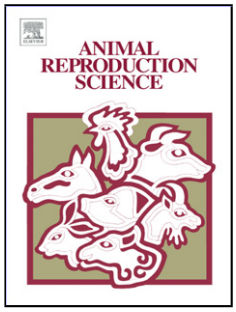

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The reproductive performance of female goats treated with melatonin is not improved after introduction to bucks displaying springtime sexual activity if these does are experiencing decreasing body weight/condition score

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Highlights

- Reproductive activity of bucks is reduced in spring when male effect is used.
- The response to the male effect decreases when females have an inadequate food supply.
- Melatonin might reduce the negative effect of decreasing body weight-condition score.
- Greater fecundity, fertility and productivity were observed in gain-melatonin does.
- Plasma glucose and IGF-1 concentrations could explain the better reproductive results.

Abstract

The aim of the present work was to determine whether treatment with melatonin modifies the reproductive response of female goats experiencing increasing or decreasing body weight (BW)/body condition score (BCS) when introduced to bucks displaying springtime sexual activity. During natural anoestrus, 53 does were isolated from bucks for a period of 42 days and distributed into two groups: 1) low BW/low BCS animals (N=24) (LLg group), which were fed 1.9 times their maintenance requirements so they would experience increasing BW and BCS; and 2) high BW/high BCS animals (N=29) (HHl group), which were fed 0.4 times their maintenance requirements so they would experience decreasing BW and BCS. Half of each group was treated, or not, with melatonin (LLg+Mel N=12, HHl+Mel N=15, LLg-Mel N=12 and HHl-Mel N=14). On 6th May they were introduced to six males, showing natural sexual activity, fitted with marking harnesses (thus permitting the detection of oestrous activity). The ovulation rate was assessed by transrectal ultrasonography and confirmed via the plasma progesterone concentration (measured twice per week in blood samples). Plasma glucose, IGF-1 and non-esterified fatty acid concentrations were also determined, along with the conception rate, fertility, prolificacy and productivity of the does. LH concentrations and LH pulsatility were also recorded in the hours around introduction to the males. 'Oestrous plus ovulation' was observed only in does treated with melatonin. A higher conception rate and greater fertility and productivity were observed among the LLg+Mel does. These females showed higher glucose and IGF-1 concentrations after the introduction of the males. LH concentrations increased after male introduction independent of all other conditions. In conclusion, the present results show that treatment with melatonin does not enhance reproductive performance in does experiencing decreasing

BW/BCS, but can improve it when does are experiencing increasing BW/body fat reserves - even when exposed to males displaying only springtime sexual activity. This might be explained by the higher blood glucose and IGF-1 concentrations of the LLg+Mel females.

Keywords: male effect, glucose, NEFA, IGF-1, progesterone, LH

Introduction

At Spanish latitudes, natural reproduction in goats is seasonal, with sexual activity occurring during autumn-winter. The photoperiod is the main environmental factor governing the seasonality of goat reproductive activity (Zarazaga et al., 2011a, b, c), and certainly in the Blanca Andaluza breed both the male (Gallego-Calvo et al., 2015a) and female (Gallego-Calvo et al., 2014) show reduced sexual activity during the lengthening days of spring. Information on the photoperiod is conveyed to the neuroendocrine system via the circadian secretion of melatonin from the pineal gland (Bittman et al., 1983). The administration of this hormone during the spring via the use of continuous, slow-release implants has been shown to advance the onset of the breeding season in goats (Chemineau et al., 1992; Williams et al., 1992). Usually, melatonin is provided following the separation of the sexes for 45 days (Zarazaga et al., 2012). When they are later brought back together, the "male effect" optimizes the female reproductive response.

The male effect can be employed to avoid seasonal anoestrus (for details see the review by Delgadillo et al., 2009), and is commonly used in extensive and semi-extensive stockraising systems in the Mediterranean. However, the results of the male effect depend greatly on factors such as breed, the type of previous isolation, the depth of anoestrus, postpartum stage, parity number, nutrition, body condition, and the degree of sexual activity displayed by males in spring (Walkden-Brown et al., 1993; Cerbito et al., 1995; Flores et al., 2000; Urrutia et al., 2003; Veliz et al., 2009; Delgadillo et al., 2009).

The nutritional status of domestic ruminants also affects their reproductive capacity. In Mediterranean extensive and semi-extensive stock-raising systems, food availability in the spring (when melatonin implants are used) can vary widely, and animals may experience an increase or reduction in body weight (BW) and body condition score (BCS) - fluctuations that can modify the reproductive response during male effect-induced spring breeding (Gallego-Calvo et al., 2015b). Variations in food availability may affect metabolic/nutritional factors such as plasma glucose, non-esterified fatty acids (NEFAs) and IGF-1.

Our group has described the interaction between nutrition and exogenous melatonin to affect LH secretion in female goats (Zarazaga et al., 2011b). In the former experiment we observed an increase in LH concentrations in ovariectomized females implanted with melatonin that showed low BW/BCS induced by a lower level of nutrition. In sheep, the ovulation rate is affected too (Robinson et al., 1991; Forcada et al., 1995; Rondón et al., 1996). All the latter authors report the effect of melatonin implantation to increase the ovulation rate more strongly in ewes with a low, rather than a high, feed intake or BCS.

It was hypothesized that, during seasonal anoestrous at Mediterranean latitudes (spring), melatonin administration might reduce the negative effect of a decreasing BW and/or BCS on the reproductive response of does when introduced to males displaying only springtime sexual activity. The present work examines: (1) how the reproductive response of does - with/without a melatonin implant - to the male effect differs depending on whether female BW and/or BCS is increasing or decreasing, and (2) whether metabolic/nutritional factors explain any of the responses observed.

Material and Methods

2.1. Study conditions

All procedures were performed by trained personnel in strict accordance with Spanish guidelines for the protection of experimental animals (RD 53/2013), and in agreement with European Union Directive 86/609. The study was conducted at the University of Huelva experimental farm (37º 20'N, 6º 54' W), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63).

