



Impact of an extract from the bran layer of rice on the nutrition of lactating mothers and growth of their exclusively breastfed infants: a longitudinal study

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ABSTRACT

Background: The nutritional composition of the bran layer of rice (RB), a globally available product of the rice milling process, has attracted attention as a nutraceutical food source. However, to capitalize on RB's nutritional properties for humans, it is necessary to achieve a level of bioavailability that increases the efficacy needed to impact the nutritional well-being of lactating mothers and their breastfed infants. To accomplish this, a hydrolyzed enzymatic extract (RBEE) with increased protein, vitamin, carbohydrate, and antioxidant bioactivity has been developed.

Objective: To determine the impact of RBEE on the nutritional status of lactating mothers (LM) and the growth of their exclusively breastfed (EBF) infants, living in food-insecure environments in rural Guatemala.

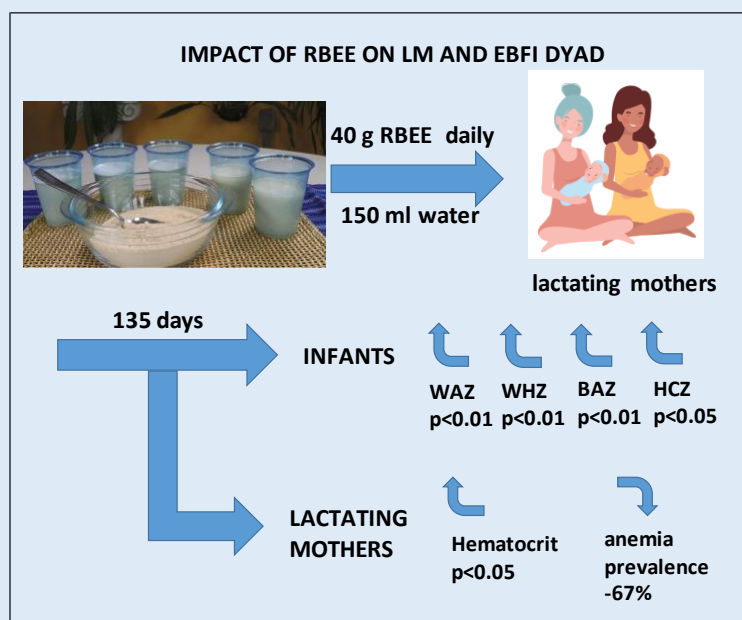
Methods: A RBEE daily ration was consumed by post-*puerperium* LM for 4.5 months of the EBF period. The nutritional impact on LMs was determined by body mass index (BMI). Anemia prevalence and packed cell volume (PCV) were obtained via hematocrit (HCT). Infant growth was determined monthly following World Health Organization (WHO) guidelines, including weight-for-length (WHZ), weight-for-age (WAZ), length-for-age (LAZ), BMI-for-age (BAZ) and head circumference-for-age (HCZ). Initial, midterm and final growth indicators were analyzed by Student t-test for independent samples.

Results: While no significant differences were found between the initial and final BMI in LMs, anemia prevalence was

significantly reduced from 12.1% to 4%. HCT results in LMs showed a significant increase ($p < 0.05$) in PCV by the study's end, from 40.55% to 41.42%. Following a student t-test analysis, infant growth indicators WHZ, WAZ and BAZ showed a highly significant ($p < 0.01$) increase. A significant ($p < 0.05$) improvement was detected in WAZ, while LAZ scores improved from -1.9 to -1.76.

Conclusions: The dietary supplementation of lactating mothers with an RBEE during the EBF period significantly impacted the mother-infant dyad, with improved growth of the exclusively breastfed infants and a reduction in anemia prevalence in mothers. Further research will include the quantity and quality of the putative increased maternal milk synthesis.

Keywords: rice bran, bioactive, bioavailability, enzyme-treated extract, functional food, infants, exclusive breastfeeding, lactating mothers, anemia, chronic, acute, malnutrition, Guatemala, growth, nutrition, children.



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INTRODUCTION

The bran layer of rice (RB) is a by-product of the rice milling process consisting of the aleuronic layer and pericarp of the kernel and is obtained when the de-husked brown rice is polished to yield white rice grain. Even though RB represents only about 8-10% of the grain's weight [1-2], it is a nutrient-rich fraction, containing 10-16% protein, 12-22% fats, 34-52% carbohydrates, and 7-16% fiber [3-4-5]. It is a source of bioactive phytochemicals with pharmacological value including tocotrienols, tocopherols, phytosterols and, efficacious antioxidants such as ferulic acid and γ -

oryzanol [6-7-8]. It is because of this phytonutrient density that RB has attracted attention as a functional food, generally defined as a natural or processed food product containing bioactive compounds which at a given dosage and through specific biomarkers, offers proven health benefits, the reduction of chronic disease and the management of symptoms [9]. However, the activation of a potent lipase that causes rancidity upon separation from the rice kernel [10], renders RB unfit for human consumption after a few hours and is thus mostly used as animal feed or discarded as a waste product [11-12]. To overcome this limitation and inactivate the lipase

enzyme, various RB stabilization techniques have been evaluated, including heat or chemical stabilization, and steaming and extrusion [12-13-14]. Stabilized rice bran was used in this research for achieving the RBEE. Even though RB contains important antioxidants and high-quality protein, its potential as a functional food is reduced by the insolubility and structural complexity of its protein [15-16] and the integrity of the nutraceutical phytochemicals [17-18], unless further hydrolyzed for increased bioavailability; up to 70% of the phenolic antioxidants can be bound to the cell walls [19]. Treatment of RB with enzymes to yield phytonutrient extracts with nutrient-dense profiles and effective nutraceutical properties has been reported [20-21-22].

