

Research Article

The Effect of Conjugated Linoleic Acid (CLA) on Male Reproductive Hormones in Mice

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Abstract: Spermatogenesis is a physiological reaction dependent to harmony of gonadotropins and steroid hormones activities. Conjugated linoleic acid is a group of long chain unsaturated fatty acids of a conjugated bound which are found in dairy products, beef and lamb. There are strong reasons that g/kg of food affects mediators involved in spermatogenesis. The aim of this study was determination of effect of various CLA doses on hormones and systemic factors of various CLA doses on hormones and systemic and local factors which affect spermatogenesis. Fifty mature male mice were divided in five groups (T₀: control, T₁: placebo and T₂-T₄: treatment groups). Each group had two replications with four mice in each replication. Samples received control diet (0 g/kg of conjugated linoleic acid) or treatment diet (0.1, 0.3 and 0.5 g/kg of CLA) which was replaced with corn oil of diet. After 30 days, blood samples were taken and level of FSH, LH and testosterone hormones were measured. Data were analyzed using ANOVA method and SPSS program and means were compared using Duncan's multiple ranges test at 5% probability level. CLA increased testosterone, LH and FSH level significantly in T₃ and T₄ groups. According to results, CLA has significant effect on secondary sexual activity of male animal which can affect spermatogenesis rate.

Keywords: CLA, mice, sexual hormones

INTRODUCTION

Conjugated Linoleic Acid (CLA) is a mixture of 28 isomers of linoleic acid (C₁₈:2) which is one of necessary fatty acids (Aydin, 2005). Among its isomers cis 9, trans11 and trans10, cis12 are very active biologically (Pariza *et al.*, 2001).

The most important way of CLA formation is dependent on *Butyrovibrio fibrosolvens* bacterium which is found only in digestive system of ruminants like cow (Fritsche and Fritsche, 1998). Breast tissue cells can produce CLA using Delta 9 desaturase enzyme (Collomb *et al.*, 2006). Thus, meat and dairy products-especially milk-are major CLA resources (Collomb *et al.*, 2006; Schmid *et al.*, 2006).

Positive various physiological effects of CLA have been shown yet. Effects like anti cancer, anti at hero sclerosis, anti osteoporosis, anti obesity, anti diabetes, lowering blood pressure, boosting the immune system and maintaining cell membrane have been reported (Nagao and Yanagita, 2005; Bhattacharya *et al.*, 2006).

Among last fifty years milk production and its efficiency has been increased considerably however reproductive efficiency has been decreased (Butler, 2003). There have been reports from United States, Europe and Australia which show milk production affected the quantity of fertility (Lucy, 2001).

As the nutrient demands for lactation exceed the nutrient intake, the associated negative energy balance increases the risk of poor reproductive performance in postpartum dairy cows. Negative linear relationship between energy balance and fertility may control nutrient intake and reproduction actions via hormonal or metabolic signals (Chagas *et al.*, 2007).

In an effort to decrease negative energy balance, fat supplements have been used to increase the energy density of the diet, although in general fat containing foods can not improve energy balance (Staples and Thatcher, 2006). Amazingly, it has been reported that some fat supplements which have high PUFA (long chain unsaturated fatty acid) caused improvement in reproductive performance, but, results of researches in this category have not been reported yet (Santos *et al.*, 2008).

Perhaps, there are some specific unsaturated fatty acids which can pass rumen without being affected by its micro organisms and be absorbed by small intestine. Maybe these fatty acids can affect reproduction tissues directly or improve it indirectly via hormones (Staples and Thatcher, 2006).

Thus, CLA can affect spermatogenesis factors. But because of origin of CLA which is in the rumen of ruminants, there are few studies on specific effect of CLA on reproduction process of these animals and

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there isn't any study on effect of CLA or endocrine and paracrine parameters on spermatogenesis factors (especially mouse).

Therefore, this study was conducted for investigating the effect of CLA concentration on spermatogenesis in mice.

MATERIALS AND METHODS

Fifty mice (Balb/C) were kept in animal house of Islamic Azad University (Khorasgan Branch) under normal condition ($24\pm 2^{\circ}\text{C}$ and 12:12 h light and darkness period) for two weeks to adapt to environment. Samples had free access to food and water. Mice were divided in five groups including T_0 : control, T_1 : control and T_2 - T_4 : treatment groups which each group had two replications with four mice in each replication. Placebo group was used for determining base level of hormones and testicle histology and control group received sunflower oil which has oleic acid. Treatment groups received 0.1, 0.3 and 0.5 g/kg of CLA in a thirty days period. After 30th day, blood samples were taken and FSH, LH and testosterone hormones were measured using Eliza, gamma counter method.

Data were analyzed using SPSS program and means were compared using Duncan's multiple ranges test at 5% probability level.

RESULTS

CLA effect on sexual hormones: Variance analysis data showed significant increase in FSH concentration in second and third treatment groups (Fig. 1). LH concentration was increased also in treatment groups which this increase was significant for second and third treatment groups (Fig. 2). CLA increased also testosterone amount of first group not significantly and for second and third groups significantly (Fig. 3).

