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Effect of Finishing Diet on Physico-Chemical and Lipolytic Parameters and Volatile Compounds Throughout the Manufacture of Dry-Cured Foal "Cecina"

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Abstract

The objective of this study was to investigate the effect of the finishing diet (1.5 kg *vs.* 3 kg of commercial feed) on the physico-chemical, colour, textural and lipolytic parameters and volatile compounds of dry-cured foal "cecina" throughout the manufacturing process. Ripening time significantly affected ($P<0.05$) all the studied parameters. Finishing diet of foals did not affect ($P>0.05$) its chemical composition. Considering physico-chemical parameters, some differences were found at several sample points. Higher red colour (a*) ($P<0.001$) and lower total work (kg·mm) ($P<0.05$) were observed in the 3 kg "cecinas" group at the end of manufacturing process. TBAR´s reached their maximum values after post-salting stage, being these results significantly higher in "cecinas" from foals feed with 3 kg of concentrate. Free fatty acids (FFA) release was significantly higher in "cecinas" from the 3 kg group, especially due to the higher content of oleic and palmitic acid. Finally, the largest amount of volatile compounds was obtained in "cecinas" from the 3 kg group, however, hexanal content was significantly higher on 1.5 group.

Keywords: Foal dry-cured "cecina"; Finishing diet; Physico-chemical parameters; Volatile compounds; Lipolysis

Introduction

Salting and drying have traditionally been used as common procedures for preserving meats, mainly based on the decrease of water activity. Nowadays, a wide variety of salted, dry-cured meat products are produced from whole meat pieces of pork, beef, goat, venison and horse. The characteristic flavour of these meats is one of the most appreciated attributes for the consumer. "Cecina" is a salted, smoked and dried meat product widely consumed in the north-west of Spain with a very similar manufacture to that used in the production of dry-cured ham. Spanish "cecina" also resembles South African "biltong", South American "charqui" and Italian "bresaola" [1]. "Cecina de León", produced from beef, and "Cecina de chivo de Vegacervera", produced from goat meat, both in the region of León (NW of Spain), have been awarded the Protected Geographical Indication (PGI) label and a guarantee mark, respectively.

Horsemeat has been historically obtained from animals that were slaughtered at the end of their working lives [2], hence the poor organoleptic and nutritional quality of this meat [3]. Consumption of horsemeat has increased in recent years in some European countries maybe due to the interest of consumers on tasting new meat products [4]. However, most of the horsemeat produced in Spain is exported to Italy and France since its consumption is still not comparable to other meat such as beef, chicken and pork [5]. Foal meat is characterized by low fat and cholesterol contents while it is rich in iron [6]. Foal meat has also a positive dietetic fatty acid profile, with a high content of unsaturated fatty acids in relation to saturated acids, and contains a greater proportion of components from the α-linolenic fatty acid family [5,7,8]. Nevertheless, it has been reported that meat quality of different animal species can be influenced in an intramuscular level by finishing diet [9-11], leading to final variations on colour traits, tenderness and fatty acid profile, and therefore conditioning consumer acceptability [12]. Studies concerning physico-chemical and sensorial characteristics of dry-cured foal "cecina" has been reported [13], as well as some works evaluating the effect of the finishing diet on fresh horsemeat quality [14,15]. However, to our knowledge, there is no information about how the finishing diet supplied to the animals influences the quality of "cecina" manufactured from foal meat.

Therefore, the aim of this work was to investigate the effect of the finishing diet of foals slaughtered at 15 months in the physico-chemical, colour and textural properties, lipolysis and volatile compounds of dry-cured foal "cecina" throughout the manufacturing process.

Materials and Methods

Animal management and foal "cecina" manufacture

For this study, twenty one foals of the "Galician Mountain" breed were used. Animals were obtained from the experimental herd of Agricultural Research Centre of Mabegondo (Marco da Curra, A Coruña, Spain). The majority of the foals were born between April and May 2010. Animals were reared with their mothers on pasture and were allowed to suck freely. Foals were weaned when they were 6-8 months old. The finishing period was 4 months (from April to August). Then, foals were fed with concentrate and pasture in the best conditions of amount and quality. Foals were fed with two different amounts of concentrate (1.5 kg of fodder/foal-day and 3 kg of fodder/foal-day).

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Table 1: Evolution of chemical composition during the manufacture process of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day).

	Fresh piece				Salting				Post-salting				Dry-ripening								Sign. feed	Sign. time
													60 days				120 days					
	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.		
Moisture (%)	76.45^{d}	76.10^{e}	0.084	ns	74.84^{d}	71.33^{d}	0.738	**	66.00^{c}	65.12^{c}	0.596	ns	44.56^{b}	43.47^{b}	0.334	ns	40.38^{a}	39.50^{a}	0.661	ns	ns	***
Fat (% of DM)	1.43^{a}	1.89^{a}	0.16	ns	1.83^{a}	1.81^{a}	0.05	ns	1.32^{a}	1.35^{a}	0.20	ns	2.20^{ab}	2.75^{b}	0.17	ns	2.89^{b}	2.95^{b}	0.06	ns	ns	***
Protein (% of DM)	86.64^{c}	86.38^{b}	0.66	ns	82.64^{b}	81.40^{ab}	0.436	ns	79.55^{a}	80.09^{a}	1.244	ns	80.87^{ab}	79.35^{a}	0.579	ns	79.02^{a}	79.41^{a}	0.317	ns	ns	***
Ash (% of DM)	9.20^{a}	8.97^{a}	0.06	*	17.59^{b}	17.39^{c}	0.27	ns	15.86^{b}	15.98^{b}	0.40	ns	16.03^{b}	16.09^{b}	0.11	ns	17.17^{b}	16.78^{bc}	0.19	ns	ns	***
NaCl (%DM)	0.54^{a}	0.70^{a}	0.08	ns	7.60^{b}	7.65^{b}	0.52	ns	8.76^{b}	10.75^{c}	0.86	ns	11.10^{c}	11.12^{c}	0.24	ns	11.46^{c}	11.47^{c}	0.19	ns	ns	***

