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Nutritional and Health Status of Autistic Children: Assessing the Efficacy of Gluten-Free High-Protein Bread in Improving Biochemical Parameters

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ABSTRACT

Background: The issue of the nutritional and health status of children with autism spectrum disorders (ASD) is intricate and encompasses dietary factors. High gluten foods consumption is a significant concern due to its potential influence on health and autism symptoms. Investigating this connection can offer valuable information for enhancing the well-being of autistic children.

Methods: This study investigated the effects of gluten-free, high-protein meals on autistic children by examining changes in biochemical markers pre and post a three-month regimen of thrice-daily consumption of chickpea and okra-based bread. The study involved 100 boys with ASD between the ages of 9 and 11. Their physical measurements, health problems, socioeconomic background, dietary habits, and metabolic index were examined.

Results: The study found common health issues in ASD children, including lethargy, solitary eating habits, decreased appetite, and bloating. In terms of body weight, 51% were within a normal range, while 48% were overweight/obese. Significant discrepancies were observed in their consumption of energy, carbohydrates, and fats when compared to standard dietary guidelines. After the dietary change, there was a marked improvement in the levels of essential minerals and vitamins. Enhanced levels of GSH, GSSG, and CoQ10, alongside reduced NADPH, NADH, and ATP levels were noted, which were likely due to increased protein and fiber in the gluten-free bread.

Conclusions: The introduction of a gluten-free and high-protein bread using chickpeas and okara demonstrated positive outcomes, enhancing essential mineral and vitamin levels. Additionally, observed biochemical changes suggest potential benefits of this dietary intervention in addressing metabolic imbalances commonly associated with ASD.

Keywords: *Okara; chickpeas; autistic children; BMI; metabolic index*

1. INTRODUCTION

Autism is a neurodevelopmental disorder that occurs through a lack of social and emotional interaction, limited verbal and non-verbal language skills, stereotypical and repetitive behaviors, self-injuring, and various other neurological symptoms [1]. Genetics has a role in the disorder's cause, together with environmental factors that develop at an early age. It is more common in boys with a ratio of 2:1 to 3:1 male to female [2]. Epidemiological research has found that environmental factors can include toxic exposure to toxins, teratogens, perinatal insults, and prenatal infections such as rubella and cytomegalovirus [3]. Parents of autistic children report problems with eating, only eating a narrow range of foods, and behavioral issues during mealtimes. Studies of autistic children's eating patterns confirm this, along with a significant occurrence of Celiac disease. This disease is an autoimmune disorder where consuming gluten (present in wheat, barley, and rye) causes the immune system to harm the small intestine. In certain cases, these feeding problems in children with autism are associated with discernible physical factors, such as atypical sensory processing, motor skill difficulties, or gastrointestinal conditions including constipation, diarrhea, abdominal bloating, or gastroesophageal reflux [4,5].

The combination of organic problems and problematic eating behaviors could be linked to an increased risk of nutritional inadequacy among ASD children. Nutritional studies examining food daily records in autistic children have identified low intakes of various nutrients including folic acid, niacin, vitamin A, vitamin B6, vitamin C, vitamin D, vitamin K, iron, zinc, and calcium when compared with Dietary Reference Intakes (DRIs) [6,7]. There has subsequently been an increased interest in diet modifications and improving gut health for ASD children. Diet modification included gluten and casein-free foods, supplementation with omega-3, and supplementation with multivitamins which can avoid celiac effects. It led to a cure of their acquired language and revealed a significant improvement in social relatedness [8,9].

Chickpeas (*Cicerarietinum L.*) provide an excellent source of protein and carbohydrates, together comprising approximately 80% of the dry seed mass; starch content varies from 41-50%. Protein quality is considered better than that of other plant seeds. Chickpeas hold most of the required essential amino acids, except for sulphur-containing amino acids which can be complemented by adding cereals to the daily diet. Starch is the major storage carbohydrate followed by dietary fiber. Their lipids are present in low amounts, but it is rich in unsaturated fatty acids such as linoleic and oleic acids. Chickpea oil contains important sterols such as β-Sitosterol, campesterol, and stigmasterol. Chickpeas are also a good source of vitamins, including riboflavin, niacin, thiamine, folate, and the vitamin A precursor β-carotene as well as minerals such as Ca, Mg, P, Fe, and K. Therefore, it can be used to develop nutritious value when added to food products and is an important food for low-income groups in developing countries and patients suffering from lifestyle diseases In the Indian subcontinent, chickpeas are ground to make flour that is used to prepare different snacks and is used in stews, soups/salads, consumed in roasted, boiled, salted and fermented forms to valuable nutrition and potential health benefits [10,11,12].

Okara is the by-product of soy milk and contains soy fiber and soy protein, which are highly nutritious. Dried okara consists of fat (10%), protein (24%), dietary fiber (52.3%), ash (4%), and considerable quantities of vitamins. The main components of okara dietary fiber are guar gum, pectin, b-glucan, carrageenan, and soluble cellulose, which could be related to cholesterol decrease in the plasma and liver. Isoflavones of okara have antioxidant properties and can be beneficial for health. It could be used as a natural ingredient or for food preparation, such as healthy bread and desserts rich in fiber and low in calories [13,14,15]. Thus, this study evaluates the health and nutritional status of autistic male children and the efficacy of chickpeas and okara bread in improving their nutritional and biochemical biomarkers.

