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Efficacy of feeding of soy fortified *Shrikhand* as functional food on thyroid hormone (T_3, T_4) and thyroid stimulating hormone (TSH) of rats

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Abstract
The present investigation was based on an ideology "Let food be your medicine and medicine be your food" and the aim was to assess the effect of soy fortified *shrikhand* on serum levels of Thyroid Hormone (T_3, T_4) and Thyroid Stimulating Hormone (TSH) in rats. The research aimed at measuring the plasma concentrations of tri-iodothyronin (T_3), thyroxin (T_4) and TSH by feeding different types of feed (control *shrikhand*, soy fortified *shrikhand* and basal feed) to 100 albino test rats. Maize, pearl millet and pigeon pea seeds (1:1:1) were given as the basal feed (G1). The other treatment combinations were basal ration supplemented with 20% whole milk *shrikhand* (G2) and 10% (G3), 20% (G4) & 30% (G5) soy fortified *shrikhand* (SS) respectively. It was observed and concluded that T_3 levels were significantly ($P<0.05$) high in control - *Shrikhand* (CS) and soy fortified *Shrikhand* (SS) than basal feed group (G_1). The T_3 levels increased ($P<0.05$) in 30% soy *shrikhand* group as compared to its 10% level of inclusion in the ration. The thyroxin (T_4) levels increased ($P<0.05$) in all SS groups than rest of the groups. The serum thyroid stimulating hormone (TSH) levels were increased ($P<0.05$) in group G_1 than SS groups.

Keywords: tri-iodothyronin (T_3), thyroxin (T_4), thyroid stimulating hormone (TSH) and soy fortified *Shrikhand*

Introduction

"Let food be your medicine and medicine be your food" was a tenet espoused by Hippocrates in approximately 400 B.C. Almost 2,500 years later, this philosophy is once again gaining momentum and importance, as it is the "food as medicine" philosophy that is the core of functional foods. Functional food may be defined as "a food product that provides specific health benefits beyond the traditional nutrients it contains" or foods containing significant levels of biologically active components that impart health benefits beyond basic nutrition (Jooyandeh *et al;* 2011) [6]. Interest in functional foods has recently increased among consumers due to greater consciousness of health and nutrition as well as the need to cure diseases and also the increasing scientific evidence of their effectiveness (Opara *et al*., 2013) [15].

There is an increasing demand to developing alternative product(s) to cow's milk due to problems with allergenicity and desire for vegetarian alternatives (Park *et al*., 2012) [16]. Soyfoods have been consumed for more than 1000 years (Golbitz, 1995) [5]. Today, the most notable features of soybean is their health benefits linked to the treatment and prevention of many chronic diseases and have become the subject of intense scientific scrutiny because of their influence on many human physiological processes both at the cellular and systemic level. They are the food factors that influence the physiological state in animals as well as human beings.

Opara *et al.* (2013) [15] suggested that extending the shelf-life of foods is one of the major objectives of fermentation with aspects such as wholesomeness, acceptability and overall quality. Shrikhand is an indigenous, semi-soft, sweetish-sour, whole milk delicious and healthful dessert particularly in western part of India and prepared from lactic acid fermentation (Nigam *et al.;* 2009; Singh *et al;* 2014) [14, 22].

Thyroid disorders are amongst the most common endocrine dysfunctions and according to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases (Araham *et al.;* 2009; Unnikrishnan and Menon2011) [1, 25]. Similarly, high frequency of occurrence has been reported in other countries as well (Rajyam *et al*., 2017) [17]. Thyroid disease is a disorder that affects the thyroid gland. Sometimes the body produces too much or too little thyroid hormone.