The nutritional composition of RB has also attracted attention as a food source with the potential to improve the nutritional well-being of vulnerable populations. Previous publications state that introducing RB into an infant's diet as a weaning food offers potential advantages, given the high fat and protein content, high digestibility, and hypoallergenic characteristics [11-23]. Protein extracts from RB are suitable to be used in protein-deficient populations [23] and the amino acid content in RB meets the requirements of young children [25]. RB along with its derivatives or components have been shown to affect diverse chronic ailments such as diabetes, cholesterol imbalance and cardiovascular disease [26-27]; other important nutraceutical effects associated with RB or its components include blockage of growth for certain cancer cells [28] and strong antioxidant properties [29-30].

Current WHO guidelines recommend EBF during the first 6 months of life [31-32]; all the infant's nutritional requirements are provided by maternal milk during this period, even the child's water needs. Appropriate solid foods should gradually become part of the infant's diet starting at 6 months old [32], even though breastfeeding can continue for up to approximately 2 years. Current efforts to prevent or mitigate infant malnutrition are

focused on a more direct nutritional intervention and only in the post-EBF period unless extraordinary circumstances (such as infant malnutrition or scarcity of maternal milk) dictate otherwise.

While it has been generally agreed that successful milk production does not necessarily depend on the mother's nutritional status [33], there is increasing evidence that breastmilk nutrient composition is affected by maternal nutritional status [34-35]. Accordingly, young children are not immune to nutrient-deficient diets among their mothers during these critically important periods of life [36]. Adequate nutrient intake that may benefit both mother and child during the time they nutritionally represent a single biological unit is of particular concern in regions of the world with high poverty levels. The purpose of this study was to determine the impact of a nutritionally enhanced RBEE obtained from stabilized RB on the nutritional status of LM and the growth of their EBF living in poor and nutritionally vulnerable communities in a rural setting.

METHODS

The field clinical trials took place in 17 communities situated within a 10-kilometer radius of the town of Comapa, in Eastern Guatemala. Comapa (coordinates N 14°06' W 89°54') is a municipality located in the Dry Corridor of Guatemala and bordering the country of El Salvador. According to the Government of Guatemala [37], 89% of Comapa's population lives in poverty (< US\$3.00/day), 44% in extreme poverty (households with <US\$1.00/day), and 79% of children under 5 years of age are chronically malnourished.

Planning field visits to Comapa started 5 months before the nutritional intervention and after having approval from the National Secretariat for Science and Technology of Guatemala (SENACYT). Communication was established with the local public Health Center to obtain their approval and collaboration for the study.

At each of the 17 locations, a woman leader was

hired to serve as coordinator/supervisor for her respective community and was placed in charge of locating pregnant women. The local coordinator was also in charge of preparing and verifying the consumption of the daily RBEE ration. Local health promoters from the Health Center were hired as project monitors and anthropometrists for the duration of the study.

As part of the planning activities, 3 training workshops were conducted with the community coordinators and the 3 local supervisors. These training activities focused on personal health and food topics including (1) proper food and water manipulation, (2) personal and infant hygiene, (3) sanitation of food surroundings and (4) protocol for the daily preparation of the RBEE beverage. All the inputs needed for the daily RBEE supplement preparation and distribution, including utensils, equipment and cleaning items were provided to every coordinator. Even though the local health promoters were trained anthropometrists, they were given a refresher course in anthropometrics taught by the project's nutritionists, following the Central America and Panama Institute of Nutrition (INCAP) guidelines [38]. Two weeks before initial registration, the anthropometric techniques were standardized following INCAP protocols [39].

Participant enrollment began in late March 2013 and continued weekly until September 2013, at a rate of 5 to 7 registrations per week. While daily consumption of RBEE beverage took place at the village of residence, all other activities, including registration, health and nutritional checkups, blood sample drawing and anthropometric measurements, took place at the local health center.

The registration procedure included: (1) a detailed oral description of the reach and details of the study, provided to the potential participating LM; (2) if participants accepted, they were asked to sign an

informed consent, followed by (3) a medical checkup of both mother and infant performed by a professional nurse of the local health center; (4) a nutritional examination of the newborns, conducted by professional nutritionists. If acute malnutrition or illness was found, infants were referred to the local health center for medical follow-up. As a last step in the registration process, a capillary blood sample was taken from the LM to evaluate for possible anemia.

Study population and sample size: The study population was comprised of post-puerperium LM and their EBF infants. The sample size (120 participants) was obtained according to the equation for finite populations found in Wayne's article [40]:

$$n=(Nz^2pq)/(d^2(N-1)+z^2pq)$$

where n =sample size; N =600, number of yearly births; pq = 0.5 for maximum variability; z =1.96, at 95% confidence level and, d = margin of error, set at 8% (0.08).

Food supplement: Forty grams daily of an RBEE fortified with seven micronutrients, added to enhance the naturally occurring levels of these vitamins and minerals, were added to meet the LM's nutritional daily needs [41]. The RBEE daily ration, which was tailored to contain more than 150 calories and greater than 5 g of protein, was delivered in 150 ml of potable bottled water and sweetened with 10 g of sugar. The 100% natural, hypoallergenic, and gluten-free RBEE is obtained through a patented process by which a unique combination of carbohydrates and proteases is added to stabilized RB (SRB) in a multistage process of centrifugation and dehydration, designed to extract and maximize the bioactive concentration of nutrients from the SRB's lipophilic and hydrophilic fractions [22]. The nutritional composition of the enhanced RBEE supplement is shown in Table 1.

