FRESH CITRUS PULP SUPPLEMENTATION EFFECTS ON WEIGHT GAIN AND PLASMA PROTEIN OF WINTERING COWS

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ABSTRACT . *Fresh citrus pulp is an energy supplement (TDN > 80%) with low protein concentration (7-8%), that is reason why it requires additional nitrogen when it is feed to growing cattle. In order to verify the effect of a citrus by-product (without nitrogen addition) on live weight gain and nitrogen plasma parameters, 80 wintering half-bred zebu cows grazed on native grassland, were used. During 2 consecutive years, twenty animals were allotted to control (C) and another 20 were supplemented (S) with fresh citrus pulp (15 ± 3 kg/animal, during 4 months). Periodic weighing and blood sampling were carried out at 0, 30, 60, 90 and 120 days. Data were statistically processed using a repeated measures design, with mean comparisons by orthogonal contrasts. In both years, S registered higher live weight change rates (492 versus 304 and 352 versus -73 g/animal/day), total protein (8.18 ± 0.41 versus 7.30 ± 0.33 and 7.95 ± 0.38 versus 7.18 ± 0.39 g/dl), albumin (3.57 ± 0.36 versus 2.92 ± 0.35 and 3.20 ± 0.37 versus 2.59 ± 0.32 g/dl), γ globulin (2.80 ± 0.46 versus 2.64 ± 0.42 and 3.23 ± 0.40 versus 3.12 ± 0.44 g/dl) and urea (0.30 ± 0.08 versus 0.24 ± 0.05 and 0.30 ± 0.05 versus 0.26 ± 0.07 g/l). Differences were significant ($p < 0.05$) in many cases. The α and β globulins fluctuated irregularly, and the albumin / globulin ratio increased in S and decreased in C. Mean comparison tests showed that in most of the cases the differences between C and S began to be significant at day 60 of the study. Proteic metabolism nutritional indicators improvement and weight gains reveal that this cheap industrial residue is useful for fattening of wintering cows, yet without nitrogen addition.*

Key Words: citrus pulp, supplementation, wintering cows, weight gain, plasma protein

EFECTOS DE LA SUPLEMENTACIÓN CON PULPA FRESCA DE CITRUS SOBRE LAS GANANCIAS DE PESO Y PROTEÍNAS PLASMÁTICAS DE VACAS DE INVERNADA

RESUMEN: *La pulpa fresca de citrus es un suplemento energético (TND > 80%) con escasa concentración proteica (7-8%), por lo que requiere nitrógeno adicional cuando se destina a la alimentación de ganado en crecimiento. El propósito de este trabajo fue verificar el efecto de este subproducto agroindustrial (sin refuerzo nitrogenado) sobre las ganancias de peso y los parámetros plasmáticos nitrogenados de vacas de invernada cruza cebú (n = 80) mantenidas sobre pastura natural. En dos años consecutivos, 20 animales operaron como controles (C) y otros 20 fueron suplementados (S) con pulpa fresca de citrus (15 ± 3 kg/animal, durante 4 meses), efectuándose pesajes y muestreos sanguíneos a los 0, 30, 60, 90 y 120 días. Con relación a C, las estadísticas finales para cada año indicaron que en S se registraron mayores cambios de peso (492 versus 304 y 352 versus -73 g/animal/día), así como más altos niveles de proteínas totales (8.18 versus 7.30 y 7.95 versus 7.18 g/dl), albúminas (3.57 versus 2.92 y 3.20 versus 2.59 g/dl), γ globulinas (2.80 versus 2.64 y 3.23 versus 3.12 g/dl) y urea (0.30 versus 0.24 y 0.30 versus 0.26 g/l), diferencias que en varios casos fueron significativas ($p < 0.05$). Los valores de α y β globulinas fluctuaron irregularmente, en tanto que la relación albúminas / globulinas aumentó en S y disminuyó en C. La mejora de los indicadores nutricionales del metabolismo proteico y las ganancias de peso revelan que este económico residuo industrial es útil para el engorde de vacas de invernada, aún sin adición nitrogenada.*

Palabras Claves: pulpa de citrus, suplementación, vacas de invernada, ganancia de peso, proteínas plasmáticas

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INTRODUCTION

Up to now, in the northeast of Argentina exist about 10 millions cattle, half of which are bred in Corrientes Province. The seasonal growth of native grassland is the main obstacle for regional livestock development, and average meat production is about 30 kg/ha/year. Most of the year, native grasses have low nutritive value, with dry matter digestibility lower than 50% and significant energetic-proteic deficiencies in winter, with protein levels of 4% and metabolizable energy rates of 1.8 to 2 Mcal/kg dry matter, DM (1).

