Effect of diet and pre-slaughter stress of beef cattle on biochemical profile and physicochemical parameters

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***Abstract*— Diet and pre slaughter stress seem to be related to the *post mortem* glycolysis and pH-T curve, with possible implications to meat quality. Forty animals from Angus breed were assigned to two different finishing diets -60 days-: grain diet and pasture diet. At slaughterhouse, both groups were divided into two subgroups: low stress and traditional stress. Diet impacted on serum insulin levels, adipose tissue color -L* and b*- and pH/T conditions of *LD* muscle. The level of stress treatment impacted on plasma glucose and lactic acid levels, adipose tissue redness and the rate of muscle anaerobic glycolysis, observed in the pH-T curve. Blood hematocrit showed an interaction of both effects. WHC of *LD* was not affected neither by diet nor by stress treatments.**

Keywords— **Diet, Stress, Biochemical Parameters, Meat Quality**

I. INTRODUCTION

It is known that muscle pH and temperature (T) interact each other during rigor development, being able to impact on several *post mortem* events in the muscle, like physical shortening and proteolytic enzymes activity [1, 2].

The rate of pH and temperature decline depends on several factors. Among them, electrical stimulation and the rate of chilling are two of the main technological -extrinsic- factors involved. Muscle levels of glycogen, initial carcasses temperature, and type of muscle are three of the main intrinsic factors involved in the development of the pH-T decline curve.

In the last years, it has been proposed that high concentrate diets in cattle lead to several metabolic modifications related to impaired insulin sensitivity, being able to affect *post mortem* glucolysis [3, 4].

Since both diet and pre-slaughter stress seem to be related to the rate of *post-mortem* glycolysis and pH-T decline curve, the aim of the present study was to evaluate the effect of different finishing diets -during 60 days- and two different pre-slaughter stress levels on some biochemical and physicochemical parameters as possible predictors of meat quality.

II. MATERIALS AND METHODS

A. Animals

The experiment was carried out in the Experimental Station INTA General Villegas (Buenos Aires, Argentina) during 60 days between June 10th 2010 and August 9th 2010. Forty steers from the Angus breed were used in the present study. During the experimental period, animals received a grain-based diet. Thereafter, they changed to the following diets:

- grain diet, 20 animals 18 months old and initial weight of 418.8 ± 17.5kg were fed with a grain diet of 39% corn silage (37% grain), 59% whole corn and 2%

mineral premix with monensin. Mean final weight was 461.9 ± 22.1 kg.

- pasture diet, 20 animals 30 months old and inicial weight of 465.2 ± 21.6 kg were fed with triticale, Triticosecale Wittmack in vegetative growth stage with a daily forage allowance equivalent to 2.5% of live weight. Mean final weight was 509.7 ± 26.5 kg.

As can be seen above, in order to unify the final weight of all animals at the slaughter time, younger animals were assigned to the grain diet while older ones were assigned to pasture diet.

When finishing diet period ended, both groups of animals were transported to the slaughterhouse a distance of 300 km in the same conditions. Once at the slaughterhouse, both groups were divided into two sub-groups according to their pre slaughter handling stress and animals were placed in separate lairage pens overnight with free access to water. In the morning, they were slaughtered under two different handling treatments:

- low stress handling (LS), animals were slaughtered first to avoid odors and noise characteristic of the slaughter. They were moved from the pen and made to wait twenty minutes in the alley next to the slaughterhouse before entering the stun box where they were stunned using a captive bolt pistol. All the process was made quietly, without yells or other actions commonly made to move up the animals (i.e. use of electric prod). Also dark zones of the race were strategically illuminated to avoid shadows.

- regular stress handling (RS), this group was slaughtered secondly, without resting and following usual procedures including yelling and use of electric prod if necessary.

B. Samples

Blood samples were collected at the exsanguination time in tubes containing and not containing anticoagulant solution (EDTA), and were immediately placed on ice. Plasma and serum were separated and stored at -20 ± 1 °C until processing for measurement of glucose, lactic acid and insulin concentrations.

