Biological Response of Rats Fed with Tofu Treated with High Hydrostatic Pressure

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Emerging technologies for food preservation have arisen in recent years, such as high-pressure (HP) hydrostatic treatment, and the biological response for this kind of food preservation is not well-known. Forty female rats (six weeks old) were used in the experiment to evaluate the biological effects of HP treatment of tofu. The animals were divided into groups that were fed with tofu (untreated), tofu treated with HP, and conventional food (as control) for 28 days. The glucose level, mineral content (calcium, potassium, zinc, and magnesium), shinbone maximum shear force, weight of the body, and weight of organs (heart, liver, spleen, and kidneys) were analyzed. The biological response for the rats was that significant differences were found in the calcium amount determined on the serum of the rats fed with untreated tofu and those fed with tofu treated with HP, and the calcium amount was lower on the rats fed with tofu treated with HP. Also, there were significant differences in the weight of the liver, and it was lower in the rats fed with tofu treated with HP. It was quite remarkable how the weight of the body and organs were smaller in the rats fed with tofu in comparison to the weight of the control rats. In the other components assayed no significant differences were found. HP produces a potential effect on tofu as it is observed in the rats response to the tofu treated with HP.

Keywords: *High pressure; tofu; weight; glucose; metabolites; heart; spleen; liver; kidneys*

INTRODUCTION

Soy food is widely recognized for its nutritional qualities, and it is being increasingly consumed in western countries because it is a good source of vegetable proteins, contains all essential amino acids required for human nutrition (Steinke, 1992), and has low fat content. Recently, this product has aroused greater interest because scientists have discovered that soy protein and its isoflavones are very beneficial to health, in ways such as lowering cholesterol levels (Sirtori et al. 1998, 1999; Anthony et al., 1996; Anderson, 1995b; Negata, 1998), treating heart disease (Tovar-Palacios et al., 1998; Honoré et al., 1997), reducing the risk of cancer (Fleming et al., 1985; Peterson and Barney, 1991; Lu et al., 1996; Yan et al., 1997; Conmally et al., 1997; Menon et al., 1998; Anderson, 1999), and causing calcium to be better utilized, thus warding off osteoporosis (Arjmandi et al., 1996; Anderson, 1995a).

The epidemiological studies involving the high soybean consuming Asian population have been based on individuals with a lifetime daily intake pattern of soybean products (Adlercreutz et al., 1993; Messina, 1995). The available data consist of a mixture of epidemiological studies examining the relationship between consumption of natural foods containing phyto-oestrogens and disease prevalence (Anderson, 1999). Thus, food processors and manufacturers of dietary supplements have become aware of the interest in the potential benefits of phyto-oestrogen consumption.

The incorporation of soybean food (such as tofu), with its phyto-oestrogens, into a western diet could be an important means for preventing and treating chronic diseases. The use of soybeans and soybean products is more attractive to people than use of drug formulations. Commonly used food preparation methods (e.g, baking, frying) do not appear to effect isoflavones (Anderson, 1999).

The advantages of preserving tofu with high pressure (HP) are decreasing the microbial population, reduction of secondary contamination that occurs during processing, and retention of good taste (Préstamo et al., 2000). However, it is still not yet known whether this high-pressure hydrostatic treatment has an adverse effect upon the tofu components.

This experiment was carried out to discover the effect on laboratory animals fed with tofu treated with HP. One group of rats was fed with tofu treated with HP, another group was fed with tofu with no treatment, and the last group, used as a control, was fed with conventional food.

MATERIALS AND METHODS

Plant Material. For this experiment we used tofu purchased from a local vegetarian shop. The samples were already preserved in plastic bags, subjected to vacuum, and stored (refrigerated).

