

ABSENCE OF BIOCHEMICALLY DEMONSTRABLE STRESS IN EARLY WEANED HALF-BRED ZEBU CALVES

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Abstract

J.A. Coppo, N.B. Mussart, M.A. Revidatti and A. Capellari. Absence of biochemically demonstrable stress in early weaned half-bred Zebu calves. Early weaning is carried out in northeastern Argentina to increase beef cattle reproductive performance. This practice reduces calves growth rate, a result attributable to stress. To verify this hypothesis, a prospective case control study was performed in 120 calves, 60 in lactation (controls, lot C) and 60 submitted to early weaning and supplemented with balanced pellets (experimental, lot E), during 120 days of follow up for 4 successive years on natural pasture. Levels of weight and stress blood indicators were measured in days 0, 7, 14, 21, 28, 60, 90 and 120. Significant cortisol increase (initial: 2.4 ± 0.6 versus final: 3.7 ± 0.9 $\mu\text{g}\cdot\text{dl}^{-1}$) and aldosterone decrease (351 ± 13 versus 291 ± 14 $\text{pg}\cdot\text{m}^{-1}$) were verified in E (time effect) during calf development. The differences between C and E (treatment effect) were not significant in any of the studied hormones. In E (versus C), less weight gains were verified (139.4 ± 11.6 versus 158.7 ± 11.7 kg, $P < 0.001$). Significant increases ($P < 0.05$) in total leukocytes (12.08 ± 1.08 versus 9.76 ± 0.90 $\text{G}\cdot\text{l}^{-1}$), neutrophils (4.12 ± 0.59 versus 3.78 ± 0.59 $\text{G}\cdot\text{l}^{-1}$) and lymphocytes (7.26 ± 0.95 versus 5.39 ± 0.76 $\text{G}\cdot\text{l}^{-1}$) were also confirmed in E, increases beginning between 7 and 28 days after weaning. No significant fluctuations were observed in monocytes, eosinophils, sodium, potassium and chloride. Modifications were attributed to combined effects of ontogeny (growth) and sympathetic alarms (catecholamines), rather than stress (cortisol, aldosterone).

Key words: calves, early weaning, stress, sympathetic alarm, blood indicators.

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INTRODUCTION

Different disturbances (stressors) can cause unspecified neuroendocrine response to correct the pathogenic agent adverse effects on homeostasis; these reactions can be fleeting or durable. In the *sympathetic alarm*, the autonomous nervous system releases catecholamines (epinephrine and norepinephrine), causing hyperglucemia, leukocytosis, neutrophilia, hyperkalemia and another metabolic changes. This state is brief and involves secretions from the adrenal medulla, that generally abates without further consequences

(Duncan and Prasse, 1986; Kaneko, 1989; Coppo, 2000; Coppo, 2001a).

When a stressor remains for a longer period of time, a more durable changes occur, characterized by hyperglucemia, leukocytosis, neutrophilia, lymphopenia, eosinopenia, hypokalemia, hypernatremia and hyperchloremia (*stress*), with adrenal medulla participation (glucocorticoids and mineralocorticoids release). This syndrome derives from a previous alarm stage (adaptation intent), and continues with a resistance stage (adaptation achieved), concluding in an exhaustion stage, with

adaptation loss and health rupture (distress) (Kent and Ewbank, 1986; Kaneko, 1989; Nockels, 1992; Niebyski *et al.*, 1997; Coppo, 2001b; Coppo, 2001c).

Stress is defined as a defense mechanism characterized by an adaptation effort, which becomes illness when aggressions are intense and durable. Contrary to the alarm stage, in the resistance phase the hypophysis-adrenal axis (cortisol, corticosterone, aldosterone) remains active because of the absence of a feedback mechanism (ACTH), because the maintenance of stress has priority as a protection device (Kaneko, 1989; Coppo, 2001b).

