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Short communication

The influence of chemical form on the effects of supplementary malate on serum metabolites and enzymes in finishing bull calves

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ABSTRACT

This study investigated the effects of free malic acid and a commercial malate salt on serum metabolic parameters in finishing-stage Belgian Blue bull calves maintained in a commercial feedlot. Serum levels of glucose, non-esterified fatty acids (NEFA), β-Hydroxibutirate (BHBT) urea nitrogen (SUN), creatinine, total protein (TSP), L-lactate, aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were monitored over 86 days in 38 animals randomly allotted to three groups: MA (supplementation of feed with 4-g DL-malic acid per kg on a dry mass (DM) basis; 14 animals), MS (supplementation of feed with 4 g of a commercial disodium/calcium DL-malic acid salt per kg DM; 14 animals), and C (controls with no malate supplement; 10 animals). All the parameters considered except SUN lay within the physiological ranges for intensively reared beef, possibly due to the high crude protein (CP) content of the diet and the forage fiber source (barley straw). However, animals fed either form of malate had lower serum L-lactate and creatinine levels than those that did not receive this supplement, and their NEFA levels fell over time instead of rising. The only parameters differing between the free acid and salt groups were SUN and BHBT, which for unknown reasons, were higher in group MA than in either controls or group MS. That SUN was higher in group MA than in group C is attributed to its favoring CP-degrading ruminal flora. That BHBT was higher in group MA than in groups MS and C could be due to that the acid form can promote butyrate synthesis in rumen.

1. Introduction

Currently, the beneficial *in vitro* effects of the organic acid on a ruminal environment (Martin and Streeter, 1995; Carro et al., 1999; Carro and Ranilla, 2003) is well known. However, the results of *in vivo* studies have been contradictory (Sanson and Stallcup, 1984; Martin et al., 1999; Carro et al., 2006; Castillo et al., 2008), probably due at least in part to differences in diet composition and/or malate dosage (Callaway et al., 1997; Castillo et al., 2004). Another factor that may influence the response to malate is the chemical form in which it is administered. *In vitro*, free malic acid and its disodium salt have similar effects, except for a lowering of pH by the former (Martin and Streeter, 1995); in a previous research performed by our group (Castillo et al., 2007) we found that the feed:gain ratio of finishing-stage bull calves has been reported to be lower with the free acid than with a commercial disodium/ calcium salt, and their blood acid–base balance was better with the salt than the free acid at a dosage of 4 g per kg of feed.

To clarify further the different influences of the free acid and the disodium/calcium salt during the finishing stage, in the study described here we examined their effects on serum levels of glucose, non-esterified fatty acids (NEFA), β-hydroxibutirate (BHBT), urea nitrogen (SUN), creatinine, total protein (TSP), L-lactate, aspartate aminotransferase (AST) and

Abbreviations: ADF, acid detergent fiber; ADG, average daily gain; AST, aspartate aminotransferase; BW, body weight; CP, crude protein; DM, dry matter; EE, ether extract; GGT, gamma glytamyltransferase; MA, malic acid; MS, malate salt; NDF, neutral detergent fiber; NEFA, non-esterified fatty acids; SUN, serum urea nitrogen; TSP, total serum protein.

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gamma glutamyl transferase (GGT) in finishing-stage Belgian Blue bull calves maintained in a commercial feedlot.

2. Materials and methods

2.1. Animals, feeding management and experimental design

Thirty-eight double-muscled Belgian Blue bull calves (122.9 ± 2.0 kg) were purchased and transported to the commercial study farm (Coren SCL, Ourense, NW Spain) at an age of 3–5 weeks. Adaptation to high-grain diets was carried out using a milk replacer (1-L/20-kg body weight (BW)) combined with a solid starter containing maize, wheat, barley, soybean meal, and vitamin–mineral premix (see footnote to Table 1); water and straw were available *ad libitum*. The compositions of the diets provided during the growing and finishing periods (14–22 and 23–35 weeks of age) are listed in Table 1; feed, water and barley straw were freely accessible at all times. Fresh feed was provided once a day at 08:00 h. Throughout the study the animals were cared for and managed in accordance with official Spanish guidelines on animal care and with EC Directive 86/609/EEC for animal experiments.