Table 1. Nutritional profile of enhanced rice bran enzymatic extract for lactating women (29)

Nutrient	Rice bran enzymatic extract typical analysis			Per ration (40 g)		
	100 g	40 g		Added	Total	% Lactating women RDA ¹
Energy (calories)	448-468	180-188			180-188	7.0
Protein (g)	12-15	4.8-6.0			4.8-6.0	5.0
Fat (g)	27.90	11.16			11.16	12.6
Carbohydrates (g)	58.40	23.36			23.36	6.4
Fiber (g)	3.00	1.20			1.20	
Vitamin A (mcg)	106.70	42.68		400.00	442.68	44.3
Thiamin (mg)	3.14	1.26			1.26	96.6
Riboflavin (mg)	2.84	1.14			1.14	71.0
Niacin (mg)	7.26	2.90			2.90	17.1
Pantothenic acid (mg)	6.29	2.52			2.52	35.9
Pyridoxin(mg)	3.51	1.40			1.40	70.2
Biotin (mcg)	5.25	2.10			2.10	6.0
Folate (mcg)	126.81	50.72		487.00	537.72	100.0
Vitamin B12 (mcg)	0.64	0.26		2.80	3.06	100.0
Vitamin C (mg)	10.62	4.25		100.00	104.25	100.0
Vitamin D (mcg)	1.91	0.76		2.50	3.26	65.3
Vitamin E (mg ET)	1.56	0.62			0.62	3.3
Tocophenols (mg)	2.11	0.84			0.84	
Tocotrienols (mg)	2.28	0.91			0.91	
Phytosterols (mg)	939.88	375.95			375.95	
Gamma-oryzanol (mg)	250.34	100.14			100.14	
Ferulic acid (mcg)	3502.50	1401.00			1401.00	
Vitamin K (mcg)	8.08	3.23			3.23	5.6
Calcium (mg)	145.70	58.28			58.28	5.8
Chromium (mcg)	283.91	113.56			113.56	252.0
Copper (mcg)	937.39	374.96			374.96	72.0
Iron (mg)	4.14	1.66		7.39	9.05	58.0
Magnesium (mg)	542.30	216.92			216.92	79.0
Manganese (mg)	12.95	5.18			5.18	2.6
Molybdenum (mcg)	35.85	14.34			14.34	28.7
Phosphorus (mg)	1366.00	546.40			546.40	78.1
Potassium (g)	1.60	0.64			0.64	12.5
Zinc (mg)	5.00	2.00		11.70	13.70	60.5

¹ Required daily allowance as recommended by the Nutrition Institute for Central America and Panamá, INCAP.

Study design: The study followed a longitudinal design and participants were enrolled approximately 45 days after birth of the infant, at which time LM's started consuming the daily supplement. Starting the intervention 45 days

after birth was done to allow LM to begin their participation at their pre-pregnancy weight. From the day of enrollment participants consumed the daily ration of the RBEE for a continuous period of 135 days until their infant became 6

months old. No specific control group was included in the design because this region of the country suffers of very high food insecurity/nutritional inequality. Having some study participants not consuming the RBEE could have (according to local health personnel) been a cause for discontent among the local population. For this reason, it was deemed sufficient to compare the change in growth indicators before, during and after the nutritional intervention window.

Growth parameters: BMI, weight and height measurements were taken from LM at the beginning and end of the study. Adult weight data was determined using a Tanita digital scale model UM061 while adult height was obtained using a SECA 213 portable stadiometer. In the case of infants, weight was taken using a Tanita pediatric digital scale, model BD 585. The supine length of infants was determined using a SECA 210 infant measuring mat while the cephalic perimeter was obtained with a SECA head circumference measuring tape. These three growth measurements were taken at registration and repeated at 33–35-day intervals for 135 days and a total of 5 measurements.

Anemia prevalence in lactating mothers: Anemia prevalence in LM was determined via hematocrit assay, one of the two most utilized tests in the world [42], also used in Guatemala's public health system. The laboratory technician of the local health center oversaw collecting the blood samples and running the tests, following WHO micro hematocrit (HCT) guidelines [43]. Using sterile Marienfeld blood lancets (Lauda-Königshofen, Germany), a capillary blood sample was collected from the index finger of the non-dominant hand of the LM into sterile 75 mm by 1.1-1.2 mm heparinized Marienfeld capillary tubes. The filled tubes were placed vertically on a holding tray containing sealant clay, to close the bottom end of the tubes. Using a micro HCT analog centrifuge Gemmy KHT-400, the blood samples were spun for 7 minutes at 12,000 rpm in 24-tube batches

for a total of twelve participants per batch, as duplicate samples were obtained for redundancy purposes. Once centrifuged, the packed cell volume (PCV) was determined using a micro hematocrit capillary PCV lineal reader.

Statistics: In infants, anthropometric measurements of length, weight and head circumference were transformed to 5 WHO growth indicators, expressed in z scores, using WHO ANTHRO version 3.2.2 [44]. Growth indicators included WHZ, WAZ, LAZ, HCZ, and BAZ. Using Infostat version 2013 [45], a Student t-test for independent samples was conducted on the five growth z scores. Prevalence of chronic and acute malnutrition was determined before and at the end of the consumption window.

In LM, weight and height data were used to determine BMI prior to and after the RBEE supplementation period and, classified according to WHO guidelines. A Student t-test for independent samples was performed on the LM before and after BMI data. Prevalence of anemia was also determined at the outset and after the nutritional intervention, applying a 37% PCV cutoff after a +1-correction factor for altitude. A Student t-test was used to detect any significant variation in the HCT values at the beginning and the end of the supplementation window.

