# Effect of Soybean on Bone Health of Male and Female Albino Rats

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**Abstract:** Litanies of health benefits are frequently attributed to soy but it's also considered as endocrine disruptors, indicating that it has the potential to cause adverse health effects as well. Consequently the present study investigated whether consumption of soybean has health benefits or adverse effects on bone health. Each sex was randomly divided into 4 groups, control group fed on the basal diet (AIN93 G),and three treated groups given 30, 60 and 90 g cooked soybeans/70 kg human body weight (b.w.) for three months . Female and Male rats showed that soybean increased serum parathyroid hormone (PTH) and decreased calcium (Ca) level in bone and serum. In the present study found that soybean have adverse effects on bone of male and female

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**Keywords:** Soybean, bone PTH, Ca, rats.

## 1. Introduction:

Considerable interest has been focused on the role of soy protein and is flavones on osteoporosis, the most common chronic bone disease and the major public health problem worldwide which is characterized by deterioration of skeletal architecture, resulting in increased bone fragility and risk of fractures. Although osteoporotic bone loss can be caused by a variety of factors, such as nutrient deficiency, chronic alcohol intake and sex steroid deficiency

Since osteoporosis is difficult to treat, early prevention of this disease is becoming of great interest. In particular, building adequate bone during period of rapid growth in early development is critical to increase peak bone mass and reduce the effects of bone loss later in life (Chen *et al.,* 2008).

Estrogen plays a critical role in the premenopausal acquisition and maintenance of peak bone mass (Dhuper *et al.,* 1990 and Sowers *et al.,* 1998). This observation in turn has led to speculation that the estrogen-like compounds (isoflavones) contained in soy reduced risk of fracture (Valachovicova *et al.,* 2004); increased bone mineral content (BMC) and bone mineral density (BMD) (Chen *et al.,* 2008).

Whereas others have observed no effects of soy on BMC and BMD of human (Anderson *et al.,* 2002 and Balk *et al.,* 2005); in mice (Erlandsson *et al.,* 2005) and in monkeys (Lee *et al.,* 2007)

In contrast Kerstetter *et al.* (2006) found that soy consumption elevated PTH which accelerates skeletal resorption and decreased bone calcium content.

## 2. Material And Methods

### Experimental Animals:-

Male and female Wister albino rats with average body weight 120 g obtained from the private market Abou-Rawash, Giza, Egypt, were used in the present study. They were kept on vegetables and water *adlibitum* for one week prior to the experiment to remove any traces of previous soybean. Following this brief adjustment period, each sex was divided into four groups (n = 12 per group).

Control group(Group I): Rats were kept on the basal diet (AIN 93 G) according to (Reeves, 1997) and water *adlibitum* for three months.

Three treated groups: Eashgroup was fed individually on the following quantities of cooked soybean 30, 60 & 90 g /70 kg human b.w. for groups II, III, and IV, respectively , daily for three months. Doses used in our study are according to Messina, 1999 and Chang *et al.,* 2008.

All three treated groups were then given the basal diet (AIN 93G) and water *adlibitum* through out the experimental period.

### Soybean Diet:

Commercial soybean seeds sample (Giza 22) obtained from the Agriculture Research Center; Giza, Egypt was used in the present study because it's the common soybean used in the manufacture of most soy foods present in local markets. Also it's used as a dietary source of proteins for poultry and livestock.

It contains 40 % protein, 20 % fat, 5 % ash and 35 % carbohydrates (soluble sugars and insoluble sugars) (Food Technology Research Institute Agriculture Research Center, Giza, 2008).