Thyroid hormones regulate metabolism—the way the body uses energy—and affect nearly every organ in the body. Too much thyroid hormone is called hyperthyroidism and can cause many of the body's functions to speed up. Too little thyroid hormone is called hypothyroidism and can cause many of the body's functions to slow down. The thyroid gland controls the body metabolism, growth, neurodevelopment and maintenance of the internal environment. The thyroid gland works under the control of thyroid stimulating hormone (TSH) from pituitary gland which in turn is under the control of thyro-tropin hormone (TRH) from hypothalamus. It secretes 93% of thyroxin (T_4) and 7% tri-iodothyronin (T_3), T_3 is almost 10 times more active than T_4 and produced mainly by the conversion of T_4 in the peripheral tissues. These hormones increase transcription of several genes, known to affect catabolism. Thyroid hormone is responsible for initiating a chain of molecular events or gene expressions when the active form of the hormone interacts with specific cell receptors and subcellular components of various organs (Lynn and Lynn, 2007; Mansoor *et al*., 2011) [9, 11].

The present research was carried out to investigate the efficacy of feeding of soy fortified *shrikhand* on the thyroid hormone (T_3, T_4 and TSH) of rats. The research measured the plasma concentration of tri-iodothyronin (T_3), thyroxin (T_4) and thyroid stimulating hormone (TSH) by feeding of different types of feed (control *shrikhand*, soy fortified *shrikhand* and basal feed) to the rats.

Materials and Methods

The standard yoghurt culture (*Lactobacillus delbrueckii ssp. bulgaricus* NCDC 009 and *Streptococcus thermophilus* NCDC 074) were obtained separately from National Collection of Dairy Culture (NCDC), Dairy Microbiology Division at NDRI Karnal, Haryana, India. The rest of the materials including cow milk were procured from the Department of Animal Husbandry & Dairying, Banaras Hindu University, Varanasi, India.

Preparation of soymilk and soy fortified shrikhand

Soymilk and soy fortified *shrikhand* was prepared according to the methods given by Singh *et al*. (2014) [22].

Selection of Experimental Animals

One hundred albino rats were obtained from Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, with informed consent from authorities and the study protocol was approved by the institute's committee on human research. The animal experiments conformed to institutional standards. The rats were kept in sterile cages and were provides with sufficient food and water. Out of 100 rats, 30 rats of similar sex, body weight and body conformation were selected at 25±1 days of age for the study. The rats having an average body weight of 32.56±0.30 gm were randomly divided into 5 groups of 6 each.

Preparation of Feed Mixtures

Maize, pearl millet and pigeon pea seeds were ground in Willy type grinder separately and mixed in the ratio of 1:1:1. In the feed 1 per cent mineral mixture (M/s Virbac Animal Health India Pvt. Ltd, Mumbai, India) was added to make basal feed (G1) and collected in a tin container. The treatment combinations were basal ration (G1), basal ration supplemented with 20% whole milk *shrikhand* (G2) and 10% (G3), 20% (G4) & 30% (G5) soy fortified *shrikhand* (SS). The basal ration contains maize, pearl millet and pigeon pea (1:1:1) along with 1 per cent mineral mixture of the total feed.

Analysis of Thyroid Hormone

The analysis of thyroid hormone (T_3, T_4 and TSH) was done as per the methods given by Mansoor *et al*. 2011 [11] using ELISA.

Statistical Analysis

Data were analysed using SAS software package (SAS, 2006) [19] Duncan's multiple range test (Montgomery, 1997) [13] was used to detect differences between treatment means.

Results and Discussion

Thyroid Hormones (TH) are essential for regulation of biological processes such as growth, metabolism, neurodevelopment and protein synthesis (Kunisue *et al*., 2011) [8]. Blood TH levels are considered to be good indicators of the nutritional status of an animal (Riis and Madsen, 1985; Todini, 2007) [18, 24]. In the present investigation, the average T_3 levels in the blood samples (Table 1) were 0.43±0.033, 0.53±0.033, 0.63±0.033, 0.73±0.033, 0.77±0.033 ng/ml in groups G_1, G_2, G_3, G_4 and G_5, respectively. The T_3 levels were significantly ($P<0.05$) high in all the *shrikhand* groups (control and soy fortified *shrikhand*) than basal fed group (Fig. 1). Irrespective of the groups, the T_3 levels in the blood increases as the levels of *shrikhand* were increased in the feed. The levels of T_3 increased ($P<0.05$) in soy fortified *shrikhand* groups as compared to control *shrikhand* group. The increase in the value was significantly ($P<0.05$) high when 30% soy *shrikhand* was added in the ration than 10% level.