2. METHODS

Subjects

This study included 100 autistic male children aged from 9-11 years. The autism center in Al-Ahsa Governorate in the Kingdom of Saudi Arabia (KSA) provided the sample from October to December 2023. The caregivers/parents signed the consent form, which provided all required information regarding the study's purpose, all measurements, and laboratory tests. Children with severe health issues and illnesses, or those taking supplements (vitamins and minerals) within the last two months, were excluded. The mother of every child completed a questionnaire to obtain data about possible ASD risk factors, including questions on socio-demographics, birth family and medical history, anthropometric measurements, food record method, 24-hour recall, and nutrient intake. Mothers were provided with fresh chickpeas and okara bread to offer their children for three meals per day as an alternative to bread and cereals containing gluten for three months. Ethical approval was granted by the Research Ethics Committee of King Faisal University (KFU-REC-2023-OCT-ETHICS1307).

Materials

Biochemical analysis kits were obtained from Alkan for pharmaceutical and chemical analyses. Chickpea seeds, soybeans, yeast, salt, shortening, wheat flour, and sugar were purchased from the local market, Al-Ahsa, KSA.

Okara flour preparation

Okara was obtained in line with [16]. Soaked soybeans (grain: water = 1:5, 14 h, at 15 °C) were ground and cooked (grain: water = 1:6, 30 min, at 100 °C) in a soymilk maker (Mester, D1158-W11QG, China). After filtration of the soybean suspension using a muslin cloth and hand squeezing, soy milk and okara were obtained. Okara was then dried to 15% moisture content on a heated surface with constant manual stirring. This was then stored in polyethylene bags at - 20˚C until used.

Chickpea flour preparation

Dust and undesirable materials were removed from the seeds by dry cleaning. Subsequently, the seeds were soaked in water for 12 h then boiled for 1h and dehydrated in an air-circulated oven at 50˚C for 48 hrs. A laboratory disc mill (Braun AG Frankfurt Type: KM 32, Germany) was used to ground the dehydrated discs into a fine powder which was then stored in polyethylene bags at - 20˚C until used.

Preparation of bread

The ingredients of the control sample bread, according to the methods of [17], were 100 g wheat flour, 5.0 g instant active dry yeast, 5.0g salt (sodium chloride), 2.0 g sugar, 3.0 g shortening and 10ml water while the gluten-free bread contained 45 g Chickpeas and 45 g okara powder replacement by wheat flour (control sample for comparison in sensory evaluation and chemical composition). The ingredients were put into a mixing bowl at 28 ±2.0°C and mixed for 6 min, then the formulated dough was manually rounded by folding 20 times and was then left to rest for 10 min. The prepared dough (125g) was placed in a lightly greased baking pan (5 :9: 8) and proved for 80 min in a cabinet at 30± 0.5 °C and 85% relative humidity; it was then baked for 20 min at 250 °C in an electric oven. The baked breads were cooled at room temperature (25± 2.0 °C) for 60 min and then packed in polyethylene bags and stored for 6 days at room temperature (25± 2.0 °C). The baked bread was presented to autistic children three times daily for three months.

Sensory evaluation of bread

The bread's organoleptic properties were measured by 20 trained judges and allocated a score from zero to 10 for color, odor, taste, texture, and general acceptance [18].

Chemical composition of bread

Moisture, protein (N x 6.25 Kjeldahl, method), fat (hexane solvent, Soxhielt apparatus), fiber, and ash were ascertained by AOAC [19]. Carbohydrate was calculated by differences, as follows: % Carbohydrates = 100 - (% moisture + % protein + % fat + % ash + % fiber). Energy value was estimated by the sum of multiplying protein and carbohydrates by 4.0 and fat by 9.0 [20].

Evaluation of nutritional status

The parents of children with autism were asked to complete questionnaires relating to the severity and symptoms of autism, and where the health problem was diagnosed by a physician [21]. Socio-demographic characteristics of autistic children were evaluated. Dietary intake and nutritional status were measured by a food frequency questionnaire and 24-hour dietary recall for three days [22]. To assess the risk of inadequate nutrient intake, a detailed description of all food and beverages consumed was recorded. The food diary data was analyzed using the computerized nutritional analysis package Dietplan6 P3 (Windows and Mac Serial No: 6918). The dietary recommended intake for nutrients was calculated using the Institute of Medicine [23] and DRI [24].

Anthropometric measurements

Anthropometric assessments measured height, weight, and BMI. BMI is calculated as weight in (kg) / height in metre^2 (kg/m^2). Underweight is when BMI <18.5kg/m^2, normal weight when BMI was 18.5-24.9kg/m^2, overweight when BMI >25-29.9kg/m^{2,} obese class 1 when BMI was 30-34.9kg/m^2, obese class 2 when BMI was 35- 39.9kg/m^2 and morbidly obese when BMI was ≥ 40kg/m^2 [25].

Laboratory biochemical assessment

After an overnight fast of 12 hours, 5 ml of blood samples were collected. All study data (laboratory samples) were given a coordinating subject code. Laboratory analyses were performed blind to subject groups: Children with autism before feeding on a modified diet and an autism group after feeding a modified diet for 3 months. In triplicates, vitamins are primarily concentrated in serum and were spun for 10 minutes in a centrifuge at 3000rpm or plasma according to the type of nutrients. Fat-soluble vitamins (A, D, E, K) were determined according to the methods of [26, 27, 28], respectively. Water-soluble vitamins such as vitamin C [29], B12, and folic acid [30] were determined. Serum Ca, Fe, Zn, K, and Na were analyzed according to the standard method of AOAC [19] by using the atomic absorption spectroscopy technique (GBC, Model 932AA, Australia). Glutathione (GSH and GSSG) was measured by [31]. ATP was estimated by [32], and NADH & NADPH were assessed according to [33] and [34] respectively. CoQ10 was determined by the methods of [35].