2.2. Animals and management

Before starting the experiment, the natural BW and BCS of the does was determined over three consecutive weeks to ensure that neither varied in any animal. On March 25th, 53 adult (4 years old), non-pregnant does in anoestrus were divided into two groups depending on their BW and BCS (Mean±SEM): 1) low BW (≤37 kg) (35.2±1.1 kg, range 25-37 kg)/low BCS (≤ 2.50) (2.45±0.03, range 1.75-2.50) (N=24); this was known as the LowLow-gain (LLg) group since its members were fed 1.9 times their maintenance requirements, ensuring they would experience increasing BW and BCS; and 2) high BW (≥38 kg) (40.6±0.5 kg, range 38-52 kg)/high BCS (≥2.75) (2.84±0.04, range 2.75-3.75) (N=29); this was known as the HighHigh-loss (HHl) group since these animals were fed 0.4 times their maintenance requirements, ensuring they would

experience decreasing BW and BCS. Both nutrition regimens were designed according to INRA standards (Morand-Fehr and Sauvant, 1988) and followed for a total of 98 days.

The feed provided was a commercial concentrate composed of oats (24.7%), maize (23.0%), peas (20.4%), barley (16.3%), lucerne pellets (12.2%) and a mineral-vitamin complement (3.4%). The nutritional values of the concentrate were 0.94 milk fodder units (UFL) and 77 g digestible protein/kg dry matter. This concentrate was offered individually once per day. In addition, barley straw, distributed to each group as a whole, provided 0.37 UFL and 25 g of digestible protein/kg dry matter. All animals had free access to water and mineral/vitamin blocks. The aim of the feeding regimens was that, at the time of introduction to the males, the does of the LLg group would have approximately the BW and BCS originally shown by the does of the HHl group, and that the does of the HHl group would have approximately the BW and BCS originally shown by the does of the LLg group.

At the start of the experiment (March 25th), 12 does from the LLg group (and 15 from the HHl group received a single subcutaneous implant containing 18 mg of melatonin (Melovine®) (CEVA Salud Animal, Barcelona, Spain) at the base of the left ear (LLg+Mel and HHl+Mel subgroups).

To determine their ovarian cyclicity before the onset of the experiment (March 25th), blood samples were collected once per week over three consecutive weeks to determine the plasma progesterone concentration. The does were deemed cyclic if their progesterone concentration was >0.5 ng/mL in at least two consecutive samples (Zarazaga et al., 2009).

2.3. Introduction to males displaying springtime sexual activity

On 6th May (D0), 42 days after the start of the treatments, six adult males (treated in no way) displaying springtime sexual activity, were fitted with marking harnesses and placed in contact with the females (three with the LLg and three with HHl does) and kept with them for the following 42 days.

2.4. Variables recorded

2.4.1. *Body weight and body condition score*

The BW and BCS were recorded weekly for all does. The BCS was examined by lumbar palpation (always by the same handler) and recorded on a scale of 0 = emaciated to 5 = very fat, with increments of 0.25 (Hervieu et al., 1991).

2.4.2. *Detection of oestrous behaviour*

Oestrous activity was recorded every day by direct visual observation of the marks left by the marking harnesses (Walkden-Brown et al., 1993).

2.4.3. *Detection of ovulation*

Ovulation and the ovulation rate were assessed by the number of corpora lutea observed in transrectal ultrasonography conducted 6-8 days after the detection of oestrus (Simoes et al., 2005). The procedure was performed using an Aloka SSD-500 (Ecotron, Madrid, Spain) apparatus connected to a 7.5 MHz linear probe. The presence of corpora lutea was confirmed by the plasma progesterone concentration in plasma sampled twice per week.

2.4.4. *Plasma samples and analysis*

Blood samples were taken twice per week from the onset of the experiment until the end of the study (June 17th) by jugular venipuncture and using tubes containing heparin. Plasma was obtained after centrifugation at 3500 rpm for 30 min; this was stored at -20°C until analysis.

Plasma progesterone was determined twice per week using a commercial enzyme-linked immunoassay (ELISA) kit (Ridgeway Science Ltd., Gloucester, UK), following the manufacturer's instructions (Madgwick et al., 2005). The mean intra and inter-assay coefficients of variation were 3.2% and 8.5% respectively. The sensitivity of the assay was 0.1 ng/mL. The percentages of females showing oestrus with or without ovulation, as well as those showing silent ovulation, were inferred from the plasma progesterone concentrations.

Plasma glucose was determined twice per week using a standard glucose enzyme assay kit (Wako, Chemicals GMBH, Germany) (sensitivity 0.25 mg/mL, intra- and inter-assay coefficients of variation 6.5% and 7.3% respectively).

NEFAs were determined twice per week by spectrophotometric assay using the NEFA-HR2 kit (Wako, Chemicals GMBH, Germany) (sensitivity 0.028 mg/mL, intra- and inter-assay coefficients of variation 5.6% and 6.4% respectively).

Plasma insulin-like growth factor- 1 (IGF-1) was determined weekly using the commercial Goat Insulin-Like Growth Factor Kit (Cusabio, Shanghai, China) (sensitivity 0.1 ng/mL, intra- and inter-assay coefficients of variation 5.4% and 5.9% respectively).

2.4.5. *LH pulsatility at male introduction*

Plasma LH could not be monitored in all the does for logistic reasons. Seven does were therefore randomly selected from each subgroup for this purpose. Blood samples were collected in 5 mL tubes containing heparin using jugular catheters; this was performed at 15 min intervals from 2 h before male introduction (control period) to 6 h after male introduction. These samples were centrifuged (3500 rpm for 30 min at 4°C) and the plasma stored at -20°C until analysis. LH was determined using the LH Detect® enzyme-linked immunosorbent assay (INRA, France), as reported by Maurel (1991). The sensitivity of the assay was 0.1 ng/mL. The intra- and inter-assay coefficients of variation in the control were 5.2% and 7.1% respectively.

LH pulses were defined according to Baird et al. (1981) as the point with two consecutive values higher than the two preceding ones, and with the highest value (pulse amplitude) exceeding the mean basal value by at least four times the coefficient of variation of the assay.

2.4.6. *Conception rate, fertility and productivity*

The conception rate (percentage of pregnant does/does mounted by the males) was determined via transrectal ultrasonography on day 45 after mounting (Schrick et al., 1993). Fertility (percentage of goats kidding/does mounted by the males), prolificacy (number of kids born per female kidding) and productivity (number of kids born per female in each mating group) were also determined.

