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The Effect of Corn Oil in Sperm Parameters and Levels of Some Hormone, Elements, and Biochemical Parameters in Serum of White Male Rabbits

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Abstract

Corn oil is a pale-yellow drying or semidrying edible oil extracted from the germ of corn and used for cooking and salad preparation. Corn oil is finding wide application at home, restaurants, hotels, hospitals, and other food industries. Nowadays it is finding wide application at all places of food preparation. The principal goal of this study was to investigate the role of corn oil on the fertility, levels of some hormone, elements, and biochemical parameters of adult male rabbits. Fifteen adult male rabbits were randomly distributed into three groups, 5 animals of each groups: Group (1) was given orally distilled water and another groups (2 and 3) of rabbits were treated orally with 2 and 2.5 ml/day of corn oil alone as vehicle. The results show that corn oil caused significant differences ($P > 0.05$) in sperm parameters and insignificant differences ($P < 0.05$) in levels of some Hormone, some trace element, and some biochemical parameters of male rabbits. It was concluded that the addition of corn oil may improve semen quality of any animal, mammals as well as human being.

Keywords: corn oil, rabbits, sperm parameters, hormone, elements, biochemical parameters.

1. Introduction

Corn is the small hard seed of any of the cereal grasses used for food. Corn oil is edible and therefore is used in the preparation of food items. It is the most widely consumed in the world because this oil is generally less expensive than most other types of vegetable oils. Recently, many researchers have discovered the strong antioxidant potential mostly in corn oil (Orhun 2013). Corn oil has generally been assumed to be biologically inert with regard to reproductive performance and developmental status (Kuperman *et al*. 2011). Sperm cells contain very high proportions of polyunsaturated fatty acids (PUFA) (Rooke *et al*. 2001), and normal spermatozoa possess a higher percentage of the most representative PUFA (C22:6 n-3) than those detected in blood serum phospholipids and in other cell membranes (Lenzi *et al*. 1996). The lipid composition, the degree of PUFA unsaturation, and the proportion of sperm PUFA have been shown to affect sperm quantity (Cerolini *et al*. 2000; Safarinejad & Safarinejad 2012). Animals cannot synthesize n-6 or n-3 fatty acids de novo because of a lack of the appropriate fatty acid desaturase enzymes. The n-6 PUFA and the n-3 PUFA therefore need to be provided in the diet as these PUFAs are essential for numerous processes including growth, reproduction, vision, and brain development (Gurr *et al*. 2000). The most important feature of lipid composition of the rabbit semen is the extremely high proportions of long chain polyunsaturated fatty acids (PUFAs) in the phospholipid fraction of spermatozoa. High PUFA proportion of the rabbit sperm is necessity antioxidant order to maintain specific membrane properties (fluidity, flexibility, etc) (Mourvaki a *et al.* 2010; Mourvaki b *et al.* 2010). It is known that the fatty acid composition of sperm membranes, especially their unsaturated components, determine their biophysical characteristics such as fluidity and flexibility as appropriate for their specific functions, including sperm motility and fertilizing capacity (Khatibjoo *et al.* 2011). Metals play a vital role as structural and functional components of protein and enzymes in cells . Each mineral plays a number of different functions in the body , the most important pathway of metals to transport into human is from soil to plant and from plant to human (Kirmani *et.al*. 2011). Some metals such as Ca, Mg and Zn have been reported to be essential for human health, whereas others such as Pb, Cd and Al have been identified as toxic. Rests of the elements are not toxic to human unless they are present in high concentrations (Nasli-Esfahani *et al*. 2011). The present study was performed to evaluate the effect of corn oil on epididymis sperm parameters, the levels of some hormone, and some biochemical parameters of male rabbits. Also the present study is concerned with the determine whether corn oil can influence the bioavailability of several elements important for the human healthy.

