

# The incidence of difficult calvings in the beef cattle in the Slovak Republic

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Calving difficulty is one of important economic traits in beef production. In the Slovak Republic calving difficulty in beef cattle is recorded on the regular basis, however, these data have not been used so far in decision making. The aim of this study was to get the first insight into the data. Data on 288 346 calvings in 5 major Slovak populations (Charolais, Limousine, Slovak Simmental, Pinzgau, Beef Simmental) during the period 1990–2017 were exploited. The primary analyses showed that there exist differences in the incidence of the difficult calvings according to the breed of the dam. The lowest rate of assisted calvings was observed in Limousine breed. Lower incidence was observed when calvings of crossbred matings were considered. Only slight associations of difficult calvings with sex of calves, parity and season of calvings were observed. Slightly higher rates of assisted calvings were observed in the group of heifers and births of males.

**Keywords:** beef cattle, calving ease, dystocia, reproduction

## 1 Introduction

Calving difficulty is considered very important reproduction trait. It affects the economics of the breeding directly through costs for the labor and veterinarian assistance and indirectly through the consequent production and fertility of the cow and calf (López de Maturana et al., 2007, Atashi et al., 2012). The incidence of the difficult calvings varies across the populations. According to De Amicis et al. (2018) incidence of difficult calvings is more frequent in dairy cattle. In the beef cattle authors reported 6.6% and 6.2% of difficult calvings in Charolais and Hereford primiparous cows (Eriksson et al., 2004), 3.7% of difficult calvings in Canadian Simmental (Jamrozik and Miller, 2014). It was showed that difference in incidence exists between primiparous and multiparous cows (Fuerst and Egger-Danner, 2003). Some authors Môtus et al. (2017), Juozaitiene et al. (2017) even reported increased incidence of difficult calvings after the second one.

In the Slovak Republic the calving difficulty in beef cattle is part of the performance recording, however, these data are not used in the genetic evaluation. This study presents the first insight into the data in order to make further decisions on its use.

## 2 Materials and methods

The data on beef cattle performance recording are collected by the Breeding Services of the SR, s.e, and provided to the Institute of Animal Production Nitra on regular basis. In this study the data on 288,346 calvings in 5 major Slovak populations (Charolais, Limousine, Slovak Simmental, Pinzgau, Beef Simmental) during the period 1990–2017 were exploited. The calvings of twins were excluded from this analysis.

The recording of the calving difficulty in the Slovak Republic is based on the four-point scale with 1 referring to easy calving (no assistance needed) and 4 referring to caesarian section, and with additional category 0 referring to unobserved calving. Based on the practice the unobserved calvings ( $n = 19,383$ ) were included in easy calvings category (Phocas and Laloë, 2004). Based on published findings (Carnier et al., 2000, Steinbock et al., 2003), distinction was made between the first and later calvings. Females, which gave first birth by the age of 3 years were considered heifers and females which gave consecutive birth (first birth record was available) were considered multiparous cows. There were 75,734 calvings, where the parity was doubtful. These were mainly cows with the first recorded birth at higher age,

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which could result from moving animals from dairy to beef herd, or abortion (not recorded).

The incidence of difficult calvings according to sex (males, females), parity (heifers, cows) and season (spring, summer, autumn, winter) was tested by chi-square test within SAS software (Statistical Analysis System, Version 9.1, 2004). Since the large sample size was used and high significant levels were expected, the Cramers` V test was also applied to obtain strength of associations.

### 3 Results and discussion

The incidence of difficult calvings according to the breed of dam is summarized in Table 1. It is obvious that the most of the calvings were recorded as easy and overall only 4.9% of calvings required some form of assistance. When comparing the incidence of difficult calvings

among the breeds (purebred) the lowest rate of calvings requiring assistance was observed in Limousine (5.1%). In other three breeds incidence of these calvings was similar (6.2–7.7%). The incidence of calvings requiring assistance was lower in all breeds when crossbred matings were compared.

Due to large sample size statistical significance of chi-square is doubtful. The Cramers` V test showed that there are only very weak associations between the difficulty of calvings and the sex of calves (0.028), parity (0.036) and season (0.011). Indeed, when comparing the calvings according to the sex of calves, only slight difference was observed between the births of males and females (5.43% vs. 4.22%). This observation is in agreement with findings, that the males are born heavier and incidence

**Table 1** The distribution of calvings

		1 (no assistance)	2 (slight assistance)	3 (major assistance)	4 (caesarian)
Charolais	Pure <sup>a</sup>	7,663	521	38	3
	Cross <sup>b</sup>	79,508	4,060	290	12
Limousine	Pure	5,966	293	24	2
	Cross	75,676	3,138	220	18
Slovak Simmental	Pure	15,212	940	34	0
	Cross	68,167	2,923	93	3
Pinzgau	Pure	3,199	241	11	1
	Cross	7,050	290	17	1
Beef Simmental	Pure	1,148	76	9	1
	Cross	11,039	443	16	0
Total		274,628	12,925	752	41

