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What effect does chocolate containing wheat germ have on children with Down syndrome's blood lipids and intelligence quotient? A randomized clinical trial.

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Abstract

Early dietary interventions for children with Down syndrome (DS) reduce the onset of various DS-related disorders and improve their quality of life. This study aimed to formulate a chocolate product supplemented with functional foods and nutrients (i.e., wheat germ, psyllium, choline, and inositol) to improve blood lipid profiles and intelligence quotient (IQ) in DS children. Seventeen children with DS (nine boys and eight girls) aged 7 to 11 years were randomly chosen from Egyptian medical facilities in Menoufia and Gharbia. The children were divided into two groups: the control group (n=8), which received standard medical treatment and 30 g/day placebo chocolate (with no supplements), and the wheat germ group (n=9), which received standard medical treatment and 30 g/day chocolate supplemented with wheat germ, psyllium, choline, and inositol. The dietary intervention lasted for 84 days. Results showed that body weight decreased significantly (P<0.001) among the WG group by 3.1 kg, while it increased by kilograms5 kg among the control group. In addition, the WG group had a significant (P<0.001) decrease in BMI of 3.1 kg/m2. WG-supplemented chocolate reduced LDH (-31.9%), total cholesterol (-16.1%), and triglycerides (-34.5%). In comparison, the same parameters (LDH, total cholesterol, and triglycerides) decreased slightly in the control group (-2.5%, -7.8%, and -4.5%, respectively). Furthermore, WG-supplemented chocolate increased verbal IQ, IQ battery, and overall IQ (+4.2%, +4.7%, and +8.6%, respectively). In conclusion incorporating of chocolate supplemented with wheat germ, psyllium, choline, and inositol in the diets of DS children resulted in significant improvements in obesity, blood lipids, and IQs.

Keywords: Choline, Psyllium, Inositol, IQ, LDH, Cholesterol, Triglycerides

1. Introduction:

DOWN SYNDROME (DS), often called trisomy 21, is the most common genetic intellectual disability in humans [1]. DS is linked to metabolic disorders, tissue dimorphism, organ abnormalities, cognitive problems, and phenotypic features [2,3]. Intellectual disability/mental retardation, Alzheimer's disease, narrow slanted eyes, a flat nose, and tiny stature are symptoms [4]. Thyroid problems, immunological anomalies, and growth abnormalities are significant effects of DS [5]. Down syndrome can cause a high IQ or modest mental disability [6]. Children with Down syndrome are often overweight [7,8]. Obese-DS had increased waist circumference (WC), % body fat, total fat mass, triglycerides, insulin, and HOMA-IR, and decreased HDLc [9]. Obese DS also had a greater metabolic syndrome prevalence at an earlier age [9]. DS patients had higher triglyceride levels but lower HDL cholesterol, apo AI, and HDL cholesterol/total cholesterol ratios [10].

Down syndrome children make dietary mistakes that cause nutritional problems [11, 12]. Children with DS prefer basic, easy-to-chew-and-swallow carbohydrate meals, and their diet lacks fresh vegetables, fruit, vitamins, minerals, and fiber [13,14]. Early dietary interventions by parents or guardians of DS children reduce or delay the onset of various DS-associated disorders and improve their quality of life [5].

Psyllium supplementation for three weeks or longer decreases blood cholesterol in adults with high cholesterol and blood glucose in people with type 2 diabetes [15]. Jalanka et al. [16] found that psyllium supplementation increased stool water, especially in constipated people. Blusztajn et al. [17] found that choline can help DS models and improves cognitive performance by reversing hypermethylation in animal neurons.

Wheat germ (Triticum aestivum L) is a flour byproduct; it is rich in tocopherols, phytosterols, and policosanols [18]. Wheat germ (WG) contains vitamins E, folate, phosphorus, thiamin, zinc, magnesium, vital fatty acids, and fiber [19]. According to studies, WG oil has antihyperglycemic and antioxidant properties [18,20]. Mohammedi [21] studied WG's effects on T2DM patients' metabolic control and oxidative stress and found that consuming WG decreased overall cholesterol and increased antioxidant capacity.

Studies indicated that a spermidine-rich wheat germ supplement improved verbal memory and inflammation [22]. More recently, Liu et al. [20] discovered that feeding hyperlipidemic rats with wheat germ for 28 days reduced blood TC and LDL cholesterol. According to Yun et al. [23], wheat germ could boost immunological function.

The main objective of this study was to formulate a chocolate product supplemented with some functional foods and nutrients (i.e. wheat germ, psyllium, choline, and inositol) to improve blood lipids profile and IQ in Down Syndrome children.

2. Subjects and Methods

2.1 Subjects

Seventeen children with DS (9 boys and 8 girls) were randomly chosen from Egyptian medical facilities (Special centers for children with down syndrome) in Menoufia and Gharbia governorates.

2.1.1 Inclusion and exclusion criteria

The inclusion criteria were: (1) age between 7 and 11 years, (2) gender; males and females, (3) residency; being born and residing in Egypt, (4) being diagnosed with down syndrome, and (5) consenting to participate and signing a consent form (Parents or guardian).

Children with the following criteria were excluded from the study; (1) DS without obesity, (2) serious diseases that may interfere with DS, (3) rigorous athletes, (4) girls who began menstruation, (5) children who take medications that may interfere with DS measures, (6) hospitalized DS children, (7) children with severe down syndrome, and (8) disabled DS children.

2.1.2 Sampling technique:

The targeted sample size was 20 DS children (aged 7 to 11 years old) plus 10% to account for missing data, overestimation and/or underestimation, and withdrawal. However, due to chocolate allergy, a lack of data decreased adherence, withdrawal, and refusal to sign the consent form; the final number of subjects was seventeen (9 boys and 8 girls).

2.1.3 Sample sitting

The study takes place in a governmental institution for children with DS in Gharbia Governorate, Egypt (Jammet Tanmeet Al Mogtama, Moatamadia, Mahla Kobra, Gharbia Governorate, Egypt) during the period from October 2021 to April 2022.

