

## Effect of a New Type of Food Supplement (Live and Healthy Nutrition) on Changes in T3, T4, and TSH Hormones in Male Rat Lead Acetate Recipient

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### Abstract

**Background:** Thyroid gland is one of the important glands of the body that regulates the body's energy metabolism. This gland is shaped like a butterfly and is located in front of the trachea. Considering the increasing prevalence of thyroid gland diseases and the lack of proper treatment of this disease, the present study was conducted to investigate the effect of new healthy and live food supplement from wheat on changes in T3, T4, and TSH hormones in rats.

**Methods:** In this research, a new and green route synthesized healthy and live food supplement. This organic biomaterial was named NBS. The NBS healthy and live food supplement had various vitamins, macro and micro molecules, and ingredients such as B1, B2, B3, B5, B6, B9, C, K, A, E, D, phosphorus, potassium, sulfur, magnesium, calcium, boron, iron, manganese, zinc, copper; omega-3, omega-6, omega-9, and etc. For induction of disease, the lead acetate was used in diet of rats. The mice received the new food supplement for nutrition at therapeutic concentrations of 125, 250 and 500 mg/kg body weight.

**Results:** According to the results, it can be concluded that food supplement (healthy and live) causes the relative return of T3, T4 and TSH levels to normal. It was determined that the concentration of 500 mg/kg has the most effective therapeutic effect on the condition of hypothyroidism.

**Conclusions:** The new food supplement (healthy and alive) has suitable therapeutic effect for treating abnormalities of thyroid parameters.

**Keywords:** Hormone, T3, T4, TSH, Food Supplement (Healthy and alive), Normal.

### Background

The thyroid gland is one of the largest endocrine glands that its thyroid hormones, thyroxin and triiodothyronine are important for the growth, evolution and metabolism of the body. Hypothyroidism is one of the most important endocrine disrupters in humans and animals caused by a deficiency of the production of hormone by the thyroid gland. Hypothyroidism is usually associated with symptoms such as tiredness, drowsiness, muscle slowness, cardiac arrest, slow heart rate, decreased cardiac output, and decreased blood volume. In addition, in hypothyroidism, wide skin symptoms such as dry skin, shrinkage, thinness, and loss of hair growth are seen [1]. Thyroid is one of the very important intracellular tubers that is responsible for regulating important body functions [2]. The thyroid and precisely secreted hormones from which the most important are T4 or thyroxine, control and coordinate important actions such as metabolic regulation, control of base metabolism, help with growth hormones, guiding neural message and reproduction. . In the absence of

these hormones, both physical and nerve growth will be disturbed [3]. This gland is one of the largest endocrine glands that is composed of two lobes. Its weight is about 25 grams and its size is larger in women, but its size increases during menstruation and pregnancy. In the central part, there is an isthmus (strait) that connects the two thyroid lobes to each other. Sometimes a third lobe can also be pulled out of an isthmus or two other main lobes. In some cases, small masses of thyroid tissue are seen around the gland that do not bind to the primary gland, and they are called thyroid adnexa [4].

The TSH hormone secreted by the anterior pituitary cells plays a central role in controlling the thyroid function and is the most useful physiological indicator of thyroid hormone activity. The main factor is determining the adjustment point in the thyroid axis of the TSH hormone. The hypothalamic TRH hormone regulates the secretion of this hormone. TRH is the major stimulant for the synthesis and secretion of TSH. Approximately 15 minutes after