<sup>a</sup> – calvings from matings of purebred bulls to females with max 12.5% of foreign blood, <sup>b</sup> – calvings from matings of purebred bulls to females with more than 12.5% of foreign blood

**Table 2** Distribution of calvings according to sex, parity and season

	1 (no assistance)	2 (slight assistance)	3 (major assistance)	4 (caesarian)
Sex				
Males	121,328	6,540	401	23
Females	153,300	6,385	351	18
Parity				
Heifers	67,982	4,403	319	20
Cows	133,735	5,863	276	14
Season				
Spring <sup>a</sup>	121,062	6,043	362	21
Summer <sup>b</sup>	55,894	2,820	150	5
Autumn <sup>c</sup>	27,428	1,201	74	1
Winter <sup>d</sup>	70,244	2,861	166	14

a – February–April, b – May–July, c – August–October, d – November–January

of difficult calvings is higher in this group (Atashi et al., 2012, McHugh et al., 2014).

The similar pattern was observed when parity of calving was considered. Slightly higher incidence of the difficult calvings was observed in heifers (6.52%) compared to multiparous cows (4.4%). Higher incidence of difficult calvings in heifers was similar to those reported in beef populations by Eriksson et al. (2004). Higher rates of assisted calvings in beef populations were reported by Phocas, F. and Laloë, D. (2004).

Negligible difference in the incidence of difficult calvings among seasons was observed. Authors (Uematsu et al., 2013; Mekonnen and Moges, 2016) reported higher incidence of difficult calvings in the winter and spring period. However, this was not observed in our study. Moreover, the lowest incidence was observed in the winter. This could result from keeping the beef cattle in barns during this period and allowing better control during calvings.

#### 4 Conclusions

The overall incidence of 4.9% was observed in the 5 major populations in the Slovak Republic. Slight differences in the incidence were observed among the breeds. Also only slight differences in the incidence were observed between the difficult calvings and the sex of calves, parity and season of the calving. Further analysis including other smaller beef populations and analysis of herd management on the calving difficulty will follow.

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## Enrichment of table eggs with selenium and lutein – our experiences

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By modifying meals for hens it is possible to influence the content of selenium and lutein in eggs, which enables the production of eggs with an increased share of the desired functional ingredients. Such eggs on the market represent enriched or functional foods that are characterized by preventive action in order to preserve the human health. The results of our research show that the composition of meals affects the content of selenium and lutein in eggs. The use of Se-yeast in hens' mixtures in the amount of 0.5 mg/kg of feed increases the selenium content in egg whites and yolks by 62.94% and 41.54% in comparison to eggs from hens fed with a conventional mixture. Addition of 400 mg/kg of lutein to a hens' mixture can enrich egg yolk with lutein by 86.93% with respect to eggs from hens fed with a conventional mixture (without the addition of lutein). By designing hens' mixtures using selenium and lutein having antioxidant activity, it is possible to produce eggs with improved nutritional value and extended shelf life.

**Keywords:** eggs, enrichment, lutein, selenium

### 1 Introduction

Egg is an excellent source of proteins, vitamins and minerals. If the feeding of hens with specially designed mixtures increases the content of some nutrient substances in eggs, then such eggs are called enriched and can be considered a functional product. Consumption of such products may have a preventative effect on the preservation of human health. Selenium and lutein are antioxidants whose content in eggs increases proportionally to their increase in hens' mixtures (Surai 2000, Kralik et al., 2009, Grčević et al., 2014, Jing et al., 2015, Fašiangová et al., 2017, Kralik et al., 2018). The importance of selenium and lutein in human nutrition is multiple. Selenium is an integral part of more than 25 selenoproteins and some antioxidative enzymes (glutathione peroxidase – GPx and superoxide dismutase – SOD) and affects regulation of various physiological functions in the body (Kryukov et al., 2003). It prevents inflammatory processes, protects against UV rays and reduces the risk of atherosclerosis (Ferencik and Ebringer, 2003). Lutein is carotenoid present in the eye lens, and has a protective function from the occurrence of cataracts. It protects against oxidative damage and blindness. Lutein

plays an important role in the prevention of age-related macular degeneration (AMD), a disease occurring in humans over the age of 65, a leading cause of blindness in the developed world. Lutein as an antioxidant reduces phototoxic damage to the protein and DNA of the eye lens (Gao et al., 2011). Given that the text outlines the range of benefits that arise when consuming eggs enriched with selenium and lutein, the aim of this paper is to show the possibilities of increasing their content in table eggs.