2.2 Methods:

2.2.1 Manufacturing of chocolate:

The suggested chocolate was produced in the El-Mostafa Sweets Factory in Elmehala El-Kobra. The factory has a strict hygiene protocol for infection control, clean equipment and machines, and smooth surfaces to avoid bacterial growth. The researcher and the employees followed the food safety instructions and used head and mouth covers and gloves.

a. Ingredients

a.1 Chocolate bars

The processed chocolate was obtained from El-Mostafa Sweets Factory in Elmehala El-Kobra city, Gharbia Governorate, Egypt.

a.2 Choline and Inositol Capsules

A Dietary supplement, 500 mg Veg Capsules, were obtained from Now Company, manufactured and quality tested by NOW FOODS 395S. Glen Ellyn Rd., Bloomingdale, IL60108, USA. This product was obtained via Amazon Company.

a.3 Wheat Germ

Natural raw grains (uncooked) were obtained from Bob's Red Mill Company, USA (via Amazon). The wheat germ was powdered and added to the chocolate.

a.4 Psyllium

Psyllium Husk (100% natural Plantago ovata Husk Fibers), the world's best-selling fiber supplement, from Imtenan Company, Obour City, Egypt

b. The technique of chocolate manufacturing:

The chocolate bar was manufactured according to standards given by Egyptian Organization for Standards & Quality (No 465-Part 3, 2005) and using the procedure described in Afoakwa [24]. The chocolate was then melted in large containers at 60 degrees Celsius to avoid losing active components (such as flavonoids) and other nutrients. The chocolate was then mixed and supplemented with the suggested ingredients (Psyllium, Choline, Inositol). A large machine with arms for stirring chocolate thoroughly mixed the ingredients. Finally, the supplemented chocolate was mixed with powdered wheat germ. The single chocolate piece weighed 15 grams and was packed in hard safety plastic to maintain the high temperature of the chocolate. Finally, the chocolate was placed in the cooling machine line and refrigerated until use (Pic 1).

Pictures (1): Chocolate manufacturing in El-Mostafa Sweets Factory in Elmehala El-Kobra City

c- Final composition of chocolate bars

The chocolate bars were prepared with standard methods and composed of chocolate as a base material, nuts, psyllium (as fiber source), choline, and inositol (table 1).

Table 1: The composition of one chocolate bar (Composition per 15 gram).

Ingredients	Regular chocolate	Wheat germ chocolate
Raw chocolate	15.0 g	11.1 g
Wheat germ	0.0 g	1.25 g
Psyllium*	0.0 g	2.5 g
Choline	0.0 g	92.0 mg
Inositol	0.0 g	92.0 mg
Total	15 g	15 g

** Source of fiber*

d- Determination of Chemical composition

The moisture and ash content of chocolate was determined according to methods described in Ranganna [25]. In contrast, the total protein, Carbohydrate, Fat, and crude fiber were determined according to methods described in AOAC [26]. At the same time, the methods described by Singleton and Rossi [27] were used to determine total phenols and total flavonoids.

e. Panel test of suggested chocolate

The 5-point Likert scale measures participants' levels of agreement with statements. The options are (1) Strongly disagree, (2) Disagree, (3) Neither agree nor disagree, (4) Agree, and (5) Strongly agree. Chocolate industry experts, ten children with DS, and their mothers evaluated the proposed chocolate in terms of its color, brightness, flavor, sweetness, texture, consistency, amount of filer, and general appeal. All evaluators' feedback was considered and implemented into the final study product.

2.2.2 Experimental Design of intervention.

All children who met the inclusion criteria were enrolled in this study, consumed the suggested formulas (Chocolate Bars), and were divided into two groups as follows:

I-Control group (n=8; 4 boys and 4 girls): DS children in this group received their standard medical treatment and placebo chocolate bars (without any supplement).

II- The wheat germ group (n=9; 5 boys and 4 girls): **DS** children in this group received standard medical treatment in addition to chocolate bars supplemented with wheat germ and other ingredients.

The intervention lasted for eighteen weeks (126 days), each child got the prescribed amount of chocolate (two bars or 30 grams/day). To curb their potential for developing a chocolate addiction, each child was given two chocolate bars per day for fourteen days, then none for seven days, and so on. Therefore, the net amounts consumed by each child were 168 bars over 12 weeks (84 days), which amounted to 2,520 grams.

2.2.3 Data collected

2.2.3.1 Anthropometric measurements:

Using standard techniques given by Gibson, 2005 [28], the body weight (kg), body height (cm), body mass index (**BMI**) (kg/m2), mid-upper arm circumference in centimeters (AC), triceps skin-fold thickness in millimeters (TSF), and mid-upper arm muscle circumference in centimeter (AMC) were measured at baseline and the end of the experiments. All obtained data were compared with standard growth charts for children with down syndrome given by CDC [29].

2.2.3.2 Nutrients intakes

Nutrient intakes were calculated using the 24-hour food recall method during the week preceding and following the dietary intervention (obtained for three different days, including one holiday). We also asked mothers about the number of meals their children ate, the types of foods they ate, and their children's appetites before and after the intervention.

The nutrient value for each type and quantity of food items was analyzed and converted into calories and macronutrients using the National Nutrition analysis software program and Egyptian food composition tables.

The adequacy of energy and macronutrient intakes was compared to standard dietary requirements. Energy requirements for each DS child were calculated using the Harries Benedict Equations provided by the Institute of Medicine, Food and Nutrition Board [2002] [30]. These equations were based on gender, age (years), physical activity, body weight (kg), and height (cm) (m).

Protein (grams/day) was calculated to be 19 g for children aged 4 to 8 years and 34 g for children aged 9 to 13 years [30]. For DS children, fat (gram/day) was calculated as 25% of total energy [31], and carbs (gram/day) were calculated by differences [30].

2.2.3.3 Biochemical Analysis:

Blood samples were collected from all of the children studied at baseline and after the dietary intervention period to determine total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), and lactate dehydrogenase (LDH). Furthermore, very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were calculated using the Lee and Nieman formula [32].

2.2.3.4 Intelligence quotient (IQ)

The IQ for children with DS and children without DS were carried out by professional psychologists using The Stanford-Binet and the Wechsler intelligence scale for children at baseline and after dietary intervention [33]

2.2.3 Statistical Analysis

The statistical package for social sciences (SPSS) version 24 was used for the statistical analysis. The string data were presented as frequency and percentage, whereas the numerical data were presented as mean SD. The significant differences between string variables were calculated using Chi2. On the other hand, the difference between the two groups was calculated using the independent sample t. test. While paired sample t. test was used to find the significance level between baseline and intervention values. The value was considered significant when the significance level was less than 0.05.

2.2.4 Ethical considerations

Down syndrome patients recruited in this study participated voluntarily and were fully informed about the objectives and methods of the study. Consent was obtained from parents. This study was ethically approved by the Scientific Research Ethics Committee (SREC), Faculty of Home Economics, Menoufia University, Egypt; Approval: 15- SREC-11-2020.