administration of TRH, the amount of TSH secretion reaches its maximum. Reducing the levels of thyroid hormones increases TSH production and exacerbates the TRH stimulation effect on TSH. Increasing levels of thyroid hormones also rapidly and directly inhibit TSH and inhibit the TRH stimulatory effect on TSH. It shows that thyroid hormones are the main regulator of TSH production. Like other pituitary hormones, TSH is secreted in rash and its secretion varies overnight [2, 4]. The maximum level of secretion of this hormone occurs at night, and because the TSH hormone fluctuation is mild, it is enough to measure the amount of hormone in the circulation. Thyroid gland hormonal disorders have a direct effect on infertility, so the study of the causes of these disorders is essential in the onset of infertility treatment. Physicians consider thyroid disorders to be two of the most common endocrine disorders, especially in women. About one to two percent of the causes and problems of fertility and the decline in women's fertility are due to endocrine problems, including the thyroid gland [2, 4]. Thyroid gland disorders in men also lead to malnutrition in pubescence at adulthood and in young age, but in general, it is much less common in men than in women. Hypothyroidism Hyperthyroidism is one of the most common diseases in diabetic patients. In plain language, the disease occurs when the thyroid gland cannot produce the hormonal body as much as it needs. Thyroid gland hormone is triiodothyronine and thyroxine. These hormones are responsible for regulating energy consumption, producing heat and facilitating growth in the body. The disease is three times more common in women than in men. Symptoms are included weakness and fatigue, sleepiness, dry, rough and cold skin, cold intolerance, decreased sweating, bradycardia or slowness, large tongue, edema (facial swelling) (myxedema), malaise, constipation, weight gain, menstrual disorders, frequent abortions, Delayed reflexes, anemia and joint and muscle pain. The disease is progressive in adults and is gradual [2, 4].

Today, herbal remedies and food supplements are widely used around the world because of their availability, relatively low cost, and because of their low side effects and effectiveness in the treatment of diseases [5, 6]. Given the adverse effects of chemical drugs and numerous reported side effects, this study aimed to investigate the effect of healthy and live food supplement from Wheat on changes in T3, T4, and TSH hormones in male rats receiving lead acetate. For this purpose, a green route synthesized new healthy and live food supplement. This organic biomaterial was named NBS. The NBS healthy and live food supplement has various vitamins, macro and micro molecules, and ingredients such as B1, B2, B3, B5, B6, B9, C, K, A, E, D, phosphorus, and etc.

The healthy and viable drug supplement in the current research may be comparable to chemical supplements. The majority of the multivitamins that are available on the market only meet the needs of the human body. In addition, special attention was paid to their regulation and balance. This highlights the importance of the balanced cellular, molecular, and metabolic function of the human body, which has often been overlooked in other chemical and herbal drugs. In general, emphasis on balance is associated with the improvement and treatment of various diseases. Another example in this regard is Ganoderma fungi, which has

recently been introduced as a therapeutic drug owing to its active compounds for the body, some of which require further investigation. These fungi contain some chemicals that are unknown to the body, including three types of toxins, which may be hazardous to liver health. In addition, the long-term consumption of this material at high doses could lead to adverse complications.

With this background in mind, no comparable foreign and domestic products have been registered that are produced in a similar manner to the processing of cereal grains in the form of a powder supplement for the disease control and treatment.

## Methods

In this experimental study, 30 Wistar rats were used in a weight range of 180-220 g. Animals in standard cages were kept at 22-25 ° C and 12 hours of light and 12 hours of darkness. Animals were tested for water. They had enough food and began experimenting with the optimal weight range. Animals were randomly divided into six random groups including:

**Group 1:** Control group that did not receive any treatment.

**Group 2:** A control group with regular food plus water containing lead acetate at a concentration of 0.5 g / liter.

**Group 3:** Treatment group number one receiving 125 mg / kg body weight of food supplement (healthy and alive) as gavage plus water containing lead acetate at a concentration of 0.5 g / liter.

**Group 4:** Treatment group number one receiving 250 mg / kg body weight of food supplement (healthy and alive) as gavage plus water containing lead acetate at a concentration of 0.5 g / liter.

**Group 5:** Group 1 therapy receiving 500 mg / kg of body weight food supplement (live and healthy nutrition) in the form of gavage plus water containing lead acetate at a concentration of 0.5 grams per liter.

**Group 6:** Healthy control group receiving drinking water plus 500 mg/ kg body weight food supplement (healthy and alive).

Animals were weighed at the end of the fourth week and then anesthetized by diethyl ether and bleeding from the heart, which was performed directly. Blood samples were centrifuged at 4000 rpm for 10 minutes in a centrifuge and after separation of serum was done using the T3, T4, TSH hormone kit, manufactured by Pars Test Company.

