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Full Length Research Paper

Dynamics of the concentration of the hormones triiodothyronine and thyroxine at the beginning of the reproductive season and the effect of application of iodine in Nelore heifers bred in pasture

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The aim of this study was to evaluate the dynamics of triiodothyronine (T3) and thyroxine (T4) hormones, their relation with body condition score (BCS) and reproductive stage of 248 Nelore heifers, and the effect of iodine application on the induction of cyclicity of 217 anoestrus heifers in pasture at the beginning of the reproductive season. At day zero (D0), an assessment of the BCS was performed. An examination of ovarian structures was performed by ultrasound (US) and the serum was collected for hormonal analysis. Anestrous animals received 27 mg of iodine or placebo (saline). After 40 days (D40), another US was performed, and BCS and hormonal determination evaluations were repeated. At D0 and D40, the cycling group had a BCS mean and a T3 concentration higher than the anestrous group. BCS and T3 and T4 concentrations influenced cyclicity and probability of pregnancy. The treatment did not affect cyclicity, pregnancy rate and number of days between the treatment and calving.

Key words: Bovine, reproduction, thyroid.

INTRODUCTION

The influence of thyroid hormones on reproduction has already been scientifically proven (Starling et al., 2005). Thyroid hormones are important in fertility, acting on ovarian follicle growth, cell proliferation and differentiation of most body tissues and fetal development (Kota et al., 2013). The hypothalamic-pituitary-thyroid and the hypothalamic-pituitary-ovarian axes act simultaneously on the control of folliculogenesis. Thyroid hormones may promote different effects on ovarian function, providing direct or indirect action on follicular development and maturation which results in the regulation of fertility (Saraiva et al., 2010).

Iodine is an essential trace element found in human and animal bodies in minute quantities. The only confirmed role of iodine is in the synthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), which

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are critical for the development of sexual and nervous system as well as the regulation of metabolism, maintaining body temperature and the energy status. Consequently, a severe iodine deficiency hinders the secretion of thyroid hormones (Samanc et al., 2010). Iodine deficiency causes reproductive failures, stillbirth, gestational age extension, abortion, delayed puberty, irregular estrus, reduced fertility and anoestrus (Blood and Henderson, 1991). Studies in pre-pubertal women report that higher concentrations of T4 are associated with precocious puberty (Wilken et al., 2016).

Thyroid hormones have been linked to the different patterns of postpartum luteal activity in dairy cows. Kafi et al., (2012) observed that the serum profile of thyroid hormones was lower in high-producing animals that showed a prolonged luteal phase, anovulation and a delayed first ovulation compared to a normal luteal activity at postpartum. Low serum levels of thyroid hormones in high production animals lead to decrease in energy metabolism; there is mobilization of body fat reserves by directing nutrients to milk production (Khatri and Bhutto, 2014).

Osorio et al., (2014) found that nutritional status is a key factor for the production of T3 and T4, and that the intake of iodine is responsible for the production of such hormones. These thyroid hormones play a crucial role in the development, growth, reproduction and production of cattle. Besides the influence of iodine, serum levels of T3 and T4 may also be influenced by a thermal stress caused by high temperatures and humidity. Júnior et al. (2003) reported that, besides causing changes to physiological and behavioral reactions, heat stress also triggers acute and chronic changes in plasma cortisol concentrations and thyroid hormones (T3 and T4). The circadian secretion rhythm of T3 and T4 is determined by TSH pulses that occur at night. T3 and T4 concentrations are variable during the day, but show significant differences at night (Teixeira et al., 2008). The concentration of iodine in the ovary has been known to be higher than in any other organ except for thyroid. The ovary iodide uptake varies according to reproductive stage. It is increased by estrogen and by a hypothyroid state, and blocked by goitrogens, substances that hinder the absorption of iodine (Slebodziński, 2005). The hypothesis of study was if the application of iodine in females without an ovarian activity is capable of causing a stimulus for the production of T3 and T4 hormones by the thyroid gland, starting cyclicity. Therefore the dynamics of the concentration of T3 and T4 was evaluated at the beginning of the reproductive stage and the effect of application of iodine on the induction of cyclicity of Nelore heifers bred in pasture.