It is commonly thought that weaning may produce stress in the calf (Lefcourt and Elsasser, 1995) and that early weaning may cause a stress that can affect alimentary conversion efficiency (Galli *et al.*, 1995). Calf gastric mucosa proteolytic activity can be reduced by high cortisol concentration (Pelletier *et al.*, 1983). In dairy calves, stress frequently causes behavior alterations (Thomas *et al.*, 2001), as well as deaths due to abomasal ulcers (Frerking *et al.*, 1996).

In an extensive system of beef cattle breeding, early weaning consists of the abrupt separation between the dam and its calf of 60-75 days of age and no less than 70 kg of live weight. By contrast, in the conventional method weaning is performed at 6-8 months, with 150 ± 15 kg live weight (Galli *et al.*, 1995). This is a practice that tends to improve the breeding index and increases the animal·ha⁻¹ load. It also increases reproductive performance, as it generates higher forage availability for reproductive function, because nursing is suppressed and calves receive artificial feeding (Arias *et al.*, 1998). In half-bred zebu beef calves, early weaning causes growth retardation and lower weight gain (Peruchena, 1992; Galli *et al.*, 1995; Arias *et al.*, 1998); these alterations are attributed *prima facie* to stress. In the northeastern Argentina area (production: 300,000 calves·year⁻¹) stress has been estimated to cause losses equivalent to US\$ 50,000,000 per year (Peruchena, 1992).

The purpose of this investigation was to determine the evolution of hematological and biochemical parameters to demonstrate the presence of an eventual stress produced by early weaning in half-bred zebu calves.

MATERIALS AND METHODS

Experimental design. A repeated measures prospective design was used, considering treatment (early weaning versus continued suckling) and time (growth, ontogeny) as independent variables. Weight and blood parameters are considered dependent variables, which were measured 8 times during lapses of 4 months (late spring and summer), in 4 successive years.

Animals. Every year, 30 nursing half-bred zebu castrated calves (60-75 days old and 60-90 kg live weight), 50% females and 50% males (castrated at 15-20 days old), clinically healthy and phenotypically homogeneous, were used. They were randomly divided into experimental (E) and control (C) groups of 15 animals each. The control calves continued with their dams, while those in group E were weaned and fed with a commercial balanced concentrated (16% crude protein, 7% fiber, 4% ether extract, ME = 2.77 Mcal·kg⁻¹ DM), at 1.5% of live weight·day⁻¹, plus pasture. Both lots kept grazing in contiguous plots (5 ha) with similar pasture (3 calves·ha⁻¹). The study was carried out on a farm in northeast Argentina, in a subtropical climate area with 1200 mm of rain per 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, at approximately 6-8 months old.

Sample collection. Weighing and blood extractions by jugular venepuncture began for both groups on day 0 and then on days 7, 14, 21, 28, 60, 90 and 120. Blood samples were taken with and without anti-coagulant (EDTA) at 7-8 h each morning. The clotted blood was centrifuged (700 g, 10 min) to obtain the serum, which was kept at 4°C until

assayed within 6 hours of extraction to avoid modifications that storage may cause.

Assay procedures: Cortisol was measured in Immulite analyzer by enzyme-immune-assay method, using the solid phase chemoluminescent technique (DPC reagents). Aldosterone was determined in gamma counter ANSR-Abbot by radio-immune-assay method, using ^{125}I marked antibodies (DPC reagents). Total leukocytes were evaluated by electronic recount in Sequoia-Turner hematological analyzer (Wiener reagents). Leukocyte differential counts were obtained by microscopy, from stained smear (Giemsa), using a Bitex-100 counter. Sodium and potassium were determined in a Metrolab-305 flame photometer (Biopur reagents) and chloride was determined by mercuric thiocyanate technique (450 nm), using a Labora Mannheim 4010 spectrophotometer.

Statistical methods: Normality of the variables' distribution was assessed using the Wilk-Shapiro (WS) test. Parametric descriptive statistics tests (mean \bar{x} , standard deviation SD, and confidence interval $\text{CI}\pm 95\%$) were calculated by conventional procedures. Correlation coefficients (r) were obtained by the Pearson procedure. Thus, analysis

of variance (Anova) for repeated measures, including the significance of the time and treatment effects, as well as its interaction were also calculated. The year of assay was considered as covariable. Following the Anova, significance of differences between C and E groups on each day was estimated by the Tukey test. All the calculations were made using the software Statistica, Version 1999. Statistical significance in this paper (P) refers to the 5% level.

