REPRODUCTIVE BIOLOGY

ORIGINAL RESEARCH

Differential effects of soy-containing diets on the reproductive tissues growth and reproductive hormone secretion in male rats

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SUMMARY

Pregnant and lactating females were fed soy-rich breeding diet. Their male progeny, immediately after weaning (postnatal day – PND 22) and after reaching puberty (PND 60), were studied to determine reproductive tissue growth and secretion of reproductive hormones. Similarly, the response of adult males to a soy maintenance diet over shorter (PND 160) and longer (PND 280) periods of time was examined. The relative weights (standardized by body weight) of the testes, epididymis and prostate, and the concentrations of luteinizing hormone (LH), testosterone and prolactin (PRL) were used as the examined end points. In rats on PND 22, no significant differences in the relative organs weights and the plasma hormones concentrations were found between the experimental and control groups. In rats on PND 60 which continued consuming a soy breeding diet, the relative tissue weights did not

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differ significantly, while the mean plasma LH and PRL concentrations were higher ($p<0.01$-0.001) compared to the controls. In rats on PND 160 fed soy maintenance diet, the higher relative testes ($p<0.01$) and epididymis ($p<0.05$) weights as well as plasma testosterone ($p<0.001$) concentration were recorded compared to the controls. In rats on PND 280 fed a soy maintenance diet, the relative weights of all reproductive tissues were similar to those of controls, however, the weight of the body and the real weights of the reproductive tissues were lower ($p<0.05$) than in the controls. The mean plasma concentrations of the reproductive hormones did not differ significantly between the two groups. In conclusion, a supplement of soy in the rat diet may affect growth and/or development of the reproductive tissues in male rats and also affect concentrations of reproductive hormones. The effects depend on the period of life when the soy diet is applied. *Reproductive Biology 2006 6 (3):275–290.*
Key words: soy meal, soy isoflavones, testes, epididymis, prostate, testosterone, LH, prolactin, male rats

INTRODUCTION

It is generally recognized that soy products are valuable components of human and animal diets. Various physiologically active compounds such as isoflavones that belong to a large family of phytoestrogens, are present in soybean and its products. Following ingestion, isoflavones, which include i.e. genistein and daidzein, are biotransformed by intestinal microflora, absorbed by enterocytes and undergo enterohepatic recycling, reaching the circulating concentrations that in some circumstances exceed the amounts of endogenous estrogens [34, 35]. The chemical structure of isoflavones and their metabolites is similar to estrogens enabling them to bind to estrogen receptor (ER) α and β [5, 16, 17]. The response to isoflavones was demonstrated in numerous biological effects at a molecular, cellular or physiological level [21, 36]. Although isoflavones have various health promoting effects [8, 33, 34], the question has been recently raised of the possible effects of feeding commercial diets containing soy to laboratory animals on the hormonal factors as well as on behavioral and social interactions [7, 13, 24].

Two main types of diets composed of natural feeds, including soy products, are fed to laboratory animals: breeding diets, designed for growth and reproduction, and the so-called maintenance diets fed to adult animals in long-term experiments. Thus, the laboratory animals may be exposed to isoflavones during different life stages including fetal, neonatal and growing period until maturity. It may be assumed, that any modifications in the development of reproductive organs or hormonal status due to isoflavones, may depend on time and duration of exposure. It was found that there is efficient transplacental passage of isoflavones [7] and that the fetuses and neonates are more sensitive than adults to estrogenic exposure resulting from maternal diet [18, 24, 42].

The objective of the study was to evaluate the effects of feeding a breeding diet containing soy products to pregnant and lactating females on: 1/ growth of the reproductive tissues and 2/ secretion of the reproductive hormones in their male progeny, immediately after weaning and after reaching puberty. Similarly, the response of adult males to a maintenance soy diet over shorter and longer periods of time was examined. The relative weights (standardized by body weight) of testes, epididymis and prostate and the concentrations of luteinizing hormone (LH), testosterone and prolactin (PRL) were used as the examined end points.