2.5. Statistical analyses

Data are presented as means±standard error. The weekly values for BW, BCS and IGF-1, and the twice weekly values for glucose and NEFAs, were examined by ANOVA with time as a repeated measure and the experimental factors (nutrition and melatonin treatment) as main factors. Differences at each week between groups were subjected to ANOVA with the experimental factors as the source of variation The Duncan test was used to detect differences between subgroups at each week. The percentage of females showing oestrous behaviour, those showing oestrus and ovulation, the conception rate, and fertility values, were compared between groups using the Fisher-Freeman-Halton exact probability test for multiple group comparisons, and the Fisher exact probability test for two-group comparisons. Ovulation rates and prolificacy were compared using the Mann Mann-Whitney U test. Productivity and the interval 'male introduction to date of showing oestrous plus ovulation' were compared using ANOVA with the treatments (nutrition and melatonin) as main factors. The Duncan test was used to detect differences between subgroups when interactions between treatments were observed.

The mean concentrations of LH before or after male introduction were analysed by ANOVA, with time as a repeated measure and the experimental treatments as main factors. ANOVA was performed to detect differences caused by all sources of variation before and after the introduction of the males. The mean number of pulses per hour experienced by each animal was calculated. ANOVA involving nutrition, melatonin treatment and the effect of introduction of the males (pre vs. post male introduction) was performed to examine their effects on the hourly pulse frequency. Significance was set at $P<0.05$. All analyses were performed using the SPSS package (Statistical Package for the Social Sciences, 2008).

Results

Body weights, body condition scores and metabolic/nutritional variables

The nutrition level provided induced an increase of 5.19±0.92 kg and 0.33±0.05 points in BW and BCS respectively in the LLg group does, and a reduction of 4.60±0.78 kg and 0.22±0.05 points in BW and BCS respectively in the HHl group does ($P<0.001$). Neither melatonin treatment nor the interaction *nutrition x melatonin* had any effect on the gain or losses of BW or BCS. The interaction *nutrition x time,* however, had a clear effect on both BW and BCS ($P<0.001$) (Fig. 1); an increase in both variables was seen in the LLg group whereas reductions were recorded for the HHl group. Neither nutrition nor melatonin treatment alone, nor the interaction *nutrition x melatonin,* nor *melatonin x time,* nor *nutrition x melatonin x time*, had any effect on BW or BCS.

Plasma glucose concentrations were higher in the LLg group than in the HHl group (77.02±0.59 mg/dL vs 69.99±0.61 mg/dL respectively; P<0.01). Moreover, these concentrations were higher in the females treated with melatonin (as a whole) (75.33±0.66 mg/dL) than in those not-treated with melatonin (as a whole) (70.94±0.56 mg/dL) (P<0.01). The interactions *nutrition x time* and *melatonin treatment x time* also had a significant influence on plasma glucose (P<0.001). One week after starting the experiment, plasma glucose fell suddenly in the HHl group, and remained lower than in the LLg group for 3 weeks (until 22nd April). It was also lower at the time of male introduction and for 1.5 weeks at the end of the experiment. Note that the glucose concentrations of the LLg and HHl groups were similar for the first week of the experiment, over the 5.5 weeks in the middle of the experiment, and for the very last sample of the experiment (Fig. 2 top). With respect to the interaction *melatonin treatment x time*, the glucose concentrations differed between the groups for five (not continuous) samples only, and were higher for the melatonin-treated females (as a whole) than the no-melatonin-treatment females (as a whole) (Fig. 2 bottom). Neither the interaction *nutrition x melatonin treatment* nor *nutrition x melatonin x time* had any effect on plasma glucose.

The NEFA concentration was higher in the HHl group than in the LLg group (9.91±0.36 mg/dL vs 22.70±0.67 mg/dL; P<0.01). Similarly, it was higher in the females (as a whole) not treated with melatonin (15.14±0.56 mg/dL vs 18.75±0.67 mg/dL in melatonin-treated females [as a whole]; P<0.01). The interactions *nutrition x time*, *melatonin treatment x time* and *nutrition x melatonin x time* had a significant influence on the NEFA concentration (*P*<0.001) (Fig. 3). It was similar in all subgroups at the very start and the very end of the experiment. By the second sample, however, higher concentrations were observed for the HHl-Mel subgroup than for the other three subgroups, which were

maintained over the next three weeks. In the fourth week their concentrations were similar to those recorded for the HHl+Mel subgroup does, and higher than those recorded for the LLg+Mel and LLg-Mel does until the eighth week. For two weeks and a half after introduction to the bucks, the NEFA concentrations of the LLg-Mel does increased, and remained higher than those of the other three subgroups for one week. Finally, the NEFA concentrations of the HHl+Mel does were higher than those of the other three subgroups for the two weeks of the experiment preceding the last sample taken. The interaction *nutrition x melatonin* treatment had no effect on NEFA concentration.

The IGF-1 concentrations were higher in the LLg group than in the HHl group (132.96±5.18 mg/dL vs 84.38±2.48 mg/dL; $P<0.01$). No effect of melatonin treatment was observed on IGF-1 concentration, while the interactions *nutrition x time*, *melatonin treatment x time* and *nutrition x melatonin x time* had a significant influence ($P<0.001$). No differences were observed between the subgroups at the start of the experiment. However, from then on, the IGF-1 concentration of the HHl+Mel subgroup showed the lowest concentrations; the highest were observed in the LLg+Mel subgroup for most of the experiment. In the fifth week after male introduction, all subgroups showed increased IGF-1 concentrations except for the HHl-Mel subgroup. The interaction *nutrition x melatonin* treatment had no effect on IGF-1 concentration.

The interaction *nutrition x melatonin treatment x male introduction* determined that the HHl-Mel does had lower IGF-1 concentrations after male introduction than before their introduction, while no such difference was seen for the LLg-Mel females (Fig. 5).

Reproductive response

Melatonin treatment significantly increased the percentage of females undergoing ovulation and showing oestrous activity plus ovulation (Table 1) ($P<0.01$) to a similar degree in both the LLg and HHl groups (Table 2). The interval between the introduction of the males and the oestrous response was shorter in the LLg+Mel subgroup than in the HHl+Mel subgroup ($P<0.05$) (Table 1). Neither nutrition nor the interaction *nutrition x melatonin* had any effect on these variables.

