

## Research Article

### Effects of Conjugated Linoleic Acid (CLA) on the Reproductive Axis (Pituitary, Thyroid, Testes) of Ram in Non-reproductive Season

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**Abstract:** Conjugated Linoleic Acid is a group of long-chain unsaturated fatty acids with more than one double bond and a mixture of 28 isomers of Linoleic acid (C 18:2) and it is counted as one of the essential acids. The main purpose of this study was to investigate the effect of CLA on some reproductive hormones in rams. In this study, six rams 3 to 4 years old with an average weight of 90 kg were selected. Rams were randomly divided into 3 groups and were treated by CLA treatment for 30 days. The first group (as a control group) didn't receive CLA, The second group received 0.5 g and the third group received 1 g of CLA. The blood testing was done on rams every 15 days using a 20 mL syringe. Data analysis was performed by SAS software. Also mean comparison was done using Duncan's test method ( $p < 0.05$ ). Obtained results showed that the serum concentration of testosterone hormone was decreased numerically as well as the concentration of FSH hormone however the concentration of LH was increased. Also the CLA had a significant effect on Leptin concentration. CLA in oral form can reduce the concentration of testosterone in rams.

**Keywords:** CLA, non-reproductive season, ram, testosterone

## INTRODUCTION

Generally fatty acids exist in two forms in nature: Saturated (without any double bonds in their structure) and unsaturated (which have double bonds in their structure). Essential Fatty Acids (EFA) are those which have is more than one double bond in their structure) and Linoleic acid is the most important one among them. Conjugated Linoleic Acid (CLA) naturally can be found in animal sources (Aydin, 2005; Khodaei *et al.*, 2009). From the perspective of biology, among the various isomers of conjugated Linoleic acid, Isomers cis-9, trans-11 (CLA c9, t11) and trans-10, cis-12 (CLA t10, c12) are remarkably active (Pariza *et al.*, 2005). Generally CLA is naturally produced in the ruminant's rumen through partial bio-hydrogenation or bacterial fermentation as the first-mediated of Linoleic acid isomerization to Stearic acid (Fritsche and Fritsche, 1998).

Conjugated Linoleic Acid content in foods varies by the type of nutrition, breeds and maturity of animal, but it usually ranges from 3 to 7 mg/g fat (Khodaei *et al.*, 2009). During recent years more attention has been paid to the beneficial effects of CLA on health

condition (Kepler *et al.*, 1966). So far, effects such as anti-obesity, anti-cancer, anti-atherosclerosis, anti-osteoporosis, anti-diabetes, lower blood pressure, protect cell membranes and increases immune function has been emphasized (Banni, 2002; Bhattacharya *et al.*, 2006). CLA also has an effect on production of Prostaglandins (Nagao and Yanagita, 2005). It has been confirmed by some researchers that Conjugated Linoleic Acid improves the efficiency of pregnancy giving birth (Park *et al.*, 1997). As certain long-chain omega-3 fatty acids increase the period of pregnancy in both animals and human beings (Ha *et al.*, 1990). Studies conducted on female rats showed that Conjugated Linoleic Acid in mice not only has no stimulatory effects on ovulation rate of mice, but also it reduced the production and of affecting (Rahman *et al.*, 2007) literature studies are proof to this matter that few researches has been allocated to the effects of Conjugated Linoleic Acid on reproduction in male ruminants (ram). Therefore, the aim of this study is to investigate the effect of CLA on the changes in pituitary, thyroid, Gonadal hormones along with some affecting localized hormones on reproduction in rams.

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**MATERIALS AND METHODS**

$$Y_{ijk} = \mu + T_i + B_j + e_{ijk}$$

In this experiment, 6 heads of Afshari breed rams with the average weight of 90 kg and age 3 to 4 years were selected. Animals were kept in groups and examined by veterinarian concerning their reproductive health condition. The rams also reported to have no infectious and parasitic diseases. The tests were performed in a private sheep ranching in Golpayegan city (Isfahan province) during the spring and summer of 2013.

