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RESEARCH PAPER

The Effect of Zinc Supplementation on Plasma Melatonin and Kisspeptin Levels in Rams

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Abstract

The study that researched the effect of zinc added to the rations of rams on kisspeptin and melatonin levels was conducted on 2 years old 12 Kivircik crossbreed rams (6 control, 6 experimental) for 1 year. In addition to the *ad libitum* dry alfalfa straw, it was given mixed feed (barley, salt and vitamin-mineral mixture as standard) supplemented with 25 mg/kg/ DM ZnO, for the control group and 125 mg/kg/ DM ZnO for experimental group per day. During the study, blood samples were taken once a month and kisspeptin and melatonin levels were measured with ELISA in plasmas obtained from blood samples. Melatonin levels were found to be between 62.8-164.5 ng/L in experimental group and 22.1-105.9 ng/L in control group. Kisspeptin levels were determined to be between 209.8-514.2 ng/L in experimental group and 92.6-356.6 ng/L in control group. Zinc supplementations showed numerical increases in kisspeptin and melatonin levels but because of the individual variations, no statistical significance was found ($P > 0.05$).

Introduction

Zinc; is an important trace element that mediates vital functions such as vitamin synthesis, hormone production, enzyme activation, energy production, reproduction and growth. It has been reported that zinc deficiency causes regression in growth and development, reproductive disorders, weakening of the immune system and histological structural disorders in organs (Haenlein and Anke 2011). Melatonin regulates reproduction in animals depending on photoperiod by inhibiting GnRH and LH in the long-day period and triggering GnRH secretion in short-day period but the relationship between melatonin and GnRH hasn't been explained clearly yet (Buchanan and Yellon, 1991, Viguie *et al*., 1995, Goodman *et al*., 2010). Kisspeptins are proteins controlled by the Kiss-1 gene and acts by binding to GPR54 receptor. It has been reported that there are 4 type of kisspeptin which have same binding sites but different amino acid sequences (Kisspeptin- 54, 14, 13 and 10) and the type that binds strongest to the receptor is kisspeptin-10 (Lee *et al*., 1996, Othaki *et al* 2001).

In recent years, kisspeptin was reported to assume an important role by transferring the melatonin signals to the GnRH neurons (Irwig *et al*., 2004, Ancel *et al*., 2012). Revel *et al.* (2006) reported that melatonin activates the reproductive axis by modulating Kiss1-R signals depending on photoperiod. Carnevalli *et al.* (2011) determined that melatonin induces kisspeptin and GnRH receptors in zebrafish. Alvarado *et al.* (2015) found that exogenously melatonin administration increased significantly Kiss-1-R expression in male sea bass in the hypothalamus after 30 days.

Researches carried out in ewes also showed that determination of Kiss-1 expression, in the long-day period was significantly lower than short-day period (Clarke *et al*., 2009b) and in short-day period Kiss-1-mRNA expression in arcuat nucleus is significantly higher than long-day period (Wagner *et al*., 2008). It has been suggested that kisspeptin, can play a role on starting melatonin-induced GnRH secretion, by transferring melatonin signals into the basal premamillar nucleus of the hypothalamus (Clarke *et al*., 2009a).

It has been found that melatonin administrations increased levels of zinc and leptin and there is an interaction between melatonin-zinc-leptin triplet (Song and Chen, 2009). Another study's results in pinealectomied mice, showed that zinc supplementation increased melatonin levels and there was an interaction between zinc and melatonin (Baltacı *et al.,* 2003). On the based of literature data, we found few references about zinc and kisspeptin. Quershi and Abbas (2013) suggested that kisspeptin-10 administration increased serum zinc, copper, cobalt and manganese levels but administration of kisspeptin-10 antagonist (peptide 234) decreased these trace elements dramatically.

In this study, based on the information given above, it was thought that zinc might effect on plasma melatonin and kisspeptin levels and aimed to investigate the effect of long-term Zn supplementation to the ration of rams on plasma melatonin and kisspeptin levels.