RESULTS AND DISCUSSION

Initial and final descriptive statistics obtained in C and E for each studied parameter during four consecutive years, are detailed in Table 1. Initial values for the breed, age, and geographical area reference (Coppo, 2001a) were statistically homogeneous to each parameter ($\text{CI}\pm 95\%$) and revealed symmetrical distribution (WS), supporting the use of parametric statistics (Steel and Torrie, 1992). Very scarce basophils detected in the leukocyte differential recount were not processed, as they were considered to be a discrete quantitative variable (almost dichotomic), the binomial distribution of which required the use of non-parametric statistics. Leukocyte concentrations are expressed in $\text{giga}\cdot\text{l}^{-1}$ ($\text{G}\cdot\text{l}^{-1}$).

Table 1. Parameters evolution ($\bar{x} \pm \text{SD}$) in control (C) and experimental (E) animals. *Evolución de los parámetros ($\bar{x} \pm \text{DE}$) en animales controles (C) y experimentales (E).*

Parameter Initial (day 0)	Final (day 120)			
	C (n = 60)	E (n = 60)	C (n = 60)	E (n = 60)
Cortisol ($\mu\text{g}\cdot\text{dl}^{-1}$)	2.2 \pm 0.5 a	2.4 \pm 0.6 a	3.4 \pm 0.8 b	3.7 \pm 0.9 b
Aldosterone ($\text{pg}\cdot\text{ml}^{-1}$)	348 \pm 12 a	351 \pm 13 a	288 \pm 11 b	291 \pm 14 b
Total leukocytes ($\text{G}\cdot\text{l}^{-1}$)	14.31 \pm 1.69 a	14.19 \pm 1.59 a	9.76 \pm 0.90 b	12.08 \pm 1.08 c
Neutrophils ($\text{G}\cdot\text{l}^{-1}$)	3.58 \pm 0.57 a	3.67 \pm 0.62 a	3.78 \pm 0.59 a	4.12 \pm 0.59 b
Lymphocytes ($\text{G}\cdot\text{l}^{-1}$)	10.22 \pm 1.45a	10.01 \pm 1.31 a	5.39 \pm 0.76 b	7.26 \pm 0.95 c
Monocytes ($\text{G}\cdot\text{l}^{-1}$)	0.44 \pm 0.08 a	0.43 \pm 0.07 a	0.31 \pm 0.09 b	0.36 \pm 0.09 b
Eosinophils ($\text{G}\cdot\text{l}^{-1}$)	0.07 \pm 0.05 a	0.08 \pm 0.06 a	0.36 \pm 0.19 b	0.33 \pm 0.15 b
Sodium ($\text{meq}\cdot\text{l}^{-1}$)	144 \pm 5 a	142 \pm 6 a	142 \pm 5 a	143 \pm 6 a
Potassium ($\text{meq}\cdot\text{l}^{-1}$)	4.53 \pm 0.46 a	4.51 \pm 0.49 a	4.50 \pm 0.49 a	4.56 \pm 0.51 a
Chloride ($\text{meq}\cdot\text{l}^{-1}$)	95.8 \pm 6.8 a	96.2 \pm 6.3 a	96.0 \pm 7.4 a	95.7 \pm 5.9 a
Weight (kg)	78.9 \pm 6.9 a	77.8 \pm 7.0 a	158.7 \pm 11.7b	139.4 \pm 11.6 c

In each line, different letters indicate significant differences between means groups ($P < 0.05$). Initial values were homogeneous in C and E. When trial concluded, significantly total leukocytes, neutrophils and lymphocytes higher values, as well as weight lower values, were registered in E.

