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The administration of supplements milk with high protein of whey increased estrogen level but did not affect testosterone level in male wistar rat (*Rattus norvegicus*)

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ABSTRACT

Introduction: Supplements milk is a high protein of *whey* that consumed by many teenagers to maintain the fitness in accordance with the advertisements in the society, the presence of hormones in milk that are not listed in its composition causes a hormonal balance disorder. The aim of this research was to prove that giving supplements milk with a high protein of *whey* in increasing estrogen levels and lowers testosterone counts in male Wistar rats *(Rattus norvegicus)*.

Methods: This research employed experimental research using a pretest-posttest control group design that used 22 white male Wistar rats *(Rattus norvegicus),* the samples were divided into 2 groups, namely the control group (P0) and the treatment group (P1) that treated by supplements milk with a high-protein of *whey* once a day for three weeks. The blood was taken before and after treatment for estrogen levels and testosterone counts.

Result: The finding indicated that a significant increase in estrogen levels before and after the administration of milk supplement with high *whey* protein 11.603 ± 0.502 ng/ ml to 16.141 ± 0.347 ng/ ml ($p<0.001$). There was no decrease in testosterone counts before and after subjects were administered by milk supplement with high *whey* protein 1.86 ± 0.325 ng/ ml to 1.69 ± 0.260ng/ ml $p = 0.220$ ($p> 0.05$).

Conclusion: Based on the results of the analysis it could be concluded that the administration of milk supplement with high *whey* protein could increase estrogen levels while decreasing testosterone in male Wistar rats *(Rattus norvegicus)*.

Keywords: *Whey* protein, Estrogen level, Testosterone

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INTRODUCTION

The aging process is influenced by various factors, generally grouped into internal and external factors. Internal factors are free radicals, reduced hormones, glycosylation, methylation, apoptosis, a decreased immune system, and genes. Whereas external factors include unhealthy lifestyles, unhealthy diets, wrong habits, environmental pollution, stress, and poverty. If these factors could be prevented, then the aging process could be prevented, slowed down, maybe even inhibited. The hormones that affected were the hormone testosterone, *growth hormone* (GH), *estrogen*, *dehydroepiandrosterone* (DHEA), melatonin and thyroid.[1]

The hormone estrogen is a hormone that has a big influence on human life.[1] In men, this hormone is produced in smaller amounts and plays a role in sperm production and libido. If there is a change in the ratio of androgen levels to estrogen in plasma, it will cause feminization symptoms such as gynecomastia.[2,3]

Lately, one of the most causing factors for aging is unhealthy consumption patterns. Along with the increasing interest of men to have a fit body, the higher the consumption of milk supplement with high whey protein. At a young age hormone levels are still in the balance, but the use of milk supplements that contain hormones that are not in accordance with needs could affect the balance of hormones in the body. Instant supplement products like this tend to have a bad impact and could accelerate the aging process.

One of the milk supplements with whey protein is milk Pro Performance AMP that is usually used to increase stamina and form muscles that could be used without physical activity. The results of the examination of hormone content in milk were carried out at the Analytical Laboratory of the Bukit Jimbaran Campus, Udayana University. The results of the analysis showed that in the whey protein of the product contained *phytoestrogens* of 2.87 pg/ 100g and *phytoprogesterone* of 1.93 pg/ 100g.

Phytoestrogens work like estrogen and are thought to show biological effects at the molecular, cellular, or physiological level. This phytoestrogen has the ability to bind to estrogen receptor (ER), with a higher bond affinity for ER-β compared to ER-α.[4] The presence of this high estrogen level feeds back negatively to luteinizing hormone (LH) which results in reduced production of testosterone.[5] When testosterone levels are reduced, it would interfere with the growth and maintenance of secondary sex organs, affect libido, aggression, mood, and aspects of cognition, including verbal memory and visual-spatial skills, etc.[5] Testosterone deficiency is also associated with the increased fat mass (especially central fat), reduced insulin sensitivity, impaired glucose tolerance, increased triglyceride levels, cholesterol and decreased high-density lipoprotein (HDL).[6]