Statistical methods

An estimation was performed of the frequency distribution and percentage of health and nutritional problems, and socio-demographic characteristics from the total samples. Data was expressed as mean ± SD and statistical differences between before and after feeding, which were analyzed for significance using the unpaired student's t-test. Statistical significance was defined as $p \leq 0.05$. Statistical analysis was carried out using SPSS version 24 (IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

Socio-demographic characteristics

Table one shows the data relating to the socio-demographic characteristics of autistic male children. In terms of mother and father's education, the highest percentage was university education followed by secondary education. For occupation, 58% of the children's mothers were employees while all the children's fathers were employees. Most autistic children were found in 10000-20000 SAR as economic status (61%). The highest percentage of autistic children used bottle feeding as delivery feeding (53%). Ninety-five percent have no psychiatric family history and five percent had a psychiatric family history which was the first consanguinity degree (60%).

Table (1): Socio-demographic characteristics of autistic male children

Socio-demographic characteristics	**Autistic children**	
	Frequency	**Percentage**
Mother education		
Illiterate	2	2.0
Read and write	12	12.0
Secondary	21	21.0
University	65	65.0
Father education		
Illiterate	1	1.0
Read and write	7	7.0
Secondary	13	13.0
University	79	79.0
Mother occupation		
Housewife	42	42.0
Employee	58	58.0
Father Occupation		
Unemployed	0	0
Employee	100	100.0
Monthly income		
Less than 10000 SAR	26	26.0
10000-20000 SAR	61	61.0
More than 20000 SAR	13	13.0
Delivery feeding		
Breast Feeding	25	25
Bottle Feeding	53	53
Mix Feeding	22	22
Family mental health		
Yes	5	4.0
No	95	96.0
If yes, what is the consanguinity degree		
First	3	60.0
Second	1	20.0
Third	1	20.0

Health and feeding problems

Table 2 shows the high percentage of reported health and feeding problems of autistic children. A high percentage of autistic children suffered from bloating, vomiting, constipation, sensitivity to flavored foods, overeating, eating alone, lethargy, sleep problems, hyperactivity, and being destructive, while the other problems recorded a frequency lower than 50%.

Table (2): Health problems of autistic male children

Health problem	Frequency	Percentage
• Bloating	76	76.0
Diarrhea	42	42.0
• Bloody stools	12	12.0
• Vomiting	53	53.0
• Constipation	57	57.0
• Gastroesophageal reflux	49	49.0
Loss of appetite	22	22.0
Sensitivity to flavored foods	69	69.0
Eats too much	84	84.0
limited diet	16	16.0
Eating alone	88	88.0
Not sensitive to pain	45	45.0
Lethargic	89	89.0
Sleep problem	91	91.0
Hyperactive	76	76.0
Destructive	63	63.0
Screams	49	49.0
Repetitive movements	41	41.0

Anthropometric parameters

Table 3 shows the anthropometric parameters of autistic children. The average age of autistic children was 10.32 ± 0.72y., the mean values of weight and height recorded 42.13± 2.64kg. and 130.76± 4.99 cm. From the obtained mean values of weight and height, the mean value of BMI was 24.64±3.41. Table 4 shows the distribution of autistic children according to BMI, with approximately half of autistic children falling within a normal weight (51%). Outcomes also found a high prevalence of being overweight (35%) followed by 13% as obese and the last percentage was underweight (1%).

Table (3): Anthropometric parameters of autistic male children

Variable	Autistic children
Age(y)	10.32± 0.72
Height (cm)	130.76± 4.99
Weight(kg)	42.13± 2.64

BMI (kg/m²)	24.64±3.41

Data are means ± SD.

Table (4): Distribution of the study subjects by BMI.

Variable	Autistic children
Underweight %	1
Normal weight %	51
Overweight%	35
Obesity%	13

Mean values of nutrient intake

Table 5 shows a summary of the mean values of 24 recall nutrient intake for three days. Autistic children had low intake of all nutrients except total carbohydrates, total fat, vitamin A, and total energy as compared with daily recommended intake (DRI). Vitamins such as Riboflavin, D, and E were recorded up 90% of DRI while, the percentage of protein, fiber, niacin, B12, zinc, and magnesium was between 60 to 76%. The other nutrients showed a low percentage of DRI (40 to 57%).

Table 5: The average intake of different macronutrients and micronutrients for autistic children compared with DRI

Nutrients	Average intake	% DRI
Total proteins (g/d)	24.95± 3.98	73.38
Total fats (g/d)	58.76± 4.67	105.83
Total carbohydrates (g/d)	200.54± 11.34	154.26
Total energy (Kcal/d)	1430.8 ± 10.02	137.5
Fiber (g/d)	9.04 ± 0.09	60.26
Niacin (mg/d)	10.09 ± 1.64	72.07
Folic acid (mcg/d)	113.68 ± 14.75	56.84
Riboflavin(mg/d)	0.64± 0.04	91.42
Vit B12 (mcg/d)	1.81 ± 0.07	60.33
Vit. A (mcg/d)	500.03 ± 15.34	100.4
Vit. C (mg/d)	40.21± 5.34	53.61
Vit. D (IU/d)	587.87± 20.43	97.98
Vit. E (mg/d)	13.65± 3.54	91.00
Iron (mg/d)	7.92 ± 1.04	56.57
Phosphorus (mg/d)	943.54 ± 17.32	75.48
Zinc (mg/d)	5.57 ± 0.87	61.88
Calcium (mg/d)	511.42 ± 18.22	51.14
Magnesium (mg/d)	249.86 ± 17.67	71.39
Fluoride (mcg/d)	100.13± 20.44	50.87

Data are means ± SD.