MATERIALS AND METHODS

All procedures were approved by the Ethics Committee on Animal Use (CEUA IF Goiano) under protocol number 012/2013. The study was conducted on a farm located in the municipality of Figueirão, Mato Grosso do Sul state, Brazil (18˚36'56.51" S and 53˚35'26.80" W). Two hundred and forty-eight (n=248) Nelore heifers were selected. They were bred in a pasture, aged 20±2 months, weighing an average of 268.7±29.4 kg. They were kept in pastures with *Brachiaria brizantha*, with an adequate natural shading and free access to water. Mineral salt (Dukamp 80/S®, Monte Aprazível, SP state, Brazil) was freely provided in covered troughs with an average daily consumption of 80 g.

During the first 40 days of the experimental period (day 0 to day 40 of the study), the temperature and humidity were measured daily. A daily index of temperature and humidity (THI) was calculated using the model defined by Thom (1959) and applied by Maturana Filho et al. (2011): THI = 0.8 x T + [(RH(%)/100) x (T - 14.4)] + 46.4; where T is maximum temperature in °C, and RH is maximum relative humidity, measured by a digital thermo-hygrometer (Instrutemp®, Belenzinho, SP state, Brazil). The rainfall between days 0 and 40 was 231 mm, and the average temperature and humidity were 25.5±1.7°C and 76±10.8%, respectively. The average THI was 91.83.

On the first day (D0) of the experiment, the animals were immobilized in a restraint snare, and a gynecological examination was performed by ultrasound (DP 2200 VET), using a linear transrectal probe at 5 mHz to verify the reproductive stage. Animals were classified according to the presence or absence of corpora lutea, into cycling (n=31) and anoestrus (n=217). No signs of stress that could affect the results of the experiment were observed during the handling of the animals.

In this management, blood samples were collected through the side coccygeal vein for laboratorial analysis of T3 and T4 levels. The collection was made through the side coccygeal vein and the blood was transferred to a sterile vial without anticoagulant, using a 120 × 40 mm needle with a 5 mL volume. Blood samples were centrifuged and serum was separated and stored at -20°C pending analysis. Serum levels of T3 and T4 were determined by electrochemiluminescence; using Cobas, 411 equipment (Roche/Hitashi®) and laboratory kits (T3: Roche Diagnostics, Indianapolis, IN, USA; T4: Roche Diagnostics, Indianapolis, IN, USA). The visual analysis of heifers was performed at the exit of the chute; a time when the animals are walking, procuring body condition score. To evaluate body condition score, a scale from 1 to 5 was used; 1 is excessively thin and 5 is excessively fat. The scores were attributed to animals according to the amount of tissue stores, especially fat and muscles, associated with specific anatomic markers (Ferguson et al., 1994). Heifers diagnosed in anoestrus were divided into two groups. The treated group (TG: 110) received an application of 27 mg (3.0 mL) of iodine solution (sodium iodide at 0.6%), and the control group (CG: 107) received 3.0 mL of placebo (saline). The iodine solution was obtained from a mixture of distilled water with Sodium Iodide PA® (PM. 149.89; Labsynth, Diadema/SP state).

On the fifth day of the study, the breeding season began with the introduction of bulls selected for breeding by an andrologic examination into the batch of heifers. The proportion bull/heifer was 1/30. Forty days (D40) after the application of the iodine and the placebo, a second gynecological examination was performed to identify anoestrus, cycling and pregnant animals. New blood samples were collected to study the dynamics of the concentrations of the hormones, T3 and T4, at the early reproductive season and the effect of the application of iodine. The procedures followed the same methodology as at the first day of the experiment. Heifers were submitted to a 90-day breeding season with bulls selected through andrologic examination. 60 after days the withdrawal of the bulls, a pregnancy diagnosis was performed by means of ultrasound, to verify the final pregnancy rate in the different experimental groups. The animals remained on the same property until calving, enabling the record of the calving dates. The interval between the start of the breeding season and the calving was

Table 1. Average concentration and standard deviation of triiodothyronine (T3) and thyroxine (T4) of three body condition score (BCS) groups of cycling and anoestrus Nelore heifers raised on pasture in the first experimental day (D0) and forty days later (D40).

