# WELL-BEING OF SUCKLING CALVES REARED IN TWO SYSTEMS IN THE CENTRAL MILKING AREA OF ARGENTINA: PHYSIOLOGICAL AND BEHAVIORAL INDI-CATORS

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#### SUMMARY

Ten Holstein calves were utilized to determine well-being indicators, in two rearing systems: traditional stake system (TS) and modified system (MS). Rectal temperature (RT), respiratory rate and heart rate (HR) were recorded at 08:00, 14:00, 20:00 and 02:00, three non consecutive days. In different days, behavior was observed, between 08:00 and 18:00. Records included: standing, laying, eating and "other". Physiological data were analyzed in a split plot in time design. Variables showing hourly fluctuations were subjected to cosinor analysis. Significant treatment effects were detected for the physiological variables (P<0.01). Significant hour effects were detected for TR and RC (P<0.01). Rectal temperature adjusted to circadian rhythms. For analyzing behaviors, contingency tables were built and data were analyzed by means of the 2 test. Significant effects of rearing system on behavior were detected (P < 0.01). The modification seemed to improve animal well-being.

Key words: Holstein calves, physiological parameters, behavior, winter, rhythm.

#### RESUMEN

#### Bienestar de terneros lactantes criados bajo dos sistemas en la cuenca lechera central de la Argentina: Indicadores fisiológicos y de comportamiento.

Diez terneros Holstein se utilizaron para determinar indicadores de bienestar en dos sistemas de crianza: tradicional en estacas (TS) y modificado (MS). Se registraron la temperatura rectal (RT), ritmos respiratorio (RR) y cardíaco (HR) a las 08:00, 14:00, 20:00 and 02:00, tres días no consecutivos. En días diferentes se observó el comportamiento, entre las 08:00 y las 18:00. Los registros incluyeron: parados, echados, comiendo y "otras". Los datos fisiológicos se analizaron en diseño en

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Manuscrito recibido el 30 de agosto de 2010 y aceptado para su publicación el 12 de noviembre de 2010.

parcelas divididas en el tiempo. Las variables que presentaron diferencias horarias se sometieron al análisis de cosinor. Se detectaron efectos del tratamiento para las variables fisiológicas (P<0,01). Las horas presentaron efecto significativo para TR and RC (P<0,01). La TR ajustó a ritmos circadianos. Para analizar el comportamiento se construyeron tablas de contingencia y se aplicó una prueba de 2. Se detectaron efectos del sistema de crianza sobre el comportamiento (P<0,01). La modificación pareció mejorar el bienestar.

Palabras clave: terneros Holstein, parámetros fisiológicos, comportamiento, invierno, ritmo.

#### INTRODUCTION

Animal well-being (AW) is not a concept easy to define. According to Broom (1986) it includes the biological functions related to health and production and the need to express the species natural behavioral patterns. It depends on several factors, such as health, housing and management, social interactions and the possibility to develop different behaviors.

Well-being of the newborn is an issue, particularly during extreme months, since the environment plays an important role during the first weeks of life. Calves are more sensible to hypothermia when winters are very cold and humid (Azzam *et al.*, 1993). As much as a 50% increase in calf deaths may be expected when low temperatures are combined with rain and wind. Therefore, improving animal comfort should be a priority in dairy systems, since it could contribute to effectively reduce the environ-mentally driven stress (Berra *et al*, 1996).

Environmental conditions in calf rearing systems are quite different from those found in the natural life of a calf growing together with its mother. In commercial farms, calves are fed either milk or milk replacer offered in amounts limited to sustain rumen develop-ment and reach early weaning. However, when weather is either too hot or too cold, animals must utilize part of the feed energy to maintain body temperature, thus decreasing the energy for growth. This could negatively affect AW.

Stress plays an important role in calf health and represents an indicator of AW. Animals can suffer either physical -hunger, thirst, tiredness, lesions, thermal extremes-, or psychological -movement restriction, mana-gement practices, sudden changesstress (Grandin, 1997).

On the other hand, rhythmicity in body temperature is an important physiological process both as a convenient and reliable marker of the operation of the biological clock (Klerman *et al.*, 2002) and as an indicator of the general health of an animal and of its energy metabolism (Cossins & Bowler, 1987).