RESULTS

Weekly participant registration began in late March 2013 and ended in early September, for a total of 174 initially present LM and their breastfed infants. If no complicating factor was identified after the initial health evaluation of both LM and infant, participants were accepted into the trials. Causes for dismissal from the field trials included (1) disease or health issues in either LM or infant, (2) mothers not exclusively breastfeeding their newborns and, (3) infants deemed too old for inclusion in the trials. After this selection process, a total of 153 participants were selected to continue in the supplementation trial. Of the initial 153 LM, 129 successfully finished the 4.5 month-long nutritional supplementation window. The main causes for participants not finishing the study included (1) inconsistency by LM on

taking the daily supplement, (2) irregularity by LM in taking infants to their scheduled anthropometric measurements or desertion from the program, (3) acute malnutrition or disease resulting in referral to the local health center for treatment and (4) families leaving their community of residence, mainly due to seasonal employment. By the end of the study all remaining LM’s had taken their daily ration without exception, as verified by the local village coordinator consumption logs.

Initial evaluation of participants: The general information and initial anthropometric measurements of both LM and EBFI are documented in Table 2. Of the initial 153 infants,

83 were female and 70 were male with an average age of 46 days. The age of the infants at registration ranged between 37 and 67 days old. The average age of participating LM was 26.9 years, with the oldest participant being 42 years of age. The initial HCT (corrected +1 for altitude) in LM averaged 40.55%, with a minimum value of 28% PCV and a maximum of 51% PCV; the initial BMI in LM was 22.5. The growth data taken among infants at registration showed that the average initial WHZ score was in the normal range at 0.17, while mean WAZ and HCZ scored below normal at -1.6 and -1.3, respectively. Average LAZ was calculated at -1.9, very close to the CM -2 cutoff point.

Table 2. General information and nutritional/growth data of participants at registration.

Age/ Anthropometry/ Growth z Scores	Lactating Mothers (n=153)			Exclusively Breastfed Infants (n=153)		
Age						
Years	26.90	±	6.6			
Days				46	±	0.04
Initial hematocrit	40.55	±	4			
Initial BMI	22.50	±	2.6			
Anthropometry						
Weight (kg)	48.70	±	6.4	3.98	±	0.71
Height (m)	1.47	±	0.05			
Length (cm)				52.3	±	2.43
Head circumference (cm)				36.3	±	1.41
Growth Indicator (z scores)						
Weight for length				0.17	±	1.3
Weight for age				-1.6	±	1.3
Length for age				-1.9	±	1.21
Head circum. for age				-1.3	±	1.14
Body mass index for age				-0.82	±	1.33

Growth of EBFI during LM supplementation: The average weight gain for infants during the trial was 3 kg, equivalent to a monthly gain of 677 g (1.49 lb). As for length, infants exhibited a growth of 10.45 cm at a rate of

2.36 cm per month, while their head circumference grew at a monthly rate of 1.14 cm, for a total of 5.11 cm in the 135 days (Table 3).

Table 3. Mean anthropometrics measurements of EBF infants during LM daily supplementation with RBEE.

Measurement	Days into LM Supplementation	Average							
		Weight (kg)		Length (cm)			Head Circumference (cm)		
0	0	3.98	± 0.71	52.31	± 2.44		36.33	± 1.41	
1	30	4.97	± 0.76	55.87	± 2.17		38.05	± 1.35	
2	67	5.91	± 0.79	58.72	± 2.28		39.44	± 1.25	
3	97	6.47	± 0.8	60.78	± 2.25		40.48	± 1.37	
4	135	6.98	± 0.87	62.76	± 2.27		41.44	± 1.31	

As Table 4 shows, an important number of infants were categorized as malnourished at the time of registration. Slightly over 7% fell in either the severe (SAM) or moderate (MAM) acute malnutrition categories. Even at the very young average age of 46 days old, 43% of the infants were chronically malnourished (LAZ ≤ -2), 18.3% of them severely. An important number of infants also exhibited retardation in cranial growth, with 25% of them exhibiting HCZ values ≤ -2. By the end of the 135-day intervention, the overall nutritional indicators had improved substantially. As shown in Table 4, no infants were acutely malnourished by the end of the study and

only 6.2% remained in the “at-risk” category, a similar prevalence to that found in BAZ. LAZ showed a slight improvement overall, decreasing the CM prevalence from 43% to 39%. However, the severe CM prevalence was reduced by 45%, affecting 18.3% of the infants at registration, compared to 10% by the end of the nutritional supplementation window. HCZ scores found that over 25% of the infants exhibited an initial delay in cranial growth, 7.2% of them severely. By the end of the field study, moderate to severe cranial growth delay had fallen to 11.6% of the infants, with only 1.5% of them in the severely undersized category according to HCZ.

