Effect of Whey protein on the fertility of Male Albino Rat

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Abstract

This research aimed to determine the effect of Whey protein on the biochemical levels of reproductive hormones in male albino rats. An adult male albino rats (n= 36) were randomly divided into three groups (12 in each group). First and second groups orally administrated with (0.8 and 1.6 g) of whey protein, respectively, and third group as control administrated with normal saline for sixty days. After administration, body weights were recorded, the blood and seminal fluid were collected for physiological and fertility studies. The results showed a significant increase ($p<0.05$) in the mean body weights of treated group compared with control group. Hormones level showed treated groups were a significantly increased ($p\leq0.05$) in the mean of testosterone concentration, a significant decrease ($p\leq0.05$) in the mean of prolactin concentration and the luteinizing hormone concentration, but there is no significant change ($p\leq0.05$) has been noticed in the mean of follicle stimulating hormone concentration, compared to the control group. The sperms concentration rate ($X10^7$) increased significantly ($p\leq 0.05$), also, sperm characteristics showed the motility of sperms increased significantly in the percentage rate ($p\leq 0.05$), whereas the dead and abnormal sperms decreased significantly ($p\leq 0.05$) in the percentage rate. Regarding the fertility for the current study revealed there was no significant increasing ($p<0.05$) in the number of births within treated groups compared with control group.

Key words: Whey protein, hormones, Rat, biochemical level, fertility.
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Introduction

Whey protein is a mixture of proteins isolated from bovine milk. Whey contains about 50% of the total milk solids, including about 100% of the lactose and about 20% of the total protein. The proteins consist of α-lactalbumin, β-lactoglobulin, and serum albumin (Solak, 2012). Whey protein components provides and their known benefits to human health (Fenelon et al. 2018). It is a high nutritional value due to the essential amino acids (Katouzian, et al. 2020). Whey, a protein compound derived from milk, is touted as a functional food with a number of health benefits. The biological components of whey exhibit a range of immune-boosting properties (Bumrungpert , 2018). Additionally, whey has the ability to act as an antioxidant, antihypertensive, anti-

neoplastic, hypo-lipid-emic, antiviral, antibacterial, and chelating agent. The primary mechanism by which whey is believed to exert its effect is the intracellular conversion of the amino acid cysteine to glutathione, which is a powerful intracellular antioxidant (Arora, Singh, and Sindhu 2019). Whey protein is an excellent protein for individuals of all ages to improve and maintain their health. Traditionally, whey protein was only used by athletes and bodybuilders to promote muscle growth (Bo, 2019). Additionally, whey protein increases muscle mass, boosts metabolism, aids in weight management, and also benefits overall health (Khaire and Gogate 2019). There are some nutritional supplements that affect the concentration and activity of hormones in the body, mention (Al-Sa'aidi, 2009) that daily oral administration of alcoholic extract of *Nigella sativa* lead to clear improvement of male rats fertility, showed a significant increase ($P<0.01$) of testosterone and luteinizing hormone and a significant decrease ($P<0.01$) of follicle stimulating hormone in treatment groups compared with control. The present study aimed to investigate the adverse effects of oral administration of Whey protein on male albino rats through the study of the levels of some biochemical parameters of male involve Testosterone, Prolactin, LH and FSH hormones. Study the fertility of treated male.

Materials and Method

Whey protein Collection

The whey protein utilized in Iraq are always imported, entire products that are made abroad. Whey protein was collected from sport center in Baghdad. In this study, different doses of whey protein were used (0.8 and 1.6) g. The doses were prepared in accordance with the human dose (2-3 g\kg of body weight) (Campbell BI, 2018).

Animal and Experimental protocols

The animals were obtained from the laboratory animal center of AL- Qadisiya University. The rats were housed in normal laboratory conditions and had free access to food and drinking water. 36 albino rats were randomly divided into 3 groups. Each group contains (12) rats in a separate cage. The dose (0.8) g was given to group 1, and (1.6) g was given to group 2, and compared to control group that given normal saline. The treatment continued for sixty days. After the experiment end, three treated male rat from each group mate with healthy female (one male with two female) for fertility study.

