Journal of Applied Biology & Biotechnology Vol. 13(3), pp. 31-37, May-June, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2025.203979

Consumption of corn and soybean sprouts enriched with egg shell in improving oxidative stress and estrogen depletion in ovariectomized rats

Siti Aminah[1*], Wulandari Meikawati[2], Sri Hartati[3], Diode Yonata[1]

[1]Department of Food Technology, Universitas Muhammadiyah Semarang, Semarang, Indonesia.
[2]Department of Public Health, Universitas Muhammadiyah Semarang, Semarang, Indonesia.
[3]Department of Agricultural Product Technology, Universitas Veteran Bangun Nusantara, Sukoharjo, Indonesia.

ARTICLE INFO

Article history:
Received on: August 16, 2024
Accepted on: January 11, 2025
Available Online: March 25, 2025

Key words:
Corn and soybean sprouts, oxidative stress, estrogen depletion, ovariectomized

ABSTRACT

The main objective of this research was to determine the effect of consumption of corn sprouts and soybean sprouts formulas enriched with eggshells as a source of calcium corn and soybean sprouts with eggshells (CSSEs), on the improvement of oxidative stress and estrogen hormone depletion in ovariectomy (OVX) rats. The animals used Sprague Dawley rats were divided into four groups, (i) without OVX (NC); (ii) OVX control (OVX-C); (iii) OVX+ethynylestradiol (OVX-E); (iv) OVX+CSSE (OVX-K). All treatments were given orally for 6 weeks. After 6 weeks, the OVX group experienced a higher increase in body weight than the NC group. The results of serum analysis showed that the control group (NC) had the highest estradiol level and the lowest in OVX-C, while the OVX-E and OVX-K groups showed an increase in serum levels of estradiol after treatment. The addition of estradiol and CSSE increased serum Ca and P. After the treatment, there was an increase in superoxidase dismutase (SOD) levels in the OVX-E and OVX-K groups, and vice versa in the OVX-C group decreased. Serum malondialdehyde (MDA) levels decreased after treatment in the OVX-E and OVX-K groups and increased in the OVX-C groups. CSSE consumption improved estrogen depletion and oxidative stress with SOD and MDA markers and increased Ca and P in postmenopausal ovariectomized rats.

1. INTRODUCTION

The incidence of bone fractures (fractures) and osteopenia (early osteoporosis) in menopausal women is becoming an increasingly serious problem. It is reported that as many as 10%–30% of women aged over 40 years in the Asia-Pacific region experience osteoporosis or postmenopausal osteoporosis (PO). Menopausal women have a 5.6 times risk of developing osteoporosis [1]. The onset of PO disease is associated with a decrease in estrogen production hormone during women's aging process. Estrogen is essential in metabolism, bone formation, parathyroid hormone, and vitamin D [2]. Estrogen will promote bone formation by increasing osteoblast activity by acclimating bone calcium resorption [3]. Estrogen deficiency in postmenopausal women causes an imbalance in the absorption and excretion of Ca and P in the blood so bones become brittle and break easily [4].

Estrogen deficiency causes an increase in oxidative stress induced by reactive oxygen species (ROS) during aging. Oxidative stress is also an important pathogenic factor of PO [5]. Lipid peroxidation is one of the most harmful effects of the presence of ROS, where the final product is malondialdehyde (MDA). Elevated serum MDA levels are known to increase bone soldering. The increase in MDA was indicated by a decrease in the activity of the antioxidant enzymes superoxidase dismutase (SOD) and glutathione peroxidase in serum [6]. Increased production of oxidative stress due to ROS accumulation has been confirmed to cause inflammatory conditions and impact bone remodeling imbalance. When the production of the hormone estrogen decreases, the function of osteoclasts, osteoblasts, and osteocytes in bone remodeling does not function. At the same time, there is an increase in nicotinamide adenine dinucleotide phosphate oxidase in the membrane and a decrease in the capacity of the antioxidant system so that bone resorption is more significant than bone formation, resulting in PO [5,7].

Hormone replacement therapy, especially for symptomatic relief of PO, has been widely used, with various contraindications reported. Among various interventions, soy phytoestrogens, in this case, isoflavones, have been confirmed to reduce oxidative stress without side effects and contribute to developing effective therapies to reduce PO symptoms [8]. Administration of isoflavone-rich soybean sprout flour to postmenopausal rats has previously been reported to increase Ca levels and improve Ca:P balance in the

*Corresponding Author
Siti Aminah, Department of Food Technology, Universitas Muhammadiyah Semarang, Semarang, Indonesia. E-mail: sitiaminah @ unimus.ac.id

sera of ovariectomized rats [9]. The granulated mixture of corn and soybean sprouts (CSSs) has a chemical composition that has the potential for bone health. CSS isoflavones have increased bone formation activity in the bone remodeling process [10]. The CSS formulation will be more complete if enriched with calcium-sourced food ingredients. Duck egg shells contain a calcium component of 10.11% [11,12], so they can be used for bone health, especially for PO sufferers.