Materials and Methods

Animals, Housing and Breeding

The study was conducted one year (from April 2017 to March 2018) with 2 years old 12 Kıvırcık crossbreed rams which were divided into two groups (6 control, 6 experimental) and housed in outdoor paddocks in Aydın Adnan Menderes University Faculty of Veterinary Medicine's farm. This study was approved by the Ethics Committee of the Experimental Medicine Research and Application Centre of Selçuk University, 2017 / 62 report.

Throughout the study, all rams were fed individually with *ad libitum* dry alfalfa straw and 150 g/day pellet formed mixed feed (barley, salt and vitamin-mineral mixture) which include 25 mg/kg/DN ZnO (Zinc oxide). According to ARC (1980), the zinc requirement has been reported to be 30 mg/kg/DM in growing lamb, and 27 mg/kg/DM in lactating sheep. Additionally to the standard ration, experimental rams were provided to take 100 mg/kg/DM ZnO. Thus, a total of 125 mg/kg /DM ZnO was given to the experimental group daily. Water was provided ad libitum.

Sample Collection and Hormone Assays

Blood samples were collected with holder in monthly intervals from vena jugularis and centrifugated for 5 min 3000 rpm and obtained plasma samples were stored at -20 C^0 for one year until the end of the study. Hormone assays performed with ELISA kits. Kisspeptin (Bioaassay Tecnologl Laboratory catalog no: E0051Sh) and melatonin levels (Bioaassay Tecnologl Laboratory catalog no: E0108Sh) were measured with ELISA reader (Biotek ELx800) at 460 nm.

Statistical Analysis

Statistical analyses were conducted with SPSS (version 21). Data's normality and homogeneity were analyzed with Kolmogorov- Smirnov and Shapiro Wilk tests and test results showed that data distribution were not normal and homogenous. Therefore, Mann-Whitney U test was used for analyzing the statistical difference of means.

Results and Discussion

Melatonin levels showed seasonal changes in both control and experimental groups. The levels of the control group were found to be 22.1 ± 14.1 ng/L in April, which started to increase (93.8 ± 45.5 ng/L) in June and decreased after September. Similar progress was observed in the experimental group; the levels of melatonin were found to be 62.8 ± 26.2 ng/L in April, 164.5 ± 61.7 ng/L in June, and the highest levels until October decreased to 46.6 ± 26.5 ng/L in November (Table 1, Figure 1). Although zinc supplementation was increased the melatonin levels of the experimental group, the differences were not statistically significant, due to individual values were highly variable. In kisspeptin levels, similar to the levels of melatonin, seasonal changes were observed in the control group. The kisspeptin levels in the control group started to rise in May and reached the highest levels in June (356.6 ± 184.6 ng/L). In the experimental group, the kisspeptin levels reached the highest level in June (514.2 ± 180.4 ng/L). The kisspeptin levels maintained with slight releases until October and decreased rapidly to 215.5 ± 124.8 ng/L in November (Table 2, Figure 2).

To our best knowledge there was no study about the effects of zinc on kisspeptin and/or melatonin in both rams and ewes in the literature.

Baltacı and Moğulkoç (2017) reported that zinc supplements increased melatonin and leptin levels in male mice with hypothyroidism (P < 0.05). Bediz *et al.* (2003), reported that melatonin production decreased in zinc deficiency, while zinc supplements increased melatonin production in rats. In another study, it was reported that melatonin supplements to rats increased plasma and small intestinal melatonin levels (Özturk *et al.,* 2008). Studies on kisspeptin and zinc were limited in the literature. However, the fact that Kiss-1 mRNA expression was higher in the short-day period compared to the long-day period (Wagner *et al.,* 2008, Clarke *et al.,* 2009b) has given rise to thought that kisspeptin was affected by melatonin. In the present study, numerical increases in melatonin and kisspeptin levels were observed in rams. However, no statistical difference was found due to the highly variable individual hormone levels (P > 0.05).

Plasma melatonin levels in the experimental group showed monthly remarkable variations like in control but the highest level of melatonin (164.5 ± 61.7 ng/L) was determined in June and showed high concentrations until October. The lowest level of melatonin was 23.1 ± 12.1 ng/L in January and remained 46.6 ± 26.5-77.7 ± 62.9 ng/L intervals in the other months (Table 1, Figure 1). Although considerable differences were seen in melatonin levels between experimental and control group, especially in April, June and October, but it wasn't found to be significant ($P > 0.05$).