Rhythmic patterns in the cardiovascular system have been described in humans (Lemmer, 1987) and in the bovine (Piccione *et al.*, 1998, 2005).

Rules of behavior are also associated to AW evaluations, since behavioral changes are usually the first stress indicators (Wilson, 1971; Merck & Co., 1979; Stephens, 1980; Curtis, 1982).

Modern production systems have imposed severe space restrictions, which produce animal suffering, mainly under extreme conditions (Faulkner & Weary, 2000; Flower & Weary, 2003; Vickers *et al.*, 2005). Ethograms help analyze animal behavior as a basis to create optimal environments for animal production (Banks, 1982; Hartsock, 1982). The objective of the present work was to describe stress indicators, both physiological and behavioral, for suckling calves reared in winter under two different systems, the control being represented by the traditional regional rearing system. The alternative system improved both the possibilities of contact between animals and the protection against cold.

## MATERIALS AND METHODS

### Experimental site and period

The trial was carried out in a commercial dairy farm located in the central Santa Fe milking area (31° 26' S, 60° 56' W) during winter 2007, between June 14th and August 17th. It covered the suckling stage, until calves reached 50 days of life. The climate of the area has been classified as transitional tempe-rate (Conde, 2000).

#### Animals and treatments

Ten Holstein calves (avg. birth weight) were randomly assigned to two treatments -traditional rearing system (TS) and modified rearing system (MS), right after birth. All born animals were subjected to a glutaraldehyde clot test to evaluate absorp-tion of colostral immunoglobulins. Only animals considered to have absorbed adequate amounts entered the trial. Health status was observed all over the trial.

#### Management

Animals in the control treatment were reared under the traditional system utilized in commercial calf rearing units in the area, the so called "stake system" (Fig. 1 a). It consists of a stake, to which calves are tied with a necklace and a 1.5 long chain and have no protection. Also, the stake has two rings to hold buckets to offer the diet components: milk and starter. Starter fed every day was adjusted according to the amount effectively consumed the previous day.

In the modified, "sliding system", calves were tied by the necklace and chain to a wire held between to stakes 10 meters apart. The chain could slide along the wire, thus improving the possibilities of contact between animals. Also, calves were protected by waterproof capes with woolen lining (Fig. 1 b).

Calves in both treatments received milk twice a day (4.0 L in the whole), starter and water.

#### Measurements

Rectal temperature (RT), was recorded once every 15 days, four times daily, at 08:00, 14:00, 20:00 and 02:00, with a clinical thermometer

Respiration (RR) and heart rates (HR) were recorded, following the same schedule, the former by counting flank movements, and the latter by means of a stethoscope.

Behavioral observations were performed using the sweep sampling technique at regular intervals, recording the activity developed by each individual (Martin & Bateson, 1991).

The time-sampling session was divided into successive 10 - 15 min long observational periods for each treatment followed by a 15 min long resting period (Martin & Bateson, 1991).

The observations were supported with video recordings and, when needed, by binocular observations.

Behavior was observed every 15 days, from 08:00, right after the morning milk supply, till 18:00, right before the afternoon milk supply. Data were collected during the light cycle, since bovines present diurnal habits (Valtorta *et al.*, 2006).

The behaviors examined were: standing,

laying, eating and other (any not considered).

Meteorological data were obtained from an automatic station located at the Facultad de Ciencias Agrarias - Universidad Nacional del Litoral, at 11 km from the commercial unit.

#### Statistical Analysis

Physiological data were analyzed in a split plot in time design (InfoStat/P, 2007), according to the model:

$$Y_{iik} = \mu + s_i + d_{ii} + h_{iik} + e_{iik}$$

where  $\mu = effect$  of the mean

si = effect of the ith rearing system, i = 1, 2dij = effect of the jth day on the ith rearing

system

hijk = effect of the kth hour from the jth day on the ith rearing system

eijk = effect of the random residual error associated with the measurement on the kth hour from the jth day on the ith rearing system.