The mean serum thyroxin (T_4) levels in the blood (Table 1; Fig. 2) were the highest in group G_5 (5.17±0.033 µg/dl) followed by groups G_4 (5.07±0.088 µg/dl), G_3 (4.97±0.067 µg/dl), G_2 (4.57±0.088 µg/dl) and G_1 (4.33±0.088 µg/dl). When the T_4 levels were compared from basal feed to soy fortified *shrikhand* groups and control *shrikhand* to soy fortified *shrikhand*, the values significantly increased ($P<0.05$) in soy fortified *shrikhand* groups. The differences in the values among the groups G_3 to G_4 and G_4 to G_5 were not significant ($P>0.05$). T_3 is almost 10 times more active than T_4 and produced mainly by the conversion of T_4 in the peripheral tissues (Mansoor *et al*., 2011)[11].

In the present investigation (Table 1; Fig. 3), the mean serum TSH levels were the highest in group G_1 (0.027±0.0033 µg/dl) followed by G_2 (0.017±0.0033 µg/dl), G_3 (0.013±0.0033 µg/dl), G_4 (0.013±0.0033 µg/dl) and G_5 (0.010±0 µg/dl). When the difference in serum TSH levels were compared between basal feed and soy fortified *shrikhand* groups, the TSH levels significantly decreased ($P<0.05$) in soy fortified *shrikhand* groups. The difference in the values from basal feed to control *shrikhand* and control *shrikhand* to soy *shrikhand* were not significant ($P>0.05$). Similar with the present findings, researches have reported that soy isoflavones may caused the increase in serum thyroxine (T_4) and triiodothyronine (T_3) levels in rodents (Balmier *et al;* 1996; Madej *et al.;* 2002; Mittal *et al;,* 2011) [2, 10, 12]. Siglin *et al*. (2000) [21] and Kunisue *et al.* (2011) [8] reported that the serum TSH levels were decreased due to increase in T_4 and T_3 levels when low cholesterol feed given to the rats. Subclinical hypothyroidism is mainly based on increased TSH level between 4.5 to 10 mU/L with normal T_3 and T_4 (Mittal *et al.;* 2011) [12]. In the presence of isoflavones, the levels of T_3 and T_4 increased (www.soynutrition.com). Soy contains higher isoflavones (Booth *et al.*; 1999; Setchell *et al.;* 2003; Belen *et al.;* 2013; Kuan-I *et al.;* 2013; Siok-Koon and Min-Tze, 2013) [3, 20, 4, 7, 23] by which in present study the levels of T_3 and T_4 increased in soy fortified groups.