## Results

Analysis of the new NBS healthy and live food powder are shown as S1.

In this study, in order to make changes in the level of T3, T4, TSH hormones, the mice received lead acetate at a concentration of 0.5 g / L, a week after acclimating to the new medium, with a normal diet. Animals were weighed at the end of the fourth week and then bleached with diethyl ether and blood samples were

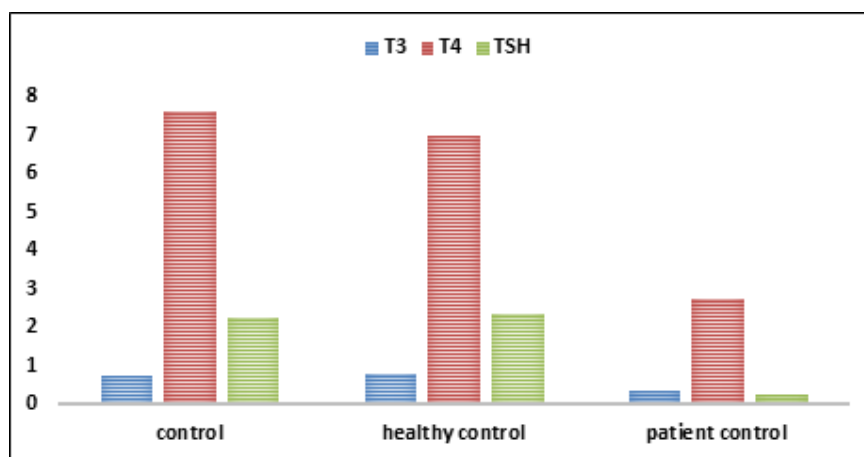
taken directly from their hearts. Centrifuges collected Blood samples for centrifugation at 4000 rpm for 10 minutes and iso-

lated serum was measured using the T3, T4, TSH and T3, T4 and TSH assay kits Figure 1.



**Figure 1:** Blood Collection from Rat Heart, Serum Separation and Sample Analysis with ELISA Reader

Hormones were measured. The average results of this test are shown in Figure 2.



**Figure 2:** Comparison of T3, T4, and TSH Hormones between the Three Experimental Control Groups, the Patient Control and the Healthy Control

In order to analysis the data in this study, we first make sure that the distribution of data is normal, using Kolmogorov-Smirnov test. In this regard, by using the information test obtained by observing Sig, it can be stated that these data have a normal distribution. Therefore, parametric tests should be used to analysis the differences between groups. In this study, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. The results of this test showed that there was a

significant difference between the groups with 5% probability level. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups. Based on the results of this table, it can be stated that the use of NBS dietary supplements causes a significant difference in the level of thyroid hormones in the studied groups in which a concentration of 125 has the lowest effect and a concentration of 500 has the greatest effect on the level of thyroid hormones (Table 1).

**Table 1:** (a) The Kolmogorov-Smirnov Test to Determine the Data Distribution Normality, (b) Results of ANOVA Test between groups and Data, and (c) The Scheffe Post Hoc Test Results to Determine the Differences between Groups

a		T3	T4	TSH
N		30	30	30
Normal Parametersa	Mean	.5707	5.0067	1.3223
	Std. Deviation	.21764	2.15614	1.02009
Most Extreme Differences	Absolute	.135	.127	.227
	Positive	.135	.112	.227
	Negative	-.123	-.127	-.138
Kolmogorov-Smirnov Z		.738	.698	1.245
Asymp. Sig. (2-tailed)		.647	.715	.090
a. Test distribution is Normal.				