Table 2 exposes the repeated measures Anova results, showing that there is statistical significance for both, *treatment* and *time* effects, except for potassium and chloride (treatment and time effects), as well as cortisol, aldosterone, monocytes, eosinophils and sodium (treatment effect). Interactions between treatment and time were not detected. Covariable *year of assay* was not significant as well. *Circadian rhythm* covariable was excluded from the design (Steel and Torrie, 1992), fixing the sample collection in a uniform morning schedule; eventual effects attributable to *post-prandial stage* were neutralized by fasting, and modifications attributable to *sex* covariable would have minimized because males had been castrated at early age (2nd-3rd week).

Table 2. Repeated measures Anova results in control (C) and experimental (E) groups.

Resultados del Andeva de medidas repetidas en grupos control (C) y experimental (E).

Parameter	Time effect (C+E)	Treatment effect (E)	day
Cortisol	increase *	irregular (NS)	-
Aldosterone	decrease **	irregular (NS)	-
Total leukocytes	decrease **	increase *	7
Neutrophils	increase *	increase *	7
Lymphocytes	decrease **	increase *	28
Monocytes	decrease *	irregular (NS)	-
Eosinophils	increase **	irregular (NS)	-
Sodium	decrease *	irregular (NS)	-
Potassium	irregular (NS)	irregular (NS)	-
Chloride	irregular (NS)	irregular (NS)	-
Weight	increase **	decrease **	7

Day: beginning of significant differences between C and E.

* $P \leq 0.05-0.01$; ** $P \leq 0.01-0.001$; NS: no significant

Calves did not register clinical alterations or deaths in either lot, such as has been reported due to stress because of abomasal ulcer. In dairy cattle such pathology has increased recently (Frerking *et al.*, 1996). An increase of bleats frequency was detected in E during the first 24 hours post-weaning. The brief

duration of this behavior would indicate a low probability that these calves have suffered a severe stress, similar to that which happen in dairy breeds, where vocalizations continue during several weeks, accompanied by other types of behavioral, postural and ambulatory dysfunctions (Thomas *et al.*, 2001). Investigations have demonstrated that half-bred zebu calves are less sensitive to social changes; they also show a shorter suckling duration, remaining less time in contact with their dams, and develop less agonistic actions with them (Das *et al.*, 2001).

Cortisol increased over time in both C and E lots, without significant differences between nursing and weaned calves (Figure 1). In E, there was a significant negative correlation between cortisol and aldosterone ($r = -0.96$, $P = 0.001$), total leukocytes ($r = -0.94$, $P = 0.004$), and lymphocytes ($r = -0.95$, $P = 0.002$), as well as significant positive correlation between cortisol and eosinophils ($r = 0.81$, $P = 0.01$), and weight ($r = 0.94$, $P = 0.001$).

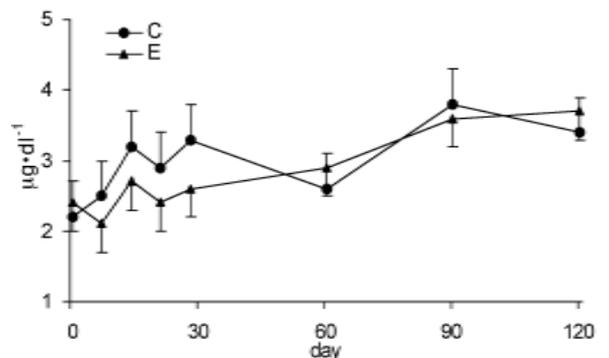


Figure 1. Cortisol evolution in control (C) and experimental (E) animals. Significant differences between C and E were not registered. Increases are attributed to ontogeny. Bars = standar desviation. *Evolución de cortisol en animales controles (C) y experimentales (E). No se registraron diferencias significativas entre C y E. Los aumentos son atribuidos a la ontogenia. Barras = desviación estándar.*