### 2 Material and methods

#### *Increasing of selenium content in eggs*

Selenium is found in nature in two forms, inorganic and organic. The inorganic form of selenium is sodium selenite, while the organic form of selenium used in poultry nutrition is Se-yeast and more recently different cereals bio-fortified with selenium. In feeding of hens for egg production, only the inorganic form of selenium was first used, while for a period of ten years organic form is used exclusively. The reason for the above was a series of studies that demonstrated better utilization of organic form of selenium from feed than inorganic form

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(Pan et al., 2007, Kralik et al., 2009, Jing et al., 2015, Kralik et al., 2016). It is known that microelements are present in traces in eggs, and mostly in egg yolk. Selenium is, however, specific in relation to other microelements because it is an integral part of a variety selenoprotein and is also incorporated into egg whites. In order to achieve the maximum increase of selenium in eggs, it is necessary to feed hens with specially prepared mixtures with increased levels of selenium during 4–5 weeks (Paton et al., 2002, Gajčević et al., 2009, Skřivan 2009, Jing et al., 2015). Skřivan (2009) states that if the inorganic form of selenium is added to feed for laying hens, it is possible to increase the selenium content in eggs from one and a half to two times compare to eggs produced without the addition of selenium to feed. However, if the organic selenium in the form of Se-yeast is added to feed, it is possible to increase the content of selenium in eggs at twice the level achieved by using sodium selenite. Many researchers in their papers point out the possibility of enriching eggs with selenium as well as placing such

eggs on the market as enriched or functional foods. Paton et al. (2002) reported that it is possible to increase the selenium content in egg whites from 80 ng-g to 150 ng-g and in yolks from 320 ng-g to 480 ng-g, by feeding hens with mixtures containing 0.1 mg·kg or 0.3 mg·kg Se-yeast for a period of 33 days. Aljamal et al. (2014) suggest that the content of selenium in eggs increases in accordance with its content in feed. Thus, when hens are fed mixtures containing 0.2 mg·kg of selenium, the selenium content in egg whites is 2.33 mg·kg of dry matter and in yolks 1.57 mg·kg dry matter. The same authors state that increased selenium content in feed (0.4 mg·kg) increases the content of selenium in the edible part of the egg (egg whites = 2.93 mg·kg of dry matter and egg yolk = 1.63 mg·kg of dry matter). Table 1 shows the results of our research aimed at enriching of eggs with selenium.

#### Increasing of lutein content in eggs

Lutein is a plant pigment found in green leafy vegetables. It is main constituent of specific eye tissues like yellow

**Table 1** The impact of selenium level and source on content of selenium in the edible part of the egg

Source of Se	Level of Se in feed (mg·kg)	Se content in egg white (mg·kg)	Se content in egg yolk (mg·kg)	Reference
Se-yeast	0.2	0.232	0.585	Gajčević et al. (2009)
	0.4	0.345	0.780	
Inorganic Se	0.2	0.181	0.573	Kralik et al. (2009)
Inorganic Se	0.4	0.230	0.757	
Se-yeast	0.4	0.345	0.783	
Bio-fortified corn	0.233	0.073	0.303	Kralik et al. (2017)
	0.426	0.207	0.626	
Inorganic Se	0.5	0.053	0.387	Kralik et al. (2018)
Se-yeast	0.5	0.143	0.662	

**Table 2** Enrichment of eggs with lutein

Type of eggs	Lutein addition (mg·kg feed)	Lutein content (mg·60 g egg)	Reference
Free range eggs	0	0.55	Grčević et al. (2014)
Table eggs (producer 1)	0	0.24	
Table eggs (producer 2)	0	0.20	
Table eggs	0	0.20	Grčević (2015)
	200	1.38	
	400	1.53	
Omega-3 enriched eggs	0	0.33	
	200	1.62	
	400	1.78	
Designed eggs (enriched with omega-3, selenium, vitamin E, lutein)	0	0.19	Kralik et al. (2018)
	200	1.61	



spot (*macula lutea*) and eye lens. Since it has antioxidant properties and effectively filters high-energy blue light, it is very important for eye health. It is found that lutein is very important for central vision and visual acuity (Landrum and Bone, 2001) and that it has a crucial role in delaying of age-related macular degeneration, a major cause of blindness in older people (Gale et al., 2001). Lutein is soluble in fats and oils so it accumulates very well in the egg yolk, and this is also a reason for its better bioavailability from eggs than from other sources, like vegetables or food supplements (Chung et al., 2004). Its content in egg yolk can be affected by the addition of increased levels of lutein to laying hens' feed. An overview of own results on enrichment of eggs with lutein is presented in Table 2. From the results shown in the Table 2 it can be noticed that already 200 mg/kg lutein is enough for significant enrichment of eggs with lutein. Many different authors have been conducting research on enriching eggs with lutein. First of them were Leeson and Caston (2004) who studied the efficacy of transferring lutein from feed to the egg. They added lutein in corn-soy diets ranging from 0 mg/kg to 1,000 mg/kg, increasing the lutein level by 125 mg/kg for each subsequent treatment. The best effect of egg enrichment was achieved by addition of 500 mg/kg of lutein to the mixture. The content of lutein in egg increased from 0.16 mg per 60 g egg in group without lutein to 1.49 mg per 60 g egg with the addition of 500 mg/kg lutein.

