF- α , IL-1 β) by modulation of macrophages and dendritic cell ability to present antigens and stimulate T-cells. Lf also increased IFN γ , which was the specific response towards Th1.¹⁶ A study examining mice with urinary tract infection due to *Escherichia coli* showed that Lf intervention orally was able to decrease the number of bacteria in the kidneys and bladder after 24 h of Lf consumption, and reduced IL-6 by urinary leucocytes.⁴⁵ A study conducted on Senegalese children receiving tetanus vaccine in stunted children aged one- to nine-years-old showed that the production of IFN γ was compromised.² It was stated that undernutrition is related to immunodeficiency even when it is mild, whether the innate or adaptive immune systems.²

¹² In our study, even though there was no significant difference in IL-6 levels before and after the intervention, Lf reduced IL-6, which is in line with other studies showing a reduction in undernutrition groups. It was found that IL-6 levels were lower in undernutrition compared to good nutrition groups (2.54 pg/mL vs. 6.02 pg/mL, $p < 0.0001$).⁴⁶ Genetic investigation showed that the IL-6 164 gene with a GG and GC genotype (mutant phenotype) was more frequent in undernourished children.⁴⁷

When the groups were examined based on the LAZ/HAZ categories, stunted subjects had higher IL-6 levels compared to normal stature and there was a significant difference compared to severely stunted. This is in line with a study in Egyptian children, where IL-6 was higher in stunted compared to normal stature children (1.6 ± 0.2 vs. 1.5 ± 0.3 pg/mL),⁴⁸ but it was decreased in malnourished compared to normal children.⁴⁶ This showed that when the children had an LAZ/HAZ score greater than or equal to -2 SD, IL-6 was increased but it decreased when children had an LAZ/HAZ score greater or equal to -3 SD. On malnutrition, the acute-phase response was attenuated, and the production of cytokines decreased. An animal study showed that IL-1 β production decreased in malnourished guinea pigs induced with endotoxins.⁷ Stunting is a form of growth failure due to long term nutritional deficiency or it is caused by chronic malnutrition or recurrent undernutrition.^{49,50} After a six-month intervention with food supplementation, stunted Bangladeshi children aged 12–18 months old experienced an IL-6 increment (from 0 [0–1.2] to 1.68 [0.83–4.7] pg/mL, $p = 0.001$),⁵¹ which contradicts this study as IL-6 levels were reduced in stunted and normal stature children. However, IL-6 was found to have increased in severely stunted children, so the post-intervention levels of IL-6 were higher in severely stunted even though there was no significant difference. Severely stunted children might undergo these immune alterations which are similar to severely acute malnutrition, so IL-6 levels were lowest at the outset but increased drastically after the intervention to surpass normal stature and stunted children. It was stated that immune function is an activity with high costs on energy demand, and in developing children the allocation of energy in immune functions may lead to a trade-off with physical growth, particularly those with exposure to infection.⁵²

A similar anomaly was also seen in the WAZ categories even though there was no significant difference. Being underweight has been used as an indicator of undernutrition due to a short-term nutritional deficiency.⁴⁹ However, an *in vitro* study using peripheral blood mononuclear cells (PBMC) taken from children suffering from protein energy malnutrition (PEM) contradicted this study, which showed an increment in IL-6 expression after stimulation with LPS,⁵³ even though it was expressed earlier, reached its peak earlier, and lasted longer than controls in rats.⁵⁴ As the immune function is costly in terms of energy, it has negative effects on growth. In children with mildly elevated immune activity, they experience a growth reduction of up to 49%,⁵² as seen in underweight children who experienced an increase in IL-6 due to the trade-off in body fat between immune function and growth.⁵²

Regarding malnutrition, lymphatic tissue, particularly the thymus, experiences atrophy, leads to a ⁵⁴ reduction in delayed-type hypersensitivity responses, followed by a reduction in levels of antibodies in severely malnourished children (≥ 3 SD of WLZ/WHZ WHO child growth standards), but it remains intact in moderate malnutrition (leucocyte and lymphocyte, high levels of immunoglobulin, particularly IgA, and acute phase response), and cytokine patterns are skewed towards a Th2-response.⁵⁵ However, our study found that IL-6 started to reduce in wasted patients, with the lowest levels in those that were severely wasted. Nutritional intervention increases IL-6 in both wasted and severely wasted, but it is reduced with good nutrition, which is in line with research that states undernutrition, even in the mildest form causes immunodeficiency.⁵⁶

⁸² Wenling C57BL/6J mice in a wasting model's study, which underwent 14 days of weight loss, showed increases of IL-10 in the malnourished group at three and at 14 days.⁵⁷ It was stated that malnutrition modifies the body's resistance against

infection, particularly the immune response. Lipopolysaccharide (LPS) injection (1.25 µg i.v.) in a protein-energy malnutrition (PEM) mouse model, showed that the circulating levels of IL-10 were increased, and high levels were found in bone marrow cells, which showed immunodeficiency.⁵⁸ This finding was in-line with a study in children with marasmic-PEM, IL-10 was significantly higher compared to controls (19.08 ± 5.93 vs 10.46 ± 3.90 pg/mL; $p = 0.000$).⁵⁹ This may be caused by the deficits of NF-κB activation. NF-κB was the major transcription pathway for proinflammatory cytokine production.⁶⁰ Using BMI as the parameter to determine malnutrition, subjects with severe malnutrition (BMI <16.5) had higher levels of IL-10 (8.0 ± 3.6 pg/mL) compared to those with moderate malnutrition (BMI = 16.5-18.4) (2.6 ± 4.3 pg/mL) and good nutrition (BMI ≥18.5) (2.8 ± 0.7 pg/mL) in adults,⁶¹ which was similar to the WAZ category where IL-10 was slightly increased in those underweight, and increased drastically in those severely underweight.

