

**EFFECT OF SEX, AGE AT SLAUGHTER AND AGING PERIOD
ON WATER-HOLDING CAPACITY AND TENDERNESS
OF MEAT OF POSAVINA HORSE BREED**

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Summary

The aim of this study was to determine water-holding capacity (thawing loss – TL and cooking loss – CL) and tenderness (Warner-Bratzler shear force, WBSF) of meat of Posavina horse breed considering sex, age at slaughter and aging period. Altogether, 12 animals of Posavina horse breed (6 males and 6 females) were involved in the study. The average age of the animals was 18 months and ranged from six to 36 months. Muscle samples for the analysis were taken from the *M. longissimus dorsi* of each carcass after the aging period of 14 days and 28 days. Water-holding capacity and tenderness of meat of Posavina horse breed were analyzed using the SAS/STAT software package version 9.4. The mean values for TL, CL, and WBSF were 8.41%, 19.24%, and 26.04 N, respectively. The results showed that TL, CL, and WBSF of meat of Posavina horse breed are not significantly affected by sex, slaughter age or aging period ($p>0.05$). This study gave a new insight into the meat quality traits of Posavina horse breed which could contribute to further necessary research to complete an understanding of horse meat quality.

Key words: Posavina horse, meat, thawing loss, cooking loss, tenderness

Introduction

Among numerous traits that determine meat quality, water-holding capacity (WHC) and tenderness of meat are important traits associated with consumer acceptance and processing technology. It is known that poor WHC result in numerous economic losses recognizable in a lower yield and nutritional value of meat (water-soluble proteins, vitamins), thus affecting the overall quality of products (Bowker, 2017.). For consumers, poor WHC of meat products results in diminished visual appeal due to excess of drip loss and inferior palatability traits related to juiciness and tenderness (Bowker, 2017.). Numerous studies confirm that consumers can determine the existence of differences in meat tenderness and are willing to pay a higher price for meat if they perceive it as more tender (Polkinghorne and Thompson, 2010.; Schroeder and Tonsor, 2011.; Listrat *et al.*, 2016.). Also, several studies have presented that quality of horse meat can also be influenced by other factors such as breed, age, sex, and aging time (Domínguez *et al.*, 2015.; Diaconu *et al.*, 2016.; Kaić *et al.*, 2018.). In latest years, due to its beneficial nutritional characteristics (low fat and cholesterol content, high protein and haem iron content, and favorable fatty acid profile), horse meat has become more popular being regarded as a “dietary” and considered as a new alternative in meat consumption (Lorenzo *et al.*, 2017.). However,

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compared to other meat producing species, there is still a lack of knowledge and information about quality traits of horse meat and factors affecting those traits. Posavina horse breed represents the recognizable transboundary autochthonous cold-blooded breed. The significant income of this breed comes from meat production although there is a lack of knowledge on their meat quality traits. In this context, the aim of this study was to determine WHC and tenderness of meat of Posavina horse breed considering sex, age at slaughter and aging period.

Materials and methods

The study was conducted as a part of the routine slaughtering and *post mortem* processing practice of horse meat in the specialized commercial butchery. Altogether, 12 animals of Posavina horse breed (6 males and 6 females) were involved in the study. The animals for the slaughter were raised at small farms with similar (traditional) husbandry practices and were monitored according to EU regulations (Commission Implementing Regulation, 2015.). The ages of the animals ranged from six months to 36 months with an average of 18 months. The animals were stunned with a captive bolt, slaughtered and dressed according to the Council Regulation (2009.). After evisceration, the carcasses were weighed and then chilled at 4 °C in a cold chamber. As a part of routine practice, the carcasses were aged 14 days under refrigerated conditions. After the aging period of 14 days, two 2-cm thick slices from *M. longissimus dorsi* (between 6th and 8th thoracic vertebrae; LT) were taken from each carcass (n=24).

The samples were prepared for Warner-Bratzler shear force (WBSF) determination and vacuum packaged. One slice of LT was immediately frozen at -20°C (14 days aged samples; first aging, n=12), whereas the others were stored under refrigerated conditions for another 14 days (altogether 28 days; second aging, n=12). At the end of the second aging period, the LT samples were frozen at -20°C. After the storage period, the samples were thawed under the conventional refrigerated conditions (at 4°C for 24 h) and used for determination of thawing loss (TL), cooking loss (CL) and WBSF. Analytical methods taken for the determination of TL, CL, and WBSF were performed as previously described Kaić et al. (2018.). The WHC and tenderness of meat of Posavina horse breed were analyzed using the SAS/STAT software package version 9.4 (SAS Inst. Inc., 2004.). Descriptive statistics of the investigated traits was analyzed using MEANS procedure, whereas the effect of sex, age at slaughter, and aging period were analyzed using MIXED procedures. The best fitted model for WHC and tenderness included the overall mean (μ), sex (i = male, female), the linear regression coefficient (β) of age in months (x_{ijk}), aging period (j = 14, 28 days) as fixed effects, and the random residual (e_{ijk}). Post hoc comparison among the least square means was assessed using a t-test. Differences were considered if $p < 0.05$.