Table 1. Monthly plasma melatonin levels of experimental and control groups, ng/L.

Months	Experimental	Control	*P* Value
April	62.8 ± 26.2	22.1 ± 14.1	0.180
May	69.3 ± 27.6	76.0 ± 55.2	0.240
June	164.5 ± 61.7	93.8 ± 45.5	0.394
July	92.0 ± 43.2	104.7 ± 53.4	0.818
August	133.1 ± 58.9	105.9 ± 56.4	0.394
September	99.5 ± 51.1	83.0 ± 54.2	0.589
October	112.8 ± 42.0	63.4 ± 52.0	0.132
November	46.6 ± 26.5	57.6 ± 50.3	0.394
December	48.3 ± 24.4	56.1 ± 50.0	0.699
January	23.1 ± 12.1	57.2 ± 51.2	0.818
February	69.8 ± 41.7	69.4 ± 60.0	0.818
March	77.7 ± 62.9	68.7 ± 58.8	1.000

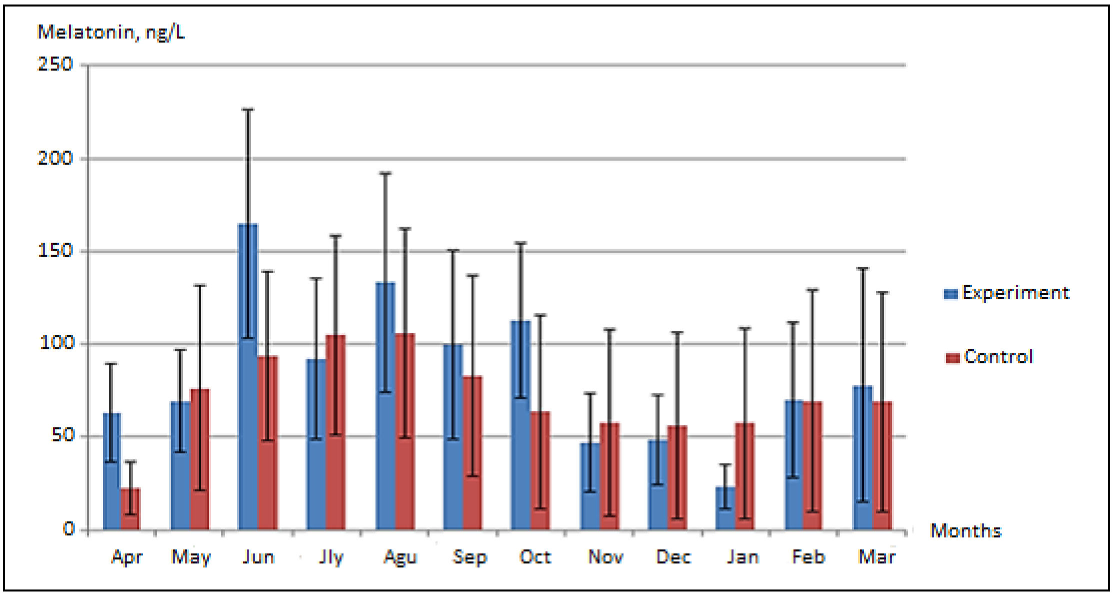

Figure 1. Monthly plasma melatonin levels of experimental and control groups, ng/L.

It can be seen that monthly kisspeptin levels of the experimental group were relaised like monthly melatonin. Kisspeptin levels were 209.8 ± 94.4 ng/L in April, and began to rise in May (293.5 ± 125.8 ng/L), the highest levels seen in June (514.2 ± 180.4 ng/L), continued to relaese in high levels until October and started to decrease in November (Table 2, Figure 2). Especially in summer and autumn months, kisspeptin levels in experimental group were quite higher than in the control but there was no statistically significance ($P > 0.05$) because of the large variations of individual values and less numbers of animals. Monthly linear increases between kisspeptin and melatonin levels suggest a relationship between these two hormones.

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Table 2. Monthly plasma kisspeptin levels of experimental and control groups, ng/L.