In the research of Leeson et al. (2007) the maximum enrichment of egg with lutein was observed at the addition of 125 mg/kg of lutein to the mixture. Lutein level in egg rose from 0.09 mg per egg to 1.67 mg per egg, while further addition of 250 mg of lutein to the mixture did not affect the increase of lutein level in the egg (1.61 mg per egg). Golzar-Adabi et al. (2010) also investigated the effect of lutein supplemented to mixtures for laying hens on the content of lutein in eggs. They added 0, 250, 500 and 750 mg/kg of lutein to mixtures based on corn and soybean. Supplementation of 250 mg/kg lutein resulted in most significant increase of lutein level in egg, which rose from 0.12 mg per 57 g in control group to 1.35 mg per 57 g of egg in experimental group. The results of the above-mentioned research confirm the fact that the addition of lutein to hens' mixtures affects the level of lutein in the eggs, with the best results being achieved with an addition of not more than 500 mg/kg of lutein.

#### 4 Conclusions

Different research in egg production has shown that it is possible to enrich eggs with selenium and lutein, while clinical studies have shown that eggs enriched with the specified ingredients can be functional foods. It is desirable to use eggs enriched with selenium and lutein

in human nutrition to increase their daily intake into the body. In addition, increasing the content of selenium and lutein in eggs has a positive effect on the quality and freshness of the eggs.

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## Effects of the tax burden on the production of fattening pigs

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The paper analyses how much tax burden affects production volume and budget revenues. The projections show what happens when a government increases the tax rate (added value tax), i.e. how it reflects on the volume of fattening pig production on the one hand and how it reflects on the level of tax revenue. Fiscal policy represents a complex area through which the state sets out the operating conditions through various instruments. In this paper, the simulation of the tax rates and the effects on the produced quantities is made on pig meat production. By imposing large taxes, taxpayers feel less incentive to make money, because whatever they earn, they go to the state. A more detailed analysis was made on a sample of fattening production of 100,000 animals. Some implications of established empirical links between variables are presented in the paper. The change in tax rate of 10% multiplies an increase in tax reported in cash in amount of 44.4%.

**Keywords:** added value tax, production of fattening pigs, tax rate, tax revenue

### 1 Introduction

The production of fatteners in the Republic of Croatia represents a very important part of the economy. Various theoretical studies indicate that at a tax rate of zero percent and at a rate of one hundred percent the tax revenue is equal to zero. With the increase in tax rates, also tax revenues increases, but after some threshold, the tax rate increase does not fill the state budget (Baumol and Blinder, 1991), but vice versa. Namely, high tax rates unstimulate people from working and saving. In a case of high tax rates, people move their economic activity to an area of the unofficial economy, or they decide for more leisure and less work. By increasing the tax rate, the state has therefore failed to reach an acceptable measure of fiscal policy: state revenues are lower, work effort is lower, and also investments and economy growth decreases. In case increasing of the factors of production increase, domestic products are not competitive and the export of agricultural products is reduced (Messere, 2009). Therefore, the aim of this paper was to show the importance of optimization of the added value tax rate in the production of fattened pigs.

### 2 Materials and methods

The calculation of the sale price of pig meat was made on the basis of 100,000 pigs on an average of 100 kg.

Following was calculated: fixed costs, variable costs, mixed costs and earnings. Furthermore, the offer of companies and the offer of the branch was analysed in accordance to the microeconomic model of Varian (2015). Final considerations will be made on the basis of a comparison of the tax rate with the level of tax revenue. This method shows the consequences for the revenue side of the budget if the tax rate varies, and how this reflects on the total volume of production. Analysed data were provided by following producers: Belje d.d., Protein Bobota Ltd., Pivac Ltd. and PIK Vrbovec and refer to average wages. In this study following methods were used: the analytical method and the case study method. Prices are expressed in Euro. The table 1 shows the average costs for the production of fatter live weight 100 kg and the calculated price per kilogram of pig meat.

**Table 1** The elements forming the cost price of pig meat

Elements of calculations	Price of fatter, live weight – 100 kg (Euro)	Price of pig meat, kg (Euro)
Fixed costs	10.67	0.11
Variable Costs	94.76	0.95
Mixed costs	12.53	0.13
Cost price	117.96	1.18

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### 3 Results and discussion

In the analysis of operating costs, the calculation of the selling price of pig meat at different rates of value added tax (hereinafter referred to as VAT) is formed. VAT is a multi-stage sales tax that is calculated at each stage of the production and sales cycle, but only on the amount of value added that was formed at that stage, not on the total production value. It should be noted here that the EU legislation allows each member to apply a reduced VAT rate to certain goods. By analysing the production of 100,000 average fatteners live weighs of 100 kg, the calculation of the sales at different rates of VAT is made, the review is given in the Table 2.