Isoflavones in CSS flour enriched with calcium from eggshells corn and soybean sprouts with eggshells (CSSEs) are believed to prevent oxidative stress and ROS accumulation through the estrogenic effect produced by its genistein and daidzein components. This formula is hoped to be an alternative to hormone replacement therapy that is safe, healthy, and economical, especially for eliminating PO symptoms. The CSSE flour formula needs to be studied further for its physiological effects in inhibiting the production of oxidative stress in postmenopausal women through increasing SOD activity in reducing serum MDA levels in postmenopausal females Sprague Dawley rats obtained through ovariectomy (OVX).

2. MATERIALS AND METHODS

2.1. Materials

Soybean seed and corn yellow seed varietal Anjasmoro were obtained from Legumes and Tubers Crops Research Institute (Malang—Indonesia), and shells of duck eggs from duck farmers (Semarang—Indonesia). Ethinylestradiol 0.05 mg/tablet—Lynoral (PT Sydna Farma, Jakarta—Indonesia), estradiol Enzyme Linked Immunosorbent Assay (ELISA) kits (DRG—Germany), daidzein, and genistein standard (Sigma-Aldrich—Germany), Ca and P reagent kit (DiaSys—Germany).

2.2. CSS Production

CSS was produced using the previous method Aminah *et al.* [13]. Corn and soybean seeds that have been sorted and washed with running water, soaked for 6 hours, drained, washed thoroughly, and drained again. Furthermore, corn and soybeans were placed on the baking sheet plastic net and given base cloth wipes, then placed in a closed room in dark conditions. Germination was performed for 36 hours and was done by spraying water every 6 hours. After the Sprouts obtained were washed, drained, and sprouts were dried in a cabinet dryer with a temperature of ± 50°C for 8 hours. Grinding the Sprouts of corn and soybeans is done using a disk mill.

2.3. Eggshell Flour Production

The shells of duck eggs were washed and shrunk manually using a hand-made size reduction of approximately (0.12–0.52 mm). Further soaking with distilled water at a temperature of 100°C for 10 minutes followed by immersion using acetic acid (CH_3COOH) with a concentration of 2 N for 3 hours at a temperature of 60°C using a water bath, each extractant has a concentration of 2 N, and the comparison of the shell with a solution of the marinade is 1: 2 (*w*/*v*). The next is lifted and rinsed clean with distilled water. Drying the eggshells using a cabinet dryer at a temperature of 50°C for ± 1 hour, then made into powder using a disk mill [13].

2.4. Diet and Experimental Model

Twenty-four female Sprague Dawley rats aged 2 months (160–180 g) were obtained from the Integrated Research and Testing Laboratory (LPPT) at the Universitas Gadjah Mada for the experiment. Rats were raised in individual cages in an air-conditioned room at a temperature of 26°C–29°C; humidity: 60%–70%, with a 12 hours cycle of light and dark. After adapting to the environment for the first week, 18 rats were ovariectomized (OVX), while six other rats were dissected without ovarian retrieval as the normal control group. The rats were anesthetized using ketamine (10%) and xylazine (2%), given as an intramuscular injection before surgery [9]. All rats were fed the AIN-93 M diet through the 1-week recovery period (Table 1). Next, the rats were randomly divided into four groups: (i) normal control (without OVX/NC); (ii) OVX control (OVX-C); (iii) OVX+ethynylestradiol (OVX-E); (iv) OVX+CSSE (OVX-K). All rats were fed a base diet of AIN-93 M. All treatments were given orally for 6 weeks, with the amount of CSSE given based on a dose of 10 µg/g (b.wt./day) of isoflavones. Treated with ethynylestradiol at 30 µg/kg b.wt./day. During the experiment, the weight of each rat was checked every 7 days.

2.5. Sample Collection

Blood samples were collected before and after treatment to analyze the biochemical serum. After an overnight fast, blood samples were taken from the rats' orbital sinus under anesthesia with an intramuscular injection of ketamine (10%) and xylazine (2%). Blood was collected in microtubes, centrifuged at 3,000 rpm for 15 minutes (Thermo Scientific, Micro Legend 12), and stored at −20°C until analyzed [9,11].

2.6. Laboratory Analysis

The proximate composition of CSSE (moisture, ash, fat, protein, and fibers) was determined according to the AOAC method [14], and carbohydrates were determined by difference. Ca content of CSSE with atomic absorption spectrometry method [14], isoflavone content of CSSE with high-performance liquid chromatography method [15]. Analysis of serum Ca and P was carried out by the Arsenazo III photometric method using a reagent kit. Serum estradiol analysis using tmhe ELISA method using the DRG Estradiol ELISA reagent kit (EIA-2693). Serum SOD levels were analyzed using the ELISA reagent kit (K335-100) method, while serum MDA levels were analyzed using the thiobarbituric acid reactive substances method as described by previous studies [16].

2.7. Statistical Analysis

Data analysis used the one-way Analysis of Variance method of difference test and continued with the least significance different post

Table 1. Nutrition composition of composite flour obtained from CSSE.

No	Components	Content
1	Moisture (g/100 g)	7.74 ± 0.33
2	Ash (g/100 g)	14.16 ± 0.05
3	Total fat (g/100 g)	13.55 ± 0.67
4	Protein (g/100 g)	17.52 ± 0.17
5	Fibers (g/100 g)	26.89 ± 0.82
6	Carbohydrate (g/100 g)	20.14 ± 0.52
7	Calcium (g/100 g)	0.093 ± 0.01
8	Genistein (mg/g)	0.089 ± 0.01
9	Daidzein (mg/g)	0.480 ± 0.01

Composition of CSSE flour is corn sprout flour and soybean sprout flour with a ratio of 1:9, which is then added 15% (*w*/*w*) eggshell flour as a source of Ca.

hoc test to determine the significant difference between treatments with a significance level of $p < 0.05$. Statistical analysis was performed using Statistical Package for the Social Sciences 22.0 software.

