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BIOCHEMICAL DEMONSTRATION OF MALNUTRITION STATE IN EARLY WEANED HALF-BRED ZEBU CALVES

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SUMMARY

The purpose of this study was to demonstrate that early weaning, rather than stress, may induce malnutrition in calves. Sixty out of 120 half-bred zebu suckling-calves (60-75 days old) were weaned at day 0 and fed with a commercial balanced diet (group E), while the rest continued suckling (group C). Blood samples were taken on days 0, 7, 14, 21, 28, 60, 90 and 120, and sera nutritional indicators were determined by conventional methods. Comparing the final results of both groups, C versus E, decreases in sera concentrations ($p < 0.05$) of albumin (3.39±0.29 versus 3.20±0.24 g/dl), urea (0.30±0.04 versus 0.25±0.03 g/l), triglycerides (0.36±0.10 versus 0.21±0.09 g/l), hemoglobin (13.8±1.1 versus 11.7±1.4 g/dl), iron (112±17 versus 92±20 ug/dl), copper (80±19 versus 59±20 ug/dl), and weight (158.7±11.7 versus 139.4±11.6 kg) were registered in group E. Differences between C and E began to be significant from day 7 to 21. Results suggest malnutrition in animals of group E. At the end of the assay, the declining tendency of nutritional indicators tended to revert to normal values. Such changes are attributed to compensatory growth. The absence of a consequential increment in sera urea, triglycerides, hemoglobin and copper concentrations, neither clinical alterations nor

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deaths, discount the existence of stress in early weaned calves.

Key words: *calves, early weaning, growth delay, malnutrition, serum biochemical indicators, stress.*

RESUMEN

DEMOSTRACIÓN BIOQUÍMICA DEL ESTADO DE DESNUTRICIÓN EN TERNEROS CRUZA CEBÚ PRECOZMENTE DESTETADOS

El propósito del estudio fue demostrar que el destete precoz, antes que el estrés, es capaz de provocar un estado de malnutrición en terneros. Sesenta terneros lactantes cruza cebú (60-75 días de edad) fueron destetados al día 0 y recibieron una dieta balanceada comercial (grupo E), en tanto que otros 60 continuaron amamantándose (grupo C). Se tomaron muestras de sangre a los 7, 14, 21, 28, 60, 90 y 120 días, y mediante métodos convencionales fueron medidos algunos indicadores nutricionales séricos. Comparando los resultados finales de ambos grupos, C versus E, en este último fueron registradas disminuciones séricas ($p < 0.05$) de las concentraciones de albúmina (3.39±0.29 versus 3.20±0.24 g/dl), urea (0.30±0.04 versus 0.25±0.03 g/l), triglicéridos (0.36±0.10 versus 0.21±0.09 g/l), hemoglobina (13.8±1.1 versus 11.7±1.4 g/dl), hierro (112±17 versus 92±20 ug/dl), cobre (80±19 versus 59±20 ug/dl) y peso (158.7±11.7 versus 139.4±11.6 kg). Las diferencias entre C y E comenzaron a ser significativas a partir de los 7-21 días. Los resultados sugieren un estado de malnutrición en el grupo E. Al final del ensayo, todos los indicadores nutricionales tendieron a revertir su tendencia declinante, cambios que son atribuidos al crecimiento compensatorio. La ausencia de aumentos resultantes en las concentraciones séricas de urea, triglicéridos, hemoglobina y cobre, ni de alteraciones clínicas y/o muertes, descartan la existencia de estrés en los terneros precozmente destetados.

Palabras clave: *terneros, destete precoz, retraso del crecimiento, desnutrición, indicadores bioquímicos séricos, estrés.*

INTRODUCTION

In northeastern Argentina, weaning is conventionally carried out at 6-8 months old, when the calf weighs 150 ± 15 kg. The early weaning, in the extensive breeding system for beef cattle, is the abrupt separation of the cow and its 60-75 day-old calf, no less than 70 kg live weight (Galli *et al*., 1995). It is a handling practice which tends to improve the reproductive performance, generating larger forage provision, since the nursing is suppressed and calves receive artificial feeding (Arias *et al*., 1998). In half-bred zebu calves, early weaning causes growth delay and smaller weight gain (Peruchena, 1992; Galli *et al*., 1995; Arias *et al*., 1998); in other countries, the average daily weight gained in early weaned calves was 550 g, compared to the 690 g in conventionally weaned calves (Bomba *et al.*, 1989). The growth delay in early weaning is *prima facie* attributed to stress (Renner, 1991; Galli *et al*., 1995; Lefcourt and Elsasser, 1995).