From this analysis it can be seen that at various tax rates (10%, 13%, 15% and 18%) tax varies. The lowest tax is 1.179.586,67 Euro, and the highest tax is 2.123.256,00 Euro. The difference between the largest and the lowest tax is 943.669,33 Euro or 55.56%. Although the difference is the largest compared to the lowest analysed 10% tax rate, the amount of tax is multiplied by 6.5 times. In continuation, at Figure 1 the offer of the branch is shown.

Presented offer of a branch was created on the basis of costs and different tax rates. The parts of the offer marked with red colour presents a taxable part. It is visible that shifts at lower tax rate are more straightforward than those where the tax rate is higher. As the tax rate increases, the bargaining tendency becomes more straightforward and vice versa, when the tax rate drops, the bids become more and more straightforward. In the branch with free entry and expulsion of goods to the market a long-term average cost curve would have to be a straight line at a price level equal to the minimum average cost. This is the long-term supply curve of a company that has constant yields on volume (Varian, 2015). Since our market is free, that is, entry and exit from the market is free, in the short run the supply branch will have a positive slope (upward), while in the long run it becomes more flat at the price level with the same average cost (Barro, 1987).

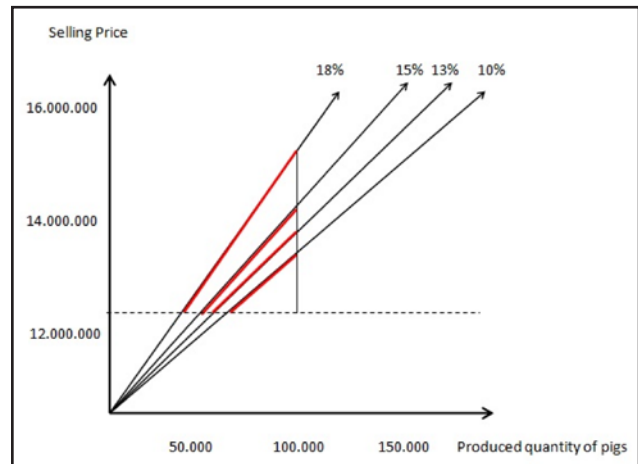


Figure 1 The offer of the branch

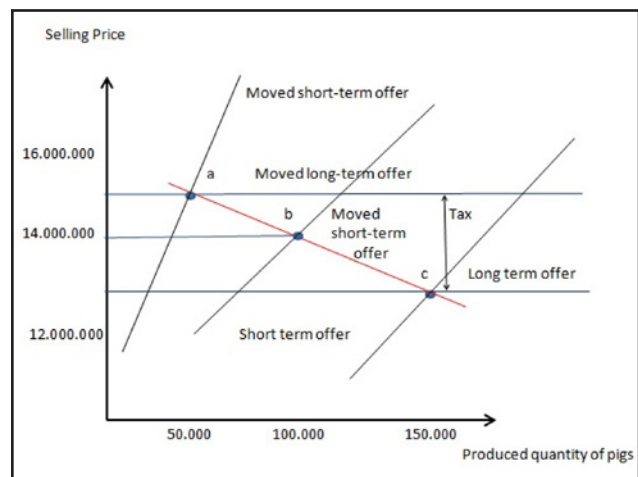


Figure 2 Changes in tax rates and impact on offer and demand of fatteners

The introduction of higher taxation faces end-consumers with higher prices of products (Samuelson, 1992).

Figure 2 shows the behaviour of the offer and demand curve due to changes in the tax rates that reflects on the changes in the prices. Blue horizontal lines represent

Table 2 Calculation of sales price of fatteners (Euro)

Elements of calculations	Calculation of sales prices at different rates of VAT			
	10%	13%	15%	18%
Fixed costs	1.066.666,67	1.066.666,67	1.066.666,67	1.066.666,67
Variable Costs	9.475.866,67	9.475.866,67	9.475.866,67	9.475.866,67
Mixed costs	1.253.333,33	1.253.333,33	1.253.333,33	1.253.333,33
Cost price	11.795.866,67	11.795.866,67	11.795.866,67	11.795.866,67
Amount of VAT	1.179.586,67	1.533.462,67	1.769.380,00	2.123.256,00
Selling Price	12.975.453,33	13.329.329,33	13.565.246,67	13.919.122,67

Made by the author according to the obtained data

long-term offer curves. Black vertical lines represent short-term offer curves. The red line is the demand curve. The point c represents the introduction of tax. At this point, the demand for 150,000 fatteners is projected, while the short-term offer curve is under 45 degrees' slope. Point b represents the pay-price limit for fatteners production, and shows that in case of introduction of tax rate 13%, the price is 13,418,787.92 Euro, the demand is reduced to 100,000 fatteners and the short-term offer becomes steeper. As the price rises higher, because of the introduction of more tax rates the market suffers the pressure, and the demand is reduced to 52,000 fatteners, the short-term curve is completely upright, the long-term curve is shifted upwards and the cross-section with the wrong demand is in point a.