3. RESULTS AND DISCUSSION

3.1. Body Weight

The average body weight of rats for 6 weeks of treatment is presented in Figure 1. All treatment groups experienced a significant increase in body weight, where the OVX-C group experienced the highest weight gain of 49.50 ± 6.22 g, and the lowest NC group was 42.75 ± 6.63 g (Table 2). In general, the ovariectomized group experienced a higher increase in body weight than the group without OVX. This was associated with increased abdominal fat in rats [17,18]. Fat catabolism in ovariectomized rats decreased due to low estrogen hormone, increasing fat stores in adipose tissue. This increase in fat in adipose tissue causes increased body weight in rats [19].

The weight gain of the OVX-K group was lower than that of the OVX-C group and slightly higher than the OVX-E group but not significant. The isoflavone component of CSSE may cause this. Isoflavones can act as pro-estrogenic components, so the available estrogen will be involved in energy metabolism by binding to estrogen receptors in the abdomen and subcutaneous fat tissue [20]. The increase in body weight (OVX-K), which was higher than the OVX-E group, probably came from the contribution of nutritional components from CSSE, both protein, fat, and carbohydrates, while in the OVX-E group, lynoral was given no nutritional elements that contributed to energy.

3.2. Serum Estradiol Levels

After 6 weeks of treatment, the NC rat group had the highest estradiol levels (35.21 ± 0.81 pg/ml), then the OVX-C group (28.03 ± 0.27 pg/ml) and OVX-K (26.54 ± 0.51 pg/ml), and the lowest in the OVX-C group (21.35 ± 0.38 pg/ml (Fig. 2)). Estradiol belongs to the group of estrogens and is an important steroid hormone during puberty, the menstrual cycle, and menopause [21]. Estrogen deficiency can occur due to two things: the effects of aging and the removal of the ovaries (OVX). Decreased estrogen production generally occurs in women when they age 40, known as pre-menopause. Estrogen itself is produced by the theca cells in the ovaries. This OVX process, which is the removal of the ovaries, results in a decrease in estrogen production. The addition of ethynyl estradiol (OVX-E) increased the serum estradiol level of rats after 6 weeks of treatment, similar to the administration of CSSE (OVX-K). Thus, CSSE flour could be hormone replacement therapy in ovariectomized rats. The isoflavone component in the CSSE formula is believed to act as a phytoestrogen. Isoflavones have a high affinity for the estrogen receptor in conditions of estrogen deficiency. It has been previously confirmed that Japanese and Chinese women who consume soy as a source of isoflavones have a lower risk of menopausal symptoms [22]. However, the addition of isoflavones will significantly affect serum estradiol levels in ovariectomized rats when the dose and duration are given at higher levels [11].

Table 2. Changes in body weight of rats pre and post treatment.

Groups	Body mass (g)		
	Pretreatment	Posttreatment	Different
NC	172.00 ± 7.87	215.75 ± 5.38	42.75 ± 6.63[a]
OVX-C	166.00 ± 5.89	215.50 ± 6.66	49.50 ± 6.22[c]
OVX-E	164.75 ± 6.38	209.50 ± 6.42	44.75 ± 6.40[b]
OVX-K	177.75 ± 8.23	223.00 ± 9.92	45.25 ± 9.25[b]

NC = normal controls; OVX-C = ovariectomized controls; OVX-E = ovariectomized + estradiol; OVX-K = ovariectomized + CSSE. Data were analyzed one-way ANOVA, values are means ± standard deviation. Different superscripts in the same colums different showed statistically significant differences ($p < 0.05$) as determined by LSD.

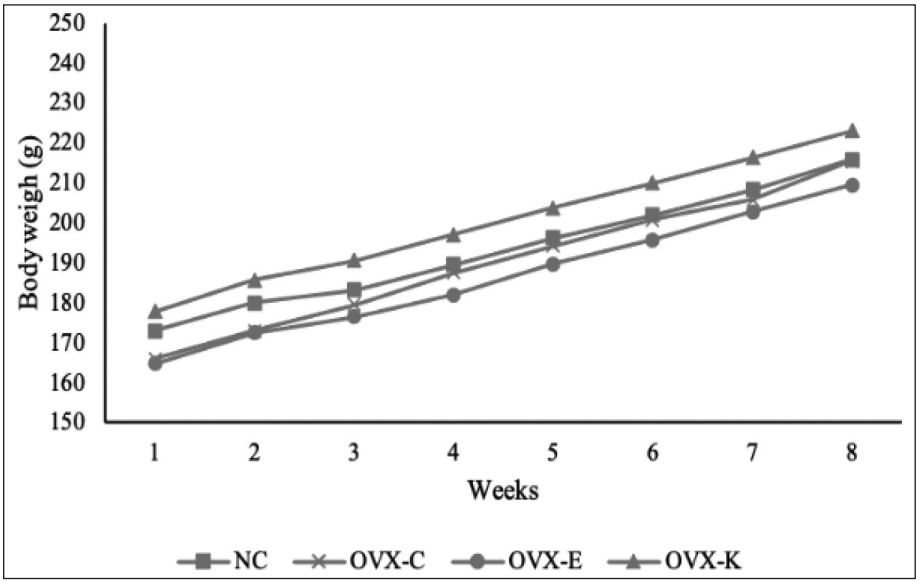

Figure 1. Effect of CSSE flour on body weight. Results are expressed as means ± standard deviation. Groups: NC = normal controls; OVX-C = ovariectomized controls; OVX-E = ovariectomized + estradiol; OVX-K = ovariectomized + CSSE.