2. Materials and Methods

2.1 Experimental animals

Fifteen (15) New Zealand White male rabbits aged 4 months and averagely weighing 1.513 g and put in cage under control of water, diet, light duration (12hour light-12hour dark). These animals were divided into 3 groups (5 animals for each group), control group was treated orally with distill water and experimental group was treated orally with 2 and 2.5ml/daily of corn oil and for 50 days. The animals were seduced after a period end of experience with chloroform then the caudal end of the epididymis was cut for sperm analysis. Blood samples were collected in tubes (plain and coated with anticoagulant). Plain tubes centrifuged for separation of serum at 3,000 rpm for 15 minutes , and sera were stored at –20 ^{0}C for determination of the level of some hormone and some biochemical measurements.

2.2 Assay of Biochemical Parameters

Total cholesterol and total protein were determined using Bio. Labo. S.A. kit (France). Zinc and iron were determined by Atomic Absorption Spectrophotometer (Shimadzu AA-6300).

2.3 Serum hormonal analysis

The anesthetized animals were immediately used for collecting blood samples from heart. The blood was drained into glass tubes, coagulated at 37ºC, centrifuged at 3,000 rpm for 15 minutes and serum was then stored at -80 ºC till the measurement of hormones. The concentration of testosterone (T) hormone was measured by R & D System kit (INC.U.S.A), and concentration of prolactin (PRL) hormone was measured by Cusabio Biotech Co. kit (LTD), while concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were analyzed using Monobind Inc. kit (U.S.A.). In all cases, serum hormone analysis was done according to the manufacture's protocol.

3. Statistical analysis

All obtained data from the analysis were normally distributed. The differences between the treated and control groups were statistically evaluated using a Student's F-test. All data are expressed as the mean values±SE, with significant values at $p<0.05$.

4. Results

Body, testis, epididymis weight for each animal; body weight was recorded after termination period. After termination, there were significant increase ($p<0.05$) in body weight of rabbits treated with 2.5 ml of corn oil compared to control group, as well as, the results showed insignificant increase in the testis and epididymis weight of corn oil groups compared to control group ($P>0.05$) (Table 1). In sperm parameters**,** the results showed a significant increase ($p<0.05$) in each of the concentration of sperm in the testis, the average number of sperm per 1 g of testicular, epididymis sperm number, sperm motility, and sperm viability in corn oil group compared to control group (Table 2). The treatment of animals with corn oil significantly increased ($p<0.05$) the percentage of progressively motile sperm (a + b), and the percentage of topical motile sperm (c), and decreased ($p<0.05$) the percentage of non-motile sperm (d) when compared with control group (Table 3). Hormonal levels, rabbits treated with corn oil showed insignificant elevation ($p>0.05$) in the level of T, PRL, FSH, and LH (figure 1, 2, 3, and 4) respectively. Figure 5 and 6 revealed that corn oil caused a significant decreased ($P< 0.05$) in serum iron, and insignificant increased ($P> 0.05$) in serum zinc of treated group compared to the control groups. Figure 7 and 8 showed insignificant decrease ($P > 0.05$) in serum total cholesterol concentration and insignificant increased ($P> 0.05$) in serum total protein of treated group when compared with control group.

5. Discussion

Our study demonstrated that corn oil affected body, testicular, and epididymis weight of rabbits. Significant increase in body weight might be due to insignificant increase in organs weight or food consumption. Taib *et al.* (2013), showed that body weight of treated groups increased throughout the experimental period. Also there were no significant differences in the initial body weights and average daily food intakes, weight gain tended to increase as the ratio of n-3/n-6 PUFAs increased (Estienne *et al.* 2008). In 2006, Cerolini *et al.*, have been showed that mean body weight and feed intake of the birds in the different treatment groups showed no significant differences, there was no interaction of corn oil on body weight and feed intake. While Wathes *et al.*

