EFFECTS OF GROWING AND WEANING ON PLASMATIC LEVELS OF ALDOSTERONE, Na^+, K^+ AND Cl^- IN HALF-BRED ZEBU CALVES

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ABSTRACT: Variations of plasmatic levels of aldosterone, Na^+, K^+ and Cl^- were registered during the growth (2^{nd} to 6^{th} month) of nursing and early weaned calves. Some reports suggest that small growing rate of calves submitted to early weaning would be due to the stress of mother-breeding separation at 2 months post-partum. Sixty out of 120 suckling half-bred zebu calves (2 months old) were weaned at day 0 receiving a commercial balanced diet, while the remainder continued suckling. Blood samples were taken at 0, 7, 14, 21, 28, 60, 90 and 120 days for laboratory determinations performed by conventional methods. Analysis of the variance for repeated measures demonstrated that differences were not significant for the treatment effect (suckling versus weaned) in any of the studied parameters. The time effect (growth) was significant ($p < 0.05$) for the decline of aldosterone and Na^+ in both lots and was attributed to ontogenic reasons. Such results turn improbable stress occurrence. Confidence intervals for this race and age (2nd and 6th month respectively) were of 346-352 and 286-292 pg/ml for aldosterone, 141-144 and 140-143 mmol/l for Na^+, 4.4-4.6 and 4.4-4.7 mmol/l for K^+, as well as 94-98 and 95-98 mmol/l for Cl^-.

RESUMEN: Efectos del crecimiento y destete sobre los niveles plasmáticos de aldosterona, Na^+, K^+ y Cl^- en terneros cruza cebú.
Se estudian las variaciones de aldosterona, Na^+, K^+ y Cl^- registradas durante el crecimiento (2° a 6° mes) de terneros índicos en lactación y destetados precozmente. Algunos reportes sugieren que la menor velocidad de crecimiento de los terneros sometidos a destete precoz se debería al estrés que implica la separación madre-cría a los 2 meses post-parto. Sesenta terneros lactantes cruza cebú de 2 meses de edad fueron destetados (día 0), suplementándose su alimentación con un balanceado comercial. Otros 60 continuaron su amamantamiento al pie de madre. En ambos grupos, las determinaciones de laboratorio se efectuaron a partir de muestras de sangre extraídas a los 0, 7, 14, 21, 28, 60, 90 y 120 días. El análisis de la variancia para medidas repetidas demostró que las diferencias no fueron significativas para el efecto tratamiento (lactantes versus destetados) en ninguno de los parámetros estudiados. El efecto tiempo (crecimiento) fue significativo ($p < 0.05$) para las declinaciones de aldosterona y sodio, que ocurrieron en ambos lotes y se atribuyen a razones ontogénicas. Tales resultados tornan improbable la ocurrencia de estrés. Los intervalos de confianza para esta raza y edad (2° y 6° mes respectivamente) fueron de 346-352 y 286-292 pg/ml para aldosterona, 141-144 y 140-143 mmol/l para Na+, 4,4-4,6 y 4,4-4,7 mmol/l para K+, así como 94-98 y 95-98 mmol/l para Cl-.

Key words: aldosterone, electrolytes, calf, growing, weaning, stress
Palabras clave: aldosterona, electrolitos, ternero, crecimiento, destete, estrés

INTRODUCTION

Calf internal environment suffers large physiologic changes from suckling stage (monogastric) to ruminant stage (polygastric). During nursing, higher plasmatic levels of glucose, fructosamine and alkaline phosphatase (Coppo *et al.*, 1996), as well as inorganic phosphorous (Corbellini, 1998), γ-globulins (Gascón, 1992), neutrophils (Jain, 1993), chylomicrons and VLDL (Cirio and Tebot, 2000), were registered.

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Data about hormonal reference values (especially aldosterone) during growing of half-bred zebu calves are not abundant; this cattle is the most numerous and economically more important in the subtropical area of the north of Argentina. In the argentine breeding extensive system of meat livestock, calves are weaned at 180 days old. Some years ago began the practice of early weaning (age: 60 days), which produces profit for their mothers (nutritional improvement and rapid return to reproductive activity). However, this practice affects calves growth rate; in day 180 they show significant small weight than those of nursing congenerous. This phenomenon is attributed to the stress caused by early weaning (Galli *et al.*, 1995).