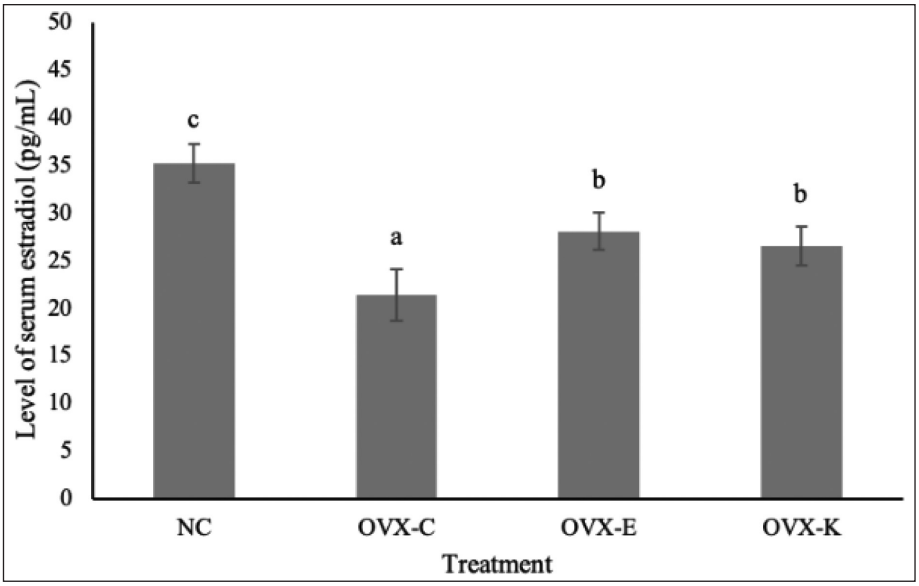

Figure 2. Effect of CSSE flour on level on serum estradiol. Results are expressed as means ± standard deviation. Groups: NC = normal controls; OVX-C = ovariectomized controls; OVX-E = ovariectomized + estradiol; OVX-K = ovariectomized + CSSE.

Table 3. Effect of CSSE flour on Ca and P levels in serum of rats after 6 weeks.

Variable	Groups			
	NC	OVX-C	OVX-E	OVX-K
Serum calcium (mg/dl)	13.20 ± 0.19^{c}	5.63 ± 0.07^{a}	12.36 ± 0.22^{b}	12.14 ± 0.14^{b}
Serum phosphorus (mg/dl)	2.81 ± 0.03^{d}	1.08 ± 0.02^{a}	2.57 ± 0.08^{c}	2.50 ± 0.07^{b}
Ca/P ratio	4.69 ± 0.11^{a}	5.21 ± 0.15^{c}	4.78 ± 0.19^{ab}	4.87 ± 0.17^{b}

NC = normal controls; OVX-C = ovariectomized controls; OVX-E = ovariectomized + estradiol; OVX-K = ovariectomized + CSSE. Data were analyzed one-way ANOVA, values are means ± standard deviation. Different superscripts in the same row showed statistically significant differences ($p < 0.05$) as determined by LSD.

3.3. Serum Ca and P Levels

Serum Ca and P levels of rats are presented in Table 3. The normal control group (NC) showed the highest mean serum Ca level (13.26 ± 0.23 mg/dl), significantly different from all groups. In the OVX group, it was seen that the administration of estradiol (OVX-E) and CSSE(OVX-K) for 6 weeks was able to increase serum Ca levels to 12.33 ± 0.26 mg/dl and 12.10 ± 0.16 mg/dl, respectively, while the OVX group with the standard diet (OVX-C) had the lowest serum Ca level (5.66 mg/dl). The same results were also seen in serum P levels after 6 weeks of treatment. The NC group had the highest mean serum P level (2.81 ± 0.03 mg/dl), which was followed by the OVX-E group (2.55 ± 0.08), the OVX-K group (2.26 ± 0.04 mg/dl), and the mean the lowest serum P level was in the OVX-C group (1.08 ± 0.02 mg/dl). Based on these data, the serum Ca/P ratio in the OVX-E (4.78/1) and OVX-K (4.87/1) groups were seen to be close to the serum Ca/P ratio in the NC group (4.69/1), while the OVX-C group was still too high (5.21/1).

