

Effect of Late Pregnancy, Parturition and Early Lactation on T3, T4 and Cortisol Level of Heifers and Cows

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Summary

The present study was carried out in the Al-Dujalah Cows Station which is located in Wasit governorate, Iraq. From 305 crossbred cows (Holstein x Friesian breeds), thirty six heifers (2.5-3.5) years old and thirty six multiparous cows (5-7) years old at different physiological conditions. The heifers and cows were divided into six equal groups depending on their physiological conditions as follows, **group 1:** Non-pregnant heifers (control), **group 2:** (Heifers in 7th month of pregnancy), **group 3:** (Heifers in 8th month of pregnancy), **group 4:** (Heifers in 9th month of pregnancy), **group 5:** (Heifers at 1-4 hours of parturition), **group 6:** (Heifers in first month of lactation). The multiparous cows were divided as that of heifers.

The blood samples (5ml) were collected from Jugular vein at 2 weeks interval during pregnancy, at parturition (1-4 hours after parturition) and first month of lactation and deposited into plastic tube without anticoagulant to obtaining the serum samples for hormonal analysis.

The study revealed the following results: A significant increase ($p \leq 0.05$) in serum (T4) level was recorded in last month of pregnancy in both heifers and cows compared with control and other studied groups and a significant decrease in T3 level was observed in 7th month of pregnancy, at parturition and first month of lactation in cows while there was a significant increase in T3 level was recorded in 9th month of pregnancy in heifers compared with control and other studied groups.

A significant increase ($p \leq 0.05$) in serum cortisol level was recorded in first month lactating animals compared with control and other studied groups in both heifers and cows, and a significant increase in serum cortisol level was observed in control, 8th and 9th month of pregnancy and at parturition in cows compared with that of heifers.

Key words: Cows late Pregnancy, Parturition, Early Lactation, T4, T3, Cortiso

تأثير الفترة المتأخرة من الحمل, وعند الولادة والمرحلة المبكرة من إنتاج الحليب على مستوى هورمونات الثايرونين ثلاثي اليود والدريين والكورتيزول في الأباكير والأبقار

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الخلاصة

أجريت الدراسة الحالية في محطة أبقار الدجيل الواقعة في محافظة واسط ومن مجموع ٣٠٥ من الأبقار المضربة (هولشتاين + فريزيان) اختيرت ٣٦ من الأباكير بعمر ٢,٥-٣,٥ سنة و ٣٦ من الأبقار متعددة الولادات بعمر ٥-٧ سنة. قسمت كل من الأباكير والأبقار إلى ست مجاميع متساوية اعتماداً على الحالة الفسلجية وكالاتي المجموعة الأولى (السيطرة) أباكير غير حوامل, المجموعة الثانية أباكير حوامل في الشهر السابع من الحمل المجموعة الثالثة أباكير حوامل في الشهر الثامن من الحمل المجموعة سابعة بعد الرابعة أباكير في الشهر التاسع من الحمل المجموعة الخامسة أباكير حوامل عند الولادة (١-٤) ساعة بعد الولادة المجموعة السادسة أباكير في الشهر الأول من إنتاج الحليب. وقسمت الأبقار متعددة الولادات كما هو الحال في الأباكير. جمعت عينات الدم بواقع ٥ مل من كل حيوان من الوريد الوداجي كل أسبوعين خلال الحمل و (١-٤) ساعة بعد الولادة وخلال الشهر الأول من إنتاج الحليب في أنابيب اختبار بلاستيكية خالية من مانع التخثر للحصول على مصل الدم لغرض قياس مستوى هرمونات الدراسة. أظهرت الدراسة الحالية ارتفاع معنوي ($P \leq 0,05$) في مستوى هورمون الدريين (T4) في الشهر الأخير من الحمل في كل من الأباكير والأبقار مقارنة بمجموعة السيطرة وبقية مجاميع الدراسة كما لوحظ انخفاض معنوي ($P \leq 0,05$) بمستوى هرمون الثايرونين ثلاثي اليود (T3) في الشهر السابع من الحمل وعند الولادة وفي الشهر الأول من إنتاج الحليب في الأبقار متعددة الولادات بينما سجل انخفاض معنوي ($P \leq 0,05$) بمستوى الثايرونين ثلاثي اليود في الشهر التاسع من الحمل في الأباكير مقارنة بمجموعة السيطرة وبقية مجاميع الدراسة. سجل ارتفاع معنوي ($P \leq 0,05$) بمستوى الكورتيزول في الشهر الأول من إنتاج الحليب في كل من الأباكير والأبقار مقارنة بمجموعة السيطرة وبقية مجاميع الدراسة. كما لوحظ ارتفاع معنوي ($P \leq 0,05$) بمستوى الكورتيزول في مصل دم مجموعة السيطرة والحوامل في الشهر الثامن والتاسع وعند الولادة في الأبقار مقارنة بالأباكير.

