EVALUATION OF OXIDATIVE STRESS IN DAIRY GOATS LACTATING NATURALLY OR BY HORMONAL INDUCTION

[EVALUACIÓN DEL ESTRÉS OXIDATIVO EN CABRAS LECHERAS EN LACTACIÓN NATURAL O INDUCIDA]

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SUMMARY

The objective was to evaluate the oxidative stress (OS) and its association with performance and serum cortisol in dairy goats under induced (IL) and natural lactation (NL). Six goats programmed to be eliminated due to reproductive problems were IL as follows: a) days 1-7, estradiol plus progesterone; b) days 8-14, estradiol; c) days 18-20, flumetasone; d) every seven days bovine somatotropin; f) on day 21 milking began. Cortisol, antioxidant capacity and Glutathione Peroxidase activity (GHS-Px) were determined in serum. During the first two weeks in milk, NL goats had higher cortisol (P<0.05) and lower (P<0.0001) antioxidant capacity than NL goats (week1: NL= 3.5±1.2, IL= 14.4±1.1nM/ml; week2: NL= 9.7±0.8, IL= 34.4±0.7nM/ml) but groups did not differ in GHS-Px activity (P>0.05). Milk yield/day between groups (NL=2.54±0.2; IL= 2.12±0.2kg) and proportion of pregnant goats (NL=100%; IL=83.3%) were similar (P>0.05). The treatment was effective as inductor of lactation; besides most of the IL animals became pregnant despite their previous reproductive problems, prolonging their productive life with a natural lactation plus the additional benefit of at least one more kid. Because the antioxidant capacity increased and cortisol decreased in IL goats, apparently their welfare was more adequate than in NL animals.

Key words: Oxidative stress, serum cortisol, induced lactation, dairy goats.

RESUMEN

El objetivo fue evaluar el estrés oxidativo (OS) y su asociación con el desempeño productivo y el cortisol sérico de cabras lecheras en lactación inducida (IL) o natural (NL). Seis cabras programadas para el desecho por infertilidad fueron IL como sigue: a) días 1-7, estradiol más progesterona; b) días 8-14, estradiol; c) días 18-20, flumetasona; d) cada siete días, somatotropina bovina; f) día 21 inició el ordeño. Las cabras NL parieron durante el tratamiento IL. Cortisol, capacidad antioxidante y actividad de la Glutation-Peroxidasa (GHS-Px) fueron cuantificados en suero. En las dos primeras semanas en ordeño, las cabras NL tuvieron más cortisol (P<0.05) y menor (P<0.0001) capacidad antioxidante que los animales IL (semana1: NL= 3.5±1.2, IL= 14.4±1.1nM/ml; semana2: NL= 9.7±0.8, IL= 34.4±0.7nM/ml), pero la actividad de GHS-Px fue similar entre grupos (P>0.05). La producción (NL= 2.54±0.2; IL= 2.12±0.2kg/día) y la proporción de cabras gestantes (NL=100%; IL=83.3%) fueron similares (P>0.05). El tratamiento indujo lactaciones de manera efectiva y la mayoría de cabras IL resultaron gestantes a pesar de los problemas reproductivos previos, prolongando su vida productiva con al menos una lactación natural. Puesto que la capacidad antioxidante aumentó y el cortisol disminuyó en cabras IL, aparentemente su bienestar fue más adecuado que en cabras de NL.

Palabras Clave: Estrés oxidativo, cortisol sérico, lactación inducida, cabras lecheras.

INTRODUCTION

Oxidative stress (OS) affects negatively the productive performance and health of ruminants (Celi, 2010; Lykkesfeldt and Svendsen, 2007). In dairy cows, OS is associated to the transition period (Spears and Wise, 2008; Sordillo and Aitken, 2009), when most cows experience a series of metabolic adjustments that result in a state of negative energy balance (Ingvartsen and Andersen, 2000). In goats, studies relative to OS during the transition interval and lactation are scarce but overall they show that under conventional diets, dairy goats experience negative energy balance in early lactation (Donnem et al., 2011), phenomenon that could explain the OS observed during their periparturient period (Celi et al., 2008). On the other hand, serum cortisol has been implicated as a mechanism that mediates effects of stress inducing factors in the low reproductive performance of dairy cows and some of the metabolic diseases observed in those animals (Stoebel and Moberg, 1982), thus cortisol has been proposed as an indicator of nonspecific stress. Elevated serum cortisol concur with OS in cows at the end of pregnancy and during parturition (Ingvartsen and Andersen, 2000), and an association between OS and circulating cortisol may exist as it was suggested by Dimri et al. (2010). An issue of concern for some sectors of society is the potential negative effect that the veterinary and production related procedures may have on farm animals welfare (Mormède et al., 2007), particularly under intensive production systems. Goats milk farming under intensive management is becoming more frequent (Celi et al., 2008), and reproductive problems is a major drawback in those systems as indicated by an annual culling rate of 14.5% due to infertility in dairy goats (Malher et al., 2001). Similarly, production of proteins of interest for humans is generated in transgenic goats more frequently than in other animal models (Houdebine, 2009), and dairy goats are usually recognized as a preferable animal model to cattle for the production of transgenic proteins in milk, due to their shorter gestation, earlier sexual maturation, and smaller size (Zhang et al., 2010). However, relative to other systems devised to produce recombinant pharmaceutical proteins, biopharming with transgenic goats offer some disadvantages (Houdebine, 2009), including a relatively long time for collection of products and elevated infertility rate. The induction of lactation by simulating the maternal hormonal changes near parturition was originally designed to reduce economic losses due to the high culling rate due to reproductive problems of heifers and cows in intensive bovine dairy systems (Centeno et al., 2012). Therefore, hormonal induction of lactation may reduce the culling rate, and make possible to obtain milk from goats with a high milk yield potential but with problems to become pregnant, prolonging their productive life. In a previous study (Mellado et al., 1996), goats induced to lactate hormonally had similar total solids and protein yield than goats under natural lactation. Based on these results and that protocols that induce lactations do not modify importantly the composition of milk from transgenic goats (Zhou et al., 2005), we tested in dairy goats programmed for leaving the farm due to reproductive problems, the effects of a protocol designed to induce lactation, with the objective of determining whether or not the oxidative stress and serum cortisol levels were different than in goats under natural lactation.