The ovulation rate was higher in the LLg+Mel subgroup ($P<0.01$) than in any other subgroup (Table 1). The LLg+Mel does showed a higher conception rate and greater fertility and productivity than any other subgroup ($P<0.05$) (Table 1).

At the time of ovulation, the BW and BCS were higher in the females of the LLg group than those of the HHl group; they were also greater in the no-melatonin-treatment females (as a whole) compared to the melatonin-treated females (as a whole) (at least $P<0.05$ in all cases) (Table 2). Also at the time of ovulation, the IGF-1 concentration was higher in the LLg group than in the HHl group, and the NEFA concentration was higher in the melatonin-treated females (as a whole) than in the no-melatonin-treatment females (as a whole).

At the time when oestrus activity became noticeable, the BW of the LLg group was higher than that of the HHl group. Finally, the plasma glucose and NEFA concentrations in the HHl group were higher than in the LLg group at this time ($P<0.05$) (Table 2).

LH secretion

Before the introduction of the males, the LH concentration fluctuated significantly over time ($P<0.01$) but the effect of time disappeared after male introduction. The LH concentrations increased to a similar degree after male introduction in all subgroups (overall mean 0.34±0.01 ng/mL before introduction vs 0.46±0.02 ng/mL after introduction; $P<0.01$).

No differences were seen between groups with respect to hourly LH pulse frequency before or after male introduction, nor did the interactions *nutrition x melatonin* nor *nutrition x melatonin x introduction of males* have any effect on this variable. However, on the whole, the hourly LH pulse frequency differed between the pre and post male introduction periods (0.02±0.02 pulses/h vs 0.12±0.03 pulses/h before and after male introduction respectively, $P<0.05$). No differences were seen in pulse amplitude in any group or subgroup (mean 1.71±0.33 ng/mL). Neither did any treatment have any effect on the time at which the first LH pulse occurred after male introduction (mean 111.00±31.08 min).

Discussion

The present results show that exogenous melatonin is unable to improve the reproductive performance of does experiencing decreasing BW/BCS, but that it can improve it in does experiencing increasing BW/BCS. This might be explained by the higher plasma glucose and IGF-1 concentrations observed in the LLg+Mel subgroup does.

The HHl-Mel subgroup showed the smallest percentage of ovulating females, while the no-melatonin-treatment females (as a whole) showed oestrous plus ovulation less commonly than the melatonin-treated females (as a whole), i.e., the nutritional regimen followed had no

influence on this outcome. However, a different relationship, between nutrition and melatonin treatment has been described in both sheep (Forcada et al., 1995) and goats (Zarazaga et al., 2011a), with a clear increase in LH concentration seen in melatonin-treated females following a restricted nutritional regimen. The present result also contrast with the findings of earlier work by our group (Gallego-Calvo et al., 2015b), in which does with decreasing BW/body fat reserves showed poorer reproductive performances than those with increasing BW/body fat reserves. This discrepancy might be explained in that, in the earlier work, the male goats displayed normal autumn-time sexual activity, while in the present work they displayed reduced, springtime sexual activity.

The overall poor reproductive response observed (percentage of ovulating females and females showing oestrous plus ovulation), determined that the conception rate, fertility and productivity results for all groups and subgroups were poor (pregnancy was achieved only in the LLg+Mel subgroup), and certainly, no differences were observed between the no-melatonin-treatment subgroups. These results contrast with previous findings reported by our group (Gallego-Calvo et al., 2015b); in the latter work, fecundity, fertility and productivity were greater among LLg does (like those of the present LLg-Mel subgroup) than among HHl does (like those of the present HHl-Mel subgroup). Again, it may be that the males in the latter experiment displayed autumn-time rather than springtime sexual activity.

From a metabolic point of view these results might be explained in two ways. First, plasma glucose concentrations were higher in the LLg than in the HHl does, and in the melatonin-treated than the no-melatonin-treatment does in general. Our group recently reported that animals increasing their body weight/body fat reserves, like those of the present LLg group, showed higher plasma insulin and glucose concentrations,

and that this might explain these animals' improved reproductive results (Gallego-Calvo et al., 2015b). Insulin concentrations were not monitored in the present study since the results were so clear in this earlier work, and because the literature clearly shows that plasma insulin concentrations increase, and better reproductive results are achieved, in does fed large amounts of concentrate (Todini et al., 2007) or when they are overfed (Tsiplakou et al., 2012). The second possible explanation lies in the fact that plasma IGF-1 concentrations became higher in the LLg+Mel, LLg-Mel and HHl+Mel subgroups compared to the HHl-Mel subgroup. Several studies suggest IGF-1 of peripheral origin to provide reproduction-promoting metabolic signals to the hypothalamo-pituitary axis. Flores et al. (2014) reported higher IGF-1 concentrations in female goats with greater food intakes compared to controls. The present clear increase in IGF-1 at four weeks after male introduction (and its maintenance for two weeks) might explain why the LLg+Mel females showed a better reproductive response; increased IGF-1 causes an increment in steroid production and speeds up follicle growth (Spicer and Echternkamp, 1995). Martinez et al. (2011) suggest that an improved oestrus response and pregnancy rate can be achieved in does via the effect of IGF-1 on follicle development.

The NEFA concentrations alone do not explain these reproductive results. However, an interaction between time and nutrition was seen: the NEFA concentration in the LLg group at 2.5-3.5 weeks following male introduction was higher than in the HHl group. The same was also noted by our group in a previous report (Gallego-Calvo et al., 2015b). It is difficult to explain why such an interaction should occur, but perhaps NEFAs are used in the synthesis of reproductive hormones. Before male introduction, the NEFA concentrations of the HHl-Mel and HHl+Mel

does were higher than in their corresponding comparison groups before male introduction. This agrees with that reported by Tsiplakou et al. (2012) and Gallego-Calvo et al. (2015b), who suggest that NEFAs are the link between energy balance and reproduction.