Different nature outbreaks (stressors) can cause unspecified neuroendocrine responses, tending to correct the adverse effects of the noxa on the homeostasis. These reactions can be fleeting or permanent. In the first case (medullo-adrenal sympathetic alarm), the autonomous nervous system liberates catecholamines (epinephrine, norepinephrine) that will lead to hyperglycemia, leukocytosis, neutrophilia, hyperkalemia, and other metabolic alterations. These changes have brief duration, and increased levels generally return to normal values without consequences (Kaneko, 1989; Lefcourt and Elsasser, 1995; Coppo, 2001c).

However, if the stressor continues with its action, more durable changes occur (cortico-adrenal stress), with glucocorticoids and mineralocorticoids release (cortisol, corticosterone, aldosterone), characterized by hyperglycemia, leukocytosis, neutrophilia, lymphopenia, eosinopenia, hypokalemia, hypernatremia, hyperchloremia and other changes. This syndrome (stress) derives from a previous alarm stage (adaptation intent), which can continue with a resistance stage (adaptation achieved), to conclude in an exhaustion stage, with loss of adaptation and unhealthy state (distress) (Kent and Ewbank, 1986; Kaneko, 1989; Nockels, 1992; Coppo, 2001c).

Stress is a defense mechanism characterized by an adaptation effort, which becomes a pathological entity when aggressions are intense and durable. Contrary to sympathetic alarm, in stress the hypophysis-adrenal axis remains active, by loss of feedback control (adrenocorticotropin), since stress will have priority in order to maintain the protection mechanisms (Kaneko, 1989; Coppo, 2001c). In calves, stress could cause behavior dysfunctions (Thomas *et al*., 2001), as well as deaths due to abomasal ulcers (Frerking *et al*., 1996).

Nevertheless, the growth delay of early weaned calves could respond to nutritional reasons, due to the abrupt feeding change that forces animals in the suckling period to ingest inappropriate food for their incomplete digestive system (Coppo, 2000). Composition and digestibility of the balanced diet which will be fed to weaned calves should also be kept in mind; important interactions exist between amino acids and minerals and among minerals themselves, which should be carefully studied when selecting different types of protein or mineral sources for animal nutrition. The availability of amino acids was greater in diets based on animal protein. The iron source affected the absorption of most amino acids, and the retention of calcium and magnesium was significantly greater when ferrous sulphate was used as iron source (Pérez-Llamas *et al*., 2001). Live weight gain after early weaning was increased (840 vs 780 g/day) when a high protein concentrate was fed (18 vs 15%); dry matter and net energy were more efficiently used in calves fed with the high protein concentrate, while protein was less efficiently converted with the higher dietary protein level (Fiems *et al*., 1998).

The purpose of this assay was to demonstrate, by means of biochemical indicators, that early weaning, rather than stress, may lead to a malnutrition state in half-bred Zebu calves.

MATERIALS AND METHODS

Experimental design. A prospective design of repeated assessment was used, considering the treatment (early weaning versus continuous suckling) and the time (growth) as independent variables, and serum concentrations of urea, copper, albumin, triglycerides, iron, and blood hemoglobin as dependent variables, which were determined on eight occasions during the four months of the study, during late spring and summer.

Animals. One hundred and twenty nursing half-bred zebu calves (60-75 days old and 60-90 kg live weight), 50% females and 50% castrated males, clinically healthy and phenotypically homogeneous, were used. They were randomly divided into experimental (E) and control (C) groups of 60 animals each, with equal numbers of females and castrated males. Animals were placed in contiguous plots with similar pasture.

Control calves continued suckling, while those of group E were weaned on day 0 and fed a commercial balanced diet (16% crude protein, 7% fiber, 4% ether extract, metabolizable energy = 2.77 Mcal/kg of dry matter), at 1.5% of live weight/day.