ANOVA						
b		Sum of Squares	df	Mean Square	F	Sig.
T3	Between Groups	1.228	5	.246	40.595	.000
	With in Groups	.145	24	.006		
	Total	1.374	29			
T4	Between Groups	123.355	5	24.671	51.649	.000
	With in Groups	11.464	24	.478		
	Total	134.819	29			
TSH	Between Groups	26.418	5	5.284	33.738	.000
	With in Groups	3.759	24	.157		
	Total	30.177	29			

Dependent Variable c	(I) VAR00006	(J) VAR00006	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
T3	ctrl	Healthy group	-.05400	.04920	.940	-.2321	.1241
		Patient group	.38400*	.04920	.000	.2059	.5621
		Nbs-125	.49000*	.04920	.000	.3119	.6681
		Nbs-250	.20600*	.04920	.016	.0279	.3841
		Nbs-500	.03800	.04920	.987	-.1401	.2161
	Healthy group	ctrl	.05400	.04920	.940	-.1241	.2321
		Patient group	.43800*	.04920	.000	.2599	.6161
		Nbs-125	.54400*	.04920	.000	.3659	.7221
		Nbs-250	.26000*	.04920	.001	.0819	.4381
		Nbs-500	.09200	.04920	.629	-.0861	.2701
	Patient group	ctrl	-.38400*	.04920	.000	-.5621	-.2059
		Healthy group	-.43800*	.04920	.000	-.6161	-.2599
		Nbs-125	.10600	.04920	.480	-.0721	.2841
		Nbs-250	-.17800	.04920	.050	-.3561	.0001
		Nbs-500	-.34600*	.04920	.000	-.5241	-.1679
	Nbs-125	ctrl	-.49000*	.04920	.000	-.6681	-.3119
		Healthy group	-.54400*	.04920	.000	-.7221	-.3659
		Patient group	-.10600	.04920	.480	-.2841	.0721
		Nbs-250	-.28400*	.04920	.001	-.4621	-.1059
		Nbs-500	-.45200*	.04920	.000	-.6301	-.2739
Nbs-250	ctrl	-.20600*	.04920	.016	-.3841	-.0279	
	Healthy group	-.26000*	.04920	.001	-.4381	-.0819	
	Patient group	.17800	.04920	.050	.0000	.3561	
	Nbs-125	.28400*	.04920	.001	.1059	.4621	
	Nbs-500	-.16800	.04920	.073	-.3461	.0101	
Nbs-500	ctrl	-.03800	.04920	.987	-.2161	.1401	
	Healthy group	-.09200	.04920	.629	-.2701	.0861	
	Patient group	.34600*	.04920	.000	.1679	.5241	
	Nbs-125	.45200*	.04920	.000	.2739	.6301	
	Nbs-250	.16800	.04920	.073	-.0101	.3461	
T4	ctrl	Healthy group	.46000	.43711	.950	-1.1223	2.0423
		Patient group	4.68000*	.43711	.000	3.0977	6.2623