Rams were randomly divided into 3 groups of 2 (control, treatment 1, treatment 2). Rams in the control group for 30 days received the diet lacking Conjugated Linoleic Acid while in the treatment groups they received diets of 0.5 and 1 g of Conjugated Linoleic Acid with the isomers of c9t11 and t10c12, respectively. Due to the steady conditions of experiment, experimental design, block design were selected randomly. Used CLA was purchased from Nanobolix Co., Turkey with the purity of 95.6% for the isomers.

**Measured variables:** For measuring the serum testosterone, FSH, LH, TSH, T3, T4, DHT, Leptin, IgE, TNF $\alpha$ , Glucose, Cholesterol, Triglycerides, LDL, HDL, AST, ALT enzymes blood sampling were done on rams in 3 sets Blood samples were taken from the rams every 15 days on days (0, 15, 30) through their jugular vein by a 20 mL syringe. Taken blood serums were separated by centrifugation (5000 rpm) and were kept in temperature -22°C till the further hormone and biochemical testing. Analysis of Hormones and Cytokines were performed through the ELISA method and the metabolic factors by auto analyzer.

**Statistical analysis:** Data analysis of obtained results was conducted by the SAS software. Comparison of means was analyzed by Duncan's test and the significant error less than 0.05 was considered. Obtained data from this survey was analyzed by a completely randomized block design using the following statistical model:

**RESULTS**

**Effect of CLA on reproductive hormones:** Analysis test results indicated that the effects of CLA on FSH in the level of 0.05 are significant and the lowest FSH serum levels were observed in the 0.5 g of CLA in dietary. Obtained analysis results on Gonadotropin LH showed that the impact of CLA treatments on serum concentration of LH at the level of 0.05 was significant (Table 1). Analysis on testosterone indicated that the effect of CLA on testosterone concentration at the level of 0.05 was not significant ( $p > 0.05$ ) and finally the obtained analysis data on Dihydroxy Testosterone (DHT) confirmed that the effect of CLA treatments on Dihydroxy testosterone at the levels of 0.05 was completely significant (Table 1).

**Effect on thyroid hormones:** Test results done on TSH the hormone from the perspective of statistically had no significant meaning. Analysis on T4 confirmed that different concentrations of CLA on Thyroxine at the level of 0.05 aren't significant ( $p > 0.05$ ). And the analysis performed on T3 indicated that concentrations of CLA are completely significant (Table 2).

**Effect on paracrine factors:** The measurements of Leptin indicated that the effect of CLA treatment on Leptin CLA at the level of 0.05 is quite significant. Data analysis table shows the effect of CLA on serum level of TNF $\alpha$  was not significant ( $p > 0.05$ ) (Table 3). The analysis related to the effect of CLA on the immunoglobulin IgE confirmed that the CLA has no significant meaning on IgE at the level of 0.05 ( $p > 0.05$ ) (Table 3).

**Effects on metabolic enzymes and biochemical factors:** Experimental results of blood's biochemical and metabolic variables confirmed that Conjugated Linoleic Acid on glucose, cholesterol, Triglycerides (TG), at the level of 0.05 had no significant meaning ( $p > 0.05$ ). But various concentrations of Conjugated

Table 1: Effect of different treatments on reproductive hormones (Commen letters in each row indicate significant differences would not be)

Variables	Control	Treatment 1	Treatment 2	S.E
FSH (miu/mL)	5.9683 <sup>a</sup>	5.9300 <sup>a</sup>	5.7850 <sup>b</sup>	0.33650
LH (miu/mL)	6.4200 <sup>a</sup>	6.5050 <sup>ab</sup>	6.6100 <sup>b</sup>	0.28900
DHT (nmol/L)	0.5217 <sup>a</sup>	0.5700 <sup>a</sup>	0.6433 <sup>b</sup>	0.01748
T (nmol/L)	5.6200 <sup>a</sup>	5.5200 <sup>a</sup>	5.4617 <sup>a</sup>	0.02104