METHODS

This research employed an experimental research using a pretest-posttest control group design that used 22 male Wistar white rats (*Rattus norvegicus*), then divided into 2 groups of 11 each, namely the control group (P0) who were administered by distilled water 2.5 cc and treatment group (P1) who were administered by supplementary milk with whey protein 0.468 gr dissolved with 2.5 cc aquades, given once a day every day, each mouse was feed 20 grams/day with the remaining food weighed again. The treatment in the control group and treatment group was given for 3 weeks. Blood was taken before and after treatment for estrogen level and testosterone count. Data analysis included descriptive analysis, data normality test, data homogeneity, comparative test and analysis of the effects of Ancova treatment (covariance analysis).

Table 1. Distribution of Estrogen Levels and Testosterone Counts

Variables	n=22
Mean of estrogen levels, ng/mL (SB)	
Group P0 *pretest*	5.300 (0.490)
Group P1 *pretest*	11.60 (0.503)
Group P0 *post-test*	13.63 (0.327)
Group P1 *post-test*	16.14 (0.374)
Mean of testosterone counts, ng/mL (SB)	
Group P0 *pretest*	1.83 (0.191)
Group P1 *pretest*	1.86 (0.326)
Group P0 *post-test* 3 weeks	1.52 (0.260)
Group P1 *post-test* 3 weeks	1.69 (0.260)

Table 2. Data Normality Test Results between Estrogen Levels and Testosterone Counts

Subject Groups	Z	P
Estrogen		
Group P0 *pretest*	0.954	0.699
Group P1 *pretest*	0.994	1.000
Group P0 *post-test*	0.960	0.771
Group P1 *post-test*	0.972	0.906
Testosterone		
Group P0 *pretest*	0.954	0.699
Group P1 *pretest*	0.994	1.000
Group P0 *post-test*	0.960	0.771
Group P1 *post-test*	0.972	0.906

RESULTS

Table 1 shows the results of estrogen and testosterone after pretest and post-test analysis after 3 weeks of treatment. There was an increase in pretest and post-test estrogen hormone levels followed by a decrease in testosterone counts in both groups

In **Table 2**, the pretest and post-test estrogen levels after 3 weeks in each group were tested for normality using the Shapiro-Wilk test. The results showed that the data were normally distributed ($p> 0.05$)

Table 3 shows that the estrogen pretest and post-test 3 weeks in each group were tested for homogeneity using the Leven's test. The results show that the data variant was homogeneous ($p> 0.05$)

In **Table 4**, the comparability analysis aims to compare the mean of estrogen levels and testosterone counts between groups after being treated as an aquadest (group P0) and administered milk supplement with high whey protein (AMP Pro Performance) (group P1) for 3 weeks. The results of the significance analysis were tested by the Independent Sample-T test. The results showed that the average estrogen level of group P0 was 13.63 ± 0.326 ng/ ml and group P1 was 16.14 ± 0.374 ng/ ml with a value of $p<0.001$. This means that the two groups after being given treatment for 3 weeks had a significantly different mean estrogen level ($p<0.001$). The mean of testosterone counts of group P0 was 1.52 ± 0.260 ng/ ml and group P1 was 1.69 ± 0.260 ng/ ml with a value of $p<0.01$. This means that the two groups, after being administered treatment for 3 weeks, had a mean of testosterone counts that was significantly different ($p <0.01$).