Oligosaccharides are soluble sugars but are not broken down by the enzymes of the digestive tract and are fermented by the micro-organisms present in the intestine, with the formation of the intestinal gas flatulence. That's why raw soybean was soaked for 12 hours at room temperature to get rid of these oligosaccharides. Also soybean was cooked at 120°C for 18 minutes in attempt to decrease the amount of the anti-nutrients present such as trypsin inhibitors, phytin, lectins, saponins, and hemaggluttinins (Sat and Keles, 2002)

**1- Analysis of hormones:**

Determination of PTH in serum was measured by enzyme-linked immunosorbent assay (ELISA) according to the method of Kao *et al.* (1992) , Ca in serum was measured by Synchron CX4 according to the method of (Prince *et al.*, 2003) were Ca in bone are extracted from tibia bone according to method of Lotinun *et al.*, 2003; Puntheeranurak *et al.*, 2006 and Charoenphandhu *et al.*, 2007, then measured by Synchron CX4 according to the method of Prince *et al.*(2003).

## 3. Results

Table (I) shows that female rats fed only on 60 and 90 g soybean / 70 kg human b.w. showed significant reduction in bone Ca++ and serum Ca++ with -23.13% and 30.00% for bone Ca++ and -14.01% and -20.88% for serum Ca++, respectively. This accompanied with significant increase in serum level of PTH with 19.75% and 35.93%, respectively.

**Table (I): Effect of soybean on serum PTH, $Ca^{++}$, and $Ca^{++}$ content in right tibia of female rats treated for three months with three different doses.**

| Parameters Groups | | PTH pg/ml) | Serum $Ca^{++}$ mg/dl) | ($Ca^{++}$) content intibiabone(mg/g) |
|---|---|---|---|---|
| Control (Group I) | Range | 5.67 — 7.15 | 11.25 — 12.11 | 19.18 — 22.00 |
| | Mean ± S.E | 6.43 ± 0.14 | 11.78 ± 0.9 | 20.40 ± 0.22 |
| Group II (30 g / 70 kg b.w.) | Range | 6.00 — 6.94 | 9.00 — 12.00 | 15.30 — 20.00 |
| | Mean ± S.E | 6.35 ± 0.11 | 11.35 ± 0.26 | 19.61 ± 0.85 |
| | % of change | -1.24 | -3.65 | -3.92 |
| | P value | N.S. | N.S. | N.S. |
| Group III (60 g / 70 kg b.w.) | Range | 6.92 — 8.84 | 9.74 — 10.54 | 14.20 — 18.00 |
| | Mean ± S.E | 7.70 ± 0.15 | 10.13 ± 0.06 | 15.68 ± 0.31 |
| | % of change | 19.75 | -14.01 | -23.13 |
| | P value | P < 0.001 | P < 0.001 | P < 0.001 |
| Group IV (90 g / 70 kg b.w.) | Range | 8.42 — 9.00 | 8.12 — 11.00 | 13.48 — 15.22 |
| | Mean ± S.E | 8.74 ± 0.07 | 9.32 ± 0.24 | 14.28 ± 0.17 |
| | % of change | 35.93 | -20.88 | -30.00 |
| | P value | P < 0.001 | P < 0.001 | P < 0.001 |
| | ANOVA | F = 82.20 P < 0.001 | F = 38.15 P < 0.001 | F = 139.45 P < 0.001 |