Food groups

The foods were grouped to represent every major food group consumed by the children (Figure 1). Grains and cereal products (6.17 servings/d) particularly bread and rice (Figure 2) were the most frequently consumed according to the food diaries. Eating snacks, sweets, and fast foods was also a common trait amongst autistic children (2.1 servings/d) and (1.51 servings/d) respectively. The mean intake of lean meats and alternatives was 2.61 servings/d. On the other hand, there was a low consumption of milk products, vegetables, and fruit groups among children (1.86 servings/d, 0.55 servings/d, and 1.13 servings/d respectively).

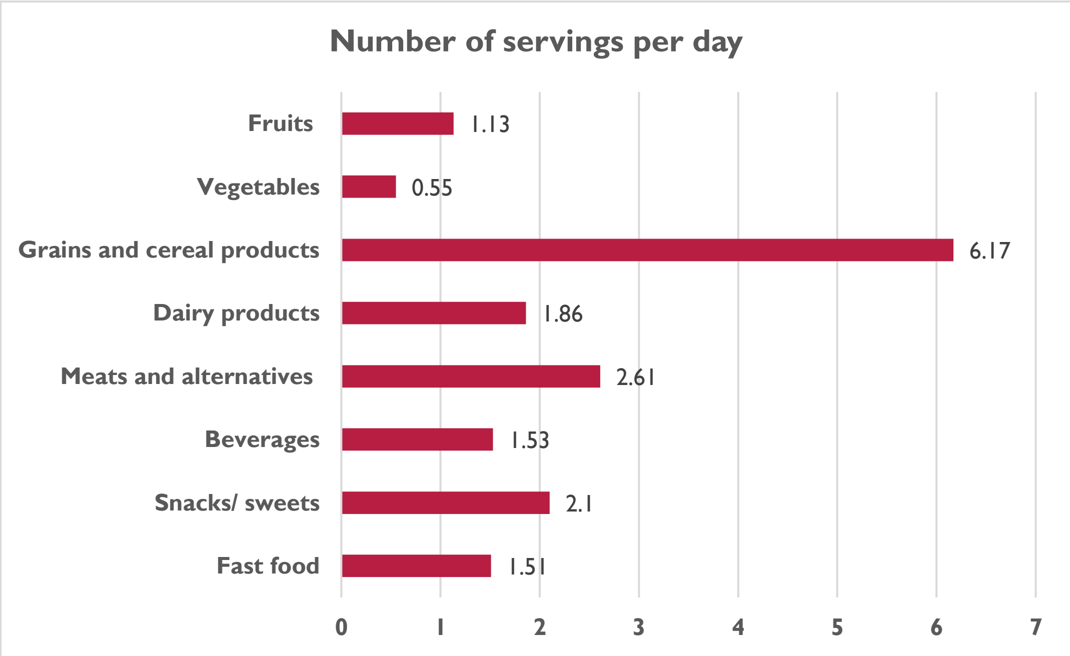

Figure1: Average daily consumption of servings in each food group for autistic children

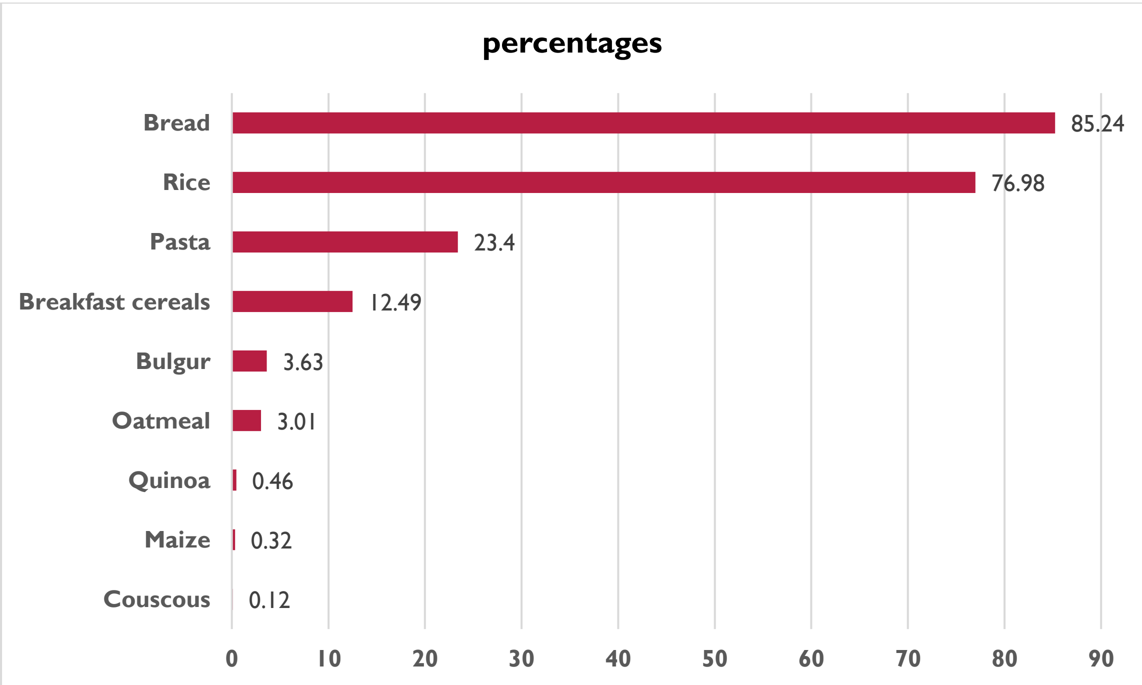

Figure2: The most consumed grains among autistic male children

The chemical composition of 100 g. tested pan bread

Table 6 shows the chemical composition of 100g of tested pan bread. The control sample was higher than the tested pan bread in moisture and total carbohydrates while the tested bread was high in the other components except total energy value which recorded non-significant changes with the control sample.

