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Effects of Conjugated Linoleic Acid on the Pituitary-Testes Axis, Thyroid Hormones and Ram Semen Quality in Non-Reproductive Season

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Abstract: Conjugated Linoleic Acid (CLA) is a group of long-chain unsaturated fatty acids with >1 double bond and a mixture of 28 isomers of linoleic acid (C 18:2) and it is counted as one of the essential fatty acids. The main purpose of this study was to investigate the effect of CLA on some reproductive hormones and ram semen quality in non-reproductive season. In this study, 6 heads of 3-4 years old rams with the average weight of 90 kg were selected. Diet rams were used by NRC. Rams were randomly divided into 3 groups and were treated by CLA for 30 days. The 1st group (as a control group) did not receive CLA. The 2nd group received 0.5 g day^{-1} and the 3rd group received 1g day^{-1} of CLA. Consumed CLA included the c9, t11 and t10, c12 isomers. The blood testing was done on rams every 15 days (0, 15 and 30) and sperm measurements were performed every 15 days using an artificial vagina. 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 (Follicle-Stimulating Hormone). However, the concentration of LH (Luteinizing Hormone) was increased. Also, the CLA had a significant effect on leptin concentration. CLA in oral form can reduce the concentration of testosterone and increase sperm motility in rams. 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. Also, CLA caused a significant increase in total motility and progressive motility of sperms in ram.

Key words: Conjugated linoleic acid, semen quality, testosterone, Duncan's test, progressive motility

INTRODUCTION

Generally fatty acids exist in 2 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 >1 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 CLA, 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).

CLA content in foods varies by the type of nutrition, breeds and maturity of animal but it usually ranges from 3-7 mg g^{-1} fat (Khodaei *et al*., 2009). During recent years more attention has been paid to the beneficial effects of CLA on health condition. 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

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production of prostaglandins (Nagao and Yanagita, 2005). It has been confirmed by some researchers that CLA improves the efficiency of pregnancy giving birth (Bhattacharya *et al*., 2006; 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 CLA in female rats not only has no stimulatory effects on ovulation rate of female rats but also, it reduced the production and of affecting (Rahman *et al*., 2007). Literature studies are proof to this matter that few researches have been allocated to the effects of CLA 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.

MATERIALS AND METHODS

In this experiment, 6 heads of Afshari breed rams with the average weight of 90 kg and age 3-4 years old were selected. Animals were kept in groups and examined by veterinarian concerning their reproductive health condition. The tests were performed in a private sheep ranching in Golpayegan City (Isfahan Province) during the Spring and Summer of 2013. Laboratory analysis of blood variables and analysis of sperm were done in Khorshid and Pars Pathobiology Lab (Humane Laboratory) in Isfahan. Rams were randomly divided into 3 groups of 2 (control, treatment 1 and 2). Diet rams were used by NRC. Rams in the control group for 30 days received the diet lacking CLA while in the treatment groups they received diets of 0.5g day^{-1} (treatment 1) and 1g day^{-1} (treatment 2) of CLA with the isomers of c9, t11 and t10, c12, respectively. 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 (Follicle-Stimulating Hormone), LH (Luteinizing Hormone), TSH, T_3, T_4, DHT (Dihydrotestosterone), leptin, IgE (Immunoglobulin E), TNF_a (Tumor Necrosis Factor alpha), glucose, cholesterol, Triglycerides (TG), LDL, HDL, AST (Aspartate Amino Transferase), ALT (Alanine Amino Transferase) enzymes blood sampling were done on rams in 3 sets blood samples were taken from the rams every 15 days on days 0, 15 and 30 through their jugular vein by a 20 mL syringe. Hormones were measured in serum samples. 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 with wave length of 450 nm and reference wave length of 630 nm and the metabolic factors by auto analyzer using DRG-Co kits made in Germany.

Sperm method: Adaptation for artificial vagina performed 3 weeks before sampling, sampling was performed every 15 days using artificial vagina. Semen samples were diluted using diluents INRA96 (IMV Technologies, L'Aigle, France). Computer assisted sperm analysis methods was used for analyzing the sperm samples in this study. Motility, velocity and concentration of sperm were also analyzed.