Day		BCS			
		≤2.0	**2.1 to 2.9**	**≥3.0**	**p-value**
D0	T3 (ng/mL)	2.71±0.08[a]	2.73±0.06[a]	2.89±0.11[a]	0.168
	T4 (ng/mL)	115.43±3.84[a]	122.73±4.02[b]	148.25±7.16[c]	≤0.001
D40	T3 (ng/mL)	2.59 ± 0.05[a]	2.50 ± 0.04[a]	2.57 ± 0.07[a]	0.905
	T4 (ng/mL)	123.41 ± 4.17[a]	131.07 ± 3.74[b]	138.63 ± 5.06[c]	0.017

*Different lowercase letters in the same line indicate statistical difference (P<0.05).

analyzed at the end of the calving season.

To perform the statistical analysis, different methodologies were used due to the nature of the study variables. For the analysis of the behavior of T3 and T4 at the two different times (D0 and D40), a Pearson's correlation was recommended to study the association between continuous variables. To evaluate differences in hormone concentrations between animals with different body condition scores as well as reproductive phases (cycling and anoestrus), an analysis of variance and a t test was used to compare means. To examine the effect of the application of iodine on cyclicity and pregnancy rate, the chi-square test was used. The comparison of means between T3 and T4 concentrations and number of days until calving of the treated and control groups was performed using analysis of variance. To study the influence of hormone levels on pregnancy rates and cyclicity, given the categorical nature of the response variables and the continuous independent variable, the logistic regression analysis using the logit function was performed. To perform the statistical analyses, the functions and packages of the statistical software R CoreTeam (2013) used a significance level of 5% probability; considering the hypothesis tests.

RESULTS AND DISCUSSION

Blood concentrations of T3 and T4 were evaluated according to different BCS, considering all animals (cycling and anoestrus) (Table 1). It is observed that average T4 concentrations in different BCS groups were statistically different at the beginning of the breeding season as well as 40 days later. Induced hypothyroidism may result in a better body weight gain and a better body condition (De Moares et al., 1998). This may explain the relative lower levels of T4 in animals with a lower BCS as a physiological mechanism to improve BCS and start cyclicity. However, results observed by Bettini et al., (2006) showed that induced hypothyroidism did not affect weight variation, super-ovulatory response and activity of the corpus luteum in crossbred heifers (½ *Bos taurus* x ½ *Bos indicus*).

Cycling and anestrous groups showed a statistical difference of 5%, regarding the averages for T3 and T4 concentrations at the beginning of the breeding season (D0). Only T4 showed no significant difference 40 days later (D40), as can be seen in Table 2. These results suggest that the hormones, T3 and T4, are related to the cyclicity of heifers bred in pastures, since the average of the concentrations were significantly higher in cycling females if compared with anoestrus animals regarding both hormones at the beginning of the breeding season. According to Saraiva et al. (2010), the hypothalamic-pituitary-thyroid and the hypothalamic-pituitary-ovarian axes act simultaneously on the control of folliculogenesis. Thyroid hormones may promote different effects on ovarian function, resulting in a regulation of fertility of bovines. The activity of iodothyronine deiodinases, enzymes responsible for the conversion of T4 into T3, is regulated by sex steroids; but the mechanisms involved are poorly defined. The heifers in this experiment were at puberty, and the lower levels of T3 found in the anoestrus group may be related to the gonadal hormones of this phase, as reported by Marassi et al., (2007).

The highest levels of T4 at D0 in the cycling group coincide with higher estrogen levels at this phase. The administration of estradiol in ovariectomized rats increased the activity of the enzyme thyroid peroxidase (TPO) (Lima et al., 2006). This enzyme catalysis' oxidation reactions and the organification of iodide in the presence of hydrogen peroxide; this suggests that estrogen stimulates the precursor reactions of T4, which is both the uptake of iodide by the thyroid and its organification.