(2007), reported that foam weight was not affected by corn oil. Mice treated with corn oil had a body weight gain of 17.6 % , the observed *in vivo* corn oil-induced increase in body weight. This may suggest that components in corn oil have the ability to cause changes in genes expression (Kildemo 2012). Binjhade & Shrivastava (2013), that received daily dose (0.2ml/day) of vehicle i.e. corn oil caused a constant increase in male *Mus musculus* body weight throughout the experiment. In agreement with Juliyana *et al*. (2011), observed no significant difference in the testis weight, width and length of corn oil control groups, our results also showed insignificant increase in testicular weight in rabbits treated with corn oil. While Taib *et al*. (2013), relieved that the relative weight of the testes was insignificantly higher in the corn oil group than control group. Testis weight was compared relative to body weight, no significant differences were found among experimental groups (Kildemo 2012). In 2013, Afolabi *et al*., founded that testicular weight (1.98±0.09) were significantly higher ($P<0.001$) in the rats treated with vehicle, that is, corn oil, as well as, our results indicate that treated with corn oil caused insignificant increase in epididymis weight compared with the control group, with $p>0.05$, as agree with Goyal *et al.* (2001), founded that absolute weight of all reproductive organs, including the testis, head and body of the epididymis, tail of the epididymis, and seminal vesicle, was significantly higher in corn oil control group. The epididymis secretes into its luminal environment, region specific proteins and glycoproteins, thus providing the favorable milieu for post testicular maturation of the sperms. Hence, alteration in the epididymal structure or function might contribute to male infertility (Thimon *et al*. 2007). In this study, the insignificant increased in the weight of reproductive organs, such as the testis and epididymis, could be due to the insignificant increased of androgen availability. The main constituents of corn oil (co): polyunsaturated fatty acids, linoleic acid and a small amount of linolenic acid (together -54%), monounsaturated oleic acid (-25%), saturated palmitic acid (-10%), saturated stearic acid (<2%), total triglyceride content (~95%), co has been used extensively without incident as a vehicle to administer test chemicals by gavage (forced ingestion) in a variety of toxicity tests and dietary studies (Kuperman *et al*. 2011). The current results also showed a significant increase in sperm concentration, sperm concentration per 1 gm of testis, sperm motility, and grade activity of treated group as compared to control group. Furthermore, corn oil significantly increased sperm viability and normal sperm morphology compared with the control group. Treated group also showed less abnormality as compared with control group. Probably this was due to insignificant increased testosterone level of rabbits given corn oil which necessary for normal sperm development. Testosterone activates genes in sertoli cells, which promote thus increases spermatogenic cells in seminiferous tubule of corn oil treated group. Motility and viability of sperm appear to be the most important parameters for the assessment of sperm fertilization capacity and the integrity of sperm membrane may play an important role on these parameters. In 2011, Adabi *et al*., noted that no effects of corn oil on sperm motility, but the greatest motility (forward motion) of spermatozoa was in corn oil groups (80.2%). An interesting finding in sperm viability assay was that corn oil increased sperm viability compared to control group. Since the application of antioxidants including corn oil has been shown to enhance sperm viability (Dormann 2003; Orhun 2013), it is reasonable to suggest that corn oil supports sperm antioxidant system to improve sperm viability. This effect may also explain increased sperm viability in corn oil group compared to control group. Khatibjoo *et al.* (2011), have been showed that the addition of cooked oil as a source of trans fatty acids to the village rooster's diet significantly increased rapid motility of spermatozoa, linearity movement. The results of present study are partially in agreement with those (Imani *et al*. 2006; Zaniboni *et al*. 2006; Afolabi *et al*. 2013) whom reported that proportion of viable spermatozoa was significantly increased in the ejaculates collected from the birds fed cooked oil. In 2008, Estienne *et al*., showed that different ratios of n-3/n-6 PUFAs had no effects on the testis index, but improved sperm quality. With an increasing n-3/n-6 PUFA ratio, sperm density and motility were increased, and the sperm deformity rate tended to decrease. More recently, it was found that boar diets fortified with n-3 rich fatty acid additives enhanced the sperm total number of average ejaculations, and the morphological integrity of sperm was improved. Furthermore, the ratio of n-3/n-6 PUFAs in boar sperm were positively correlated with sperm motility, viability, normal morphology, and normal plasma membranes (Amin *et al*. 2011), and excessive n-3 PUFA supplementation decreased the sperm density and motility in the experiment, which indicated the importance of the n-6/n-3 PUFA ratio in sperm quality. Al-Daraji *et al*. (2010), found the proportion of n-3 fatty acids in spermatozoa from Japanese male quail fed corn oil 4.3% and that of n-6 fatty acids was 33.3%. In addition, it was reported that diets containing different lipid sources changed the lipid contents of sperm, mainly affecting the sperm head and body membranes (Bongalhardo *et al*. 2009). In all species, phospholipids are the major lipid component of spermatozoan membranes. In addition, they contain large amounts of polyunsaturated fatty acids. PUFAs of the n-3 and n-6 series are essential fatty acids, because they cannot be synthesized in vertebrates and must be provided in the diet (Parks & Lynch 1992). Since the 19th century, many researchers have reported that lipids are a basic component of semen, contributing to the membrane structure of spermatozoa, the metabolism of the sperm cells, and their ability to capacitate and