Nutritional intervention increases IL-10 significantly in children aged 12–60 months old with moderate and severe malnutrition receiving curd (milk product) compared to leaf protein concentrate (LPC) (from 30.9 ± 29.5 to 67.4 ± 96.2 pg/mL vs. 29.2 ± 25.8 to 31.5 ± 24.9 pg/mL). Based on Gomez criteria for malnutrition severity, children with mild malnutrition had lower IL-10 compared to children with severe malnutrition. It was higher in subjects aged more than two years old compared to two- to five-year-olds due to a balancing pro-inflammatory response to minimize tissue damage.⁶² In malnourished children, IL-10 was found to be reduced, while in line with this study, IL-10 was depressed in severely wasted subjects.⁶³ However, the level of IL-10 was still normal in severely stunted or severely underweight children. The reduction is due to a deficiency in the number and functional Th cells, which may be caused by incomplete differentiation of T lymphocyte precursors and steroid-induced lympholysis.⁶³ Another study of malnourished children due to inadequate food intake (anorexia nervosa) and diarrhoea receiving nutritional intervention in the form of milk and yogurt, showed increased IFNγ production post intervention,⁶⁴ which is in-line with this study on severely wasted subjects. However, in undernutrition subjects and weight faltering subjects, IL-10 tends to reduce, and a higher reduction was seen in undernutrition subjects, which showed that before intervention undernutrition subjects may experience immune alterations, as seen in the IL-6/IL-10 ratio, which was higher in undernutrition group-1, but lower in group-2. At post intervention, almost all the group had a similar value, ranging from 0.43 to 0.47. Adipose tissue is the main storage for nutrients, which can sense that nutrients are inadequate by releasing adipokines (particularly leptin) to control cellular metabolism and immune function. So, undernutrition has a direct impact on adipose tissue (volume and number), and directly influences the immune system. Leptin not only mediates glucose and lipid metabolism but also immune function, by stimulating activation, proliferation and production of pro-inflammatory cytokine (IL-6, TNF-α, monocytes, macrophages, dendritic cells, and NK cells). Leptin also promotes T-cell activation and development towards Th-1 and Th-17 cell subset which is proinflammatory. Regarding undernutrition, there was leptin depletion and in contrast adiponectin is produced, resulting in the polarization towards M2 or an alternative macrophage which then secretes IL-10 and IL-1Rα. This limits the activation of the NF-κB pathway, and reduces both T-cells or B-cells. Moreover, cortisol hormone restrains the generation of the proinflammatory immune response, so the ability of macrophages and neutrophils to infiltrate the infection site was also restrained. Proinflammatory cytokine production is also reduced, but anti-inflammatory cytokines (IL-10 and IL-33) are increased.⁵

IL-6 has been used as a potential biomarker to identify patients receiving anti-inflammatory therapies as it is secreted widely as a response to pathological states such as infection, inflammation and cancer. IL-10 acts as an anti-inflammatory response, it is secreted as the response to dampen pro-inflammatory bursts and minimize tissue damage. The balance of IL-6 and IL-10 is an important biomarker reflecting the homeostasis of the immune response. In Covid-19 patients, each point increment of the IL-6/IL-10 ratio was associated with a 5.6 times more severe outcome.⁵ In children with pneumonia, the IL-6/IL-10 ratio at 9.61 determines those with severe pneumonia to those with mild disease (sensitivity 76.5% and specificity 93%).⁶⁵

Conclusions

Lactoferrin in ONS intervention improved immune response homeostasis by balancing IL-6 and IL-10 and improved the IL-6/IL-10 ratio, not only body weight but also body length.

Consent

Written informed consent for publication of the patients' details was obtained from the parents of the patients.

Data availability

Underlying data

Figshare: Underlying data for 'Effect of Lactoferrin in Oral Nutrition Supplement (ONS) towards IL-6 and IL-10 in Failure to Thrive Children with Infection', <https://www.doi.org/10.6084/m9.figshare.21813975>.²²

This project contains the following underlying data:

- Data file: Table 1: Data for Manuscript Effect of Lactoferrin in Oral Nutrition Supplement (ONS) towards IL-6 and IL-10 in Failure to Thrive Childre.xlsx
- Data archive 1: Elisa IL-6 Pre Intervention.rar
 - The concentration of IL-6 ng per L, pre intervention.pdf
 - Result of OD + Code.pdf
 - Result of OD Excel.xls
 - Result of OD.pdf
 - Standard curve.pdf
 - Sample scheme & Standard.pdf
- Data archive 2: Elisa IL-6 Post Intervention.rar
 - The concentration of IL-6, ng per L.pdf
 - Result of OD + Code.pdf
 - Result of OD Excel.xls
 - Result of OD.pdf
 - Standard curve.pdf
 - Sample scheme & Standard.pdf
- Data archive 3: Elisa_IL-10 Pre Intervention.rar
 - Result of concentration IL-10, pg per ml.pdf
 - Result of OD + Code.pdf
 - Result of OD Excel.xls
 - Result of OD.pdf
 - Standard curve.pdf
 - Sample scheme & Standard.pdf
- Data archive 4: Elisa_IL-10 Post Intervention.rar
 - Result of calculation concentration pg per ml.pdf
 - Result of OD + Code.pdf
 - Result of OD Excel.xls
 - Result of OD.pdf

- Standard curve.pdf
- Sample scheme & Standard.pdf

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Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver \(CC0 1.0 Public domain dedication\)](#).