High-Pressure Treatment. The tofu samples to be treated with HP were immersed in a steel container (100-mm diam, 300-mm height, 2.35 L vol), filled with a low-compressibility fluid (water). The water acted as the pressurizing medium. A thermocouple submerged in the pressure fluid measured the temperature during treatment. The apparatus (ACB GEC Alsthom, Nantes, France) was capable of achieving a maximum pressure of 500 MPa. The temperature was held constant using a water bath. Temperature and pressure were recorded in a Lab Tech notebook program (Laboratory Technologies Corporation, Wilmington, MA). The HP conditions used in this

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10.1021/jf0004659 CCC: $19.00
Published on Web 09/20/2000

Table 1. Serum Glucose Expressed as 100 Percent in Control Samples in Comparison with Serum Glucose of the Rats Fed with Untreated Tofu and Tofu Treated with HP

food	number of samples	median	standard deviation	standard error of media
tofu (untreated)	10	94.75	12.83	4.05
tofu (treated with HP)	10	106.00	26.95	8.52

Table 2. Broken-strength (newtons) in Shinbone, as 100 Percent in Control Samples and in Comparison with the Rats Fed with Untreated Tofu and Rats fed with Tofu Treated with HP

food	number of samples	median	standard deviation	standard error of media
tofu (untreated)	11	78.61	31.59	9.52
tofu (treated with HP)	13	110.99	47.52	13.18

work were chosen from previous works (Arroyo et al., 1997, 1999). The samples were subjected to high pressure of 400 MPa at 20 °C for 30 min. In each experiment, the indicated pressure was achieved within 2−3 min, held for 30 min, and then released to atmospheric pressure within 5−8 min. Then, this treated tofu was refrigerated and used to feed the rats.

Animals and Diet. We used an in vivo rat method to determine the differences between samples treated with HP and samples with no treatment. The animal subjects were female rats. Forty, six-week-old, female Wistar Hannover rats were used in this experiment. The rats were housed with five in each cage, and the group distribution was done randomly. The caged rats were in a room with controlled light (12 h, 8:00−20:00), temperature (22 ± 1 °C), and humidity (60−65%). The animals had free access to food and water. The animals were kept in accordance with the laboratory animal guidelines of the Facultad de Biologia, Universidad Complutense de Madrid, Spain, concerning the care and use of animals. Body weight of each of the rats was recorded weekly.

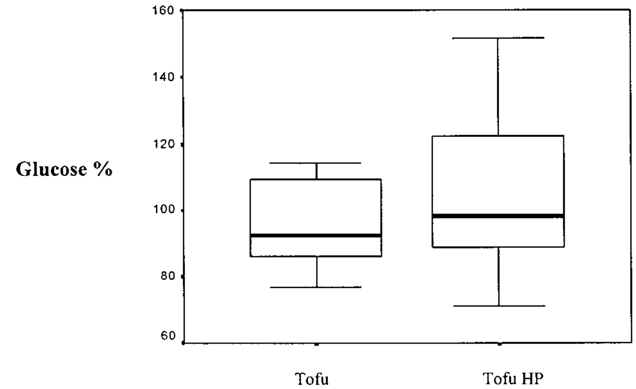

Figure 1. Glucose content expressed in percent, as determined in the serum of the rats fed with untreated tofu and the rats fed with tofu treated with HP. The control rats glucose content was considered as 100 percent.