Before the first feeding of growing diet the calves were allotted randomly to one of the three experimental groups: MA (supplementation of feed with DL-malic acid (from A. Pintaluba, SA, Reus, Spain) at 4 g/kg of DM; 14 animals), MS (supplementation of feed with disodium/calcium malate (Rumalato®, from Norel and Nature, Madrid, Spain) at 4 g/kg of DM; 14 animals), and C (controls with no malate supplement; 10 animals). Supplement was given since the first day of the growing period. These groups were kept apart and fed as groups (which prevented the recording of individual daily intake).

Table 1
Ingredients and chemical composition of the diets supplied in the present study.

	Growing	Finishing
Ingredient (g/kg DM)		
Barley	326	305
Rye	50	60.0
Wheat	100	100
Corn	100	100
Molasses	25	25
Palm oil (98% bypass)	18	20
Palm kernel oil	–	40
Soybean meal, 44% CP	151	96
DDGS [a]	70	80
Corn gluten feed	100	100
Wheat bran	–	42
Soybean hulls	32	11
Vitamin/Mineral premix [a]	28	21
Chemical composition (g/kg DM)		
CP	166	155
CF	50	50
ADF	66	72
NDF	190	216
EE [b]	41	47
NFC [c]	545	531
Starch	350	350
Ash	58	51

[a] Vitamin and mineral premix contained per kg DM premix: 10.000 IU of vitamin A, 2.000 IU of vitamin D, 10 IU of vitamin E, 0.4 mg of Co, 16 mg of Cu, 25 mg of Fe, 2 mg of I, 110 mg of Mn, 0.3 mg of Se, and 120 mg of Zn.
[b] EE: ether extract content.
[c] NFC: non-fiber carbohydrates calculated as 100 (CP + ash + NDF + EE).

2.2. Measurements and analyses

Samples of concentrate were collected at the beginning of each stage and analyzed. Starch, ether extract and ash values were determined as described by Castillo et al. (2007). Before blood sampling, the calves were examined for clinical signs of metabolic disturbances (Lorenz, 2004). Blood samples were collected by jugular venous puncture (Vacutainer® tubes without EDTA) between 09:00 and 11:00 h on the finishing period: on day 0 (at the beginning of the fattening diet) and on days 3, 7, 23, 53 and 86 (the last day, prior to slaughter). Samples were centrifuged (2000 *g* for 20 min) and the plasma was immediately frozen (−20 °C) for pending analysis. Metabolic parameters were assayed using standardized kits (RAL Técnica para el Laboratorio, Spain, for glucose, urea, creatinine and AST; Gesellschaft für Diagnostica und Biochemica Mbh, Germany, for TSP; Randox Laboratories Ltd., UK, for NEFA and BHBT; and Spinreact, Spain, for L-lactate and GGT).

2.3. Statistical analyses

Data were checked for normal distribution using the Shapiro–Wilks test and were subjected ANOVA, with *group* (TR) as the fixed main effect, *sampling date* (T) as a repeated-measured effect and a T × TR term included in the model. All statistical analyses were performed using SPSS 12.1. The criterion for statistical significance was $P \leq 0.05$; P values between 0.05 and 0.1 were considered near significant.

3. Results

Table 2 lists metabolic data for each group during the study. All parameters except AST showed a significant influence of sampling date. Comparing the final with the initial values, TSP rose in all groups; glucose and SUN fell appreciably in groups C and MS but much less in group MA; and creatinine fell slightly in groups MA and MS but not in group C. L-lactate increased during the first 3–7 days and decreased following the third day, and GGT behaved somewhat similarly in groups C and MS (which showed a considerable fall in GGT following day 53) but not in group MA (which had higher final than initial GGT levels). BHBT increased in the first 3 days with fluctuations after this; note that the C and MS groups had similar values while MA had higher concentrations, although without statistical relevance.

The only parameter for which the T×TR ANOVA term was statistically significant was NEFA, which at the end of the study was slightly higher than at the beginning in group C and slightly lower in groups MA and MS. The group only had a statistically significant effect on L-lactate ($P<0.05$), SUN ($P<0.001$) and creatinine ($P<0.001$). Lactate and creatinine were higher in untreated than in malate-treated animals, but the levels in groups MA and MS were similar. SUN values were significantly higher in group MA than in group MS ($P<0.001$), and near significantly higher than in group C.