MATERIAL AND METHODS

Animals and diets

Wistar rats were used in two experiments. The animals were maintained in a light-and temperature-controlled environment (14-h light, 10-h dark cycle; temperature 22ºC) with food and water freely available. Breeding and maintenance diets were formulated to cover nutrient requirement of rats for growth, gestation and lactation, and that of adult rats, respectively (National Research Council, NRC [23]). Experimental diets contained solvent soybean meal (SBM) and soy protein concentrate (breeding diet), or SBM only (maintenance diet) as the main sources of supplementary protein. In soy-free control diets, whey protein concentrate (Lactopol S.A., Poland) and whole egg powder (Ovopol S.A., Poland) were substituted for soy products (tab. 1). A content of essential amino acids in the experimental and control diets, including lysine, threonine and tryptophane covered rat requirements according to NRC [23]. Although different crude protein content was noted in the breeding diets (tab. 1), their nutritional value, defined as available protein content - the product of crude protein content and net protein utilization (NPU), was similar. NPU was determined as a product of protein digestibility and biological

Table 1. Proximate composition[1] of breeding (Experiment 1) and maintenance (Experiment 2) diets (g/kg)

Components	Breeding diets		Maintenance diets	
	Control 1	Exp. 1	Control 2	Exp. 2
Cereals[2]	765	695	790	710
Soy products	-	180[3]	-	120[4]
Milk and egg products[5]	130	40	60	-
Other components[6]	45	45	60	50
Sugar beet pulp	20	-	50	80
Minerals and vitamins	40	40	40	40
Metabolizable energy, MJ/kg	14.3	14.4	12.5	12.6
Crude protein	172	199	157	162
Available protein[7]	146	149	nd	nd

[1]detailed composition is a proprietary formulation; [2]corn, wheat, oat, wheat middlings; [3]commercial solvent soybean meal (SBM; 140 g) and soy concentrate Soycomil (40 g); [4]SBM; [5]whey protein concentrate (Lactopol S.A., Poland) and whole egg powder (Ovopol S.A., Poland); [6]fodder yeast, ground linseed; [7]crude protein content × net protein utilization; nd. – not determined; see text for details

value in the N balance experiments (Pastuszewska et al., unpublished data) and calculated according to Pellet and Young [27]. Within each type, soy-containing and control diets had the same metabolizable energy value (tab. 1).

The total concentrations of isoflavones in our experimental breeding and maintenance diets were estimated according to data by Wang and Murphy [40]. The mean concentrations of these compounds in the soy-derived foods i.e. 2200 µg/g in SBM and 73 µg/g in protein concentrate [40] were taken for the estimation. Thus the total isoflavone concentration was about 311 µg/g and 264 µg/g in the experimental breeding and maintenance diets, respectively.

Experiment 1

Two groups of female rats were fed during the growing period, gestation and lactation a soy meal containing (experimental 1) or soy-free (control 1) breeding diets. Twenty-four males, born within each group, were weaned at the age of 22 days. Twelve rats from the experimental group and twelve controls were sacrificed by decapitation at weaning on postnatal day (PND) 22 and others were fed respective maternal diets until PND 60. Before decapitation, all animals were weighed and anesthetized by CO_2 inhalation. Blood was collected by cardiac puncture, centrifuged in heparinized tubes and plasma was stored at -20 ºC until hormone assays were performed. The testes, epididymis and prostate were dissected and weighed (real weights). The real tissues' weights were next standardized by body weight, as the relative weights.

Experiment 2

Sixty-day old rats (n=40), fed standard commercial chow from weaning at PND 22, were divided into two groups: fed soy meal-containing (experimental 2) or soy-free (control 2) maintenance diets. From each group, ten rats were decapitated at PND 160 and 10 at PND 280 and the same procedures as in the Experiment 1 were applied.

Analytical techniques

The plasma LH and PRL concentrations were determined by radioimmunoassay (RIA) methods [15] using specific standards and antibodies supplied by Dr A.F. Parlow and NIDDK (Baltimore, MD, USA). The assay sensitivity was 49 pg/ml for both LH and PRL and intra-assay coefficients of variation were 8.5% and 9.5%, respectively. The plasma concentration of testosterone was assayed by a direct RIA method, routinely used in our laboratory [15] with a sensitivity of 6.25 pg/ml.

