Efficacy of feeding of soy fortified *Shrikhand* as functional food on thyroid hormone (T₃, T₄) 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 (T₃, T₄) and Thyroid Stimulating Hormone (TSH) in rats. The research aimed at measuring the plasma concentrations of tri-iodothyronin (T₃), thyroxin (T₄) 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 T₃ levels were significantly (P<0.05) high in control - *Shrikhand* (CS) and soy fortified *Shrikhand* (SS) than basal feed group (G1). The T₃ levels increased (P<0.05) in 30% soy *shrikhand* group as compared to its 10% level of inclusion in the ration. The thyroxin (T₄) 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 G1 than SS groups.

Keywords: tri-iodothyronin (T₃), thyroxin (T₄), 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 are 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 (T4) and 7% triiodothyronine (T3). T3 is almost 10 times more active than T4 and produced mainly by the conversion of T4 in the peripheral tissues. These hormones increase transcription of several genes, known to affect catabalism. 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 soy fortified shrikhand on the thyroid hormone (T3, T4 and TSH) of rats. The research measured the plasma concentration of tri-iodothyronin (T3), thyroxin (T4) 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

Soy milk 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 provided 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 32.5±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 (T3, T4 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 T3 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 G1, G2, G3, G4 and G5, respectively. The T3 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 T3 levels in the blood increases as the levels of shrikhand were increased in the feed. The levels of T3 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 (T4) levels in the blood (Table 1; Fig. 1) were the highest in group G5 (5.17±0.033 µg/dl) followed by groups G4 (5.07±0.088 µg/dl), G3 (4.97±0.067 µg/dl), G2 (4.57±0.088 µg/dl) and G1 (4.33±0.088 µg/dl). When the T4 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 G1 to G4 and G4 to G5 were not significant (P>0.05). T3 is almost 10 times more active than T4 and produced mainly by the conversion of T4 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 G1 (0.027±0.0033 µg/dl) followed by groups G2 (0.017±0.0033 µg/dl), G3 (0.013±0.0033 µg/dl), G4 (0.013±0.0033 µg/dl) and G5 (0.010±0.0 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, researchers have reported that soy isoflavones may caused the increase in serum thyroxine (T4) and triiodothyronine (T3) 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 T4 and T3 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 T3 and T4 (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) 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](image1)

![Fig 2: Impact of different feeding groups on the blood $T_4$ of albino rats](image2)

![Fig 3: Impact of different feeding groups on the blood TSH of albino rats](image3)

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>$T_3$ (ng/ml)</th>
<th>$T_4$ (µg/dl)</th>
<th>TSH (µ IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.43±0.033a</td>
<td>4.33±0.088b</td>
<td>0.027±0.0033c</td>
</tr>
<tr>
<td>G2</td>
<td>0.53±0.033b</td>
<td>4.57±0.088c</td>
<td>0.017±0.0033d</td>
</tr>
<tr>
<td>G3</td>
<td>0.63±0.033c</td>
<td>4.97±0.067d</td>
<td>0.013±0.0033e</td>
</tr>
<tr>
<td>G4</td>
<td>0.73±0.033d</td>
<td>5.07±0.088ed</td>
<td>0.013±0.0033f</td>
</tr>
<tr>
<td>G5</td>
<td>0.77±0.033e</td>
<td>5.17±0.033f</td>
<td>0.010±0g</td>
</tr>
</tbody>
</table>

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

### Feed combination in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Constitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Basal feed + 1% mineral mixture</td>
</tr>
<tr>
<td>G2</td>
<td>Basal feed + 20% CS</td>
</tr>
<tr>
<td>G3</td>
<td>Basal feed + 10% SS</td>
</tr>
<tr>
<td>G4</td>
<td>Basal feed + 20% SS</td>
</tr>
<tr>
<td>G5</td>
<td>Basal feed + 30% SS</td>
</tr>
</tbody>
</table>

### Conclusion

The tri-iodothyronin ($T_3$) levels were significantly (P<0.05) high in groups CS and SS than group G1. The $T_3$ levels significantly increased (P<0.05) in SS groups as compared to group CS. 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 $T_4$ 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 G3 than SS groups.

### Conflict of interest

The authors declare no conflicts of interest.

### References


11. Mansoor R, Rizvi SSR, Kausar W, Aslam F and Huda ST, Comparison of TSH, T4 and T3 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.