The treatment effect analysis in the treated group in the form of aquadest (group P0) and administered by milk supplement with high whey protein (AMP Pro Performance) (group P1) was tested based on

Table 3. Data Homogeneity Test Results between Intergroup Levels

Subjects Group	F	P
Estrogen levels *pretest*	0.033	0.858
Estrogen levels *post-test*	0.179	0.677
Testosterone counts *pretest*	0.066	0.293
Testosterone counts *post-test*	2.233	0.872

Table 4. Average Comparison of Estrogen and Testosterone Levels between Groups after Treatment 3 Weeks (*post-test*)

Variable	Subject Groups	Mean Levels (ng/ml)	SD	t	P
Estrogen	Group P0	13.63	0.326	16.700	<0.001
	Group P1	16.14	0.374		
Testosterone	Group P0	1.52	0.260	3.960	0.01
	Group P1	1.69	0.260		

Table 5. Between group comparison of estrogen level at pretest vs after treatment.

Source of Variation	Number of squares	df	variant	Variant ratio (f)	p
Estrogen Pretest	0.000014	1	0.000014	0.000	0.992
Treatment	0.766	1	0.766	5.896	0.025
Error	2.469	19	0.130		
Corrected Total	36.896	21			

Table 6. Comparison of testosterone level at pretest and post-treatment

Source of Variation	Number of squares	df	variant	Variant ratio (f)	p
Testosterone Pretest	0.126	1	0.126	10.940	0.180
Treatment	0.163	1	0.163	2.516	0.129
Error	1.230	19	0.065		
Corrected Total	1.507	21			

the average estrogen and testosterone levels of each group before being given treatment (pretest) and after being given treatment for 3 weeks (3-week post-test). The results of the analysis using Ancova (covariance analysis) presented in Table 5 show a value of p> 0.05, which means that there was no influence of high estrogen value differences on the increase of hormone after controlling the estrogen pretest values in the two groups.

Table 6 shows the value of p> 0.05, which means there was no effect of the difference in the initial value of testosterone against the increase after controlling the value of testosterone pretest in the two groups did not find significant differences in the final results of the treatment effect.

DISCUSSION

Milk Supplements with High Protein Increase Estrogen Hormone

The results of this research indicate that before the treatment (pretest) the mean of estrogen level of the P0 group was 5.300 ± 0.490 ng/ ml and the P1 group was 11.603 ± 0.502 ng/ ml (p <0.05). After treatment for 3 weeks, the mean estrogen level of the P0 group was 13.639 ± 0.326 ng/ ml and the P1 group was 16.141 ± 0.347 ng/ ml (p <0.01). A paired t-test showed that in both the P0 and P1 groups there was a significant increase in estrogen levels (p <0.01) but in the P0 group there was a significantly greater increase (p <0.01). This indicates that administering whey protein (group P1) for 3 weeks has been able to increase estrogen levels. The normal level of estrogen in male Wistar rats is 0.020 - 0.100 ng/ ml (ALPCO, 2013b). This means that the increase in estrogen levels in the control group (P0) and the treatment group (P1) exceeded the normal limits of estrogen levels in Wistar rats.

Increased estrogen levels in group P1 from 11.603 ± 0.502 ng/ ml to 16.141 ± 0.347 ng/ ml. This is because the whey protein in the product contains phytoestrogens of 2.87 pg/ 100 g and *phytoprogesterone* of 1.93 pg/ 100 g. Administering food ingredients containing estrogen would obviously increase the levels of these hormones in the blood. The results of this research showed similar results to those using Pediasure and Nutrisure liquid substitute milk.[7] Pediasure milk has been shown to contain estrogen of 4.87 pg/ g and progesterone of 5.11 pg/ g, and the treatment of this milk in weaning mice during 21 days can increase estrogen levels from 0.04 ± 0.008 ng/ml to 0.05 ± 0.006 ng/ml. Besides that, it also uses Nutrient milk which is proven to contain estrogen at 4.98 pg/ g and progesterone at 5.84 pg/ g, and the effects of this treatment in weaning mice for 21 days could increase estrogen levels from 0.04 ± 0.008 ng/ ml to 0.06 ± 0.011 ng/ ml. Margo (2015) also conducted research on Morinaga BMT soya milk which contained 12.09 mg/ 100gr phytoestrogens which resulted in an increase in estrogen levels of 48.09% compared to controls.