P= probability N.S. = non significant S.E= standard error

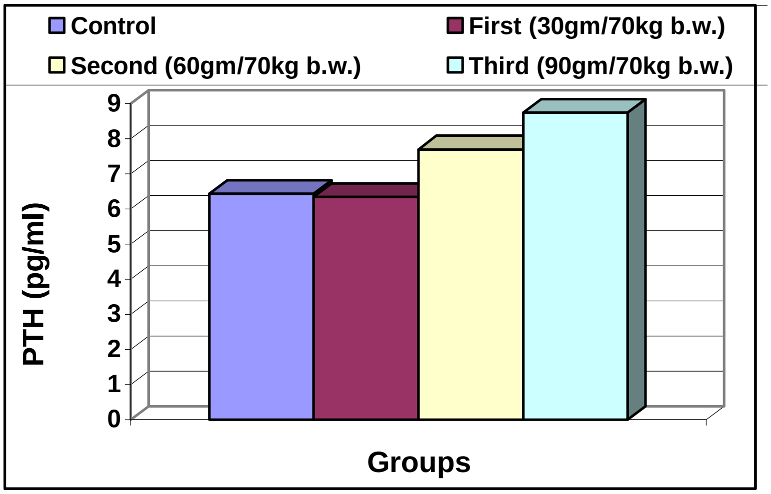


**Fig (1a): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum PTH level of female rats**

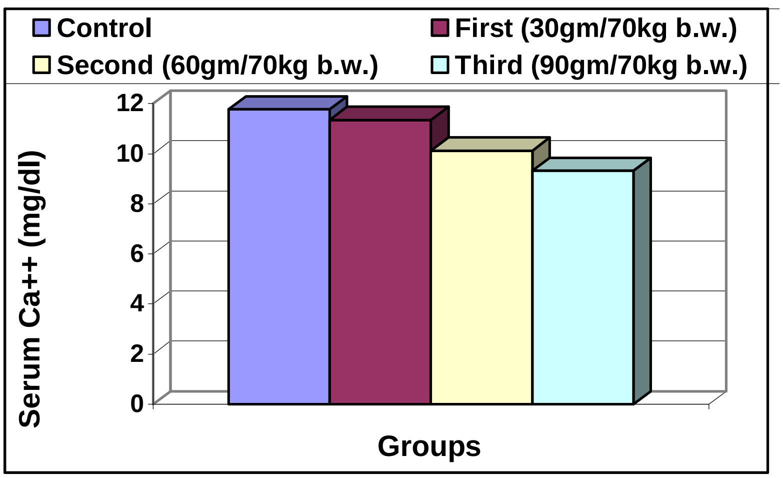


**Fig (1b): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum $Ca^{++}$ level of female rats**

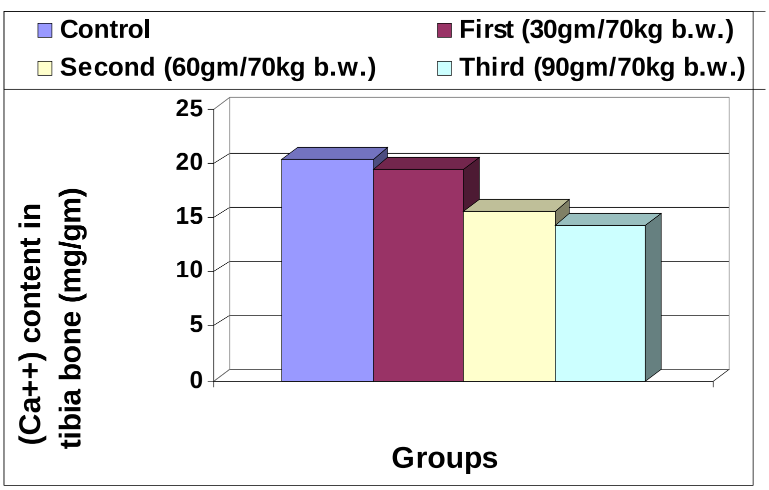


**Fig (1c): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on $Ca^{++}$ content in tibia of female rats**

Table II shows that bone Ca++ content and serum Ca++ level decreased significantly in male rats fed only on 60 and 90 g soybean/70 kg human b.w. with (-15.80% and -17.82%) for bone Ca++ content and (-8.22% and -19.00%) for serum Ca++ level respectively. This was associated with significant increased in serum level PTH of rats fed only on 90 g soybean/70 kg human b.w. with (23.23%).

**Table (II): Effect of soybean on serum PTH, $Ca^{++}$, and $Ca^{++}$ content in right tibia of male rats treated for three months with three different doses.**