Sign: Significance, *** (P < 0.001), ** (P < 0.01), * (P < 0.05), ns (not significant); SEM: Standard error of the mean.
$^{a-e}$Different letters in each parameter, within the same day of processing and finishing diet, indicate significant differences at P < 0.05.

Forty knuckles with an average weight of 2.83 ± 0.50 kg, were selected and the "cecinas" were manufactured as described by Lorenzo (2014) [13]. Samples were taken as fresh, after salting, after post-salting and after 60 and 120 days of drying–ripening. At each sample point, four pieces were analysed.

Chemical composition

Moisture, fat, protein (Kjeldahl N × 6.25) and ash were quantified according to the ISO recommended standards [16-19]. Total chlorides were quantified according to the Carpentier–Vohlard official method [20]. Results were expressed as g/100 g of dry matter.

pH, water activity, TBARs values and colour parameters

The pH of samples was measured using a digital pH-meter (Thermo Orion 710 A+, Cambridgeshire, UK) equipped with a penetration probe. Water activity was determined using a Fast-lab (Gbx, Romans sur Isére Cédex, France) water activity meter, previously calibrated with sodium chloride and potassium sulphate. Colour measurements were carried out using a CM-600d colorimeter (Minolta Chroma Meter Measuring Head, Osaka, Japan). The homogeneous mass obtained from the grounded of each sample was measured three times for each analytical point. CIELAB space: lightness (L^*), redness (a^*) and yellowness (b^*) were obtained. Before each series of measurements, the instrument was calibrated using a white ceramic tile. Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid (TBARs) method of Vyncke (1975) [21] with the modification that samples were incubated at 96ºC in a forced oven (Memmert UFP 600, Schwabach, Germany). Thiobarbituric acid reactive substances (TBARs) values were calculated from a standard curve of Malonaldehyde (MDA) and expressed as mg MDA/kg sample.

WB and texture profile analysis

Seven meat pieces of 1 × 1 × 2.5 cm (height × width x length) were removed parallel to the muscle fibre direction and were completely cut using a Warner-Bratzler (WB) shear blade with a triangular slot cutting edge (1 mm thick). Maximum shear force, shear firmness and total necessary work performed to cut the sample were obtained. Textural profile analysis (TPA) test measured in a texture Analyser (TA.XT.plus of Stable Micro Systems, Vienna Court, UK) by compressing to 60% with a compression probe of 19.85 cm^2 of surface contact at a compression speed of 3.33 mm/s and recording speed was also 3.33 mm/s. Hardness (kg), cohesiveness, springiness (mm) and chewiness (kg*mm) were obtained using the computer software (Texture Exponent 32 (version 1.0.0.68), Stable Micro Systems, Vienna Court, UK).

Free fatty acid content

Total intramuscular lipids were extracted from 50 g of each minced sample, according to Folch et al. (1957) [22] procedure. Free fatty acids were separated from fifty milligrams of the extracted lipids using aminopropyl (NH_2) mini-columns as described by García-Regueiro et al. (1994) [23]. This fraction was transesterified with a solution of boron trifluoride (14%) in methanol, according to Carreau and Dubacq (1978) [24] and the fatty acid methyl esters (FAMEs) were stored at -80°C until chromatographic analysis. Separation and quantification of FAMEs was determined following Lorenzo (2014) [13].

Volatile compounds

The volatile compounds profile was studied in dry-cured foal "cecinas" taken at the end of the ripening period. The extraction of the volatile compounds was performed using Solid-Phase Microextraction (SPME). A SPME device (Supelco, Bellefonte, PA, USA) containing a fused-silica fibre (10 mm length) coated with a 50/30 mm thickness of DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) was used for HS-SPME extraction. Separation and quantification of the volatile compounds was determined following Lorenzo (2014) [13]. Results for each volatile compound were expressed as AU (area units) x10^6/g dry matter.

Statistical Analyses

All statistical analysis was performed using SPSS package (SPSS 19.0, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was carried out for all variables considered (processing time and finishing feed). A Duncan´s test was performed to compare the mean values for processing time at a significance level of $P<0.05$. Analysis of variance using the General Linear Model (GLM) was performed, including in the model the variables finishing feed and processing time, to show the significance of time of processing. Correlations between variables were determined by correlation analyses using the Pearson's linear correlation coefficient.