The study was carried out in a farm located northeastern Argentina, in a subtropical climate area with 1200 mm annual rains and natural pastures of perennial grasses with 6% of crude protein in the summer. This region is dedicated to an extensive breeding system of beef cattle, and calves are usually weaned in summer, at approximately 6-8 months old.

Sample collection. Collection of blood samples and weight determination began for both groups on day 0 and were repeated on days 7, 14, 21, 28, 60, 90 and 120. Blood samples were taken by jugular venepuncture with and without anticoagulant (EDTA), at 7-8 AM each day. The clotted blood was centrifuged (700g, 10 min) to obtain serum, which was kept at 4ºC until assayed within 6 hours of extraction to avoid the alterations that storage may cause.

Assay procedures. Assays were performed at 37ºC in a Labora Mannheim 4010 photometer, using disposable semi-micro cuvettes of 10 mm light path. Serum concentrations of urea (urease technique, 546 nm) and copper (batocuproin method, 436 nm) were measured (Pesce and Kaplan, 1990) using Boehringer Mannheim reagents. The concentrations of serum albumin (bromide-cresol-sulphophthalein method, 625 nm), serum triglycerides (lipase-peroxydase technique, 546 nm), serum iron (PBTS procedure, 560 nm) and blood hemoglobin (cyanmethaemoglobin method, 540 nm) were measured (Pesce and Kaplan, 1990) using Wiener reagents.

Statistical methods. The normality of the distribution of the obtained values was assessed using the Wilk-Shapiro (WS) test. Parametric descriptive statistics (mean x, standard deviation SD and confidence interval CI±95%) were calculated by conventional procedures; correlation coefficients (r) were obtained by the Pearson procedure (Steel and Torrie, 1992).

Analysis of variance (ANOVA) for repeated measures was calculated, including the significance of 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. All the calculations were made using the *Statistica* software, Version 1999. Statistical significance in this paper refers to 5% level ($p < 0.05$).

RESULTS

Table 1 shows initial and final descriptive statistics for groups C and E of each parameter studied in the assay. All initial values were statistically homogeneous (CI±95%) and revealed symmetrical distribution (WS). Supplement consumption was complete and calves did not register deaths or clinical alterations in any group, except for an increase in the number of bleats («calls»), detected in group E during the first 24 hours post-weaning.

Table 1. Evolution of studied parameters (x ± SD) in control (C) and experimental (E) groups

Parameter	Initial (day 0)		Final (day 120)	
	C (n = 60)	E (n = 60)	C (n = 60)	E (n = 60)
Albumin (g/dl)	3.29 ± 0.28 a	3.31 ± 0.26 a	3.39 ± 0.29 b	3.20 ± 0.24 c
Urea (g/l)	0.22 ± 0.03 a	0.23 ± 0.03 a	0.30 ± 0.04 b	0.25 ± 0.03 a
Triglycerides (g/l)	0.42 ± 0.13 a	0.43 ± 0.12 a	0.36 ± 0.10 b	0.21 ± 0.09 c
Hemoglobin (g/dl)	12.1 ± 1.3 a	12.4 ± 1.2 a	13.8 ± 1.1 b	11.7 ± 1.4 c
Iron (ug/dl)	109 ± 17 a	113 ± 20 a	112 ± 17 a	92 ± 20 b
Copper (ug/dl)	82 ± 18 a	78 ± 21 a	80 ± 19 a	59 ± 20 b
Weight (kg)	78.9 ± 6.9 a	77.8 ± 7.0 a	158.7 ± 11.7 b	139.4 ± 11.6 c

In each line, different letters indicate significant differences between means groups ($p < 0.05$).

Table 2 shows the results of the ANOVA repeated measures. It can be observed that there was statistical significance for both treatment and time effects, except for albumin (effect of time). Interactions between treatment and time were not detected. Circadian rhythm covariable was excluded of the design, fixing the sample collection with a uniform morning schedule. Eventual effects

Table 2. Results of the repeated measures ANOVA between groups C and E

Parameter	Effect of time (C+E)	Effect of treatment (E)	day
Albumin	irregular (NS)	decrease *	21
Urea	increase *	decrease *	14
Triglycerides	decrease *	decrease **	7
Hemoglobin	increase *	decrease *	14
Iron	decrease *	decrease **	14
Copper	decrease *	decrease **	21
Weight	increase **	decrease **	7

day: beginning of significant differences between C and E (Tukey test).
* $p \leq 0.05$-0.01 ; ** $p \leq 0.009$-0.001 ; NS: not significant

attributable to post-prandial stage were neutralized by fast, and modifications attributable to sex covariable would have minimized because males were castrated immediately after birth.