fertilize the female gamete. In birds, the lipid composition of spermatozoa has an influence on fertility (Ansah & Buckland 1982). Spermatozoa are rich in phospholipids (about 80% of total lipids) and may be quite sensitive to the availability of dietary PUFAs (Anderson & Conner, 1994). However, Cerolini *et al*. (2006), reported both n-3 and n-6 rich diets affect the semen production. In spite of the fact that highly variable results have been reported for the effect of n-6 rich diets on spermatozoa production, a positive effect on semen volume and total sperm number (Cerolini *et al*. 2000). Blesbois *et al*.(1993), showed that variation in sperm concentration is reflected in the degree of motility of spermatozoa. Both n-3 and n-6 polyunsaturated rich diets improve the progressive movement of the male gametes. The percentage of dead sperm was less in the corn although there was no effect of corn oil on the percent of abnormal sperm per ejaculate was also excellent motility. The effects of corn oil treatment on T, PRL, FSH, and LH in serum were analyzed. The concentrations of T, PRL, FSH and LH in serum increased insignificantly in the corn oil treated group. The increased concentrations of serum testosterone could result from increases in the number of leydig cells and/or the repair of their structure. Regulation of male reproductive system occurs via a negative feedback loop involving the hypothalamus, anterior pituitary and testicles, which is referred to as the HPT axis. The gonadotropin releasing hormone (GnRH) is from the hypothalamus. Gonadotropin including FSH and LH from the pituitary are affected by a negative feedback from testicular hormones including testosterone and other sexual hormones (Hayes *et al*. 2001). The mechanism of feedback control of FSH is regulated by a sertoli cell product called inhibin B (Meachem *et al*. 2001). Under such a situation, the sertoli cells are found to produce less inhibin B, and then FSH released from the pituitary is increased significantly due to a negative feedback action (Boepple *et al*. 2008). Veldhuis *et al*. 2009, founded that by increasing the ratio of n-3/n-6 PUFAs, the concentrations of GnRH, FSH, LH, and T increased. In males, the hypothalamus secretes GnRH, which binds to GnRH receptors on the gonadotropic cells to stimulate the release of FSH and LH into the circulation. LH stimulates the interstitial cells located in the testes to produce testosterone, and FSH plays a role in spermatogenesis. The putting rams on a high energy diet increased GnRH pulse frequency, testicular mass, and sperm production (Martin *et al*. 1994). In 2013, Yan *et al*., reported that intake of an appropriate n-3/n-6 PUFA ratio in the diet of rats increased sperm characteristics and enhanced the structure integrity of testis and sperm, thereby improving reproductive performance, which may be related to changes in hormone metabolism. These findings provide a sound basis that a balanced n-3/n-6 PUFA ratio will be beneficial to male reproduction. Therefore, there is a necessity to determine an appropriate n-3/n-6 PUFA ratio in man and different male animals in the future. In 2001, Goyal *et al*., have been shown the mean plasma T concentration and LH concentration were (2.3–2.6 ng/ml) and (0.18–0.42 ng/ml) in the corn control groups respectively. Also lipid saturation did not change concentrations of serum prolactin during diestrus or proestrus (Clintion *et al*. 1984). Minerals bioavailability was measured by the habitual consumption of foods such as wheat, rice, corn and soy and in a study of the Chinese population showed that the amounts of phytate and fiber in these foods enabled the formation of insoluble compounds that decreased the iron bioavailability (Ma *et al*. 2005). Dietary fat positively affects perhaps the iron absorption by the chelating action of fatty acids (Bueno *et al*. 2013). Numerous interactions exist between the different trace elements affecting absorption via the gastrointestinal tract. Factors affecting bioavailability of trace elements include the actual chemical form of the nutrient (eg., organic form of iron is better absorbed than the ionic form), antagonistic ligands (eg., zinc absorption is decreased by phytate and fiber; iron absorption is decreased by fiber), facilitatory ligands (eg., zinc absorption is aided by citric acid or iron absorption is increate by amino acids or fermented products), and competitive interactions (eg., iron depresses the absorption of copper, and zinc; zinc depresses copper absorption and vice versa)(Sriram *et al*. 2009). Our results indicate that corn oil has a positive influence on zinc and iron elements. In humans, a diet rich in polyunsaturated fatty acids like linoleic acid can reduce iron retention and balance as compared to highly saturated fatty acids (Lukaski *et al*. 2001) and in turn affecting mineral status. According to some researchers, olive oil may exert certain influence on iron status and utilization which may be related to certain alterations in iron absorption or fatty acid composition of cellular membranes (Milin *et al*. 2001). Iron is essential also for normal collagen synthesis acting as cofactor for prolyl-hydroxylase as reported before in various models of iron overload state (Poli & Parola 1997). However, dietary fat may alter absorption and utilization of iron either in human or animal models, it founded a significant decrease in serum iron along with increase in testosterone level in the group that received dietary iron only (control group). It mean that the iron overloaded diet enhances oxidative stress and inflammation leading to decreased spermatogenesis and testosterone secretion (testicular function). Therefore, supplementation of dietary fats can modulate iron effect (Elseweidy *et al*. 2013). Morsi (2013), whom stated that wheat germ which composed of 10 g corn oil (10% fat), is associated with increasing zinc serum levels in rat fed wheat germ. The results of the biochemical analysis are provided in figures 7 and 8. However, the levels of serum total cholesterol and serum total protein were insignificantly decreased and insignificantly increased in the treated group compared with untreated group