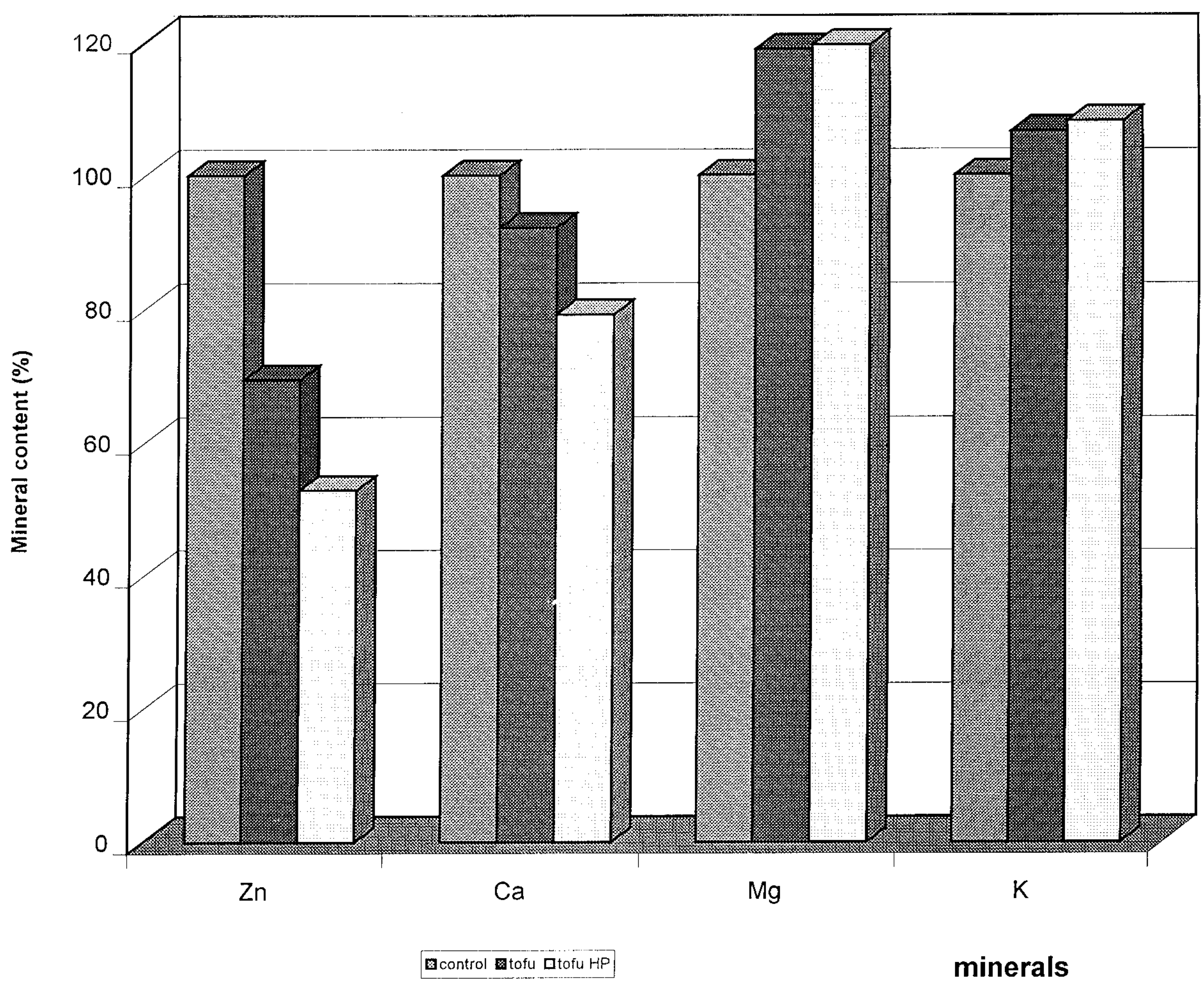

Figure 2. Mineral content (calcium, magnesium, potassium, and zinc, expressed in percent), determined in the serum of the rats fed with untreated tofu and those fed with tofu treated with HP. The control rats mineral content was considered as 100 percent.