The plasma LH concentration and hourly pulse frequency were modified only by the introduction of the males; neither the nutritional regimen followed nor treatment with/without melatonin had any effect. The lack of effect of increasing or decreasing BW or BCS before male introduction might be explained by the does' previous body fat reserves. Those of the HHl group might have been high enough to prevent any negative effect of undernutrition during the experimental period, while the better nutrition received by the LLg group during the experimental period may have been sufficient to maintain the LH concentration seen. The lack of any apparent effect of melatonin on the LH concentration before male introduction might be explained by a masking effect of nutrition. The introduction of the males - which only displayed springtime sexual activity - increased both the LH concentration and the hourly pulse frequency slightly in all does. Certainly, male reproductive condition is known to affect the strength of the male effect (Flores et al., 2000). The present conditions were trialled since stock-raisers often try to induce mating outside of the natural breeding season, yet rarely take into account the sexual condition of their bucks.

5. Conclusions

Exogenous melatonin fails to improve the reproductive performance of does experiencing decreasing BW/BCS (HHl), but does lead to improvements if their BW/body fat reserves are increasing (LLg). This may be explained by the higher concentrations of glucose and IGF-1 observed in the LLg+Mel females.

Conflict of interest

None of the authors has any financial or personal relationship with any other person or organisation that might inappropriately influence or bias the content of this paper.

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Figure Captions

Figure 1. Change in body weight (BW, top) and body condition score (BCS, bottom) in LLg does (blank symbols) fed 1.9 times their maintenance requirements and then subjected to the male effect, and in HHl does (filled symbols) fed 0.4 times their maintenance requirements and then subjected to the male effect. *P<0.05; **P<0.01; ***P<0.001. The arrow indicates the moment of male introduction (week 6).

Figure 2: Change in plasma glucose concentrations (mg/dL) in: ***top*** - LLg does (□) fed 1.9 times their maintenance requirements, and HHl does (■) fed 0.4 times their maintenance requirements; ***bottom*** - treated with melatonin (◆) or not treated with melatonin (♢). *P<0.05; **P<0.01; ***P<0.001. The arrow indicates the moment of male introduction (week 6).

Figure 3: Change in plasma NEFAs (mg/dL) in: LLg+Mel does (♢) and LLg-Mel does (□) fed 1.9 times their maintenance requirements, and HHl+Mel does (▲) and HHl-Mel does (■) fed 0.4 times their maintenance requirements. Different letters in the same week reflect significant differences between subgroups at P<0.05. The arrow indicates the moment of male introduction (week 6).

Figure 4: Change in plasma IGF-1 (ng/mL) in: LLg+Mel does (♢) and LLg-Mel does (□) fed 1.9 times their maintenance requirements, and HHl+Mel does (▲) and HHl-Mel does (■) fed 0.4 times their maintenance requirements. Different letters in the same week reflect significant

differences between subgroups at P<0.05. The arrow indicates the moment of male introduction (week 6). Different letters in the same week reflect significant differences between subgroups at P<0.05. The arrow indicates the moment of male introduction (week 6).

Figure 5: Insulin growth factor-1 (IGF-1, ng/mL) concentrations before (blank bar) and after (solid bar) male introduction to females fed 1.9 times their maintenance requirements and implanted with melatonin (LLg+Mel), to females fed 1.9 times their maintenance requirements but not implanted with melatonin (LLg-Mel), to females fed 0.4 times their maintenance requirements and implanted with melatonin (HHl+Mel), and to females fed 0.4 times their maintenance requirements and not implanted with melatonin (HHl-Mel).

Figure 1.