Results and Discussion

Chemical composition and physico-chemical parameters

The evolution of chemical composition during the manufacture process of "cecina" from foals fed with two different amounts of concentrate is shown in Table 1. Fresh knuckles showed an average

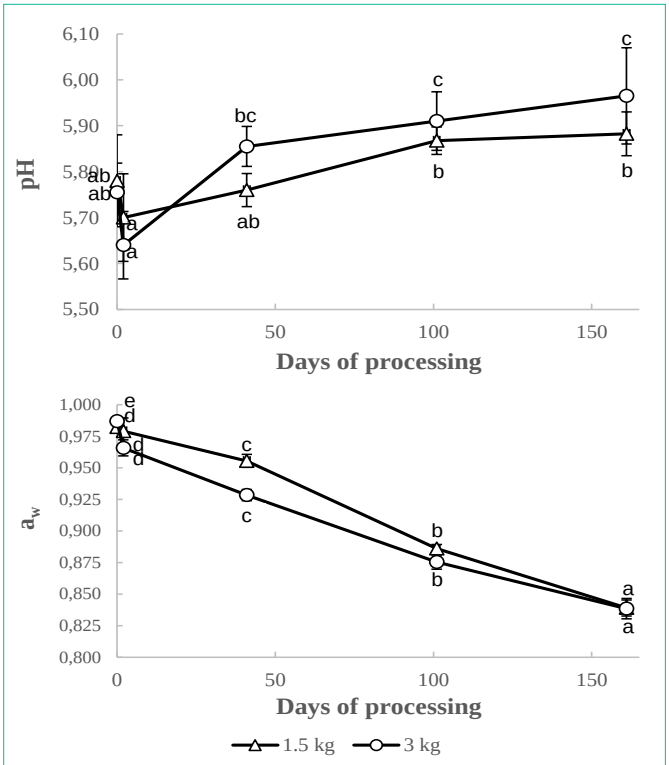

Figure 1: Evolution of pH and water activity during the manufacture process of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day). [a-e]Different letters in each parameter, within the same day of processing and finishing diet, indicate significant differences at $P < 0.05$.

moisture content about 76%. This content significantly decreases (P<0.001) throughout the manufacturing process reaching mean values of 40% after 120 days of dry-ripening, similar to that found in foal "cecina" [13] and in beef "cecina" [25]. Nevertheless, these moisture values were slightly lower than those reported in other beef "cecina" studies [26,27]. Intramuscular fat and protein contents (% of DM) were significantly (P<0.001) affected by processing time, increasing (from 1.77% in the fresh piece to 2.9% after 120 days of ripening) and decreasing (from 86.5% in the fresh piece to 79.2% after 120 days of ripening), respectively. Ash content, due to the incorporation of NaCl during salting stage, significantly increased (P<0.001) from mean values of 9% in fresh knuckles to values around 16% for the rest of the studied stages. NaCl (%DM) increment occurred during the whole process, but especially after salting, with an increment over 7%. As a result of salt diffusion, NaCl content increased to reach the final values of 11.47%. The NaCl content resulted inversely correlated with moisture content (r=-0.748, P<0.01) and positively with ash content (r=0.808, P<0.01). Final values of NaCl were slightly lower than those described on a previous study carried out in our laboratory (between 12-13%) [13]. Even though the salting time was exactly the same in both works (0.25 days/kg), the samples of the current study (where concentrate was used in the finishing diet) presented a higher fat content (2.8% *vs*. 1% in the present study and in Lorenzo, 2014 [13], respectively). As a result, a lower penetration of salt through the pieces was obtained due to the lower diffusion coefficient of salt in the fat than in the lean tissue [28]. Similar NaCl contents were found in beef "cecina" (around 8%) by García et al. (1997) [25] and Molinero et al. (2008) [26], while Molinero et al. (2008) [27] obtained higher contents (around 14%). A few significant differences (P<0.05), and only at the beginning of the manufacture process, were found between finishing diets for moisture, fat and ash content. These differences were also reported when *longissimus dorsi* (LD) muscle of these animals was studied [14].

The evolution of pH and water activity during the manufacture process of "cecina" from foals fed with two different amounts of concentrate is shown in Figure 1. Similar pH values around 5.8 were found in fresh knuckles [13]. These initial values significantly decrease (P<0.05) during the salting stage to 5.6, increasing then throughout the rest of manufacturing process to mean values of 5.9. Only after post-salting stage significant differences were observed (P<0.05), revealing a faster increment of pH in "cecinas" from the 3 kg group. Similar values on the final product were obtained in beef "cecina" [26,27,29] but lower than in foal "cecina" with no finishing diet [13].