Energetic-proteic supplementation using inexpensive regional by-products was identified as one of the main demands of beef cattle producers in a survey conducted by INTA (2). The area has plenty of citrus plantations, as well as fruits juice factories. Citrus pulp is a by-product of citric juice industries. It has high energy value as ruminant food (3, 4). However, due to limited protein concentration, it is necessary to add nitrogen from another source when the diet is being balanced for growing animals (5).

Cow-calf operations based on British x Zebu (BxZ) cattle predominates in the region; this rustic crossbreed is more efficient using nutrients than others bovine breeds (6). In such systems, older and reproductive unsuccessful cows are normally culled after fattening in late autumn, as a low price category ("*conserve*", for canned meat). In order to optimize economic yield, the current strategy consists of improving the animals winter feed through supplementation, thus obtaining a *fattened* cow in early spring, when the price of this type of cattle increases (7).

The purpose of this study was to verify live weight change (LWC) and potential modifications of some nitrogen biochemical indicators in BxZ wintering cows grazing native grassland and supplemented exclusively with citrus by-products (without addition of nitrogen), in a subtropical area characterized by scarce quality and quantity of feed.

MATERIAL AND METHODS

The studies were carried out in the Bella Vista Department, of the Corrientes province in Argentina. A total of 80 BxZ wintering cows were maintained in two paddocks with a homogeneous quantity and quality of native grassland (NG). It was mainly composed with *Paspalum notatum, Paspalum dilatatum, Desmodium sp, Trifolium sp, Andropogon lateralis, Sorghastrum agrostoide, Schizachirium spicatum, Aristida sp., Vicia sp., Acacia coven, Celtis spinosa*, and *Geofroea decorticans*. Twenty cull-type cows were supplemented for 4 months (autumn-winter) with 15 ± 3 kg/animal/day of fresh citrus pulp (supplemented group, S), while the remaining 20 (control group, C) did not receive any supplementation. The same experimental design was repeated the following year, with animals of similar characteristics.

In both years, studies began in late autumn (June). Both groups were set stocked (0.6 animal/ha) on NG, which had an average pasture cover of 2,200 and 1,800 kg of DM/ha in the 1st and 2nd year, respectively. DM *in vitro* digestibility (IVD), crude protein (CP) and energy density (ED) of NG were 48%, 4-6% and 1.7-1.8 Mcal ME/kg DM. The pasture offer (DM per animal) was 3,600 kg in the first year and 3,000 kg during the second year.

The nutritional characteristics of offered citrus pulp were: DM 21%, CP 7.6%, crude fiber 17.7%, ether extract 4.5%, ash 4.5%, nitrogen-free extract 65.7%, phosphorous 0.17%, calcium 0.54%, sodium 0.03%, potassium 0.50%, magnesium 725 mg/kg, manganese 15 mg/kg, zinc 78 mg/kg, iron 83 mg/kg and copper 15 mg/kg, and 3.62 Mcal of gross energy per kg DM. Average IVD was 87%.

During both years, animals were weighed, and jugular venepuncture blood samples were collected at days 0, 30, 60, 90 and 120 of the trial. Clotted blood was centrifuged (700 g, 10 min) in order to obtain serum, which was kept at 4 ºC until assayed. Total protein (biuret method, 540 nm, Wiener reagents) and urea (urease technique, 546 nm, GT-Lab reagents) were measured in a Gilford-Beckman photometer. Protein fractions were separated by electrophoresis in a Chemar CHF-I-3 apparatus, on cellulose acetate support (Bio-systems), veronal buffer (Merck), amido-schwartz coloration (Biopur), and evaluated by using a Citocon 440 densitometer (8, 9).

Statistically, initial homogeneity was verified by the overlapping of confidence intervals (95% CI) and the normality of distribution was assessed using the Wilk-Shapiro test (WS). Parametric descriptive statistics included indicators of central tendency (arithmetic mean, x) and dispersion (standard deviation, SD). Analysis of variance (ANOVA) for repeated measures was calculated, including the significance of the treatment and time effects, as well as the interaction between them.