respectively. This finding may be a result of that diets rich in saturated fatty acids lead to high levels of serum cholesterol, whereas diets rich in unsaturated fatty acids tend to be associated with lower levels. The mechanism by which these changes in serum cholesterol are induced by dietary fat is not known. One possible explanation for these effects would be either inhibition of cholesterol biosynthesis by unsaturated fats or stimulation of synthesis by saturated fats. The effects on synthesis, if any, could, in turn, be either the result of a direct action of the dietary fat on the biosynthetic system or of a secondary adjustment to any change in the rate of oxidation and excretion of cholesterol and its metabolites (Avigan & Steinberg 1965). While men with normal cholesterol levels receiving 4 g of n-3 fatty acids/d for 10 wk did not display any decrease in cholesterol levels (Brilla & Landerholm 1990). Serum cholesterol concentrations are normally low in horses but are increased when vegetable oil is added to the diet. The cholesterol response of the corn oil-fed horses in this study is consistent with the findings of Orme *et al*. (1997). Therefore, it was hypothesized that dietary lipid source (fish oil or corn oil) would affect serum concentrations of triglycerides, cholesterol, and individual fatty acids of horses undergoing a conditioning program (O'Connor *et al*. 2007; Mohamed *et al*. 2010). Saynor & Gillott (1992) supplemented hyperlipidemic humans with 1.8 g of eicosapentaenoic acid/day and showed that cholesterol concentrations were decreased in hypercholesterolemic subjects after 3 months. However, cholesterol concentrations were unchanged after 3 months of fish oil or corn oil supplementation in subjects that began the study with normal cholesterol. The type of dietary fat also influences serum cholesterol concentrations in rabbits, the substitution of corn oil for coconut fat in the diet reduces serum cholesterol concentration in rabbits (Kritchevsky 2001). It has recently shown in rabbits that the hypocholestrolemic effect of corn oil versus coconut fat was greater with higher concentrations of fat in the diet (Alhaidary *et al*. 2010). Because omega 3 fatty acids are essential in growth and development throughout the life cycle, they should be included in the diets of all humans. Omega-3 and omega 6 fatty acids are not interconvertible in the human body and are important components of practically all cell membranes. Whereas cellular proteins are genetically determined, the polyunsaturated fatty acid composition of cell membranes is to a great extent dependent on the dietary intake (Simopoulos 1991). In 2013, Afolabi *et al.*, founded the rats treated with vehicle, that is, corn oil had higher serum total protein about (0.99±0.03).