MATERIALS AND METHODS

Animals and general management

This experiment was approved by the Institutional Subcommittee for Caring of Experimental Animals (Subcomité Institucional para el Cuidado de Animales en Experimentación, FMVZ-UNAM). The study was performed from January to November, 2008 in an experimental farm located in the central plateau of Mexico, at 20° 36´ North and 99° 56´ West. The location altitude is 1,920 meters over the sea level and the mean annual values for temperature and rainfall are 17.5°C and 388 mm, respectively. Multiparous dairy goats (n=11) were housed in pens designed for optimal comfort; each treatment group was in a separate pen. Housing consists in a circular unit and pens are projected in a radial form from the center. Pens are trapezoidal and the external side (15 m long) gives access to a feeder bunk for 34 animals/pen; the inner side of pens (3 m long) gives access to the management area and lateral sides are 23 m in length. The shade covers the central management unit and extends for 7 m into each pen inner area which is equipped with automatic drinker; floor in the shaded area and near the feeder bunk is cemented and the rest is dust. Animals were milked twice a day (0700 and 1700 h) in a tandem parlor equipped with 4 milking units. Feed was offered two h prior each milking time. Diet consisted in a completely mixed ration based on ingredients from the same lot to minimize variation throughout the experiment. Ingredients were alfalfa hay, corn silage, ground corn, commercial concentrate and a minerals-vitamins mix. Proximate analysis and atomic spectroscopy of mixed ration samples indicated that the chemical composition of 1 kg of diet (dry matter) was CP, 19.8%; NEl, 2.97 Mcal; ADF, 24.4%; NDF, 33.7%; Ca, 10.95 g; P, 3.26 g; Vit. A, 10.50 IU; Vit. E, 0.04 IU; Vit. D, 105 IU; Se, 0.38 mg; Mn, 1063.7 mg; Zn, 1331 mg; Fe, 1967.02 mg; Cu, 8.96 mg; Co, 6.2 mg; and I, 87.33 mg. Based on feed left over from each pen, we estimated an average individual intake of 3kg/animal/day. During the reproductive season (Figure 7), goats estrus was synchronized by installing an intravaginal device (CIDR: Progesterone

0.3 g; Pfizer, New Zealand) which remained *in situ* for 11 days; additionally an intramuscular injection was applied containing eCG (400 IU/animal; Foligon, Intervet, Mexico). Goats were separated by breed and were exposed for five consecutive days, along with the rest of the farm herd, to bucks previously evaluated for adequate libido and fecundity and equipped with marker aprons. Mounting activity was observed every 12 h and crayon marks were recorded. Seven days later, bucks were penned again with does and remained with them for seven days. Pregnancy was determined by ultrasonography (ultrasound Aloka 550, 7.5 Mhz probe, Aloka Co., Walinford, CT, USA) between 45 and 60 days after the beginning of the mating period.

Treatments

Six multiparous, non-pregnant goats (3 Alpine and 3 Toggenburg; 2.8 parturitions) that had been programmed for culling due to reproductive problems, received a hormonal treatment for induction of lactation (IL). Additionally, 5 does (3 Alpine and 2 Toggenburg; 2.6 parturitions) of natural lactation (NL) that kidded during the application period of the IL treatment, were considered as control animals. Hormonal treatment given to IL animals consisted of: a) from days 1 to 7 a subcutaneous injection (SC) of estradiol cipionate (0.05 mg/kg; ECP, Pfizer, Mexico) plus SC progesterone (0.6 mg/kg; Progesterona, Fort Dodge, Mexico); b) from days 8 to14 SC estradiol cipionate (0.05 mg/kg); c) from day 18 to 20 an intramuscular injection of flumetasone (0.25 mg/day; Fluvet, Fort Dodge, Mexico); d) on days 1, 7, 14 and 21 a SC administration of bovine recombinant somatotropin (125 mg/goat; Lactotropina, Elanco, Mexico).