Weight measurements

Body weight was measured before and after experiment by using a scale equipped balance.

Blood Collection

After the end of the treatment period, blood samples were collected by stab-heart method, immediately before killing the animals, samples were collected with syringe and blood samples were placed in a sterile bendroff tubes in a centrifuge at 2000 rpm for 10 minute to separate the serum. The concentration of hormones testosterone, prolactin, FSH and LH were measured (Al-Warid, 2012).

Seminal fluid collection

Seminal fluid samples were collected from the distal region of the epididymis (cauda) according to the method of Mali et al. (2002). Seminal fluid samples were used for evaluation of sperm concentration, motility, dead and abnormal sperms (Narayana et al, 2005). An average of sperms was counted on slide under a microscope.

Statistical Analysis

Statistical Analysis of data was performed using SAS (Statistical Analysis System) version (26) 2019. One way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. ($p\leq0.05$) was considered statistically significant.

Result

Body Weights

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The results showed a significant increase ($p<0.05$) in the mean body weights of treated group with concentration (0.8 g) and the second treated group with concentration (1.6 g) of whey protein compared with the control group. The highest value of body weight (382.00±10.45) at the dose was (1.6 g) for the group treated with whey protein compared with the control group (271.91±4.39) (Table.1).

Table (1): Effect of different doses of whey protein on the body weights in rats before and after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of body weight (g) before treatment	Mean ± SE of body weight (g) after treatment
Control	12	177.16 ± 4.64	271.91 ± 4.39a
Group 1	12	175.08 ± 4.48	327.66 ± 6.61b
Group 2	12	178.75 ± 5.55	382.00 ± 10.45c
LSD	28.293		43.626*

The means with different letters within the same column differ significantly from each other.
*: The mean difference is significant at the (P≤ 0.05) level.

Effect on the level of hormones in male rats:

Testosterone hormone

The results showed a significant increase ($p\leq0.05$) in the mean testosterone concentration of male rats treated with whey protein and doses (0.8 and 1.6 g) compared to the control group. The highest percentage of the hormone was (4.273 ± 0.150) at the dose (1.6) g compared to the control group which reached (3.333 ± 0.107) (Table.2).

Table (2): Effect of different doses of whey protein on the testosterone hormone concentration in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of testosterone hormone concentration (ng/ml)
Control	5	3.333 ± 0.107a
Group 1	5	3.562 ± 0.091ab
Group 2	5	4.273 ± 0.150c
LSD	0.736	

The means with different letters within the same column differ significantly from each other. *: The mean difference is significant at the (P≤ 0.05) level.

Prolactin hormone

The results in table (3) showed a significant decrease ($p\leq0.05$) in the average of prolactin hormone concentration of treated animals with whey protein. The lowest percentage of the hormone was (13.188 ± 0.458) at the dose (1.6) g compared to the control group, which reached (16.094 ± 0.244).

Table (3): Effect of different doses of whey protein on the Prolactin hormone concentration in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of Prolactin hormone concentration (mIU/ml)
Control	5	16.094 ± 0.244a
Group 1	5	14.434 ± 0.299b
Group 2	5	13.188 ± 0.458c
LSD	2.133	

The means with different letters within the same column differ significantly from each other.
*: The mean difference is significant at the (P≤ 0.05) level.

Luteinizing hormone (LH)

The statistical results appeared that there was non-significant decrease in the percentage of the LH hormone in the treated groups, the lowest percentage (3.369±0.217) in the dose (0.8) g and (3.535±0.361) in the dose (1.6) g were recorded respectively compared to the control group, which was (4.261±0.276) (Table.4).

Table (4): Effect of different doses of whey protein on the LH hormone concentration in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of LH hormone concentration (mIU/ml)
Control	5	4.261 ± 0.276
Group 1	5	3.369 ± 0.217
Group 2	5	3.535 ± 0.361
LSD	1.796	

The means with different letters within the same column differ significantly from each other. *: The mean difference is significant at the (P≤ 0.05) level.