Extended data

Figshare: Extended data for 'Effect of Lactoferrin in Oral Nutrition Supplement (ONS) towards IL-6 and IL-10 in Failure to Thrive Children with Infection', <https://www.doi.org/10.6084/m9.figshare.22210798.v2>.²³

This project contains the following extended data:

- Informed consent: Essential information for potential research participants (WHO-CIOMS 2016)
- Airlangga University: Ethical clearance
- [ClinicalTrials.gov](#): Protocol registration
- [ClinicalTrials.gov](#): Completed study
- Study protocol

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Reporting guidelines

Figshare: TREND checklist for 'Effect of Lactoferrin in Oral Nutrition Supplement (ONS) towards IL-6 and IL-10 in Failure to Thrive Children with Infection', <https://www.doi.org/10.6084/m9.figshare.22210798.v2>.²³

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References

- Belliemi CV: **The Golden 1,000 Days**. *J. Gen. Pract.* 2016; **04**(02): 1–4. [Publisher Full Text](#)
- Gaayeb L, Sarr JB, Cames C, et al.: **Effects of malnutrition on children's immunity to bacterial antigens in northern Senegal**. *Am. J. Trop. Med. Hyg.* 2014; **90**(3): 566–573. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Stephens C: **Burden of infection on growth failure**. *J. Nutr.* 1999; **129**(2 SUPPL): 534S–538S. [Publisher Full Text](#)
- Hossain M, Nahar B, Haque MA, et al.: **Serum Adipokines, Growth Factors, and Cytokines Are Independently Associated with Stunting in Bangladeshi Children**. *Nutrients*. 2019; **11**(8): 1827. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gwela A, Mupere E, Berkley JA, et al.: **Undernutrition, Host Immunity and Vulnerability to Infection among Young Children**. *Pediatr. Infect. Dis. J.* 2019; **38**(8): E175–E177. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uauy R: **Improving linear growth without excess body fat gain in women and children**. *Food Nutr. Bull.* 2013; **34**(2): 259–262. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ling PR, Smith RJ, Kie S, et al.: **Effects of protein malnutrition on IL-6-mediated signaling in the liver and the systemic acute-phase response in rats**. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 2004; **287**(4): R801–R808. [PubMed Abstract](#) | [Publisher Full Text](#)
- U'Dhuibhir P, Collura N, Walsh D: **Complete Oral Nutritional Supplements: Dietitian Preferences and Clinical Practice**. *J. Diet Suppl.* 2019; **16**(1): 40–50. [PubMed Abstract](#) | [Publisher Full Text](#)
- Collins C, Tucker C, Walton C, et al.: **Pharmacy Technician Review of Oral Nutritional Supplements (ONS) within Care Homes**. *Pharmacy*. 2019; **7**(1): 28. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lo ALC, Abdul Malek MR, Zulkifli N, et al.: **Factors Affecting Compliance Rate towards Oral Nutritional Supplements Intake Among Geriatric Patients in Hospital Kuala Lumpur**. *J. Sains Kesihatan Malaysia*. 2019; **17**(01): 115–124. [Publisher Full Text](#)
- Philipson TJ, Snider JT, Lakdawalla DN, et al.: **Impact of oral nutritional supplementation on hospital outcomes**. *Am. J. Manag. Care*. 2013; **32**(2): S6–S7. [Publisher Full Text](#)
- Yeung SSY, Lee JSW, Kwok T: **A Nutritionally Complete Oral Nutritional Supplement Powder Improved Nutritional Outcomes in Free-Living Adults at Risk of Malnutrition: A Randomized Controlled Trial**. *Int. J. Environ. Res. Public Health*. 2022; **19**(18). [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hubbard GP, Fry C, Sorensen K, et al.: **Energy-dense, low-volume paediatric oral nutritional supplements improve total nutrient intake and increase growth in paediatric patients requiring nutritional support: results of a randomised controlled pilot trial**. *Eur. J. Pediatr.* 2020; **179**(9): 1421–1430. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