Results obtained in this half-bred Zebu calf (*Bos indicus*) study confirmed the cortisol increasing ontogenic tendency reported in European breed

cattle (*Bos taurus*). The latter show a variation in plasmatic cortisol concentration from 0.5-2.7 $\mu\text{g}\cdot\text{dl}^{-1}$ in the nursing stage to 5-15 $\mu\text{g}\cdot\text{dl}^{-1}$ in adult cattle (Verde, 1992). In cattle, stress increases cortisol plasmatic concentration, which remains at this level during prolonged periods because of the loss of a feedback mechanism (Kent and Ewbank, 1986). From basal levels of $10\pm 4 \mu\text{g}\cdot\text{dl}^{-1}$, cortisol increased to $28\pm 4 \mu\text{g}\cdot\text{dl}^{-1}$ in stressed cows (Domingues *et al.*, 1997). In cold exposure stress, plasma cortisol, glucose, lactate, urea, T_3 , T_4 and triglycerides significant increases were verified in Zebu calves (Godfrey *et al.*, 1991). Plasma cortisol also rises in calf dehorning stress (Morisse *et al.*, 1995). In rats, immobilization stress elevates glucocorticoids from basal values ($1-4 \mu\text{g}\cdot\text{dl}^{-1}$) to more than 50 $\mu\text{g}\cdot\text{dl}^{-1}$ in 24 hours. These values do not drop to initial concentrations for up to 20 days (Nieblyski *et al.*, 1997).

On the other hand, regrouping and relocation carried out in dairy calves didn't elevate basal cortisol levels, and there was not clear evidence that these maneuvers stressed calves (Veissier *et al.*, 2001). Between calves tethered in stalls versus calves untethered in pens, neither cortisol plasma modifications, nor another stress indicators were verified (Wilson *et al.*, 1999). In other studies, weaning neither would have produced cortisol elevations in beef calves (Lefcourt and Elsasser, 1995). In this assay, the scarce and continued cortisol increments occurred in both, weaned and unweaned groups, should be attributed to ontogenesis rather than to an eventual stress.

Aldosterone (Figure 2) showed a declining tendency, which was inversely proportional to the age increase in both C and E lots (time effect significant), without statistical differences between sucking and weaned calves (treatment effect not significant). In E, significant correlations between aldosterone and total leukocytes ($r = 0.88$, $P = 0.003$), lymphocytes ($r = 0.92$, $P = 0.001$), eosinophils ($r = -0.93$, $P = 0.001$) and weight ($r = -0.95$, $P = 0.001$), as well as cortisol (*ut supra*), were verified.

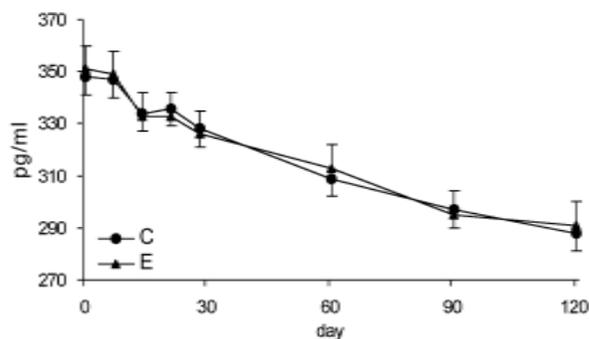


Figure 2. Aldosterone evolution in control (C) and experimental (E) animals. Significant differences between C and E were not registered. If stress should have happened, aldosterone should be increased. Bars = standar desviation.

Evolución de aldosterona en animales controles (C) y experimentales (E). No se registraron diferencias significativas entre C y E. Si se hubiera presentado estrés, la aldosterona debería haberse elevado. Barras = desviación estándar.

Aldosterone concentrations would be falling in first stages of calf life (Safwate *et al.*, 1982). In newborn calves, aldosterone may rise values of $533\pm 159 \text{ pg}\cdot\text{ml}^{-1}$, which would have diminished to $246\pm 56 \text{ pg}\cdot\text{ml}^{-1}$ toward the eighth day of life (Kaneko, 1989), suggesting an ontogenic decrease, just as the change verified in calves of the present assay.