The serum albumin concentration (Fig. 1) began to differ between C and E in the third week of the assay, revealing slightly augmentative tendency in C and markedly falling tendency in E. Such decline was reverted in the 2nd month, although final serum albumin concentrations in E were significantly lower than those in C (effect of treatment). In the early weaned group, serum albumin concentration significantly correlated with serum urea (r = -0.75, p = 0.03), blood hemoglobin (r = 0.80, p = 0.01), and serum iron concentrations (r = 0.79, p = 0.01).

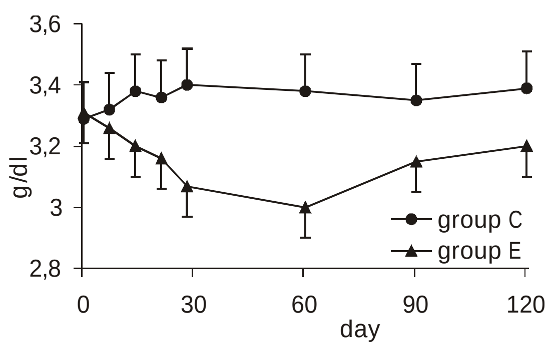

Fig. 1. Evolution of the serum albumin concentration in groups C and E

Serum urea concentration showed upward tendency (effect of time) in both groups (Fig. 2), but there were differences between C and E groups (effect of treatment), which statistical significance started from the second week of assay, with lower values in weaned calves. In E, the concentration of serum urea significantly correlated with serum albumin (*ut supra*), and serum triglycerides concentrations (r = -0.78, p = 0.04), as well as weight (r = 0.70, p = 0.05).

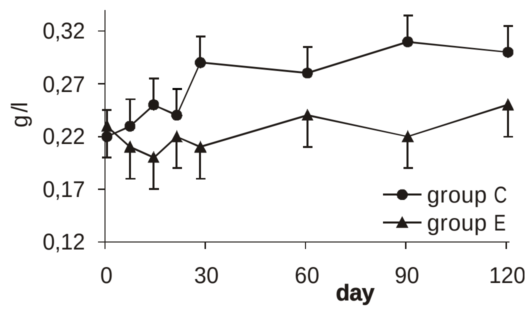

Fig. 2. Evolution of serum urea concentration in groups C and E

The concentration of serum triglycerides decreased in both C and E groups, being markedly different in early weaned calves (Fig. 3), resulting time and treatment effects both significant. The differences between C and E were significant from the first week of assay. In E, concentration of serum triglycerides was significantly correlated with serum urea (*ut supra*) and serum iron concentrations (r = 0.82, p = 0.01).

Blood hemoglobin concentration showed slightly increasing tendency in group C, and evident descending tendency in group E (Fig. 4). Differences between C and E began to be significant from the second week of assay (effect of treatment). In the first month, group E registered changes in the fall of blood hemoglobin