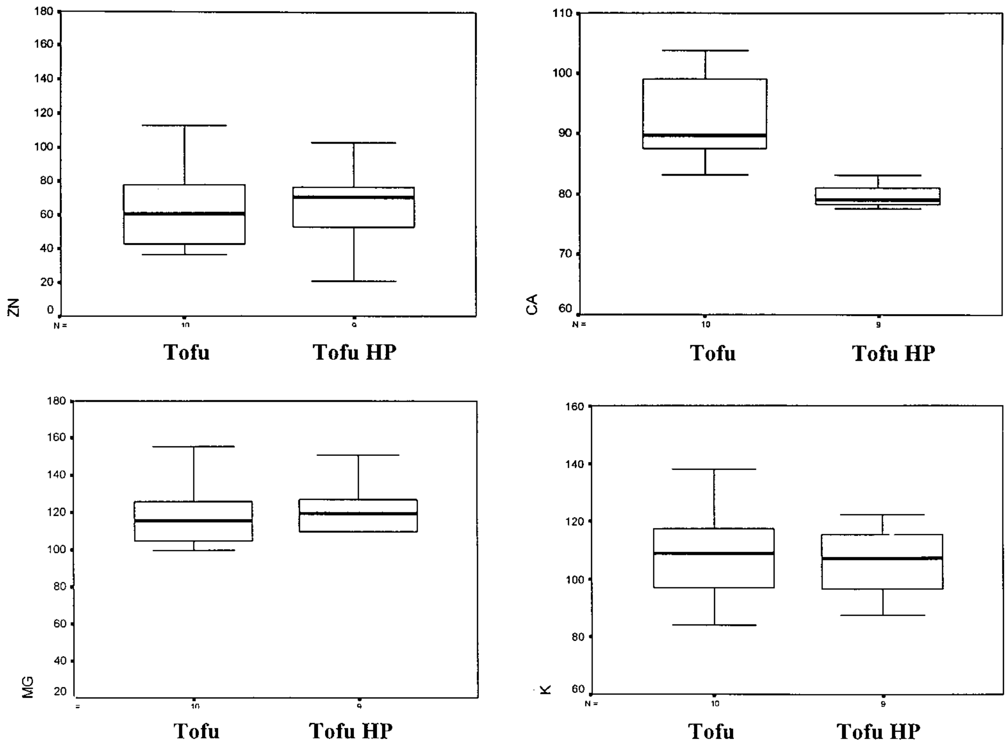

Figure 3. Statistical results of mineral content (calcium, potassium, and zinc, expressed in percent), determined in the serum of the rats fed with untreated tofu and those fed with tofu treated with HP. The control rats mineral content was considered as 100 percent.

The experiment was carried out in two parts, and 20 rats were used in each experiment. In the first group 10 control rats were fed with conventional food and 10 rats were fed with tofu. In the second group 10 control rats were fed with conventional food and 10 rats were fed with tofu treated with high pressure. The rats were fed for 28 days. The conventional food contained 17 g of protein, 3 g of fat, 4 g of cellulose, 5 g of ash, 7500 units of vitamin A, 1500 units of vitamin B3, 30 mg of vitamin E, and 30 mg of copper. Tofu made with soybeans and nigary (salt) contained 12 g of protein, 9 g of fat, and l g of carbohydrates.

After the animals were fed for 28 days, they were killed by decapitation (guillotined), and the whole organs (liver, heart, kidneys, and spleen) and shinbone were rapidly removed. All organs were weighed. The blood was collected and the sugar content was immediately analyzed. Then, the blood was refrigerated to analyze the mineral content in the blood serum (Ca^{2+}, Mg^{2+}, K^{+}, and zinc) by atomic absorption.

Atomic Absorption. Metabolite content of the rat blood serum was determined with an atomic absorption spectrophotometer apparatus (PerkinElmer, model 5100 PC), using an air-acetylene flame and operating in the emission mode without lamp K and Na measurements. A multi-element hollow cathodic lamp was used for Ca and Mg. The values were expressed in mg/mL.

Sugar Content. The sugar content was determined using a sensor as the measuring instrument (Accutrend Sensor Complete Meter, Boehringer Mannheim Corporation, Bruxelles, Belgium), in the 10−600 mg/dL range.

Instron Measurements. The shinbone maximum shear force expressed in newtons was determined with a Warner-Bratzler shear device, on an Instron food testing instrument (model 4500). Three replicate measurements were conducted on each shinbone.

RESULTS AND DISCUSSION

The results of glucose content measurements, in the serum of the rats fed with untreated tofu and the rats fed with tofu treated with HP, are depicted in Table 1. The measurements of the control samples were considered as 100 percent. Compared with the control samples, the glucose content decreased in the serum of the rats fed with the untreated tofu. However, glucose content increased in the serum of the rats fed with tofu treated with HP. The statistical results (*T* proof for independent samples in equal media) showed that there are no significant differences in glucose amount for both treatments. Yet, there are significant differences in the standard deviation as depicted in Figure 1.

The rat shinbones were used to measure bone strength with a Warner Bratzler shear device in an Instron machine. The control samples were considered as 100 percent. The broken-strength in the shinbone of rats fed with tofu decreased. However, in the rats fed with tofu treated with HP the values were closer to those of the controls (Table 2). The bones of the rats fed with tofu treated with HP were harder than those from the rats fed with untreated tofu. The statistical studies showed that there are no significant differences at $P \geq 0.05$ (*T* proof for independent samples in equal media) in the broken-strength for both treatments.

The metabolite (Ca^{2+}, Mg^{2+}, zinc, and K^{+}) amounts were determined in the serum of the rats and it was observed that Mg^{2+} and K^{+} amounts were higher in the tofu-fed rat samples than in the control samples. Yet, Ca^{2+} and zinc amounts were lower , as depicted in Figure 2. The control samples were considered as 100 percent. The statistical analysis showed (*T* proof for independent samples in equal media) that there are significant differences ($P \geq 0.01$) in the Ca^{2+} amounts in the serum of the rats for both treatments (Figure 3). Nonetheless, no significant differences were found in the Mg^{2+}, zinc, and K^{+} amounts in the serum for both treatments. This means that calcium was affected by the HP treatment.