Water activity significantly decreased (P<0.001) along the

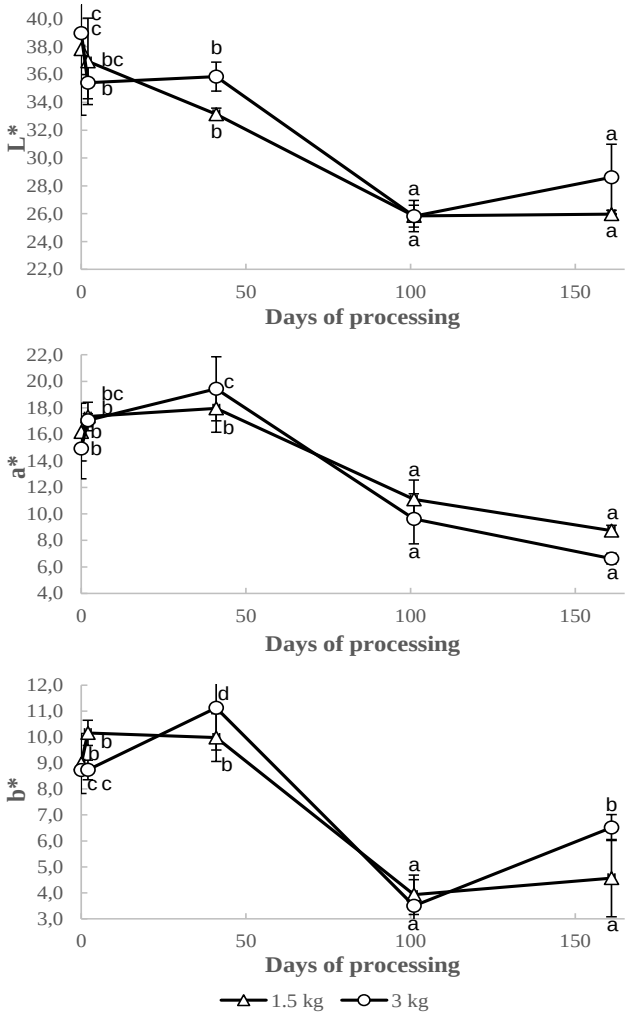

Figure 2: Evolution of colour parameters (L^*, a^* and b^*) during the manufacture process of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day). [a-d]Different letters in each parameter, within the same day of processing and finishing diet, indicate significant differences at $P < 0.05$.

manufacturing process, from mean initial values of 0.98 in fresh knuckles to 0.84 in dry-cured foal "cecina". This behaviour was the same than the previously seen for moisture content, being positive and significantly correlated to a_w (r = 0.975, P<0.01). The decrease in water activity in dry-cured meat products is one of the main factors contributing to their microbial stability. This decrease is achieved essentially by sodium chloride addition to the meat and by dehydration during the ripening process [30]. As the last physico-chemical parameters discussed above, some sample points showed some differences for a_w when finishing diets where compared. Similar a_w values were reported in analogous products [13,25,27].

The evolution of lightness (L*), redness (a*) and yellowness (b*) during the manufacture process of "cecina" from foals fed with two different amounts of concentrate is shown in Figure 2. Colour parameters stayed constant during the first stages of processing (salting and post-salting) and then significantly decreased (P<0.05) through the 60 days of dry-ripening. After 120 days of dry-ripening, L*, a* and b*-values remained practically unchanged, except b*-values of "cecinas" from the 3 kg group which, in fact, incremented. Colour parameters decrease is probably related to moisture loss and salt concentration increase [13]. These two parameters were significantly correlated to L*-value (r=0.895, P<0.01 and r = -0.860, P<0.01, respectively), a*-value (r=0.768, P<0.01 and r = -0.657, P<0.01, respectively) and b*-value (r=0.804, P<0.01 and r = -0.731, P<0.01, respectively). Different studies in "cecina" reported a similar trend for lightness (L*-values) [26] and for all the colour parameters [13,31].

The evolution of textural parameters (Warner-Bratzler test) and TPA test during the manufacture process of "cecina" from foals fed with two different amounts of concentrate is shown in Table 2. Regarding to the Warner Bratzler (WB) parameters, shear force in fresh knuckles was significantly affected by feed, being higher in the 1.5 kg group than in the 3 kg one (2.91 and 1.87 kg/cm^2, respectively). The values remained constant during the manufacturing process until the 60 days of dry-ripening were reached, then this parameter sharply increased (P<0.05) to mean values of 9.9 kg/cm^2, finding no significant differences (P>0.05) between the two finishing diets. On the contrary, firmness gradually increased (P<0.001) during all the studied stages, from higher initial mean values (P<0.01) in the 1.5 kg group than in the 3 kg one (0.80 and 0.52 kg/s, respectively) to final mean values of 2.2 kg/s, being not affected by concentrate amount (P>0.05) in the rest of the stages. Finally, a significant effect (P<0.001) of concentrate amounts was obtained for the total work at the beginning (fresh knuckles) of the manufacturing process, being higher for the 1.5 kg "cecinas" (30.93 kg·mm) than for the 3 kg ones (20.25 kg·mm). These values significantly decrease (P<0.05) during the first stages of the process, to increase then, during the dry-curing stage, obtaining again higher values (P<0.05) for the 1.5 kg "cecinas" (57.19 kg·mm) than for the 3 kg ones (51.32 kg·mm). A similar tendency throughout the manufacturing process was also found in foal "cecina" [13], although the final values were lower for shear force and firmness. As regards the texture profile analysis, a significant effect of concentrate was not observed (P>0.05) for these parameters in most of the sample points. Hardness and chewiness slightly decreased until the first 60 days of dry-ripening and from then markedly increased (P<0.05) until the end of ripening. Springiness values significantly (P<0.01) decreased along the whole manufacturing process, while cohesiveness increased (P<0.001) throughout the different stages. A similar trend for all TPA test parameters was reported by Lorenzo (2014) [13] in foal "cecina", but higher values of chewiness and lower of hardness were obtained in this work. The differences in hardness could be due to the fact that foal "cecinas" from Lorenzo (2014) [13] presented lower moisture content (35.3%) since hardness is negative correlated with moisture content (r=−0.494, P<0.01) in the present work. Our results for TPA test were in general higher than those found in beef "cecina" [26,27,31].

The evolution of the TBARs value (mg malonaldehyde/kg of sample) during the manufacture process of "cecina" from foals fed with two different amounts of concentrate is shown in Figure 3. Finishing diet significantly (P<0.05) affected the TBARs results (data not shown). TBARs values increased progressively after salting and post-salting stages (P<0.05), from initial values around 0.13 mg malonaldehyde/kg of sample in both types of "cecina" studied. Values in the 3 kg group were significantly higher than in the 1.5 kg group

Table 2: Evolution textural parameters during the manufacture process of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day).