Measurements and samples

Individual body weight (BW) and body condition (BC; 1 to 5; 1=extremely thin, 5=obese) were recorded weekly from day 1 of hormonal treatment to day 140 of lactation. Individual milk yield was measured weekly during the whole lactation. Serum was obtained from blood samples taken from a jugular vein during early (weeks 1 to 4) and mid lactation of goats (weeks 11 and 15).

Laboratory analysis

Serum cortisol was determined by a solid phase radioimmunoassay (Coat-A-Count, Siemens Health Diagnostics Inc., Los Angeles, CA, USA); based on one assay, the C. V. was 5.6% (control serum mean$\pm$SD= 34$\pm$1.9 ng/ml). Ferric Reducing Antioxidant Power (FRAP spectrophotometry assay) was used to quantify the non-specific antioxidant capacity (Benzie et al., 1996). To determine the specific Glutathione Peroxidase (GHS-Px) enzyme activity in serum, we used a modified coupled assay described by Lawrence and Burk (1976).

Design and statistical analysis

Data from continuous variables were examined by analysis of variance for fixed effects models either for one way analysis (duration of lactation, milk yield/lactation, milk yield in the lactation previous to the experiment) or for repeated measures over time (milk yield/week, changes in BW and BC transformed to Arc-sine, cortisol, FRAP and GHS-Px). In the repeated measures analysis, the model included effects of treatment, sample and their interaction; both, one way ANOVA and repeated measures analysis were performed by the PROC GLM (SAS, 2002). The correlation of changes in serum cortisol levels and variations of FRAP, GHS-Px, body measurements and milk yield related variables was determined by the PROC CORR (SAS, 2002). Homogeneity of groups (breed and kidding number) was determined by the Fisher´s Exact Test (Agresti, 1992). During the lactation that preceded the experiment, goats assigned to IL had a lower milk yield than NL goats. Similarly, IL goats had lower BW and BC at the beginning of the experimental lactation than NL animals. However, when covariance analysis were performed, only milk yield in previous lactation was significant as a covariant ($P<0.01$), thus this variable but not BW or BC was used as a covariance to analyze all data relative to milk production collected during the experimental lactation.

RESULTS

As it was indicated above, IL goats had lower ($P<0.05$) BC and BW on the first day of milking than NL goats; in addition IL animals produced less milk ($P<0.01$) during the lactation previous to the experiment than NL goats (Table 1).

Animals from both treatments had similar pregnancy rate and milk production/day of lactation. However NL goats had a longer lactation and higher total milk yield/lactation (Table 2). Nevertheless, the analysis of repeat measures over time of data relative to milk yield (Figure 1) did not identify an interaction between treatments and samples ($P>0.05$).

From the beginning of the study, IL goats were lighter and had lower BC than the NL goats, however as the lactation progressed, both BW and BC of IL animals increased (Figures 2 and 3), and were similar to those of NL goats from week 5 through 30 of lactation. In both body measurements, no effects of treatment were detected ($P>0.05$) but they were influenced by day of

measurement (P<0.05) and by the interaction between treatments and day of measurement (P<0.01).

Table 1.- Milk yield (kg) during the previous lactation, as well as body condition and body weight (kg) on the day of first milking in goats that were in natural (NL) or induced lactation (IL). Data are presented as mean ± standard error.

Treatment	Milk lactation	Body condition	Body weight
NL	531.4±32.5[a]	3.2±0.25[c]	62.7±2.0[a]
IL	337±35.6[b]	2.3±0.23[d]	47.9±1.9[b]

[a, b] Within column, distinct letters indicate difference between means (P<0.01).
[c, d] Within column, distinct letters indicate difference between means (P<0.05).

No effect of sample (P>0.05) was detected in serum cortisol, however an effect of treatment and the interaction of treatment by sample was found ((P<0.05); thus concentrations of serum cortisol were higher in the NL animals than in IL goats during the first two weeks of lactation; afterward levels of cortisol were similar between groups (Figure 4). Variations in serum concentrations of cortisol were not correlated (P>0.05) with changes in FRAP and GHS-Px values or with variations in milk yield or body measurements related data; however when the analysis was performed within treatment, only in the NL goats a negative correlation was found of cortisol with milk yield per lactation (r = -0.40; P<0.05) and lactation length (r = -0.38; P<0.05). Additionally in the same group of goats, a positive correlation (r = 0.54; P<0.01) was observed between GHS-Px and lactation length.