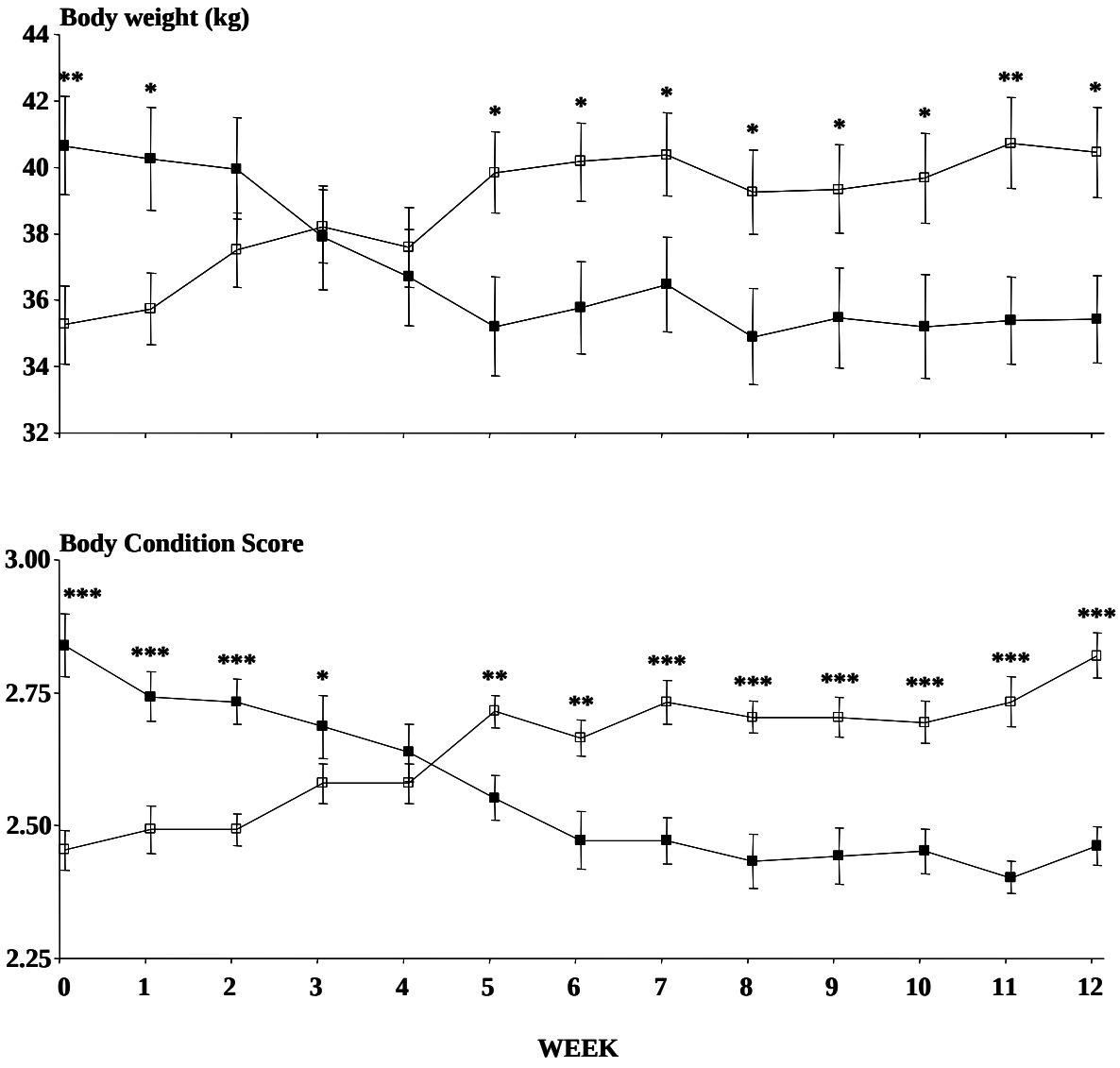


Figure 2.

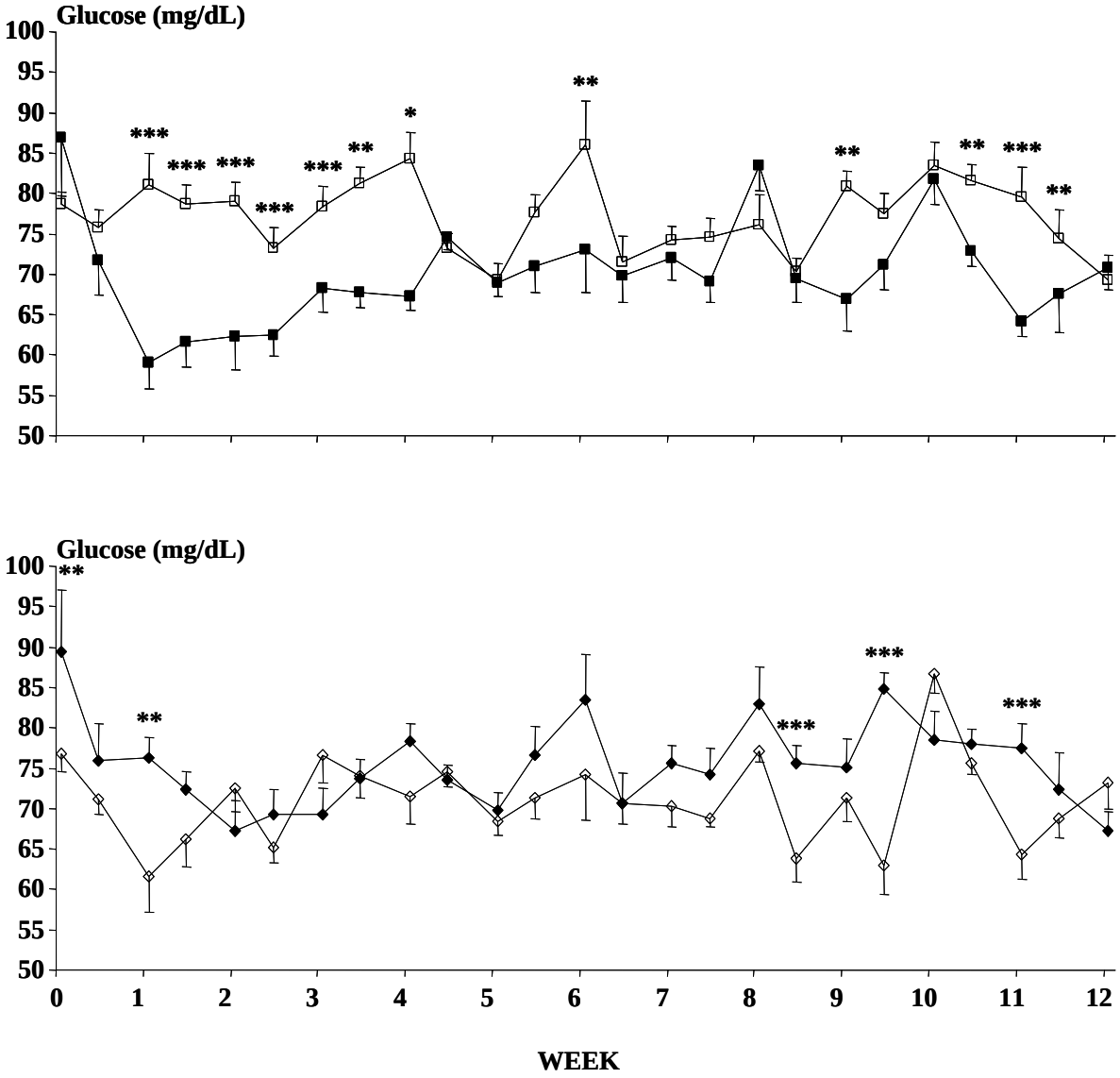
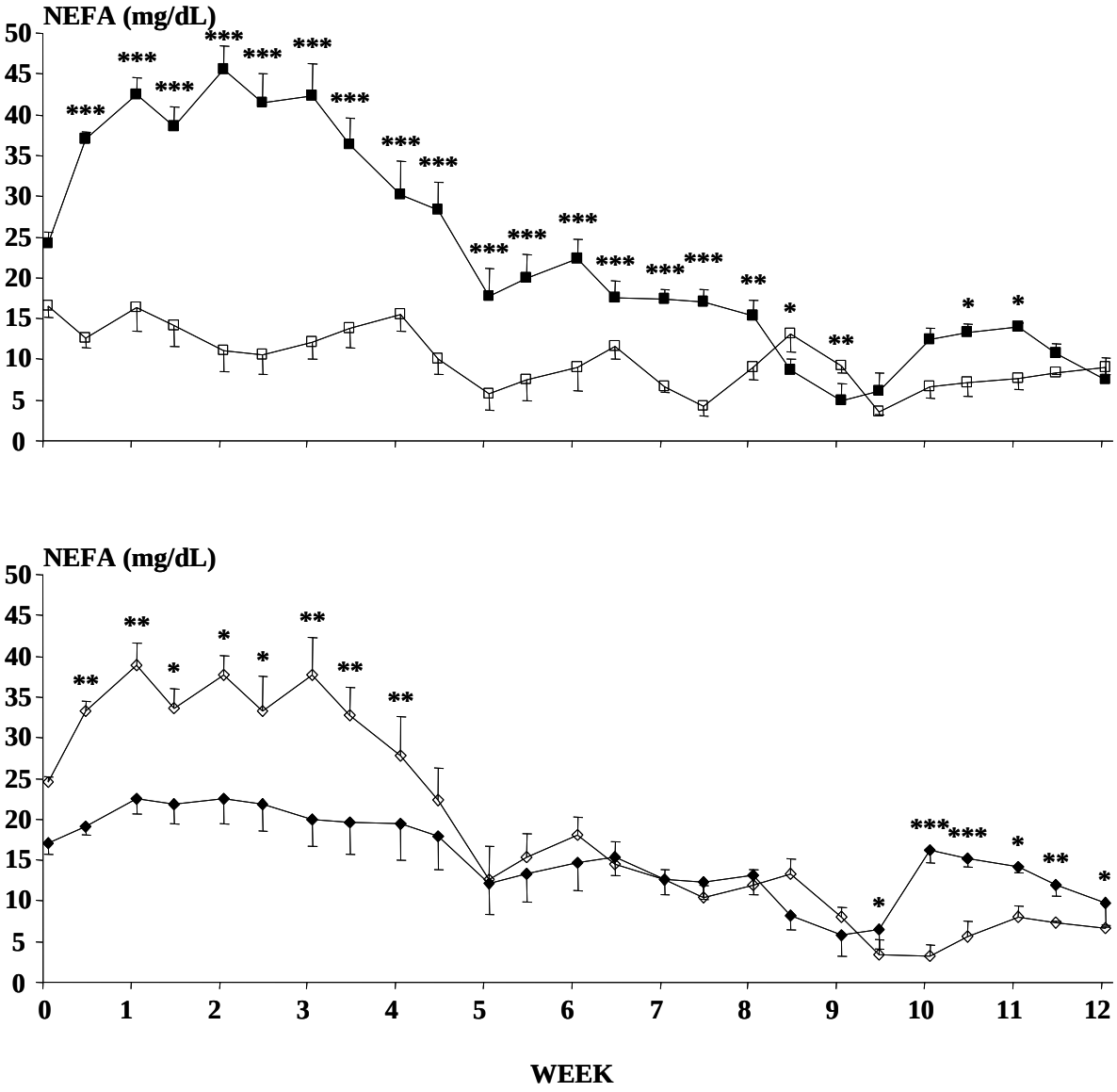


