HISTIDINE DIPEPTIDES AND FREE AMINO ACIDS OF BEEF FROM CATTLE RAISED UNDER CONTRASTING FEEDING SYSTEMS AND PRE-SLAUGHTER MANAGEMENT

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Abstract – Diet and pre-slaughter stress are key factors that may affect the biochemical processes during the conversion of muscle into meat. The aim of the present study was to evaluate the effect of two different pre-slaughter stress managements (reduced-stress handling, RH and conventional handling, CH) in Angus steers, raised under contrasting feeding systems (grain, GS and pasture, PS) on histidine dipeptides content and free amino acids profile. Dipeptides and free amino acids were analyzed by ion exchange chromatography. The concentration of free amino acids was not different neither among feeding systems nor among pre-slaughter stress managements. The concentration of histidine dipeptides was higher in CH than in RH for both production systems applied. Histidine dipeptides could act as tissue protectors and as markers of acute stress in animals.

Keywords—Histidine Dipeptides, Free Amino Acids, Pre-Slaughter Stress, Feeding Systems.

I. INTRODUCTION

The histidine dipeptides, carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-L-1-methylhistidine) are simple compounds naturally occurring in vertebrate animal tissues (1). Both dipeptides show antioxidant activity acting as metal chelators and free-radical scavengers (2). These antioxidative peptides play many roles, such as prevention of diseases and aging related to oxidative stress (3, 4, 5). The amount of these dipeptides is largely higher in the skeletal muscle than in other tissues, especially in muscles with a glycolytic metabolism (6). Nevertheless, its content may vary with the animal species (7), age (8), and/or diet (1, 2).

Muscle enzymes contribute to the generation of free amino acids *post mortem*, which improve the nutritional value and affect flavor, being able to enhance taste and aroma (9).

Despite the fact there are not enough studies in bovine muscles, results published by Hammarqvist (10) have shown that the human muscle protein catabolism increase by the administration of stress hormones.

Other authors found that, in human, an infusion of a triple combination of stress hormones into healthy volunteers produces changes in muscle amino acid metabolism similar to those observed immediately after surgical trauma (11).

Post mortem muscle metabolism may vary significantly with animal nutrition and pre-slaughter stress. Both of them are influenced by pre-slaughter muscle glycogen and muscle metabolism respectively (12).

The impact of these stress factors on the final content of histidine dipeptides and free amino acid profile of meat is not well understood. Thus, the aim of the present research was to evaluate the content of carnosine and anserine and free amino acids in beef from cattle raised under contrasting feeding systems and subjected to different pre-slaughter management.

II. MATERIALS AND METHODS

Animals

The experiment was carried out at the Experimental Station INTA (National Institute of Agriculture Technology) General Villegas (Buenos Aires, Argentina) according to the procedures stated by SENASA (13) in Argentina.

Forty steers from the Angus breed were selected for the study.

Animals were randomly raised under two contrasting feeding systems: grain- and pasture-based systems. The endpoint chosen was determined by fat cover.

Grain-based system (GS): 20 animals fed on a grain diet (39 % corn silage, 59 % whole corn and 2 % mineral premix with monensin) to a final mean weight of 461.9 ± 22.1 kg

Pasture-based system (PS): 20 animals fed on triticale (triticosecale Wittmack) with a daily forage allowance equivalent to 2.5 % of live weight, to a final mean weight of 509.7 ± 26.5 kg.

Once dorsal adipose tissue depth reached 6 mm in both feeding systems, the two groups were transported simultaneously to a distance of 300 km to the slaughterhouse. Upon arrival, both groups were randomly divided into two sub-groups: RH (reduced stress handling) and CH (conventional handling). They were placed in separate lairage pens overnight with free access to water.

Animals from RH subgroup were slaughtered first to reduce their exposure to odors and noise. Animals waited twenty minutes in the alley next to the slaughterhouse before entering the race to stun box. The process was made to be quiet, without yelling or other actions commonly used to move the animals up. Also dark zones of the race were strategically illuminated to avoid shadows. Animals from CH subgroup were slaughtered after the previous, without waiting in the alley and following usual procedures including yelling and, eventual use of electric prods. Shadows and slaughter odors and noise were not minimized during the pre slaughter handling of this subgroup submission.

Samples

Left carcasses were chilled in the abattoir at 4 ± 1 °C for 24 h. Four-ribs blocks (10th to 13th rib) were removed after measuring ultimate pH, each rib was vacuum packaged individually and maintained under -20 ± 1 °C until analysis of histidine dipeptides and free amino acids.