#### 4 Conclusions

The research results indicate that taxable income varies at different tax rates. Based on the analysis of the offer and demand relationships, the long-term and the short-term period it could be concluded that at higher tax rates the offer curve is upward and vertically set, and crosses the demand curve in the higher part of the chart. Otherwise, when the tax rate is low, the short term offer curve is more straightforward and crosses the demand curve in the lower part of the chart resulting in more favourable entrepreneurial conditions.

When determining the tax rate, its creators must be very vigilant and well defined in the short and long term, in order for the fiscal measures to be stimulating and in function of the growth of the economy. State tax policy is a powerful weapon that can act on the market. It is important to emphasize that the state can also have negative consequences if it wants to raise the tax rate above the realistic framework.

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## Consumers' sensory analysis of beef hamburger and tartare

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In the last years, consumers preferences are more likely to purchase processed-meat products instead of single meat cuts. To adapt to the new demand, beef industry needs to study consumers' sensory perception of the newly developed products to ensure their acceptability. This study aimed to examine consumers' perception on sensory characteristics of two commercial processed-meat products (hamburger and tartare) from three different types of meat (Holstein-Italian bull, Charolaise bull, and Charolaise heifer). Sensory consumer test was conducted on a non-trained panel of 64 participants for each product to assess color, texture, odor, tenderness, juiciness, salty taste, flavor and overall satisfaction using a 1 (very low) to 7 (very high) intensity scale. Sensory data of each product was analyzed through a linear mixed model including meat type and order of presentation as fixed effects, and participant and residual as random effects. Chemical composition was determined by standard methods in 9 samples of each product (3 samples/type of meat). For hamburgers, protein ranged from 15.9 to 17.0% and fat from 9.1 to 12.9%. For tartare, protein ranged from 20.1 to 20.3%, and for fat from 3.2 to 5.2%. For hamburger, participants perceived differences ( $P < 0.05$ ) in color, tenderness and juiciness between meat types, being the Charolaise bull the most appreciated ( $P < 0.05$ ). For tartare, panelists reported differences ( $P < 0.05$ ) in color, texture and tenderness, being the Charolaise heifer the most appreciated ( $P < 0.05$ ). Our results suggested that the type of meat used related to the fat content can modify consumers' sensory perception of processed-meat products.

**Keywords:** bull, heifer, meat quality, processed-meat

### 1 Introduction

In the last decades, changing lifestyles have led to shifting consumer's preferences to purchase more processed-meat (or partially-prepared) products than fresh meat (Resurreccion, 2004; Grunert, 2006). While the poultry industry has been adapted to the new consumers' demands, beef sector has remained more unchanged, gradually losing its share of the meat market (Resurreccion, 2004). Therefore, the beef industry seeks to develop newly processed or partially-prepared products but understanding consumers' sensory perception of these products is fundamental to ensure their acceptability.

Variation in meat sensory quality depends on a wide range of productive (e.g., breed, sex, age, slaughter weight, and diet) and technological factors (e.g., management, refrigeration and aging time). Campo et al. (1999) and Monsón et al. (2005) have reported a breed effect on quantity of residue after chewing, tenderness, overall odor and acid flavor, and Chambaz et al. (2003) for

tenderness and juiciness. Heifers deposit more fat than bulls, and their meat presented better characteristics in terms of eating quality because a greater amount of fat and unsaturated fatty acids in the meat is closely related to a better sensory evaluation (Venkata et al., 2015). Indeed, Bureš and Bartoň (2012) reported leaner carcasses in bulls than heifers, and in a sensory panel, meat from heifers was perceived as more tender and more acceptable. Therefore, the aim of this study was to examine consumers' perception of sensory characteristics of two commercial processed-meat products (hamburger and tartare) from three different types of meat (Holstein-Italian bull, Charolaise bull, and Charolaise heifer) using consumer tests.

### 2 Materials and methods

Hamburgers and tartare were provided by AZOVE Carni s.r.l. (Ospedaletto Euganeo, Italy), which is an important meat industry in the Veneto Region (North-East Italy), in individual skin packaging and cold stored at 4 °C. Three different types of hamburgers and tartare were

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prepared from different meat types regarding beef breed and sex [(i) Holstein-Italian bull (HIB), (ii) Charolaise bull (CB) and (iii) Charolaise heifer (FH)] following AZOVE Carni s.r.l. internal recipe. All the animals were reared in the associated herds of AZOVE Carni s.r.l.; the HIB were born in Italian commercial dairy farms while the CB and CH were imported from French herds and reared and slaughter in Italy. Information about management practice and feeding condition can be retrieved from Gallo et al. (2014). The hamburgers were obtained from the flank steak and the tartare from the boneless roast.