Figure 3.



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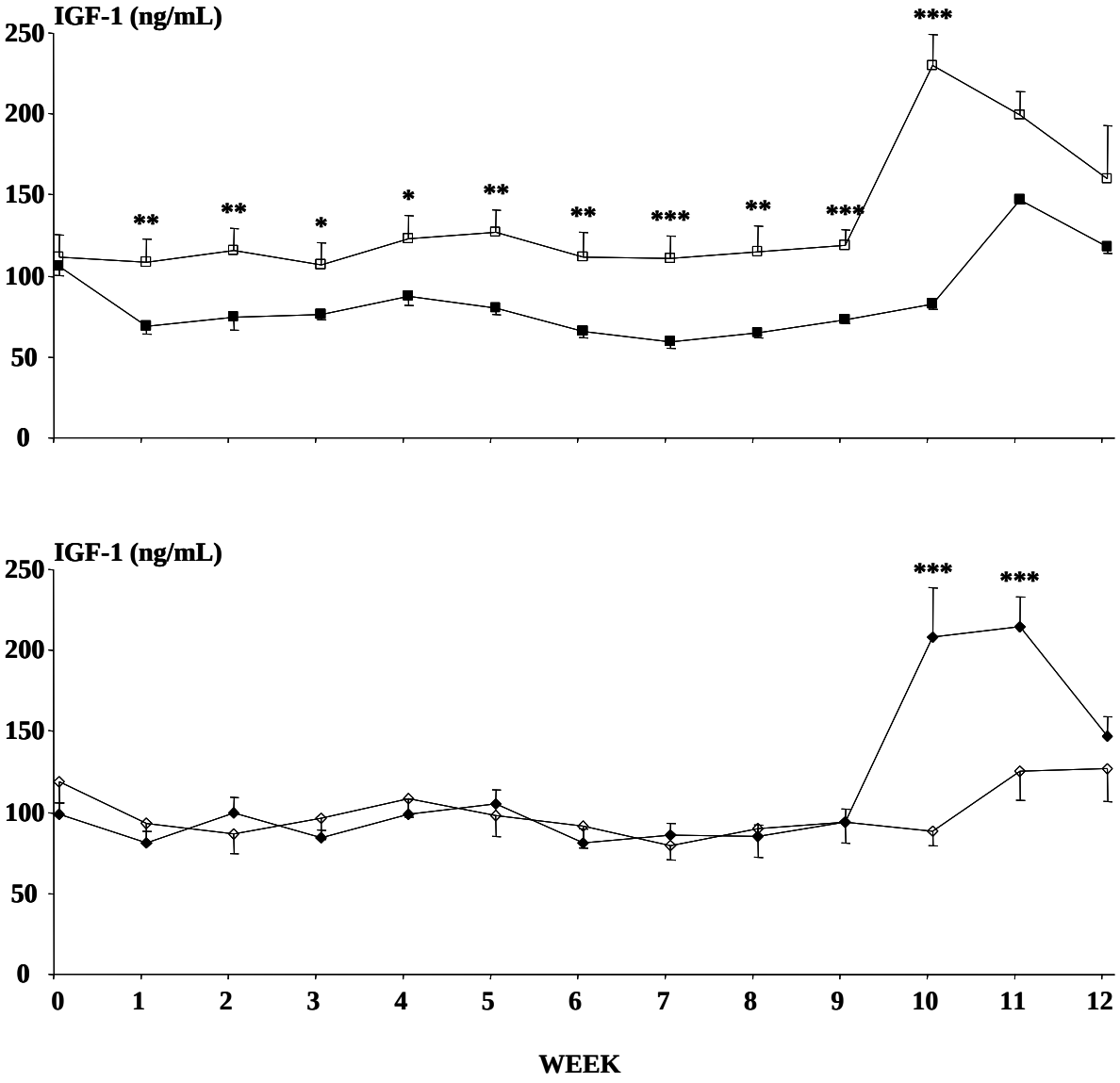
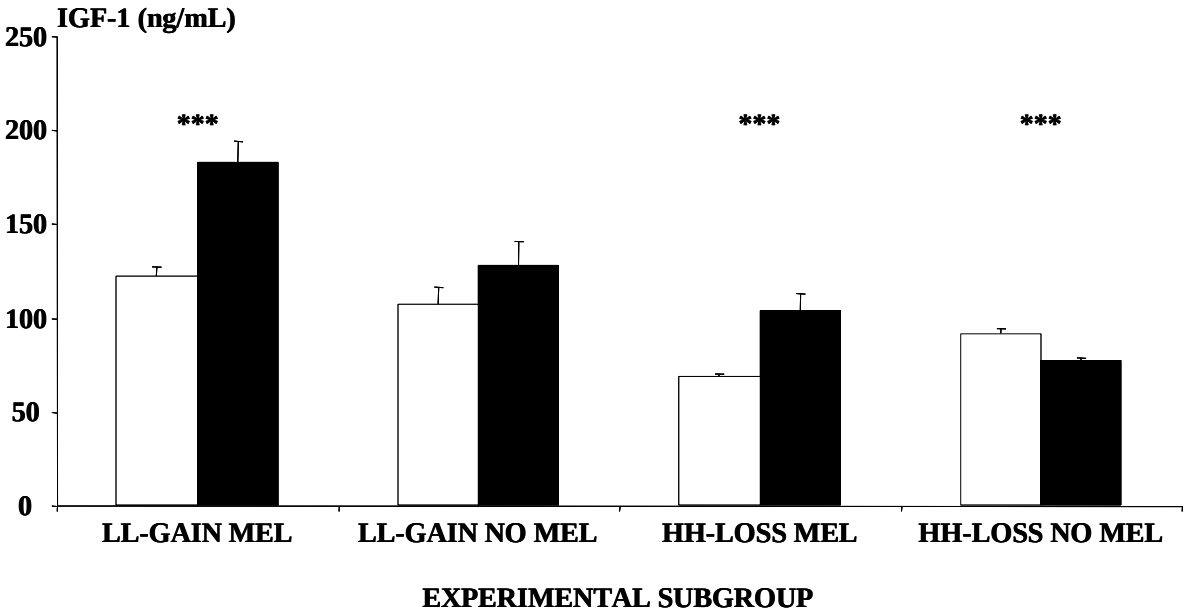


