OPINION LEADER

Improving carnosine content in pork meat through Histidine supplementation

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Abstract

Carnosine is a dipeptide formed by one molecule of L-Histidine and one of β-Alanine naturally found in muscles. Reducing crude protein in swine diets reduces the availability of the amino acid histidine, and might compromise carnosine synthesis in the muscles, affecting meat quality. 2440 fattening pigs were allocated by sex and body weight in a commercial farm, and assigned to control (CON) or histidine (HIS, supplemented with 3 kg of L-Histidine HCl) groups. Samples from the *Longissimus dorsi* and the *Semitendinosus* muscles were obtained at the slaughterhouse to analyse the carnosine content and meat quality parameters, such as pH, drip loss, intramuscular fat and oleic acid. Carnosine content was greater ($P < 0.05$) in the *Longissimus dorsi* of HIS pigs compared with CON animals, whereas the only numerical increase was observed in *Semitendinosus* of HIS pigs. Additionally, pH at 24 h post-mortem was higher in the HIS group than in CON, as well as drip loss was lowered ($P < 0.05$) by the supplementation of L-Histidine. Interestingly, an interaction between treatment and sex was observed for drip loss, being this parameter greatly lowered ($p < 0.05$) in females from the HIS group compared to males from the same treatment. Consequently, supplementing L-Histidine to the feed of fattening pigs increases carnosine content in the muscles and improves parameters related to meat quality.

Background

During the last decades, significant advances have been achieved in animal feeding, primarily focused on improving the productive efficiency of animals, the their health and reducing their negative environmental impact. Currently, due to the great challenge of improving the sustainability of our livestock production systems, reducing the crude protein in the diets is still an important objective. In this scenario, crystalline amino acids are the essential tools that allow us to work with the ideal protein concept and continue to reduce nitrogen emissions. The ideal protein concept has always been clearly related to productivity without considering other parameters, such as meat quality. In fact, it is well- known that some nutrients and ingredients of the diet may affect meat quality and composition in monogastric species (fat profile, vitamins, minerals). Nevertheless do we know the impact of reducing crude protein and some amino acids in the diet on meat quality and composition? Considering that producing meat to nourish people is actually our ultimate goal, meat and its quality should be evaluated both organoleptically and nutritionally.

Carnosine is a dipeptide formed by one molecule of L-Histidine and one of β-Alanine that is found naturally in vertebrate animals' muscles (Boldyrev et al., 2013). Its main function is to act as a buffer during muscle contraction, but it also has properties as an antioxidant and a chelating agent (Boldyrev et al., 2013). In addition, the beneficial effects of carnosine on human health as a powerful antioxidant, hypotensive and cardioprotective molecule have been extensively studied and demonstrated (Boldyrev et al., 2013; Sale et al., 2013; Solana-Manrique et al., 2022.). It is important to note that the most important source of carnosine in the human diet comes from the consumption of meat and fish, since carnosine is not found in vegetable sources of protein (Boldyrev et al., 2013).

On the other hand, the carnosine content has been related to different parameters involved in the quality of the meat, such as pH, colour, water retention and even meat tenderness (Ma et al., 2010; Cong et al., 2017). Different studies have shown that it is possible to increase the carnosine content in chicken meat by supplementing the diet with L-Histidine, and also how this increase has led to positive effects on its quality (Kai et al., 2014; Kralik et al., 2015). On the contrary, although Ma et al. (2010) showed that supplementing the diet with carnosine improved the quality parameters of pork meat, no study has shown that supplementing pig diets with L-Histidine increases the carnosine content of meat.

In order to study the effects on carnosine content and meat quality parameters in pigs supplemented with L-Histidine in the diet, this study was carried out on a commercial farm using pigs during the growing and finishing phases.

Materials and methods

A total of 2,440 pigs (Duroc genetics) were used, 1,220 animals per treatment, Control (CON) and HIS group, 610 neutered males and 610 females per treatment. Both groups received the same four feed formulas during the growing and finishing phases, adapted to the animal's nutritional requirements. Pigs from HIS group were supplemented with 3 kg per ton of L- Histidine HCl (BestAmino™ L-Histidine – CJ BIO) during the last 12 weeks before slaughtering. Samples of every batch of concentrate were taken from CON and HIS treatments to analyse crude protein, fat, and the aminogram, confirming adequate inclusion of L-Histidine in the HIS treatment. The animals arrived at the commercial farm with 20 kg of body weight (BW) and were raised until approximately 130 kg of final BW.

The animals were allocated in two identical buildings of the same farm, one per treatment, and were distributed according to their BW and sex in 80 pens per treatment. Feed in pellet form and fresh water were supplied ad libitum throughout the study.

The animals were weighed upon arrival at the farm and, at the end of the study, at the slaughterhouse, and feed consumption was recorded for the whole trial period. Thus, average daily gain (ADG), feed consumption (ADFI) and feed conversion ratio (FCR) were calculated.

Health status, mortality, and possible signs of disease in the animals were daily checked and recorded.