On the other hand, aldosterone levels would increase in stress. In horses, stress provoked by a high resistance competition (endurance) causes plasma aldosterone ascension, which remains high for more than 5 days (Schott *et al.*, 1996). In rats, stress causes aldosterone increases from basal values lower than $300 \text{ pg}\cdot\text{ml}^{-1}$ up to $1,000 \text{ pg}\cdot\text{ml}^{-1}$ (Nieblyski *et al.*, 1997); excesses of this hormone generates hypernatremia and hypokalemia (Kaneko, 1989). Absence of aldosterone elevations in the assayed calves diminishes the probability of the existence of stress.

Total leukocytes exhibited declining tendency over time; at the end they were higher in E than C (Figure 3). In E, white blood cells were significantly correlated with lymphocytes ($r = 0.99$, $P = 0.001$), neutrophils ($r = -0.82$, $P =$

0.05), eosinophils ($r = -0.76$, $P = 0.02$) and weight ($r = -0.83$, $P = 0.01$), as well as cortisol and aldosterone (*ut supra*). In both lots, total leukocytes evolution is attributed to ontogeny, because their blood concentrations decreases from 11-12 $G \cdot l^{-1}$ to 6-8 $G \cdot l^{-1}$ in adults (Jain, 1993). In Zebu cattle it would diminish from 11-15 $G \cdot l^{-1}$ (calf) to 6-9 $G \cdot l^{-1}$ (cow) (Coppo, 2001a). Total white blood cells count during stress is not affected according to some authors (Kent and Ewbank, 1986). On the contrary, other authors state that it would rise (Kaneko, 1989).

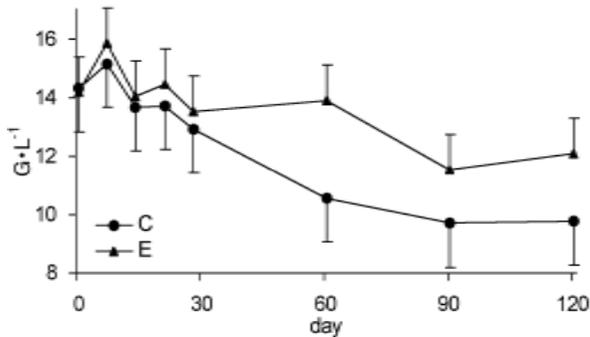


Figure 3. Total leukocytes evolution in control (C) and experimental (E) animals. In C, decrease is attributed to ontogeny. In E, decrease would be less marked due to concomitant sympathetic alarms (early weaning, diet change). Bars = standar desviation.

Evolución de leucocitos totales en animales controles (C) y experimentales (E). En C la disminución es atribuida a la ontogenia. En E la disminución sería menos acentuada debido a las concomitantes alarmas simpaticas (destete precoz, cambio de dieta). Barras = desviación estándar.

This disagreement might arise due to the definition of the “stress” term, because it is sometimes used to describe another phenomenon, known as “sympathetic alarm”. This phenomenon (physiologic leukocytosis) is transitory and may start in adrenal medulla (catecholamines discharge), similar to those that take place in anxiety and excitement, characterized by neutrophilia and lymphocytosis. Stress would cause a reaction of adrenal cortex (cortisol discharge), being more durable and mainly

characterized by neutrophilia, lymphopenia and eosinopenia (Duncan and Prasse, 1986; Coppo, 2001b). In beef European breed calves, weaning produces epinephrine and norepinephrine elevations, without big cortisol variations (Lefcourt and Elsasser, 1995). This argues in favor of the existence of sympathetic alarm (due to dam-calf separation, feeding change) rather than stress for in calves of present assay.

Leukocyte differential counts support this hypothesis. Neutrophils increased in both C and E lots, probably due to neutrophilic peaks instead of a persistent neutrophilia. Such increases were mild in C and they are attributed to sympathetic alarm (physical activity, contention, weigh determination, blood extraction). Sympathetic alarm was likely more intense in E (treatment effect significant), perhaps due to additional factors such as maternal absence and abrupt feeding change (Duncan and Prasse, 1986; Lefcourt and Elsasser, 1995; Coppo, 2001b).