Figure 5.



Tables

Table 1. Reproductive results, and body weight (BW), body condition score (BCS), glucose (mg/dL), NEFA (mg/dL) and IGF-1 (ng/mL) concentrations at ovulation, and time of oestrous, following the introduction of males displaying springtime sexual activity. The LLg does were fed 1.9 times their maintenance requirements; the HHl does were fed 0.4 times their maintenance requirements. Some females in each group were treated with melatonin (+MEL) while some received no such treatment (-MEL).

	LLg (N=24)		HHl (N=29)	
	+MEL (n=12)	-MEL (n=12)	+MEL (n=15)	-MEL (n=14)
Females showing ovulation (%)	33ab	25ab	60a	0c
Females showing oestrous plus ovulation (%)	33ab	0c	40a	0c
Interval male introduction - oestrus (days)	6.0 ± 0.0a		17.5 ± 3.3b	
Ovulation rate	2.0 ± 0.0a		1.0 ± 0.0b	
Conception rate (%)	33a		0b	
Fertility (%)	33a		0b	
Prolificacy (kids born per female kidding)	1.0 ± 0.0		0.0 ± 0.0	
Productivity (kids born per female in the group)	0.33 ±0.33a	0.00 ±0.00b	0.00 ±0.00b	0.00 ± 0.00b

a, b: Different letters in the same row reflect significant differences at $P<0.05$.

Table 2. Body weight (BW), body condition score (BCS), glucose (mg/dL), NEFA (mg/dL) and IGF-1 (ng/mL) concentrations at ovulation, and time of oestrous, after the introduction of males displaying springtime sexual activity. LLg does were fed 1.9 times their maintenance requirements; HHl does were fed 0.4 times their maintenance requirements. Some females in each group were treated with melatonin (+MEL) while some received no such treatment (-MEL).

	LLg (N=24	HHl (N=29)	+MEL (N=27)	-MEL (N=26)
BW at ovulation	39.7 ± 0.8A	32.2 ± 1.6B	34.0 ± 1.3a	42.0 ± 1.2b
BCS at ovulation	2.75 ± 0.03a	2.50± 0.00b	2.58 ± 0.03a	2.75± 0.03b
Glucose (mg/dL) at ovulation	81.06 ± 8.64	94.41 ± 10.61	84.66 ± 8.35	105.50 ± 9.48
NEFA (mg/dL) at ovulation	5.27 ± 1.24	10.51 ± 2.06	9.71 ± 1.45a	1.77 ± 0.75b
IGF-1 (ng/mL) at ovulation	126.72 ± 11.25A	65.04 ± 1.90B	91.36 ± 11.47	94.89 ± 7.83
BW at oestrus	39.0 ± 1.00A	35.0 ± 2.2B	36.6 ± 0.8	
BCS at oestrus	2.75 ± 0.02	2.63 ± 0.06	2.68 ± 0.04	
Glucose (mg/dL) at oestrus	68.28 ± 3.82A	77.15 ± 1.72B	73.60 ± 1.76	
NEFAs (mg/dL) at oestrus	6.88 ± 0.92a	14.85 ± 2.23b	11.66 ± 1.83	
IGF-1 (ng/mL) at oestrus	132.57 ± 8.21	107.60 ± 11.17	117.56 ± 7.63	

Different lower case letters in the same row reflect significant differences at $P<0.05$.

Different upper case letters in the same row reflect significant differences at $P<0.01$.