For chemical composition a total of 9 hamburgers and 9 tartare (3 of each type of meat) were analyzed in the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Italy). Dry matter was determined at 103 °C for 24 h and ash content was measured gravimetrically by igniting samples in a muffle furnace at 550 °C for 4 h (AOAC, 2000). Protein content and ether extract were determination by Kjeltec 2300 and Soxhlet 255 Foss Tecator (Foss Electric A/S, Hillerød, Denmark), respectively (AOAC, 2000). Physical traits – pH, drip loss and color [lightness ( $L^*$ ), red index ( $a^*$ ), yellow index ( $b^*$ ), saturation index (SI)] – were analyzed according to De Marchi et al. (2009). In hamburgers cooking loss and tenderness was evaluated after cooking the hamburger in a hot water bath (75 °C, 45 min). In tartare, tenderness was measured on the raw product. Color traits were determined using the colorimeter Minolta CM-600d (Konica Minolta, Japan) averaging 5 readings for each hamburger sample, and 3 readings for each tartare sample. Color was measured when the pack was opened.

The consumer test was conducted in the Sensory Analysis Laboratory (AnSen-LAB) of DAFNAE to judge color, texture, odor, tenderness, juiciness, salty taste, flavor and overall acceptability on a 7-point scale from very low (1) to very high (7) intensity. A total of 128 non-trained panelists were recruited from a database of students of the University of Padova in Legnaro (Padova, Italy) after compiling a survey to determine their socio-demographic characteristics and acceptability to consume beef hamburger and tartare. Gender equality was assured (55% female and 45% male), and the average age was 21.34 (standard deviation, 2.10). Panelists were randomly split into 8 groups of 16 panelists each. Hamburger and tartare were tested in 2 consecutive days. One day, 4 groups analyzed the hamburger, and the next day, the other 4 groups evaluated the tartare. In each session (30 min) the methodology was explained to the group before starting. Hamburgers were cooked in a hot water bath (75 °C, 60 min) before each session. All samples were presented to the panel at room temperature. For

each product, the order of presentation of the samples was: group 1, HIB-CB-CH; group 2, HIB-CH-CB; group 3, CB-HIB-CH; group 4, CH-CB-HIB. Spring water and unsalted bread were provided to clean their mouths between samples.

Consumer test data was analyzed using the PROC MIXED of SAS ver. 9.4 (SAS Institute Inc., Cary, NC). Each product was analyzed separately considering the type of meat (HIB, CB and CH) and the order of presentation (1 to 4) as fixed effects, and participant and residual as random factors. Multiple comparisons of least squares means were performed for the fixed effects using Bonferroni's test. Differences were considered significant at  $P < 0.05$ , unless otherwise indicated.

### 3 Results and discussion

Chemical composition and physical traits within each type of product, were similar among the type of meat used (Table 1). The greatest fat content for CH in tartare could be due to the greater fat deposition of heifers than bulls (Venkata et al., 2015). However, that relation was not observed for hamburgers probably due to a lack of standardization of the trimming process between the different types of meat. The statistical model revealed that for hamburgers the order effect was only significant for the assessment of color, whereas the meat type effect was significant for color, tenderness, juiciness and overall satisfaction. On the other hand, for tartare samples, the order was significant for odor and tenderness, while meat type was significant for color, texture, tenderness and overall satisfaction.

For the hamburgers, color was darker in HIB and CH than in CB hamburger, whereas tenderness, juiciness and overall satisfaction were greater in CB than in HIB and CH ( $P < 0.05$ ; Table 2). The brighter color perceived for the CB is in agreement with the greater  $L^*$  and lower SI reported in Table 1. For tartare, color and texture of Italian-Holstein differed from Charolaise (CB and CH), whereas tenderness and overall satisfaction differed between bull and heifer meat ( $P < 0.05$ ; Table 2). The greater color score for HIB agreed with the lower  $L^*$  and greater SI presented in Table 1.

Consumers perceived greater scores for color (darker) and texture (grainier) in meat as worse sensory quality, which suggests the lower suitability of HIB meat to produce tartare. Venkata et al. (2015) have reported that as fat increases, meat brightness does, which could explain the brighter color in CB hamburgers. Also, fat is considered an important contributor to tenderness and juiciness (Venkata et al., 2015) which could explain the greater tenderness, juiciness and overall acceptability of CB for hamburgers and CH for tartare.