Table (6): The chemical composition of 100 g. control and tested pan bread

Constitutes	Control sample Mean ± SD	Gluten-free bread Mean ± SD
Moisture	33.21±2.45 [a]	22.56±1.96 [b]
Protein	12.08±1.87 [b]	19.15±1.04 [a]
Fat	2.07±0.44 [b]	4.45±0.86 [a]
Ash	1.23±0.08 [b]	4.75±0.23 [a]
Fiber	1.86±0.11 [b]	12.65±1.22 [a]
Total carbohydrates	49.55±6.53 [a]	37.44±6.73 [b]
Energy kcal	265.15±8.03 [a]	266.41±9.54 [a]

Data are means ± (SD). Values were significantly different compared to the control sample at $P \leq 0.05$. The same letter means non-significant.

The sensory evaluation of the technological pan bread

Table 7 shows an evaluation of sensory properties (color, texture, flavor, appearance, and overall acceptability). It is revealed that the control sample's sensory properties had the highest scores and there were significant changes with modified bread, except for flavor (odor and taste) and overall acceptability.

Table (7): The sensory properties of the gluten-free pan bread

Samples of pan bread	Sensory properties							
	Color (10 degree)		Texture (10 degree)		Flavor (10 degree)		Appearance (10 degree)	Overall acceptability (10 degree)
	Internal color	Eternal color	Homogeneity of pores	Size of pores	Taste	Odor		
Control bread	4.79 ± 0.08 [a]	4.71 ±0.11 [a]	4.76 ± 0.03 [a]	4.77 ± 0.21 [a]	4.73 ± 0.34 [a]	4.79 ± 0.88 [a]	9.67 ± 0.09 [a]	9.71 ± 0.61 [a]
Gluten-free bread	4.03 ± 0.26 [b]	4.07 ± 0.89 [b]	3.97 ± 0.87 [b]	4.07 ± 0.75 [b]	4.33 ± 0.76 [a]	4.27 ± 0.69 [a]	9.23 ± 0.91 [b]	9.37 ± 0.44 [a]
LSD	0.44	0.63	0.74	0.51	0.55	0.61	0.37	0.43

Data are means ± (SD). Values were significantly different compared to the control sample at $P \le 0.05$. The same letter means non-significant.

Mean values of some minerals and vitamins before and after feeding bread product

Table 8 presents the mean values of some minerals and vitamins for autistic children before and after feeding bread products. Autistic children before feeding on tested products with chickpeas and okara had a significantly high percentage of serum sodium, plasma, Vit. A and serum total Vit E from the normal range while the other tested nutrients were lower. This improved after feeding for three months, the differences were significant, and it is expected this improvement will increase by increasing the period of product consumption.

Table (8): Mean values of some vitamins and minerals for autistic male children before and after feeding chickpea and okara bread

Variables	Before consumption	% Normal	After consumption	% Normal
Sodium-Serum mEq/l	0.48±0.05 [a]	160.004	0.30±0.02 [b]	100.002
Iron-Serum µg/dl	40.97± 2.86 [b]	47.63	51.64± 2.12[a]	60.03
Zinc-RBC µg/g	4.01±0.23 [b]	32.87	6.97±0.53 [a]	57.13
Calcium-Serum (mg/dl)	3.79±0.23 [b]	38.28	8.99 ±1.02 [a]	90.80
Potassium-Serum mEq/L	139.23±5.22 [b]	45.79	172.08± 3.05[a]	56.59
Vit. A (plasma) µg/100 ml	77.04± 2.74 [a]	103.40	67.88± 4.02 [b]	91.11
Folic Acid (serum) µg/l	9.01±1.11 [b]	34.65	17.99±1.08 [a]	69.18
Vit B12 (plasma) µg/l	286.33± 9.77 [b]	35.96	341.99±6.92 [a]	42.95
Vit C(plasma) mg/100 ml	0.41±0.09 [b]	24.40	0.89±0.08 [a]	52.97
Vit D3(25-hydroxy in plasma) µg/l	10.01±1.09 [b]	31.77	19.01±2.33 [a]	60.33
Total Vit E (serum) mg/100 ml	1.03±0.08 [a]	104.04	0.96±0.12 [b]	96.97
Vit K (plasma) ng/l	201.98± 12.55 [a]	85.94	215.05±4.93 [b]	91.50

Data are means ± (SD). Values are significantly different compared to before treatment ≤0.05. The same letter means non-significant.

Mean values of some metabolic production before and after feeding pan bread product

Table 9 shows a summary of the average levels of metabolic production in autistic children before and after feeding the tested product. Children with autism had significantly elevated oxidative stress, as evidenced by decreased GSSG/GSH ratio (glutathione is the primary antioxidant in the body), increased plasma nitrotyrosine, and significantly decreased CoQ10 (plasma) as compared to the same volunteers after consumption of modified bread.