6. Conclusion

In conclusion, intake of corn oil is potentially useful in increasing the fertility of male rabbits by increasing sperm concentration, motility, grade activity, viability, and reduced abnormality. It is good solvent for reproductive system research studies. However, further works are needed to better understand the exact mechanism leading to the changes of the spermatogenic cells due to the administration of corn oil.

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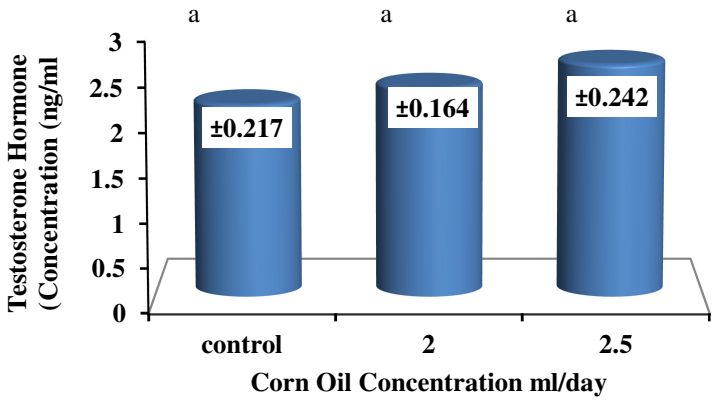

Figure 1.The Level of Testosterone Hormone in Different Groups of Male Adult Rabbits Same Letters Indicate Inignificant at P>0.05, Mean±SE, N=5

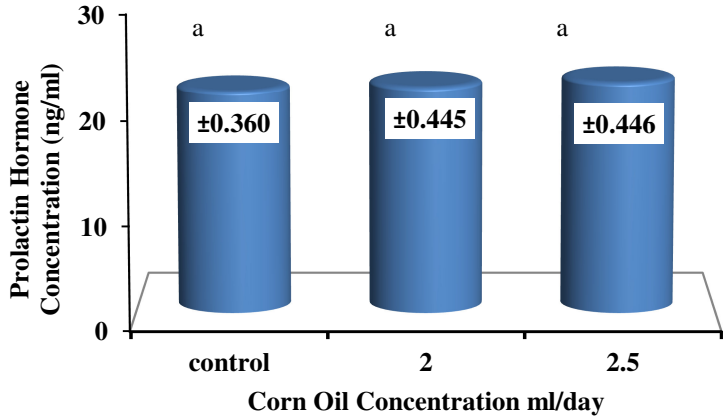

Figure 2. The Level of Prolactin Hormone in Different Groups of Male Adult Rabbits

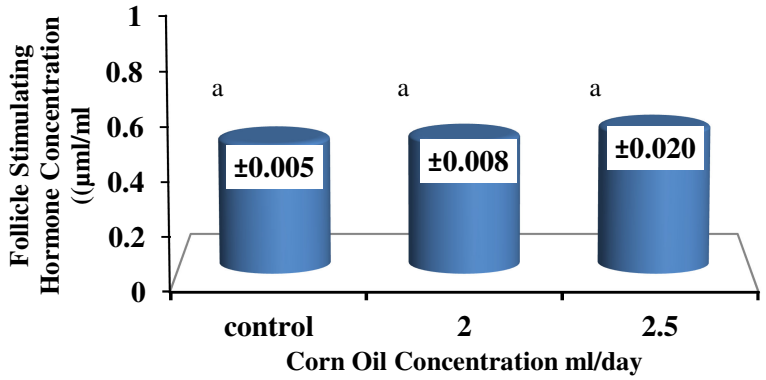

Figure 3. The Level of Follicle Stimulating Hormone in Different Groups of Male Adult Rabbits