The OVX process has been shown to increase the excretion of Ca and P through urine, reducing serum Ca and P levels [4,23]. The decrease in serum Ca and P levels in ovariectomized rats was associated with a decreased ability of estrogen to absorb Ca and P in the intestine. Hence, the excretion of Ca and P in feces and urine increased significantly [17]. Estrogen deficiency after OVX has been shown to accelerate the development of PO [23]. Isoflavone components in CSS flour and Ca components in duck eggshell flour have the potential to be estradiol in increasing serum Ca levels. Isoflavones in CSS indirectly affect calcium absorption because they have activity such as the hormone estrogen [9]. This increase in calcium absorption will further increase calcium homeostasis [24,25]. Isoflavones, especially genistein, increase serum mineral levels while decreasing mineral excretion in the urine [23].

The calcium component of duck eggshell flour in the CSSE formula may contribute to the provision of Ca. The availability of Ca in the CSSE formula is in the ideal proportion. This is illustrated by the serum Ca levels in the OVX-K group, which are not significantly different from the OVX-E group. Giving too high Ca is also a concern, considering postmenopausal women experience decreased calcium absorption in milk. So, unabsorbed Ca causes harmful side effects such as kidney stones in the heart and brain [26]. However, if Ca is too low, it will trigger Ca reabsorption in bone by osteoblast cells, increasing the risk of osteoporosis [27]. Decreased intestinal calcium absorption is related to estrogen deficiency [28], possibly contributing to accelerated bone density loss.

The addition of CSSE for 6 weeks significantly affected serum P levels of ovariectomized rats. Although the serum P level of the OVX-K group was slightly lower than that of the OVX-E and NC groups, the effect was very positive. The added duck eggshell flour may contribute to the supply of P [13], and the presence of genistein and daidzein can also contribute to the increased absorption of P in the intestine so that the level of P in the serum increases [11]. Calcium and

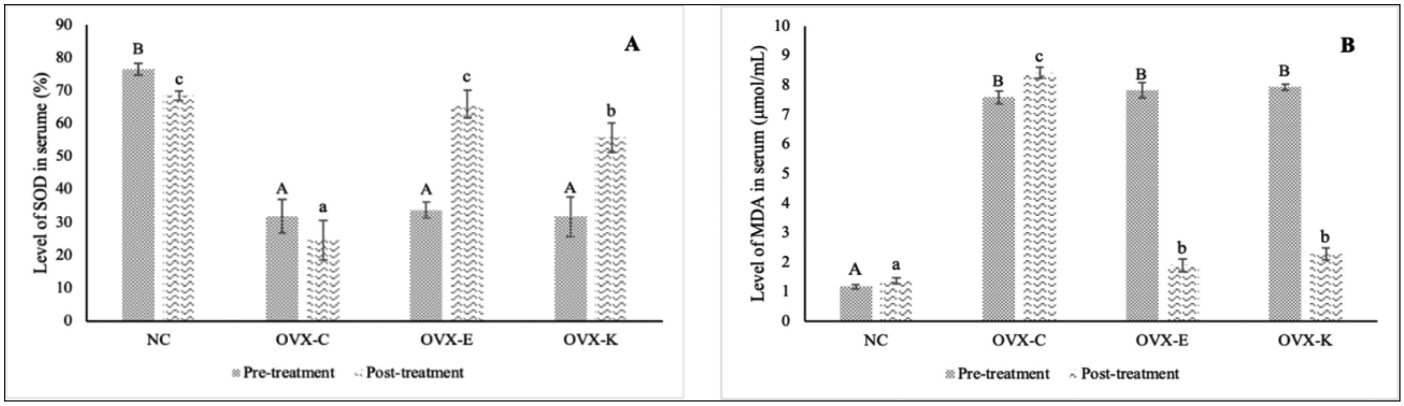

Figure 3. Effect of CSSE flour on level of SOD serum (A) and MDA serum (B). Results are expressed as means ± standard deviation. Groups: NC = normal controls; OVX-C = ovariectomized controls; OVX-E = ovariectomized + estradiol; OVX-K = ovariectomized + CSSE.

phosphorus are ingredients used for bone mineralization so that their adequacy and availability in the body can help prevent a decrease in bone density, which can result in brittleness, uncomplicated fracture, or osteoporosis.

3.4. Activities of Antioxidant Enzymes SOD

The SOD activity of rat serum before and after treatment can be seen in Figure 3A. Serum SOD activity in ovariectomized rats ranged from 30.38% to 32.69%, lower than in control rats (without OVX) (75.77%). After 6 weeks of treatment, the serum SOD activity of rats in the OVX-E group (67.75% ± 0.93%) was not different from that in the NC group (69.12% ± 0.58%), while the OVX-K group had a slightly lower serum SOD activity (59.30% ± 1.37%). Rats undergoing OVX are known to have decreased blood antioxidants and increased oxidative stress markers. SOD enzyme expression is known after OVX so oxidative stress occurs. The activity of SOD and glutathione peroxidase in rats decreased after 1 day of OVX [29]. Decreased SOD activity in OVX rats led to an accumulation of O_2, which was shown to inhibit antioxidant enzymes. SOD is responsible for the dismutation of O_2 into hydrogen peroxide, which is more reactive than O_2 [30]. The decrease in SOD in ovariectomized rats is exacerbated by failure in estrogen production, resulting in post-OVX hormone deficiency [31,32]. Estrogen deficiency conditions can stimulate the production of cytokines in peripheral blood cells and increase the concentration of interleukin-6 associated with postmenopausal oxidative stress in women [33]. Estrogen has been shown to protect important organs from oxidative damage due to its antioxidant abilities [34].