Statistical analysis: Data analysis of obtained results was conducted by the SAS98 software. Data analysis was performed using the sperm of SCA (Sperm Class Analyzer) software (Company Micro-Optic, Spain). Comparison of means was analyzed by Duncan's test and the significant error <0.05 was considered.

RESULTS

Effect of CLA on reproductive hormones: Analysis test results indicated that the effects of CLA on FSH are significant and the lowest FSH serum levels were observed in the 0.5 g day^{-1} of CLA in dietary. Obtained analysis results on gonadotropin LH showed that the impact of CLA treatments on serum concentration of LH was significant (Table 1).

Analysis on testosterone indicated that the effect of CLA on testosterone concentration 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 was completely significant (Table 1).

Effect on thyroid hormones: Test results done on TSH the hormone from the perspective of statically had no significant meaning ($p>0.05$). Analysis on T_4 confirmed that different concentrations of CLA on thyroxine were not significant ($p>0.05$) and the analysis performed on T_3 indicated that concentrations of CLA are completely significant (Table 2).

Table 1: Effect of different treatments on reproductive hormones (mean+SE)

Variables	Control	Treatment 1	Treatment 2	SEM
FSH (μ mL^{-1})	5.9683^a	5.9300^a	5.7850^b	0.3365
LH (μ mL^{-1})	6.4200^a	6.5050^{ab}	6.6100^b	0.2890
DHT (nmol L^{-1})	0.5217^a	0.5700^a	0.6433^b	0.01748
T (nmol L^{-1})	5.6200^a	5.5200^a	5.4617^a	0.02104

Common letters in each row indicate significant differences

Table 2: Effect of different treatments on thyroid hormone (mean+SE)

Variables	Control	Treatment 1	Treatment 2	SEM
TSH (nmol L^{-1})	0.1835[a]	0.17700[b]	0.1800[b]	0.00195
T_3 (nmol L^{-1})	0.9917[a]	1.0467[a]	1.1183[b]	0.01689
T_4 (nmol L^{-1})	28.2833[a]	29.0333[a]	27.800[a]	0.26743

Table 3: Effect of different treatments on paracrine factors (mean+SE)

Variables	Control	Treatment 1	Treatment 2	SEM
Leptin (ng mL^{-1})	5.3833[a]	5.3900[a]	5.5483[b]	0.02412
TNF_α (Pg mL^{-1})	2.2500[a]	2.1317[a]	2.0533[a]	0.04397
IgE (ng mL^{-1})	14.5100[a]	14.2233[a]	14.2317[a]	0.30117

Table 4: Effect of different treatments on blood enzymes and metabolic factors (mean+SE)

Variables	Control	Treatment 1	Treatment 2	SEM
AST (UL^{-1})	85.5000[a]	86.3167[a]	88.4000[b]	0.47553
ALT (UL^{-1})	26.7333[a]	28.8500[b]	28.3000[b]	0.41200
Glucose (g dL^{-1})	66.4083[a]	70.4967[a]	66.0850[a]	1.17700
Cholesterol (mmol mL^{-1})	3.5083[a]	3.5683[a]	3.4550[a]	0.46750
HDL	27.2333[a]	29.6333[a]	32.0800[b]	0.69676
LDL	37.8333[a]	37.6500[a]	32.8500[b]	0.95572
TG (nmol L^{-1})	1.2800[a]	1.1900[a]	1.2800[a]	0.01872

Table 5: Computer assisted sperm analysis on sperm motility and speed

Variables ($\mu m\ sec^{-1}$)	Control (%)	Treatment 1 (%)	Treatment 2 (%)	Concentration ($M\ mL^{-1}$)
Motile	83.85[a]	86.06[ab]	88.91[b]	2,524.08
Progressive	43.88[a]	45.20[ab]	47.99[b]	1,320.79
Non-progressive	39.97[a]	37.70[a]	36.90[a]	1,203.29
Rapid	43.54[a]	45.80[ab]	47.90[b]	1,310.66
Medium	5.92[a]	6.99[a]	7.55[b]	178.27
Slow	34.39[a]	36.60[a]	35.50[a]	1,035.16
Immotile	16.15[a]	15.00[a]	13.90[a]	486.18

Common letters in each row indicate significant differences

Effect on paracrine factors: The measurements of leptin indicated that the effect of CLA treatment on leptin CLA is quite significant ($p>0.05$). According to the data analysis, Table 3 shows the effect of CLA on serum level of TNF_a was not significant ($p>0.05$).