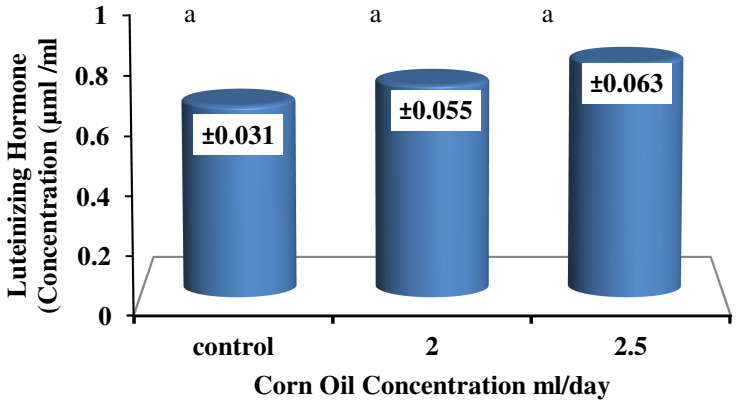

Figure 4. The Level of Luteinizing Hormone in Different Groups of Male Adult Rabbits

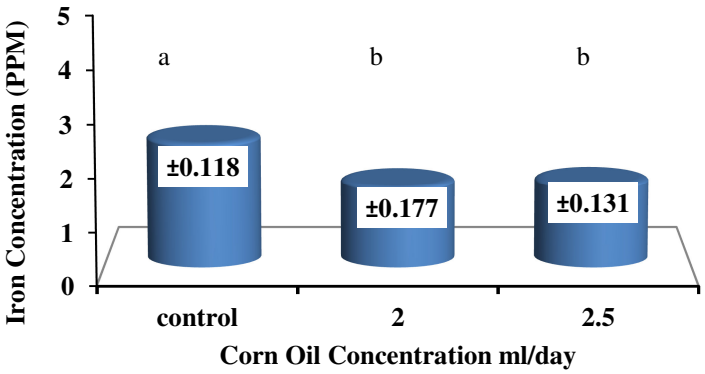

Figure 5. The Mean Values of Iron (Fe) in Serum Sample of Different Groups of Male Adult Rabbits Different Letters Indicate Significant at P<0.05, Mean±SE, N=5

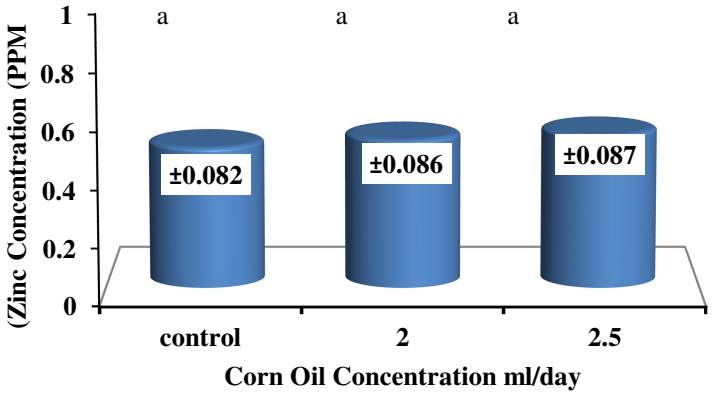

Figure 6. The Mean Values of Zinc (Zn) in Serum Sample of Different Groups of Male Adult Rabbits