Results and discussion

Table 1. shows descriptive statistics for TL, CL, and WBSF of meat of Posavina horse breed. The average TL value found in our study is greater (8.41%) than the TL reported by Tateo *et al.* (2008.) in the LT muscle (5.81%) of Italian Heavy Draft horse. Among the numerous factors that could have influenced on these differences, different aging/freezing/thawing conditions might have played a crucial role. Kim and Brad Kim (2017.) reported that each aging, freezing and/or thawing process by itself, in sequence or in combination may result in different quality characteristics of meat. The samples of LT reported by Tateo *et al.* (2008.) were vacuum-packaged 72 h *post mortem* and stored for 10 days. After that, the samples were thawed

at 2°C to 5°C during a period of 24 h. Contrary, our samples were aged separately 14 and 28 days, vacuum-packaged, stored at -20°C until the end of the trial and thawed at 4 °C during a period of 24 h. The relatively high variability in the TL of our samples (CV= 28.17%) could be due to the differences in damaged levels to muscular cells ultrastructure during frozen storage.

The average CL found in our study was lower (19.24%) than the CL value (25.4%) reported by Tateo *et al.* (2008.). These differences could be explained with a greater amount of water in our samples released when measuring TL. In addition, according to Franco *et al.* (2011.), this result could be due to the different conditions during thermal process. They explained how small differences in the control of cooking temperature over a long time could affect the final CL values, and that the rigorous control of a process is necessary when comparing research results. Tateo *et al.* (2008.) measured CL when an internal temperature of 70 °C had been attained in the sample for 3 min. Contrary, our CL was measured when the endpoint temperature of 75 °C had been reached in the sample.

The WBSF mean value was 26.4 N and ranged from 16.35 N to 43.53 N. This results revealed that according to the tenderness categories classification proposed by Belew *et al.* (2003.), the meat of Posavina horse breed can be considered from “very tender” (WBSF <3.2 kg) to “intermediate” (3.9<WBSF<4.6 kg). The variability in WBSF (CV= 26.37%) could be due to the random variation and needs to be confirmed by a larger number of samples.

Table 1 Descriptive statistics for thawing loss (TL), cooking loss (CL), and Warner-Bratzler shear force (WBSF) of Posavina horse breed

Tablica 1. Opisna statistika kala odmrzavanja (TL), kala kuhanja (CL) i Warner-Bratzler sile presijecanja (WBSF) mesa posavskog konja

	N	Mean	Std	CV, %	Min	Max
TL, %	24	8.41	2.368	28.17	4.97	13.92
CL, %	24	19.24	3.217	16.72	12.39	24.97
WBSF, N	24	26.04	6.867	26.37	16.35	43.53

n: number of samples – broj uzoraka; mean – aritmetička srednja vrijednost; Min. - Maks.: minimum and maximum – minimalna i maksimalna vrijednost; CV: coefficient of variability – koeficijent varijabilnosti

Effect of sex, age at slaughter and aging period on TL, CL, and WBSF of meat of Posavina horse breed is shown in table 2. Regarding the influence of sex, no significant differences for TL were found (p=0.056). The TL values ranged from 7.27% in females to 9.54% in males. In agreement with this, Tateo *et al.* (2008.) and Kaić *et al.* (2018.) also did not find significant differences for TL between meat from male and female horses. The TL was not significantly different with increasing age (p=0.312; β =0.07 % per month). De Palo *et al.* (2013.) and Kaić *et al.* (2018.) also did not found significant differences in TL among the meat of foals slaughtered at different ages. Although is known that the aging period could have a major effect on meat quality, the literature data shows that the optimum aging period for foal meat has not been defined yet. In addition, Kim and Brad Kim (2017.) indicated that different combinations of the aging period before freezing could improve the water-holding capacity of meat, particularly TL. Our results for the aging period (14 vs 28 days) did not reveal significant differences in TL among the LT muscles (p=0.241).

The CL values showed that there is no significant difference in meat from male (19.2%) and female (19.3%; $p=0.910$) animals. Contrary to that, Tateo *et al.* (2008.), Lorenzo *et al.* (2013.), and Kaić *et al.* (2018.) found differences in CL values between the sexes, with greater values found in meat from males. An increase in age was not followed by an increase of CL values ($p=0.107$; $\beta=0.15$ % per month). In agreement with our result, Franco *et al.* (2011.) also did not find differences in CL values among foals slaughtered at the age of 9 months and 12 months. Concerning the aging period, the results show that there is no significant difference in CL values between the meat aged 14 and 28 days ($p=0.388$).