Aldosterone is involved in the stress when sympathetic alarms activate the hypothalamus-pituitary-adrenal axis. Following glucocorticoids action, aldosterone promotes water and salt conservation (Na^+, Cl^-), in expense of K^+ loss (Dickson, 1999). Their action is singularly important for the argentine northeastern cattle, because soil and pasture are deficient in Na^+, possessing K^+ in excess (Coppo and Coppo, 1999). This mineralocorticoid also participates in heat acclimatization, protecting against excessive NaCl losses through sweat (Guyton and Hall, 1998), mechanism very important in this cattle race (*Bos indicus*), characterized by segregating 480 ml/hour/m^2, greater than european races (*Bos taurus*), which lose only 40 ml/hour/m^2 (Castaño, 1995).

Because the possibility that early weaning was able to cause stress and increase aldosterone levels, the aim of this work was to verify eventual changes of the hormone plasmatic concentrations and its associate electrolytes (Na^+, K^+, Cl^-) during growth (60 to 180 days) of early weaned half-bred zebu calves, versus nursing controls. The latter would allow obtain aldosterone reference intervals for this race calves, as well as to verify ontogenic changes of the hormone and its associate electrolytes, between 2 and 6 months of life.

MATERIALS AND METHODS

Experimental design.- A prospective design of repeated assessments was used, considering treatment (early weaning versus continued suckling) and time (growth) as independent variables, and plasmatic values of aldosterone, Na^+, K^+ and Cl^- concentrations as dependent variables, which were determined 8 times during 4 months of study, in late spring and summer.

Animals.- One hundred and twenty nursing half-bred zebu calves (60-70 days old and 60-90 kg weight), 50% females and 50% castrated males, clinically healthy and phenotypically homogeneous, were used. They were randomly divided in experimental (E) and control (C) groups of 60 animals each, with equal numbers of females and castrated males in each group, which stayed in contiguous plots with similar pasture.

The control calves continued consuming their mother's milk, while those of group E were weaned on day 0 and fed a commercial balanced diet (16% crude protein, 7% fiber, 4% ether extract, 0.08% Na^+, 0.08% Cl^-, ME = 2.77 Mcal/kg DM), at 1.5% of live-weight/day.

The study was carried out on a farm in the northeast of Argentina, in a subtropical area with 1200 mm of rain/year and natural pastures of perennial grasses

with 6% of crude protein in summer. The region is dedicated to extensive breeding of beef cattle, and calves are usually weaned in summer, approximately at 6-8 months old.

Sample collection.- The collection of samples began in day 0 for both groups and then in day 7, 14, 21, 28, 60, 90 and 120. Blood samples were taken without anti-coagulant at 7-8 h each morning, by jugular venepuncture. The clotted blood was centrifuged (700g, 10 min) to obtain serum, which was kept at 4°C until assayed within 6 hours of extraction.

Assay procedures.- Aldosterone was determined by radioimmunoassay (RIA), using ^{125}I marked antibodies, competitive technique in tubes with stuck antigens, read in gamma counter ANSR-Abbot, reagents DPC Lab. Sodium and potassium were measured by flame photometry in Metrolab 305-D, using Biopur reagents. Chloride was assayed by spectrophotometry (450 nm, 37°C), by mercuric thiocyanate technique (Wiener reagents), in a Labora Mannheim 4010 photometer (Henry *et al.*, 1991).

Statistical methods.- The normality of the distribution of the laboratory values was assessed using the Wilk-Shapiro test. Parametric descriptive statistics tests (mean, standard deviation and ±95% confidence interval) were calculated by conventional procedures. Analysis of variance (Anova) for repeated measures was calculated by the software *Statistica* Version 1999, including significance of the time and treatment effects. Following the Anova, the significance of differences between groups C and E on each day was estimated by the Tukey test. Statistical significance in this paper refers to the 5% level (Steel and Torrie, 1992).