Months	Experimental	Control	P Value
April	209.8 ± 94.4	92.6 ± 54.0	0.485
May	293.5 ± 125.8	238.7 ± 170.5	0.394
June	514.2 ± 180.4	356.6 ± 184.6	0.394
July	391.4 ± 181.4	291.4 ± 146.8	0.394
August	421.6 ± 174.6	330.9 ± 187.0	0.485
September	396.8 ± 179.7	268.6 ± 191.4	0.310
October	493.5 ± 187.2	216.4 ± 193.7	0.065
November	215.0 ± 124.8	187.8 ± 169.8	0.310
December	154.8 ± 70.3	131.4 ± 113.5	0.310
January	218.7 ± 155.6	215.0 ±197.0	0.310
February	288.3 ± 160.3	223.3 ± 199.2	0.240
March	242.9 ± 195.9	220.5 ± 199.7	0.589

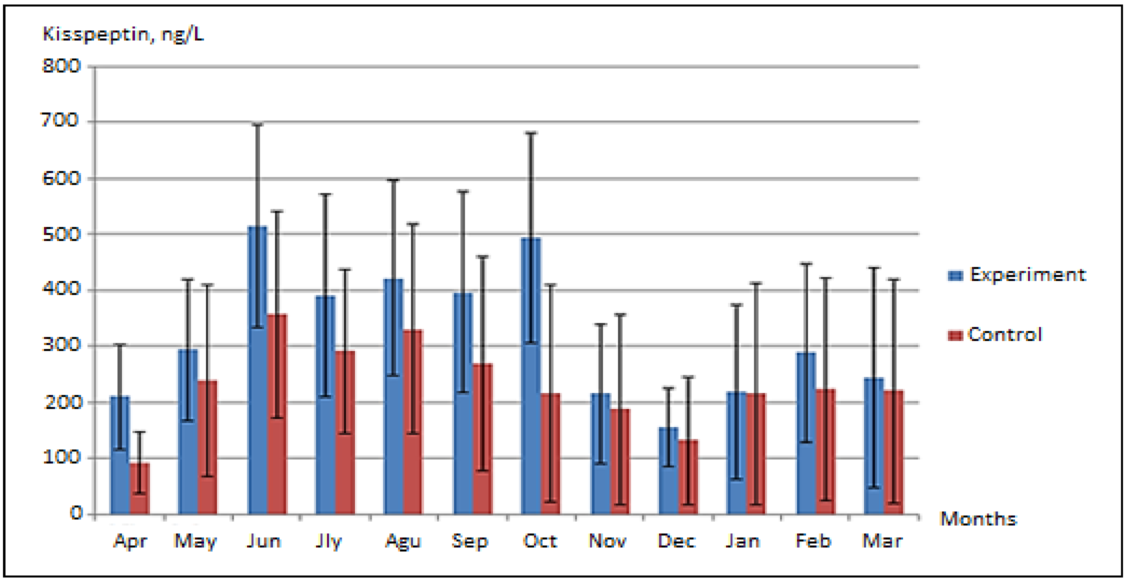

Figure 2. Monthly plasma kisspeptin levels of experimental and control groups, ng/L.

On the other hand, findings indicating a significant relationship between zinc and melatonin (Baltacı *et al.*, 2003, Bediz *et al.*, 2003, Song and Chen, 2009, Özturk *et al.,* 2008, Baltacı and Moğulkoç, 2017) and the data that kisspeptin acts a transporter role between GnRH and melatonin (Goodman *et al.,* 2010, Irwig *et al*., 2004, Ancel *et al.,* 2012, Revel *et al.,* 2006, Wagner *et al*., 2008, Clarke *et al.,* 2009a) and the study about kisspeptin-10 and zinc interaction (Quershi and Abbas, 2013) support the idea that there may be a relationship between kisspeptin and zinc in rams.

Conclusion

Although zinc supplements increased melatonin and kisspeptin levels especially in September and October, there was no statistical difference due to the fact that individual hormone levels were very variable.

(*P* > 0.05). It has been thought that kisspeptin and melatonin hormone studies must be carried out with large number of animals to get a define result.

Author contributions

All authors contributed that first author; sample collection, hormone assays, writing, while second author hormon assays writing- editing- statistical analysis.

Conflicts of interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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