		Nbs-125	5.14000*	.43711	.000	3.5577	6.7223
		Nbs-250	3.24000*	.43711	.000	1.6577	4.8223
		Nbs-500	1.08000	.43711	.330	-.5023	2.6623
	Healthy group	ctrl	-.46000	.43711	.950	-2.0423	1.1223
		Patient group	4.22000*	.43711	.000	2.6377	5.8023
		Nbs-125	4.68000*	.43711	.000	3.0977	6.2623
		Nbs-250	2.78000*	.43711	.000	1.1977	4.3623
		Nbs-500	.62000	.43711	.842	-.9623	2.2023
		Patient group	ctrl	-4.68000*	.43711	.000	-6.2623
	Patient group	Healthy group	-4.22000*	.43711	.000	-5.8023	-2.6377
		Nbs-125	.46000	.43711	.950	-1.1223	2.0423
		Nbs-250	-1.44000	.43711	.091	-3.0223	.1423
		Nbs-500	-3.60000*	.43711	.000	-5.1823	-2.0177
		Nbs-125	ctrl	-5.14000*	.43711	.000	-6.7223
	Nbs-125	Healthy group	-4.68000*	.43711	.000	-6.2623	-3.0977
		Patient group	-.46000	.43711	.950	-2.0423	1.1223
		Nbs-250	-1.90000*	.43711	.012	-3.4823	-.3177
		Nbs-500	-4.06000*	.43711	.000	-5.6423	-2.4777
		Nbs-250	ctrl	-3.24000*	.43711	.000	-4.8223
	Nbs-250	Healthy group	-2.78000*	.43711	.000	-4.3623	-1.1977
		Patient group	1.44000	.43711	.091	-.1423	3.0223
		Nbs-125	1.90000*	.43711	.012	.3177	3.4823
		Nbs-500	-2.16000*	.43711	.003	-3.7423	-.5777
		Nbs-500	ctrl	-1.08000	.43711	.330	-2.6623
Nbs-500	Healthy group	-.62000	.43711	.842	-2.2023	.9623	
	Patient group	3.60000*	.43711	.000	2.0177	5.1823	
	Nbs-125	4.06000*	.43711	.000	2.4777	5.6423	
	Nbs-250	2.16000*	.43711	.003	.5777	3.7423	
	TSH	ctrl	Healthy group	-.26000	.25029	.952	-1.1660
Patient group			1.83400*	.25029	.000	.9280	2.7400
Nbs-125			1.75200*	.25029	.000	.8460	2.6580
Nbs-250			1.54000*	.25029	.000	.6340	2.4460
Nbs-500			-.20000	.25029	.985	-1.1060	.7060
Healthy group		ctrl	.26000	.25029	.952	-.6460	1.1660
		Patient group	2.09400*	.25029	.000	1.1880	3.0000
		Nbs-125	2.01200*	.25029	.000	1.1060	2.9180
		Nbs-250	1.80000*	.25029	.000	.8940	2.7060
		Nbs-500	.06000	.25029	1.000	-.8460	.9660
Patient group		ctrl	-1.83400*	.25029	.000	-2.7400	-.9280
		Healthy group	-2.09400*	.25029	.000	-3.0000	-1.1880
		Nbs-125	-.08200	.25029	1.000	-.9880	.8240
		Nbs-250	-.29400	.25029	.922	-1.2000	.6120
		Nbs-500	-2.03400*	.25029	.000	-2.9400	-1.1280
Nbs-125		ctrl	-1.75200*	.25029	.000	-2.6580	-.8460
		Healthy group	-2.01200*	.25029	.000	-2.9180	-1.1060
		Patient group	.08200	.25029	1.000	-.8240	.9880
		Nbs-250	-.21200	.25029	.980	-1.1180	.6940

	Nbs-250	Nbs-500	-1.95200*	.25029	.000	-2.8580	-1.0460
		ctrl	-1.54000*	.25029	.000	-2.4460	-.6340
		Healthy group	-1.80000*	.25029	.000	-2.7060	-.8940
		Patient group	.29400	.25029	.922	-.6120	1.2000
		Nbs-125	.21200	.25029	.980	-.6940	1.1180
	Nbs-500	-1.74000*	.25029	.000	-2.6460	-.8340	
	Nbs-500	ctrl	.20000	.25029	.985	-.7060	1.1060
		Healthy group	-.06000	.25029	1.000	-.9660	.8460
		Patient group	2.03400*	.25029	.000	1.1280	2.9400
		Nbs-125	1.95200*	.25029	.000	1.0460	2.8580
Nbs-250		1.74000*	.25029	.000	.8340	2.6460	

\*. The mean difference is significant at the 0.05 level.

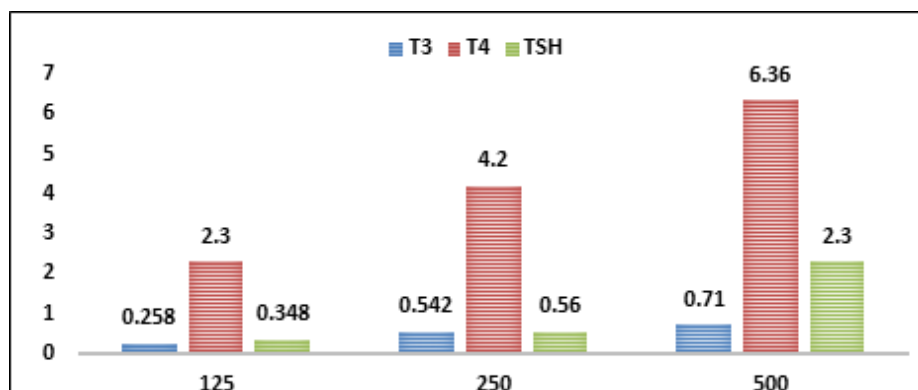
Considering the results of measuring the level of T3, T4, TSH hormones in the studied rats in both healthy controls and control group and the comparison of the mean results of these two groups, it can be stated that the use of lead acetate significantly reduces levels of T3, T4, and TSH hormones. These results suggest the induction of secondary hyperthyroidism. Therefore, it can be stated that the test method is used correctly and the method of work induces thyroid dysfunction (hyperthyroidism).