Table 1. Descriptive statistics (x ± SD) in supplemented (S) and control (C) animals.
Tabla 1. Estadísticas descriptivas (x ± DE) en animales suplementados (S) y controles (C).

parameter	lot	year 1		year 2	
		initial (n = 20)	final (n = 20)	initial (n = 20)	final (n = 20)
live weight (kg)	C	406.5 ± 33.2	443.0 ± 28.5 *	383.8 ± 37.6	375.0 ± 35.3
	S	428.0 ± 39.3	487.1 ± 31.8 *	390.5 ± 30.8	432.8 ± 25.9 *
total protein (g/dl)	C	7.40 ± 0.32	7.30 ± 0.33	7.36 ± 0.35	7.18 ± 0.29 *
	S	7.31 ± 0.26	8.18 ± 0.41 *	7.42 ± 0.34	7.95 ± 0.38 *
albumin (g/dl)	C	3.08 ± 0.20	2.92 ± 0.35	3.07 ± 0.28	2.59 ± 0.32 *
	S	3.05 ± 0.27	3.57 ± 0.36 *	2.89 ± 0.23	3.20 ± 0.37 *
α globulin (g/dl)	C	0.73 ± 0.10	0.75 ± 0.13	0.65 ± 0.11	0.61 ± 0.08
	S	0.77 ± 0.08	0.83 ± 0.12	0.62 ± 0.09	0.65 ± 0.13
β globulin (g/dl)	C	0.96 ± 0.13	0.99 ± 0.19	0.88 ± 0.14	0.86 ± 0.11
	S	0.94 ± 0.18	0.98 ± 0.12	0.85 ± 0.09	0.87 ± 0.10
γ globulin (g/dl)	C	2.63 ± 0.40	2.64 ± 0.42	2.76 ± 0.33	3.12 ± 0.44 *
	S	2.55 ± 0.31	2.80 ± 0.46 *	3.06 ± 0.41	3.23 ± 0.40 *
alb./glob. ratio	C	0.71 ± 0.08	0.67 ± 0.12	0.72 ± 0.09	0.56 ± 0.07 *
	S	0.71 ± 0.10	0.77 ± 0.08	0.64 ± 0.10	0.67 ± 0.11
urea (g/l)	C	0.33 ± 0.04	0.24 ± 0.05 *	0.31 ± 0.04	0.26 ± 0.07 *
	S	0.34 ± 0.04	0.30 ± 0.08	0.32 ± 0.06	0.30 ± 0.05

* final data statistically different from the initial ($p < 0.05$)

Following the ANOVA, the significance of differences between groups C and S on each day was estimated by orthogonal contrasts. Correlation was obtained by Pearson method (10). Statistical significance in this paper refers to the 5% level ($p < 0.05$).

RESULTS AND DISCUSSION

Descriptive statistics of supplemented and control groups during two years are shown in *Table 1*. Obtained values are in agreement to those reported with similar crossbreed, age, feeding type and geographical area (11). For each parameter, initial values were statistically homogeneous (CI ± 95%) and distribution (WS) was close to the normal (10).

Total LWC was significantly higher in S than in C, in both assays. During the first year, climatic benign winter promoted a higher pasture availability (2,200 kg DM/ha), so the LWC were higher in S (492 g/animal/day) than in C (304 g/animal/day). During the second year (harsh), pasture yield was lower (1,800 kg DM/ha) and control cows lost weight (-73 g/animal/day) while S showed only gains of 352 g/animal/day.

Treatment and time effects were significant in both groups, while the interaction treatment by time was not significant. Means comparisons by orthogonal contrasts revealed that LWC differences between C and S began to be significant at day 30 in both, the first and second year. *Figure I* shows the average LWC registered at every sampling date, standing out the biggest final gains in S.

Previous studies (12) had shown that when supplementing wintering half-bred zebu cows with brewery residues, there were positive LWC in supplemented groups (314 g/animal/day) and losses in control groups (-128 g/animal/day). Furthermore, in northeastern Argentina, a study using the same type of cattle supplemented with cottonseed showed increases of 292 g/animal/day, whereas controls only gained 51 g/animal/day