3. Results

Table 2 describes the chemical composition of the suggested chocolates. Total solids were 29.46 grams, ash was 0.48 grams, protein was 2.58 grams (9.25% of DRI), fat was 10.98 grams (19.0% of DRI), carbs were 12.71 grams (3.52% of DRI), fiber content was relatively high at 2.71 grams (9.96% of DRI), and total calories were 160.0 kcal (7.71% of DRI). It contains 40.23 mg of total phenols and 2.49 mg of total flavonoids as active ingredients.

Table 2: Chemical composition of chocolate supplemented with wheat germ.

	Per 30 g chocolate	Chocolate with Wheat germ	% DRI
1	Total solids	29.46 g	-
2	Ash	0.48 g	-
3	Fat	10.98 g	19.0%
4	Protein	2.58 g	9.25%
5	Fiber	2.71g	9.96%
6	Total carbohydrate	12.71 g	3.52%
7	Total calories*	160.0 kcal	7.71%
8	Total phenols	40.23 mg	-
9	Total flavonoids	2.49 mg	-

** Calculated by multiplying protein by 4, carbohydrate by 4, and fat by 9.*

As shown, results in Table 3 did not reveal any significant differences between studied groups concerning age, which is 9.0±1.1 years for the control group and 9.8±1.1 years for the wheat germ group.

The independent sample t-test revealed no significant differences between the two groups at the baseline in terms of body height. The same trend was seen after the dietary intervention period. The paired sample t-test, on the other hand, revealed that all of the children in the study gained body height, and the values increased significantly across the two groups.

Concerning body weight, the independent sample t-test revealed no significant differences between the studied groups' pre-intervention. However, the body weight of the WG group after the dietary intervention period was significantly lower than the control group (35.3±4.0 vs. 42.6±5.1 kg and $P<0.01$). The paired sample t-test revealed that the body weight of the control group increased insignificantly by 0.5 kg, while it decreased significantly ($P<0.001$) among the WG group by 3.1 kg (- 8.1%).

Concerning BMI, although the BMI of the WG group was lower than the control group (26.0±1.1 vs. 29.2±4.9 kg/m2), the independent sample t-test revealed no significant differences between the two groups prior to intervention. Post-intervention, the BMI of the WG group was significantly ($P<0.05$) lower than the control group (22.9±1.0 vs. 28.2±4.4 kg/m2). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a highly significant ($P<0.001$) decrease in BMI by 3.1 kg/m2, meanwhile, the BMI decreased significantly ($P<0.01$) by 1.0kg/m2 among the control group.

Concerning arm circumference (AC), the mean value of the WG group was significantly ($P<0.05$) higher than the control group (29.8±1.5 vs. 26.3±3.4 cm) prior to intervention. Post-intervention, the AC of the WG group was also higher than the control group, but the difference was not significant4 kg/m2). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a highly significant (P<0.001) decrease in AC by 1.8 cm; in contrast, the AC increased significantly (P<0.05) by 1.0 cm among the control group.

Regarding triceps skinfold thickness (TSF), the mean value of the WG group was insignificantly higher than the control group (29.8±2.9 vs. 28.4±4.0 mm) prior to intervention. Post-intervention, the TSF of the WG group was lower than the control group, but the difference was not significant. The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.01) decrease in TSF by 1.8 mm, in contrast, the TSF increased insignificantly by 1.0 mm among the control group.

Regarding arm muscle circumference (AMC), the mean value of the WG group was significantly (P<0.01) higher than the control group (20.4±1.3 vs. 17.3±2.2 cm) prior to intervention. Post-intervention, the AMC of the WG group was also higher than the control group, but the difference was not significant. The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.001) decrease in AMC by 1.2 cm. In contrast, the AC increased insignificantly by 0.7 cm among the control group.

Table 3: Anthropometric indices (mean±SD) of wheat germ groups and control positives group after 18 weeks of dietary intervention.

		Control(n=8)		Wheat (n=9)		Independent t.test	
		mean	±SD	mean	±SD	t	Sig.
Age (years)		9.0	±1.1	9.8	±1.1	-1.48	0.160
Height (cm)	Pre	119.8	±14.2	121.4	±6.9	-0.31	0.765
	Post	123.8	±13.3	123.9	±6.8	-0.03	0.980
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-3.15	0.016*	-21.21	0.000***		
Weight (kg)	Pre	42.1	±5.6	38.4	±4.7	1.47	0.162
	Post	42.6	±5.1	35.3	±4.0	3.35	0.004**
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-2.00	0.086	6.72	0.000***		
BMI (kg/m2)	Pre	29.2	±4.9	26.0	±1.1	1.80	0.111
	Post	28.2	±4.4	22.9	±1.0	3.32	0.011*
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		3.98	0.005**	10.63	0.000***		
AC (cm)	Pre	26.3	±3.3	29.8	±1.5	-2.71	0.023*
	Post	27.3	±3.4	28.0	±1.4	-0.57	0.579
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		3.59	0.011*	8.00	0.000***		
TSF (mm)	Pre	28.4	±4.0	29.8	±2.9	-0.84	0.417
	Post	29.4	±4.0	28.0	±1.9	0.93	0.369
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		0.54	0.371	3.83	0.005**		
AMC (cm)	Pre	17.3	±2.2	20.4	±1.3	-3.56	0.003**
	Post	18.0	±2.2	19.2	±1.2	-1.35	0.206
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		0.87	0.188	6.12	0.000***		

** P<0.05, ** P<0.01, and *** P<0.001*

A paired sample t-test was used to determine the significant differences between pre and post-intervention values.

Table 4 presents the mean and standard deviation of macronutrients intakes of studied groups before and after the intervention.

In terms of calories pre-intervention, the values of the WG group and control group were very close (1525.3±177.4 vs. 1547.4±269.2 kcal/day), and the independent sample t-test revealed no significant differences between the two groups. Post-intervention, the calories intakes of the WG group was insignificantly lower than the control group (1544.4±147.9 vs. 1400.5±260.5 kcal/day). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.001) decrease in calories intake by 146.9 calorie (-9.5%), meanwhile, it increased insignificantly by only 19.1 (+1.3%) among the control group.

Regarding daily protein intakes pre-intervention, the values of the WG group and control were very close (52.6±6.0 vs. 51.5±8.4 g/day) and this trend did not change after dietary intervention (53.3±5.7 vs. 54.6±11.5 g/day). The paired sample t-test did not reveal any significant differences between values pre and post intervention for both control and WG groups.