Table (9): Mean values of some metabolic indices for autistic male children before and after feeding modified pan bread

Metabolic markers	Before consumption	% Normal	After consumption	% Normal
CoQ10(plasma) µg/ml	0.46± 0.04 [a]	76.67	0.51± 0.09 [b]	85.004
NADH(RBC) nmol/ml	24.07± 6.83 [a]	126.25	20.12± 5.99 [b]	105.53
NADPH(RBC) nmol/ml	35.47±8.44 [a]	118.23	29.06±2.56 [b]	96.86
ATP (plasma) nmol/l	19.33± 2.77 [a]	113.71	16.98±1.06 [b]	99.89
GSH (Reduced plasma glutathione) nmol/ml	2.57± 0.33 [b]	62.68	3.21±0.12 [a]	78.29
GSSG (Oxidized glutathione) nmol/ml	0.230± 0.01 [b]	62.17	0.304±0.07 [a]	82.17

Data are means ± (SD). Values are significantly different compared to before treatment $P \leq 0.05$. The same letter means non-significant.

4. DISCUSSION

Autism spectrum disorders (ASDs) represent a group of neurodevelopmental disorders typified by impairments in verbal and non-verbal communication, social withdrawal and stereotypical behaviours, which may or may not be associated with cognitive deficits, self-injurious behaviours and other neurological comorbidities**.** So that, the current study aimed to evaluate the healthy and nutritional status of autistic male children. The first factor which evaluated was socio-demographic characteristics of autistic male children, it was found that 58% of the children's mothers were employees while 100% of the children's fathers were employees, and 61% of autistic children were found in 10000-20000 SAR as economic status. 53% of autistic children used bottle feeding as delivery feeding and 95% have no psychiatric family history. The authors in [36] concur with the obtained results; they observed positive correlations between the diagnosis of ASD and paternal or maternal education. They also found that living in areas with higher socioeconomic indicators are more likely to be diagnosed with ASD than children in a lower socioeconomic development [37]. The authors in [38] revealed that breastfeeding was found not to affect the severity of autism, and this contradicts the finding of [39] who reported that breastfeeding may protect vulnerable children from ASD. Likewise, [40] stated that there is a great risk of developing ASD if the child was not breastfed in the first six months. Also, the results reveal that there is no relationship between neuro-developmental disorders family history and ASD. The results of [41] were in contrast with the obtained data which observed that there was a link between elevated risk of ASD and neurological and mental illness history in the family.

In this study, another factor examined was the health problems faced by autistic children. The results showed that 91% suffered from sleep problems, 89% were lethargic, 89% ate alone and 88% ate high amounts of food. This in addition to bloating, vomiting, constipation, sensitivity to flavored foods, hyperactivity, and being destructive. For that reason, nutritional status is essential for identifying human activities and developmental progress. Different feeding problems and sensory integration dysfunction faced by the parents of their autistic children affected the nutritional status of the children. It also has deleterious effects on brain development and cognitive processing [42]. Limited diet, food refusal, or loss of appetite is particularly high in children with autism [9]. The authors in [43] found that children with ASD have difficulty with correctly identifying taste and olfactory sensations, with an over or under-responsiveness to sensory stimuli. This may add to the high prevalence of feeding difficulties among this population.

Regarding the anthropometric parameters of autistic children. The average age of autistic children was 10.32 ± 0.72y and, the mean value of BMI was 24.64±3.41. The obtained data showed that 51%, 35%, 13% and 1% of autistic children had normal weight, overweight, obese and underweight respectively. Along the same line, [44] found that the age of children with autism is related to nutritional problems such as food refusal and food obsession. Also, [45] discovered a relationship between age and food preferences. Consequently, the effect of age on nutritional behavior in children with autism should not be ignored as the details produced by these studies can help with the management of children with autism. The other studied anthropometric measurements such as height, weight, and BMI and found very mixed results [46]. This has been associated with an increased risk of being overweight/obese and underweight, nutritional changes, and abnormal feeding behaviors [1]. Consistent with this study, a meta-analysis from different continents confirmed that, although children with ASD have a greater likelihood of having a normal body weight (52%), the remaining were more likely to be obese, overweight, or underweight (21.8%, 19.8%, and 6.4% respectively) [42]. The study of [47] showed that a high BMI value among autistic children is linked to greater caloric intake, preference for low-nutrition foods, and lower physical activity levels. Additionally, [48] reported that idiosyncratic eating behaviors, reduced opportunities for physical, and recreational activities, the use of medications (such as antipsychotics), and genetics may be contributing to this excessive weight gain in children with ASD.

For the evaluation of nutritional status of autistic children, it was reported that they had low intake of all nutrients except total carbohydrates, total fat, vitamin A, and total energy as compared with daily recommended intake (DRI). The other nutrients showed a low percentage of DRI (40 to 57%). Vitamins such as Riboflavin, D, and E were recorded at up to 90% of DRI, while the percentage of protein, fiber, niacin, B12, zinc, and magnesium ranged between 60% to 76%. Other nutrients showed lower percentages of DRI (ranging from 40% to 57%). The results concur with previously published reports of [41] and [49] whereby various nutritional deficiencies have been described, such as limited food preferences or specific food/texture aversions and food selectivity which is common in children between the ages of 3 and 11 years, including picky eating and food refusal. Autistic children were also found to have poor protein intake and nutrient deficiencies, including fiber, vitamin D, vitamin E, calcium, vitamin B12, and iron deficiencies. The authors in [50] proposed that children with autism have poorer bone density compared to a control group of the same age because of poor calcium intake where dairy has been removed from their child's diet as part of the popularity of the gluten-free casein-free diet [51].