14. Satue-Gracia MT, Frankel EN, Rangavajhyala N, et al.: **Lactoferrin in infant formulas: Effect on oxidation.** *J. Agric. Food Chem.* 2000; **48**(10): 4984–4990.
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Johnston WH, Ashley C, Yeiser M, et al.: **Growth and tolerance of formula with lactoferrin in infants through one year of age: Double-blind, randomized, controlled trial.** *BMC Pediatr.* 2015; **15**(1): 1–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Drago-Serrano ME, Campos-Rodríguez R, Carrero JC, et al.: **Lactoferrin: Balancing ups and downs of inflammation due to microbial infections.** *Int. J. Mol. Sci.* 2017; **18**(3).
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Presti S, Manti S, Parisi GF, et al.: **Lactoferrin: Cytokine modulation and application in clinical practice.** *J. Clin. Med.* 2021; **10**(23).
[Publisher Full Text](#)
18. Zimecki M, Wlaszczyk A, Zagulski T, et al.: **Lactoferrin lowers serum interleukin 6 and tumor necrosis factor α levels in mice subjected to surgery.** *Arch. Immunol. Ther. Exp (Warsz).* 1998; **46**(2): 97–104.
[PubMed Abstract](#)
19. Berthon BS, Williams LM, Williams EJ, et al.: **Effect of Lactoferrin Supplementation on Inflammation, Immune Function, and Prevention of Respiratory Tract Infections in Humans: A Systematic Review and Meta-analysis.** *Adv. Nutr.* 2022; **13**: 1799–1819.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Dewi EK, Nindya TS: **Hubungan Tingkat Kecukupan Zat Besi Dan Seng Dengan Kejadian Stunting Pada Balita 6-23 Bulan.** *Amerta Nutr.* 2017; **1**(4): 361.
[Publisher Full Text](#)
21. IDEXX Laboratories: **ELISA Technical Guide.** 2018; 1–30.
[Reference Source](#)
22. Widjaja NA, Hamida A, Purnomo MT, et al.: **Effect of oral nutritional supplements on IL-6 and IL-10 in failure to thrive children with infection.** [Dataset]. *figshare.*
[Publisher Full Text](#)
23. Widjaja NA, Hamida A, Purnomo MT, et al.: **Ethical Clearance and Study Protocols of The Effect of Lactoferrin in High Calorie Formula on IL-6 and IL-10 in Children With Failure to Thrive and Infection.** [Dataset]. *figshare.*
[Publisher Full Text](#)
24. Titaley CR, Ariawan I, Hapsari D, et al.: **Determinants of the Stunting of Children in Indonesia: A Multilevel Analysis of the 2013 Indonesia Basic Health Survey.** *Nutrients.* 2013; **11**: 1160.
25. Akombi BJ, Agho KE, Hall JJ, et al.: **Stunting and severe stunting among children under-5 years in Nigeria: A multilevel analysis.** *BMC Pediatr.* 2017; **17**(1): 1–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. El Kishawi RR, Soo KL, Abed YA, et al.: **Prevalence and associated factors influencing stunting in children aged 2-5 years in the Gaza Strip-Palestine: A cross-sectional study.** *BMC Pediatr.* 2017; **17**(1): 1–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Syahril E, Mappaware NA, Akib M, et al.: **Analysis of Obstetric Medical Determinant Factors toward Mothers with the Stunting Children.** *Green Med. J.* 2020; **2**(3): 88–100.
[Publisher Full Text](#)
28. Farahata TM, Ragabb S, Salamaa AA, et al.: **Prevalence of stunted growth in children less than 5-year old in Qalyoubia governorate.** *Menoufia Med. J.* 2018; **30**: 1089–1092.
[Publisher Full Text](#)
29. Bahagia Febriani AD, Daud D, Rauf S, et al.: **Risk factors and nutritional profiles associated with stunting in children.** *Pediatr. Gastroenterol. Hepatol. Nutr.* 2020; **23**(5): 457–463.
[Publisher Full Text](#)
30. Prawirohartono EP, Nurdianti DS, Hakimi M: **Prognostic factors at birth for stunting at 24 months of age in rural Indonesia.** *Paediatr. Indones.* 2016; **56**(1): 48–56.
[Publisher Full Text](#)
31. Wamani H, Åstram AN, Peterson S, et al.: **Boys are more stunted than girls in Sub-Saharan Africa: A meta-analysis of 16 demographic and health surveys.** *BMC Pediatr.* 2007; **7**: 1–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. Thurstans S, Opondo C, Seal A, et al.: **Boys are more likely to be undernourished than girls: A systematic review and meta-analysis of sex differences in undernutrition.** *BMJ Glob. Heal.* 2020; **5**(12): 1–17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Bork KA, Diallo A: **Boys are more stunted than girls from early infancy to 3 years of age in rural Senegal.** *J. Nutr.* 2017; **147**(5): 940–947.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Zhang Z, Li F, Hannon BA, et al.: **Effect of oral nutritional supplementation on growth in children with undernutrition: A systematic review and meta-analysis.** *Nutrients.* 2021; **13**(9).
[Publisher Full Text](#)
35. Soliman A, De Sanctis V, Elsididig S, et al.: **Impact of oral nutritional supplements (ONS) on growth outcomes and IGF-1 level in underweight older children and young adolescents (5–14 years) with short stature and no systemic disease: High versus normal calories density formula.** *Acta Biomed.* 2021; **92**: e2021320–e2021329.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Ochoa TJ, Chea-Woo E, Campos M, et al.: **Impact of Lactoferrin Supplementation on Growth and Prevalence of Giardia Colonization in Children.** *Clin. Infect. Dis.* 2008; **48**(12): 1881–1883.
37. Pham DT, Hoang TN, Ngo NT, et al.: **Effect of Oral Nutritional Supplementation on Growth in Vietnamese Children with Stunting.** *Open Nutr. J.* 2019; **13**(1): 43–52.
[Publisher Full Text](#)
38. Krusel ML, Zimecki M, Actor JK: **Lactoferrin in a context of inflammation-induced pathology.** *Front. Immunol.* 2017; **8**(NOV).
[Publisher Full Text](#)
39. Motoki N, Mizuki M, Tsukahara T, et al.: **Effects of Lactoferrin-Fortified Formula on Acute Gastrointestinal Symptoms in Children Aged 12–32 Months: A Randomized, Double-Blind, Placebo-Controlled Trial.** *Front. Pediatr.* 2020; **8**(May): 1–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Björnsjö M, Hernell O, Lönnerdal B, et al.: **Immunological Effects of Adding Bovine Lactoferrin and Reducing Iron in Infant Formula: A Randomized Controlled Trial.** *J. Pediatr. Gastroenterol. Nutr.* 2022; **74**(3): E65–E72.
[Publisher Full Text](#)
41. Gelgel KTP, Sudipa PH, Mufa RMD: **Lactoferrin Effect on Piglets in Terms of Body Weight and Number of Bacteria.** *EAS J. Vet. Med. Sci.* 2021; **3**(6): 58–61.
[Publisher Full Text](#)
42. Hwang SA, Wilk KM, Bangale YA, et al.: **Lactoferrin modulation of IL-12 and IL-10 response from activated murine leukocytes.** *Med. Microbiol. Immunol.* 2007; **196**(3): 171–180.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Hwang SA, Krusel ML, Actor JK: **Lactoferrin augments BCG vaccine efficacy to generate T helper response and subsequent protection against challenge with virulent Mycobacterium tuberculosis.** *Int. Immunopharmacol.* 2005; **5**(3): 591–599.
[PubMed Abstract](#) | [Publisher Full Text](#)
44. Hwang SA, Wilk KM, Budnicka M, et al.: **Lactoferrin enhanced efficacy of the BCG vaccine to generate host protective responses against challenge with virulent Mycobacterium tuberculosis.** *Vaccine.* 2007; **25**(37–38): 6730–6743.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. Haversen LA, Engberg I, Baltzer L, et al.: **Human lactoferrin and peptides derived from a surface-exposed helical region reduce experimental Escherichia coli urinary tract infection in mice.** *Infect. Immun.* 2000; **68**(10): 5816–5823.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
46. Mărginean CO, Man L, Pitea AM, et al.: **The assessment between IL-6 and IL-8 and anthropometric status in malnourished children.** *Romanian J. Morphol. Embryol.* 2013; **54**(4): 935–938.
[PubMed Abstract](#)
47. Mărginean CO, Banescu C, Voidazan S, et al.: **IL-6/572 C/G, 190 C/T, and 174 G/C gene polymorphisms in children's malnutrition.** *J. Pediatr. Gastroenterol. Nutr.* 2014; **59**(5): 666–673.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Abd El-Maksoud AM, Khairy SA, Sharada HM, et al.: **Evaluation of pro-inflammatory cytokines in nutritionally stunted Egyptian children.** *Egypt Pediatr. Assoc. Gaz.* 2017; **65**(3): 80–84.
[Publisher Full Text](#)
49. Soliman A, De Sanctis V, Alaaraj N, et al.: **Early and long-term consequences of nutritional stunting: From childhood to adulthood.** *Acta Biomed.* 2021; **92**(1): e2021168–e2021112.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
50. Sari I, Marwidah M, Amin MA, et al.: **Relationship Of Chronic Energy Deficiency During Pregnancy With Stunting In Toddlers Aged 6-60 Month.** *J. Life Birth.* 2022; **6**(1): 1–10.
51. Sakr Y, Dubois MJ, De Backer D, et al.: **Persistent-microcirculatory alterations are associated with organ failure and death in patients with septic shock.** *Crit. Care Med.* 2004; **32**(9): 1825–1831.
[Publisher Full Text](#)
52. Urlacher SS, Ellison PT, Sugiyama LS, et al.: **Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists.** *Proc. Natl. Acad. Sci. U. S. A.* 2018; **115**(17):