Data from FRAP analysis, indicating antioxidant capacity, were not affected (P>0.05) by treatment but they were significantly altered (P<0.001) by sample and the interaction between treatments and samples. Accordingly, the general antioxidant capacity (Figure 5) was considerably lower (P<0.01) in NL goats during weeks 1, 2 and 4 of lactation but in week 3 they had a higher value than IL goats (P<0.01); later FRAP values were parallel but again in week 11 they were greater in NL goats (P<0.01). As in FRAP, data of GHS-Px activity were not affected by treatment (P>0.05) but they were significantly affected by sample (P<0.06) and the interaction between treatments and samples (P<0.01). Consequently GHS-Px values in serum (Figure 6) were similar in both groups during the first three weeks of lactation but the NL animals had higher GHS-Px values on week 4 (P<0.01), whereas the IL goats had higher values on week 15 than NL goats (P<0.05).

DISCUSSION

It was expected that IL and NL animals would differ in body measurements at the beginning of milking and in milk yield during the lactation previous to the experiment as randomization procedure was not used to assign goats to lactation treatments, rather IL animals were those that had been programmed for elimination of the herd. Similarly the NL goats were animals that kidded during the time when IL treatments were applied. Nevertheless the use of milk production in the previous lactation allowed us to adjust for those differences and to examine the effects of lactation type without bias. Under these conditions, IL and NL goats were similar in reproductive performance and milk production per day of lactation. However, milk yield during lactation as well as lactation length were greater in NL than in IL goats. Our results are consistent with a report (Mellado et al., 1996) in which goats hormonally induced to lactate equaled the NL animals in at least one of the variables associated with productive performance.

Table 2.- Productive and reproductive performance of goats during a natural (NL) or induced lactation (IL); data related to milk yield are mean ± standard error.

Treatment (kg)	Pregnant (%)	Milk/day (kg)	Lactation length (day)	Milk/lactation (kg)
NL	100.0 (5/5)*	2.54±0.2	248.8±3.8[a]	638.4±36.8[a]
IL	83.3 (5/6)*	2.12±0.2	232.6±4.4[b]	502.3±42.1[b]

[a, b] Indicates difference between means (P<0.05). * Inside the parenthesis: pregnant goats/exposed goats

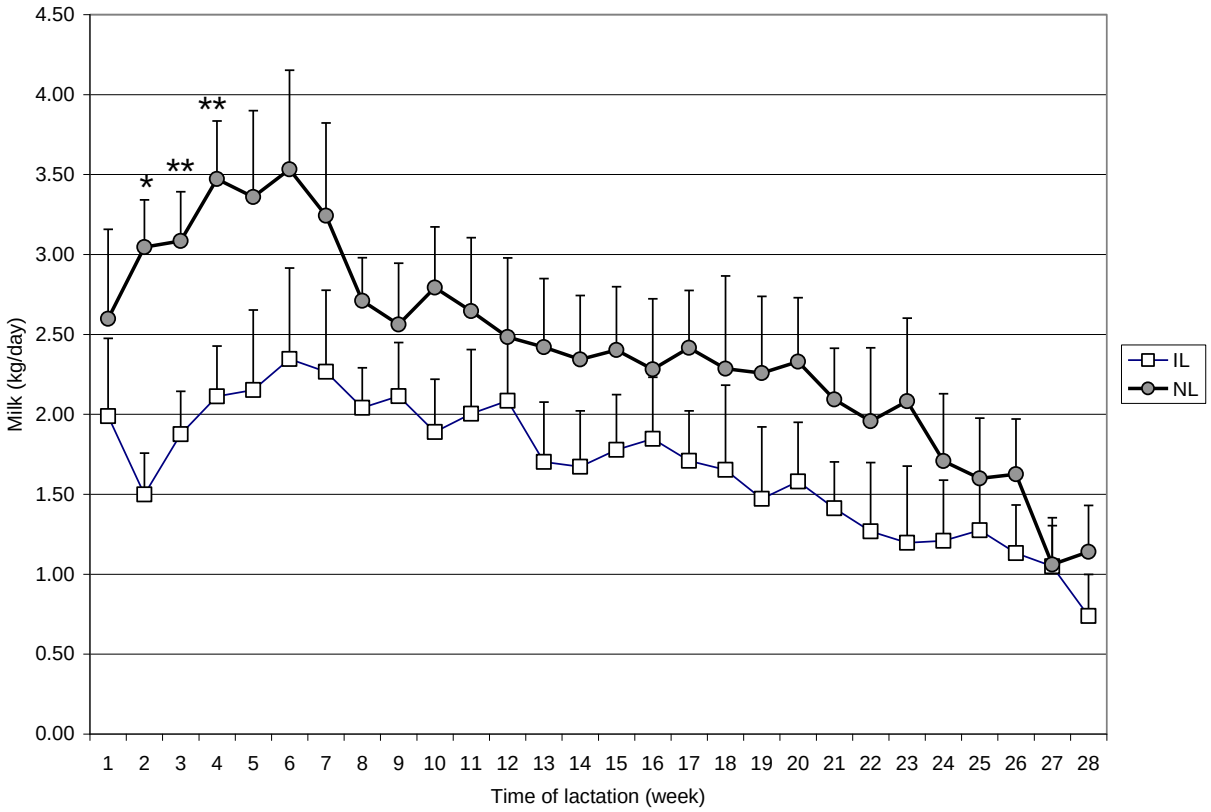

Figure 1.- Milk yield in goats during a natural (NL) or induced lactation (IL); each point is the weekly mean ± standard error. * and ** indicate difference between contemporary means (P<0.01 and P<0.05, respectively). Treatment (P>0.05); Time (P<0.01); Treatment x Time (P<0.01).