Increased oxidative stress indicates damage to bone tissue. Estrogen deficiency stimulates ROS to activate osteoclasts, which results in oxidative bone damage [16]. Therefore, a decrease in the SOD enzyme indicates an increase in oxidative stress due to bone tissue damage. Administration of CSSE(OVX-K) restored serum SOD activity close to the control group (NC) and ethynylestradiol (OVX-E). This shows that the use of natural phytoestrogens as a strategy to improve oxidative stress conditions in postmenopausal women has very potential. The estrogenic activity of isoflavones is associated with their ability to increase SOD enzyme activity [35]. Isoflavones have been reported to be very strong in scavenging free radicals in diabetic patients [36], and genistein in isoflavones has been confirmed to reduce free radical-induced tissue injury through antioxidant activity [37]. Therefore, many postmenopausal women consider phytoestrogens, in this case isoflavones, as dietary supplements taken as natural alternatives to conventional hormone therapy [38].

3.5. Serum Levels of MDA

Serum MDA levels of rats before and after treatment can be seen in Figure 3B. The MDA serum levels of rats before treatment were 1.28 mol/ml (NC) and 7.66–8.05 mol/ml in the OVX group. OVX accelerates oxidative stress, as indicated by increased serum MDA levels. Several studies have shown an indication of an antioxidant imbalance in some mammals after OVX, and this can be seen from the MDA concentration, which significantly increased after 24 hours of OVX [39,40]. OVX treatment reduces lipid peroxidase concentrations, contributing to oxidative stress [41–43].

Treatment with ethynylestradiol (OVX-E) and CSSE (OVX-K) for 6 weeks significantly reduced the rat serum MDA to a near normal (NC) group. These results align with those reported in previous studies [44], that the phenolic ring of soy isoflavones is known to modulate the expression of prooxidant and intracellular antioxidant enzymes in suppressing excessive ROS formation. In addition, the scavenging effect of soy isoflavones in decreasing serum MDA levels involves ROS-mediated activation of NF-κB/TNF-a signaling [45]. The potent antioxidant of isoflavone polyphenols can reduce serum MDA to close to normal levels. The presence of isoflavones will neutralize excessive ROS from the effect of OVX, thus bone repair will take place more quickly [46].

4. CONCLUSION

Consumption of CSSE for 6 weeks in ovariectomized rats caused: 1) significantly increased body weight of treated rats compared to the normal control group; 2) The OVX-K and OVX E groups experienced a significant increase in serum estrogen, Ca, and phosphorus than the control group. 3). Oxidative stress markers SOD showed an increase in the OVX-K and OVX E groups, and both groups experienced a significant decrease in serum MDA levels. In general, the results of this study indicate that consumption of CSSE can improve estrogen depletion and oxidative stress to help maintain bone health in postmenopausal women.

5. ACKNOWLEDGMENTS

Thank you to the Director General of Higher Education and the University of Muhammadiyah Semarang for the support provided for the implementation of this research.

6. LIST OF ABBREVIATIONS

AOAC, Association of Official Analytical Chemists; Ca, Calcium; CSS, Corn and soybean sprouts; CSSE, Corn and soybean sprouts with eggshells; ELISA, Enzyme Linked Immunosorbent Assay; MDA, Malondialdehyde; P, Phosphorus; ROS, Reactive oxygen species; SPSS, Statistical Package for the Social Sciences; SOD, Superoxidase dismutase.

7. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

The study protocol was approved by the Institutional Ethics Committee with approval number 184/V/2017/KomisiBioetik.

9. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

10. FUNDING

There is no funding to report.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Chandran M, Brind'Amour K, Fujiwara S, Ha YC, Tang H, Hwang JS, *et al.* Prevalence of osteoporosis and incidence of related fractures in developed economies in the Asia Pacific region: a systematic review. Osteoporos Int 2023;34:1037–53; doi: http://doi.org/10.1007/s00198-022-06657-8
2. Aloia JF, Dhaliwal R, Shieh A, Mikhail M, Islam S, Yeh JK. Calcium and vitamin D supplementation in postmenopausal women. J Clin Endocrinol Metab 2013;98:1702–9; doi: http://doi.org/10.1210/jc.2013-2121
3. Kini U, Nandeesh BN. Physiology of bone formation, remodelling, and metabolism. In: Fogelman, Gnanasegaran G, Wall H (eds.). Radionuclide and hybrid bone imaging, Springer, Berlin, Germany, pp 29–57, 2012.
4. Unis U, Hamza M, Abdelzaher E. Comparison of the effects of sitagliptin and estrogen on ovariectomy induced osteoporosis in rats. Int J Pharm Res 2015;5:10–8.
5. Callaway DA, Jiang JX. Reaxtive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. J Bone Min Metab 2015;33:359–70; doi: http://doi.org/10.1007/s00774-015-0656-4
6. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. Med Sci Mon 2004;10:141–7.
7. Zhou T, Gai Z, Gao X, Li L. The potential mechanism of exercise combined with natural extracts to prevent and treat postmenopausal osteoporosis. J Healthc Eng 2021;2021:2852661; doi: http://doi.org/10.1155%2F2021%2F2852661
8. Marinho DZ, Calio ML, Santos MA, Ko GM, Teixeira CP, Carbonel AF, *et al.* Evaluation of the isoflavones and estrogen effects on the rat adrenal. Glynecol Endocrinol 2017;33:811–5; doi: http://doi.org/10.1080/09513590.2017.1318371
9. Aminah S, Suparmo, Naruki S, Wuryastuty H. Consumption of elicited soybean sprout flour increasescalcium level in serum of ovariectomized rats. Univ Med 2017;36:94–101; doi: http://doi.org/10.18051/UnivMed.2017.v36.94-101
10. Aminah S, Yusuf M. Efisiensi pakan, berat badan dan panjang tulang tikus yang mengkonsumsi KEJALE selama 6 minggu. 2nd Univ Res Coloq 2015;2015:451–9. (Indonesia).
11. Aminah S, Suparmo, Naruki S, Wuryastuty H. Soybean sprouts inhibit bone turnover in ovariectomized rats. Pak J Nutr 2017;16:666–71; doi: http://doi.org/10.3923/pjn.2017.666.671
12. Yonata D, Aminah S, Hersoelistyorini W. Kadar kalsium dan karakteristik fisik tepung cangkang telur ungags dengan perendaman berbagai pelarut. Jurnal Pangan dan Gizi 2017;7:82–93.
13. Aminah S, Meikawati W, Rosidi A. Nutrition retention of product based on soybean sprouts flour and corn sprout flour enriched with duck eggshell. IOP Conf Ser: Earth Environ Sci 2019;292:012009.
14. AOAC. Official methods of analysis of the association of official analytical chemists. AOAC Inc., Arlington, VA, 2005.
15. Penalvo JL, Nurmi T, Adlercreutz H. A simplified HPLC method for total isoflavones in soy products. Food Chem 2014;88:297–305; doi: http://doi.org/10.1016/j.foodchem.2003.12.028
16. Wakf AME, Hassan HA, Gharib NS. Osteoprotective effect of soybean and sesame oils in ovariectomized rats via estrogen-like mechanism. Cytotechnology 2014;66:335–43; doi: http://doi.org/10.1007%2Fs10616-013-9580-4a
17. Park Y, Moon HJ, Paik DJ, Kim DY. Effect of dietary legumes on bone-specific gene expression in ovariectomized rats. Nutr Res and Pract 2013;7:185–91; doi: http://doi.org/10.4162/nrp.2013.7.3.185
18. Shin JA, Yang SJ, Jeong SI, Park HJ, Choi YH, Park EM. Activation of estrogen receptor β reduces blood–brain barrier breakdown following ischemic injury. Neuroscience 2013;235:165–73; doi: http://doi.org/10.1016/j.neuroscience.2013.01.031
19. Jones MEE, Thorburn AW, Britt KL, Simpson ER. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci 2000;97:12735–40; doi: http://doi.org/10.1073/pnas.97.23.12735
20. Joyner JM, Hutley LJ, Cameron DP. Estrogen receptors in human preadipocytes. Endocrine 2001;15:225–30; doi: http://doi.org/10.1385/endo:15:2:225
21. Ma X, Zhang C, Deng J, Song Y, Li Q, Guo Y, *et al.* Simultaneous degradation of estrone, 17β-Estradiol and 17α-Ethinyl estradiol in an aqueous UV/H2O2 system. Int J Environ Res Public Health 2015;12:12016–29; doi: http://doi.org/10.3390%2Fijerph121012016
22. Levis S, Griebeler ML. The role of soy foods in the treatment of menopausal symptoms. J Nutr 2010;140:2318–21; doi: http://doi.org/10.3945/jn.110.124388
23. Miao Q, Li JG, Miao S, Hu N, Zhang J, Zhang S, *et al.* The bone-protective effect of genistein in the animal model of bilateral ovariectomy: roles of phytoestrogens and PTH/PTHR1 against post-