Variations of the parameters showing significant hourly differences were subjected to cosinor analysis (Halberg *et al.*, 1972). When fitness to a cosenoidal variation was detected, data were fit to the following equation (Nelson *et al.*, 1979):

$$P_{n} = M + A \cos (\omega t + \Phi)$$

where: Pp = Physiological parameter (TR, RR, CR)

M = mesor (value around which the oscillations are recorded)

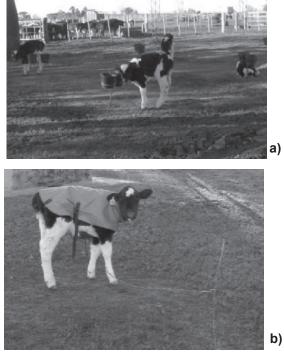


Fig. 1: Calves in two different rearing systems in Central Argentina: a) Traditional "stake" system; b) Modified "sliding" system, with improved possibilities of contact between animals and a protective cape.

A = amplitude (half the difference between maximum and minimum values)

 $\Phi$  = acrophase (time of recording of the maximum values, both in hours and in radians)

 $\omega$  = angular frequency (radians / time unit. The complete 24 h cycle is represented by 4 radians)

t = time, in hours

For analyzing behavioral data, contingency tables were built, and the differences were analyzed by the  $X^2$  method, as proposed by Mader *et al.* (2007).

## RESULTS

Average birth weight was  $32.3 \pm 4.09$ 

and  $30.0 \pm 2.00$  kg for calves in TS and MS, respectively.

Table 1 shows the meteorological data recorded during the days when the physiological data were taken. Table 2 shows general means for the RT, HR and RR recorded in treatments TS and MS.

Table 3 presents the data corresponding to significance of effects in the split plot in time analysis of all data. Day of measurement affected all parameters, and the results are presented in table 4.

During the first measuring day, which was the coldest (table 1), RT for calves in TS was almost 1°C lower than that recorded in MS. On the other hand, during the last measuring day, differences were detected for HR and

Table 1: Minimum temperature (mT), maximum temperature (MT), relative humidity (RH), solar radiation (SR) and precipitation (Pp) recorded during three physiological parameters measuring days in a trial where two different calf rearing systems, traditional stake and modified sliding, were compared.

Day	mT (°C)	MT (°C)	RH (%)	SR ww/m2)	Pp (mm)
1	-0.7	6.9	86	184	
2	-0.5	16.6	62	174	
3	5.8	12.0	97	82	1

Table 2. Overall mean rectal temperature (RT), heart rate (HR) and respiration rate (RR) values for calves reared in two systems: traditional stake (TS) and modified sliding (MS).

Treatment	RT (°C)	HR (be <i>a</i> ts/min)	RR (breaths/min)
TS	38.3±0.8 <sup>a</sup>	65.6± 18.0 <sup>a</sup>	20.8±4.41 <sup>a</sup>
MS	38.7±0.5 <sup>b</sup>	71.4±23.0 <sup>b</sup>	25.1±7.79 <sup>b</sup>

a, b Within column, indicate significant differences (P<0.01)

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RR, but not for RT.

Hour of measurement affected all variables. When data were analyzed to detect the existence of rhythmic variations, the results presented in table 5, 6 and 7 were obtained. Rectal temperature adjusted to circadian rhythms two out of the three measuring days. Table 8 presents the meteorological data corresponding to the behavioral data collection days.

A significant relationship (P < 0.01) between behaviors and the rearing system was detected. Figure 2 shows the percentage distribution of the time devoted to each activity.

Table 3: Split plot in time analysis effects for rectal temperature (RT, °C), heart rate (HR, beats/ min) and respiration rate (RR, breaths/min) for calves reared under two systems: control and modified.

Parameter	Effects				
i didinotor	Rearing system	Day	Hour		
RT	0,0072	< 0,0001	< 0,0001		
RR	0.031	0,003	0.0408		
HR	0,033	< 0,0001	< 0,0001		

Table 4: Average values recorded for rectal temperature (RT,  $^{\circ}C$ ), heart rate (HR, beats/min) and respiration rate (RR, breaths/min) for calves reared under two systems: control (TS) and modified (MS), during the different days of measurement.