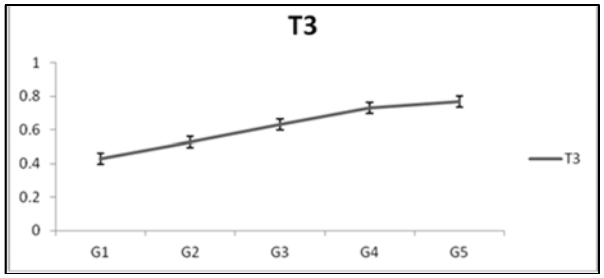

Fig 1: Impact of different feeding groups on the blood T_3 of albino rats

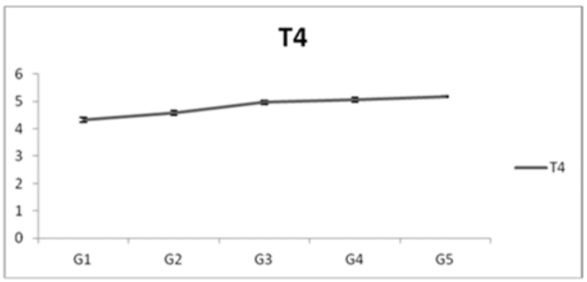

Fig 2: Impact of different feeding groups on the blood T_4 of albino rats

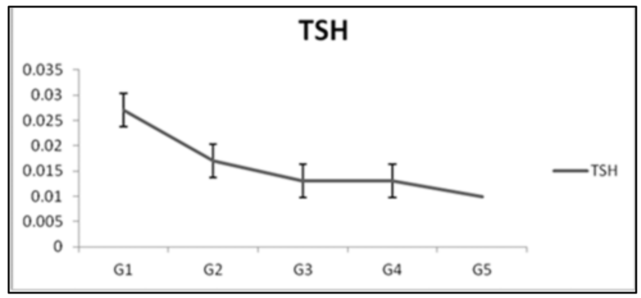

Fig 3: Impact of different feeding groups on the blood TSH of albino rats

Table 1: Impact of different feeding groups on the hormonal characteristics of the albino rats

Groups	T_3 (ng/ml)	T_4 (µg/dl)	TSH (µ IU/ml)
G_1	0.43±0.033[a]	4.33±0.088[a]	0.027 ±0.0033[a]
G_2	0.53±0.033[b]	4.57±0.088[b]	0.017±0.0033[ab]
G_3	0.63±0.033[c]	4.97±0.067[c]	0.013± 0.0033[b]
G_4	0.73±0.033[d]	5.07±0.088[cd]	0.013± 0.0033[b]
G_5	0.77±0.033[d]	5.17±0.033[d]	0.010± 0[b]

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, $P<0.05$)

Feed combination in different groups

Group	Constitution
G1(Basal feed)	Maize: pearl millet: pigeon pea(1:1:1) + 1% mineral mixture
G2	Basal feed + 20% CS
G3	Basal feed + 10% SS
G4	Basal feed + 20% SS
G5	Basal feed + 30% SS

Conclusion

The tri-iodothyronin (T_3) levels were significantly ($P<0.05$) high in groups CS and SS than group G_1.The T_3 levels significantly increased ($P<0.05$) in SS groups as compared to group CS. The T_3levels increased ($P<0.05$) in 30 % soy *shrikhand* group as compared to its 10% level of inclusion in the ration. The T_4 levels increased ($P<0.05$) in all SS groups than rest of the groups. The T_4 value ranged from 4.33±0.088 to 5.17±0.033 µg/dl during experimentation. The serum TSH levels were increased ($P<0.05$) in group G_1 than SS groups.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Araham R, Murugan VS, Pukazhvanthen P and Sen SK, Thyroid disorders in women of Puducherry. Indian J Clinical Biochem. 2009; 24(1): 52- 59.
2. Balmir F, Staack R, Jeffrey E, Jimenez MDB, Wang L and Potter SM. An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters, J Nutri. 1996; 126: 3046 - 3053.
3. Booth C, Hargreaves DF, Hadfield JA, McGown AT, Potten, CS, Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis *in vitro*, British J Cancer. 1999; 80: 1550.
4. Belén F, Benedetti S, Sánchez J, Hernández E, Auleda JM, Prudêncio ES, Petrus JCC and Raventós M, Behavior of functional compounds during freeze concentration of tofu whey, J Food Eng. 2013; 116: 681-688.
5. Golbitz P, Traditional soy foods: processing and products, Soyatech, Inc., Bar Harbor, ME, USA, J Nutri. 1995; 125(3S): 570S-576S.
6. Jooyandeh H, Soy Products as Healthy and Functional Foods, Middle-East J Sci Res. 2011; 7(1):71-80.
7. Kuan-I C, Yi-Chen L, Chia-Wei L, Roch-Chui Y, Cheng-Chun C and Kuan-Chen C, Enrichment of two