Results and discussion

Aldosterone: Descriptive statistics for plasmatic aldosterone are shown in Table 1. The distribution was approximately normal, with wide individual ranges. Confidence intervals revealed that both lots belonged to the same statistical population. Differences between nursing and early weaned calves in each date of sampling were not significative. Aldosterone levels of 533 ± 159 pg/ml have been reported in newborn european calves; this concentration declines to 246 ± 56 pg/ml in the eighth day of life (Tasker, 1989), suggesting an ontogenic decrease. In the present assay, our means were lightly higher than the last level; this difference may be attributed to the race (indian breed) and geographical area (subtropical climate). In both lots, the mineralocorticoid showed a decrescent tendency directly proportional to the growth advance (Fig. 1). The Anova for repeated measures revealed that the time effect was significant (aldosterone declined in both lots) but the treatment effect was no significant (differences between unweaned and early weaned animals were not detected). This fact would allow think that stress was absent in early weaned calves; stress increases the plasmatic aldosterone levels in different animals (Capponi and Rossier, 1996; Fuller and Lin-Tio, 1996). In rats, the basal value of this hormone (smaller than 300 pg/ml), rises to 1000 pg/ml in the acute immobilization stress (Gauna *et al.*, 1994; Niebylski *et al.*, 2000). During stress, the episodic pulsatility of corticosteroids secretion (circadian rhythm) is interrupted, and hormones will remain increased during more prolonged periods (Carcagno, 1995).

Table 1: Descriptive statistics for aldosterone (pg/ml) in control (C) and experimental (E) groups

Day	Mean ± Standard Deviation		Wilk-Shapiro (C + E)	Confidence Interval		Range (C + E)	Signif. (p)
	C	E		C	E		
0	348 ± 12	351 ± 13	0.996	345-351	347-354	320-382	NS
7	347 ± 11	349 ± 11	0.997	344-350	346-352	319-377	NS
14	334 ± 9	333 ± 8	0.996	332-337	331-336	313-357	NS
21	336 ± 11	333 ± 12	0.995	332-339	330-337	298-363	NS
28	328 ± 11	326 ± 11	0.974	325-331	323-329	304-349	NS
60	309 ± 12	313 ± 11	0.995	306-313	310-316	281-339	NS
90	297 ± 9	295 ± 12	0.992	295-300	292-297	269-322	NS
120	288 ± 11	291 ± 14	0.995	285-291	287-294	261-323	NS

Critical value for Wilk-Shapiro test ($n > 50$, $p < 0.05$): 0.947.
p: significance ($p < 0.05$) of differences between C and E (Tukey post-Anova of repeated measures), NS: no significant.

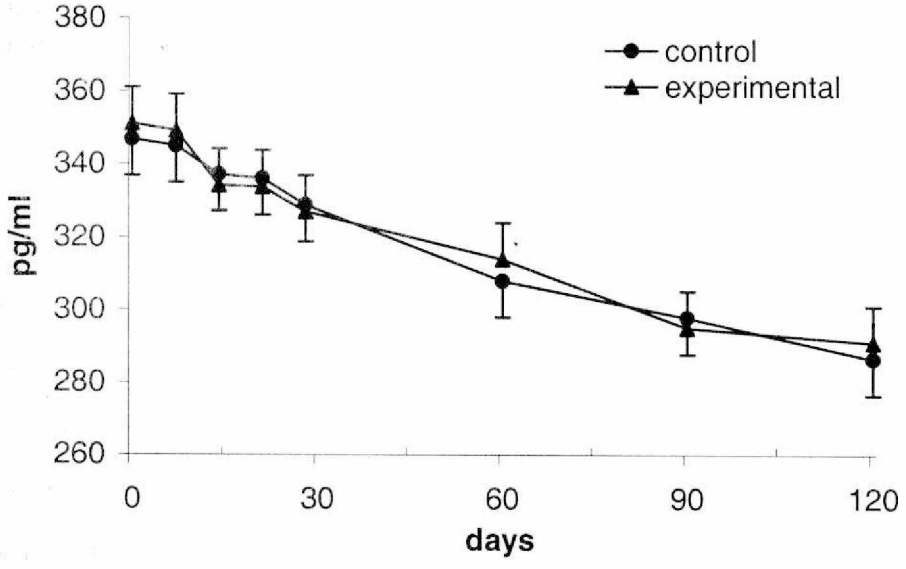

Fig. 1: Aldosterone evolution in plasma of unweaned (control) and early weaned (experimental) calves.
Results are expressed as means ± standard deviation. Analysis of the variance for repeated measures revealed that time effect was significant ($p < 0.05$) but treatment effect was no significant.

Table 2: Descriptive statistics for sodium (mmol/l) in control (C) and experimental (E) groups.