The body weight of the rats increased after 4 weeks of the experiment. In the rats fed with untreated tofu

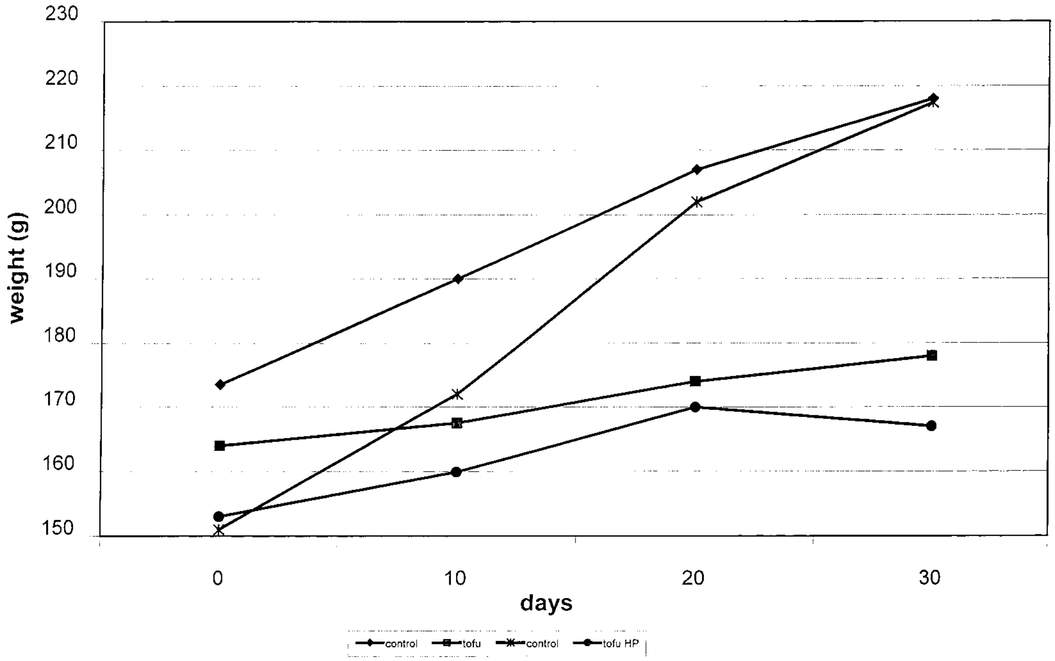

Figure 4. The increase in body weight of the rats fed with conventional food, untreated tofu, and tofu treated with HP for 28 days.

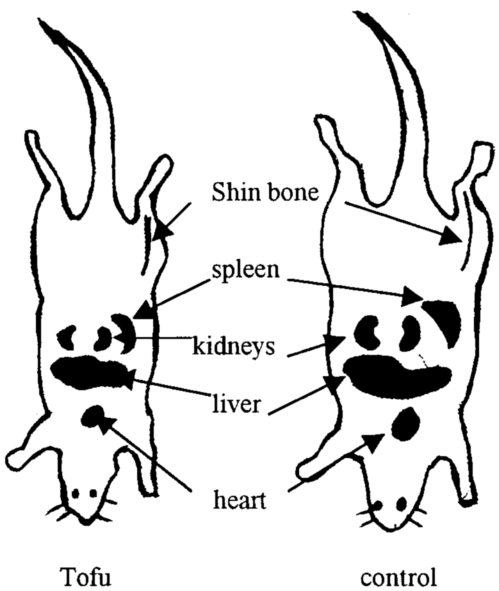

Figure 5. Drawing of rats showing how the body, heart, liver, kidneys, and spleen weights of the rats fed with tofu were smaller than those of the control rats fed with a conventional diet.

and those fed with tofu treated with HP the body weight increase was remarkably less in comparison with that of the control rats (Figure 4). Considering that the control rats have the normal weight (100 percent), the rats fed with tofu weighed only 29.23% and the rats fed with tofu treated with HP weighed only 26.08%. The weights of the organs analyzed (heart, liver, spleen, and kidneys) also decreased in both treatments in comparison with the control samples, as shown in Figure 5. The statistical studies (*T* proof on independent samples in equal media) showed that there are significant differences ($P \geq 0.05$) in liver weight between both treatments. However, no significant differences were found in body, spleen, heart, and kidneys weight between both treatments (Figure 6). Therefore, among the organs, the liver is the one that was more affected by the tofu treated with HP.