Fortunato et al., (2014) also observed the stimulatory effect of estrogen on the thyroid function in rats by increasing the uptake of iodide through the activity of TPO and by increasing the biosynthesis of thyroid hormones. These reports coincide with the dynamics of T3 and T4 studied in this research, in which the concentrations showed a positive relation with BCS and cyclicity. Table 3 shows the mean concentrations of T3 and T4 hormones of 248 Nelore heifers bred in pasture at the beginning of the breeding season and forty days later, regardless of the reproductive stage observed in gynaecological examinations. It is observed that the means of T3 concentrations differed significantly after 40 days, showing a decrease in concentration. This indicates a possible increase in the activity of iodothyronines, enzymes responsible for T4 deiodination, stimulated by gonadotropins secreted during the follicular period. The THI may also influence this change in the T3 concentration

Table 2. Mean and standard deviation of the concentration of the hormones triiodothyronine (T3) and thyroxine (T4) in Nelore heifers, cycling and anoestrus, raised on pasture at the beginning (D0) of the breeding season and forty days later (D40).

Day		Cycling (n=31)	Anoestrus (n=217)	p-value
D0	T3(ng/mL)	3.08 ± 0.15[b]	2.71 ± 0.05[a]	0.023
	T4(ng/mL)	151.14 ± 7.50[b]	122.66 ± 3.01[a]	0.048
		Cycling (n=187)	Anoestrus (n=58)	p-value
D40	T3(ng/mL)	2.75 ± 0.11[b]	2.50 ± 0.03[a]	0.003
	T4(ng/mL)	**140.35 ± 7.58[a]**	**128.79 ± 2.58[a]**	**0.464**

* Different letters in the same line indicate statistical difference (P<0.05).

Table 3. Mean and standard deviation of the concentrations of triiodothyronine (T3) and thyroxine (T4) of Nelore heifers at the beginning of the breeding season (D0) and 40 days later (D40).

Hormones	D0	D40	p-value
T3 (ng/mL)	2.77 ± 0.05[b]	2.54 ± 0.03[a]	≤0.001
T4 (ng/mL)	126.26 ± 2.89[a]	130.25 ± 2.47[a]	0.312

* Different letters in the same line indicate statistical difference at 5%.

Table 4. the breeding season of the control group and of the group treated on the first day of the experiment (D0) and 40 days (D40) after treatment with iodine.

Group	D0	D40
T3		
Treated (ng/mL)	2.69 ± 0.05 [bB]	2.46 ± 0.04 [aA]
Control (ng/mL)	2.73 ± 0.08 [aA]	2.55 ± 0.04 [aA]
T4		
Treated (ng/mL)	120.41 ± 3.82 [aA]	126.32 ± 3.48 [aA]
Control (ng/mL)	126.20 ± 4.78 [aA]	131.50 ± 3.82 [aA]

* Different lowercase letters in the same column indicate that there was statistical difference between groups, different uppercase letters in the same line indicate statistical difference between times (P<0.05)

found at D40. In the first 40 days of this experiment, temperature and humidity data were collected daily, resulting in an average THI of 91.83; this reflects high temperatures and humidity during this period. High environmental temperatures cause heat stress, which leads to an increased blood cortisol concentration and a decreased concentration of thyroid hormones (Starling et al., 2005). Consistent with these results is Coelho et al*.,* (2008) who observed that thermal stress promotes significant decreases only in the T3 plasma concentrations of goats; while T4 levels, in all breeds studied, remained unchanged.

Teixeira et al. (2008) reported this same behaviour in empty mares, in which variations in T3 and T4 levels were observed while maintaining a balanced correlation. Upon evaluating the effect of the application of iodine on heifers in anoestrus at the beginning of the breeding season, it was observed that the average concentration of T3 was statistically lower at D40 for the treated group (Table 4). The treatment with iodine may have influenced the serum level of T3 after 40 days. T3 levels reflect the functional status of the peripheral tissue more than the secreting performance of the thyroid gland. Table 5 shows the percentage of heifers cycling after 40 days, the pregnancy rate at the end of the breeding season and the average of the interval from the treatment calving. To study the effect of the application of iodine on animals that were gaining, losing or maintaining the body condition score, the animals were grouped according to the body condition score as observed between D0 and D40. Table 6 shows the results of mean T3 concentrations of different groups and their respective treatments during the two periods evaluated. The results in Table 6 show that animals that gained BCS during the first forty days of experiment and received iodine decreased the average concentration of T3. Table 7

Table 5. Cyclicity rate 40 days after treatment, pregnancy rate at the end of the breeding season and average number of days of treatment until delivery (LI) of the heifers from the control group and treated with iodine.