Statistical analysis

The significance of differences in the weights of the reproductive tissues and in the concentrations of the hormones between rats fed a soy containing diet and control diet were determined by the non parametric ANOVA rank Kruskal-Wallis test within each group of age. The relative testes, epididymis and prostate weights and plasma concentrations of the hormones are expressed as a mean±SEM.

RESULTS

Experiment 1

In rats of PND 22, no significant differences were found between the experimental and control groups in the relative weight of testes (tab. 2) as well as in the mean plasma concentrations of LH, testosterone and PRL (fig. 1). The weights of epididymis and prostate were not recorded in this age group.

In rats of PND 60 which continued consuming an experimental breeding diet, the relative testes, epididymis and prostate weights did not differ significantly from the controls (tab. 2). The significant differences between these groups were recorded in the mean plasma LH and PRL concentrations: ($p<0.01$-0.001, fig. 1). Only an increased tendency in

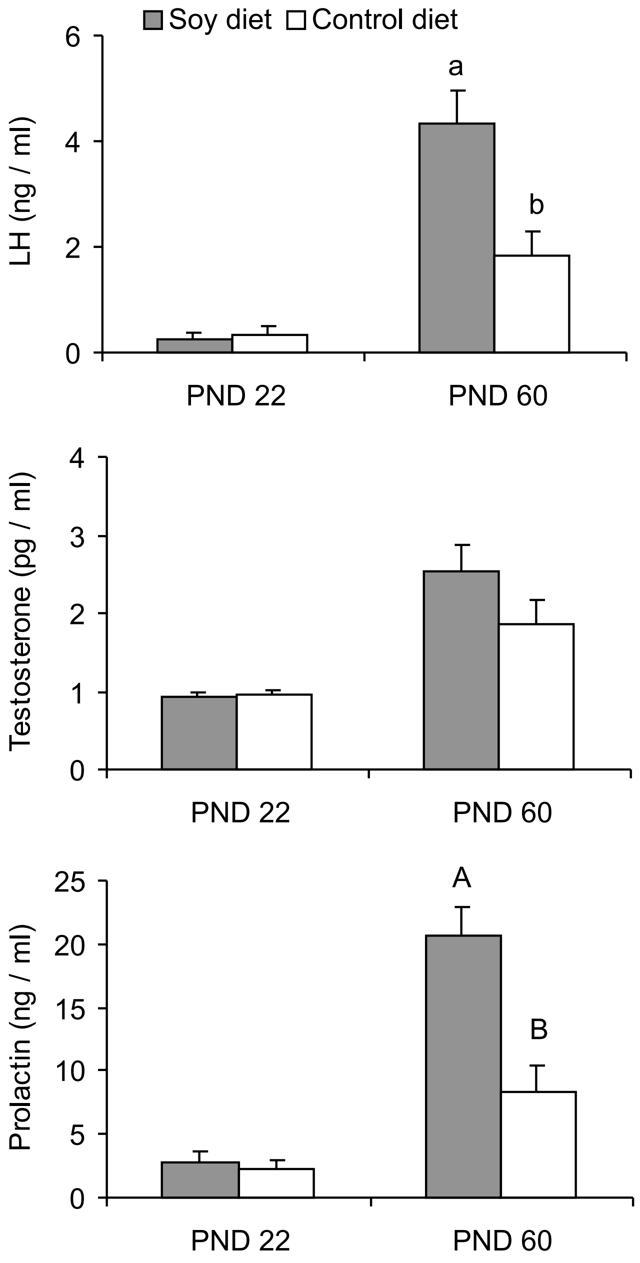

Figure 1. Mean (±SEM) plasma LH, testosterone and prolactin concentrations in rats on postnatal days (PND) 22 and 60 born to dams fed soy-containing or soy-free (control) breeding diets. ab: $p<0.01$; AB: $p<0.001$.