| Parameters / Groups | | PTH pg/ml)( | Serum $Ca^{++}$ mg/dl)( | ($Ca^{++}$) content in tibia bone mg/gm)( |
|---|---|---|---|---|
| Group I (Control) | Range | 5.43 — 6.80 | 12.00 — 12.31 | 19.95 — 22.75 |
| | Mean ± S.E | 6.20 ± 0.16 | 12.05 ± 0.10 | 20.82 ± 0.28 |
| Group II (30 g / 70 kg b.w.) | Range | 5.10 — 6.85 | 10.50 — 12.44 | 17.00 — 22.00 |
| | Mean ± S.E | 6.04 ± 0.17 | 12.20 ± 0.49 | 20.01 ± 0.36 |
| | % of change | -2.58 | 1.24 | -3.89 |
| | P value | N.S. | N.S. | N.S. |
| Group III (60 g / 70 kg b.w.) | Range | 6.00 — 6.90 | 9.50 — 12.44 | 14.33 — 19.60 |
| | Mean ± S.E | 6.46 ± 0.10 | 11.06 ± 0.89 | 17.53 ± 0.50 |
| | % of change | 4.19 | -8.22 | -15.80 |
| | P value | N.S. | P < 0.01 | P < 0.001 |
| Group IV (90 g / 70 kg b.w.) | Range | 7 — 8.45 | 9.00 — 10.12 | 14.02 — 20.90 |
| | Mean ± S.E | 7.64 ± 0.18 | 9.76 ± 0.37 | 17.11 ± 0.78 |
| | % of change | 23.23 | -19.00 | -17.82 |
| | P value | P < 0.001 | P < 0.001 | P < 0.001 |
| | ANOVA | F = 20.05<br>P < 0.001 | F = 36.98<br>P < 0.001 | F = 13.62<br>P < 0.001 |