Follicle Stimulating hormone (FSH)

The statistical results appeared that there was non-significant decrease in the mean percentage of the FSH hormone in the treated groups, (3.289 ± 0.287) in the dose (0.8) g and (3.013 ± 0.292) in the dose (1.6) g were recorded respectively compared to the control group, which was (3.353 ± 0.280) (table 5).

Table (5): Effect of different doses of whey protein on the FSH hormone concentration in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of FSH hormone concentration (mIU/ml)
Control	5	3.353 ± 0.280
Group 1	5	3.289 ± 0.287
Group 2	5	3.013 ± 0.292
LSD	1.770 NS	

The means with different letters within the same column differ significantly from each other. NS: No significant. *: The mean difference is significant at the (P≤ 0.05) level.

Changes in sperm characteristics:

Concentration of sperms

The results showed increased in the sperms concentration rate ($X10^7$), as the highest percentage of sperms concentration reached (19.00 ±1.00) in the dose (1.6) g/kg compared with the control group, which amounted to (17.200 ± 0.86) (Table.9).

Table (9): Effect of different doses of whey protein on the percentage of sperms count rate ($X10^7$) in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of concentration ($X10^7$)
Control	5	17.200 ± 0.86 a
Group 1	5	18.200 ± 1.06 a
Group 2	5	19.00 ± 1.00 a
LSD	6.038	

The different letters within the column indicate the presence of significant differences. (P≤ 0.05).

Sperm motility percentage:

Through the experiment, it was noticed that the motility of sperms increased significantly in the percentage rate (p≤ 0.05), as the highest percentage of sperms motility reached (92.40 ± 1.02) in the dose (1.6) g, compared to the control group, which amounted to (87.00 ± 1.7) (Table. 6).

Table (6): Effect of different doses of whey protein on the percentage of sperm motility in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of motility (%)
Control	5	87.00 ± 1.70 a
Group 1	5	91.60 ± 1.07 b
Group 2	5	92.40 ± 1.02 bc
LSD	8.283	

The different letters within the column indicate the presence of significant differences. (P≤ 0.05)

The dead sperm percentage

The results showed that the dead sperms decreased significantly in the percentage rate (p≤ 0.05), as the lowest percentage of dead sperms reached (13.80 ± 0.73) in the dose (1.6) g compared with the control group, which amounted to (17.00 ± 1.00) (Table.7).

Table (7): Effect of different doses of whey protein on the percentage of dead sperms in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of dead sperms (%)
Control	5	17.00 ± 1.00 a
Group 1	5	14.20 ± 0.58 b

Group 2	**5**	**13.80 ± 0.73 bc**
LSD	**4.878**	

The different letters within the column indicate the presence of significant differences. (P≤ 0.05)

Percentage of abnormal sperm

The results showed the abnormal sperms decreased significantly in the percentage rate (p≤ 0.05), as the lowest percentage of abnormal sperms reached (14.80 ± 0.73) in the dose (1.6) g compared to the control group, which amounted to (18.80 ± 0.86) (Table.8).

Table (8): Effect of different doses of whey protein on the percentage of abnormal sperms in rats after treatment, and its comparison with control group animals.

Groups	N	Mean ± SE of abnormal sperms (%)
Control	**5**	**18.80 ± 0.86 a**
Group 1	**5**	**15.00 ± 0.70 b**
Group 2	**5**	**14.80 ± 0.73 bc**
LSD	**4.747**	

The different letters within the column indicate the presence of significant differences. (P≤ 0.05)

Effects on Fertility:

At the end of the experiment and after mating processes (1 treated male with 2 healthy females), the results showed that there was no significant increasing (p<0.05) in the number of births. The number of the births was (9.666 ± 0.666) at the dose (1.6) g, which is the highest dose resemble to the control group, which reached (8.166 ± 0.477) while the lowest mean number was (9.000 ± 0.577) in the dose (0.8) g (Table.10).