Day	Treatment	RT	HR	RR
1	TS	37.6±0.7 <sup>a</sup>	56.7 ± 8.2 <sup>b</sup>	19.6 ± 3.2 <sup>a</sup>
1	MS	38.6 ± 0.7 <sup>b</sup>	63.0 ± 11.9 <sup>b</sup>	21.4± 3.95ª
2	TS	38.5 ± 0.8 <sup>b</sup>	54.2 ± 9.7 <sup>a</sup>	20.6 ± 4.5ª
2	MS	38.7±0.5 <sup>b</sup>	54.8 ± 8.0 <sup>a</sup>	22.6± 3.2ª
3	TS	38.6 ± 0.5 <sup>b</sup>	80.7± 20.2 <sup>c</sup>	22.2 ± 5.1ª
3	MS	38.7 ± 0.2 <sup>b</sup>	94.0 ± 23.0 <sup>d</sup>	31.2± 10.1 <sup>b</sup>

a, b, c, d Within column, indicate significant differences (P<0.05)

Day	Variable	Treatment	Mesor	Amplitude	Acrophase	P<
1	тр	ST	37,2	0,62	17:43	0,005
	TR	SM	38,4	0,8	16:20	0,006
_	тр	ST	38,5	1,02	18:52	0,01
2	TR	SM	38,6	0,56	18:40	0,05
3	TD	ST	38,6	0,4	14:08	0,50
5	TR	SM	38,6	0,28	17:43	0,24

Table 5: Circadian analysis of the values recorded for rectal temperature (RT,  $^{\circ}$ C) for calves reared under two systems: traditional stake (TS) and modified sliding (MS).

Table 6. Circadian analysis of the values recorded for heart rate (HR, bests/min) for calves reared under two systems: traditional stake (TS) and modified sliding (MS).

Day	variable	Treatment	Mesor	Amplitude	Acrophase	P<
1	DO	ST	56,7	6,96	16:47	0,15
1	RC	SM	62,3	10,9	09:20	0,11
	_	ST	55,3	9,46	02:24	0,65
2	RC	SM	61,8	5,86	16:44	0,96
2		ST	80,6	21,7	15:28	0,10
3	RC	SM	93,8	28,4	21:56	0,24

*Table 7: Circadian analysis of the values recorded for respiration rate (RR, breaths/min) for calves reared under two systems: traditional stake (TS) and modified sliding (MS).* 

Day	variable	Treatment	Mesor	Amplitude	Acrophase	P<
	RR	ST	19,5	3,1	06:56	0,37
1	ĸĸ	SM	20,6	3,2	20:48	0,13
	RR	ST	20,6	3,1	17:10	0,48
2	ΓΓ	SM	22,7	3,6	17:12	0,57
	RR	ST	21,2	3,6	09:40	0,47
3	ĸĸ	SM	32,1	8,26	06:56	0,98

Calves in TS and MS were laying 65 and 67% of the time, respectively. On the other hand, animals in TS were standing longer than those in MS (28 vs. 22%).

Calves in MS spent 5 % of their time in social activities (agonistic and non agonistic interactions), while their mates in TS only devoted 2 % of their time to those activities. The main activities were social licking and sniffing. No ludic behavior was recorded in TS, while in MS calves were occasionally running with their tails up, kicking with their two rear legs or hitting their heads with no menacing intention.

Finally, time spent eating the started feed was 4 % for TS and 7 % for MS.

Table 8: Minimum temperature (mT, °C), maximum temperature (MT, °C), relative humidity (RH, %), solar radiation (SR, W/m2) and wind speed (WS, km/h) recorded during three behaviour observation days in a trial where two different calf rearing systems, traditional stake and modified sliding, were compared.

Day	mT (°C)	MT (°C)	RH (%)	SR (W/m <sup>2</sup> )	WS, km <i>l</i> h
1	-0.1	9.8	98	118	0.82
2	2.3	23.2	71	192	3.09
3	-0.3	14.4	84	121.4	4.87

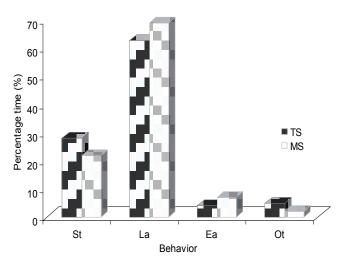


Figure 2. Behaviors recorded in calves reared in two different systems in Central Argentina: Standing (St); Laying (La); Eating (Ea); Other (Ot; any not considered). TS: Traditional "stake" system; MS: Modified "sliding" system, with improved possibilities of contact between animals and a protective cape.