- E3914–E3921.
[Publisher Full Text](#)
53. Malavé I, Vethencourt MA, Chacón R, *et al.*: **Production of interleukin-6 in cultures of peripheral blood mononuclear cells from children with primary protein-calorie malnutrition and from eutrophic controls.** *Ann. Nutr. Metab.* 1998; **42**(5): 266–273.
[Publisher Full Text](#)
54. Lyoumi S, Tamion F, Petit J, *et al.*: **Induction and modulation of acute-phase response by protein malnutrition in rats: Comparative effect of systemic and localized inflammation on interleukin-6 and acute-phase protein synthesis.** *J. Nutr.* 1998; **128**(2): 166–174.
[PubMed Abstract](#) | [Publisher Full Text](#)
55. Rytter MJH, Kolte L, Briend A, *et al.*: **The immune system in children with malnutrition - A systematic review.** *PLoS One.* 2014; **9**(8): e105017.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
56. Bourke CD, Berkley JA, Prendergast AJ: **Immune Dysfunction as a Cause and Consequence of Malnutrition.** *Trends Immunol.* 2016; **37**(6): 386–398.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
57. Monk JM, Woodward B: **Elevated blood interleukin-10 levels and undiminished systemic interleukin-10 production rate prevail throughout acute protein-energy malnutrition in the weanling mouse.** *Cytokine.* 2009; **47**(2): 126–131.
[PubMed Abstract](#) | [Publisher Full Text](#)
58. Fock RA, Vinolo MAR, Crisma AR, *et al.*: **Protein-energy malnutrition modifies the production of interleukin-10 in response to lipopolysaccharide (LPS) in a murine model.** *J. Nutr. Sci. Vitaminol. (Tokyo).* 2008; **54**(5): 371–377.
[Publisher Full Text](#)
59. Wahyudi T, Puryatni A, Hernowati TE: **Relationship between Cysteine, Interleukin (IL)-2, And Interleukin (IL)-10 in Children with Marasmus Type Malnutrition.** *J. Trop. Life Sci.* 2016; **6**(1): 53–58.
[Publisher Full Text](#)
60. Anstead GM, Chandrasekar B, Zhang Q, *et al.*: **Multinutrient undernutrition dysregulates the resident macrophage proinflammatory cytokine network, nuclear factor- κ B activation, and nitric oxide production.** *J. Leukoc. Biol.* 2003; **74**(6): 982–991.
[PubMed Abstract](#) | [Publisher Full Text](#)
61. Takele Y, Adem E, Getahun M, *et al.*: **Malnutrition in healthy individuals results in increased mixed cytokine profiles, altered neutrophil subsets and function.** *PLoS One.* 2016; **11**(8): 1–18.
[Publisher Full Text](#)
62. Dewan P, Kaur IR, Faridi MMA, *et al.*: **Cytokine response to dietary rehabilitation with curd (Indian dahi) & leaf protein concentrate in malnourished children.** *Indian J. Med. Res.* 2009; **130**(1): 31–36.
[PubMed Abstract](#)
63. Chandra RK: **Numerical and functional deficiency in T helper cells in protein energy malnutrition.** *Clin. Exp. Immunol.* 1983; **51**(1): 126–132.
[Reference Source](#)
64. Solis B, Nova E, Gómez S, *et al.*: **The effect of fermented milk on interferon production in malnourished children and in anorexia nervosa patients undergoing nutritional care.** *Eur. J. Clin. Nutr.* 2002; **56**: S27–S33.
[PubMed Abstract](#) | [Publisher Full Text](#)
65. de Brito R d CCM, Lucena-Silva N, Torres LC, *et al.*: **The balance between the serum levels of IL-6 and IL-10 cytokines discriminates mild and severe acute pneumonia.** *BMC Pulm. Med.* 2016; **16**(1): 121–170.
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Rachael Anyim