Table 2: Effect of different treatments on thyroid hormone (Commen letters in each row indicate significant differences would not be)

Variables	Control	Treatment 1	Treatment 2	S.E
TSH (nmol/L)	0.18350 <sup>a</sup>	0.17700 <sup>b</sup>	0.1800 <sup>b</sup>	0.00195
T <sub>3</sub> (nmol/L)	0.99170 <sup>a</sup>	1.04670 <sup>a</sup>	1.1183 <sup>b</sup>	0.01689
T <sub>4</sub> (nmol/L)	28.2833 <sup>a</sup>	29.0333 <sup>a</sup>	27.800 <sup>a</sup>	0.26743

Table 3: Effect of different treatments on paracrine factors (Commen letters in each row indicate significant differences would not be)

Variables	Control	Treatment 1	Treatment 2	S.E
Leptin (ng/mL)	5.38330 <sup>a</sup>	5.39000 <sup>a</sup>	5.54830 <sup>b</sup>	0.02412
Tnf $\alpha$ (Pg/mL)	2.25000 <sup>a</sup>	2.13170 <sup>a</sup>	2.05330 <sup>a</sup>	0.04397
Ige (ng/mL)	14.5100 <sup>a</sup>	14.2233 <sup>a</sup>	14.2317 <sup>a</sup>	0.30117

Table 4: Effect of different treatments on blood enzymes and metabolic factors (Commen letters in each row indicate significant differences would not be)

Variables	Control	Treatment 1	Treatment 2	S.E.
AST(U/L)	85.5000 <sup>a</sup>	86.3167 <sup>a</sup>	88.4000 <sup>b</sup>	0.47553
ALT(U/L)	26.7333 <sup>a</sup>	28.8500 <sup>b</sup>	28.3000 <sup>b</sup>	0.41200
Glocose (g/dl)	66.4083 <sup>a</sup>	70.4967 <sup>a</sup>	66.0850 <sup>a</sup>	1.17700
Cholestrol (mmol/mL)	3.50830 <sup>a</sup>	3.56830 <sup>a</sup>	3.45500 <sup>a</sup>	0.46750
HDL	27.2333 <sup>a</sup>	29.6333 <sup>a</sup>	32.0800 <sup>b</sup>	0.69676
LDL	37.8333 <sup>a</sup>	37.6500 <sup>a</sup>	32.8500 <sup>b</sup>	0.95572
TG (nmol/L)	1.28000 <sup>a</sup>	1.19000 <sup>a</sup>	1.28000 <sup>a</sup>	0.01872

Linoleic Acid on HDL was highly significant ( $p>0.05$ ) same as the LDL ( $p>0.05$ ) (Table 4). Results gained by the analysis on liver enzyme Alanine Amino Transferase (ALT) showed that the effect of CLA on this enzyme at level of 0.05 has no significant meaning however the analysis on liver enzyme Aspartate Amino Transferase (AST) suggested quite a significant meaning on the effect of CLA on this enzyme ( $p>0.05$ ) (Table 4).

### DISCUSSION

Nutrition is one of the affecting agents on the hormones and local factors. Since CLA by itself exists in the daily diet it may has an influence reproductive variables.

During present study, a reduction in FSH concentrations was observed which was similar to other researches (Yamasaki *et al.*, 2003) meanwhile it didn't conform to the study (Modaresi *et al.*, 2013). Lowering effects of FSH may primarily be as a result of inhibin hormone inhibitor in which the impact of negative feedback lead to the reduction of FSH. Moreover, Leakage of FSH which has a pulsed manner lowers the concentration of this hormone.

