Effect of Fed on Wheat Germ on Serum Minerals, Detoxification Enzymes and Immunological Indicators of Rats

Azza AL-Adle Ahmed Morsi

Faculty of Education, Hail University, Saudi Arabia

Abstract: Wheat germ is acting as one of the most good multiple vitamins and multiple mineral foods along with its additional high antioxidants content. This investigation was used about six levels of wheat germ which were 5%, 10%, 15%, 20%, 25%, and 30%, to form phytogenic diets for studying their effects on twenty eight Sprague-Dawley white male albino rats. Blood samples were collected for using to determine the serum minerals [Ca, P, Mg, Fe, Zn, and I], Bone minerals [Ca, Fe, P, and Zn], detoxification enzymes [GSH, GSHP, GSSGR, GST, g-GT, and catalase $x10^4$], and immunological indicators [Total immunoglobulins, IgG, T_4, and T_8]. The results were revealed that the highest values of serum calcium, phosphorus, iron, zinc, and iodine for level six of wheat germ were showed very high significant increase ($P< 0.001$) than control group. Whereas, the highest values of bone iron for Groups of rats were received 20%, 25% and 30% of wheat germ respectively, which showed very high significant increased ($P< 0.001$) than control group. Also, the results were showed that the highest values of GSH, GSHP, GSSGR, GST, and catalase $x10^4$ were for rats fed on different level of wheat germ, which showed very high significant increased ($P< 0.001$) than control group.

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1. Introduction:

Wheat germ is very high in protein. It contains around 28 percent protein and has more protein than can be found in most meat products. The human body needs protein in order to repair tissue damage and to help minerals and nutrients reach our cells. The amount of nutrients that are contained within wheat germ seems endless. It contains more potassium and iron than any other food source. Also found in great quantities are riboflavin, calcium, zinc magnesium and vitamins A, B1 and B3. Vitamins B1 and 3 are very important to maintain energy levels and maintain healthy muscles, organs, hair and skin. Wheat germ is one of the highest foods in B-complex vitamins that play good role in metabolism and handling stress. It is especially high in B-6 and folic acid which lower homo-cysteine levels and preventing arterial damage. B-vitamins are needed for energy, digestion, nerves, muscles, skin and hair, organs growth and repair of tissues **(Vos, 2000).** It is rich abundantly in protein (28.9 %). High quality protein is absolutely essential for repair of tissues and for carrying minerals and other nutrients in to cells **(Sidhu, 2001).** Another important vitamin found in wheat germ is vitamin E. Vitamin E is a very important antioxidant. It is helpful in preventing the body's aging process and also to prevent heart disease. Vitamin E also helps to prevent blood clots and is needed to strengthen the body's immune system **(Al-Hooti *et al.*, 2002).** Wheat germ is the embryo of the wheat kernel. It is separated from wheat being milled for flour. The germ is only a very small part of the kernel, approximately 2 ½ percent in total. The word germ does not have anything to do with bacteria; it simply refers to germination. The germ is the reproductive part that germinates and forms the wheat grass. Wheat germ is sodium and cholesterol free, and dense in nutrients. It is a good source of Co-enzyme Q10 (Ubiquinone) and PABA (Para-amino benzoic acid). Wheat germ contains naturally occurring polyunsaturated fat. Two tablespoons (50 gm) of wheat germ contains 65 calories, 6 grams of protein, 2 grams of unsaturated fat, and 2 grams of fiber **(Berbel-Garcia, 2004).** Wheat germ has been found to be very beneficial in order to keep the body in tip top condition. It is used by athletes in their diet to improve cardiovascular function and improve endurance levels. Body builders will also add wheat germ to their diets in order to bulk up and maintain the nutritional levels they need to perform because wheat germ contains fat, proper cold storage is necessary to prevent spoilage**.** There are no known side effects to wheat germ consumption at normal dietary levels**.** Because of its high oil content, improperly stored wheat germ can become rancid. So, store it in sealed glass jars and keep it refrigerated **(Paula, 2006).** The main objective of present study was studying the effect of different levels of wheat germ on bone and serum minerals and immunological indicators, after knowing its active constituents and its biological activities.