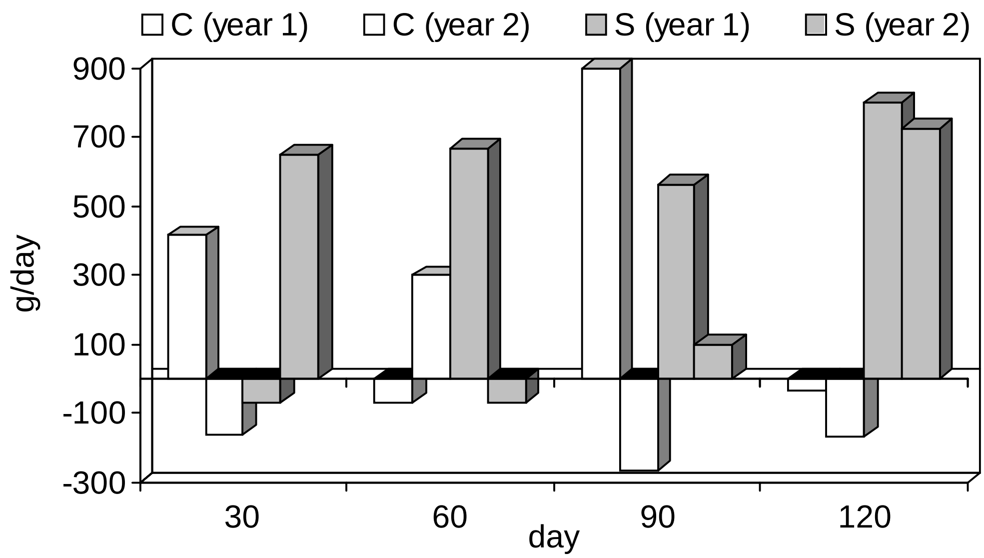

Figure I. Weight gain evolution in supplemented (S) and control (C) animals.
Figura I. Evolución de la ganancia de peso en animales suplementados (S) y controles (C).

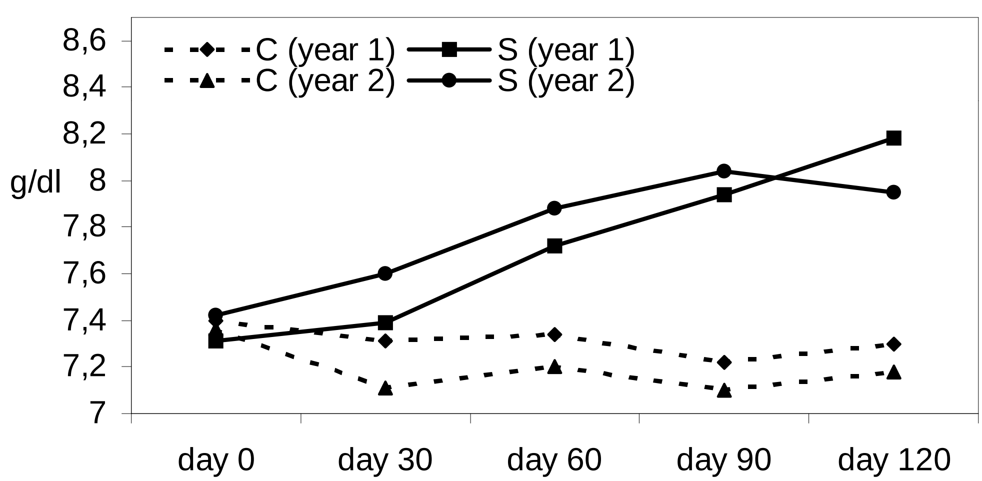

Figure II. Total protein evolution in supplemented (S) and control (C) animals.
Figura II. Evolución de proteínas totales en animales suplementados (S) y controles (C).

(13). By offering increased amounts of citrus pulp (14) or citric residues mixed with other complements (15), some researchers obtained weight gains from 236 to 1100 g/animal/day.

Total protein (TP) decreased in C during both years *(Table 1)*, although only in the 2nd year differences were statistically significant ($p < 0.05$). S increased significantly TP in both experiments. There was no time x treatment interaction for these parameters. Differences between treatments were significantly stood out after 60 days of assays *(Figure II)*. Similar results as TP were showed by albumins in both assays. However, differences between C and S began to be statistically significant at day 30 (year 1) and day 60 (year 2) (Figure III).

Serum protein makes up the cow nutritional chemistry panel (8). To synthesize albumin, fibrinogen, and 50% of globulins, the liver requires amino acids, coming directly or indirectly from the diet (11, 16), and immune system needs be able to