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## DISCUSSION

High significant effects of the rearing system were detected for all the physiological parameters under analysis.

Lower critical temperature for the newborn calf is 15°C and it decreases to -2°C by the time animals reach one month of age (Christopherson, 1985). During the first measuring day, temperature was quite low (table 1) and calves were very young. This combination could have contributed to the results shown in table 4. Animals reared in the traditional system presented slight hypothermia. This condition would be reached when rectal temperature fell below 37.7°C (Butler *et al.*, 2006). This result indicates that the provision of the woolen lined cape could contribute to improve the well-being status of the young calf.

During the last measuring day there were no differences in RT in response to treatment. During that day, a persistent drizzle was recorded at all measuring times. This condition may have affected the lack of effect of the capes on RT. Wet, cold weather can be rough on calves born in winter. Precipitation adds to the negative effects of low temperature (Azzam, 1993; Buttler *et al.*, 2006).

Respiration rate was lower during the coldest days, especially in animals in TS. Scibilia *et al.* (1987) reported average RR of 19.4 and 25 breaths/min at -4 and 10°C, respectively. They considered that reduced RR may be a mechanism to reduce evaporative heat loss, and that those low RR indicate that calves were stressed. Again, in the present study, the provision of capes impro-ved calves response.

Circadian rhythmicity in physiological processes in animals has been described for a multitude of variables (Piccione & Caola, 2002; Dunlap *et al.*, 2004; Refinetti, 2005). In the present work, RT, RR and HR presented daily fluctuations. However, only RT adjusted to circadian rhythms two out of the three measuring days.

Piccione *et al.* (2003) found that calves exhibit a robust daily rhythm of body temperature. However, they observed that calves presented a daily oscillation lower than 1°C when they were younger than 52 days. In the present study, calves in TS showed daily RT amplitude higher than 1°C. Their trial was performed in summer, when RT could be expected to decrease to a lesser extent during the night period, because of the hotter conditions.

The acrophase of the daily temperature rhythm was recorded before sunset, in agreement with previous results (Piccione *et al.*, 2003; Hahn *et al.*, 1990; Araki *et al.*, 1987).

Heart and respiration rates daily variations did not adjust to circadian rhythms. Piccione *et al.* (2010) found that HR adjusted to circadian rhythms in puppets of different breeds only after they were 2-month old. They concluded that it looks like the biological clock controls at first the oscillation of a few variables and, later on, the daily patterns of other variables seem to be controlled in a hierarchical order.

Calves in MS were standing for a longer time. Animals could lose more heat by conduction when in direct contact with the floor (Leadley *et al*, 2006). The capes provided to animals in this treatment could reduce those losses.

Works done by Chua *et al.* (2002), comparing calves housed either individually or in small groups showed that animals were laying 70% of the time. Also, Holstein cows were laying 51% of the time (Vitela *et al.*, 2005). Those results agree with the ones presented here.

Social activities, mainly licking and sniffing, were more frequent in MS calves. These behaviors would not be enhanced in TS, because of the lack of possibility of contact between animals. Social licking presents several functions, including animal recognition in the herd, hierarchy establishment and cleaning (Fraser & Broom, 1990).

Some ludic activities, such as calves running with their tails up, kicking with their two rear legs or hitting their heads with no menacing intention, were recorded in MS. This behavior is normal in the young bovine (UCO, 2002). Early social interaction is important for the development of calf normal socialization behavior (Jensen *et al.*, 1997). Also, social activities may affect calves health and performance (Chua *et al.*, 2002).

Animals in both groups spent only a short time eating their starter, in disagreement with the results presented by UCO (2002), who informed that calves devoted 22% of their time to eating. Probably the results could be affected by the observational period and also by the rearing system, since they presented data corresponding to calves reared either in groups or individually in hutches.

#### CONCLUSION

The provision of a protective cape would render more comfort to calves reared in winter, as evaluated by the rectal temperature, thus improving calves well-being. The modifications introduced to the rearing system do not seem to alter the daily fluctuations of the analyzed physiological indicators.