Despite that NL goats had higher BW and BC during early lactation, serum cortisol levels remained elevated during the first two weeks of milking when compared with IL animals. The increased serum cortisol in NL animals may be attributed to the homeorhetic mechanisms that occur during the final stage of gestation and beginning of lactation (Bauman and Currie, 1980), when accelerated growth of fetuses takes place along with stress induced by parturition, phenomena that are not experienced by IL goats. Those effects may be combined with the rapid increment in milk production in NL animals versus the slow increments in milk yield observed in IL goats. It has been reported that high serum concentrations of cortisol are one of the mechanisms inherent to lactation and indicate the metabolic response induced for the increased demands of nutrients imposed by the milk synthesis process (Bauman and Currie, 1980; Tucker, 2000).

The results from the FRAP assay in serum indicate that the NL goats were exposed to an increased risk of OS during the first two weeks postpartum, and the profile describing the variation in the antioxidant capacity follows a similar pattern to that recorded in dairy goats fed with energy deficient diets (Celi et al., 2010).

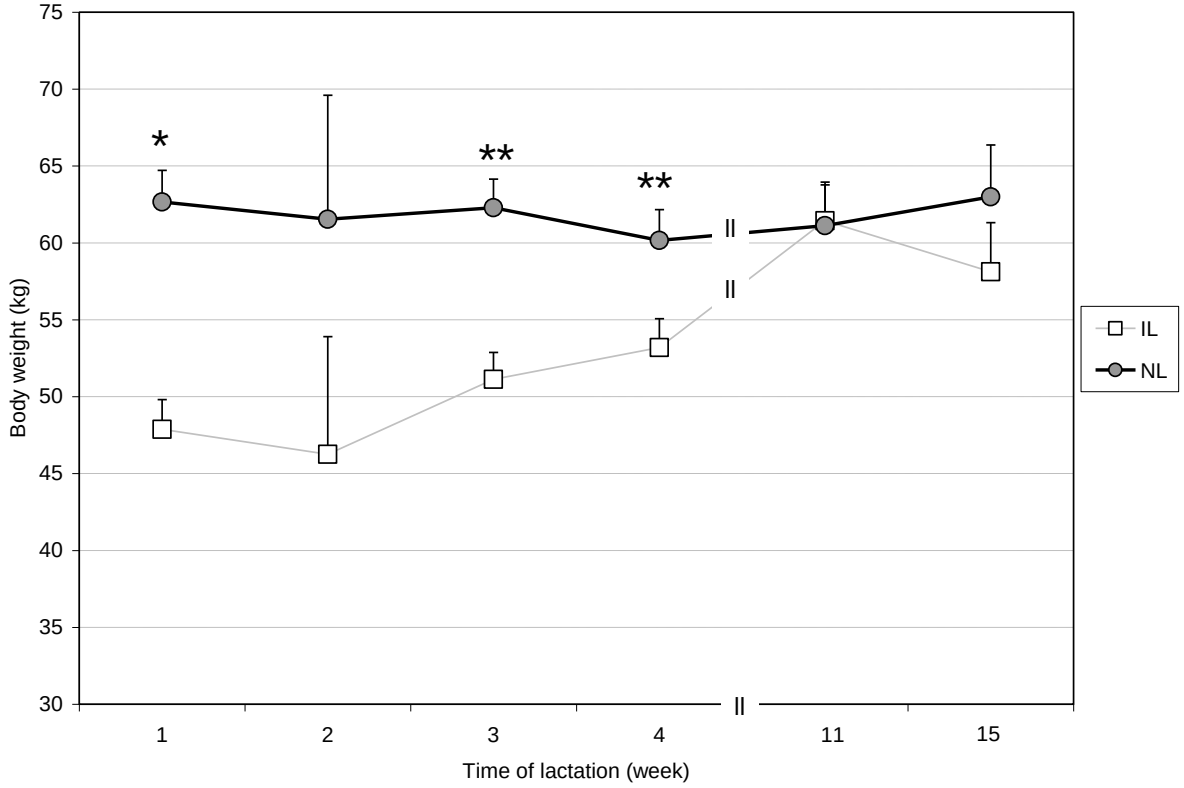

Figure 2.- Changes in body weight of goats during a natural (NL) or induced lactation (IL); each point is the weekly mean ± standard error at early (weeks 1 to 4) and mid lactation (weeks 11 and 15). * and ** indicate difference between contemporary means (P<0.01 and P<0.05, respectively). Treatment (P>0.05); Time (P<0.05); Treatment x Time (P<0.01).