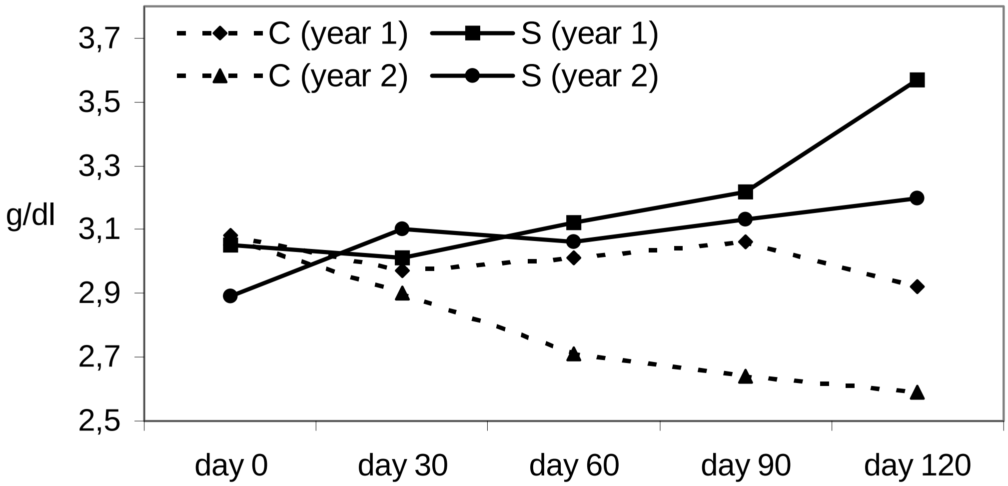

Figure III. Albumin evolution in supplemented (S) and control (C) animals.
Figura III. Evolución de albúminas en animales suplementados (S) y controles (C).

elaborate the remaining 50% of globulins (antibodies). Plasmatic decreases of total protein in C should be related to the pasture winter impoverishment (17) and increases in S to the additional nitrogen supplied (18).

Less food availability in winter could cause the decline of this parameter in C (17), while the enhance in S should necessarily be by the supplementation: albuminemia changes directly proportional to the protein intake (8, 19), decreasing in subnutrition, malabsorption, hepatopathy and other illness (8, 9).

There was not significance ($p < 0.05$) in α globulins for treatment, time, or treatment x time effects. Orthogonal contrasts did not detect differences between means. The levels of this proteic fraction usually are modified by inflammations, hepatic, renal and other dysfunctions (16). α globulins include the high density lipoproteins (HDL), in charge of the cholesterol reverse transport system; dietary lipid excess increases HDL in ruminants (11, 20). Evidently, the quantity of citrus pulp ether extract (4%) was not enough to alter the proteinogram in S.

No treatment, time, or treatment x time effects were detected for β globulins. However, they presented a trend similar to those of LWC. β globulins are important from a nutritional point of view, because they make the cholesterol direct transport (LDL), and mobilize heavy metals (Fe, Zn, Cu), vitamin D and some hormones (11).

γ globulins increased significantly in S, in both years. In the case of C, they dropped in the 2nd year ($p < 0.05$), with no change in the first trial. Treatment and time effects were significant, but not their interaction. Significant differences between means started at day 60. Besides giving viscosity to the plasm, generating coloidosmotic pressure, and participating in the acid-base balance, γ globulins operate as antibodies and, like others seric proteins, they could be altered by subnutrition (8, 11).

The albumins / globulins ratio decreased in C (significantly during the second year). Although protein content of citrus pulp was low, when a more climatically rigorous winter was presented (lower quantity and quality of NG), supplementation was able to maintain the albumins / globulins ratio.

Urea was initially homogeneous in both groups of each assay (IC ± 95%), but progressively decreased along the trials in C ($p < 0.05$). Treatment and time effects were significant. Treatment effect began to be different at 60 and 30 days, for the 1st and 2nd year, respectively (results not shown).

Plasma urea level is able to indicate the quantity of nitrogen contained in diet; thus, its decrease in C should be related with CP deficit (19). Citrus pulp addition attenuated the decrease of urea (9). During cattle growth (calves, steers, heifers), citrus pulp should be accompanied with urea to increase the nitrogen supply, avoiding the fall of ruminal ammonium (21). This fact would not be so important in fattening animals as wintering cows, because just the citrus pulp supplement (7.6% CP) was able to keep stable blood urea values.

In S, LWC significantly correlated with TP ($r = 0.98$, $p = 0.0003$) and albumin ($r = 0.93$, $p = 0.02$), while albumin revealed positive lineal association with TP ($r = 0.90$, $p = 0.03$), during the 1st year. In the 2nd one, significant correlation between LWC and albumin ($r = 0.97$, $p = 0.004$) was registered in S.

Secondary effects attributed to the supplement were not verified. It is known that citrus pulp could produce ruminal parakeratosis in calves (22); in adult ruminants, it could cause diarrhea (18) and carry viscerotropic pesticides (23), as well as hemorrhage-causing aflatoxins like citrinin (24). In birds it causes hepatomegaly, with up to 97% mortality (18). References about hepatic alterations induced in ruminants by citrus residue ingestion, such as those reported for other agroindustrial byproducts as cottonseed (25), were not found.

In conclusion, the results of this study suggest that citrus pulp, without nitrogen addition, is able to increase weight gain and plasma concentrations of total protein, albumin and γglobulin in supplemented wintering cows ($p < 0.05$). Increase of weight correlated significantly with plasma increases of total protein (first year) and albumin (both years). The supplementation with this agroindustrial residue is able to lessen plasma urea decrease by the winter pasture impoverishment, and did not cause undesirable secondary effects.

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