Parameter	Control	Treated
Cyclicity rate (%)	73.33[a]	73.39[a]
Pregnancy rate (%)	85.71[a]	79.82[a]
LI (Mean±standard deviation)	338.37±3.68[a]	337.61±3.58[a]

* Equal letters in the same line indicate that there was no statistical difference (P<0.05).

Table 6. Mean and standard deviation of the concentration of triiodothyronine (T3) of anoestrus Nelore heifers in the groups gaining, maintaining and losing body score condition between the first day (D0) of the experiment and forty days (D40) later, treated with iodine and placebo.

Groups	Treatment	D0 (ng/mL)	D40 (ng/mL)	p-value
Gaining	Iodine (n=49)	2.70±0.08 [aB]	2.41±0.05 [aA]	0.00
	Placebo (n=39)	2.62±0.14 [aA]	2.47±0.07 [aA]	0.35
Maintaning	Iodine (n=51)	2.72±0.09 [aA]	2.51±0.06 [aA]	0.07
	Placebo (n=55)	2.75±0.10 [aA]	2.60±0.06 [aA]	0.22
Losing	Iodine (n=10)	2.54±0.15 [aA]	2.45±0.15 [aA]	0.69
	Placebo (n=13)	3.12±0.33 [aA]	2.60±0.12 [aA]	0.16

* Different lowercase letters in the same column indicate that there was statistical difference between groups, different uppercase letters in the same line indicate statistical difference between times (P<0.05).

Table 7. Mean and standard deviation of the concentration of thyroxine (T4) of anoestrus Nelore heifers in the groups gaining, maintaining and losing BCS between the first day (D0) of the experiment and forty days (D40) later, treated with iodine and placebo.

Groups	Treatment	D0 (ng/mL)	D40 (ng/mL)	p-value
Gaining	Iodine (n=49)	119.3±6.0[acA]	126.6±5.2[aA]	0.36
	Placebo (n=39)	119.2±7.4[acA]	123±6.4[aA]	0.69
Maintaining	Iodine (n=51)	115.2±4.5[aA]	127.7±5.4[abA]	0.08
	Placebo (n=55)	125.5±5.8[abcA]	133±5.0[abA]	0.33
Losing	Iodine (n=10)	150.1±17.2[cA]	115.4±6.8[aA]	0.07
	Placebo (n=13)	149.9± 20.5[bcA]	150.4±11.5[bA]	0.98

* Different lowercase letters in the same column indicate that there was statistical difference between groups, different uppercase letters in the same line indicate statistical difference between times (P<0.05).

shows the results in relation to T4. Animals that gained BCS had lower mean concentrations of T4. These results agree with De Moraes et al*.,* (1998), who reported that induced hypothyroidism may result in improved weight gains and increased body condition. Table 8 shows the percentages of cycling and pregnant animals forty days after treatment and sixty days after the withdrawal of the bulls.

The results showed no statistical difference between heifer groups. This is different from Ferreira et al*.,* (2013) who observed an influence of BCS on the pregnancy rate of Nellore cows calved in pasture and subjected to timed artificial insemination, in which females with a lower body condition had lower pregnancy rates compared to those with a better condition (BCS ≥ 3). This study investigated the effect of the application of iodine on the plasma concentrations of thyroid hormones. However, there may be other effects on granulosa cells, such as an increased response to FSH and an improvement of follicle quality, as described by Cecconi et al*.,* (2004). Spicer et al*.* (2001) suggest that thyroid hormones may have direct stimulatory effects on the ovarian function of cattle, acting at the level of granulosa and theca cells. Figure 1 shows the influence of T3 and T4 concentrations on the

Table 8. Percentage of cycling and pregnant heifers forty days after the beginning of the experiment (D40) and final pregnancy in each group.