2. Material and Methods

2.1. MATERIAL:

Investigated sample is dried wheat germ, which are used after knowing her active constituents and her biological activities. This investigation is used about six levels of wheat germ, which are used as individuals to form phytogenic diets for studying their effects on improvement of immunological indicators, serum and bone minerals concentration. These levels include 5%, 10%, 15%, 20%, 25%, and 30%.

2.1.1. Basal Diet:

The basal diet (Rice protein-basal diet) was composed of 11.8 g of rice protein (10% protein), 10 g corn oil (10% fat), 4 g mixture of minerals (4% minerals), 1 g mixture of vitamins (1% vitamins), 4 g bran (4% fiber), and corn starch up to 100 g **(Jerome *et al.*, 2002)**. The minerals mixture which was used in this experiment as recommended by **Hegsted *et al.* (1941),** and the vitamins mixture as recommended by **Muller (1964).**

2.1.2. Experimental Animals (Rats):

Twenty eight Sprague-Dawley white male albino rats, each weighing 70-90 g, were used in this investigation. All rats were housed in special cages under controlled conditions every day, the animals were observed for the external appearance shape, color, and distribution of hair and physical activity. All rats were fed on the control diet for six consecutive days before the beginning of the experiment for adaptation. Diets were presented to rats in special non-scattering feeding containers to avoid loss of food and contamination. Tape water was provided to rats by mean of plastic pipes circle projecting through wire cages.

2.2. Methods:

2.2.1. Preparation of Wheat Germ:

Wheat germ was milled with other compounds by using a precession mill to give a powder and was kept in clean plastic bag in a cool dry location for using, and dark location for reduce oxidation of their contents. Refrigeration will vastly prolong the working life of essential oils. Wheat germ is used extensively in animal feeds, but for human consumption, wheat germ cereals and wheat germ oil are the two most popular preparations of the grain. A jar of vacuum-packed wheat germ can be safely stored up to one year unopened. To increase fiber and nutrients in bread and cereal recipes, wheat germ may be used to replace 0.5-1 cup of regular flour**.**

2.2.2. Grouping Of Experimental Animals (Rats):

The rats were divided into two main groups each one consists of four healthy rats as following:

1- Group (1) (Control group): Fed on basal diet only

2- Group (2) (wheat germ levels):

This group was furtherly subdivided into six sub-groups as follows:

- **2.1. Group (A):** Fed on basal diet plus 5 % of wheat germ.
- **2.2. Group (B):** Fed on basal diet plus 10 % of wheat germ.
- **2.3. Group (C):** Fed on basal diet plus 15 % of wheat germ.
- **2.4. Group (D):** Fed on basal diet plus 20 % of wheat germ.
- **2.5. Group (E):** Fed on basal diet plus 25 % of wheat germ.
- **2.6. Group (F):** Fed on basal diet plus 30 % of wheat germ.

2.2.3. Biological Evaluation:

During the experimental period (28 days), the consumed diet was recorded everyday feed intake, and body weight was recorded every three day. Biological evaluation of the different diets was carried out by determination of body weight gain, percentage of body weight gain and feed efficiency ratio **(Chapman *et al.*, 1959),** using the following formulas:

2.3. Biochemical Analysis:

At the end of the experiment, rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal vein. Blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. Serum was carefully separated and transferred into dry clean eppendorf tubes and kept frozen at 20°C till analysis. And biochemical analysis was procedures in Institute of Nutrition-Cairo-Egypt. Blood samples were used for determination of the concentration following parameters:

2.3.1. Determination of Serum Minerals:

Serum minerals (Ca, P, Mg, Fe, Zn, & I) were determined by **Barnett (1973)**; **Tietz (1983)** and **Liu *et al.* (2007).**

2.3.2. Determination of bone minerals:

Bone minerals (Ca, P, Fe, & Zn) were determined by **Barnett (1973)**; **Tietz (1983)** and **Liu *et al.* (2007).**

2.3.3. Determination of detoxification enzymes:

Detoxification enzymes (GSH, GSHP, GSSGR, GST, g-GT, & Catalase x_{10}^{4}) were determined by sandwich ELISA quantification according to the described method of **Chikako *et al.* (1996).**

2.3.4. Determination of immunological indicators:

Immunological indicators were examined in animal serum for immuno-globulins and both of CD^4 and CD^8 by enzyme linked immunosorbent assay (ELISA) according to the described methods and guideline of kits manufacturer (Toxo-ISAGA IgM; bioMérieux) **(Desmonts *et al.*, 1981)**. All the

commercially available tests were performed according to the recommendations given by the manufacturers.