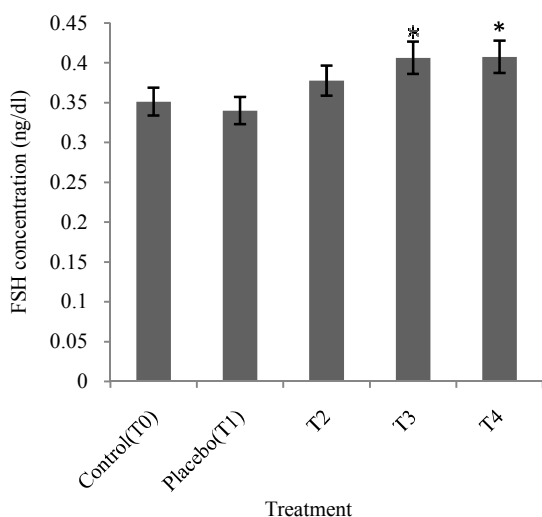


Fig. 1: Mean comparison of the FSH concentration of studied groups

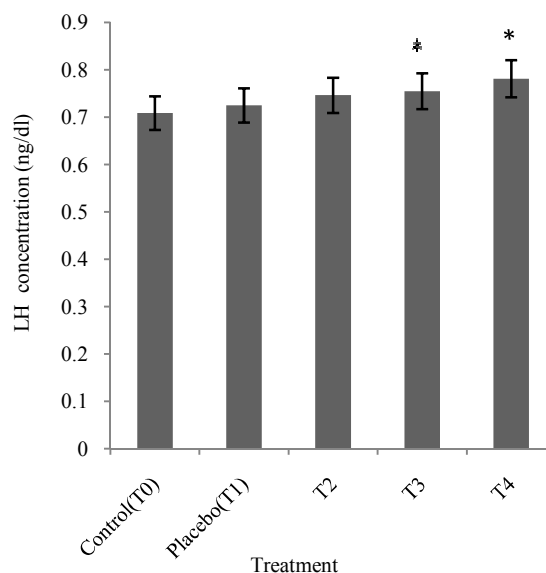


Fig. 2: Mean comparison of the LH concentration of studied groups

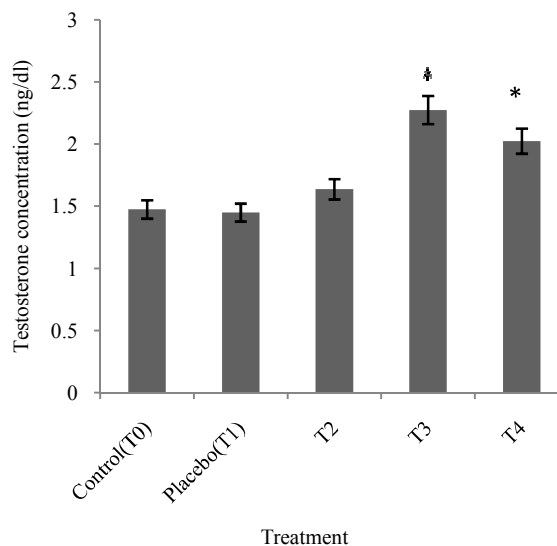


Fig. 3: Mean comparison of the testosterone concentration of studied groups

DISCUSSION

Spermatogenesis is dependent of hypothalamic-pituitary-testicular axis performance. Local factors and sometimes immunologic factors are very important here. Nourishment is from affecting factors of these hormones and factors. Since CLA exist in diet it may affect reproduction parameters. There isn't any study about CLA effect on spermatogenesis affecting factors of non-ruminant animals and then we tried to do this. Present study on mouse showed that measured factors were increased with increase in CLA concentration. The most important androgen of male sex is

testosterone which spermatogenesis and stimulation of sertoli cells activity are from its effects. Testosterone was affected by CLA of diet and was increased in serum. Gonadotropine releasing hormone of hypothalamus increases LH and FSH secretion (by affecting the anterior pituitary) and therefore simulates testosterone secretion (Santos *et al.*, 2008).

According to results of this study Fig. 1 to 3, simultaneous increase of serum level of testosterone, LH and FSH hormones how effect of CLA on hypothalamic-pituitary-testicular axis. This effect can be because of increase in testosterone level in serum. So, increase in testosterone level in this study has been secondary effect of gonad stimulating hormones especially LH. Also, increase in FSH, LH and testosterone can be due to existing compounds in CLA which affect hypothalamic-pituitary-testicular axis and increase mentioned hormones (Collomb *et al.*, 2006). This axis itself can be affected by concentration controlling factors (negative and positive). By reduction in stem cells and spermatocytes feedback effect of inhibin will be decreased and then FSH will be increased (Squires, 2003).

CONCLUSION

According to results of this study, conjugated linoleic acid can simulate spermatogenesis rate of mouse and increase production and concentration of its affecting parameters and as cells are decreased and sperm production is reduced, CLA can compensate it via increase in sexual activity affecting parameters.

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