Lymphocytes diminished in both C and E lots, due to ontogenic reasons (time effect significant); for these leukocytes, decreases from 6.8 $G \cdot l^{-1}$ (calf) to 4.5 $G \cdot l^{-1}$ (adult) have been described (Jain, 1993). As a counter-argument to stress existence, it should be kept in mind that lymphocytes decreases were more marked in C than E, the opposite of that predicted (Kent and Ewbank, 1986; Duncan and Prasse, 1986; Kaneko, 1989). Further, strict “lymphopenias” did not happen; only decreases occurred that remained within the reference interval (Coppo, 2001a). Monocytes declination can also be attributed to development, because calves would possess 0.7 $G \cdot l^{-1}$ in contrast to 0.4 $G \cdot l^{-1}$ in adults (Jain, 1993).

In E, neutrophils significantly correlated to lymphocytes ($r = -0.79$, $P = 0.04$) and eosinophils ($r = 0.77$, $P = 0.04$), as well as total leukocytes (*ut supra*). Significant lineal associations between lymphocytes and eosinophils ($r = -0.89$, $P = 0.01$), weight ($r = -0.84$, $P = 0.01$), cortisol, aldosterone, total leukocytes and neutrophils (*ut supra*), were verified. Monocytes did not show correlation with

another studied parameters. Eosinophils significantly correlated with cortisol, aldosterone, total leukocytes, neutrophils and lymphocytes (*ut supra*), as well as weight ($r = 0.81$, $P = 0.01$).

If stress had occurred, eosinophils should have decreased or disappeared (Duncan and Prasse, 1986, Kaneko, 1989; Coppo, 2001b). On the contrary, they increased (Figure 4), just as would be expected for ontogenic reasons (Coppo, 2001a). Eosinophils would register elevations from nursing stage ($0.06\text{--}0.08\text{ G}\cdot\text{l}^{-1}$) to maturity ($0.6\text{ G}\cdot\text{l}^{-1}$) (Jain, 1993). Eosinopenia absence supports the hypothesis of nonexistent stress in E, taking into account the significant correlations established between the ontogeny and sympathetic alarm indicators. Other authors have not found blood leukocyte differential count modifications that indicate stress, in calves submitted to painful situations (Wilson *et al.*, 1999).

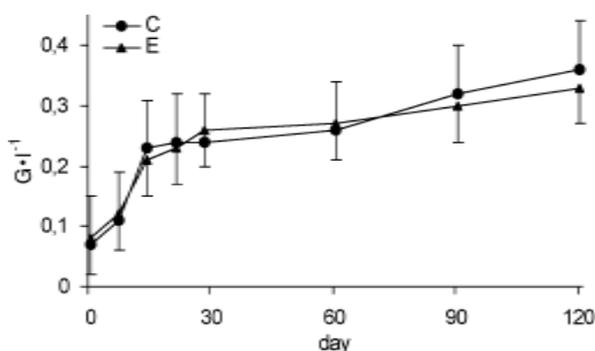


Figure 4. Eosinophils evolution in control (C) and experimental (E) animals. Significant differences between C and E were not registered. If stress had existed, eosinophils should have decreased or disappeared. Bars = standar desviation.

Evolución de eosinófilos en animales controles (C) y experimentales (E). No se registraron diferencias significativas entre C y E. Si hubiera existido estrés los eosinófilos deberían haber disminuido o desaparecido. Barras = desviación estándar.

Sodium decreased in both C and E lots (time effect significant), without correlations to other studied parameters. Early weaning did not cause significant differences between C and E (treatment

effect not significant), which would indicate absence of stress, because sodium should increase due to the aldosterone effect, as has been observed in rats (Niebylski *et al.*, 1997). Aldosterone evolution would not correlate to sodium and potassium input and output in pre-weaning calves (Safwate *et al.*, 1982).

Potassium and chloride revealed light and irregular modifications, without statistical significances or correlations with other parameters. These electrolytes would not vary by growth progress (Coppo, 2001a). Hypokalemia and hyperkalemia would be the response of this cation to stress; the hyperaldosteronemia of stress would also generate plasma chloride elevation, but this effect would be light because chloride would be diluted by the water simultaneously absorbed (Kaneko, 1989; Nockels, 1992; Coppo, 2001a).