The latter, it was expected that due to the decrease in testosterone levels, concentration of FSH increases via the respond of negative feedback. However probably the existence of some compounds in unsaturated fatty acids caused the reduction in this hormone (Mazur and Adiercrutz, 1988) and CLA intake can enhance the second messengers which this incremental effect leads to an increase in amount of inhibins and thus reduction in FSH. Research shows that many local factors have an influence on production and secretion of LH. During this study it was observed that CLA increased the concentration of LH which corresponded to the study (Modaresi *et al.*, 2013) but in comparison with study (Khodaei *et al.*, 2009) showed the reverse effect. Incremental effect of CLA on LH is was expected to be as a result of nitric oxide effect which causes enhancement in GnRH and eventually increases the LH (Squires, 2003; Tamanini *et al.*, 2003).

The results were a proof on this matter that that testosterone concentration has decreased which was in accordance with the study (Modaresi *et al.*, 2013). This phenomenon may happen due to an increase in LH as it is the stimulating factor in production and secretion of

testosterone. It stimulates Leydig cells in the testes for secretion of testosterone. Also as another reason to this phenomenon reduction in number of FSH hormone receptor or the sensitivity of these receptors can be referred to that prevents the synthesis and secretion of testosterone on Leydig cells (Mazur and Adiercrutz, 1988). Dihydrotestosterone, is one of the main testis hormones and its synthesis and secretion site is the testes itself. Taking CLA increases the concentration of these hormones which can be justified with the high concentrations of LH and its impact on Leydig cells. Leydig cells are responsible for stimulating the production and secretion of these hormones from the testis.

One of the other measured variables was thyroid hormones. These hormones regulate the two main processes: first they have an effect on metabolic way, oxygen consumption, basic metabolism rate and regulate the carbohydrates, fat and protein metabolisms. Secondly, they have an impact on cell differentiation and tissue maturation and indirectly on the growth. In current study, T3 which is a hormone with the ability to bind with the nuclear receptors resulting the gene expression, increased significantly. Several researches suggest that the lack of these hormones can lead to the low fertility. Leptin was discovered for the first time in 1994 by a researcher named Jeffrey Friedman. Leptin, is a significant hormone with rather important effects important effects in regulation of body weight, metabolism and reproduction. reproductive impacts of Leptin causes the sexual development in both male and female and these effects are caused by the ability of Leptin in increasing the secretion of Gonadotropin-releasing hormones. Effect of Leptin on GnRH pulses takes place by inhibiting neuropeptide Y (Flint *et al.*, 2002).

It is possible that the natural ligand CLA is the reason behind increasing the Leptin level. Due to is a natural ligand CLA, this ligand is the Peroxisome Proliferators-Activated Receptors (ppars) existing in cells. Some studies indicates that activation of PPAR significantly decreases the Leptin's gene expression (Derecka *et al.*, 2008). Meanwhile it has been shown that CLA merged with cell membrane phospholipids alters Leptin production (Yamasaki *et al.*, 2003). LDL, Triglycerides, cholesterol and HDL significantly decreased however HDL had a significant increase which was parallel to the results of study (Modaresi

*et al.*, 2013). It can be justified by the usage of unsaturated fatty acids (37, 38). Moreover, several further studies indicates that CLA reduces atherosclerosis scars in rodents, blood cholesterol, triglycerides, LDL and increases the HDL in number of animal models. Decreasing the concentration of triglycerides probably is related to the effects of CLA on decreasing the activity of Adenylate cyclase enzyme and the sensitive to lipase hormone by insulin (Nagao and Yanagita, 2005).

In current study liver enzymes were also measured. Liver is considered the most important site for metabolism. Under normal circumstances these enzymes exist inside the liver cells but as any injury happens to the liver they are released into the bloodstream. The most sensitive ones among these enzymes are Alanine aminotransferase and Aspartate aminotransferase which are responsible for the catalysis of chemical reactions in cells. During this research Aspartate aminotransferase enzyme increased significantly which can be justified by the intake of unsaturated fatty acids.

### CONCLUSION

According to the obtained results in this survey, CLA is knowingly effective on reproduction. In rams it caused an increase in Dihydroxy testosterone concentrations however it reduced the total testosterone concentration.

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