2.4. Statistical Analysis:

Statistical analysis were performed by using computer program statistical package for social science **(**SPSS**),** and compared with each other using the suitable tests **(SPSS, 1998)**.

3. Results and Discussion

3.1. Effect of different levels of wheat germ intake on serum minerals

Table (1) explained the effect of different levels of wheat germ intake on serum minerals. As for serum calcium, the mean values of all groups were higher than control group, which were 1.9±0.01, 2.1±0.03, 2.2±0.01, 2.3±0.02, 2.4±0.03, 2.5±0.03, and 2.8±0.03 mmol/l respectively. The results refer to that group six was very high significant differences $P < 0.001$, when compared with control group.

The same table showed that the mean values of serum phosphorus of all groups were higher than control group, by mean 0.71±0.2, 0.72±0.3, 0.74±0.1, 0.78±0.2, 0.82±0.3, 0.85±0.3, and 0.86±0.3 mmol/l respectively. The results were showed that group six very high significant differences $P< 0.001$, comparing to control group. While, the mean values of serum magnesium of all groups were higher than control group, which were 0.71±0.02, 0.73±0.03, 0.74±0.01, 0.78±0.03, 0.80±0.02, 0.84±0.02 and 0.84±0.02 mmol/l respectively. Table was referred to that group five and six referring to very high significant differences $P < 0.001$, when compared with control group. However, the mean values of iron of all groups were higher than control group, by mean 60.2±3.3, 62.15±2.02, 64.10±4.55, 67.29±1.5, 71.25±1.10, 76.25±1.10, and 78.25±1.10 ug/dl respectively. As well as, group six points to very high significant differences $P < 0.001$, comparing with control group. As for zinc**,** the mean values of all groups were higher than control group, which were 13.4±0.2, 13.6±0.1, 13.8±0.2, 14.2±0.1, 14.8±1.0, 15.3±1.0, and 15.8±1.0 umol/dl respectively. These results were revealed that group five and six referred to very high significant differences $p < 0.001$, when compared with control group.

On the other hand, the mean values of iodine of all groups were higher than control group, by mean 8.3±0.1, 8.4±0.2, 8.5±0.1, 8.7±0.3, 9.3±0.2, 9.4±0.2, and 9.7±0.2 umol/dl respectively. According the results from the table was illustrated that group one and group two were very high significant $P < 0.001$, comparing with control group. From these data, it could be concluded that there are direct relationship between intake dose of wheat germ and increase of serum levels of minerals. Our results are going in the same line with that reported by **Morita *et al.* (2008)** whom stated that calmodulin is associated with increasing calcium serum levels in rat fed wheat germ. On the other hand, calmodulin which is available in rich quantity in wheat germ is associated also with improvement of brain tissue development and memory enhancement **(Manabe, 2008)**.

Table (1): Effect of different levels of wheat germ intake on serum minerals

Parameters	Control group	5% of W.G	10% of W.G	15% of W.G	20% of W.G	25% of W.G	30% of W.G
Serum.Ca (mmol/l)	1.9±0.01^a	2.1±0.03^a	2.2±0.01^b	2.3±0.02^b	2.4±0.03^c	2.5±0.03^c	2.8±0.03^c
Serum. P (mmol/l)	0.71±0.2^a	0.72±0.3^a	0.74±0.1^a	0.78±0.2^b	0.82±0.3^c	0.85±0.3^c	0.86±0.3^c
Serum. Mg (mmol/l)	0.71±0.02^a	0.73±0.03^a	0.74±0.01^a	0.78±0.03^b	0.80±0.02^c	0.84±0.02^c	0.84±0.02^c
Serum. Fe (µg/dl)	60.2±3.3^a	62.15±2.02^a	64.10±4.55^b	67.29±1.5^b	71.25±1.10^c	76.25±1.10^c	78.25±1.10^c
Serum. Zn (µmol/dl)	13.4±0.2^a	13.6±0.1^a	13.8±0.2^a	14.2±0.1^b	14.8±1.0^c	15.3±1.0^c	15.8±1.0^c
Serum. I (µmol/dl)^1	8.3±0.1^a	8.4±0.2^a	8.5±0.1^a	8.7±0.3 ^{b}	9.3±0.2^c	9.4±0.2^c	9.7±0.2^c

Values are expressed as the mean ± SE.