menopausal osteoporosis. Int J Mol Sci 2012;13:56–70; doi: http://doi.org/10.3390/ijms13010056
24. Arjmandi BH, Smith BJ. Soy isoflavones' osteoprotective role in postmenopausal women: mechanism of action. J Nutr Biochem 2002;13:130–7; doi: http://doi.org/10.1016/s0955-2863(02)00172-9
25. Cai DJ, Zhao Y, Glasier J, Cullen D, Barnes S, Turner CH, *et al.* Comparative effect of soy protein, soy isoflavones, and 17β-estradiol on bone metabolism in adult ovariectomized rats. J Bone Miner Res 2005;20:828–39; doi: http://doi.org/10.1359/jbmr.041236a
26. Picherit C, Bennetau-Pelissero C, Chanteranne B, Lebecque P, Davicco MJ, Barlet JP, *et al.* Soybean isoflavones dose-dependently reduce bone turnover but do not reverse established osteopenia in adult ovariectomized rats. J Nutr 2001;131:723–8; doi: http://doi.org/10.1093/jn/131.3.723
27. Qureshi HJ, Hussain G, Jafary ZA, Bashir MU, Latif N, Riaz Z. Calcium status in premenopausal and postmenopausal women. J Ayub Med Coll Abbottabad 2010;22:143–5.
28. Picherit C, Coxam V, Bennetau-Pelissero C, Kati-Coulibaly S, Davicco MJ, Lebecque P, *et al.* Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. J Nutr 2000;130:1675–81; doi: http://doi.org/10.1093/jn/130.7.1675
29. Anadol E, Yarim GF, Gultiken N, Kazak F. Effect of ovariohysterectomy on some oxidative stress markers in the rat. Harran Univ Vet Fak Derg 2016;5:1248.
30. Muthusami S, Ramachandran I, Muthusamy B, Vasudevan G, Prabhu V, Subramaniam V, *et al.* Ovariectomy induces oxidative stress and impairs bone antioxidant system in adult rats. Clin Chim Acta 2005;360:81–6; doi: http://doi.org/10.1016/j.cccn.2005.04.014
31. Sankar P, Zachariah B, Vickneshwaran V, Jacob SE, Sridhar MG. Amelioration of oxidatve stress and insulin resistance by soy isoflavones (from Glycine max) in ovariectomized Wistar rats fed with high fat diet: the molecular mechanism. Exp Gerontol 2015;63:67–75; doi: http://doi.org/10.1016/j.exger.2015.02.001
32. White RE, Gerrity R, Barman SA, Han G. Estrogen and oxidative stress: a novel mechanism that may increase the risk for cardiovascular disease in women. Steroids 2010;75:788–93; doi: http://doi.org/10.1016/j.steroids.2009.12.007
33. Kim OY, Gerrity R, Barman SA, Han G. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy nonobese women. AGE 2010;34:415–25; doi: http://doi.org/10.1016/j.steroids.2009.12.007
34. Sener G, Arbak S, Kurtaran P, Gedik N, Yegen BC. Estrogen protects the liver and intestines against sepsis-induced injury in rats. J Surg Res 2005;128:70–8; doi: http://doi.org/10.1016/j.jss.2005.02.019
35. Xiao Y, Zhang S, Tong H, Shi S. Comprehensive evaluation of the role of soy and isoflavone supplementation in humans and animals over the past two decades. Phytother Res 2018;32:384–94; doi: http://doi.org/10.1002/ptr.5966
36. Umeno A, Horie M, Murotomi K, Nakajima Y, Yoshida Y. Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. Molecules 2016;21:708; doi: http://doi.org/10.3390/molecules21060708
37. Han RM, Tian YX, Liu Y, Chen CH, Ai XC, Zhang JP, *et al.* Comparison of flavonoids and isoflavonoids as antioxidant. J Agric Food Chem 2009;57:3780–5; doi: http://doi.org/10.1021/jf803850p
38. Poluzzi E, Piccinni C, Raschi E, Rampa A, Recanatini M, Ponti FD. Phytoestrogens in postmenopause: the state of the art from a chemical, pharmacological and regulatory perspective. Curr Med Chem 2014;21:417–36; doi: http://doi.org/10.2174/09298673113206660297
39. Gunay A, Gunes N, Gunay U. Effect of ovariohysterectomy on lipid peroxidation and levels of some antioxidants and biochemical parameters in bitches. Bull Veter Inst Pulawy 2011;55:695–8.
40. Serin G, Kiral F, Serin I. Acute effect of ovariohysterectomy on lipid peroxidation and some antioxidant levels in dogs. Bull Veter Inst Pulawy 2018;52:251–3.
41. Tang Z, Wang Y, Zhu X, Ni X, Lu J. Exercise increases cystathionine-γ-lyase expression and decreases the status of oxidative stress in myocardium of ovariectomized rats. Int Heart J 2016;57:96–103; doi: http://doi.org/10.1536/ihj.15-099
42. Mudron P, Herzog K, Holtershinken M, Rehage J. Effects of abdominal surgery on thiobarbituric acid reactive substances and plasma anti-oxidative capacity in dairy cows. J Vet Med A Physiol Pathol Clin Med 2017;54:441–4; doi: http://doi.org/10.1111/j.1439-0442.2007.00992.x
43. Koźlik J, Przybylowska J, Mikrut K, Zukiewicz-Sobczak WA, Zwolinski J, Piatek J, *et al.* Selected oxidative stress markers in gynecological laparoscopy. Wideochir Inne Tech Maloinwazyjne 2015;10:92–100; doi: http://doi.org/10.5114%2Fwiitm.2014.47449
44. Abdelrazek HMA, Mahmoud MMA, Tag HM, Greish SM, Eltamany DA, Soliman MTA. Soy isoflavones ameliorate metabolic and immunological alterations of ovariectomy in female wistar rats: antioxidant and estrogen sparing potential. Oxid Med Cell Long 2019;2019:1–13; doi: http://doi.org/10.1155%2F2019%2F5713606
45. Domazetovic V, Marcucci G, Iantomasi T, Brandi ML, Vincenzini MT. Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab 2017;14:209–16; doi: http://doi.org/10.11138%2Fccmbm%2F2017.14.1.209
46. Karin M, Delhase M. The IκB kinase (IKK) and NF-κB: key elements of proinflammatory signalling. Sem Immunol 2000;12:85–98; doi: http://doi.org/10.1006/smim.2000.0210

How to cite this article:
Aminah S, Meikawati W, Hartati S, Yonata D. Consumption of corn and soybean sprouts enriched with egg shell in improving oxidative stress and estrogen depletion in ovariectomized rats. J Appl Biol Biotech. 2025;13(3):31–37. DOI: 10.7324/JABB.2025.203979.