The changes introduced to the rearing environment, allowing more movement and contact between calves, as well as the provision of protective capes seemed to improve animal well-being, as evaluated through behavioral variables.

#### REFERENCES

- ARAKI, C.T.; R. M. NAKAMURA & L. W. G.. KAM. 1987. Diurnal temperature sensitivity of dairy cattle in a naturally cycling environment. J Therm Biol. 12:23-26
- AZZAM, S.M.; J.E. KINDER; M. K. NIEL-SEN; L. A. WERTH; K. E. GREGORY; L.V. CUNDIFFT & R. M. KOCH. 1993. Environmental effects on neonatal mortality of beef calves. J Anim Sci 71(2): 282-290.
- **BANKS, E. M.** 1982. Behavioral research to answer questions about animal welfare. J. Anim. Sci. 54:434.
- BERRA, G.; J. E.CARRILLO & L. ESTOL. 1996. Enemigo № 1: El Frío. Infotambo. Pag. 60-64 - Año X :90
- **BROOM, D. M.** 1986. Indicators of poor welfare. British Veterinary Journal, 142: 524 - 526.
- BUTLER, L.; R. DAY & C. WRIGHT. 2006. Cold stress and newborn calves. South Dakota Cooperative Extension Services. http:// agbiopubs.sdstate.edu/articles/ExEx2050. pdf Accessed July 21st 2008
- CHRISTOPHERSON, R. J. 1985. Management and housing of animals in cold environments. In: Yousef, M.K. (Ed.), Stress Physiology in Livestock, Vol. II. Ungulates. CRC Press, Inc., Boca Raton, Florida, USA. pp. 175-194.
- CHUA, B.; E. COENEN; J. VAN DENLE & D. M. WEARY. 2002. Effects of Pair Versus Individual Housing on the Behavior and Performance of Dairy Calves. J. Dairy Sci. 85:360-364.
- **CONDE, J.** 2000. Mapa climático de Köppen. http://www.terra.es/personal/jesusconde. Accessed November 25th 2007
- COSSINS, A. R. & K. BOWLER. 1987. Temperature Biology of Animals. London, Chapman & Hall.
- **CURTIS, S. E.** 1982. Measurement of stress in animals.Proc. Symp. on Management of Food Producing Animals 1: 1.

- DUNLAP, J. C.; J. J. LOROS & P. J. DE-COUR-SEY. 2004. Chronobiology: Biological timekeeping. Sunderland, MA: Sinaur Associates
- FAULKNER, P. M. & D. M. WEARY. 2000. Reducing pain after dehorning in dairy calves. J.Dairy Sci. 83:2037-2041.
- **FLOWER, F. & D. M. WEARY.** 2003. The effects of early separation of the dairy cow and calf. Anim. Welfare 12: 339-348.
- FRASER, A.F. & D. M. BROOM. 1990. Farm Animal Behaviour and Welfare (3rd edit.). Bailliere Tindall: London. Pp: 353
- **GRANDIN, T.** 1997. Assessment of Stress During Handling and Transport. J. Anim. Sci. 75:249-257
- HAHN, G. L.; R. A. EIGENBERG; J. A.
  NIENA-BER & E.T. LITTLEDIKE.
  1990. Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. J Anim Sci. 68:2658-2665
- HALBERG, F.; E. A. JOHNSON; W. NEL-SON; W. RUNGE & R. SOTHERN. 1972. Autorhythmometry procedures for physiological self measurements and their analysis. Physiol. Teacher 1: 1-11
- HARTSOCK, T. G. 1982. Ethological approach to farm animal behavior research. J. Anim. Sci. 54:447.
- **INFOSTAT/P.** 2007. Software estadístico. Estadística y Diseño F.C.A. Universidad Nacional de Córdoba.
- JENSEN, M. B.; K. S. VESTERGAARD; C.C. KROHN & L. MUNKSGAAR. 1997. Effect of single versus group housing and space allowance on responses of calves during open-field tests. Appl. Anim. Behav. Sci. 54:109-121.
- KLERMAN, E. B.; H. B.GERSHENGORN; J. F. DUFFY & R. E. KRONAUER. 2002. Comparisons of the variability of three markers of the human circadian pacemaker. J Biol Rhythms. 17:181-193