	Fresh piece				Salting				Post-salting				Dry-ripening									Sign. time
													60 days				120 days					
	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	1.5 kg	3 kg	SEM	Sign.	Sig. feed	
Textural Parameters																						
Shear force (kg/cm^2)	2.91^{a}	1.87^{a}	0.27	**	2.55^{a}	3.28^{ab}	0.29	ns	1.82^{a}	2.44^{ab}	0.40	ns	2.54^{a}	4.39^{b}	0.59	ns	11.05^{b}	8.65^{bc}	1.33	ns	ns	***
Firmness (kg/s)	0.80^{a}	0.52^{a}	0.08	**	0.57^{a}	0.73^{a}	0.05	ns	0.43^{a}	0.51^{a}	0.09	ns	0.48^{a}	0.75^{a}	0.09	ns	2.12^{b}	2.24^{b}	0.25	ns	ns	***
Total work (kg·mm)	30.93^{c}	20.25^{a}	2.678	***	26.30^{bc}	26.49^{a}	1.339	ns	13.84^{a}	19.51^{a}	3.194	ns	23.09^{ab}	32.09^{ab}	3.557	ns	57.19^{d}	51.32^{b}	3.943	*	ns	**
TPA test																						
Hardness (kg)	6.32^{b}	4.73^{b}	0.56	ns	4.81^{b}	1.87^{a}	0.67	*	2.16^{a}	1.66^{a}	0.14	ns	1.67^{a}	2.83^{ab}	0.30	*	15.35^{c}	17.37^{c}	1.33	ns	ns	***
Springiness (mm)	0.75^{cd}	0.66^{abc}	0.03	ns	0.66^{bc}	0.74^{c}	0.02	*	0.83^{d}	0.69^{bc}	0.05	ns	0.62^{ab}	0.59^{ab}	0.01	*	0.53^{a}	0.53^{a}	0.01	ns	ns	**
Cohesiveness	0.36^{a}	0.33^{a}	0.02	ns	0.31^{a}	0.44^{b}	0.04	ns	0.49^{b}	0.44^{b}	0.01	**	0.58^{c}	0.57^{c}	0.01	ns	0.52^{bc}	0.50^{bc}	0.01	ns	ns	***
Chewiness (kg·mm)	2.44^{b}	1.57^{a}	0.27	ns	1.45^{a}	0.77^{a}	0.20	ns	1.07^{a}	0.74^{a}	0.09	*	0.97^{a}	1.57^{a}	0.15	*	7.74^{c}	8.30^{b}	0.59	ns	ns	***

Sign: Significance, *** (P < 0.001), ** (P < 0.01), * (P < 0.05), ns (not significant); SEM: Standard error of the mean.
$^{a-d}$Different letters in each parameter, within the same day of processing and finishing diet, indicate significant differences at P < 0.05.

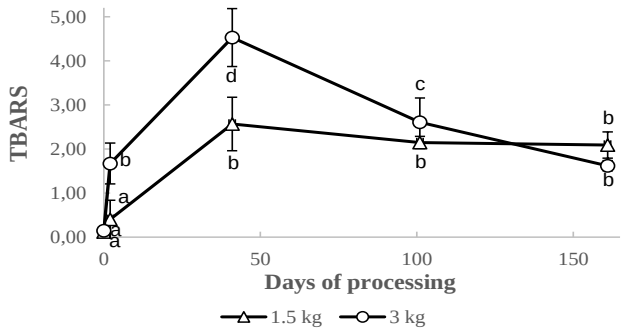

Figure 3: Evolution of TBARS value (mg malonaldehyde/kg of sample) during the manufacture process of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day). [a-d]Different letters in each parameter, within the same day of processing and finishing diet, indicate significant differences at $P < 0.05$.

at the end of salting and post-salting stages ($P<0.05$ and $P<0.01$, respectively: data not shown), reaching maximum values of 4.19 and 2.57 mg malonaldehyde/kg of sample after post-salting, respectively. Then, during dry-ripening process, the TBARs values slowly decreased to the end of manufacturing process reaching final mean values of 1.89 mg malonaldehyde/kg of sample, without any significant differences ($P>0.05$) between the two finishing diets compared. The higher values obtained after post-salting in the 3 kg group could not be entirely explained considering the fact that intramuscular fat content was similar in the two groups of concentrate. Moreover, when the fatty acid content of the *longissimus dorsi* muscle of foals from this study [9] is considered, a significantly higher content of PUFA is found in foals from the 1.5 kg finishing diet, being this PUFA more susceptible to oxidation [32]. The final values found in foal "cecina" by Lorenzo *et al.* (2014) [13] showed twice the content of the results of this study. Our data are similar to those found by other authors in dry-cured sausages [32,33] and slightly higher than in dry-cured ham [34].

Free fatty acid content

The evolution of free fatty acid content (expressed as mg/100 g of fat) during the manufacture process of "cecina" from foals fed with two different amounts of concentrate is shown in Table 3. Free Fatty Acids (FFA) were analysed during ripening process as indicators of the lipolysis in dry-cured foal "cecina". Lipolysis is directly involved in flavour formation during ripening of cured products. FFA are released as a result of endogenous or microbial lipases activities. The total FFA content increased significantly throughout the ripening process ($P<0.001$), from 348-349 mg/100 g of fat in the raw pieces to 2609-3133 mg/100 g of fat at the end of the ripening stage for the "cecinas" from animals feed with 1.5 and 3 kg of concentrate, respectively.