Table (2): Effect of different levels of wheat germ intake on bone minerals:

Table 2 explain the effect of different levels of wheat germ intake on bone minerals. The results were showed that the mean values of bone calcium of all groups were higher than control group, by mean 11.12±0.2, 11.13±0.3, 11.16±0.1, 11.18± 0.2, 11.19± 0.1, and 11.20± 0.1 mmol/l respectively, except group one was lower than control group, which was 11.11± 0.1 mmol/l. However, group six was referred to very high significant differences $P < 0.001$, when compared with control group.

Respecting to the results of this table we can observe that the mean values of bone iron of groups four, five, and six were higher than control group, which were 1.2±0.02, 1.3±0.03, 1.3±0.02, and 1.3±0.02 ug/dl respectively. But, the mean values of group one, two, and three were equaled with control group, by mean 1.2±0.01 ug/dl. Although, groups four, five, and six point to very high significant differences $P < 0.001$, comparing with control group.

Refer to the mean values of bone phosphorus, the results were illustrated that all groups were higher than control group, which were 112.16±20.56, 115.18±22.35, 115.10±11.20, 119.10±9.35, 129.16±8.56, 132.16±2.50, and 135.16±2.50 mmol/l respectively. Corresponding to the results from this table, group six was revealed very high significant differences $P< 0.001$, when compared with control group.

Point to the results of the same table explain that the mean values of bone zinc of groups three, four, five, and six were higher than control group, by mean 0.53±0.06, 0.54±0.06, 0.55±0.04, 0.57±0.01, and 0.58±0.01 umol/dl respectively. In contrast, group one and two were equaled with control group, which were 0.53±0.03 and 0.53±0.02 umol/dl. However, group six was showed very high significant differences $P < 0.001$, comparing with control group.

Kunzelmann *et al.*, (2004) found that wheat germ lectins did not inhibit electrogenic Na (+) absorption dose dependently in both colon and trachea but other cereal lectins do this inhibitory effect. The inhibitory effects on Na (+) absorption were suppressed by the sugar mannose, by inhibition of phosphor-lipase C (PLC) and protein kinase C (PKC). Thus, nutritional phytohem-agglutinins block salt absorption in a PLC- and PKC-dependent manner, probably by inhibition of the epithelial Na (+) channel (ENaC). CaM can also make use of the calcium stores in the endoplasmic reticulum, and the sarco-plasmic reticulum. **Lee *et al.* (2002)** and **Lin *et al.* (2004)** were observed that CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. CaM can bind up to four calcium ions, and can undergo post-translational modifications, such as phosphorylation, acetylation, methylation and proteolytic cleavage, each of which can potentially modulate its actions. These observations were agreeable with our results.

Table (2): Effect of different levels of wheat germ intake on bone minerals

Parameters	Control group	5% of W.G	10% of W.G	15% of W.G	20% of W.G	25% of W.G	30% of W.G
Bone. Ca (mmol/l)	11.12±0.2[a]	11.11±0.1[a]	11.13±0.3[a]	11.16±0.1[a]	11.18±0.2[a]	11.19±0.1[a]	11.20±0.1[a]
Bone. Fe (ug/dl)	1.2±0.02[a]	1.2±0.01[a]	1.2±0.01[a]	1.2±0.02[a]	1.3±0.03[a]	1.3±0.02[a]	1.3±0.02[a]
Bone. P (mmol/l)	112.2±20.6[a]	115.2±22.4[a]	115.1±11.2[a]	119.1±9.4[a]	129.2±8.6[b]	132.2±2.5[b]	135.2±2.5[b]
Bone. Zn (umol/dl)	0.53±0.06[a]	0.53±0.03[a]	0.53±0.02[a]	0.54±0.06[a]	0.55±0.04[a]	0.57±0.01[a]	0.58±0.01[b]

Values are expressed as the mean ± SE.

Effect of different levels of wheat germ intake on detoxification enzymes:

Table (3) showed the effect of different levels of wheat germ intake on detoxification enzymes. As for GSH enzyme, the mean values of all groups were higher than control group, which were 2.64± 0.4, 2.98±0.2, 3.20±0.3, 3.60±0.1, 3.94±0.2, 4.15±0.1, and 4.35±0.1u/ml respectively. But, group six was revealed very high significant differences $P < 0.001$, when compared with control group.