Concerning fat intake pre-intervention, the value of the WG group was higher than control group (41.9±11.2 vs. 47.2±14.5 g/day), and the independent sample t-test revealed no significant differences between the two groups. Post-intervention, the fat intake of the WG group was insignificantly higher than the control group (41.5±11.6 vs. 48.2±15.2 g/day). The paired sample t-test revealed no significant differences for both control and WG groups.

Regarding carbohydrate intake pre-intervention, the values of the control and WG groups were very close (234.4±38.6 vs. 229.1±38.8 g/day) and there was no significant differences between the two groups. Post-intervention, the carbohydrate intake of the WG group was significantly (P<0.001) lower than the control group (187.2±30.9 vs. 239.5±33.8 g/day). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.001) decrease in carbohydrate intake by 41.9 (-19.3%), meanwhile, it increased insignificantly by 5.1 (+2.2%) among the control group.

As for fiber intake pre-intervention, the value of the WG group was significantly (P<0.01) higher than control group (3.9±1.1 vs. 5.1±1.1 g/day). Post-intervention, the fiber intake of the WG group was also and significantly (P<0.001) higher than the control group (8.3±1.1 vs. 3.7±1.0 g/day). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.001) increase in fiber intake by 3.2 62.7%), meanwhile, it decreased insignificantly by 0.2 (-5.1%) among the control group.

Table 4: Energy and macronutrient intakes (mean±SD) of wheat germ groups and control positives group after 18 weeks of dietary intervention.

		Control(n=8)		Wheat (n=9)		Independent t.test	
		mean	±SD	mean	±SD	t.value	Sig.
Calories	Pre	1525.3	±177.4	1547.4	±269.2	0.3	0.787
(kcal/day)	Post	1544.4	±147.9	1400.5	±260.5	-1.9	0.074
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-1.4	0.170	30.2	0.000***		
Protein (g/day)	Pre	52.6	±6.0	51.5	±8.4	-0.4	0.671
	Post	53.3	±5.7	54.6	±11.5	0.4	0.712
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-1.5	0.166	-1.9	0.083		
Fats (g/day)	Pre	41.9	±11.2	47.2	±14.5	1.2	0.262

		Control(n=8)		Wheat (n=9)		Independent t.test	
		mean	±SD	mean	±SD	t.value	Sig.
	Post	41.5	±11.6	48.2	±15.2	1.4	0.176
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		1.2	0.242	-0.8	0.426		
Carbohydrate (g/day)	Pre	234.4	±38.6	229.1	±38.8	-0.4	0.706
	Post	239.5	±33.8	187.2	±30.9	-4.5	0.000***
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-1.5	0.164	10.4	0.000***		
Fiber (g/day)	Pre	3.9	±1.1	5.1	±1.1	-3.2	0.003**
	Post	3.7	±1.0	8.3	±1.1	-12.2	0.000***
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		1.3	0.225	-37.3	0.000***		

** P<0.05, ** P<0.01, and *** P<0.001*
A paired sample t-test was used to determine the significant differences between pre and post-intervention values.

Results in Table 5 show the mean and standard deviation of lactate dehydrogenase (LDH) and blood lipids of studied groups pre and post-intervention.

Concerning LDH, although the value of the WG group was slightly lower than the control group (697.8±56.6 vs. 738.6±83.0 U/L), the independent sample t-test revealed no significant differences between the two groups prior to intervention. Post-intervention, the LDH of the WG group was sharply and significantly (P<0.01) lower than the control group (475.2±147.2 vs. 720.0±66.0 U/L). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.05) decrease in LDH by 222.6 U/L (-31.9%), meanwhile, the LDH decreased insignificantly by only 18.6 U/L (-2.5%) among the control group.

Regarding Total cholesterol, although the value of the WG group was lower than the control group (198.8±15.3 vs. 217.9±16.4mg/dL), the independent sample t-test revealed no significant differences between the two groups prior to intervention. Post-intervention, the Total cholesterol of the WG group was significantly (P<0.05) lower than the control group (166.7±24.2 vs. 200.9±25.0 mg/dL). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.05) decrease in Total cholesterol by 32.1 mg/dL (-16.1%), meanwhile, the total cholesterol decreased insignificantly by 17.0 mg/dL (-7.8%) among the control group.

Concerning Triglycerides, although the value of the WG group was slightly higher than the control group (165.3±33.3 vs. 160.4±11.4 mg/dL), the independent sample t-test revealed no significant differences between the two groups prior to intervention. Post-intervention, the Triglycerides of the WG group were sharply and significantly (P<0.05) lower than the control group (108.3±37.6 vs. 153.2±14.0 mg/dL). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.05) decrease in Triglycerides by 57.0 mg/dL (-34.5%), meanwhile, the Triglycerides decreased insignificantly by only 7.2 mg/dL (-4.5%) among the control group.

Regarding HDL, the value of the WG group was significantly (P<0.05) lower than the control group (43.3±6.1 vs. 49.3±3.5 mg/dL) prior to intervention. Post-intervention, the HDL value of the WG group was also lower than the control group (46.3±8.2 vs. 50.1±7.0 mg/dL). However, the independent sample t-test revealed no significant differences between the two groups. Intervention with chocolate supplemented with wheat germ caused an increase in HDL value by 3.0 mg/dL, but this difference was not significant; in addition, the HDL value of the control group increased by 0.8 mg/dL, but this increase was not significant as well.

As for LDL, the value of the WG group was insignificantly lower than the control group (122.4±8.1 vs. 136.3±17.9 mg/dL) prior to intervention. Post-intervention, the LDL value of the WG group was also lower than the control group (108.0±22.1 vs. 126.3±13.9 mg/dL); however, the independent sample t-test revealed no significant differences between the two groups. Intervention with chocolate supplemented with wheat germ caused an increase in LDL value by 14.0 mg/dL, but this difference was not significant; in addition, the LDL value of the control group increased by 10.0 mg/dL, but this increase was not significant as well.

Table 5: Lactate dehydrogenase (LDH) and blood lipids parameters (mean±SD) of wheat germ groups and control positives group after 18 weeks of dietary intervention.