This increase in estrogen levels was also due to the high process of converting testosterone to estrogen

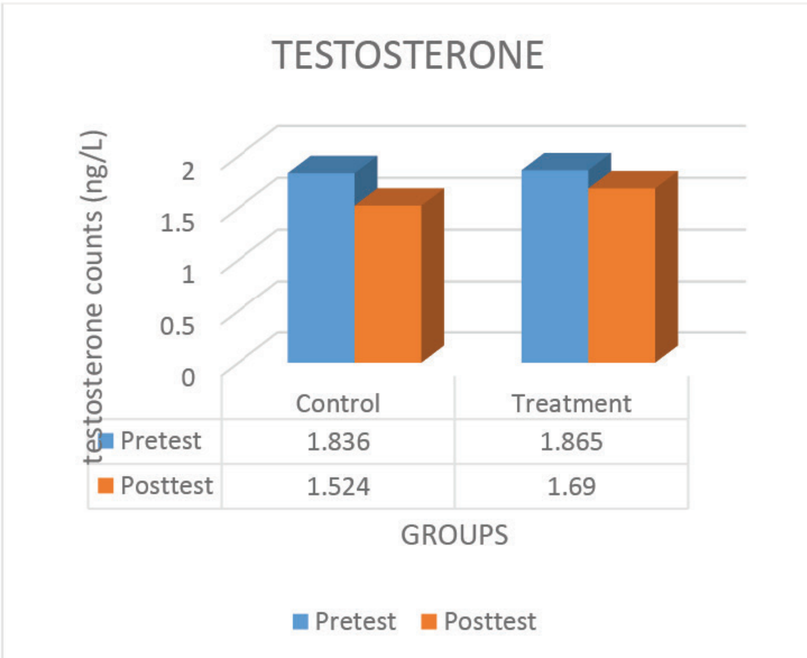

Figure 1. Graph of Changes in Estrogen Levels Before and After Treatment between Groups.

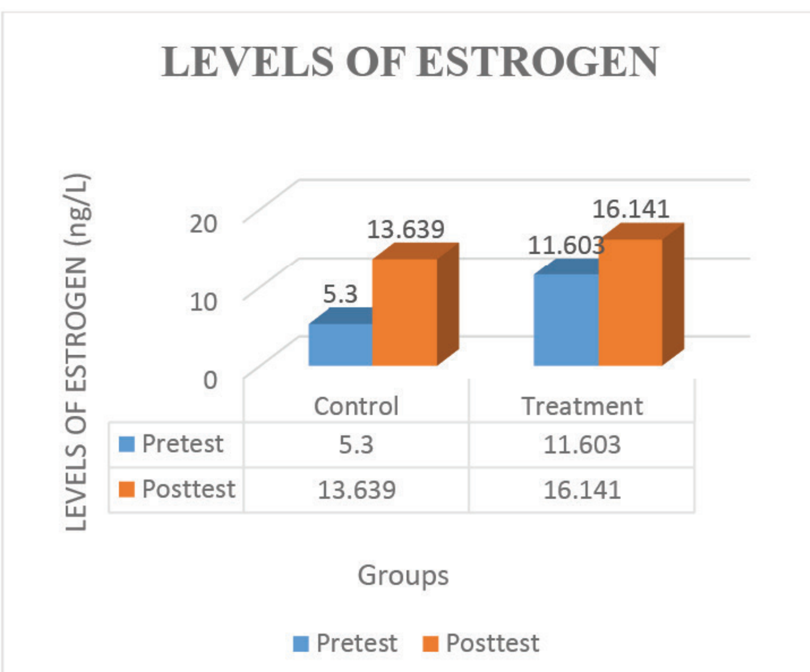

Figure 2. Graph of changes in Estrogen Level after treatment between groups.