Respect to the amount of concentrate in finishing diet, major differences between two batches were observed at the end of the ripening process since FFA release was significantly higher in the "cecinas" from animals that received 3 kg than in those receiving 1.5 kg of concentrate. This is due to the "cecinas" from animals that received 3 kg of concentrate, respect to 1.5 kg, showed a greater content of palmitic (1261.1 *vs.* 1060.4 mg/100 g of fat) and palmitoleic (96.3 *vs.* 69.6 mg/100 g of fat) acids, and especially a higher content of oleic acid (399.3 *vs.* 719.2 mg/100 g of fat). However, the content of linolenic acid was lower in "cecinas" from this batch (37.6 *vs.* 56.1 mg/100 g of fat).

In raw pieces from both batches, FFA maintained the relationship MUFA>SFA>PUFA as previously observed [13]. During ripening process the increase in SFA was 11-15 fold, 3-5 fold in MUFA and 7-8 fold in PUFA. At the end of ripening, in "cecinas" of the animals fed with 1.5 kg of concentrate, individual FFA followed this order: palmitic>stearic>linoleic>oleic, while from foals fed with 3 kg of concentrate, the order changed: palmitic>oleic>stearic>linoleic. The sum of these four fatty acids represented over 91% of total FFA in both cases. A similar profile was previously described in dry-cured meat products [13,35].

There are several studies describing FFA increase during the manufacturing process of dry-cured products [32,35]. In our study it was found that the major release of FFA occurred during dry-ripening stage, which coincides with described in cured "lacón" [36] and in a previous work of foal "cecina" [13]. Total average content of FFA was similar to those described in pork sausages [33,35] and in foal "cecinas" [13], however, lower values were described in other products [32,37]. This variability is normal since the FFA content depends on many factors, such as the raw materials used, the length and conditions of the process, and the activity and specificity of lipases [35].

As just mentioned, the content of all individual fatty acids increased throughout the ripening process. As regards, the least abundant fatty acids, arachidonic and eicosapentaenoic acids were only found in the last day of ripening with final concentrations varying from 16.9-20.9 and 30.9-25.2 mg/100 g of fat, respectively. Linolenic acid content increased after the post-salting stage (between 80.59 and 120.49 mg/100 g of fat), and then suffered a gradual decrease until the end of the dry-ripening process (between 37.33 and 56.06 mg/100 g of fat). This degradation was also observed in other dry-cured products [13,32]. However, other authors described an increase on the content of this fatty acid throughout the ripening process [33,36]. It is well known that the amount of each fatty acid depends on the release rate and the oxidative degradation [13]. In this case, linolenic acid decrease may be related to the high susceptibility of this fatty acid to oxidize. This hypothesis is supported by the fact that PUFA are more subjected to oxidation than SFA and MUFA [32], however TBARs results revealed a higher oxidation of this samples after the post-salting stage, but it was no detected in the dry-ripening stage. Lizaso et al. (1999) [38] also reported that SFA/UFA ratio increased during fermentation; however Martín-Sánchez et al. (2011) [35] observed a decrease in this ratio. Neutral lipids are the most abundant lipid fraction in intramuscular fat. In our study SFA are liberated in greater proportions than MUFA or PUFA, indicating that release is preferably originated from triglycerides fraction since it is richer in SFA fatty acids.

Volatile compounds

Volatile compounds, expressed as area units (AU) × 10^6/g of dry matter, of "cecina" from foals fed with two different amounts of concentrate, after 120 days of dry-ripening, are shown in Table 4. The SPME technique is not normally used for absolute quantifications, but when the same exact extraction methodology is employed, this technique permits to compare relative amounts between samples. A

Table 3: Evolution free fatty acid content (mg/100 g of fat) during the process of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day).