A portion of *Longissimus dorsi* muscle was removed for water holding capacity (WHC) measurements in the interval of 2 hours *post mortem* and was processed immediately. Spectrocolorimeter analysis of adipose tissue was measured within 2 h *post mortem* as well.

C. Methods

Hematocrit (CPV) was measured immediately in blood samples containing EDTA as anticoagulant by the micro-hematocrit method. Plasma glucose and lactic acid concentrations were measured using commercial enzymatic kits (Wiener, Rosario, Argentina and Randox Labs Ltd., UK, respectively). Serum insulin levels were measured using commercial kit provided by DiaSource (INS-EASIA, DiaSource S.A., Belgium)

Muscle pH was measured in the *Longissimus dorsi* (*LD*) muscle at the 12 th rib level at 3, 6 and 24 h using a portable pHmeter (Thermo Orion model 420, USA) with a standardized combination electrode. Simultaneously, and in the same muscle, temperature was measured (2 cm near to the place of pH measurement) using temperature data loggers (Maxim, CA, USA) programmed to record information each minute.

Data representing pH and temperature at 3, 6 and 24 h *post mortem* were calculated and are expressed as mean ± standard deviation.

Perirenal fat color was measured by a portable reflectance spectrophotometer (BYK Spectro guide 4570gloss, UK) according to CIELab scale and D65-artifitial daylight illuminant. Measurements were done at 4 ± 1 ºC.

Water-holding capacity (WHC) was determined according to the compression method described by Pla Torres [5].

III. RESULTS AND DISCUSSION

Blood hematocrit and the concentrations of glucose, lactic acid and insulin are shown in Table 1. CPV values showed significant interaction between diets and stress levels. In general, pasture fed animals showed higher CPV values when compared to grain fed animals. This finding could be possibly explained due to the increased stress displayed in these animals in the abattoir installations as a result of lower human contact than their grain fed counter mates. Among pasture fed animals, those treated with TS showed the higher CPV values. Unexpectedly, animals fed with grain diet and belonging to TS, displayed the lower CPV values. A possible explanation is that differences in the levels of acute stress, applied to grain-fed animals, were not enough to produce changes in CPV in these animals, which are supposed to be more accustomed to human contact and handling.

As can be seen in Table 1, all the animals displayed hyperglycemia at slaughter. Animals belonging to TS from both finishing diets displayed the higher values of plasma glucose. Lactic acid levels in plasma displayed a similar behavior than glucose. Taken together, these results support the idea that the traditional handling resulted more stressful than the low stress handling, with comparable impact on both finishing diets.

Regarding serum insulin levels, no significant difference was found with respect to stress levels. Interestingly, insulin levels were higher in animals fed with pasture during the last 60 days. Bearing in mind that high energy diets (i.e. grain diets) are more prone to induce increase in blood insulin, this finding could be possibly more related to the age and weight of the animals than to the diet [6, 7].

Table 1. Blood hematocrit, plasma glucose, lactic acid and serum insulin levels, from animals belonging to different finishing diets and stress levels.

	Pasture		Grain		Significant Effect	p- value
	LS	RS	LS	RS		
CPV (%)	43.6 a	45.3 a	41.1 a,b	38.0 b	diet x stress	0.013
Glucose (mM)	5.31 b	6.15 a	5.76 b	6.17 a	stress	0.020
Lactate (mg%)	60.0 b	66.8 a	61.7 b	65.5 a	stress	0.031
Insulin (µUI/mL)	42.2 a	42.8 a	28.9 b	32.8 b	diet	0.009

Values are expressed as Mean.
NS: non significant differences

WHC of *LD* assayed at slaughterhouse was not affected neither by finishing diet nor by stress level (Table 2). Fat from pasture-fed animals showed a high β-carotene content that increases the yellow coloration and produces an overall lower reflectance intensity. These characteristics are reflected in a higher b* parameter and a lower L* parameter compared to grain-fed animals. Interestingly, a* parameter showed a significant effect associated to stress. This difference should presumably be attributed to incomplete exsanguinations of the capillaries of adipose tissue [8]. Further research is needed in relation to this topic.