Table 4. Percentage (%) of EBFI (n=153) in each z-score category according to initial and final nutritional evaluations

Growth Indicator	z-Score (%)							
	≤ -3	-2.99	-1.99	-0.99	0.01	1	2	≥3
		&	&	&	&	&	&	
weight-for-length (WHZ)								
Initial	1.3	5.9	9.8	24.8	32.7	18.3	7.2	
Final			6.2	26.3	33.3	27.2	6.2	0.8
weight-for-age (WAZ)								
initial	17.1	17.1	26.1	30.5	8.5	0.7		
Final	0.8	10.1	34.1	29.4	20.2	5.4		
length-for-age (LAZ)								
Initial	18.3	24.8	35.3	16.3	4.6	0.7		
Final	10.1	29.5	38.7	18.6	3.1			
head circum.-for-age (HCZ)								
Initial	7.2	18.3	32.1	31.3	10.4	0.7		
Final	1.5	10.1	41.8	32.6	11.7	2.3		
BMI-for-age (BAZ)								
Initial	9.2	9.2	22.2	28.8	23.5			
Final			8.5	34.2	31.1	21.7	3.7	0.8

A summary of descriptive statistics for the 5 tested growth indicators is documented in Table 5. Based on a percentile scale, the most impressive gain was obtained by BAZ with an improvement of 41.9 percentile points at the end of the study. Both WHZ and WAZ showed similar

gains with 13.44 and 16.29 percentile points, respectively. HCZ exhibited a 5.71 percentile points increase, ending at the 15.39 percentile mark, while LAZ improved by 1.05 points.

Table 5. Summary of descriptive statistics for EBFI growth indicators at 0, 67 and 135 days.

Anthropometric Measurement	Growth Indicator	z Score			Percentile
		Mean ± sd	Median		
0 (0 days)	WHZ	0.17 ± 1.3	0.32	56.75	
2 (67 days)	WHZ	0.63 ± 1.11	0.68	73.53	
4 (135 days)	WHZ	0.53 ± 0.99	0.46	70.19	
0 (0 days)	WAZ	-1.6 ± 1.3	-1.39	5.48	
2 (67 days)	WAZ	-0.94 ± 1.19	-0.89	17.36	
4 (135 days)	WAZ	-0.78 ± 1.04	-0.9	21.77	
0 (0 days)	LAZ	-1.9 ± 1.21	-1.85	2.87	
2 (67 days)	LAZ	-1.72 ± 1.25	-1.72	4.27	
4 (135 days)	LAZ	-1.76 ± 1	-1.72	3.92	
0 (0 days)	HCZ	-1.3 ± 1.14	-1.16	9.68	
2 (67 days)	HCZ	-1.05 ± 1.18	-1.14	14.69	
4 (135 days)	HCZ	-1.02 ± 0.91	-1.11	15.39	
0 (0 days)	BAZ	-0.82 ± 1.33	-0.63	20.61	
2 (67 days)	BAZ	0.14 ± 1.14	0.13	55.57	
4 (135 days)	BAZ	0.32 ± 1.01	0.19	62.55	

Statistical analysis determined that the most significant growth occurred during the first half of the trial, between days 0 and 67 of the supplementation (Table 6). The Student t-test for independent samples showed highly significant gains ($p < 0.01$) for WHZ, WAZ and BAZ during the first half of the daily supplementation, but no statistically

significant differences were found in their rate of growth during the second half of the trial, between days 67 and 135. No significant improvement in the rate of growth was found in the LAZ at any stage of the trial. In contrast, the head circumference (HCZ), showed a significant ($p < 0.05$) growth at the end of the intervention period.

Table 6. Student T-test results for z-scores of 5 growth indicators at 0, 67 and 135 days.

Growth Indicator	Statistical Group		n		Mean		Mean 2 Mean 1	T	p-value
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2			
WLZ	0	67	153	128	0.17	0.63	0.46	-3.15	0.002
WLZ	0	135	153	129	0.17	0.53	0.36	-2.64	0.009
WLZ	67	135	128	129	0.63	0.53	-0.1	0.75	0.455
WAZ	0	67	153	128	-1.6	-0.94	0.66	-4.41	<0.0001
WAZ	0	135	153	129	-1.6	-0.78	0.81	-5.85	<0.0001
WAZ	67	135	128	129	-0.94	-0.78	0.15	-1.1	0.272
LAZ	0	67	153	128	-1.9	-1.72	0.18	-1.25	0.213
LAZ	0	135	153	129	-1.9	-1.76	0.14	-1.05	0.293
LAZ	67	135	128	129	-1.72	-1.76	-0.04	0.32	0.752
HCZ	0	67	153	128	-1.3	-1.05	0.24	-1.73	0.084
HCZ	0	135	153	129	-1.3	-1.02	0.28	-2.26	0.024
HCZ	67	135	128	129	-1.05	-1.02	0.03	-0.26	0.798
BAZ	0	67	153	128	-0.82	0.14	0.96	-6.44	<0.0001
BAZ	0	135	153	129	-0.82	0.32	1.13	-8.12	<0.0001
BAZ	67	135	128	129	0.14	0.32	0.17	-1.29	0.199

Nutritional status of lactating mothers

Body mass index: The average BMI at registration for LM was 22.5 kg/m² (± 2.6). Of the 4.6% underweight LM before supplementation, all the cases fell under the mild thinness category with BMIs between 17 and 18.49 kg/m² (Table 7). Slightly over 80% were in a normal BMI range while 15% were overweight with only one obesity case.

By the end of the field trials, the prevalence of underweight LM had increased to almost 11%, 13 of them exhibiting mild thinness and one moderate thinness. Sixty-nine percent of the LM fell within the normal category while 26 of 129 LM were overweight, equivalent to 20.2% of the participants. The average BMI by the end of the intervention was 22.8 (±3.2) kg/m².

Table 7. Body mass index of LMs (WHO metrics) during the 135-day RBEE nutritional supplementation window.