Refer to the results in the same table, the mean values of catalase $x10^4$ enzyme of all groups were higher than control group, by mean 36.2±4.12, 38.1±4.11, 40.6±4.10, 42.1±5.12, 44.3±4.13, 46.20±5.19, and 48.1±5.19 u/ml respectively. While, group six was referred to very high significant differences $P < 0.001$, comparing with control group. As for GSHP enzyme, the mean values of all groups were higher than control group, which were 0.38±0.06, 0.38±0.02, 0.39±0.01, 0.39±0.05, 0.42±0.01, 0.45±0.03, and 0.48±0.03 u/ml respectively, except group one was equaled with control group, by mean 0.38± 0.02 u/ml. Although, group six was point to very high significant differences $P < 0.001$, when compared with control group.

For the GSSGR enzyme, the mean values of all groups were higher than control group, which were 0.50±0.06, 0.52±0.03, 0.54±0.02, 0.55±0.06, 0.57±0.04, 0.58±0.01, and 0.59±0.01 u/ml respectively. As well as, group six was point to very high significant differences $p < 0.001$, corresponding to control group. Regarding to GST enzyme, the mean values of all groups were higher than control group, by mean 22.3±1.3, 23.4±1.5, 24.4±1.1, 24.9±1.0, 25.4±1.3, 25.5±1.6, and 25.6±1.6 u/ml respectively. From these results, group six was point to very high significant differences $P < 0.001$, comparing to control group. Refer to g-GT enzyme, the mean values of all groups were higher than control group, which were 0.56±0.04, 0.58±0.01, 0.59±0.02, 0.60±0.01, 0.62±0.01, 0.63±0.02, and 0.63±0.02 u/ml respectively. While, group five and six were referred to very high significant differences $P < 0.001$, when compared with control group.

Wu *et al.*, (2001) were suggested that the activity of blood and liver glutathione peroxidase (GSH-Px) and peroxidase dismutase (SOD) were increased. This suggests revealed that the effects of flavonoids of wheat germ on inducing peroxidase might be one of the chemical prevention mechanisms on mammary tumors. These suggest were agreeable with our results.

Table (3): Effect of different levels of wheat germ intake on detoxification enzymes

Parameters	Control group	5% of W.G	10% of W.G	15% of W.G	20% of W.G	25% of W.G	30% of W.G
GSH (u/ml)	2.64±0.4[a]	2.98±0.2[a]	3.20±0.3[b]	3.60±0.1[b]	3.94±0.2[b]	4.15±0.1[c]	4.35±0.1[c]
Catalase x_{10}^4 (u/ml)	36.2±4.12[a]	38.1±4.11[a]	40.6±4.10[a]	42.1±5.12[b]	44.3±4.13[b]	46.2±5.19[c]	48.1±5.19[c]
GSHP (u/ml)	0.38±0.06[a]	0.38±0.02[a]	0.39±0.01[a]	0.39±0.05[a]	0.42±0.01[b]	0.45±0.03[c]	0.48±0.03[c]
GSSGR (u/ml)	0.50±0.06[a]	0.52±0.03[a]	0.54±0.02[a]	0.55±0.06[b]	0.57±0.04[b]	0.58±0.01[c]	0.59±0.01[c]
GST (u/ml)	22.3±1.3[a]	23.4±1.5[a]	24.4±1.1[a]	24.9±1.0[b]	25.4±1.3[b]	25.5±1.6[b]	25.6±1.6[b]
g-GT (u/ml)	0.56±0.04[a]	0.58±0.01[a]	0.59±0.02[b]	0.60±0.01[b]	0.62±0.01[b]	0.63±0.02[b]	0.63±0.02[b]

Values are expressed as the mean ± SE. **GSH:** Glutathione. **GSHP:** Glutathione peroxidase. **GSSGR:** Glutathione reductase. **GST:** Glutathione s-transferase. **g-GT:** gamma-glutamyl transpeptidase.