Introduction

Pregnant dairy cows are at a light risk of metabolic and reproductive disorders and oxidative stress is considered to be involved in these events (Turk *et al.*, 2005). Pregnancy and lactation are physiological statuses considered to modify metabolism in animals (Krajnicakova *et al.* 2003; Iriadam, 2007).

The last 2-3 months of gravidity are extremely critical in cattle due to stress of pregnancy. The following detrimental factors may negatively

influence pregnant breeding cows and therefore the fetus, qualitatively not fully valued feed ration, shortened dry period and a number of health disturbances (diarrhea, anemia, hepatodystrophy, ketosis, mastitis, acid-base balance disturbances, intrauterine infections (Slanina *et al.* 1977). The hormonal activity of thyroid gland has an important role in the transitional period for determining cell metabolism of lipid and carbohydrates and the lactation course itself by its thyroid hormones (Nikolic *et al.* 1997). Thyroid

hormones, triiodothyronine (T3) and thyroxin (T4) have a major role in differentiation, growth and development of animals (Bani Ismail *et al.* 2009).

Cortisol is a well-accepted indicator for stress in animals and man. The releases of adrenocorticotrophic hormone from the pituitary gland occur in late pregnancy, (Kulbery *et al.* 2002). The cortisol values were varied according to pregnancy, parturition and lactation as stresses (Henze *et al.* 1994).

The present study is aimed to investigate the effect of late pregnancy, parturition and early lactation on some hormones level of crossbred cows and heifers.

Materials and Methods

Experimental Animals:

The present study was carried out in the Al-Dujalah cow's station (picture 1) which is located in Wasit governorate-Iraq, about 40 kilometers southeast of the city of AL-Kut. From 305 crossbred cows (Holestine X Friesian breeds), thirty six heifers and thirty six adult cows of different ages and physiological conditions were selected for this study. These cows were under the control of the veterinary health by veterinarians working at the station. Pregnancy was diagnosed based on the records of the station and to confirm the pregnancy and determine the age of the fetus, rectal palpation was used. All animals were maintained and housed under similar conditions of feeding and management. The animals were fed twice a day with green fodder and concentration mixture, and water was supplied ad libitum.

Experimental Design:

The study included two experiments as follows:

The First Experiment

Thirty six heifers, (2.5-3.5) years old were divided into 6 groups, 6 animals in each group. The grouping was done depending on their physiological conditions as follows:

1. Group 1: non-pregnant heifers (control).
2. Group 2: pregnant heifers (in the 7th month of pregnancy).
3. Group 3: pregnant heifers (in the 8th month of pregnancy).
4. Group 4: pregnant heifers (in the 9th month of pregnancy).
5. Group 5: parturient heifers (1-4 hours after parturition).
6. Group 6: postpartum heifers in first month of lactation.

The Second Experiment

Thirty six adult multiparous cows (5-7) years old were divided into 6 equal groups. The grouping was done depending on their physiological conditions like that of the first experiment.

Collection of Blood Samples

All blood samples were collected between 8-10 hours am in order to standardize the time of collection which may affect certain blood parameters. Blood samples (5) ml were collected from jugular vein puncture at 2 weeks interval and at parturition during the experimental period from all animals. The collected blood from each animal deposited into tube without anticoagulant and allowed to clot at room temperature. Then the tubes were centrifuged at 3,000 rpm for 15 minute and the serum samples were stored at -20 °c until used for biochemical analysis.

Total Triiodothyronine (T3) and Thyroxin (T4) Concentrations

For determination of serum T3 and T4 level, Kit of (Monobind Inc. lake forest CA 92630, USA) was used.