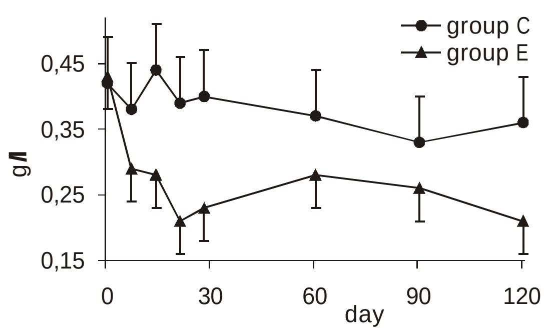

Fig. 3. Evolution of triglycerides concentration in groups C and E

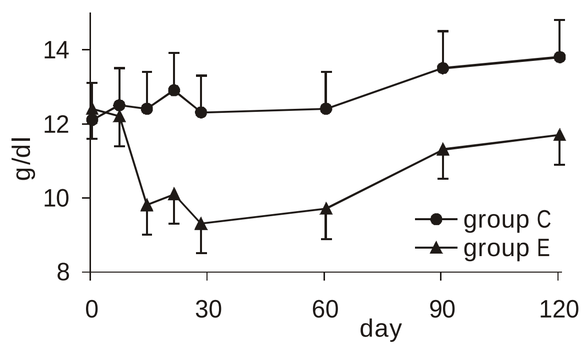

Fig. 4. Evolution of blood hemoglobin concentration in groups C and E

concentration, which began to increase although it never reached the levels of group C. In E, the concentration of blood hemoglobin significantly correlated with serum albumin and serum iron concentrations (*ut supra*).

Group C did not register significant variations of the serum iron concentration, which values were similar at the beginning and the end of the assay. On the contrary, in group E this trace-element progressively declined during the first month, and then changed this tendency, finishing the assay with lower values than initial ones (Fig. 5). Differences between C and E (effect of treatment) began

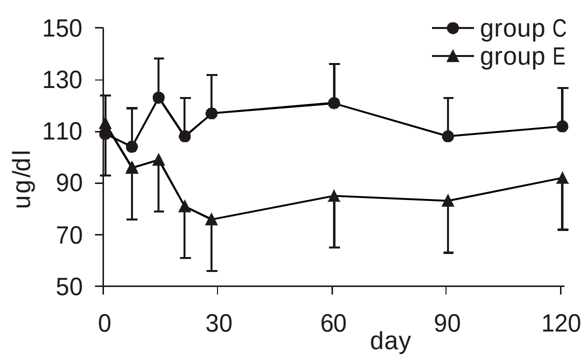

Fig. 5. Evolution of serum iron concentration in groups C and E

to be significant from the second week. In E, the concentration of serum iron significantly correlated with serum copper (r = 0.77, p = 0.02), and serum albumin, blood hemoglobin and serum triglycerides concentrations (*ut supra*).

The serum copper concentrations diminished in both C and E groups, slightly in suckling calves and markedly in early weaned animals (Fig. 6). Differences between C and E groups began to be significant from the third week. In E, the serum copper concentration significantly correlated with the serum iron concentration (*ut supra*), and showed an increased tendency starting from the first month of the assay.

Weight was increasing through time in both C and E groups, with significantly higher values in C than E (Fig. 7). In total, suckling

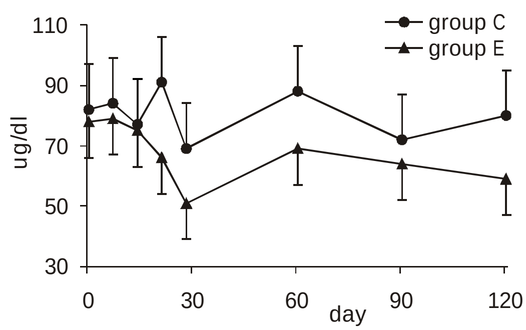

Fig. 6. Evolution of serum copper concentration in groups C and E

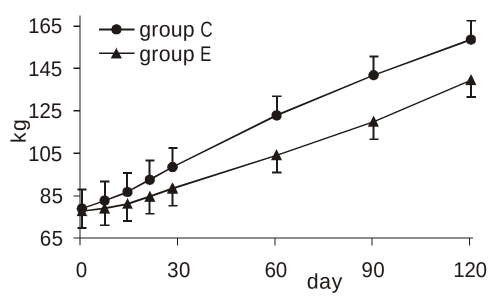

Fig. 7. Evolution of live weight in groups C and E

calves revealed 79.9 kg live weight gain (666 g/animal/day), as long as those early weaned only increased 61.6 kg (513 g/animal/day). The ANOVA detected significance for both time (ontogenic increase) and treatment effects (growth delay in weaned calves). Mean comparison test revealed that differences between C and E began to be significant from the first week ($p < 0.05$), being highly significant ($p < 0.001$) from day 14 until the end of the assay. In lot E, weight was positively correlated with serum urea concentration *(ut supra)*.