As a result of this, we could say that there are differences between the rats fed with untreated tofu and those fed with tofu treated with HP. Yet, the differences were only significant for the liver weight and calcium amount.

The glucose values were in a normal range for all rats. The apparent glucose increases in the blood serum of rats fed with tofu treated with HP are not significant in comparison to the glucose of the rats fed with tofu. This increase could be due to HP effects on the tofu carbohydrates, making them more accessible in the intestinal gut and resulting in the glucose levels increasing. Another explanation could be that there are some phytate remains in the tofu (Doell et al., 1981) and they could be destroyed by the HP. As it is well-known, phytate inhibits the action of a number of enzymes important for digestion, including pepsin, (Vaintraub and Bulmaga, 1991), trypsin (Sing and Krikorian, 1982), and alpha amylase (Sharma et al., 1978). HP could destroy the remaining phytate that acts as an inhibitor of these enzymes, especially alpha amylase. It could be that more glucose is absorbed during digestion and as a result the glucose level was increased in the samples treated with HP.

The same explanation could be given for the results on the broken-strength in the shinbone, where the rats fed with tofu treated with HP presented harder bones. Analysis of the metabolite shows significant differences between calcium values determined in the serum of the rats fed with untreated tofu and the others fed with tofu treated with HP. The calcium decrease is in accordance with the decrease in the shinbone broken-strength in the rats fed with tofu. However, there is no agreement with the rats fed with tofu treated with HP where the broken strength increased, although the calcium content decreased significantly. The explanation for this calcium decrease could be that calcium is also involved in other processes.