Groups	Treatment	Cycling (%) D40	Pregnant (%) D40	Final pregnancy (%) 60 days after the withdrawal of bulls
Gaining	Iodine (n=49)	81.63[a]	16.32[a]	79.59[a]
	Placebo (n=39)	74.36[a]	7.69[a]	79.48[a]
Maintaining	Iodine (n=51)	66.67[a]	5.88[a]	82.35[a]
	Placebo (n=55)	76.36[a]	7.27[a]	89.09[a]
Losing	Iodine (n=10)	60[a]	10[a]	70[a]
	Placebo (n=13)	53.84[a]	0[a]	84.61[a]

* Different lowercase letters in the same column indicate that there was statistical difference between groups (P<0.05).

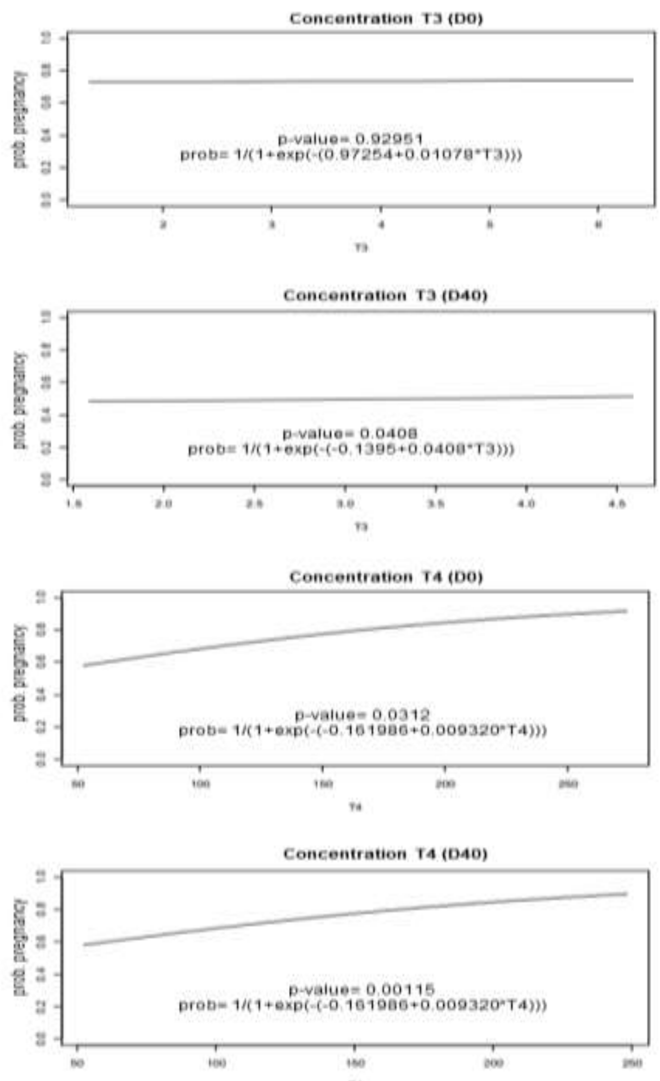

Figure 1. Influence of hormones concentrations of triiodothyronine (T3) and thyroxine (T4) at the beginning of the breeding season (D0) and forty days after (D40) on the probability of pregnancy of heifers raised on pasture 60 days after the late mating season.

probability of pregnancy at D0 and D40. It is noted that only the concentration of T3 at D0 was not significant, while the increase in other concentrations early in the breeding season increases the probability of pregnancy. These results reaffirm the importance of thyroid hormones to reproduction.

Conclusion

Thyroid hormones levels are related to the cyclicity of heifers, and they influence the probability of pregnancy and the treatment with iodine do not have effect on cyclicity, pregnancy rate and number of days between treatments and calving of Nelore heifers bred in pasture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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