Effect of different levels of wheat germ intake on immunological indicators:

Table (4) revealed the effect of different levels of wheat germ intake on immunological indicators. As for the mean values of total immunoglobulins of all groups were higher than control group, which were 1140.6±50.2, 1150.6±60.1, 1190.13±30.3, 1250.2±30.1, 1360.2±45.32, 1380.2±45.2, and 1400.2±50.1 u/ml respectively. Regarding to the results group six was referred to very high significant differences $P < 0.001$, when compared with control group. The same table illustrate that the mean values of IgG of all groups were higher than control group, by mean 650.5±30.5, 700.2±25.3, 750.2±20.6, 800.6±10.52, 850.3±25.3, 855.3±20.4, and 860.3±30.3 u/ml respectively. From the results we can observe that group six was point to very high significant differences $P < 0.001$, comparing with control group.

Point to the mean values of CD_4 (T_4) of group three, four, five and six were higher than control group, which were 2500.9±20.6, 2600.1±60.4, 2750.2±40.6, 2775.2±35.5, and 2800.2±30.5 u/ml respectively. But, the mean values of group one and two were lower than control group, by mean 2500.2±10.4 and 2500.1±50.2 u/ml respectively. However, group six showed very high significant differences $P < 0.001$, corresponding to control group.

According to the mean values of CD_8 (T_8), the results were revealed that all groups were higher than control group, by mean 1200.8±90.1, 1300.53±65.03, 1350.53±40.02, 1400.5±25.1, 1500.6±30.54, 1550.6±35.5, and 1600.6±40.5 u/ml respectively. From this table we can observe that group six was referred very high significant differences $P < 0.001$, when compared with control group. Our results indicate that wheat germ feeding is associated with improvement of general immunity indices through intake level- dose manner. This finding is agreed and supported with reported by **Telekes *et al.* (2007)**. They reported that wheat germ has significant anti-inflammatory efficacy confirmed by plethysmography, histology, and real-time PCR. Beside its immunomodulatory effect, wheat germ is reported to have clear anti-cancer effect. They added that wheat germ is possesses unique cancer-fighting characteristics when taken orally in fresh form and can inhibit metastatic tumor dissemination and proliferation during and after chemotherapy, surgery, or radiation. Benefits of wheat germ have been shown in various human cancers, in cultures of in vitro grown cancer cells, in the prevention of chemical carcinogenesis, and also in some autoimmune conditions. Special references are made for its safety, including its co-administration with anticancer drugs, as well as for its immunomodulatory activity, its molecular targets, and its use in cancer clinical trials. In addition, **(Chignola *et al.*, 2002)** reported that wheat germ has powerful anti-cancer properties through its immunomodulatory pathways.

Table (4): Effect of different levels of wheat germ intake on immunological indicators

Parameters	Control group	5% of W.G	10% of W.G	15% of W.G	20% of W.G	25% of W.G	30% of W.G
Total Ig (u/ml)	1140.6 ±50.2[a]	1150.6 ±60.1[a]	1190.13 ±30.3[a]	1250.2 ±30.1[a]	1360.2 ±45.32[a]	1380.2 ±45.2[b]	1400.2 ±50.1[b]
IgG (u/ml)	650.5 ±30.5[a]	700.2 ±25.3[a]	750.2 ±20.6[a]	800.6 ±10.52[b]	850.3 ±25.3[b]	855.3 ±20.4[b]	860.3 ±30.3[b]
CD_4 (T_4) (u/ml)	2500.9 ±20.6[a]	2500.2 ±10.4[a]	2500.1 ±50.2[a]	2600.1 ±60.4[a]	2750.2 ±40.6[b]	2775.2 ±35.5[b]	2800.2 ±30.5[b]
CD_8 (T_8) (u/ml)	1200.8 ±90.1[a]	1300.53 ±65.03[a]	1350.53 ±40.02[a]	1400.5 ±25.1[a]	1500.6 ±30.54[b]	1550.6 ±35.5[b]	1600.6 ±40.5[b]

Values are expressed as the mean±SE. **IgG:** Immunoglobulin-G. **CD^4:** Cluster of differentiation marker for helper T cells. **CD^8:** Cluster of differentiation marker for suppressor T cells.

References

Barnett, R.N. (1973): Calcium determination. Am. J. Clin. Pathol, 59:83.

Berbel-Garcia, A. (2004): *"Coenzyme Q 10 improves lactic acidosis, strokelike episodes, and epilepsy in a patient with MELAS". Clin. Neuro-Pharmacology 27: 187-191.*

Chapman, D.G.; Castilla, R. and Champbell, J.A. (1959): Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. Can J. Biochem. Physio, 37: 679-686.