Animals were slaughtered in 4 consecutive weeks, 305 animals per treatment per week. At slaughterhouse, carcass weight was recorded individually, and samples from 44 animals were taken from the *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles to analyse carnosine content by HPLC, following the protocol described in Table 1. Additionally, 100 muscle samples of LD from 100 animals per treatment (25 per week) were taken to analyse pH and drip loss at 24 hours post-sacrifice, along with intramuscular fat (IMF) content. From the same 100 animals, oleic acid was measured by NIR in the tip of the ham.

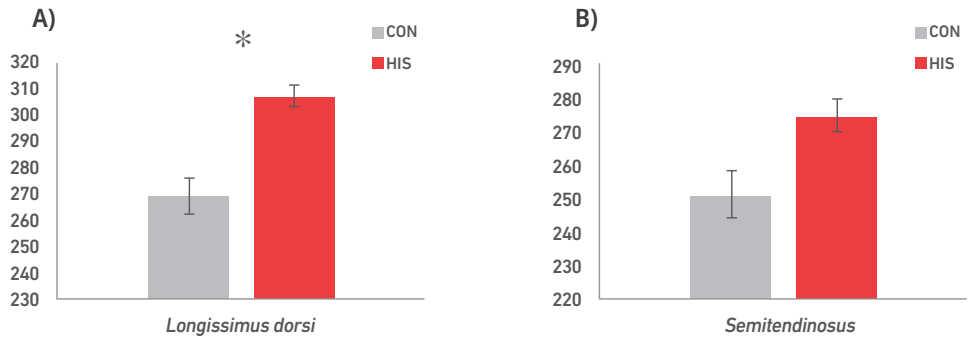
Table 1. Animals sampled at the slaughterhouse to obtain 100 g of muscle from *Longissimus dorsi* and 100 g from *Semitendinosus* muscle to analyse carnosine content.

Treatment	Week 1	Week 2	Week 3	Week 4*	TOTAL
CON	3	3	3	3	12
HIS	8	8	8	8	32
Total	11	11	11	11	44

* During the last week of sampling, due to logistic problems at the slaughterhouse, it was not possible to obtain the samples from the ST muscle of 11 animals (3 CON and 8 HIS) to analyse carnosine content. Consequently, only 33 samples from this muscle were finally analysed.

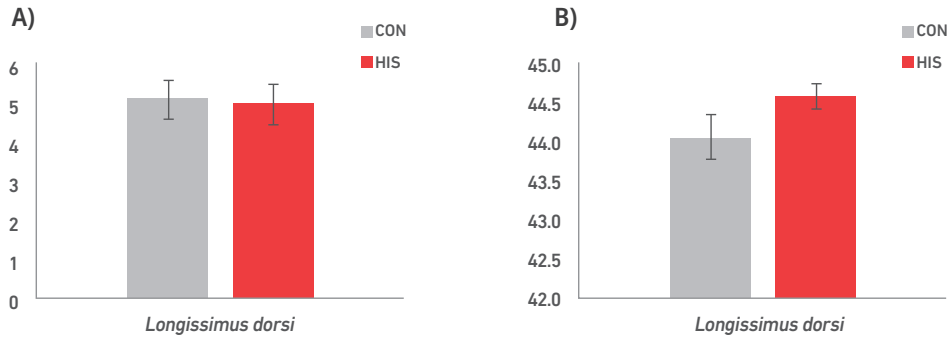
Results

No negative impact of adding 3 Kg extra HIS per ton of feed was detected for ADG, ADFI, and FCR, neither for final BW nor carcass weight at the end of the study.
Carnosine content was greater (P < 0.05) in LD of HIS pigs (307,03 ± 4,240 mg / 100 muscle) compared with CON animals (269,04 ± 6,925mg / 100g muscle) (Figure 1A), whereas only a numerical difference was observed in the ST (274,29 ± 4,826mg / 100g muscle and 250,56 ± 7,817mg 100g muscle for HIS and CON pigs, respectively) (Figure 1B).



* Means statistically different (P < 0.05); error bars represent the SE between groups

Figure 1. Carnosine content in *Longissimus dorsi* (A) and *Semitendinosus* (B) muscles of CON and HIS animals.



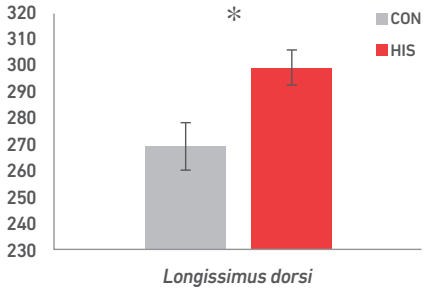
* Means statistically different (P < 0.05); error bars represent the SE between groups.

Figure 2. IMF (%) content (A) and Oleic acid (% of total fat) (B) in *Longissimus dorsi* of CON and HIS animals.

L-Histidine supplementation increased (P < 0.05) the pH in the LD muscle of pigs (5.68 ± 0.023 and 5.58 ± 0.032 for HIS and CON pigs, respectively) measured 24 h post-mortem (Figure 3).