Day	Mean ± Standard Deviation		Wilk-Shapiro (C + E)	Confidence Interval		Range (C + E)	Signif. (p)
	C	E		C	E		
0	144 ± 5	142 ± 6	0.981	142-145	140-144	129-155	NS
7	143 ± 7	143 ± 7	0.977	141-144	140-144	125-157	NS
14	142 ± 6	141 ± 6	0.990	140-143	139-143	128-156	NS
21	141 ± 7	142 ± 6	0.986	139-143	140-143	128-155	NS
28	143 ± 6	140 ± 7	0.970	141-144	138-142	127-155	NS
60	140 ± 7	142 ± 7	0.987	137-141	139-144	125-156	NS
90	141 ± 6	141 ± 7	0.985	139-142	138-143	125-155	NS
120	142 ± 5	143 ± 6	0.980	140-143	141-144	130-155	NS

Critical value for Wilk-Shapiro test ($n > 50$, $p < 0.05$): 0.947.
p: significance ($p < 0.05$) of differences between C and E (Tukey post-Anova of repeated measures), NS: no significant.

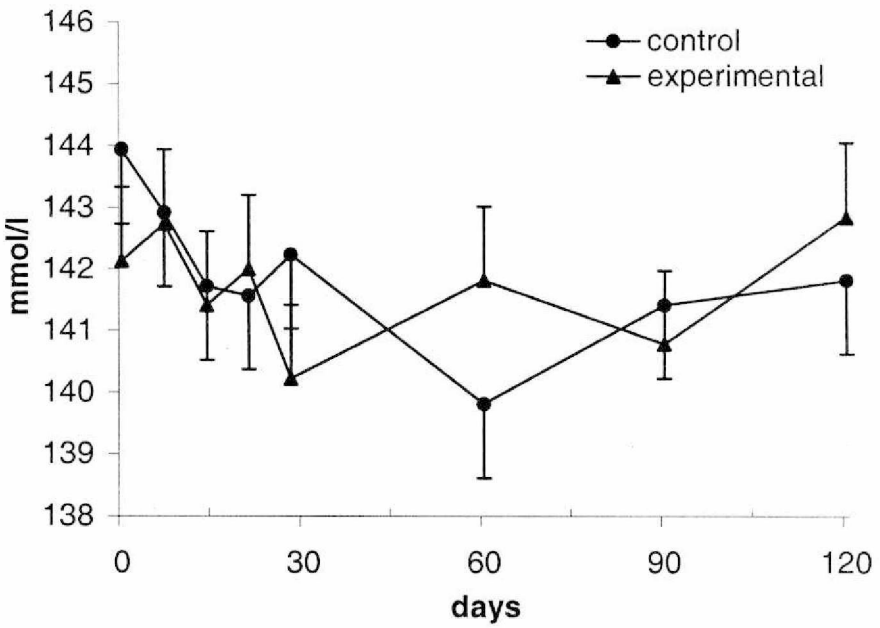

Fig. 2: Sodium evolution in plasma of unweaned (control) and early weaned (experimental) calves.
Results are expressed as means ± standard deviation. Analysis of the variance for repeated measures revealed that time effect was significant ($p < 0.05$) but treatment effect was no significant.

Sodium: the range of its physiological fluctuations was wide, with gaussian distribution in all cases (Table 2). The means comparison was not significant. In each sampling date, the superposition of confidence intervals covered the arithmetic means. This revealed smaller dispersion than those reported for calves in other publications: 124 to 150 mmol/l (Dürr and Kraft, 1980) and 126 to 146 mmol/l (Coles, 1986). Figure 2 suggests a slight declining tendency of calves natremia in both lots; such tendency is statistically confirmed by the time effect significance ($p < 0.05$) of the Anova for repeated measures. Treatment effect was not significant, adding another argument to discard stress; in this syndrome the plasmatic sodium should be increased (Bensi *et al.*, 1997; Carrizo and Morales, 1990). In rats, acute immobilization stress reduced sodium renal excretion and decreased sodium intake, with decline of the urine volume (Niebylski *et al.*, 2000).