DISCUSSION

The lack of clinical alterations and/or deaths is an argument for the stress absence. In calves, this entity frequently causes mortality due to abomasal ulcers (Frerking *et al.*, 1996). The brief duration lapse of increment in the bleats frequency in lot E (24 hours) would move away the probability that these calves suffered a severe stress as which would happen in dairy breeds, where vocalizations would continue during several weeks and they would be accompanied by another behavioral, postural and ambulatory dysfunctions (Thomas *et al.*, 2001). Investigations have demonstrated that rustic half-bred zebu calves would not be very sensitive to social changes; they would show a smaller suckling duration, they would remain less time in contact with their dams, and they would develop less agonistic actions with them (Das *et al.*, 2001).

The initial values of the studied parameters framed in the breed, age, and geographical area reference intervals (Coppo, 2001b). Taking into account that experimental calves received high protein dietary supplementation (16%), the registered hypoalbuminemia should be related to inadequate use (scarce digestibility) rather than insufficient supply (Coppo, 2001b). Unfortunately, manufacturers did not provide detailed information about composition and digestibility of the balanced diet used in this study.

Serum albumin concentration would significantly decrease during malnutrition (Slobodianik *et al*., 1999); alimentary restriction would early alter their serum levels (Jain, 1993). This biochemical parameter significantly correlated with the increase of weight in supplemented calves, revealing itself as a good nutritional indicator (Marcos and Beltramino, 1984).

The serum albumin concentration would not be altered in the stress, because cortisol would cause an increment in the synthesis in the liver, but it would also cause albumin catabolic degradation (Kaneko, 1989). No modifications on the albuminemia attributable to stress of transportation in calves were found (Kent and Ewbank, 1986). Final inflection of serum albumin concentration curve in weaned group could be associated to an adaptation of the digestive tract of the calf to the new diet, turning more efficient the complex proteins metabolization, phenomenon related to compensatory growth (Ryan *et al*., 1993; Phillips *et al*., 2001).

Progressive increase of serum urea concentration in C should be attributed to ontogeny (Marcos and Beltramino, 1984), but decreases in E, very marked during the first weeks after weaning, should be related to scarce nitrogen supply, because cattle serum urea level declines when dietary protein lacks (García, 2000; Coppo, 2001b). Urea is a nutritional indicator related to protein intake (Slobodianik *et al*., 1999). Higher dietary protein increased serum urea concentration in early weaned double-muscled calves (Fiems *et al*., 1998). On the other hand, the adrenal hyperactivity (stress) would cause increment of the serum urea concentration (Godfrey *et al*., 1991; Gómez Piquer, 1992).

Decrease of serum triglycerides concentration in group C could be due to alimentary reasons, because at this age calf accelerates its transition from suckling to ruminant, consuming progressively less quantity of milk, which concentration of triglycerides is high (Marcos and Beltramino, 1984). The marked decline of triglycerides in E should be related to a malnutrition state (Coppo, 1990). In the same way, plasma nonesterified fatty acids declined with age and were lower in early weaned calves (Quigley *et al.*, 1991). These results contradict the existence of an eventual stress state, in which the concentration of serum triglycerides should be high (Mitchell *et al*., 1989; Godfrey *et al*., 1991; Jain, 1993).

Blood hemoglobin concentration increases during calf growth, being this the reason why the ascending tendency of this parameter in group C, should be attributed to ontogeny (Krizanovic *et al*., 2001). The concentrations of the respiratory pigment would be 10 g/dl in the newborn, 11 g/dl at 3 months old, and up to 15 g/dl in adult cattle (Jain, 1993).

Fall of hemoglobin concentration in E should be attributed to malnutrition, because its appropriate synthesis depends on the balance of the nutrients of the diet, which decrease in alimentary deficiencies (Jain, 1993; Coppo 2001b). The final increase would be related to the compensatory growth changes (Ryan *et al*., 1993). If stress had existed, cortisol would have caused increment of the erythropoiesis and increase of the blood hemoglobin concentration (Kaneko, 1989; Gómez Piquer, 1992). In horses, the stress would rise up to 15% this concentration (Jain, 1993).

The serum iron concentration would be slightly affected by the ontogenia. The fall of this element in group E should be attributed to nutritional reasons, because iron alimentary lack may cause decrease of iron plasma concentration (Gomez Piquer, 1992; Corbellini, 1998; Coppo, 2001b). Decline of serum iron concentration have been described in the inflammatory stress (Nockels, 1992), but it would not have happened in the road transportation stress of the calf (Kent and Ewbank, 1986).