Cortisol hormone Concentration

Jconcentration in serum or plasma by microplate enzyme immunoassay, kit of (Monobind Inc. lake forest CA 92630, USA) was used.

Statistical analysis:

Analysis was done using analysis of variance (one way ANOVA) through SPSS computer

package version 11. The differences are considered to be significant at ($p < 0.05$), and the differences between means were done by LSD, (Steel & Torrie, 1980). All data expressed throughout as mean \pm SD.

Results

The results of the T3 levels are represented in Table (1). In heifers a significant increase ($p \leq 0.05$) in T3 level was recorded in 9th month pregnant group compared with control and other studied

groups and there was a significant decrease ($p \leq 0.05$) in T3 level recorded in 7th month pregnant, parturient and early lactating groups compared with control and other groups.

There was a significant decrease ($p \leq 0.05$) in T3 level of 7th month pregnant, parturient and 1st month lactating cows compared with control.

No significant difference was observed between both heifer and cows in all studied reproductive stages.

Table (1): The serum (T3) hormone level ($\mu\text{g/dl}$) in Heifers and cows during late pregnancy, parturition, and early lactation (Mean \pm SD)

Phys. state Animals	Non-pregnant (Control)	Month of pregnancy			At parturition 1-4 h.	1 st month of lactation
		7 th	8 th	9 th		
Heifers	0.99 \pm 0.12 Ab	0.88 \pm 0.18 Ac	1.02 \pm 0.18 Ab	1.16 \pm 0.12 Aa	0.88 \pm 0.16 Ac	0.89 \pm 0.16 Ac
Multiparous Cows.	1.07 \pm 0.2 Aa	0.93 \pm 0.29 Ab	1.05 \pm 0.24 Aa	1.18 \pm 0.16 Aa	0.74 \pm 0.20 Ac	0.92 \pm 0.15 Ab

n=6

The different small letters refer to significant differences at ($p \leq 0.05$) among groups in horizontal row. The different large letters refer to significant differences at ($p \leq 0.05$) between groups in vertical column.

n= refer to the number of the animals in each group.

Phys.state refer to physiological state of animals

Table (2) showed that there was a significant increase ($p \leq 0.05$) of T4 concentration in 9th month pregnant heifers compared with non pregnant control, 7th, 8th, month pregnant, parturient, and first month lactating heifers groups. While no significant ($p \leq 0.05$) difference in T4 values were

recorded in parturient and 1st month lactating heifers compared with control group.

The same patterns were observed in cows. No significant difference was observed between both multiparous cows and heifers in all studied groups.

Table (2): The serum T4 level (ng/ml) of heifers and cows during late pregnancy, parturition, and early lactation. (Mean±SD).

Phys.state Animals	Non-pregnant (Control)	Month of pregnancy			At parturition 1-4 h.	1 st month of lactation
		7 th	8 th	9 th		
Heifers	3.38±0.5 Ac	5.26±0.93 Ab	5.67±0.49 Ab	6.67±0.48 Aa	3.28±0.64 Ac	3.65±0.59 Ac
Multiparous cows.	3.56±0.65 Ae	5.45±0.71 Ac	6.08±0.60 Ab	6.92±0.30 Aa	3.41±0.40 Ae	4.30±0.9 Ad

n=6

The different small letters refer to significant differences at ($p \leq 0.05$) among groups in horizontal row. The different large letters refer to significant differences at ($p \leq 0.05$) between groups **cows** in vertical column. **n**= refer to the number of the animals in each group
Phys. state refer to physiological state of animals

The results indicated that a significant increase ($p \leq 0.05$) in cortisol level was recorded in parturient heifers compared with control and other studied groups (Table 3). On other hand no significant difference was observed in cortisol level between 7th month pregnant heifers and control. In cows serum cortisol level was increased significantly ($p \leq 0.05$) at parturition compared with control and other studied groups in cows,

while a significant decrease ($p \leq 0.05$) in cortisol levels were recorded of 7th month ,8th month pregnant and first month lactating cows compared with control and 9th month pregnant cows. In comparison between cows and heifers a significant ($p \leq 0.05$) increase in of cortisol levels were observed in control, 8th and 9th month pregnant and parturient groups than that of heifers.