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Widjaja et al.'s manuscript highlights growth failure in children (diagnosed with infectious diseases) and whether lactoferrin in oral nutrition supplementation improves their immune responses, captured here via changes in pro-inflammatory and anti-inflammatory cytokines, interleukin-6 and interleukin-10, respectively. Participating children received lactoferrin in oral nutrition supplementation for a period of 90 days, which the authors claim resulted in increased body length and weight, as well as decreased interleukin-10 and interleukin-6 (though the latter was not statistically significant between pre- and post-intervention). Furthermore, children with severe growth stunting experienced a larger decrease in interleukin-6 compared to children of normal stature.

As a whole, the authors' manuscript is very detailed and well written, evidenced by their thorough references to the current literature. The methods for data collection are written clearly for replication and the study is well executed. However, the paper could benefit from some reorganizing. For example, "stunting" is defined on page 12 in the middle of paragraph 3 but should be discussed much earlier in the paper (i.e., in the introduction). The statistics presented also need a significant overhaul. See "Major Comments" and "Minor Comments" for additional details.

Major Comments:

1. The introduction section should be expanded to include functions/relevance of interleukin-10 (not just IL-6 and lactoferrin), definitions of stunting versus wasting, and outline the growth standards used. It could also benefit from highlighting the study scope and setting. For example, in the first paragraph in the discussion section (page 11), it is unclear why the authors discuss the prevalence of stunting in other countries when these have not been previously addressed. It might be better to move this information to the introduction to improve the background information presented and flesh out the current relevance to

Indonesia in particular. It could be useful to state the rationale for conducting this study in Indonesia, what infectious disease burdens are prevalent in the area (e.g., urinary tract infections and tuberculosis are mentioned in the methods but then COVID-19 and pneumonia are in the discussion) and whether these may be confounding variables in analyses. If available within the data they collected, the authors could also address cultural considerations since part of the intervention includes dietary counseling and dietary supplementation of animal protein, as well as whether participants are still breastfeeding and how this might impact the generated results.

2. While the authors have cited the literature, they have not stated what hypotheses may be driving their analyses and what results they expect to yield. These should be foregrounded in the introduction for readers.
3. Methods for data collection, including anthropometrics and blood draws, seem appropriate. However, it would have been good to see a discussion on the volume of blood drawn and whether it was a fasting draw, as well as information on time of day for collections since some bioactive properties (e.g., IL-6) can exhibit diurnal variation, thereby confounding the results. The authors have only one sentence detailing their statistical analyses and superscripts in corresponding tables, which severely limits the replicability of their quantitative methods. It would be useful to expand on their methods here - including the results of normality and homogeneity tests and clearly describing which tests were performed on which variables, so that there is no confusion as to how analyses were conducted.
4. Clarification on the results presented would be helpful; as currently written, there seem to be mismatches between the statistics and the authors' reports/concluding statements. We wonder if, perhaps, different tests are reported in the text versus in the tables; if so, the results yielded in those tests should match those in the tables/text. For example, in the abstract the authors suggest that IL-10 was significantly reduced after intervention. However, the closest value to statistical significance is p -value = 0.076 presented in Table 5. Additionally, they state that their intervention improved the IL-6/IL-10 ratio. However, there are no statistically significant differences in pre- and post-intervention ratios, as seen in Table 6. Convention is moving beyond solely using $p < 0.05$ as a threshold for data interpretation, so if that is the case here, the authors should disclose their cutoff point for determining "significant" results. Furthermore, the authors should remodel their tables to not only include p -values but also to include the comprehensive test results, test statistics, and effect sizes/magnitude of the effect. The p -values, means, and standard deviations presented do not convey a complete picture of the story written in the abstract and in the paper itself. Additionally, presenting SD as a \pm value suggests symmetry of data without actually testing for it. It could be useful if the authors instead did repeated measures ANOVA of pre- and post-intervention within group changes for IL-6 and IL-10, as well as present the tests of normality and homogeneity and the results of those tests. Additionally, clarification on the specific growth curves being used and how it was determined that Oral Nutrition Supplement intervention improved body weight and length/height could also be useful. For example, Table 1/paragraph 1 states that age, body weight, and height age were lower in group 1 versus group 2. However, the groups were broken up by age, so these changes could largely be innate and not necessarily linked to ONS supplementation. Table 2 presents a similar issue. Were the authors comparing these metrics to external growth