	Fresh piece				Salting				Post-salting				Dry-ripening									
													60 days				120 days					
	1.5 kg	3 kg	SEM	*Sign.*	1.5 kg	3 kg	SEM	*Sign.*	1.5 kg	3 kg	SEM	*Sign.*	1.5 kg	3 kg	SEM	*Sign.*	1.5 kg	3 kg	SEM	*Sign.*	*Sig. feed*	*Sign. time*
C14	0.00	0.00			0.00	0.00	0.00		8.40^{a}	0.00	1.59	ns	0.00	0.00			33.71^{b}	39.99	2.29	ns	ns	***
C16	99.17^{a}	86.54^{a}	4.89	ns	104.74^{a}	83.64^{a}	6.29	ns	176.84^{b}	263.10^{b}	16.74	ns	432.40^{c}	482.63^{c}	13.96	ns	1060.43^{d}	1261.13^{d}	43.63	**	***	***
C16:1n-7	16.44^{a}	15.74^{a}	0.35	ns	12.28^{a}	21.28^{a}	1.80	***	22.91^{b}	33.42^{b}	2.38	ns	23.53^{b}	33.57^{b}	2.55	ns	69.66^{c}	96.35^{c}	5.72	**	***	***
C17	0.00	0.00			0.00	0.00	0.00		0.92^{a}	0.00	0.18	ns	0.00	0.00			6.86^{b}	7.59	0.34	ns	ns	***
C18	39.03^{a}	26.08^{a}	2.67	**	33.84^{a}	25.27^{a}	1.81	**	75.38^{b}	74.67^{b}	1.00	ns	193.31^{c}	181.91^{c}	5.27	ns	480.94^{d}	464.27^{d}	12.28	ns	ns	***
C18:1*n*-9	124.91^{a}	145.93^{a}	6.77	ns	123.75^{a}	114.34^{a}	7.32	ns	240.24^{b}	336.74^{b}	20.87	ns	285.81^{b}	315.62^{b}	18.19	ns	399.25^{c}	719.22^{c}	62.71	***	***	***
C18:2*n*-6	68.73^{a}	74.96^{a}	3.61	ns	85.99^{a}	63.48^{a}	5.31	*	190.59^{b}	194.60^{b}	2.84	ns	442.56^{c}	316.85^{c}	25.57	ns	454.47^{c}	461.82^{d}	13.19	ns	***	***
C18:3*n*-3	0.00	0.00			73.35^{b}	40.52^{a}	6.36	***	120.44^{c}	80.59^{b}	7.97	ns	70.45^{b}	49.44^{a}	5.48	ns	56.06^{a}	37.33^{a}	4.03	**	***	***
C20:4*n*-6	0.00	0.00			0.00	0.00			0.00	0.00			0.00	0.00			16.90	20.88	1.66	ns	ns	***
C20:5*n*-3	0.00	0.00			0.00	0.00			0.00	0.00			0.00	0.00			30.92	25.21	1.85	ns	ns	***
Total FFA	348.27^{a}	349.25^{a}	8.96	ns	433.94^{a}	348.52^{a}	21.37	*	835.73^{b}	983.12^{b}	29.97	ns	1448.05^{c}	1380.01^{c}	32.31	ns	2609.20^{d}	3133.80^{d}	104.99	***	***	***
SFA	138.19^{a}	112.63^{a}	6.60	*	138.57^{a}	108.91^{a}	7.76	*	261.54^{b}	337.77^{b}	14.98	ns	625.72^{c}	664.54^{c}	15.43	ns	1581.94^{d}	1772.99^{d}	40.61	**	***	***
UFA	210.08^{a}	236.63^{a}	7.48	ns	295.37^{b}	239.61^{a}	13.70	*	574.19^{c}	645.35^{b}	15.88	ns	822.34^{d}	715.47^{c}	28.30	ns	1027.26^{e}	1360.81^{d}	66.65	***	***	***
MUFA	141.35^{a}	161.66^{a}	6.69	ns	136.03^{a}	135.62^{a}	7.14	ns	263.15^{b}	370.16^{b}	22.99	ns	309.34^{b}	349.19^{b}	19.44	ns	468.91^{c}	815.57^{c}	67.57	***	***	***
PUFA	68.73^{a}	74.96^{a}	3.61	ns	159.34^{b}	103.99^{a}	11.02	***	311.03^{c}	275.20^{b}	7.60	ns	513.01^{d}	366.29^{c}	29.72	ns	558.35^{e}	545.25^{d}	14.49	ns	***	***

Sign.: Significance, *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$), ns (not significant); SEM: Standard Error of the Mean.
SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.
[a-e]Different letters in each parameter, within the same day of processing and finishing diet, indicate significant differences at $P < 0.05$.

total of 31 volatile compounds were isolated and tentatively identified from the two batches of "cecina", including 2 aldehydes, 15 esters, 9 aliphatic and 2 aromatic hydrocarbons and other 3 compounds such as 1 ketone, 1 furan and 1 alcohol. The sum of the total volatile compounds was 25,791 and 29,122 AU×10^6/g of dry matter in the 1.5 and 3 kg groups, respectively.

Esters were the most abundant compounds and represented 83.2 and 86.2% of the total chromatographic area in the 1.5 and 3 kg group, respectively. Probably, esters arise from the esterification of several alcohols and carboxylic acids [39], although Ramírez and Cava (2007) [40] did not exclude that the action of microorganisms might be involved in the formation of esters. In line with this, esters can modulate the global flavour due to their low odour thresholds, imparting fruity notes, mainly those formed from short-chain acid [41], whereas esters with long-chain acids have a slight fatty odour [42]. Most abundant compounds were methyl 2-methyl- butanoate and methyl hexanoate. Significant differences between groups were found for methyl acetate ($P<0.05$) and methyl octanoate ($P<0.01$), with higher amounts in the 1.5 and in 3 kg groups, respectively. A similar percentage of esters and the same major compounds were obtained in dry-cured "cecina" by Lorenzo (2014) [13]. Furthermore, most of the esters obtained in this work were previously found in different dry-cured meat products [32,33].

Aliphatic hydrocarbons were the second most important compounds, but they only represented 8.6 and 9.8% of the total chromatographic area in the 1.5 and 3 kg group, respectively. Among these compounds, undecane was the most abundant. Significantly higher amounts of octane ($P<0.05$), tridecane ($P<0.01$) and tetradecane ($P<0.01$) were obtained in 3 kg group of "cecinas", while 5-undecene, 9-methyl- ($P<0.01$) content was higher in the 1.5 kg group. Aromatic hydrocarbons only represented over a 0.7% and only ethylbenzene and p-xylene were detected. Aliphatic hydrocarbons have a high threshold value so they do not contribute significantly to the aroma of dry-ripened meat products, in this sense aromatic hydrocarbons could play an important role [40]. Aliphatic hydrocarbons with less than 10 carbons atoms arise mainly from lipid oxidation, while those with longer chains could be accumulated in the fat depots of the animal, probably from the feeding [43].