Table 2. The relative (standardized by body weight) weights of testes, epididymis and prostate (mean±SEM) of male rats born to dams fed control or soy meal-containing breeding diets after weaning at PND 22 and following exposure to the same maternal diets until PND 60

Age	PND 22		PND 60	
	control diet	soy diet	control diet	soy diet
Testes	0.75 ± 0.03	0.79 ± 0.03	0.96 ± 0.03	1.01 ± 0.01
Epididymis	-	-	0.21 ± 0.01	0.22 ± 0.01
Prostate	-	-	0.29 ± 0.01	0.29 ± 0.01

PND: postnatal day

testosterone concentration was observed in rats fed experimental breeding diet as compared with the controls.

Experiment 2

Rats of PND 160 fed experimental maintenance diet had significantly ($p<0.05-0.01$) higher relative testes and epididymis weights than in the controls (tab. 3). While no significant differences were found between these groups in the relative weight of prostate, the real weight of prostate

Table 3. The relative (standardized by body weight) weights of testes, epididymis and prostate (mean±SEM) of male rats fed control or soy meal-containing maintenance diets from PND 60 until PND 160 and PND 280

Age	PND 160		PND 280	
	control diet	soy diet	control diet	soy diet
Testes	0.69 ± 0.02^{A}	0.78 ± 0.02^{B}	0.66 ± 0.01	0.63 ± 0.03
Epididymis	0.26 ± 0.01^{a}	0.30 ± 0.01^{b}	0.24 ± 0.01	0.23 ± 0.01
Prostate	0.31 ± 0.02	0.30 ± 0.03	0.37 ± 0.01	0.35 ± 0.01

ab: $p<0.05$; AB: $p<0.01$ (significant differences within age groups); PND: postnatal day

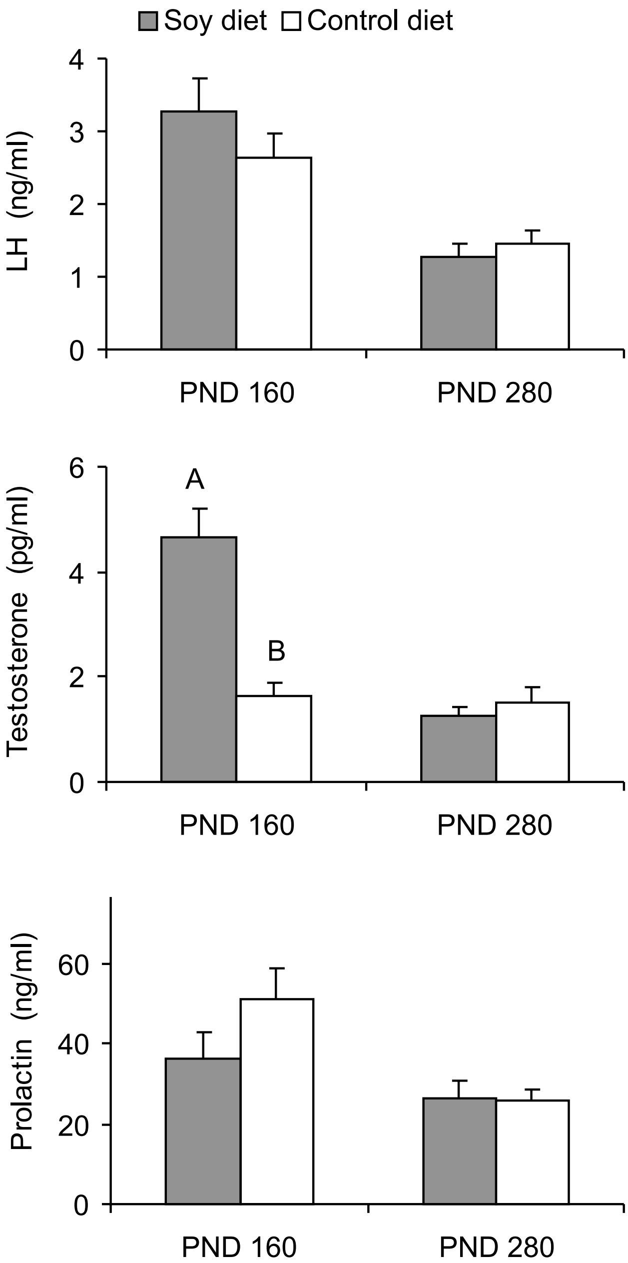

Figure 2. Mean (±SEM) plasma LH, testosterone and prolactin concentrations in rats on postnatal days (PND) 160 and 280, fed soy-containing or soy-free (control) maintenance diets. AB: $p<0.001$.

was significantly ($p<0.05$) lower in the experimental group (data not shown). No significant differences in the mean plasma LH and PRL concentrations were recorded between the experimental and control groups (fig. 2). Only plasma testosterone concentration was significantly ($p<0.001$) higher in rats fed an experimental maintenance diet, compared with the controls (fig. 2).