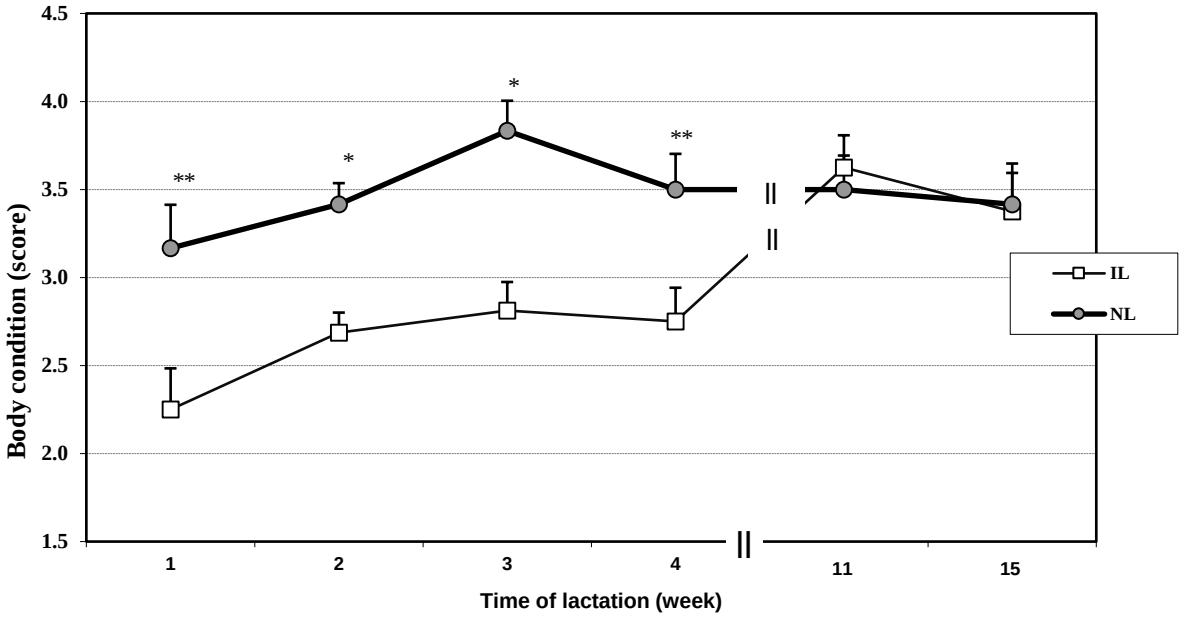

Figure 3.- Changes in body condition of goats during natural (NL) or induced lactation (IL); each point is the weekly mean ± standard error at early (weeks 1 to 4) and mid lactation (weeks 11 and 15). *, ** indicate difference between contemporary means (P<0.01 and P<0.05, respectively). Treatment (P>0.05); Time (P<0.05); Treatment x Time (P<0.01).

Relative to NL animals, IL goats showed a higher general antioxidant capacity during the two first weeks of lactation, an observation that according to our best knowledge is informed for the first time. The high levels of serum cortisol observed here may indicate an association with the decreased antioxidant capacity; this is possible because corticosterone fed to rats daily during four weeks, decreased the repair capacity of the hepatic mitochondrial antioxidant system (Caro et al., 2007). It is also possible that the NL goats in this study had some dietary antioxidant deficiencies because they were fed with forages preserved as hay or silage, and there are reports that at least β-carotene is low in this type of diets (Spears and Weiss, 2008). Goats fed high-energy diets (Celi et al., 2010) had an elevated antioxidant capacity in comparison with the insufficiently fed goats, and was similar to that recorded in IL animals in the present study; thus it is feasible that the relationship between ingestion and nutrients demand in these goats, due to the slower increase in milk production, was more balanced than in NL animals. Perhaps for the same reason, cortisol was lower and the general antioxidant capacity was higher in IL than in NL goats.