- LEADLEY, S. 2006.Calf blankets. http:// www.atticacows.com/docu-mentView. asp?docID=535 Accessed 11 April 2008
- LEMMER, B. 1987. Cardiovascular medications. In: Kuemmerle, H.P., G. Hitzenbergerand H. Spitzy (Eds.) Klinische Pharmakologie, ecomed fourth ed. Landsberg, pp. 1-14.
- MADER, T. L.; M. S. DAVIS & J. B. GAU-GHAN. 2007. Effect of sprinkling on feedlot microclimate and cattle behavior. Int. J. Biometeorol. 51: 541-551.
- MARTIN, P. & P. BATESON. 1991. La medición del comportamiento. Alianza Universidad. Versión española de Fernando Colmenares, 1º edición. Ed. Alianza. Madrid - España. Pp: 215.
- MERCK & CO. 1979. Behavior. In: O. H. Siegmund (Ed.) The Merck Veterinary Manual. p 1450. Merck and Co., Inc., Rahway, NJ.
- NELSON, W.; Y. LIANG TONG; JUNG-KUEN LEE & F. HALBERG. 1979. Methods for cosinor rhythmometry, Chronobiologia 6: 305-323
- PICCIONE, G. & G. CAOLA. 2002. Biological rhythm in livestock. J Vet Sci 3: 145-157
- PICCIONE G; E. GIUDICE; F. FAZIO & J.P. MORTOLA. 2010. The daily rhythm of body temperature, heart and respiratory rate in newborn dogs. J Comp Physiol B DOI 10.1007/s00360-010-0462-1
- PICCIONE, G.; F. GRASSO & E. GIUDICE. 2005. Circadian rhythm in the cardiovascular system of domestic animals. Res. Vet. Sci. 79: 155-160
- PICCIONE, G.; G. CAOLA & R. REFINET-TI. 2003. Daily and estrous rhythmicity of body temperature in domestic cattle. BMC Physiology, 3. http://www.biocentral. com/1472-6793/37 Accessed September 30th 2007
- PICCIONE, G.; C. CASTALDO; E. GIU-DICE & G. CAOLA. 1998. Studio della periodicità circadiana di alcuni parametri elettrocardiografici nella bovina. Atti della

Società Italiana di Buiatria 30: 313-319.

- **REFINETTI, R.** 2005. Circadian physiology. 2nd ed. Boca Raton FL: CRC Press
- SCIBILIA, L. S.; L. D. MULLER; R.S. KEN-SINGER; T. F. SWEENEY & P. R. SHELLENBERGER. 1987. Effect of environmental temperature and dietary fat on growth and physiological responses of newborn calves. J Anim Sci 70: 1426-1433
- STEPHENS, D. B. 1980. Stress and its measurement in domestic animals: A review of behavioral and physiological studies under field and laboratory situations. Adv. Vet. Sci. Comp. Med. 24:179.
- UCO (Universidad de Córdoba, Producción Animal), 2002. http://www.uco.es/organiza/departamentos/prodanimal/economia/ aula/img/pictorex/06\_07\_02\_TEMA\_8.pdf Accessed 7 April 2008.

VALTORTA, S. E.; M.C. SCAGLIONE;

P. ACOSTA; J. E. CORONEL; H. R. BEL-DOMENICO & J. C. BOGGIO. 2006. Daily rhythms in blood and milk lead toxicokinetics following intravenous administration of lead acetate to dairy cows in summer. Int. J. Biometeorol. 50: 133-138

- VICKERS, K. J.; L. NIEL; L. M. KIEHL-BAUCH & D. M. WEARY. 2005. Calf response to caustic paste and hot-iron dehorning using sedation with and without local anesthetic. J. Dairy Sci. 88: 1454-1459.
- VITELA, I.; C. CRUZ VÁZQUEZ & J. SO-LANO. 2005. Comportamiento de vacas Holstein mantenidas en un sistema de estabulación libre, en invierno, en zona árida, México. Arch. Med. Vet., 37: 23-27.
- WILSON, W. O. 1971. Evaluation of stressor agents in domestic animals. J. Anim. Sci. 32:578.