Table (10): Effect of different doses of whey protein on the number of births in rats after treatment, and its comparison with control group animals.

Groups	No. of treated males	No. of the females	Mean ± SE of birth (no.)
Control	**3**	**6**	**8.166 ± 0.477**
Group 1	**3**	**6**	**9.000 ± 0.577**
Group 2	**3**	**6**	**9.666 ± 0.666**
LSD			**3.490 NS**

The different letters within the column indicate the presence of significant differences, (P≤ 0.05). N: The number of female rats, no.: number of births, NS: No Significant.

Discussion

The present study aimed to investigate the effect of whey protein on the reproductive hormone of male albino rats. Body weight showed a significant increase (p<0.05) in the mean body weights of treated groups. The random intake of whey protein supplements without consulting the doctor led to an increase in body weight of the experimental animals, is most likely increase in muscle protein or in water retention within the body, or both. This result is in agreement with several previous studies like (Cawood, 2012) who study on the effect of high protein oral nutritional supplements (ONS) on human, he reported and explain the increases in body weight due to nutritional supplement ingestion. Other study suggested body weights were increased in rats fed supplemental conjugated linoleic acid (Nall, 2009). The study was conducted with a swine

model that dietary L-arginine supplementation may increase muscle gain (Tan, 2009). While (Solerte, 2008) indicated that nutritional supplements with the oral amino acids mixture significantly increased whole-body lean mass in elderly subjects. Testosterone hormone showed a significant increase ($p \leq 0.05$) in the mean concentration of male rats treated with whey protein. The increase in testosterone concentration may be due to the effect of whey protein during stimulation of Leydig cells in the testes as well as LH stimulation to product testosterone in Leydig cells, which in turn may act on Sertoli cells. Many studies improved the effect of the supplements on testosterone production, (Zamir, 2021) emphasized significantly increase in testosterone hormone in the men that supplemented with vitamin D. Branched chain amino acids BCAA proteins supplementation resulted in a significant increase in testosterone hormone concentration in men (Sharp, 2010). Prolactin hormone showed a significant decrease ($p \leq 0.05$) in the concentration average of treated animals with whey protein. The decrease in prolactin concentration may be due to the effect of whey protein according to (Beulens, 2004) from the results of the current study observed the presence of a significant decrease in prolactin hormone in cows after a-lactalbumin and carbohydrate supplement diets, because the secretion of prolactin is stimulated by serotonergic neurons terminating in the hypothalamus, and prolactin secretion is shown to be effected by a-lactalbumin and carbohydrate supplement diets. Different readings of the Luteinizing hormone (LH) concentration were observed, excess use of proteins may disturb the endocrine function by affecting the function of the hypothalamus-pituitary-gonads. From the results of the current study observed the presence of a significant decrease in Luteinizing hormone (LH) in cows after vitamins C and E supplement used according to (Uzun, 2009), because vitamins may induces pathological changes in the testicular tissues. Soy protein powder supplementation in male caused significantly decreased LH concentrations (Goodin, 2007), because soy isoflavones possess some estrogenic activities, it is postulated that soy may exert an effect on the hypothalamic-pituitary-gonadal axis to reduce-regulate androgen synthesis. However, one recent study reported reduced levels of the luteinizing hormone (LH) in twelve athletes, who were consuming high doses of whey protein supplementation daily, before and after exercise according to (Rahman, 2018) suggesting defective in pituitary gland function. No significant ($p<0.05$) in the average of Follicle Stimulating hormone (FSH) concentration of treated animals with whey protein. There are some studies that support the effect of Rosmarinic acid intake on the serum concentrations of certain reproductive hormones in men, Rosmarinic acid showed no significant differences for FSH concentration (Khaki, 2012), Rosmarinic acid can balance sex hormones in rats. Other studies documented, effects of ingestion of soy protein containing various concentrations of isoflavones showed no significant differences were found for follicle-stimulating hormone (Persky, 2002). And affecting the synthesis of hormones involved in reproduction by disturbing the pituitary-testis axis (Mehrdad, 2013). The results showed increased in the sperms concentration rate ($X10^7$), LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and indirectly stimulates spermatogenesis via testosterone. Because testosterone is necessary for sperms concentration. Increased testosterone level caused activates Sertoli cells, which promote differentiation of spermatogonia. FSH maintains sperm-forming cells and thus maintains sperms concentration. Some studies documented that the lysine, methionine, threonine, tryptophan and valine in the diets through the addition of synthetic amino acids supplementation to boar