Classification	BMI (kg/m ²) Main cut-off points	Lactating Mothers			
		Initial Number (n=153)	%	Final Number (n=129)	%
Underweight	<18.50	7	4.6%	14	10.9%
Severe thinness	<16.00	0		0	
Moderate thinness	16.00 - 16.99	0		1	
Mild thinness	17.00 - 18.49	7		13	
Normal range	18.50 - 24.99	123	80.4%	89	69.0%
Overweight	≥25.00	23	15.0%	26	20.2%
Pre-obese	25.00 - 29.99	22		21	
Obese	≥30.00	1			
Obese class I	30.00 - 34.99			5	
Obese class II	35.00 - 39.99				
Obese class III	≥40.00				

Anemia prevalence (hematocrit): The average HCT results, as seen in **Figure 1**, were equal to 40.55% PCV before taking the RBEE supplement. After the 135-day supplementation period, the average HCT value had increased to 41.42%, a statistically (p<0.05) significant increase according to the Student t-test for independent samples. In addition, the standard deviation had decreased from 3.9% to 2.9%, showing a tighter grouping of HCT results.

According to the initial HCT results, 12.1% of LM (n=174) were categorized as anemic with a < 37% HCT result. By the end of the supplementation period, the prevalence of anemia had been reduced to 4% (n=123). If the borderline results were included (HCT ≤ 37), the initial prevalence of anemia increased to 21.2% of the registered LM, decreasing to 11.4% by the end of the nutritional intervention.

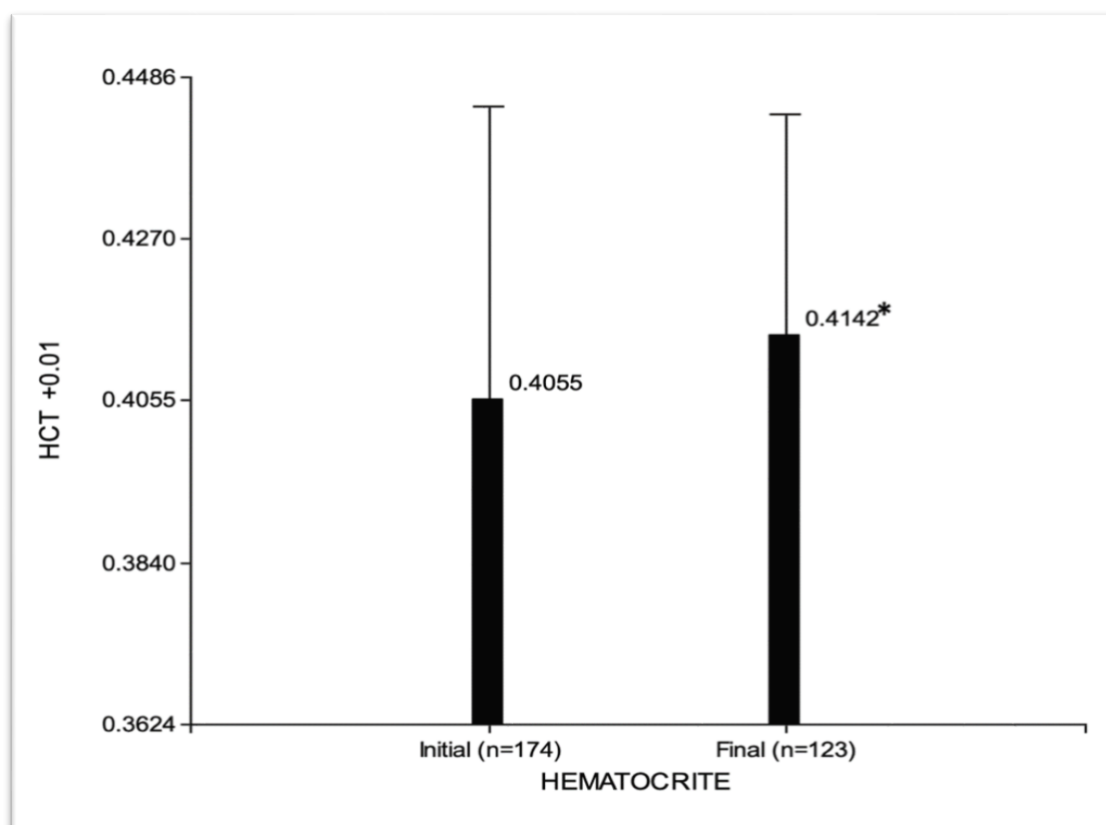


Figure 1. Hematocrit results for blood samples taken at registration of participants (initial) and at the end of the RBEE supplementation window (Final). *Indicates a statistically significant difference at 5%, (p -value = 0.0297), according to the student T-test for independent samples. Hematocrit values were adjusted by +.01, given the 1,200-1,300 m above sea level altitude of the study communities.

DISCUSSION

Comapa is one of the most nutrient deficient and impoverished municipalities in the Eastern dry corridor of Guatemala. Neighboring El Salvador, the communities included in this study are in mountainous terrain with little water available and poor soils. This scarcity in natural resources is one important reason why nearly 50% of its population lives in extreme poverty and stunting is common. The average height of the lactating women in the study, equal to 1.47 m is very close to the overall Guatemalan average for women (1.48 m) [46] which are reported to be the shortest women in the world [47]. Thirty-one percent ($n=153$) of the adult women participants exhibited heights below 1.45 m, an important threshold as probabilities of birth complications is increased in short women [48],

particularly with women below the above-mentioned height [49].