		Control(n=8)		Wheat (n=9)		ANOVA	
		mean	±SD	Mean	±SD	F	sig
LDH (UL/L)	Pre	738.6	±83.0	697.8	±56.6	1.02	0.332
	Post	720.0	±66.0	475.2	±147.2	3.76	0.008**
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		1.94	0.100	3.61	0.015*		
Total cholesterol	Pre	217.9	±16.4	198.8	±15.3	2.15	0.055
(mg/dL)	Post	200.9	±25.0	166.7	±24.2	2.49	0.030*
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		1.51	0.182	3.15	0.026*		
Triglycerides (mg/dL)	Pre	160.4	±11.4	165.3	±33.3	-0.31	0.761
	Post	153.2	±14.0	108.3	±37.6	2.51	0.034*
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		1.51	0.205	2.74	0.041*		
HDL (mg/dL)	Pre	49.3	±3.5	43.3	±6.1	2.22	0.048*
	Post	50.1	±7.0	46.3	±8.2	0.91	0.384
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-0.52	0.620	-1.00	0.363		
LDL (mg/dL)	Pre	136.3	±17.9	122.4	±8.1	1.84	0.100
	Post	126.3	±13.9	108.0	±22.1	1.81	0.097
Paired Sample t-test		t.value	Sig	t.value	Sig		
		1.59	0.163	1.92	0.113		

** P<0.05, ** P<0.01, and *** P<0.001*
A paired sample t-test was used to determine the significant differences between pre and post-intervention values.

Table 6 presents the mean and standard deviation of various intelligence quotient (IQ) types of studied groups before and after the intervention.

In terms of verbal IQ (VIQ) pre-intervention, the values of the WG group and control group were very close (46.5±0.5 vs. 46.3±0.9), and the independent sample t-test revealed no significant differences between the two groups. Post-intervention, the VIQ of the WG group was significantly (P<0.001) higher than the control group (50.5±0.5 vs. 46.8±0.5). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.001) increase in VIQ by 4.0 (+8.6%), meanwhile, it increased insignificantly by only 0.5 (+1.1%) among the control group.

In terms of non-verbal IQ pre-intervention, the value of the WG group was significantly (P<0.05) higher than the control group (50.5±0.5 vs. 48.0±2.3). Post-intervention, the non-verbal IQ of the two groups did not change and the paired sample t-test did not any significant differences between values pre and post intervention.

Concerning IQ battery pre-intervention, the values of the WG group and control group were very close (53.0±2.3 vs. 53.8±2.7), and the independent sample t-test revealed no significant differences between the two groups. Post-intervention, the IQ battery of the WG group was insignificantly higher than the control group (55.5±0.5 vs. 53.8±2.7). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.05) increase in IQ battery by 2.5 (+4.7%), meanwhile, it did not change among the control group.

Regarding Overall IQ pre-intervention, the value of the WG group was significantly (P<0.05) higher than the control group (47.5±0.5 vs. 44.8±2.7). Post-intervention, the Overall IQ of the WG group was significantly (P<0.001) higher than the control group (49.5±0.5 vs. 45.3±2.2). The paired sample t-test revealed that chocolate supplemented with wheat germ caused a significant (P<0.001) increase in Overall IQ by 2.0 (+4.2%), meanwhile, it increased significantly (P<0.05) by 0.5 (+1.1%) among the control group.

Table 6: Intelligent quotient (IQ) (mean±SD) of the positive control group and intervention groups.

		Control(n=8)		Wheat (n=8)			
		Mean	±SD	Mean	±SD		
Verbal IQ (VIQ)	Pre	46.3	±0.9	46.5	±0.5	-0.68	0.506
	Post	46.8	±0.5	50.5	±0.5	-15.00	0.000***
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-2.65	0.033*	-10.58	0.000***		
Nonverbal IQ	Pre	48.0	±2.3	50.5	±0.5	-3.04	0.017*
	Post	48.0	±2.3	50.5	±0.4	-3.08	0.017*
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		0.00	1.000	0.00	1.000		
IQ battery	Pre	53.8	±2.7	53.0	±2.3	0.61	0.554
	Post	53.8	±2.7	55.5	±0.5	1.825	0.108
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		0.00	1.000	-3.04	0.019*		
Overall IQ	Pre	44.8	±2.7	47.5	±0.5	-2.87	0.022*
	Post	45.3	±2.2	49.5	±0.5	-5.34	0.001***
Paired Sample t-test		*t.value*	*Sig*	*t.value*	*Sig*		
		-2.65	0.033*	-5.29	0.001***		

** P<0.05, ** P<0.01, and *** P<0.001*
A paired sample t-test was used to determine the significant differences between pre and post-intervention values.

4. Discussion:

This study was designed to determine how down syndrome (DS) outcomes in children with DS are affected by feeding supplemented black chocolate with some ingredients known to have health benefits. Chocolate was produced by adding wheat germ (2.5 g), choline (184 mg), inositol (184 mg), and psyllium (5 g). Seventeen DS children were included in the study, and they were divided into two groups: the control group (n=8), which received 30 g of chocolate without any additional ingredients or supplements, and the wheat germ group (n=9), which received 30 g of chocolate with wheat germ. The anthropometric indices, LDH, blood lipids, and IQ, were assessed before and after the intervention, which lasted for 18 weeks.

The findings showed that, compared with the control group, the DS children who consumed wheat germ chocolate experienced significant weight loss, and their BMI changed significantly.

These findings can be explained primarily by changes in calorie and macronutrient intake among DS children who consumed supplemented chocolate. The results of Table (4) revealed a significant decrease in the amount of calories and carbohydrates consumed after eating supplemented chocolate and a significant increase in fiber intake among this group. Also, the mothers of the WG group reported a decrease in the number of meals and foods consumed and a decrease in their children's appetite. At the same time, there was no change in the control group's children.

Secondly, these could be explained by the favorable effects attributed to psyllium, which is rich in water-soluble dietary fiber, representing about 70.0% of total fibers [16]. Studies demonstrated that psyllium supplementation, when used as a snack for type 2 diabetes patients, has decreased body weight [34]. Moreover, Togawa et al. [35] discovered that feeding psyllium caused a significant decrease in the relative weight of the fat tissue depots in mice fed a high-fat diet (HFD) plus psyllium. Furthermore, Deng et al., [36] discovered that Psyllium husk aids in weight loss by suppressing appetite.

Also, one study [37] suggested that supplementing choline above an animal's recommended allowance may help to reduce body fat gain in growing kittens. Therefore choline may help in reducing body weight.

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme found in every cell of the human body. This enzyme converts glucose to pyruvic acid during aerobic glycolysis. When the body is subjected to oxidative stress or injury, LDH may be produced, elevating its serum level. Extracellular leaking of this enzyme implies cell injury or death [38]. As a result, the greater concentration in DS children poses a health risk. The current investigation found that the DS group's LDH levels were significantly higher before dietary intervention. This study showed that providing wheat germ-supplemented chocolate to DS children significantly lowered LDH. These findings were consistent with those of Alamery et al. [39], who investigated the effect of wheat germ oil on LDH concentrations and discovered that it had a positive effect. As a result, this chocolate supplemented with wheat germ may protect DS children from a variety of health concerns connected with high LDH.