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increased sperms concentration (Dong, 2016). The other study demonstrated that that supplementing Vitamin E can have a positive role in improving semen quantity via protecting testicular cell membrane and mitochondria from antioxidant abilities (Yue D, Yan L, 2010). The results of the current study are in line with what was stated in several recent studies, which stated that vitamin C caused an increase in fertility almost and a relatively increase in sperm producing cells in rats (Vijayprasad, 2014) (Shabanian, 2017), because increased testosterone promote sperm concentration. Whereas, the sperm characteristics were noticed that the motility of sperms increased significantly, the reason for this is due to the high percentage of testosterone, which is responsible for the formation of sperm in the testes and for the secretory activity of the organs and gonads, which in turn make up the seminal fluid that contains the materials necessary for the movement of sperm. When the percentage of testosterone hormone is high, this affects the components of the semen and then leads to an increase in the movement of sperm. Study mentioned the selenium and vitamin E supplementation resulted in a significant increased sperm motility and viability (Keskes-Ammar, 2003) due to decrease in an oxidative stress marker (malondialdehyde). It also agreed with the studies (Shalaby, 2004) and (Cito, 2020) which stated that the concomitant administration of α-tocopherol and simvastatin to hypercholesterolemic rats markedly increased fertility index and sperm motility and viability associated with a significant reduction of sperm cell abnormalities, it is improved their reproductive efficiency and produced additional protection. The dead of sperms decreased significantly in the percentage rate ($p \leq 0.05$), may be due to whey protein has the ability to act as an antioxidant, and prevent sperm DNA damage. Study documented that vitamin C supplementation in infertile men with idiopathic oligozoospermia induced a significant increase in the percentage of normal spermatozoa, as well as, decreased in the dead sperm (Eslamian, 2017) because it is improved their reproductive efficiency. Another study by Greco and colleagues (2005) noticed that treatment with vitamin E and vitamin C resulted decreased sperm DNA damage in men. The abnormal sperms decreased significantly in the percentage rate ($p \leq 0.05$), because testosterone is necessary for normal sperm development. Increased testosterone level caused activates sertoli cells, which promote differentiation of spermatogonia. Some studies confirmed that vitamin C supplementation improved normal sperm morphology in men (Cyrus, 2015) due to regulates testis function. Other study performed that 2-month supplementation of vitamin E and vitamin C resulted in a significant reduction of sperm DNA damage in men (Greco, 2005) because it act as antioxidant treatment. Supplementation of vitamin E to mice groups declined lipid peroxidation, depressed the percentage of sperm abnormality, and increased the activity of antioxidant enzymes. The role of nutritional supplements in reducing oxidative stress-related effects on spermatogenesis (Acharya, 2008). After mating the treated male rats with healthy females, there was no significant ($p<0.05$) in the number of births. The number of births did not change between the different concentrations and the control group. This study agreed with (Ketheeswaran, 2020) who suggested that the highest recommended human dose of whey protein supplementation do not significantly impair fertility in male mice. One study demonstrated that oral administration of L-arginine had no effect whatsoever on sexual competence of male rats (Ratnasooriya, 2001).

Conclusion:

The whey protein used in this study cause severe effects on weights, hormones and seminal fluid. So, further studies are necessary to investigate the chemical compositions of whey protein and their effects on organs.

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