In rats of PND 280, no significant differences in the relative weights of the studied reproductive tissues (tab. 3) and in the mean concentrations of the reproductive hormones (fig. 2) were recorded between the experimental and control groups. However, the weight of the body and real weights of the reproductive organs were significantly ($p<0.05$) lower in rats fed the experimental diet, than in the controls (data not shown).

DISCUSSION

The results of the present study indicate that soy-rich diet fed to male rats may affect the growth and/or development of the reproductive tissues and also change the secretion of LH, testosterone and PRL. The influence of such a diet is diverse and dependent on the age and/or stage in which animals were under the impact of phytoestrogens. It should be pointed out that the most significant changes in the concentrations of the studied hormones were recorded before and during puberty.

Soybeans and soy-based foods, i.e. soybean meal, are an excellent source of protein and are used extensively in feeds for all classes of livestock and in preparation of the laboratory animal diets. They are also a rich source of the biologically active isoflavones, especially genistein and daidzein [6, 40]. The soy protein concentrate, additionally used in our experimental breeding diet, supplied proteins of high nutritional value, which are crucial for the proper growth and development of the neonates and young animals. Due to the processing used during manufacture of a concentrate, it contains reduced levels of isoflavones in comparison with soybean meal [40]. As estimated above, the amounts of isoflavones in our experimental breeding and maintenance diets were close to the lower range of the total isoflavone content of the commercial rodent diets containing soy as evaluated by

Brown and Setchell [7]. On the other hand, the ground linseed, which may be an additional source of phytoestrogens – lignans, was included in the same amounts into the experimenal and control diets. However, the effects of lignans on the reproductive functions, although possible [36], are not well recognized. Due to the small and similar amounts of linseed in our diets, the effect of lignans on the examined end points could be comparable and of minor importance.

In the numerous studies, the effects of biological activity of soy-derived phytoestrogens were widely described as reproductive disturbances resulted in the action of these compounds within the brain and/or peripheral tissues [21, 25, 26, 33, 42]. During animal development, one of the most critical period is the organogenesis, when the fetus is highly susceptible to toxic agents [30]. In rats, this process occurs between days 7-17 of gestation [11]. A further sensitive period in the development occurs during the rapid acceleration of brain growth, which in rats corresponds to the first 3-4 weeks of postnatal life [11]. For the most part, no changes in the relative reproductive tissues weights were found in our male rats of PND 22 and PND 60 born to dams fed soy-rich breeding diet from their childhood and next through gestation and lactation. This is partly in agreement with the study by Weber et al. [41] who found that rats fed a diet containing soy-derived isoflavones showed no change in prostate weight when measured before puberty. Although Fritz et al. [12] demonstrated dose-dependent reduction in androgen receptor and ERα and ERβ expression in the prostate of male rats exposed to genistein throughout gestation to PND 70, no differences in the histopathology or weight of the reproductive tract were evident between treatment and control groups. No differences in reproductive organs' weights or histology were also observed in studies by Kang et al. [14]. Also male rabbits, whose mothers received a supplement of soy meal or soy isoflavones, throughout gestation and lactation, had similar morphology of genital organs and sexual behavior to the control group [9].

The obtained results, however, contrast with some other data. Odum et al. [24] showed a reduction of testes and epididymis weights in young (PND 68) rats fed a soy-containing diet. Wisniewski et al. [42], in turn, showed that exposure to genistein during gestation and lactation demasculinizes the reproductive system in male rats. In females, more visible estrogenic effects of soy isoflavones on reproductive organs were reported – an increased uterine weight and advanced vaginal opening in immature animals [10, 20, 39].