WG groups' total cholesterol and triglycerides significantly decreased after receiving chocolates supplemented with wheat germ. Also, the LDL decreased, and HDL increased, but the changes were not significant.

In agreement with current findings, Liaqat et al. [40] assessed the relationship between wheat germ interventions and metabolic indicators based on biomarkers of cholesterol and triglycerides. They observed a relationship between wheat germ interventions and the control of metabolic markers, including blood lipids. According to Salehi-Sahlabadi et al. [41], consuming wheat germ may boost overall antioxidant capacity and reduce the total serum cholesterol and triglyceride levels. Also, Arshad et al. [42] found that wheat germ oil containing natural -tocopherol alone or in combination with -lipoic acid increased levels of antioxidant enzymes superoxide dismutase, catalase, and glutathione reductase while significantly lowering total plasma cholesterol, LDL, and triglycerides and significantly raising HDL.

Adding psyllium contributes to the improvement of blood lipid profile among DS children. However, psyllium supplementation, when used as a snack for type 2 diabetes patients, has been shown to increase HDL and decrease LDL cholesterol [34].

Also, a study by Brum et al. [43] indicated that when taken together with LDL-lowering statins, psyllium reduced LDL cholesterol, equivalent to doubling the statin medicine dose. Moreover, Deng et al., [36] discovered that Psyllium husk aids in weight loss by suppressing appetite. The researchers assessed the effects of psyllium husk treatments on mice with high-fat diet (HFD)-induced obesity utilizing obesity-related indices, metabolism indices, and gut microbiota. Orlistat was more effective at weight loss, whereas psyllium husk lowered serum, liver cholesterol, and triglyceride levels. The expression of CYP7A1 in the liver and the structures of fecal bile acids, on the other hand, differed between the two medicines. According to studies [44,45], psyllium is known for its beneficial effects in lowering low-density lipoprotein, attenuating blood sugar levels in diabetes, and preventing constipation.

According to Lim and Lee [46], psyllium seed husk (PSH) consumption decreases myocardial (MI) I/R injury in rats by suppressing apoptotic cascades via gene expression modification of multiple genes located upstream of apoptosis. Nevertheless, Williams [47] found that eating psyllium for three weeks or longer often lowers blood cholesterol levels in those with high cholesterol.

Regarding the four types of intelligence quotient, feeding chocolate supplemented with wheat germ resulted in a notable and significant rise in VIQ. People with Down syndrome may have significant cognitive impairments with an intelligence quotient (IQ) ranging from 30 to 70 [48,49].

When Alzheimer's disease (AD) rats were fed a dyslipidemia diet supplemented with 30% wheat germ, all the studied biochemical and nutritional parameters significantly improved to different degrees. The researchers concluded that wheat germ was protective against rats' Alzheimer's model [50].

Mohammadi et al., [51] investigated the effects of wheat germ consumption on mental health and brain-derived neurotrophic factor (BDNF) in patients with type 2 diabetes mellitus (T2DM). Their findings revealed that wheat germ consumption for 12 weeks could significantly reduce stress and depression in T2DM patients.

Also, this improvement in IQs may be due to choline, the memory vitamin, as Velazquez et al. [52] discovered that offspring of choline-supplemented dams performed much better than un-supplemented Ts65Dn mice in the murine model of DS and AD. Their results imply that adding more choline to the maternal diet has significant translational potential for DS.

5. Conclusion:

The DS children who consumed wheat germ chocolate experienced significant weight loss. Feeding wheat germ-supplemented chocolate to DS children significantly lowered LDH, total cholesterol, triglycerides, and LDL-c. On the other hand, administration of WG-supplemented chocolate increased HDL-c and IQs. However, this improvement proved that chocolate supplemented with wheat germ, psyllium, choline, and inositol has a favorable impact on the health status of DS children.

6. Study Limitations

The major limitation of this study was the small sample size, which may have been caused by the fact that there were only two institutes for children with these disabilities in the Governorates of Menoufia and Gharbia. There were approximately

80 children with DS in each cohort. However, children between the ages of 7 and 11 comprised approximately 30 percent of the total population at that institution, and we contacted the parents of all children in the target age range. Children with DS whose parents signed the consent form were enrolled in the study, and those were the final participants.

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Conflict of interest

This research holds no conflict of interest.

Author contribution

Hassan collected the data, manufactured the chocolate, carried out the chemical composition, and administer the chocolate to DS children. El Shafie, diagnosed the children, supervised the IQ test, carried out the blood analysis, and measured the anthropometry. Both authors contributed to writing and editing the article.

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References:

[1] World Health Organization. Genes and Human Disease 2017, USA. Available at http://www.who.int/genomics/public/geneticdiseases/en/index1.html

[2] Doğan E, Çekal N, Körükçü Ö. A study on the examination of eating behaviors and eating habits of individuals with Down syndrome. *Journal of Human Sciences*. 2020 May 20;17(2):684-99. doi:10.14687/jhs.v17i2.5966. [Google Scholar]

[3] O'Neill KL, Shults J, Stallings VA, Stettler N. Child-feeding practices in children with down syndrome and their siblings. *The Journal of Pediatrics*. 2005 Feb 1;146(2):234-8. doi: 10.1016/j.jpeds.2004.10.045 [PubMed] [Google Scholar]

[4] Patterson D. Molecular genetic analysis of Down syndrome. *Human Genetics*. 2009 Jul;126(1):195-214. doi: 10.1007/s00439-009-0696-8. [PubMed] [Google Scholar]

[5] Mazurek D, Wyka J. Down syndrome-genetic and nutritional aspects of accompanying disorders. *Roczniki Państwowego Zakładu Higieny*. 2015;66(3). PMID: 26400113 [PubMed] [Google Scholar]

[6] Määttä T, Tervo-Määttä T, Taanila A, Kaski M, Iivanainen M. Mental health, behaviour and intellectual abilities of people with Down syndrome. *Down syndrome research and practice*. 2006 Jan 1;11(1):37-43. doi: 10.3104/reports.313 [PubMed] [Google Scholar]

[7] Kus A, Sadowska L, Mysłek M. Usprawnianie korekcyjne dzieci i młodzieży z zespołem Downa w świetle dyshar- monii rozwoju somatycznego. *Postęp Reh* 2002; 16: 65-76. (abstract).