The second cause was the risk of inadequate intake of Vitamin D [52]. The low intake of magnesium in autistic children caused side effects including diarrhea, hypotension, and a decrease in reflexes [53]. Autistic children frequently use high-fat supplements, and commercial preparations such as fried potatoes and high-fat foods can contain high Vitamin A, and this leads to risk for vitamin A toxicity. Vitamin A toxicity exhibits symptoms that include headache, seizures, vomiting, abdominal pain acutely, eczema, skin erythema, conjunctivitis, and musculoskeletal tenderness chronically and may be associated with osteoporosis [54]. A study by [55] has shown that nutritional deficiencies of folic acid, occurring during this early period of brain and spinal cord development can lead to neural plate or tube defects. A lack of nutrients including energy, protein, omega 3, etc.; deficiencies in serum iron indicators and calcium, and vitamins B12, B9, and D levels were reported in autistic children. Understanding food preferences and nutritional requirements can guide comprehensive planning interventions for ASD children [50].

In the case products, groups consumed by the children, it could be observed that grains and cereal products particularly bread and rice were the most frequently consumed according to the food diaries while milk products, vegetables, and fruit groups were the lowest consumption among children. These findings align with previously published data by [56] who found that children with autism had poorer food variety scores and a wider range of food choices compared to typical healthy children. The study of [57] reported that autistic children were found to consume significantly fewer daily servings of fruits and vegetables (3.1 versus 4.4, p=0.006) and significantly more daily servings of sweetened beverages (2.6 versus 1.7, p=0.03) and snack foods (4.0 versus 3.0, p=0.01) than typically developing children. The authors in [58] noted that autistic children prefer energy-dense, nutrient-deficient foods and reject fruits, vegetables, and whole grains. The WHO/FAO report recommends consuming 400 grams (i.e. five servings -80 grams as one serving) of fruits and vegetables daily to prevent non-communicable diseases, as well as prevent and alleviate several micronutrient deficiencies [59].

The results of [41] showed that some children with autism seemed to be equally as flexible in their food choices as normal children, while others were much more limited. Regarding bread, our findings are consistent with [60] showing that autistic children prefer to eat bread several times throughout the day, 63.89% state that the children consume one to two daily servings, and 23.61% consume three to four servings. However, several studies indicate that autistic children with gut problems may have an allergy or high sensitivity to foods containing gluten or casein like bread and milk which may lead to nutritional deficiency and both gastrointestinal and neurobehavioral symptoms in children with ASD [61]. For this reason, one of the objectives of this study was to determine the effect of okara and chickpea bread on the nutritional status of autistic children.

From that, there is growing interest in possible dietary involvement in the aetiology and treatment of Autistic Spectrum Disorders (ASD). The most popular dietary intervention is the gluten free diet. Hence the current study aimed to prepare pan bread from free gluten sources as okara and chickpea which recorded lower than control bread in moisture and total carbohydrates and higher in protein, fat, ash and dietary fiber with significant differences whereas it was nonsignificant in case of energy value. The control sample's sensory properties had the highest scores and there were significant changes with modified bread, except for flavor (odor and taste) and overall acceptability. Recent evidence has revealed that nutrition can support individuals with autism. The results were in the same range as [62, 63] and [64] who discovered that chickpea products had a lower moisture content, despite having gluten-free properties. The carbohydrate (%) in chickpea snacks was 30.43 to 45.87% and the protein (%) content of chickpea snack samples was 18.33% to 30.95%. The study of [65] also reported that chickpea-based baked food products provide an excellent nutrient source and contain 105-526 Kcal of energy. The high fiber in chickpeas is a low glycemic index food and contains high soluble fiber which may help increase healthy gut bacteria, prevent the overgrowth of unhealthy bacteria, and reduce the risk of some digestive conditions, such as irritable bowel syndrome. Since they are a good source of choline, chickpeas may help to support brain function. They also contain zinc and iron which protect against depression, anxiety, and iron deficiency anemia [10,11]. The sample contained okara which is a promisingly good source of fiber, ash, and protein that is superior to most of the food usually consumed, the proteins and ash content was in the range of (18–30%) and (3–4%) respectively [66]. Okara protein is one of the most important vegetable protein resources due to its functional properties and high nutritional value [67].

Wheat contains gluten proteins which are formed from gliadins (prolamins) and glutenins (glutelins). In ASD cases, gluten causes serious damage to small intestine mucosa differentiated by inflammation, lymphocytic infiltration, villous flattening, crypt hyperplasia, diarrhea, and abdominal pain, which are typical gastrointestinal symptoms of diagnosed active celiac disease [68,69]. From the obtained results, the tested sample had near values of sensory properties compared to the control bread. This is because the chickpea flour acts as a good binding factor and renders smooth light-yellow texture to the dough on kneading with water. The chickpeas proteins are mainly globulins (salt-soluble proteins) and albumins (water-soluble proteins). The fractional composition data of these proteins confirmed that chickpea products can be used in food preparation technology with gluten-free content for its good functional properties such as solubility, water and oil absorption capacity, emulsifying, foaming, and gelling [70,71]. The authors in [62] stated that kneading the dough from chickpea flour resulted in fundamental changes in dough rheology and led to an increase in chickpeas' water-soluble proteins.