Chignola, R.; Rizzi, C.; Vincenzi, S.; Cestari, T.; Brutti, N.; Riviera, A.P.; Sartoris, S.; Peruffo, A.D. and Andrighetto, G. (2002): Effects of Dietary wheat germ deprivation on the immune system in Wister rats: a pilot study. Int. Immunopharmacol., 2 (10): 1495-1501.

Chikako, K.; Koh-Ichi, S.; Shyoji, K.; Takayoshi, M. and Sota, H. (1996): A simplified method for the estimation of glutathione peroxidase activity and selenium concentration in bovine blood. J. Dairy Sci. 79 (9): 1543-1548.

Desmonts, G.; Naot, Y. and Remington, J. S. (1981): Immunoglobulin M-immunosorbent agglutination assay for diagnosis of infectious diseases: Diagnosis of acute congenital and acquired Toxoplasma infections. J. Clin. Microbiol. 14: 486-491.

Hegsted, D.; Mills, R. and Perkins, E. (1941): Salt mixture. J. Biol. Chem., 138: 459.

Jerome, B.; Elyett, G.; Edmond, R.; Andrzej, M. and Yaves, R. (2002): Substituting honey for refined CHO protects rats from hypertriglyceridemic and prooxidative effects of fructose. J. of Bio. Chem. France.

Kunzelmann, K.; Sun, J.; Schreiber, R. and Konig, J. (2004): Effects of dietary lectins on ion transport in epithelia. Br. J. Pharmacol.; 142 (8): 1219-1226.

Lee, H.W.; Yang, W.; Ye, Y.; Liu, Z.R.; Glushka, J. and Yang, J.J. (2002): Isolated EF-loop III of calmodulin in a scaffold protein remains unpaired in solution using pulsed-field-gradient NMR spectroscopy. Biochim. Biophys. Acta 1598, 80-87.

Lin, Y.; Rudrum, M.; Van der Wielen, R.P.; Trautwein, E.A.; McNeil, G.; Sierkma, A. and Meijer, G.W. (2004): Wheat germ policosanol failed to lower plasma cholesterol in subjects with normal to mildly elevated cholesterol concentrations. Metabo. 53 (10): 1309-1314,

Liu, L.E.; Ding, L.; Qi, M.; Han, X.L. and Zhang, H.Q. (2007): Determination of trace elements in new food sources by flame atomic absorption spectrophotometry. Spectrosc Spect Anal. 27 (7): 1436-1439.

Manabe, T. (2008): Molecular mechanisms for memory formation. Brain Nerve; 60 (7): 707-715.

Morita, M.; Iguchi, A. and Takemura, A. (2008): Roles of Calmodulin and Calcium/Calmodulin-Dependent Protein Kinase in Flagellar Motility Regulation in the Coral Acropora Digitifera. Mar. Biotechnol. (NY). 26.

Muller, A. (1964): Vitamin mixture. J. Biol. Chem. 150: 305.

Paula, F.M. (2006): Wheat germ. Reavley, Nocola. The New Encyclopedia of Vitamins, Minerals, Supplements, and Herbs. New York: M. Evans and Company, Enotes.com LLC.

Sidhu, J.S. (2001): Studies on the development of pan bread using raw wheat germ. J. of Food Qual. 24 (3): 235-247.

SPSS (1998): Statistical package for social science, computer software, Ver.10. SPSS Company. London, UK. Statistics version 1.0 copyright 1995. Analytical software windows version 95.

Telekes, A.; Resetar, A.; Balint, G.; Blazso, G.; Falkay, G.; Lapis, K.; Raso, E.; zende, B.; Ehrenfeld, M.; Shoenfeld, Y. and Hidvegi, M. (2007): Fermented wheat germ extract (avemar) inhibits adjuvant arthritis. Ann. N. Y. Acad. Sci.; 1110: 348-361.

Tietz, N.W. (1983): In clinical guide to laboratory, tests. W.B Saunders Company. Philadelphia, p 384.

Vos, E. (2000): Whole grains and coronary heart disease. Am. J. Clin. Nutr. 71: 1009.

Wu, B.; Xu, G.; Zhao, X. and Ren, X. (2001): Anti-oxidation of flavones of wheat germ on mammary tumor of rats. Am. J.; 30 (4): 215-217.

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