[8] Jimenez L, Cerda J, Alberti G, Lizama M. High rates of overweight and obesity in Chilean children with Down syndrome. *Revista medica de Chile*. 2015 Apr 1;143(4):451-8. doi: 10.4067/S0034-98872015000400006. [PubMed] [Google Scholar]

[9] Yahia S, El-Farahaty R, Abdel-Hady EG, Shoaib R, Ramadan R, Salem N. Serum adiponectin, body adiposity and metabolic parameters in obese Egyptian children with Down syndrome. *Journal of Pediatric Endocrinology and Metabolism*. 2021 Nov 1;34(11):1401-10. doi: 10.1515/jpem-2021-0324 [PubMed] [Google Scholar]

[10] Pueschel SM, Craig WY, Haddow JE. Lipids and lipoproteins in persons with Down's syndrome. *J Intellect Disabil Res.* 1992 Aug;36 (Pt 4):365-9. doi: 10.1111/j.1365-2788.1992.tb00535.x. [PubMed] [Google Scholar]

[11] Wrzochal A, Gładyś-Jakubczyk A, Suliga E. Evaluation of diet in preschool-age children with Down syndrome–preliminary examination. *Medical Studies/Studia Medyczne.* 2019;35(2):128-38. [CrossRef] [Google Scholar]

[12] Hassan, S., El Shafie, A., Ismail, M. The Prevailing Nutritional Problems among Children with Down Syndrome; Case-Control Study. *Journal of Home Economics - Menoufia University,* 2022; 32(4): 43-65. doi: 10.21608/mkas.2022.156958.1162. [PubMed] [Google Scholar]

[13] Smarkandy MM, Mohamed BA, Al-Hamdan AA. Nutritional assessment and obesity in Down syndrome children and their siblings in Saudi Arabia. *Saudi Med J* 2012; 33: 1216-1221. PMID: 23147880 [PubMed] [Google Scholar]

[14] Goluch-Koniuszy Z, Kunowski M. Glycemic index and glycemic load of diets in children and young people with Down's syndrome. *Acta Sci Pol Technol Aliment* 2013; 12: 181-194. [Google Scholar]

[15] Gibb RD, McRorie JW Jr, Russell DA, Hasselblad V, D'Alessio DA. Psyllium fiber improves glycemic control proportional to loss of glycemic control: a meta-analysis of data in euglycemic subjects, patients at risk of type 2 diabetes mellitus, and patients being treated for type 2 diabetes mellitus. *Am J Clin Nutr.* 2015 Dec;102(6):1604-14. doi: 10.3945/ajcn.115.106989. [PubMed] [Google Scholar]

[16] Jalanka J, Major G, Murray K, Singh G, Nowak A, Kurtz C, et al. The effect of psyllium husk on intestinal microbiota in constipated patients and healthy controls. *International journal of molecular sciences.* 2019 Jan 20;20(2):433. doi: 10.3390/ijms20020433 [PubMed] [Google Scholar]

[17] Blusztajn JK, Slack BE, Mellott TJ. Neuroprotective Actions of Dietary Choline. *Nutrients.* 2017 Jul 28;9(8):815. doi: 10.3390/nu9080815. [PubMed] [Google Scholar].

[18] Ghafoor K, Özcan MM, AL-Juhaımı F, Babıker EE, Sarker ZI, Ahmed IA, et al. Nutritional composition, extraction, and utilization of wheat germ oil: A review. *European Journal of Lipid Science and Technology.* 2017 Jul;119(7):1600160. [Google Scholar]

[19] Cohen, Allen Carson. Insect Diets: Science and Technology. *CRC Press.* ISBN 0-8493-1577-8. Retrieved 9 July 2009. [Google Scholar]

[20] Liu C, Sun Y, Yang L, Chen Y, Ji R, Wang H, et al. The Hypolipidemic and Antioxidant Activity of Wheat Germ and Wheat Germ Protein in High-Fat Diet-Induced Rats. *Molecules.* 2022 Mar 31;27(7):2260. doi: 10.3390/molecules27072260. [PubMed] [Google Scholar]

[21] Mohammedi Z. Allelopathic activity of thymol on seeds germination and effect on seedling properties at the non-allelopathic dose. *American Journal of Essential Oils and Natural Products.* 2021;9(1):01-5. [Google Scholar]

[22] Schwarz C, Benson GS, Horn N, Wurdack K, Grittner U, Schilling R, et al. Effects of Spermidine Supplementation on Cognition and Biomarkers in Older Adults With Subjective Cognitive Decline: A Randomized Clinical Trial. *JAMA New Open.* 2022 May 2;5(5):e2213875. doi: 10.1001/jamanetworkopen.2022.13875. [PubMed] [Google Scholar]

[23] Yun L, Wu T, Li Q, Zhang M. Dietary supplementation with purified wheat germ glycoprotein improve immunostimulatory activity in cyclophosphamide induced Balb/c mice. *Int J Biol Macromol.* 2018 Oct 15;118(Pt A):1267-1275. doi: 10.1016/j.ijbiomac.2018.06.199. [PubMed] [Google Scholar]

[24] Afoakwa EO. Chocolate science and technology. *John Wiley & Sons*; 2016 Jun 13. [Google Scholar]

[25] Ranganna S. Handbook of analysis and quality control for fruit and vegetable products. *Tata McGraw-Hill Education*; 1986. [Google Scholar]

[26] AOAC. Association of official Analytical chemists-Official Methods of analysis (18thed.).Washington, DC. *AOAC* 920.185.2005.