Regarding to aldehydes, only two compounds were detected: hexanal and heptanal. Significant differences ($P<0.05$) between both feeding groups were obtained for hexanal with higher values in the 1.5 kg group (1412.5 AU×10^6/g of dry matter) than in the 3 kg one (298.58 AU×10^6/g of dry matter). Aldehydes are typical products produced by lipid oxidation, and hexanal is mainly formed from *n*-6 fatty acids like linoleic and araquidonic acids. Aldehydes have low odour threshold values and play an important role in flavour of dry-ripened meat products [40]. Differences between the two batches of "cecinas" were not observed when TBARs index and *n*-6 free fatty acids were studied at the end of the ripening. The low amount of aldehydes detected could be related to the extraction method and SPME fibre type [44].

Within the group of "other compounds", 2-heptanone was the only ketone detected in this product, with a similar content in the two batches of "cecina", representing over a 0.89% of the total chromatographic area. To this regard, ketones, especially 2-ketones, are considered to have a great influence on the aroma of meat and meat products as they are present in large amounts and have a peculiar aroma, such as ethereal, green, tropical fruit, nutty, dry-cured ham-like or toasted [45].

Table 4: Volatile compound (10^6 AU/g of sample) of "cecina" from foals fed with two different amounts of concentrate (1.5 and 3 kg of fodder/foal-day), after 120 days of dry-ripening.

			120 days of dry-ripening			
	IK	R	1.5 kg	3 kg	SEM	*Sign. feed*
Aldehydes						
Hexanal	843	m, k, s	1412.50	298.58	301.44	*
Heptanal	925	m, k	95.00	63.75	15.83	ns
Aliphatic hydrocarbons						
Octane	800	m, k, s	69.52	115.12	11.76	*
Nonane, 3-methyl-	947	m, k	45.53	55.21	11.47	ns
Decane	1000	m, k, s	481.82	567.15	85.03	ns
Undecane	1100	m, k	754.99	1106.94	133.08	ns
5-Undecene, 9-methyl-	1129	m, k	267.92	183.03	21.59	**
Dodecane	1200	m, k, s	544.70	572.75	20.21	ns
Tridecane	1300	m, k	52.88	210.00	38.89	**
Tetradecane	1400	m, k, s	0.00	26.24	5.63	**
Pentadecane	1500	m, k, s	6.76	7.83	0.91	ns
Aromatic hydrocarbons						
Ethylbenzene	881	m, k	38.60	53.09	4.45	ns
p-Xylene	888	m, k	123.16	161.11	11.69	ns
Esters						
Methyl acetate	579	m, k	2078.06	1273.13	240.30	*
Methyl 2-methyl- propanoate	713	m, k	1639.48	1486.67	175.08	ns
Methyl butanoate	758	m, k	2308.82	2400.14	276.41	ns
Methyl 2-methyl- butanoate	812	m, k	5449.39	8212.86	765.07	ns
Methyl pentanoate	856	m, k	594.85	531.63	57.45	ns
Methyl 3-methyl-pentanoate	903	m, k	47.03	49.20	5.70	ns
Methyl hexanoate	932	m, k	6331.06	6769.32	570.71	ns
Methyl 3-hexenoate	941	m, k	75.57	76.58	7.40	ns
Methyl heptanoate	1044	m, k	637.07	792.79	89.35	ns
Methyl 2-heptenoate	1108	m. k	63.12	76.42	11.41	ns
Methyl octanoate	1161	m, k	1446.58	2470.13	246.59	**
Methyl 2-octenoate	1220	m, k	31.67	34.76	5.24	ns
Methyl nonanoate	1268	m, k	147.49	175.28	22.67	ns
Methyl decanoate	1367	m, k	578.17	729.94	86.08	ns
Methyl dodecanoate	1547	m, k	33.40	31.16	5.22	ns
Other compounds						
2-Heptanone	920	m, k	226.53	264.56	26.71	ns
Furan, 2-pentyl-	992	m, k	141.10	228.75	20.52	*
Benzyl alcohol	1124	m, k	68.57	98.21	19.36	ns

Sign: Significance, *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$), ns (not significant); SEM: Standard error of the mean. AU: area units resulting of counting the total ion chromatogram (TIC) for each compound. KI: kovats index calculated for DB-624 capillary column (J&W scientific: 30 m × 0.25 mm id, 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector. R: Reliability of identification: k: kovats index in agreement with literature (Lorenzo, 2014 [13]; Gómez & Lorenzo, 2013 [32]; Flores et al., 1997 [46]; Lorenzo & Domínguez, 2014 [47]); m: mass spectrum agreed with mass database (NIST05); s: mass spectrum and retention time identical with an authentic standard.

Conclusion

As expected, all the parameters studied showed a significant evolution along the manufacturing process. On the other hand, although the finishing diet supplied to the foals did not exhibit a significant effect on most of these parameters, TBARs values and FFA contents were higher in the 3 kg group after salting and post-salting stages and at the end of the ripening, respectively. Moreover, a higher

total amount of volatile compounds was obtained for "cecinas" from the 3 kg group, in spite of a significant higher amount of hexanal was found in 1.5 kg group.

In conclusion, the results derived from the present work suggest that the finishing diets evaluated in this study did not differ as much as to show a significant effect on the quality of this dry-ripened foal meat product.

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