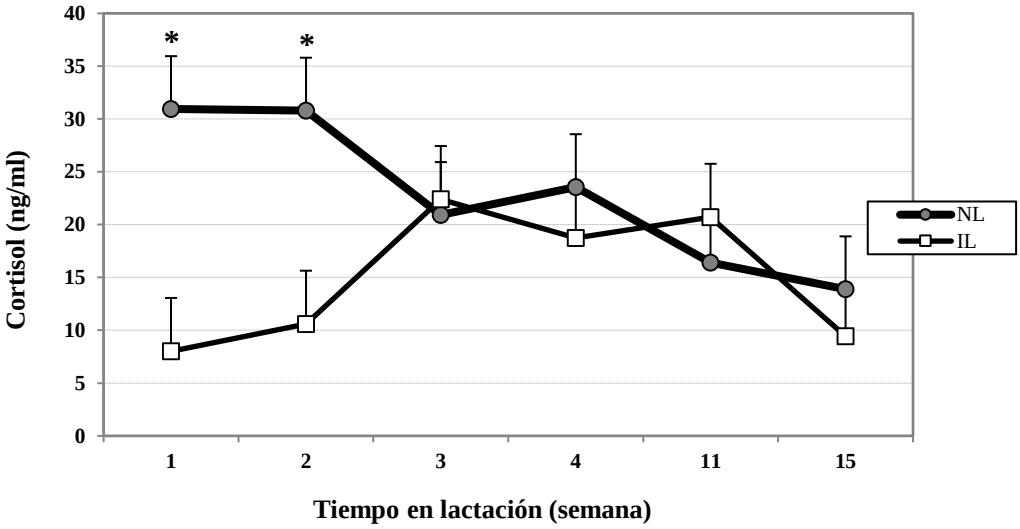

Figure 4.- Serum concentrations of cortisol in goats during natural (NL) or induced lactation (IL); each point is the weekly mean ± standard error during early (weeks 1 to 4) and mid lactation (weeks 11 and 15). * indicates difference between contemporary means (P<0.05). Treatment (P<0.05); Time (P>0.05); Treatment x Time (P<0.05).

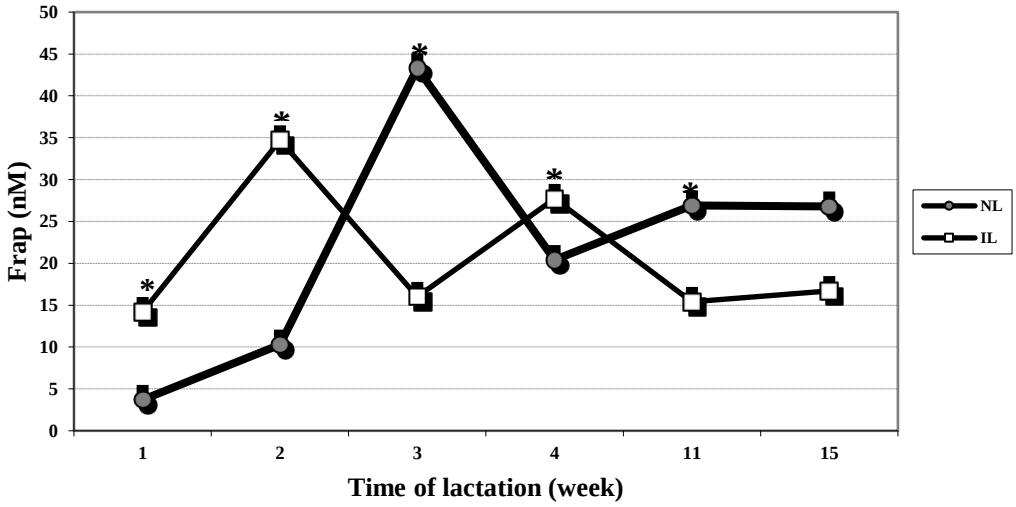

Figure 5.- Global antioxidant activity in goats during natural (NL) or induced lactation (IL); each point is the weekly mean ± standard error during early (weeks 1 to 4) and mid lactation (weeks 11 and 15). * indicates difference between contemporary means (P<0.01). Treatment (P>0.05); Time (P<0.001); Treatment x Time (P<0.001).

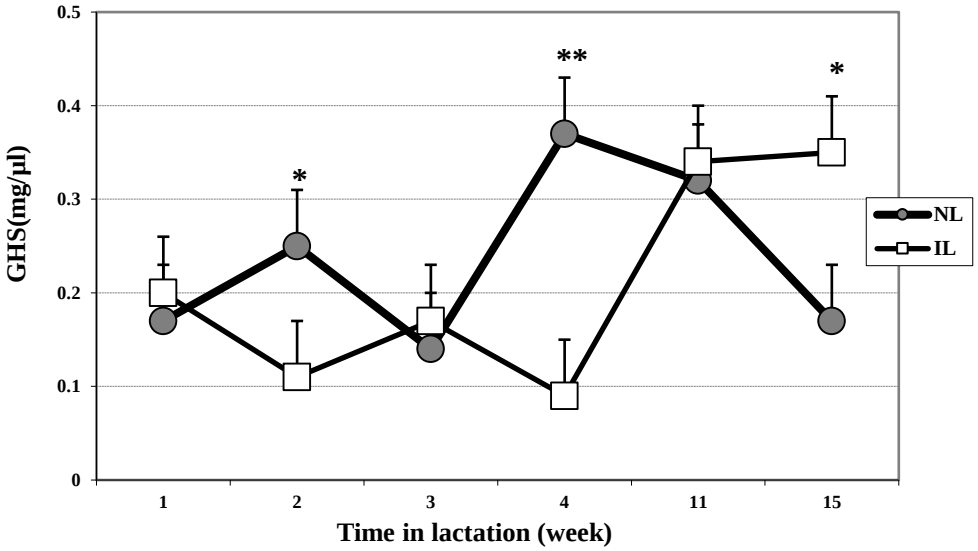

Figure 6.- Glutathione Peroxidase activity in goats during natural (NL) or induced lactation (IL); each point is the weekly mean ± standard error during early (weeks 1 to 4) and mid lactation (weeks 11 and 15). *, ** indicate difference between contemporary means (P<0.05 and P<0.01, respectively). Treatment (P>0.05); Time (P<0.06); Treatment x Time (P<0.001).