standards? If so, those standards are not clearly identified here (aside from mentioning WHO Anthro). If not, how were the authors able to confirm whether the growth is an actual improvement of health due to the ONS intervention and not the result of normal growth over the three-month period? Perhaps, it may also be useful to add the rationale in age group cutoffs (i.e., group 1 vs. group 2).

Minor Comments:

1. **Sentence Structure:** In the introduction section's first paragraph (page 3), the authors can remove "still", as it presupposes the audience is aware of growth failure as a historic health problem. In paragraph two, the second to last sentence serves no clear purpose and should either be expanded or removed. The first 2 sentences in the third paragraph can be consolidated a bit. The first sentence in the last paragraph on page 11 ("Lactoferrin...act accordingly and limits tissue damage") doesn't seem to make sense with the last clause. This may be due to missing words or punctuation.
2. **Typographical Errors and Points of Clarification:** In the discussion section, "secrets" should be "secretes" in the twelfth paragraph (page 13). "Main Complains", in Table 1, should be "Main Complaints". Furthermore, "Main Complaints" were not addressed in the methods, so it is unclear how these complaints were documented/assessed (e.g., via survey at the beginning of study) and whether these complaints occurred during the course of the trial. "Liquid pups" should also be corrected in the table. Weight faltering ("normo-weight") is unclearly defined in the table and in the text of the paper. In the far right column of Table 6, the column is labeled as Δ IL-10 but should be Δ IL-6/IL-10. It could also be useful if the authors clarified in the methods how animal protein supplementation was conducted, the rationale behind it, and how its application was measured.

Overall, the study design and work presented have academic merit, as well as public health implications. If, for example, a relatively inexpensive intervention demonstrates positive health outcomes, this is a valid justification for using it in potentially low-income, nutrient impoverished, or high disease-burden settings. However, for the reasons stated above, it is a little difficult to accurately assess whether there is a reduction in IL-6 and if this is indeed directly related to the intervention.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Our combined areas of expertise involve human milk, maternal-infant health, biomarkers of stress (e.g., cortisol) and immunity/immune responses (e.g., cytokines, antibodies), anthropometry, human skeletal growth and plasticity, and biological specimen collection (including blood draws, saliva and milk collections), as well as enzyme immunoassay estimations.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 29 August 2023

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Vinutha U. Muktamath

University of Agricultural Sciences, Dharwad, India

The study is novel and addresses the most important issue of poor nutritional status leading to infant mortality and morbidity in infants and toddlers. Appropriate statistical tool and methodology is adopted. However the title can be more specific as effect of lactoferrin on growth and stunting in children. And the sample size will be 80 and 160 as the same sample will be used for pre and post test.

The interpretation and discussion part needs to be rewritten as the present study is measuring only IL-6 and IL-10 levels in failure to thrive children. No where the results indicate the effect on immune system like reduction in morbidity status and increase in weight and length by morbidity status. More reviews can be added to support the effect of lactoferrin in oral nutrition on growth of children. By discussing the studies by other researchers on improvement of immune system cannot be used to conclude as "Lactoferrin in ONS intervention improved immune response homeostasis by balancing IL-6 and IL-10". Better to restrict to the results of present study

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Assessment of children, Differently abled and elderly

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Publication

39 Noriko Motoki, Masaru Mizuki, Teruomi Tsukahara, Momoko Miyakawa et al. "Effects of Lactoferrin-Fortified Formula on Acute Gastrointestinal Symptoms in Children Aged 12–32 Months: A Randomized, Double-Blind, Placebo-Controlled Trial", *Frontiers in Pediatrics*, 2020

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Publication

56

Lulu Zhang, Huanjie Zhang, Yutian Miao, Sijia Wu, Haiqing Ye, Yuan Yuan. "Protective effect of allicin against acrylamide-induced hepatocyte damage in vitro and in vivo", Food and Chemical Toxicology, 2012

Publication

57

Shen-An Hwang, Kerry J. Welsh, Marian L. Kruzel, Jeffrey K. Actor. "Lactoferrin Augmentation of the BCG Vaccine Leads to Increased Pulmonary Integrity", Tuberculosis Research and Treatment, 2011

Publication

58

Ying Li, Lezhen Dong, Zhishen Mu, Lingyi Liu, Junsi Yang, Zufang Wu, Daodong Pan, Lianliang Liu. "Research Advances of Lactoferrin in Electrostatic Spinning, Nano Self-Assembly, and Immune and Gut

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Microbiota Regulation", Journal of Agricultural and Food Chemistry, 2022

Publication

59

Yudi Her Oktaviono, Hanang Anugrawan Ahmad, Makhyan Jibril Al Farabi, Parama Gandi et al. "Enhancement of EPC migration by high-dose lisinopril is superior compared to captopril and ramipril", F1000Research, 2021

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Alina, S.. "THU0326 Relationship between Regular Exercise, Functional Capacity and Quality of Life in Patients with Fibromyalgia", *Annals of the Rheumatic Diseases*, 2014.