Potassium: their variations were narrow (Table 3). Calves kalemia showed symmetrical distribution and confidence intervals indicative of the same origin population. Significant differences between control and experimental lots were not registered. The values were slightly lower than those reported for calves of european races: 4.1 to 5.5 mmol/l (Dürr and Kraft, 1980) and 4.5 to 6.9 mmol/l (Coles, 1986). Potassium plasmatic levels oscillated irregularly, without clear ontogenic tendency (Fig. 3). Treatment and time effects were not significant. Kalemia should have diminished in the event of stress (Nockels, 1992; Tasker, 1989; West, 1993). This effect is attributed to hyperaldosteronism (Coppo, 2001); in dogs, stress causes hypokalemia, antidiuresis and antinatriuresis (Koepke *et al.*, 1986).

Table 3: Descriptive statistics for potassium (mmol/l) in control (C) and experimental (E) groups.

Day	Mean ± Standard Deviation		Wilk-Shapiro (C + E)	Confidence Interval		Range (C + E)	Signif. (p)
	C	E		C	E		
0	4.53±0.46	4.51±0.49	0.962	4.41-4.64	4.37-4.63	3.7-5.4	NS
7	4.48±0.48	4.53±0.52	0.975	4.35-4.60	4.39-4.66	3.5-5.5	NS
14	4.55±0.43	4.45±0.51	0.970	4.44-4.66	4.31-4.58	3.5-5.5	NS
21	4.49±0.49	4.53±0.46	0.972	4.36-4.61	4.41-4.65	3.7-5.5	NS
28	4.43±0.52	4.46±0.47	0.984	4.29-4.56	4.33-4.58	3.4-5.5	NS
60	4.48±0.42	4.44±0.48	0.987	4.37-4.59	4.31-4.57	3.5-5.5	NS
90	4.57±0.46	4.51±0.53	0.968	4.44-4.69	4.37-4.64	3.3-5.4	NS
120	4.50±0.49	4.56±0.51	0.973	4.37-4.62	4.42-4.69	3.5-5.5	NS

Critical value for Wilk-Shapiro test ($n > 50$, $p < 0.05$): 0.947.
p: significance ($p < 0.05$) of differences between C and E (Tukey post-Anova of repeated measures), NS: no significant.

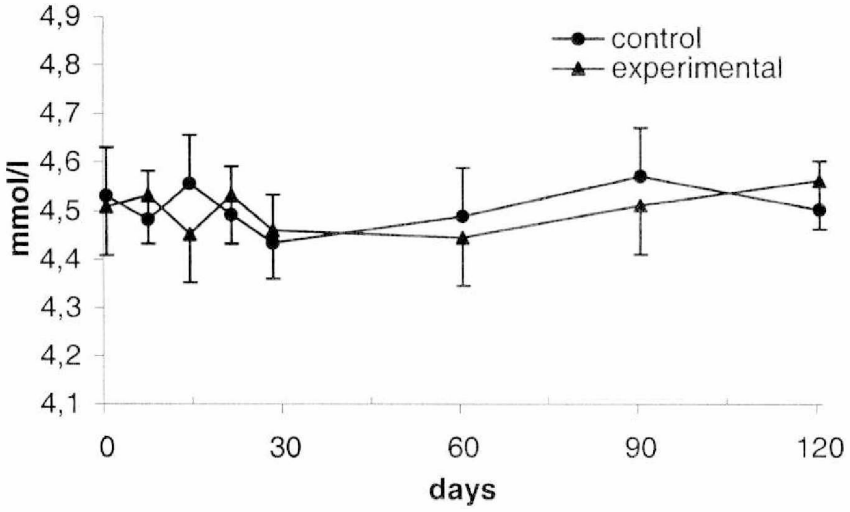

Fig. 3: Potassium evolution in plasma of unweaned (control) and early weaned (experimental) calves.
Results are expressed as means ± standard deviation. Analysis of the variance for repeated measures revealed that both the time and treatment effects were no significant.

Chloride: the variations of this anion are described in Table 4; the values distribution was normal, without significant differences between nursing and weaned calves. The obtained means were slightly lower than those (104 mmol/l) reported in other works (Coles, 1986; Tasker, 1989). Tendencies attributable to the ontogeny were not detected (Fig. 4). The analysis of the variance for repeated measures revealed no significance for both, time and the treatment effects. In the stress, aldosterone would promote high chloride resorption, secondary to sodium resorption (Guyton and Hall, 1998).

Table 4: Descriptive statistics for chloride (mmol/l) in control (C) and experimental (E) groups.