Table 2. WHC of *LD* and color parameters of adipose tissue from animals belonging to different finishing diets and stress levels.

	Pasture		Grain		Significant Effect	p- value
	LS	RS	LS	RS		
WHC (%)	51.8	54.2	50.9	52.3	NS	p>0.05
L*	74.00 b	73.92 b	75.18 a	80.54 a	diet	0.016
a*	5.19 a	4.30 b	5.46 a	2.22 b	stress	0.003
b*	21.47 a	20.55 a	17.26 b	16.83 b	diet	p>0.001

Values are expressed as Mean.
NS: non significant differences

The pH-T decline curve can be seen in the Figure 1. Non significant interaction between diet and stress treatment was observed. Nevertheless, a different curve pattern with a more pronounced slope in the first 3-6 *post mortem* hours can be seen in animals treated with TS when compared to those with LS treatment.

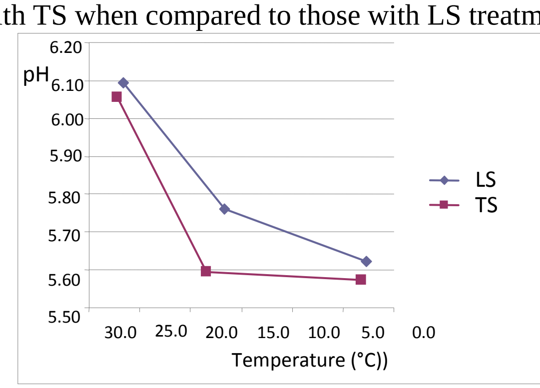

Fig. 1. pH-T decline curve measured in *LD* from animals belonging to different finishing diets and stress levels.

Values of pH and T at 3 h and 24 h can be seen in Table 3. It clearly shows an effect of diet, where pasture-fed animals displayed increased temperature and decreased pH of *LD* at both 3 h and 24 h *post mortem*. Despite core body temperature was not measured in the present study, the increased muscle temperature could be possibly due to an increased effect of the stress in pasture-fed animals. This effect would stimulate blood circulation and muscle metabolisms leading to a faster anaerobic

Table 3. pH/T slope in the 3-6h *post mortem* and pH and T values at 3 and 24 h *post mortem* in *LD* from animals belonging to different finishing diets and stress levels.

	Pasture		Grain		Significant Effect	p- value
	LS	RS	LS	RS		
pH 3h	5.97 b	5.81 b	6.13 a	6.11 a	diet	0.005
T (ºC) 3h	27.42 a	27.92 a	26.49 b	26.31 b	diet	0.035
pH 24h	5.57 b	5.49 b	5.67 a	5.64 a	diet	0.002
T (ºC) 24h	3.08 a	3.32 a	2.44 b	2.72 b	diet	0.006

Values are expressed as Mean.
NS: non significant differences

utilization of glucose and the consequent decreased of muscle pH observed in these animals. In this regard, it is possible to think that increased muscle temperature would increase the activity of glycolytic enzymes. Moreover, bearing in mind that pasture-fed animals presented increased insulin serum levels, it would be possible to think that an increased effect of insulin on the *post mortem* glycolic rate could also be involved. Diet and acute *pre mortem* stress impacted on several biochemical and physicochemical parameters in beef cattle. Diet impacted on serum insulin levels, adipose tissue color and pH/T conditions of *LD* muscle. On the other hand, the level of stress treatment impacted on plasma glucose and lactic acid levels, adipose tissue redness and the rate of muscle anaerobic glycolysis, observed in the pH-T curve. CPV showed an interesting behavior as an interaction of both effects. WHC of *LD* were not affected neither by diet nor by stress treatments.

IV. CONCLUSIONS

Different finishing diets during a short period of time and acute *pre mortem* stress were involved in changes both in the *in vivo* body metabolism and in the *post mortem* anaerobic utilization of glucose in the *LD* muscle. Under the experimental conditions used, no consequences were observed in fresh meat quality assessed by pH values.

Further studies considering the analysis of mentioned metabolisms changes on possible consequences on the conditioning quality of meat should be carry on in the future.

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