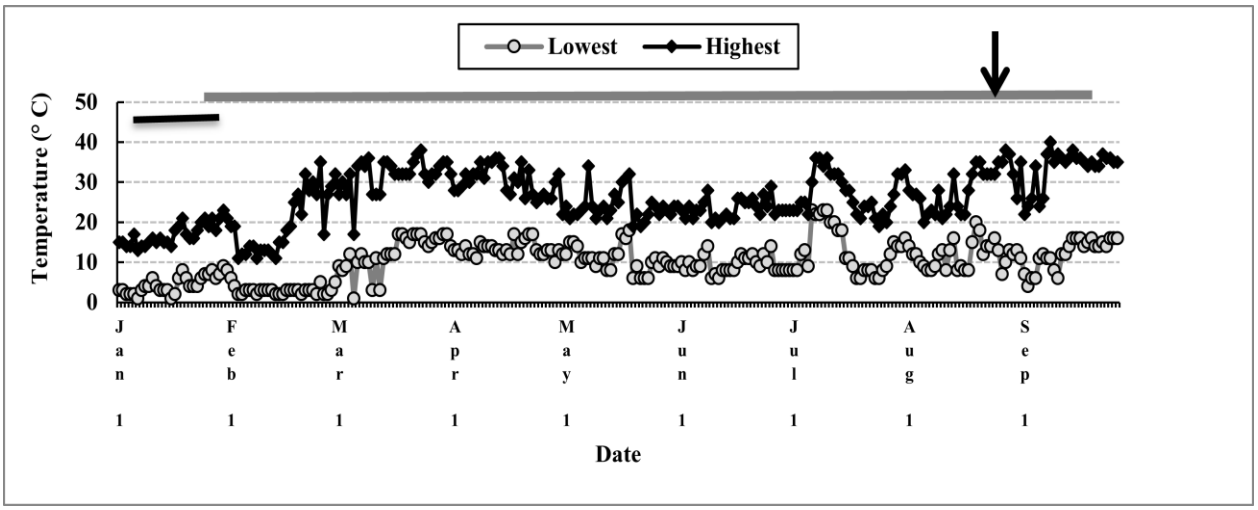

Figure 7.- Daily highest and lowest temperature recorded from January to September, 2008 in the experimental site. The black bar indicates intervals of treatment application for goats induced to lactate and parturitions of natural lactation animals. The gray bar shows the milking interval and the black arrow indicates the beginning of breeding.

In contrast with the general antioxidant capacity, the specific antioxidant function of GSH-Px was similar between IL and NL animals during the first three weeks of lactation. However, GSH-Px was higher in week four of lactation in NL than in IL goats but became higher again in IL goats in samples collected on week 15. Perhaps the proximity of the lactation peak in NL animals evoked a higher production of reactive oxygen metabolites and the consequence was the increment of GSH-Px activity in week four. However, data from this study do not provide evidence to explain the differences between groups detected at mid lactation. It may be speculated that an interaction between physiological state and environmental temperature (Figure 7) occurred since samples from weeks 11 (33°C and 17°C; highest and

lowest temperature, respectively) and 15 (32°C and 18°C; highest and lowest temperature, respectively) were collected in April and May, when maximal mean temperatures during the day were frequently above 30°C and minimal temperatures were the highest during the experimental interval. This is possible because in studies carried out in a Mediterranean, semiarid region, lactating goats presented a moderate OS during the hottest season relative to values recorded during cooler seasons (Di Trana et al., 2006). It should also be noticed that NL and IL animals differed from the phenotypic standpoint since in the lactation previous to this experiment, milk yield was significantly higher in the former; thus metabolic capacity could be different between groups as it has been suggested for high producing dairy cows in comparison with mediocre yielders (Fenwick, et al., 2008; Bauman and Currie, 1980); consequently the differences relative to the antioxidant capacity recorded here may be associated, at least partially, with the productive potential of goats. To clarify these questions, it is necessary to study the variations of antioxidant capacity in IL and NL goats under different scenarios of environmental conditions and genetic background.

Despite the differences found relative to cortisol, GHS-Rx activity and the antioxidant capacity, goats from both groups did not show evidence of reproductive or health problems; so the OS recorded, even in animals with the lowest antioxidant values, could be considered as mild. All physiological, metabolic and performance data generated in the present work, allow us to say that IL treatment do not affect negatively the goat's welfare, as it was documented in IL primiparous Holstein cows whose cortisol concentrations in serum and hair were significantly lower than in the contemporary NL controls (González-de-la-Vara et al., 2008).

CONCLUSIONS

In summary, relative to NL animals, IL goats had similar milk yield/day of lactation and reproductive performance but during the first two weeks of lactation had lower circulating levels of cortisol and higher antioxidant capacity, thus our conclusion is that the protocol for induction of lactation is not damaging for goats health and that apparently the IL goats welfare was more adequate than in NL goats.

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