Day	Mean ± Standard Deviation		Wilk-Shapiro (C + E)	Confidence Interval		Range (C + E)	Signif. (p)
	C	E		C	E		
0	95.8±6.8	96.2±6.3	0.975	93.9-97.5	94.5-97.8	85-115	NS
7	95.6±7.1	95.9±6.4	0.971	93.8-97.5	94.3-97.6	85-115	NS
14	96.1±7.1	95.7±6.9	0.951	94.2-97.8	94.1-97.7	85-114	NS
21	95.4±6.5	96.3±7.3	0.962	94.3-97.7	94.4-98.2	85-115	NS
28	95.7±7.3	95.6±6.6	0.961	94.1-97.9	94.0-97.4	85-113	NS
60	95.6±6.6	95.9±6.3	0.975	93.9-97.3	94.3-97.5	85-113	NS
90	95.3±6.5	95.8±5.7	0.971	93.2-96.5	94.4-97.4	85-112	NS
120	96.0±7.4	95.7±5.9	0.969	94.9-97.8	94.6-97.5	84-112	NS

Critical value for Wilk-Shapiro test ($n > 50$, $p < 0.05$): 0.947.
p: significance ($p < 0.05$) of differences between C and E (Tukey post-Anova of repeated measures), NS: no significant.

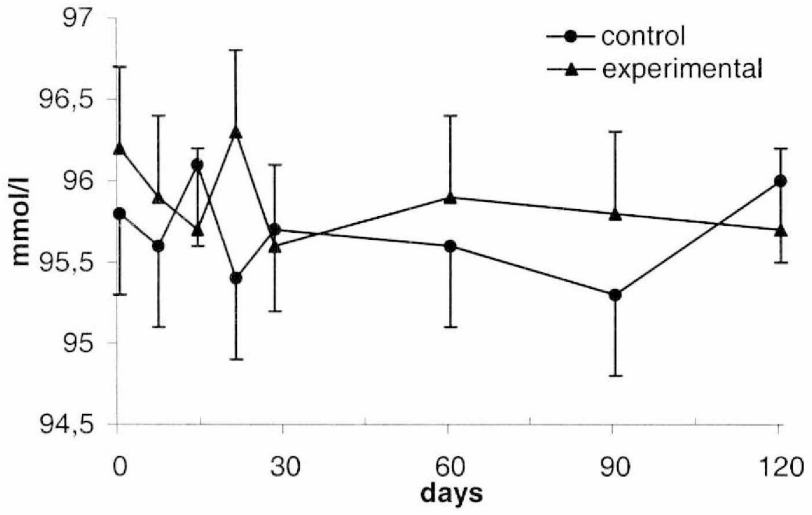

Fig. 4: Chloride evolution in plasma of unweaned (control) and early weaned (experimental) calves.
Results are expressed as means ± standard deviation. Analysis of the variance for repeated measures revealed that both the time and treatment effects were no significant.

Ontogenic variations: the confidence intervals of the studied parameters (control and experimental means for each age), are detailed in Table 5. No significant difference between sex was revealed by covariance analysis. These reference values could optimize the interpretation of internal environment changes in half-bred zebu calves.

Table 5: Physiological variations due to ontogeny in unweaned and early weaned calves.

confidence interval ± 95%	aldosterone pg/ml	sodium mmol/l	potassium mmol/l	chloride mmol/l
month 2	346-352	141-144	4.4-4.6	94-98
month 3	324-330	139-143	4.3-4.6	94-98
month 4	308-315	138-142	4.3-4.6	94-97
month 5	294-298	138-142	4.4-4.7	93-97
month 6	286-292	140-143	4.4-4.7	95-98

In conclusion, half-bred zebu calves growth was characterized by decreases of aldosterone (marked) and sodium (scarce), meanwhile potassium and chloride did not reveal large differences in regard to the adult reported values. The plasmatic levels of aldosterone, sodium, potassium and chloride were not affected by the early weaning, therefore is unlikely that this practice can produce stress in these strong and rustic calves. In previous works on this kind of calves was demonstrated that early weaning was unable to increase other stress indicators like cortisol, glucose, fructosamine,

leukocytes and neutrophils. Decreases of calcium, γ-globulins, lymphocytes and eosinophils neither was verified. On the other hand, several nutritional markers were affected, therefore the smaller weight gains in calves submitted to early weaning would be related with the composition and digestibility of the supplied balanced food, rather than stress (Coppo *et al.*, 1999).

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