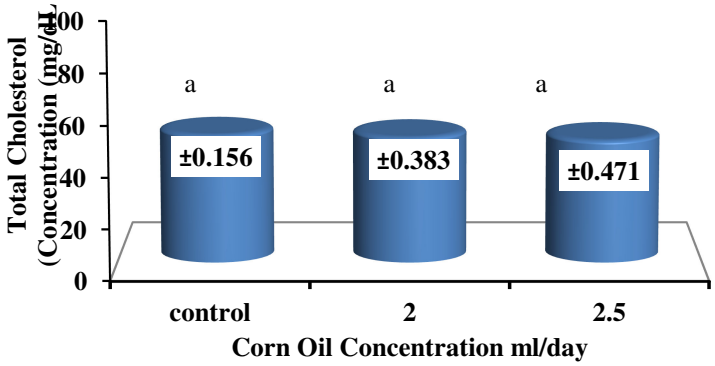

Figure 7. Effect of Different Doses of Corn Oil on Total Cholesterol Concentration in Male Adult Rabbits

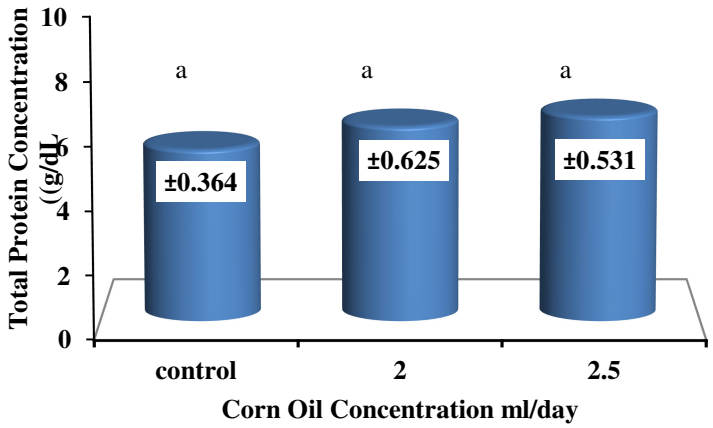

Figure 8. Effect of Different Doses of Corn Oil on Total Protein Concentration in Male Adult Rabbits

Table 1. Body, Testis, and Epididymis Weight of Adult Male Rabbits

	Control group	2ml Corn oil	2.5 ml Corn oil
Body weight (kg)	1.401±0.009a	1.425±0.013ab	1.460±0.015b
Testis weight (g)	2.366±0.316a	2.388±0.065a	2.389±0.065a
Epididymis weight (g)	1.270±0.060a	1.365±0.057a	1.368±0.316a

Different Letters Indicate Significant at P<0.05, Mean±SE, N=5

Table 2. Effect of Corn Oil Dose 2 and 2.5 ml/day in Sperm Parameters of Adult Male Rabbits

	Control group	2ml Corn oil	2.5 ml Corn oil
The concentration of sperm in the testis	29.50±1.020a	31.50±1.701ab	34.30±0.593b
The average number of sperm per 1 g of testicular	11.86±0.439a	13.10±0.358ab	13.90±0.359b
Sperm concentration in the tail of the epididymis (million/ml)	66.00±1.019a	71.00±0.489b	72.20±0.593b
The percentage of sperm motility in the tail of the epididymis	62.40±1.664a	72.40±0.727b	74.6±0.829b
The percentage of sperm viability in the tail of the epididymis	69.00±0.400a	71.60±1.252ab	75.80±1.820b
The percentage of sperm abnormality in the tail of the epididymis	25.40±0.727a	23.20±0.438b	22.20±0.593b

P<0.05, Different Letters Indicate Significant at P<0.05, Mean±SE, N=5

Table 3. Effect of Corn Oil Dose 2 and 2.5 Ml/Day in Grade Activity of Adult Male Rabbits

	Control group	2ml Corn oil	2.5 ml Corn oil
The percentage of progressively motile sperm (a ± b)	49.40±1.513a	56.00±0.633b	59.00±0.633b
The percentage of topical motile sperm (c)	13.00±0.316a	14.20±0.522ab	15.60±0.456b
The percentage of non-motile sperm (d)	37.60±1.664a	29.80±0.867b	25.40±0.830c

P<0.05, Different Letters Indicate Significant at P<0.05, Mean±SE, N=5