While no substantial change was found in the BMI of women, significant improvement ($p<0.05$) was found in the average HCT among LM by the end of the nutritional intervention period. Along with this significant improvement in HCT, a 67% reduction in the prevalence of anemia was achieved by the end of the study. Even though no statistically significant differences were found in the average BMI of LM at the beginning and end of the nutritional window, there was a 6.3% increase in the underweight category among LM by the end of the study, more than doubling the initial underweight prevalence. This decline in the nutritional status among some of the LM could be a consequence of the increased nutritional needs of the EBFI at 6 months, a demand too great for

the most impoverished and nutrient-deprived mothers in the study.

The length (average 52.3 cm) of participating EBFI at the very young age of 46 days old shows that 43% of them exhibited moderate (24.8%) or severe (18.3%) growth retardation. Since birth length was not available, it is not possible to precisely determine whether these infants were born stunted (their health cards didn't necessarily record length-at-birth), showing instead lengths taken weeks or even 1 month later. The size of an infant at the time of birth is an important metric with stunting at birth (from intra-uterine growth failure) affecting up to 50% of newborns [50-51]. Length-at-birth is an important anthropometric indicator that increases the risk of stunting in the first 12 months of life [52], in later childhood and into adult life [53] and can be affected by retardation in intrauterine growth due to maternal weight gain or deficient BMI during pregnancy [51]. A hereditary component for short stature between mother and child is also partially accountable for the short length exhibited by infants, as strong associations have been found between maternal height and neonatal length [54-55].

At registration (day 0), 43% of infants in the study exhibited a $LAZ \leq -2$, in contrast to the national average of 37.2% as reported in the 2014-15 National Child and Maternal Health Survey [56]. Even though no statistically significant differences were found between the average LAZ at registration (-1.9) and the end of the supplementation window (-1.76), there was an improvement in the average length of infants, particularly among the severely affected by CM. This initial mean LAZ (day 0 of intervention) obtained when infants were on average 1.5 months old, is similar to results in studies conducted among Mayan women living in western Guatemala, where LAZ at birth was -1.0 and by 3 months of age it had deteriorated to -2.26 [57]. In contrast to those studies, at 3.75 months of age the Comapa infants showed a mean LAZ of -1.72 an

improvement of 0.18 standard deviations, which remained practically unchanged until infants reached the end of the intervention window at 6 months old. A breakdown of the chronic undernutrition categories of EBFI at the beginning of the study showed that 18.3% and 24.8% of them were afflicted by severe ($LAZ \leq -3.0$) and moderate ($LAZ \leq -2.0$) chronic undernutrition, respectively. By the end of the study, the prevalence of severe chronic undernutrition had fallen to 10.1% of the participants, a 45% reduction. The moderate malnourished prevalence increased from 24.8% initially to 29.5% by the end of the field study which can be explained by a probable catch-up-growth in the shift of infants from the severe to the moderate undernutrition categories.

According to WHO growth charts for infants between 0 and 6 months of age, both boys and girls grow an average of 11 cm in the 1.5 months to 6 months period. With the RBEE supplementation reported in this paper, infants grew an average of 10.45 cm, a length increase very close to the expected normal growth. In the first half of the study, between 1.5 (46 days) and 3.75 (113 days) months old, infants grew an average of 6.41 cm; in the second 67 days of the supplementation window (ages 3.7 to 6 months old) infants grew 4.04 cm, a growth rate very close to the normal 4.5 cm.

According to the HCZ, WAZ and BAZ metrics, growth delays were also found in a substantial number of infants at registration. At the end of the study, head growth delay ($HCZ \leq -2.0$) was reduced by 55%, from the initial 25.5% of participating infants to 11.6%. Furthermore, only 1.5% of the participants showed a severe delay ($HCZ \leq -3$) in cephalic perimeter growth, an 80% reduction from the initial 7.2% prevalence at the beginning of the study, an important result given that recent studies have reported a negative impact in cognition at 2 and 5 years old in children with $HCZ \leq -3$ at 2 years of age [58]. Student t-test analysis showed a statistically significant ($p < 0.05$) improvement in head growth by the time infants were 6 months old (at the end of the study

window). A strong correlation between cranial circumference and brain volume has been previously reported [59-60], enhancing the importance of these findings. Further, a strong cerebral development during infancy is critical because brain growth observes a 100% increase in volume in the first 12 post-term months [61] and much of the brain's structural and functional development occurs during this period of life [62-63], including a fast integration of sensorial and motor skills, as well as 'socially-driven circuitry' [64].

A highly significant improvement was found in WAZ and BAZ scores among participating infants at the end of the study. Thirty-four percent of the infants were found to be moderately or severely WAZ-compromised at the beginning of the intervention period, but this prevalence was reduced to 11% by the study's end. At registration, 18% of the infants showed BAZ scores ≤ -2 , with no compromised infants at the end of the intervention.

In all three growth indicators where highly significant ($p < 0.01$) improvement was found (WHZ, WAZ, BAZ), the main growth boost took place in the first half (0-67 days) of the study (Table 6), as no significant differences were found when the growth indicators were contrasted during the second half of the study (68-135 days). Further analysis of the data shows that in the first half of the nutritional window, infants gained an average of 1.93 kg, almost twice the weight infants gained (1.07 kg) during the second half of the field study. The overall average weight gain (3.0 kg) during the 135 days of the nutritional window was greater than the expected normal weight gain shown in WHO's 0 to 6 months growth charts, equivalent to 2.6 kg and 2.8 kg for girls and boys, respectively. Even though overall there was a highly significant improvement in WHZ, WAZ and BAZ among infants, some children showed slower growth rates. A possible explanation for the slower weight gain and growth seen in some children may be because care and hygiene knowledge vary tremendously among impoverished populations, particularly among those with

minimal education. There is evidence suggesting that poor childcare and hygiene may affect growth and development of infants [65-66]

An explanation for the accelerated weight gain and normal growth exhibited by EBFI, particularly during the first half of the study, can probably be found in the feedback offered by mothers. During the monthly anthropometric measurements and physical evaluations, LMs were regularly asked if they could notice any significant impact from the consumption of the daily RBEE ration. Many mothers claimed increased milk production starting in the 10 to 15-day range after having initiated the supplementation. The community coordinators all agreed that increased milk quantity allowed for more frequent and longer breastfeeding periods and was an important factor in the improvement observed in their infants. Even though no specific data was obtained regarding breastmilk output in LMs, this is a significant observation because 24-hour dietary recalls and food frequency questionnaires (results not shown) demonstrated the participating breastfeeding mothers did not have any significant changes in their diet throughout the nutritional intervention.