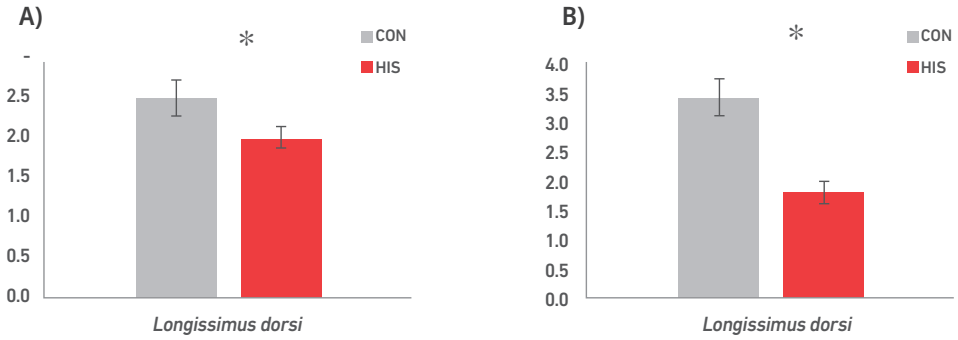
Additionally, samples of LD muscle from HIS pigs evinced lower (P < 0.05) drip loss (1.96 ± 0.119 %) than CON (2.49 ± 0.188 %) at 24 h post-mortem (Figure 4A).

Moreover, an interaction (P < 0.001) between treatment and sex was found, so this parameter was analysed separately for males and females. Thus, a greater difference was found for females, being drip loss of HIS females (1.78 ± 0.205 %) clearly lower (P < 0.05) than for CON females (3.39 ± 0.330 %) (Figure 4B).



* Means statistically different (P < 0.05); error bars represent the SE between groups.

Figure 3. pH in *Longissimus dorsi* of CON and HIS animals.



* Means statistically different (P < 0.05); error bars represent the SE between groups.

Figure 4. Drip loss (%) in the Longissimus dorsi in combined male and female (A) and for CON and HIS groups only in females (B).

Discussion

Although different studies performed in broilers have demonstrated the improvement in carnosine content of meat by increasing L-Histidine in the diet (Kai et al., 2014; Kralik et al., 2015), to the best of our knowledge, it has not been previously demonstrated in swine. Our results prove that supplementing feed with L-Histidine increases carnosine content in different muscles of pigs. However, this increment has been greater in LD muscle (about 14%) than in ST muscle (about 9.4%). Typically, carnosine content differs between muscles (Mora et al., 2008).

Additionally, Realini et al. (2013) described that LD contains more protein and lower percentage of fat than ST, that could explain the difference in carnosine content and the lower response to L-Histidine supplementation in this muscle.

D'Astous-Pagé et al. (2017), carried out an amazing study investigating the relationship between carnosine content and meat quality in three different breeds of pigs. Interestingly, they found that carnosine content and carnosine related enzymes were modulated by breed, and an improvement in meat quality parameters was observed with higher carnosine content in the muscles. In agreement with D'Astous-Pagé et al. (2017), our results show greater post-mortem pH in LD muscle containing higher carnosine content (HIS pigs). Furthermore, the solid buffering capacity of carnosine would explain the softer drop in the post-mortem pH, which also related to an improvement in water holding capacity, and involved in the reduction observed in drip loss of the LD muscle at 24h post-mortem (D'Astous-Pagé et al., 2017).

On the other hand, sex and age interaction with carnosine has been found in humans and murine skeletal muscles (Peñafiel et al., 2003; Lievens et al., 2021). In the present study, a numerical difference in the content of carnosine has been observed, being higher in males than in females, although there was no interaction between sex and treatment. Surprisingly, an interaction between sex and drip loss has been found, and females supplemented with L-Histidine evinced a more significant reduction in drip loss than males subjected to the same treatment. Our data do not allow us to provide an explanation for this observation, but it could be hypothesised that some mechanisms differ for these parameters between males and females and should be further investigated.

Finally, it should be pointed out that L-Histidine supplementation did not affect IMF and oleic acid content, both parameters positively related to meat quality, especially with organoleptic characteristics of some meat products with high added value, such as ham.

Conclusions

Supplementing L-Histidine to the feed of fattening pigs increases carnosine content in the muscles and improves meat quality parameters, such as pH and drip loss, without impairing the performance of the animals. Reducing crude protein in the diet of pigs decreases histidine content of feed and might negatively affect meat quality parameters and the nutritive value of meat by reducing the carnosine content. Therefore, in in measuring the SID histidine needs of pigs, it is essential to look both at growth and at other important physiological functions of histidine such as incorporation in carnosine and being an important precursor in the synthesis of histamine and hemoglobin.

Although much more research is needed to establish the accurate dose and the length of supplementing period before slaughtering to have an optimised impact on meat quality parameters, crystalline L-histidine would be a key tool to improve meat quality in pigs, both from an organoleptic and nutritional point of view.

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