by aromatization of testosterone from Leydig cells and germ cells. Germinal cells produce more estrogen than Leydig cells. In the testis, there was a conversion of testosterone to estradiol through the mechanism of aromatization of cytochrome P450 which causes high estrogen concentrations in the testicular fluid and seminal.[8]

The estrogen content in this supplement milk may be caused by the feeding of cattle sourced from plants, especially soybean straw. It is used to increase cow's milk production to 75% of the regular production.[8] Several other studies have shown that administration of whey protein did not affect estrogen levels in the blood. Research conducted by Kraemer et al. (2013) in 10 men aged 20-24 years showed that giving 20 grams of whey protein for 14 days did not increase estrogen levels.

High estrogen levels in men could affect reproductive health.[9] In men, high estrogen levels could cause gynecomastia, the enlargement of breast glands, generally occurs in men with excess weight. Increased estrogen levels in men also trigger prostate cancer, low sperm levels in semen, breast enlargement, infertility, and erectile dysfunction.[9,10] Therefore, the provision of high-whey protein supplements (AMP Pro Performance) is not good for men's health. Increased estrogen levels in the control group of this research may be due to factors from technical errors in estrogen analysis.

Supplements Milk with High Whey Protein (AMP Pro Performance) do not reduce Testosterone Hormones

The results of this research indicate that before the treatment (pretest) the average testosterone level of group P0 was 1.83 ± 0.190 ng/ ml and group P1 was 1.86 ± 0.325 ng/ ml ($p > 0.05$). After treatment for 3 weeks, the average testosterone level in group P0 was 1.52 ± 0.260 ng/ ml and group P1 was 1.69 ± 0.260 ng/ ml ($p > 0.01$). Paired t-test analysis showed that both in the P0 group there was a decrease in testosterone counts ($p < 0.01$) and P1 a decrease in testosterone counts was not significant ($p > 0.01$). Normal counts of male testosterone in Wistar rats are 0.66 - 5.4 ng/ ml.[11] This means that a decrease in testosterone counts in both the control group (P0) and the treatment group (P1) is still at the normal limit.

In group P1 there was a decrease in testosterone counts but not significantly. This is because the research time was only 3 weeks and the phytoestrogen level in the whey protein supplement was 2.87 pg/ ml, therefore, it requires a longer time to cause negative feedback from estrogen. This was inversely proportional to research conducted in Iran, finding that giving whey protein supplements for 3 weeks could increase blood testosterone when compared with the placebo.[12] Other studies have shown that giving whey protein for 3 months has significantly increased testosterone counts.[13] Therefore, a research conducted by Kalman et al., (2007) found that by giving whey protein, testosterone/ estradiol ratio increased.

An interesting finding in this research was that increased levels of estrogen in the blood as a result of the content of phytoestrogens and phytoprogesterone in this supplement do not reduce testosterone counts. The theory states that estrogen is negative feedback on GnRH and then decreases testosterone counts. High levels of estrogen should be followed by a decrease in testosterone counts.[14] However, from the results of this research, there was no significant reduction in testosterone counts which might be due to the 3-week research period. Phytoestrogens work like estrogen and were thought to show biological effects at the molecular, cellular, or physiological level. This phytoestrogen had the ability to bind to estrogen receptor (ER), with a bond affinity higher than ER-β compared to ER-α, this bond will facilitate the entry of phytoestrogens from the cytoplasm to the nucleus so that it could affect the transcription of DNA and RNA.[15]

CONCLUSION

Based on the results of this research, the conclusions are as follows: Administering milk or high whey protein supplement (AMP Pro Performance) orally increases estrogen hormone levels in male white rats *(Rattus norvegicus)*. and Administering milk or whey protein supplements (AMP Pro Performance) orally does not reduce testosterone counts in white mice *(Rattus norvegicus)*.
Further research in humans is needed in the form of observational studies (case-control) to be used as confirmation of the benefits and side effects of this supplementary milk product, and repeat testing with the same dosage and treatment by minimizing the possibility of technical error of examination.

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