The tested bread sample contained okara; the okara dry matter is nutritious and mainly contains protein, dietary fiber such as guar gum, pectin, b-glucan, carrageenan, soluble cellulose, and free in casein [16]. Okara is characterized by a light-yellow color, mild and neutral flavor, and low energy potential. It is rich in bioactive compounds such as antioxidants polyphenol isoquercetin; isoflavones and phytoestrogens; vitamins K, B1, B2, B3, B6, and B9 (folates); and minerals (Mg, K, N, Ca, P, Fe, Mn, Zn, and Na). Okara lacks cholesterol and lactose, which can have a significant health benefit for consumers with health risks. Okara can have a significant impact on the structure of the gluten-free matrix and the volume of bread through its high protein and dietary fiber content which can affect the rheological properties of bakery products. Additionally, dietary fiber is now often added to bakery products to prolong their freshness, which is based on their ability to retain water. The use of okara in the formulation of gluten-free bread with chickpeas is mainly where a product has high nutritional value, good sensory properties, and is free of gluten to be suitable for autistic children [16,67,72].

Autistic children before feeding on tested products with chickpeas and okara had a significantly high percentage of serum sodium, plasma, Vit. A and serum total Vit E from the normal range while the other tested nutrients were lower. This improved after feeding for three months, the differences were significant, and it is expected this improvement will increase by increasing the period of product consumption. The results concur with those of [52], whereby the mean intakes of some fat-soluble vitamins and minerals in autistic children exceeded the reference ranges. The authors in [73] found that most children with ASD did not, in general, meet reference ranges of vitamins, especially water-soluble vitamins and minerals such as calcium and iron. For elements, the results revealed that autistic children lacked vital elements compared to their normal range, consistent with [74] whereby serum Ca levels were significantly lower compared to healthy control children. The study of [75] showed that children with autistic disorder suffered from Zn deficiency, which is a trace element that has a vital role in cognitive development, healthy neurological functioning, and heavy metal detoxification when compared to healthy controls and this may be due to associated poor food selectivity, often seen in ASD children [76].

The authors in [11] showed that, compared to non-consumers, those who consumed chickpeas and/or hummus have higher nutrient intakes of dietary fiber, polyunsaturated fatty acids, vitamin A, vitamin E, vitamin C, folate, magnesium, potassium, calcium, and iron, which help promote fullness, keep appetite under control, weight management, bone health, and muscle strength [77]. Okara is also high in vitamins A, C, thiamin, riboflavin, niacin, pantothenic acid, vitamin B6, biotin, folate, and vitamin B12 and it is also a good source of magnesium, calcium, zinc, potassium, and iron. Thus, it could help with the prevention of diabetes, cardiovascular disease, obesity, and improve intestinal health through prebiotic functions, especially in ASD [67,72].

According to the average levels of metabolic production in autistic children before and after feeding the tested product. Children with autism had significantly elevated oxidative stress, as evidenced by decreased GSSG/GSH ratio and increased plasma nitrotyrosine, and significantly decreased CoQ10. GSSG is reduced to GSH by glutathione reductase, which requires NADPH. NADPH levels were significantly elevated in children with autism [78]. The higher total choline levels in children with autism could indicate an impairment in the conversion of choline to acetylcholine. The decreased plasma ATP, NADH, and NADPH may represent impairments in the formation of NADH from niacin. ATP is the primary energy source for many metabolic reactions, and decreased levels of plasma ATP after feeding may be related to decreased energetic foods and increased fiber sources [79,80]. NADH is mainly involved in catabolic reactions (mitochondrial function and energy metabolism) whereas NADPH is involved in anabolic reactions (reductive biosynthesis and antioxidation) [81].

Feeding the children gluten-free bread with chickpeas and okara resulted in an improvement in the metabolic index because of the chickpeas' content, including fiber, protein, folate, and B vitamin, which is required for protein metabolism, and maintaining cell health and function. Also, chickpeas contain biologically active compounds such as flavonoids, especially isoflavones, with a massive number of antioxidants such as vitamin E and vitamin C which protect the body from the harmful effects of free radicals and help to increase glutathione levels [77]. Okara is also high in vitamins A and C, as well as antioxidants which are polyphenols, including flavonoids and isoquercetin. A diet high in polyphenols could help to improve heart health by reducing the risk of blood clots and oxidative damage [16,77]. Evidence reveals that a gluten-free diet can reduce the beneficial gut bacteria populations, together with an increase in opportunistic pathogens and immune-suppressive effects. Some evidence supports this diet being used for the amelioration of ASD symptoms, whereby the nutritional status of the intestinal diet decreases urine peptides, decreases GI symptoms, and improves behavior [82]. Gluten and casein-free diets improved the symptoms of ASD in children showing improvement in "communication, stereotyped movements, aggressiveness, language, hyperactivity, tantrums, and signs of attention deficit hyperactivity disorder compared to the control group" [8].

5. CONCLUSION

The results lead to the conclusion that there is an increased prevalence of malnutrition, increased weight, and BMI status for autistic children. A low intake was found of protein, fiber, and micronutrients (iron, calcium, vit. C, E, K, folic acid, B12). Energy-dense foods such as cereal groups, snacks, and sweets formed the highest consumption among the food groups, while the dairy groups, fruit, and vegetables were the lowest. Pan bread intervention, by using chickpeas and okara, three times for three months helped to prevent side effects from gluten, and malnutrition and improved the nutrient intake within the age group. It also improved the child's metabolic biomarkers, minerals, and vitamins index. Therefore, increasing the period of consumption of modified pan bread may reduce the severity of ASD and its related symptoms.

HUMAN ETHICS AND CONSENT TO PARTICIPATE DECLARATIONS

The caregivers/parents signed the consent form, which provided all required information regarding the study's purpose, all measurements, and laboratory tests.

Ethical approval was granted by the Research Ethics Committee of King Faisal University (KFU-REC-2023-OCT-ETHICS1307) Saudi Arabia.

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The author has declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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