[27] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*. 1965 Jan 1;16(3):144-58. [Google Scholar]

[28] Gibson RS. Principles of nutritional assessment. *Oxford University Press*, USA; 2005. [Google Scholar]

[29] CDC. Growth charts, Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). http://www.cdc.gov/growthchart

[30] Trumbo P, Schlicker S, Yates AA, Poos M; Food and Nutrition Board of the Institute of Medicine, The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc*. 2002 Nov;102(11):1621-30. doi: 10.1016/s0002-8223(02)90346-9 [PubMed] [Google Scholar]

[31] American Health Association. Dietary Recommendations for Healthy Children. Available from: https://www.ahajournals.org/doi/10.1161/circulationaha.105.169251

[32] Lee RD, Nieman DC. Nutritional Assessment, *Mosby-Year Book*. Inc., St. Louis, MO USA. 1996. [Google Scholar]

[33] Frandsen AN, Higginson JB. The Stanford-Binet and the Wechsler intelligence scale for children. *Journal of Consulting Psychology*. 1951 Jun;15(3):236. doi: 10.1037/h0059816 [PubMed] [Google Scholar]

[34] Noureddin S, Mohsen J, Payman A. Effects of psyllium vs. placebo on constipation, weight, glycemia, and lipids: A randomized trial in patients with type 2 diabetes and chronic constipation. *Complementary therapies in medicine*. 2018 Oct 1;40:1-7. doi:10.1016/j.ctim.2018.07.004 [PubMed] [Google Scholar]

[35] Togawa N, Takahashi R, Hirai S, Fukushima T, Egashira Y. Gene expression analysis of the liver and skeletal muscle of psyllium-treated mice. *British journal of nutrition*. 2013 Feb;109(3):383-93. doi: 10.1017/S0007114512001250 [PubMed] [Google Scholar]

[36] Deng Z, Meng C, Huang H, Song S, Fu L, Fu Z. The different effects of psyllium husk and orlistat on weight control, the amelioration of hypercholesterolemia and non-alcohol fatty liver disease in obese mice induced by a high-fat diet. *Food & Function*. 2022;13(17):8829-49. doi: 10.1039/d2fo01161a. [PubMed] [Google Scholar]

[37] Verbrugghe A, Rankovic A. Dietary choline in feline nutrition and its role in obesity prevention and liver health. *In Journal of the American Oil Chemists Society* 2022 oct 1 (vol. 99, pp. 90-90). [Google Scholar]

[38] Narang AP, Greval RS, Chopra H, Kalra CS. The role of two enzymes (LDH and PHI) and a tumour marker (CEA) in the prognostic evalution of head and neck malignancy. *Indian Journal of Otolaryngology and Head & Neck Surgery*. 2001 Jan;53(1):76-80. doi:10.1007/BF02910990 [PubMed] [Google Scholar]

[39] Alamery S, Zargar S, Yaseen F, Wani TA, Siyal A. Evaluation of the Effect of Wheat Germ Oil and Olmutinib on the Thioacetamide-Induced Liver and Kidney Toxicity in Mice. *Life* (Basel). 2022 Jun 15;12(6):900. doi: 10.3390/life12060900. [PubMed] [Google Scholar]

[40] Liaqat H, Jeong E, Kim KJ, Kim JY. Effect of wheat germ on metabolic markers: a systematic review and meta-analysis of randomized controlled trials. *Food Sci Biotechnol.* 2020 May 22;29(6):739-749. doi: 10.1007/s10068-020-00769-9. [PubMed] [Google Scholar]

[41] Salehi-Sahlabadi A, Kord-Varkaneh H, Kocaadam-Bozkurt B, Seraj SS, Alavian SM, Hekmatdoost A. Wheat germ improves hepatic steatosis, hepatic enzymes, and metabolic and inflammatory parameters in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled, double-blind clinical trial. *Phytother Res.* 2022 Jul 17. doi: 10.1002/ptr.7553. [PubMed] [Google Scholar]

[42] Arshad MS, Anjum FM, Khan MI, Shahid M. Wheat germ oil and α-lipoic acid predominantly improve the lipid profile of broiler meat. *J Agric Food Chem.* 2013 Nov 20;61(46):11158-65. doi: 10.1021/jf4041029. [PubMed] [Google Scholar]

[43] Brum J, Ramsey D, McRorie J, Bauer B, Kopecky SL. Meta-analysis of usefulness of psyllium fiber as adjuvant antilipid therapy to enhance cholesterol lowering efficacy of statins. *The American journal of cardiology.* 2018 Oct 1;122(7):1169-74. doi:10.1016/j.amjcard.2018.06.040 [PubMed] [Google Scholar]

[44] Cassidy YM, McSorley EM, Allsopp PJ. Effect of soluble dietary fibre on postprandial blood glucose response and its potential as a functional food ingredient. *Journal of Functional Foods.* 2018 Jul 1;46:423-39. [Google Scholar]

[45] Jane M, McKay J, Pal S. Effects of daily consumption of psyllium, oat bran and polyGlycopleX on obesity-related disease risk factors: A critical review. *Nutrition.* 2019 Jan 1;57:84-91. doi: 10.1016/j.nut.2018.05.036 [PubMed] [Google Scholar]

[46] Lim SH, Lee J. Supplementation with psyllium seed husk reduces myocardial damage in a rat model of ischemia/reperfusion. *Nutrition Research and Practice.* 2019 Jun 1;13(3):205-13. doi: 10.4162/nrp.2019.13.3.205. [PubMed] [Google Scholar]

[47] Williams PG. The benefits of breakfast cereal consumption: a systematic review of the evidence base. *Advances in nutrition.* 2014 Sep;5(5):636S-73S. doi: 10.3945/an.114.006247 [PubMed] [Google Scholar]

[48] Chapman RS, Hesketh LJ. Behavioral phenotype of individuals with Down syndrome. *Ment Retard Dev Disabil Res Rev.* 2000;6:84–95. doi: 10.1002/1098-2779(2000)6:2<84::AID-MRDD2>3.0.CO;2-P [PubMed] [Google Scholar]

[49] Hamburg S, Lowe B, Startin CM, Padilla C, Coppus A, Silverman W, et al. Assessing general cognitive and adaptive abilities in adults with Down syndrome: a systematic review. *J Neurodev Disord.* 2019 Aug 30;11(1):20. doi: 10.1186/s11689-019-9279-8 [PubMed] [Google Scholar]

[50] Yaseen AA, Al-Okbi SY, Hussein AM, Mohamed DA, Mohammad AA, Fouda KA, et al. Potential protection from Alzheimer's disease by wheat germ and rice bran nano-form in rat model. *Journal of Applied Pharmaceutical Science.* 2019 Mar 10;9(1):067-76. [Google Scholar]

[51] Mohammadi H, Karimifar M, Heidari Z, Zare M, Amani R. The effects of wheat germ consumption on mental health and brain-derived neurotrophic factor in subjects with type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled trial. *Nutr Neurosci.* 2022 Jan;25(1):46-53. doi: 10.1080/1028415X.2019.1708032. [PubMed] [Google Scholar]

[52] Velazquez R, Ash JA, Powers BE, Kelley CM, Strawderman M, Luscher ZI, et al. Maternal choline supplementation improves spatial learning and adult hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis.* 2013 Oct;58:92-101. doi: 10.1016/j.nbd.2013.04.016 [PubMed] [Google Scholar]