Analysis

Carnosine, anserine and free amino acid content was analyzed in triplicate. Samples were treated with 0.6M Trichloroacetic acid (TCA) in order to denature proteins (14), previous the determinations.

Determination of histidine dipeptides: supernatant solution was 20-fold diluted using KH_2PO_4 90mM pH 5 and transferred into vials for chromatographic analysis. Standard anserine and carnosine were purchased from Sigma Chemical Co. Histidine dipeptides levels were determined using the HPLC method described by Sri Kantha (15). The assay was performed by a HPLC Shimadzu equipped with a UV detector. The assay parameters were: Zorbax 300-SCX column, eluent 90 mM KH_2PO_4 solution containing 10% methanol. The flow rate was 1 mL/min and the injection volume 20µL. The chromatographic runs were carried out at 55◦C, and detection was set up at 210 nm.

Determination of free amino acid: The pH of the supernatant solution was adjusted to 2.2 before the analyses by Ion Exchange Chromatography. The chromatography of the extracts was carried out in an amino acid analyzer Biochrom 30 (Biochrom Ltd) equipped with a cationic exchange column. L-Norleucin was used as internal standard and an eighteen pure amino acids solution was used to identify the amino acid composition.

Statistical Analysis

Data were subjected to analysis of variance using the SPSS® v12 statistical software. The two factorial design considered feeding systems and pre-slaughter management conditions with two levels (GS – PS and RH – CH, respectively) and their interaction (16). T-test was used as means of comparison. The level of significance was set at 0.05 for all tests.

III. RESULTS AND DISCUSSION

Table 1 shows the anserine and carnosine content in LD muscles of studied animals.

Table 1. Anserine (**A**) and Carnosine (**C**) content (mg/g) of beef from cattle raised under contrasting feeding systems and pre-slaughter management.

	GS		PS	
	CH	RH	CH	RH
A	0.243±0.097 aB	0.153±0.059 bB	0.319±0.106 aA	0.209±0.113 bA
C	1.633±0.527 a	1.000±0.384 b	1.711±0.554 a	1.343±0.610 b

Values are expressed as mean ± SD. Lowercase letters in different cells indicate management effect. Capital letters in different cells indicate feeding system effect. ($p < 0.05$).

As can be seen, animals from both contrasting feeding systems (GS and PS) exposed to RH showed lower concentration of anserine and carnosine than those exposed to CH ($p<0.05$). On the other hand, the animal feeding system displayed a significant effect in anserine levels ($p<0.05$). Thus, pasture-based animals showed increased content of anserine in LD muscle.

No interaction effect was observed between feeding system and pre-slaughter management.

Regarding this issue, it could be assumed that CH practice (for both feeding systems) led to increased levels of these dipeptides in order to protect the muscle tissue. This protection may occur due to the antioxidant and free radical-scavenging functions of carnosine, which can take place during acute stress in + management.

Although there is little literature regarding dipeptides in stressed animals, our results agree with those of other authors who have found that carnosine content in whole human muscle has been correlated with high-intensity exercise (17). Published data also related higher concentrations of these dipeptides in athletes participating in anaerobic sports (18, 19). These correlations suggest that increased levels of dipeptides found in our work would be a consequence of an increased anaerobic glycolysis involved in CH-animals before slaughter.

Animals from pasture-based system showed higher values of anserine ($p<0.05$) than animals from grain-based system, independently of pre-slaughter management. Other authors (20) found that feeding location is a significant source of variation for carnosine, and anserine.

Our results could be associated to the increased anxiety and dehydration displayed by these animals (data not shown) during transport or at the abattoir installations, since pastured-fed animals would be less accustomed to human contact than grain-fed ones.

Table 2 shows the total concentration of free amino acids of LD muscles of animals studied.

Table 2. Free Amino acid content (mg/100g) of beef from cattle raised under contrasting feeding systems and pre-slaughter management.

GS		PS	
CH	RH	CH	RH
119.87±6.14	127.20±22	127.40±4.06	115.55±48.01

As can be observed, no significant differences were found neither between feeding systems nor pre-slaughter management.

IV. CONCLUSION

The values of anserine and carnosine in LD muscle were associated to feeding system and pre-slaughter management at abattoir installations. The free amino acids content did not differ among treatments. This finding contributes to the understanding of muscle metabolism of histidine dipeptides and suggests new markers of acute stress in animals. Accordingly, possible uses should be further studied in meat quality.

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