Weight increased constantly during the time of the assays, with significant higher values in C than in E. In total, suckling calves showed 79.9 kg (666 g/animal/day) live weight gain. Early weaned calves only increased 61.6 kg (513 g/animal/day). Anova detected significance for both time (ontogenic increase) and treatment effects (growth delay in weaned calves). Mean comparison tests revealed that differences between C and E began to be significant from the first week ($P < 0.05$), and highly significant ($P < 0.001$) from the day 14 until the end of assays, when live weights were 158.7 and 139.4 kg respectively. In E, weight is positively correlated with cortisol, aldosterone, total leukocytes, lymphocytes and eosinophils (*ut supra*).

Other biochemical measures carried out simultaneously lead to the conjecture that the smaller growth rates of early weaned calves is due to nutritional reasons rather than stress, because indicators of nutrition state (urea, albumin, total protein, triglycerides, cholesterol, Cu, P, Mg, Fe, erythrocytes) were significantly lower in E than in C (Coppo, 2000). These modifications could be attributed to nutritional imbalances caused by the abrupt change of the feeding method and/or because of deficiencies of the balanced dietary

supplement. Glucose and fructosamine oscillations in E would endorse the existence of a sympathetic alarm, rather than stress (Coppo, 2001b; Coppo, 2001c).

In conclusion, biochemical and hematological results do not demonstrate stress associated to early weaning (adrenal cortex reaction), although some of the present data would indicate the presence of sympathetic alarm (adrenal medulla reaction).

RESUMEN

Para incrementar la performance reproductiva del ganado para carne, en el nordeste argentino se efectuó destete precoz. Esta práctica provoca menor velocidad de desarrollo de los terneros, circunstancia que se atribuye al estrés. Para verificar tal hipótesis, en 4 años sucesivos fueron realizados ensayos de 120 días de duración, sobre pastura natural, empleando 120 terneros: 60 en amamantamiento (controles, lote C) y 60 sometidos a destete precoz y suplementados con alimento balanceado (experimentales, lote E). Los exámenes fueron realizados a los 0, 7, 14, 21, 28, 60, 90 y 120 días, consistiendo en pesajes y determinaciones hemáticas de parámetros indicadores de estrés, bajo un diseño de medidas repetidas. Durante el desarrollo de los terneros, en E se incrementó el cortisol (inicial: $2,4 \pm 0,6$ versus final: $3,7 \pm 0,9$ $\mu\text{g}\cdot\text{dl}^{-1}$) y disminuyó la aldosterona (351 ± 13 versus 291 ± 14 $\text{pg}\cdot\text{m}^{-1}$), en ambos casos significativamente (efecto tiempo). Al final, no hubo diferencias significativas entre C y E (efecto tratamiento) para ninguna de las hormonas estudiadas. Culminados los ensayos, en E (versus C) fueron verificadas menores ganancias de peso ($139,4 \pm 11,6$ versus $158,7 \pm 11,7$ kg) ($P < 0,001$) y significativos aumentos ($P < 0,05$) de leucocitos totales ($12,08 \pm 1,08$ versus $9,76 \pm 0,90$ $\text{G}\cdot\text{l}^{-1}$), neutrófilos ($4,12 \pm 0,59$ versus $3,78 \pm 0,59$ $\text{G}\cdot\text{l}^{-1}$) y linfocitos ($7,26 \pm 0,95$ versus $5,39 \pm 0,76$ $\text{G}\cdot\text{l}^{-1}$). Las diferencias significativas entre C y E se iniciaron entre los días 7 y 28 post-destete. No se registraron variaciones significativas de monocitos, eosinófilos, sodio, potasio ni cloruro. Las modi-

ficaciones se atribuyen a los efectos conjuntos de ontogenia (crecimiento) y alarmas simpáticas (catecolaminas), antes que al estrés (cortisol, aldosterona).

Palabras claves: terneros, destete precoz, estrés, alarma simpática, indicadores hemáticos.

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