Scarce variations in serum copper concentration would be registered during calf growth (Kaneko, 1989; Gomez Piquer, 1992; Coppo, 2001b). In cattle, stress could cause elevation of the serum copper concentration, due to cortisol release by leukocytary interleukines (Nockels, 1992). In this assay, the decrease of serum copper concentration in early weaned calves is an argument in contrast to the stress existence, and it should be attributed to nutritional deficiencies. The copper plasma level is a good indicator of copper intake (Marcos and Beltramino, 1984), and it would diminish in cattle dietary deficiencies (Gómez Piquer, 1992; Corbellini, 1998).

In early weaning assays carried out in northeastern Argentina, results are unanimous when affirming that these animals (half-bred zebu calves supplemented with balanced pellets), compared to those conventionally weaned (on natural pasture), gain less weight (Peruchena, 1992; Galli *et al*., 1995; Arias *et al*., 1998). Weight differences between C and E increased until day 90 and then decreased toward day 120. In E, all the nutritional indicators registered final upward variations, which should be interpreted as an improvement in the general state of calves. They started from day 21 (triglycerides), and then sequentially appeared on day 28 (urea, hemoglobin, iron, copper), day 60 (albumin) and 90 (weight).

Final increased tendencies in E should be related to the recovery ability verified after inadequate feeding periods; it is a capacity to develop more intense and faster growth (compensatory growth), thanks to mechanisms like hyperphagia and greater metabolic efficiency (Ryan *et al.*, 1993; Phillips *et al.*, 2001). In calves restricted on feeding at early age, the potential for subsequent compensatory growth is high and practically independent of the severity of such restriction (Berge, 1991).

Apparently, ruminal metabolic development, as evidenced by changes in fermentation products, was faster in early rather than conventionally weaned calves (Anderson *et al.*, 1987). The early weaning would have a positive influence in the development of ruminal metabolism, which would be demonstrated by the higher concentrations of volatile fatty acids in rumen fluid (Bomba *et al.*, 1989). Calves submitted to early weaning and fed with high quantities of hay and concentrates, revealed larger development of the ruminal epithelium than those of conventional weaning (Zitnan *et al*., 1999). Early weaned calves showed faster growth and larger fatty deposition, when fed with highly concentrated diets (Fluharty *et al*., 2000). In early weaning, live weight gains would improve by administering a balanced diet, or by adequating the quality of its composition and digestibility; this could imply an increment in production costs (Arias *et al*., 1998).

Supporting the hypothesis that smallest growth rate for early weaned calves would be due to alimentary reasons rather than stress, other nutritional indicators (total protein, cholesterol, inorganic phosphorous, magnesium, erythrogram) also declined in E, with significantly high correlation rates (Coppo, 2000). On the contrary, indicators for cortico-adrenal stress (cortisol, aldosterone, lymphocytes, eosinophils, sodium, potassium, chloride, aspartate aminotransferase, alkaline phosphatase) were not modified (Coppo, 2001d). High initial values for total leukocytes and neutrophils (Coppo, 2001c), as well as increases of plasma glucose concentrations in E (Coppo, 2001a), could probably be attributed to medullo-adrenal sympathetic alarms caused by handling, blood sampling, feeding system change and separation of the calves from their dams.

The absence of significant increments in serum fructosamine concentration (Coppo, 2001a) would indicate that glucose changes would have been transitory hyperglycemic peaks resulting from alarms, rather than prolonged hyperglycemia due to stress (Kaneko, 1989). In European beef calves, weaning would have produced elevations of epinephrine and norepinephrine, without large variations of cortisol (Lefcourt and Elsasser, 1995). On the other hand, in the cold exposure stress, Zebu calves increased serum cortisol, glucose, lactate, urea, triglycerides, thyroxine and triiodothyronine concentrations (Godfrey *et al*., 1991).

In conclusion, the findings suggest that decreases of weight and blood nutritional indicators verified in calves submitted to early weaning should be attributed to alimentary imbalances rather than stress, caused by the abrupt change of the feeding system and/or by nutritional deficiencies of the balanced supplement.

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