The WBSF values indicated that there are no significant differences among males and females ($p=0.754$). Other studies, carried out on horse meat, also confirmed that there is no significant effect of sex on WBSF values (Tateo *et al.*, 2008.; Franco *et al.*, 2011.; Kaić *et al.*, 2018.). An increase of age was not followed by an increase in WBSF values ($p=0.854$; $\beta=0.04$ N per month). Contrary, Sarriés and Beriain (2006.) reported different WBSF values in LT muscle for 16-month-old foals (3.65 N) and 24-month-old foals (4.53 N). They explained that these differences could be due to a reduced gain rate of the older foals, which could be associated with lower protein synthesis and protein degradation. Based on their results they suggested that the meat of 24-month-old foals should be aged for a longer period than the meat from the younger 16-month-old foals. The increased aging period in the present study did not show significant differences in WBSF values ($p=0.314$). Although most studies agree that an increased aging period results in an improvement in tenderness, some of them indicate that aging may not be advantageous in all muscles (Kim and Brad Kim, 2017.). In addition to that, Kaić *et al.* (2018.) also confirmed that regarding the increased aging period, there were no significant differences in WBSF values among different muscles.

Table 2 Effect of sex, age at slaughter and aging period on thawing loss (TL), cooking loss (CL), and Warner-Bratzler shear force (WBSF) of Posavina horse breed

Tablica 2. Utjecaj spola, dobi pri klanju i dužine zrenja na kalo odmrzavanja (TL), kalo kuhanja (CL) i Warner-Bratzler silu presijecanja (WBSF) u mesu posavskog konja

	Sex / Spol		Age at slaughter / Dob pri klanju				Aging period / Zrenje					
	M	F	LSM	SE	p-value	$\beta \pm SE$	p-value	14	28	LSM	SE	p-value
TL, %	9.54	7.27	0.718	0.056	0.07 \pm 0.063	0.312	7.85	8.96	0.647	0.241		
CL, %	19.2	19.3	0.98	0.910	0.15 \pm 0.087	0.107	18.7	19.8	0.89	0.388		
WBSF, N	26.6	25.48	2.28	0.754	0.04 \pm 0.202	0.854	27.5	24.5	2.06	0.314		

M: males – muški; F: females – ženski; β = regression coefficient for age in months – koeficijent regresije; N= Newton – Njutn.

Conclusion

This study gave a new insight into the meat quality of Posavina horse breed. The results showed that thawing loss, cooking loss and Warner Bratzler shear force of meat of Posavina horse breed are not significantly affected by sex, slaughter age or aging period. It is expected that this study will contribute to further necessary research to complete an understanding of the meat quality of Posavina horse breed.

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UTJECAJ SPOLA, DOBI PRI KLANJU I DUŽINE ZRENJA NA SPOSOBNOST ZADRŽAVANJA VODE I MEKOĆU MESA POSAVSKOG KONJA

Sažetak

Cilj ovog rada bio je utvrditi sposobnost zadržavanja vode (kalo odmrzavanja – TL i kalo kuhanja – CL) i mekoću mesa (Warner-Bratzler sila presijecanja, WBSF) posavskog konja obzirom na spol, dob pri klanju i dužinu zrenja. U istraživanje je sveukupno bilo uključeno 12 životinja (6 muških i 6 ženskih) pasmine posavski konj. Prosječna dob životinja bila je 18 mjeseci (od 6 do 36 mjeseci). Uzorci mesa potrebni za daljnju analizu izuzeti su s najdužeg lednog mišića (*M. longissimus dorsi*) nakon zrenja od 14, odnosno 28 dana. Dobiveni podaci analizirani su pomoću SAS/STAT softverskog paketa verzija 9.4. Utvrđene srednje vrijednosti za TL, CL i WBSF su bile kako slijedi: 8,41%, 19,24% i 26,04 N. Rezultati istraživanja ukazuju da spol, dob pri klanju i dužina zrenja značajno ne utječu na sposobnost zadržavanja vode i mekoću mesa posavskog konja ($p > 0,05$). Istraživanjem su dobivene nove spoznaje o kakvoći mesa posavskog konja koje bi svakako trebale pridonijeti budućim istraživanjima u svrhu potpunijeg razumijevanja kakvoće konjskog mesa.

Ključne riječi: posavski konj, meso, kalo odmrzavanja, kalo kuhanja, mekoća