Interestingly, our results showed that clear effects of a soy-containing diet occurred in young animals with reference to the reproductive hormones secretion. While the marked elevation of testosterone concentration could result in the increased LH secretion, the highly elevated plasma PRL concentration seems to be a clear estrogenic response to soy isoflavones [32]. The mechanism, by which isoflavones modulate LH and PRL secretion is not substantially recognized. It is possible that the apparent stimulatory effect of an experimental diet on the secretion of these pituitary hormones results in the action of isoflavones within the central nervous system (CNS). However, very little data concerning this aspect is available, especially related to rodent males. Ashby et al. [1] demonstrated that soy-based formulae elicited estrogenic effects in an ovariectomized mouse model and suggested participation of the CNS in these effects *via* stimulation of the hypothalamic gonadotropin-releasing hormone and subsequent production of exogenous estrogens mediated by gonadotropins released from the pituitary gland. The stimulatory effect of genistein on prolactin secretion following infusion of this isoflavone directly into the CNS was, in turn, showed in ovariectomized ewes [31]. The data obtained in our rat model are most of all in agreement with a suggestion that the CNS – gonadal axis of males is highly sensitive to phytoestrogenic compounds during development [33].

A stimulatory effect of a soy-containing diet on the growth of the testes and epididymis and in consequence on the testosterone secretion was observed in rats on PND 160, during the period corresponding to full sexual activity. It could be attributed to the low levels of circulating isoflavones following treatment with the experimental diet. As suggested by Attanasova et al. [3], the effect of low levels of potent estrogens can result in significant advancement of testicular/spermatogenic development at puberty. The apparent selectivity of action of soy/phytoestrogens and its estrogenic potency could perhaps be related to the reported relatively high affinity of phytoestrogens for ERß [17] which is expressed in both Sertoli and germ cells [28]. On the other hand, the accumulating concentration of isoflavones in the organism could increase the plasma concentration of testosterone by displacement of this hormone from α-fetoprotein – a blood glycoprotein which bounds circulating sex hormones in rats [4].

Contrary to our results, some data exist, showing adverse effects of soy diet or soy-derived isoflavones on the genital organs and testosterone secretion in adult rodent males [37, 41]. According to Odum et al. [24], administration of different diets to rats can affect in a different way the timing of both male and female sexual development. It is worth noting, that the opposite effect, although not significant in the relative weights, was observed in prostate of our rats on PND 160. It is in agreement with the studies by Weber et al. [41] who showed that after puberty when androgen levels increase, prostate weights decreased in rats fed a phytoestrogen-containing diet. Even as short as after ten days of exposure, a soy meal containing diet could affect the androgenic response to testosterone propionate in prostate [38]. As further demonstrated, such an effect could result in the action of equol, the main metabolite of soy-derived isoflavone daidzein, which blocked the androgenic influence of testosterone/5α-dihydrotestosterone within the prostate cells [19].

A lack of the significant differences in the relative reproductive organs' weight between the experimental and control group of rats on PND 280 may be improperly interpreted, since a clear and significant decrease in the body and real reproductive organs weights was recorded in rats fed a soy-rich diet. A decrease in the body weight was reported earlier in both male and female rodents exposed to different doses of soy or soy-derived isoflavones and was explained, in part, by an increased locomotor activity and/or lesser food intake [9, 22, 41]. On the other hand, reproductive organs' diminished weight, especially that of prostate, strongly support the protective effects which isoflavones exert in male reproductive disorders such us benign prostatic hyperplasia and prostate cancer [2, 29]. Concomitantly, clearly diminished reproductive hormone concentration in both groups of rats on PND 280 could indicate a parallel decrease in both the secretory activity of the CNS – gonadal axis and in its sensitivity to endogenous steroids and/or soy-derived isoflavones during aging.

In conclusion, a supplement of soy in a rat diet may affect growth and/ or development of the reproductive tissues in male rats and also change the concentrations of the reproductive hormones. The effects depend on the period of life when the phytoestrogenic diet is applied. The obtained data also support an opinion that choice of diet may alter functioning of estrogenic/androgenic compounds in the clinical studies and skew estrogen/ androgen-sensitive experimental end points.

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