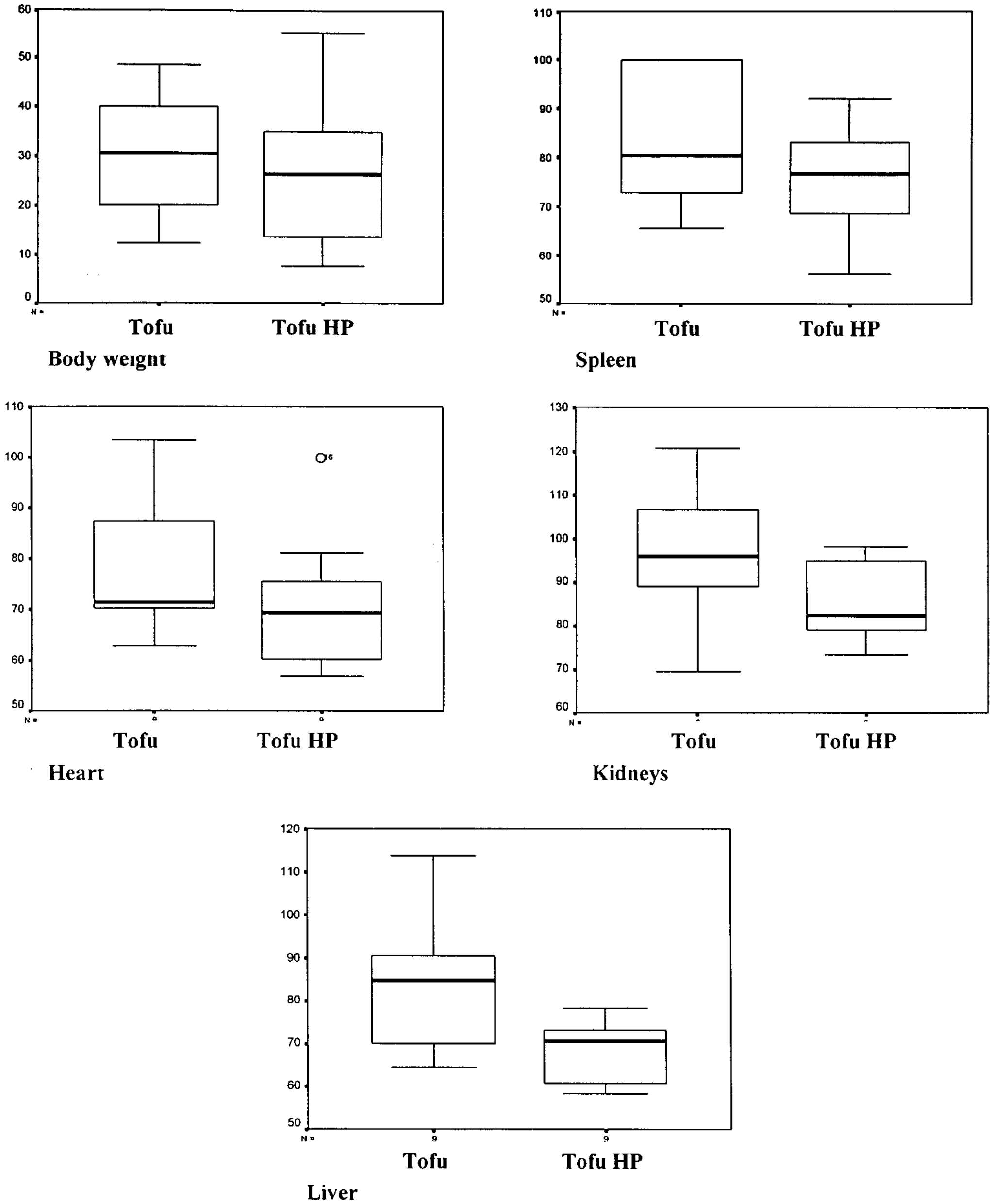

Figure 6. Statistical results of the weight of body, heart, liver, kidneys, and spleen determined on rats fed with untreated tofu and those fed with tofu treated with HP. The control rats fed with conventional food were considered as 100 percent.

What is very remarkable is the loss of weight in the rats fed with treated tofu in comparison with the weight gain of the control rats. The explanation for this is controversial. Some authors say that soy has protease inhibitors (DiPietro and Liener, 1989; Liener, 1994) and lectins (Liener, 1991) and that they are associated with the anti-nutritional process (Liener, 1994). These protease inhibitors could be responsible for the small size of the rats fed with tofu in comparison with the control rats. However, this is not clear enough because soy milk has to be heated when making tofu and the protease inhibitors should be eliminated. There are other soy components such as saponins, which are characterized by their hemolitic activity and foaming properties. In soybeans, five saponins have been identified (Oakenful, 1981). They are heat stable and have an adverse effect on the growth of animals (Ishaaya et al., 1969). However, there are other explanations for this like the effects of the soy isoflavones such as genistein, which is a specific inhibitor of tyrosine kinase (an important enzyme in transmembrane signal transduction) (Akiyama et al., 1987) and seems to play an important role in cell proliferation (Huang et al., 1992). This inhibitory power of genistein and daidzein is also related to the prevention of cancer growth (Peterson and Balnes, 1981), and they interact with the receptors of the estrogens in human cancer. (Martin et al., 1978).

Genistein and daizein are not destroyed with HP treatment (Préstamo and Morales, unpublished data). With HP treatment the tofu is more dispersed (Préstamo et al., 2000) and more components could become more accessible. Furthermore, the rats fed with tofu treated with HP are smaller than the rats fed with untreated tofu. Thus, the soy isoflavonoides could partly explain the weight loss observed in the rats fed with tofu in our experiments. However, more work has to be done to prove the relationship between the weight loss and the action of the isoflavonoids. This work is now under research in our group.

CONCLUSIONS

The biological response of the rats to the tofu treated with HP and untreated tofu gave as a result that significant differences were found between the calcium amounts determined in the serum of the rats of both diets, and it was lower on the rats fed with tofu treated with HP. Also, there are significant differences in the weight of the liver, and it was lower in the rats fed with tofu treated with HP. It appears that hydrostatic high-pressure treatment produces a potential effect on tofu as it is observed in the rats biological response to tofu treated with HP.

ACKNOWLEDGMENT

The authors thank Pilar Luna for all her help. They also thank Eva Morcillo and Maria Jesus Gaflan for their help and their care of the animals.

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Received for review April 12, 2000. Revised manuscript received July 31, 2000. Accepted July 31, 2000.

JF0004659