### Study of T3, T4, and TSH Hormones Levels in Rats Treated With Food Supplement

In the study of the interaction of food supplement (healthy and

alive nutrition) on thyroid parameters, it is concluded that the studied herbal medicine does not have a negative effect on thyroid function.

According to the results of this test, it can be concluded that food supplement (healthy and alive) causes normalization of levels of T3, T4, and TSH hormones. In the study of the interaction of the concentrations of dietary supplement, it was determined that the concentration of 500 mg/ kg has the most effective therapeutic effect and 125 mg/ kg has the least effect on the condition of hypothyroidism. The average results of this test are shown in Figure 3.



**Figure 3:** Comparison of the Therapeutic Effect of Dietary Powder (healthy and alive) on the Average of T3, T4, and TSH Hormones between the Three Groups Receiving Different Concentrations of Dietary Powder (Healthy and Alive)

### Discussion

Ferreira et al. Showed that the use of herbal flavonoids inhibited the peroxidation of the thyroid enzyme and the use of herbs containing flavonoids led to hypothyroidism [7]. Carolina et al. Also showed that flavonoids are in polyphenolic and secondary plant metabolites, inhibiting the activity of the thyroperoxidase enzyme and thus lowering the level of thyroid hormone that will further increase TSH [8]. The thyroxidase enzyme acts on the pathway of biosynthesis of thyroid hormones, the role of iodide oxidation ( $I^-$ ) in the presence of  $H_2O_2$ . This enzyme also plays a role in the final stages of the formation of thyroxine (T4) and iodo-thiourein (T3). Regarding the results of dietary intake of lead, it can be concluded that the diet has been subjected to secondary doses of hypothyroidism in doses because of decreasing

TSH as well as decreasing T3 and T4. TSH is a glycoprotein hormone that activates all stages of biosynthesis and secretion of thyroid hormones. In fact, TSH increases the activity of the iodine pump, increases the biosynthesis of iodo-oligosilum, increases the activity of the thyroxidase, and increases the biosynthesis of the thyroglobulin. Reducing T3 and T4 plasma concentrations secretes the thyrotropin-releasing hormone from the hypothalamus and subsequently secretes the TSH from the pituitary. In other words, after administration of a lead-fed diet, the hypothalamus-pituitary axis is also healthy and has a primary disorder in the thyroid and biosynthesis of thyroxine (T4) and iodo-urethane (T3) hormones and iodide oxidation ( $I^-$ ) [7, 8]. In another study aimed at investigating the effect of aqueous solution of 20% Aloe vera gel on thyroid hormones, it was found

that aqueous solution of 20% Aloe vera has a role in reducing thyroid activity [2]. It has also been shown that quercine reduces the expression of the receptor of thyrotropin, thyroid peroxidase, and thyroglobulin genes, and in vivo conditions impair thyroid function [9].

### Conclusion

The results of this study indicate that normal food intake (normal and healthy nutrition) normalizes normal levels of T3, T4, and TSH hormones. In addition, the interaction of the concentrations of dietary supplement indicated that the concentration of 500 mg/ kg had the most effective therapeutic effect and the concentration of 125 mg/ kg showed the least effect on the condition of hypothyroidism. The new food supplement (healthy and alive) has suitable therapeutic effect for treating abnormalities of thyroid parameters.

### Ethics approval and consent to participate

All procedures involving animals were approved by the Ethics Committee of the Tarbiat Modares University Medical School and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

### Funding

Not applicable.

### Authors' Contributions

Ak performed the statistical analysis and wrote the first draft of the manuscript. AB helped analyze and interpret the data and contributed in preparing the draft. Project development per-

formed by AK and AM. All authors read and approved the final manuscript.

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