isoflavoneaglycones in black soymilk by using spent coffee grounds as an immobiliser forb-glucosidase, Food Chemi. 2013; 139: 79-85.
8. Kunisue T, Kannan KC, Fisher JW and Tanabea S, Method for Analysis of Thyroid Hormones in Perchlorate administered Rats by Liquid Chromatography-Tandem Mass Spectrometry. *In:* Potential Application to Samples Stored in es-Bank of Ehime University, Interdisciplinary Studies on Environmental Chemistry-Environmental Specimen Bank, Eds., T. Isobe, K. Nomiyama, A. Subramanian and S. Tanabe. 2011, 43-50.
9. Lynn WR, Lynn JA, Hypothyroidism is easily overlooked. Practioner. 2007; 22(4):224-231.
10. Madej A, Persson E, Lundh T and Ridderstrale Y: Thyroid gland function in ovariectomized ewes exposed to phytoestrogens, J Chromato B Analytical Technol Biomed Life Sci. 2002; 777: 281-287.
11. Mansoor R, Rizvi SSR, Kausar W, Aslam F and Huda ST, Comparison of TSH, T_4 and T_3 Levels in Primary Hypothyroidism in relation to Gender and age in a Tertiary Care Hospital, Annals Pak Insti Medical Sci. 2002; 7(4):186-190.
12. Mittal N, Hota D, Dutta P, Bhansali A, Suri V, Aggarwal N, Marwah RK and Chakrabarti A, Evaluation of effect of isoflavone on thyroid economy & autoimmunity in oophorectomised women: A randomised, double-blind, placebo-controlled trial. Indian J Medical Res, 2011; 133(6):633-640.
13. Montgomery DC (1997): Design and Analysis of Experiments, 4th edition, John Wiley & Sons, New York.
14. Nigam N, Singh R. and Upadhayay PK, Incorporation of Chakka by Papaya Pulp in the Manufacture of Shrikhand. J Dairying Foods Home Sci. 2009; 28(2):115-118.
15. Opara CC, Ahiazunwo NJ and Okorie O, Production of soy-yoghurt by fermentation of soymilk with *Lactobacillus* isolated from Nunu, International J Sci Eng Investigations. 2009; 2(12):01-05.
16. Park SY, Lee DK, An HM, Kim JR, Kim MJ, Cha MK, Lee SW, Kim SO, Choi KS, Lee KO and Ha NJ, Producing functional soy-based yogurt incubated with *Bifidobacterium longum* spm1205 isolated from healthy adult Koreans, Biotechnol Biotechnolo Equip. 2012; 26(1):2759-2764.
17. Rajyam, PL, Ramulu R. and Shyamala R, The incidence of hypothyroidism in a tertiary care hospital. J Microbiol Biotechnol Res. 2017; 4(1):34-36.
18. Riis PM and Madsen A, Thyroxine concentration and secretion rates in relation to pregnancy, lactation and energy balance in goats, J Endocrinol. 1985; 107:421-427.
19. SAS. SAS/STAT Software: Changes and enhancements through release, SAS Institute Inc., Cary, NC, 2006.
20. Setchell KDR, Brown NM, Desai PB, Zimmer-Nechemias L, Wolfe BE, Jakate AS, Creutzinger V and Heubi JE, Bioavailability, disposition and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. J Nutri. 2003; 133:1207-1211.
21. Siglin JC, Mattie DR, Dodd DE, Hildebrandt PK and Baker WH, A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate. Toxicolo Sci. 2000; 57:61-74.
22. Singh D, Singh J, Kumar S. and Verma, T, Microbiological Evaluation of Soy Fortified Shrikhand by Using Response Surface Methodology. International J Applied Biol Pharmaceutical Technol. 2014; 5(1):1-7.
23. Siok-Koon Y and Min-Tze L, Effect of ultrasound on bioconversion of isoflavones and probiotic properties of parent organisms and subsequent passages of *Lactobacillus*, LWT - Food Sci Technol. 2013; 51:289-295.
24. Todini L, Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors, Animal. 2007; 1(7):997-1008.
25. Unnikrishnan AG and Menon UV, Thyroid disorders in India: An epidemiological perspective. Indian J Endocrinol and Metab. 2011; 15(2):S78.