P= probability N.S.= non significant S.E= standard error

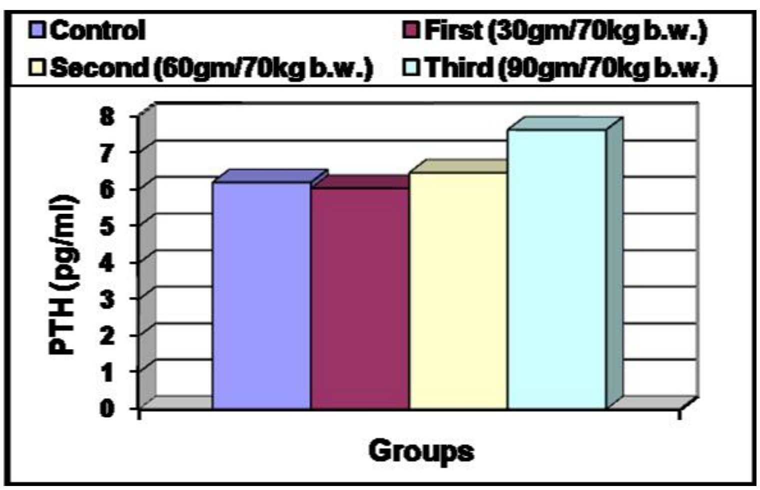


**Fig (2a): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum PTH level of male rats**

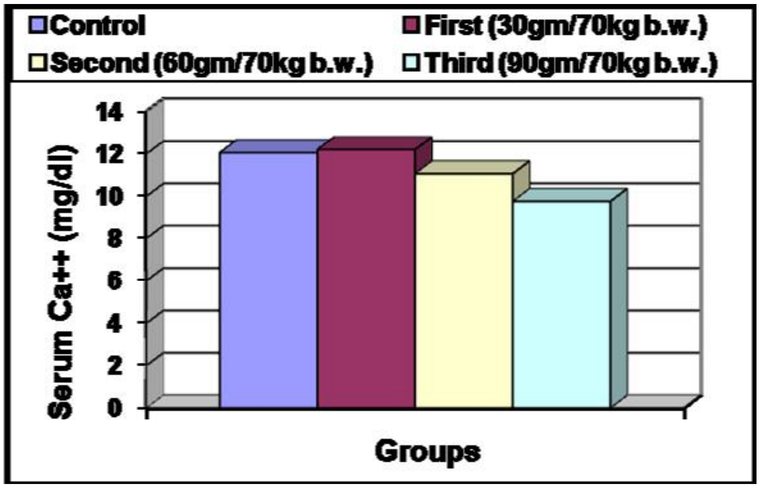


**Fig (2b): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on serum Ca++ level of male rats**

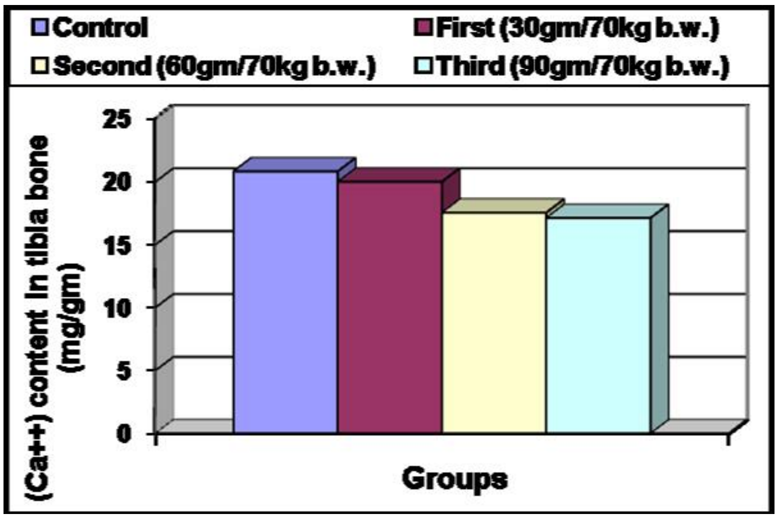


**Fig (2c): Effect of 30, 60 and 90 gm soybean/70 kg b.w. on Ca++ content in tibia bone of male rats**

**4. Discussion**

The present data indicated that female and male rats fed on only 60 and 90 g soybean/ 70 kg human b.w. showed significant reduction in Ca++ in bone and serum , this was accompanied with a significant increase in serum level of PTH.

The present study runs in full agreement with Kerstetter *et al.* (2005) who found that soy consumption elevated PTH which accelerates skeletal resorption and decreased bone calcium content.

The decrease in bone calcium during consumption of soy-based diet may be due to impaired intestinal calcium absorption, the high phytic acid content of soy foods may play a role. Phytic acid, inositol hexaphosphate, is a phosphorus-rich compound that occurs naturally at very high levels in soy foods. Phytic acid strongly chelates multivalent metal ions, particularly zinc, calcium, and iron, resulting in the formation of insoluble salts that are poorly absorbed in the gastrointestinal tract (Zhou and Erdman, 1995). Several investigations showed that phytic acid interferes with calcium absorption (Greer and Krebs, 2006).

Another interpretation of decreased calcium content in bone may be due to both anti-estrogenic and anti-androgenic actions of isoflavones. It is well known that E2 is not only essential for bone growth, but also for maintenance of skeletal integrity as shown by skeletal changes following sex steroid deficiency in rodents study (Vandenput *et al.*, 2004).

In addition, the regulation of male skeletal growth and maturation appears to be a tale of two sex hormone (androgen and estrogen) signaling pathways. Animal studies as well as relevant clinical observations provide evidence that estrogen and ERα activation stimulate longitudinal growth and induce epiphyseal growth plate closure at the end of puberty, whereas androgens appear to have no effect on longitudinal growth via the androgens receptor (AR). In contrast, androgens and AR activation play a dominant role in the acquisition of male trabecular bone moreover both androgen and E2 are essential in the maintenance of skeletal integrity in males (Callewaert *et al.,* 2009).

Adverse effects of soy on bone health may be interpreted due to its high protein content. The previous studies observed that high-protein diets induce a significant decline in intestinal calcium absorption with an accompanying fall in urinary calcium reabsorption and a rise in PTH (Kerstetter *et al.,* 2005).

Park *et al.* (2008) cited that estrogen receptors modulate tubular calcium reabsorption in the kidneys and urinary calcium excretion significantly increases with the decrease in estrogen, indicating that renal tubular reabsorption of calcium decreases in estrogen deficiency state. So it's logic to hypothesis that the anti-estrogenic and/ or weak estrogenic action of isoflavones may be a reason for decreased calcium level in bone and serum.

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