Publication

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70

Anca Meda Georgescu, Claudia Bănescu, Iudita Badea, Valeriu Moldovan et al. "IL-6 gene polymorphisms and sepsis in icu adult romanian patients: a prospective study", *Revista Romana de Medicina de Laborator*, 2017

Publication

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71

Angamuthu Selvapandiyan, Ranadhir Dey, Sreenivas Gannavaram, Ines Lakhal-Naouar, Robert Duncan, Poonam Salotra, Hira L. Nakhasi. "Immunity to Visceral Leishmaniasis Using Genetically Defined Live-Attenuated Parasites", *Journal of Tropical Medicine*, 2012

Publication

<1 %

72

Kunjung Ashadi, Oktia Woro Kasmini Handayani, Hari Setijono, Tri Rustiadi, Sugiharto, Soegiyanto. "Experienced vs Inexperienced Disabled Swimmers: Training

<1 %

Load and Recovery in Preparation Period",
Physical Education Theory and Methodology,
2023

Publication

73

Reem Gaafar, Abdel Moniem Mukhtar,
Suzanne Marlowe Minarcine. "Factors
associated with stunting in children under-5 in
Sudan A secondary analysis of the second
Sudan Household Health Survey, 2010",
Research Square Platform LLC, 2022

Publication

<1 %

74

Richard Ivey, Marko Kerac, Michael Quiring,
Hang T. Dam, Susie Doig, Emily DeLacey. "The
Nutritional Status of Individuals Adopted
Internationally as Children: A Systematic
Review", Nutrients, 2021

Publication

<1 %

75

Rytter, Maren Johanne Heilskov, Lilian Kolte,
André Briend, Henrik Friis, and Vibeke Brix
Christensen. "The Immune System in Children
with Malnutrition—A Systematic Review",
PLoS ONE, 2014.

Publication

<1 %

76

Takeuchi, S.. "Genipin prevents fulminant
hepatic failure resulting in reduction of
lethality through the suppression of TNF-@a
production", Hepatology Research, 200512

Publication

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| 84 | Giulio Giustarini, Laura Kruijssen, Manon van Roest, Rob Bleumink et al. "Tissue influx of neutrophils and monocytes is delayed during development of trovafloxacin-induced tumor necrosis factor-dependent liver injury in mice", Journal of Applied Toxicology, 2018 Publication | <1 % |

85

Leonor Rodríguez, Elsa Cervantes, Rocío Ortiz. "Malnutrition and Gastrointestinal and Respiratory Infections in Children: A Public Health Problem", *International Journal of Environmental Research and Public Health*, 2011

Publication

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86

Maria Drago-Serrano, Rafael Campos-Rodríguez, Julio Carrero, Mireya de la Garza. "Lactoferrin: Balancing Ups and Downs of Inflammation Due to Microbial Infections", *International Journal of Molecular Sciences*, 2017

Publication

<1 %

87

Mărginean, Cristina Oana, Claudia Bănescu, Septimiu Voidăzan, and Carmen Duicu. "The IL-6 572 C/G, 190 C/T, and 174 G/C Gene Polymorphisms in Children's Malnutrition :", *Journal of Pediatric Gastroenterology and Nutrition*, 2014.

Publication

<1 %

88

Yueting Tang, Jiayu Sun, Huaqin Pan, Fen Yao et al. "Aberrant cytokine expression in COVID-19 patients: Associations between cytokines and disease severity", *Cytokine*, 2021

Publication

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89

theses.hal.science

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90

Alkarimi, Heba A, Richard G Watt, Hynek Pikhart, Amal H Jawadi, Aubrey Sheiham, and Georgios Tsakos. "Impact of treating dental caries on schoolchildren's anthropometric, dental, satisfaction and appetite outcomes: a randomized controlled trial", BMC Public Health, 2012.

Publication

<1 %

91

Claire D. Bourke, Kelsey D. J. Jones, Andrew J. Prendergast. "Current Understanding of Innate Immune Cell Dysfunction in Childhood Undernutrition", Frontiers in Immunology, 2019

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92

Hwang, S.A.. "Lactoferrin augments BCG vaccine efficacy to generate T helper response and subsequent protection against challenge with virulent Mycobacterium tuberculosis", International Immunopharmacology, 200503

Publication

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93

Michał Zimecki, Jeffrey K. Actor, Marian L. Kruzel. "The potential for Lactoferrin to reduce SARS-CoV-2 induced cytokine storm", International Immunopharmacology, 2021

Publication

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94

Nutrition and Oral Medicine, 2014.

Publication

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95

Pei-Ra Ling, Robert J. Smith, Susanne Kie, Patricia Boyce, Bruce R. Bistran. "Effects of protein malnutrition on IL-6-mediated signaling in the liver and the systemic acute-phase response in rats", American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2004

Publication

<1 %

96

Samuel A.M. Martin. "Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (*Salmo salar*)", BMC Genomics, 2010

Publication

<1 %

97

Evy Yuniastuti, Lusiani Rusdi, Muhammad Syahrir Azizi, Riwanti Estiasari et al. "Effect of atorvastatin on subclinical atherosclerosis in virally-suppressed HIV-infected patients with CMV seropositivity: a randomized double-blind placebo-controlled trial", F1000Research, 2021

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