Table (3): The serum cortisol level ($\mu\text{g}/\text{dl}$) in heifers and cows during late pregnancy, parturition, and early lactation (Mean±SD).

Phys.state Animals	Non-pregnant (Control)	Month of pregnancy			At parturition 1-4 h.	1 st month of lactation
		7 th	8 th	9 th		
Heifers	10.92±1.03 Bd	10.78 ±0.7 Ad	11.82±0.89 Bc	13.50±0.84 Bb	14.52 ±0.64 Ba	13.82±1.06 Ab
Multiparous Cows.	15.87±1.39 Ab	11.43±0.55 Ad	13.30±0.67 Ac	15.57±1.25 Ab	17.20±1.01 Aa	13.82±1.95 Ac

The different small letters refer to significant differences at ($p \leq 0.05$) among groups in horizontal row. The different large letters refer to significant differences at ($p \leq 0.05$) between groups in vertical column. **n**= refer to the number of the animals in each group.
Phys.state refer to physiological state of animals

Discussion

The serum levels of thyroxin (T^r) and Triiodothyronine (T^t) as reported in the Tables (1) and (2) respectively are in agreement with Huszenicza, *et al.* (2002) who found that high levels of thyroid hormones were recorded in late pregnancy which were followed by a significant decrease in the periparturition period and the level of T^4 were found to be lower in the earliest day of lactation than in late lactation in cows.

The values of T^3 found in the present study (Table 1) indicated that T^3 level decreased significantly in parturient and first month lactating heifers and cows. These results are corresponded to the findings of Reist *et al.* (2002) and Pezzy *et al.* (2003) whom reported that the concentrations of thyroid hormones were reduced in the blood of dairy cows in the transitional period, with a markedly declined (T^3) in blood shortly before and after calving, which may occurs due to negative energy balance and high lipid mobilization.

The serum T^3 levels observed in the present study increased significantly in first month lactating cows compared with parturient cows. The T^3 levels are well within the range as has been observed by Liesegang *et al.* (2006). Cows in postpartum negative energy balance respond to decrease the levels of T^3 & T^4 and increase the level of r T^3 (Mc Guire *et al.* 1991; Yambayamba *et al.* 1996). At the onset of lactation appears to be a homeostatic adaptation to a decreased food supply, similar to the situation with fasting or energy malnutrition, the plasma concentrations of T^3 & T^4 were in their nadir in the early postpartum days possibly owing to increased metabolic clearance of thyroid hormones in peripheral tissues to suppressed secretory capacity of the thyroid gland. In the supporting the idea also TRH-induce T^4 & T^3 responds less pronounced in the second weeks of lactation than before calving or 3 months postpartum (Tveit *et al.* 1990; Huszenicza, *et al.* 2002).

The significant increase of T^r & T^t levels in the present study during late pregnancy may be occurs due to a high estrogen levels during advanced pregnancy which stimulate production of

thyroid hormone-binding globulin, leading to a rise in levels of bound T^3 & T^4 and simultaneous drop in levels of free T^3 & T^4 , with the drop in thyroid-binding globulin following delivery, levels of T^3 & T^4 drop (Learoyd *et al.* 1992).

The results of serum cortisol level of multiparous cows and heifers are given in Table 3. The results showed that cortisol concentration during pregnancy reached maximum level at parturition and then declined significantly in first month of lactation. Similarly Hendrick *et al.* (1998) who found that the serum cortisol levels were increased with parturition approached, with peak levels at parturition, the peak cortisol levels in late pregnancy may be as result of placental production of corticotrophin releasing hormone and fall abruptly at delivery.

The results observed in the present study are also accordance with the findings of Kulberg *et al.* (2002) who reported that serum cortisol levels increased as parturition approached, with peak level at parturition and slightly decreasing levels were found postpartum. Similarly high levels of cortisol were also observed in dairy heifers during the six weeks prior to delivery (Kornmatitsuk *et al.*, 2004).

The significant increase of cortisol level with advanced pregnancy may be occur due to the stress of pregnancy which causes release of adrenocorticotrophic hormone from the pituitary gland in late pregnancy, induces synthesis and secretions of glucocorticoids from the adrenal cortex (Kulberg *et al.*, 2002)

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