The analysis related to the effect of CLA on the immunoglobulin IgE confirmed that the CLA has no significant meaning on IgE ($p>0.05$) (Table 3).

Effects on metabolic enzymes and biochemical factors: Results of blood biochemical and metabolic variables confirmed that CLA on glucose, cholesterol, Triglycerides (TG) had no significant meaning ($p>0.05$). But, various concentrations of CLA on HDL was highly significant ($p<0.05$), as same as the LDL (Table 4).

Results gained by the analysis of 2 liver enzymes including ALT and AST showed that the effect of CLA on this enzymes has no significant meaning, however the analysis on liver enzymes AST suggested quite a significant meaning on the effect of CLA on this enzymes ($p>0.05$) (Table 4).

Sperm results: Results showed CLA caused significant increase in the levels of total and progressive motility of sperms in the sample compared to the control group (Table 5).

DISCUSSION

Spermatogenesis is dependent of hypothalamic-pituitary-testicular axis performance. Local factor and sometimes immunologic factors are very important here. Nutrition is one of the affecting agents on the hormones and local factors. Since, CLA buy itself exists in the daily diet it may has an influence reproductive variables. There is not any study about CLA effect on the reproductive axis, thyroid hormones and ram semen quality in non-reproductive season and then we tried to do this. During present study, a reduction in FSH concentrations was observed which was similar to other researches (Yamasaki *et al*., 2003), meanwhile it did not confirm 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. 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 Adlercreutz, 1998) 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. Researches show 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 of Modaresi *et al*. (2013) but in comparison with study, Khodaei 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 nRH and eventually increases the LH (Squires, 2003; Chatterjee *et al*., 1997; Tamanini, 2003).

The results were a proof on this matter that testosterone concentration has decreased which was in accordance with the study. 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. Dihydro testosterone is one of the main testis hormones and its synthesis and secretion site is the testes itself.

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 2 main processes: 1st they have an effect on metabolic way, oxygen consumption and 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, T_3 which is a hormone with the ability to bind with the nuclear receptors to regulating the gene expression, increased significantly. Several researches suggest that the lack of these hormones can lead to the low fertility (Gyton and Hall, 2006).

Leptin significantly increased which was in contradiction with the results of other researchers did (Modaresi *et al*., 2013; Harris *et al*., 2001; Castaneda-Gutierrez *et al*., 2009). Leptin was discovered for the 1st time in 1994 by a researcher named Jeffrey Friedman. Leptin is a significant hormone with rather important effects in regulation of body weight, metabolism and reproduction. Reproductive impacts of leptin cause 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 (GnRH). Effect of leptin on GnRH pulses takes place by inhibiting neuropeptide Y (Kosior-Korzecka and Bobowiec, 2006).

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 Proliferactors-activated receptors (PPars) existing in cells (Flint *et al*., 2002). Some studies indicate 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 (Lichtenstein and Schwab, 2000; Storlien *et al*., 1997). 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 (Nagao and Yanagita, 2008). 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 (Barbosa *et al*., 2009).

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 blood stream. The most sensitive ones among these enzymes are ALT and AST which are responsible for the catalysis of chemical reactions in cells. During this research, AST enzymes increased significantly which can be justified by the intake of unsaturated fatty acids, the effect of CLA on liver enzymes articles were found. Essential fatty acids are involved in the production of sex hormones and sexually active person keeps. According to studies, the composition of fatty acids in the sperm of one of the major factors affecting fertility is considered. Thus, fatty acids can be impaired in many infertile men, the sperm are moving and infertility helps. CLA is likely to result in increased total motility and progressive motility and fertility effects.

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. Also, CLA caused a significant increase total and progressive motility of sperms in the sample compared to the control group and thus increase fertility in the ram.

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