Another important factor that may contribute to the increased milk production and improved growth among infants is the increased bioavailability of the phytonutrients embodied in the RBEE consumed by the LM. The enhanced RBEE supplement utilized in this study has been documented to contain important amounts of macronutrients and micronutrients, with increased bioavailability resulting from the dual (carbohydrates and proteases) enzymatic process applied to stabilized RB [22].

Galactagogues, defined as substances or medications that may help assist, maintain, or increase maternal milk synthesis [67] have elicited interest among health professionals because low maternal milk production is one of the main causes of discontinuation of breastfeeding [68]. This anecdotal evidence requires

further clinical research to confirm the galactagogic properties of the RBEE. It is our observation that the testimonials offered by LMs and their village monitors, supported by the significant improvement in growth shown by infants in the study, confer value to the hypothesis that the increased synthesis of maternal milk could indeed be a bioactive property associated with the RBEE and must be addressed in future research

The quality of maternal milk produced by breastfeeding mothers under RBEE supplementation is another topic for future research. Some nutrients important to brain growth can be impacted by deficient maternal diets, including B-complex vitamins, vitamins A, D, K, iodine, zinc, iron [34] and fatty acids. The importance of human milk fatty acids for the development of the early childhood (first year) brain has been established and poor fatty acid nutrition in the mother could result in low fatty acid contents in breast milk which in turn, could alter neurological development in infants [69]. During pregnancy and infancy, long-chain polyunsaturated fatty acids play a key role in both neurogenesis and neuroplasticity, a crucial stage in brain growth and the development of motor, cognitive and socio-emotional skills [70]. In recently published work, breast milk-fed infants were found to show better cognitive and gross motor skills at 2 years of age [71], compared to formula-fed young children. These results provide further evidence in support of the long-held consensus that breastfed infants perform better on IQ and cognition tests [72]. Because cognitive, motor abilities and social skills deficiencies are some of the most important consequences associated with proteic-caloric undernutrition, future research of RBEE on the growth and development of early childhood must include cognitive, motor skill and behavioral assessments of the participating young children.

The findings in this study suggest that formulating infant nutrition supplements with RBEE to be consumed by the lactating mothers during EBF may have a

significant effect on the fight against chronic or acute malnutrition in nutritionally deficient populations. Rice bran in its raw form is readily available worldwide and the technology to transform rice bran into RBEE can be transferred and implemented in most developing countries. Government interest in incorporating the RBEE food supplement into their nutritional initiatives should be achievable once results of RBEE on growth and cognitive development have been validated. Further, future research should address the impact of RBEE (taken by LMs) not only during the EBFI but throughout the breastfeeding period. It is also possible that a smaller dosage could still have a significant positive effect on infant growth and the LMs well-being, therefore the daily amount of RBEE to be administered should also be addressed in future research. Current discussions [9] in the U.S.A. consider dosage a fundamental step in the categorization and regulation of functional foods.

CONCLUSIONS

The dietary supplementation of breastfeeding mothers with RBEE during the 46 to 180 days post-partum period had a significant positive impact on the mother-infant dyad, as the observed increase in breast milk production favored an increased growth of their exclusively breastfed infants. The increased breastmilk output reported by LMs may be a result of the increased bioavailability of nutrients and phytochemicals in the RBEE enzymatic extract, which in turn increased the bioactive potency of the supplement. Infant growth indicators, including WHZ, WAZ, and BAZ, showed highly significant gains, while HCZ showed a significant but more moderate increase. The threefold reduction in infants with a compromised weight for their age at the study's end shows the strong positive impact of the RBEE supplementation on the growth and well-being of EBF infants. In lactating mothers, anemia prevalence was significantly reduced while blood packed cell volume counts were significantly increased.

List of Abbreviations: RB: bran layer of rice, SRB: stabilized rice bran, RBEE: bran layer of rice enzymatic extract, LM: lactating mothers, EBF: exclusively breastfed infants, BMI: body mass index, PCV: packed-cell volume, HCT: hematocrit, WHO: World Health Organization, EBF: exclusive breastfeeding, INCAP: Institute of Nutrition for Central America and Panama (INCAP), WHZ: weight-for-length, WAZ: weight-for-age, LAZ: length-for-age, HCZ: head circumference-for-age, BAZ: body mass index-for-age, SAM: severe acute malnutrition, MAM: moderate acute malnutrition, CM: Chronic malnutrition (CM)

Competing Interests: The authors are members of Sustainable Nutrition International, a 501(c)3 organization, which provided the rice bran enzymatic extract used in this research and assisted in the development of research protocols and program oversight.

Authors' Contributions: G.E.S., G.H.S. and L.R.M. designed research; G.E.S conducted research and analyzed data; G.E.S. and G.H.S. wrote the paper: All authors read and approved the final manuscript.

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