**Table 1** Chemical composition (dry matter basis) and physical traits of hamburger and tartare for each type of meat<sup>a</sup>

	Hamburger			Tartare		
	HIB	CB	CH	HIB	CB	CH
Chemical composition (%)						
Dry matter	29.7(0.16)	32.3(0.60)	30.3 (0.88)	26.6(0.26)	27.3(0.38)	28.8(0.08)
Protein	17.0(0.47)	15.9(0.33)	16.2 (0.32)	20.1(0.06)	20.3(0.11)	20.3(0.09)
Fat	9.10(0.20)	12.9(0.33)	10.2 (0.97)	3.20(0.08)	3.26(0.16)	5.20(0.06)
Ash	2.00(0.03)	1.99(0.05)	2.07 (0.03)	2.19(0.03)	2.21(0.04)	2.14(0.02)
Physical traits <sup>a</sup>						
Drip l. (%)	1.54(0.12)	1.31(0.16)	1.58(0.21)	1.06(0.01)	0.77(0.01)	1.30(0.01)
TEND (N.g <sup>-1</sup> )	19.6(0.51)	14.9(0.24)	18.9(0.67)	11.4(0.82)	6.70(0.55)	6.50(1.96)
Color						
L*	40.8(0.45)	43.8(0.27)	41.7(1.48)	37.5(0.71)	39.5(0.28)	39.1(0.94)
a*	15.5(0.95)	14.7(0.40)	15.7(0.31)	16.8(0.29)	15.4(0.16)	16.9(0.14)
b*	9.30(1.03)	10.3(0.20)	10.4(0.31)	11.3(0.37)	10.5(0.06)	11.2(0.85)
SI (%)	18.1(1.32)	17.9(0.38)	18.9(0.08)	20.2(0.01)	18.6(0.16)	20.3(0.59)
CL (%)	32.1(0.10)	29.2(1.20)	32.7(0.10)	n.c.	n.c.	n.c.

<sup>a</sup> HIB – Holstein-Italian bull; CB – Charolaise bull; CH – Charolaise heifer; b TEND – tenderness calculated for hamburger after cooking, and in the raw product for tartare; L\* – lightness; a\* – red index; b\* – yellow index; SI – saturation index; CL – cooking loss. Color traits were calculated when the package was opened. n.d., not calculated  
 Values are mean (standard deviation)

**Table 2** Consumers' test sensory analysis of hamburger

	Hamburger			Tartare		
	HIB	CB	CH	HIB	CB	CH
Color	3.59 <sup>a</sup>	2.98 <sup>b</sup>	3.39 <sup>a</sup>	5.28 <sup>a</sup>	4.40 <sup>b</sup>	4.67 <sup>b</sup>
Texture	3.66	3.62	3.79	4.46 <sup>a</sup>	4.08 <sup>ab</sup>	3.77 <sup>b</sup>
Odor	3.07	3.09	3.14	3.69	3.90	3.92
Tenderness	3.78 <sup>b</sup>	4.95 <sup>a</sup>	3.78 <sup>b</sup>	4.50 <sup>b</sup>	4.69 <sup>b</sup>	5.87 <sup>a</sup>
Juiciness	2.83 <sup>b</sup>	4.09 <sup>a</sup>	3.38 <sup>b</sup>	3.52	3.49	3.78
Salty taste	3.04	3.19	3.10	2.73	2.76	3.12
Flavor	3.50	3.65	3.62	3.44	3.25	3.69
Overall acceptability	3.81 <sup>b</sup>	4.71 <sup>a</sup>	4.03 <sup>b</sup>	3.85 <sup>b</sup>	3.77 <sup>b</sup>	4.42 <sup>a</sup>

<sup>a, b, c</sup> – Values within a row for each product (Hamburger or Tartare) with different superscript letters differ significantly at  $P < 0.05$

Consumers' test sensory analysis of hamburger (Table 2): 64 participants and tartare 64 participants for each type of meat (HIB, Holstein-Italian bull; CB, Charolaise bull; CH, Charolaise heifer). Attributes were assessed on a 7-point scale from very low (1) to very high (7) intensity. Values are least squares means.

#### 4 Conclusions

Our results indicated that consumers' sensory perception of processed meat products may differ as a function of the type of meat (breed and sex) used to prepare them.

Charolaise bull and heifer were the most appreciated meat for hamburgers and tartare, respectively, and their greatest tenderness and juiciness evidenced a close relation with fat content of the product. However, further investigation is needed to discriminate the breed and sex effect from the fat content and fatty acid profile effect.

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## Evaluation of somatic cells in milk of ewes as possible physiological level

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The physiological values of SCC in sheep's milk are still under discussion. The aim of study was to describe the frequency of distribution of ewes on the basis of their individual SCC. The ewes were divided into the five SCC groups (somatic cell count) on the basis of individual SCC (G1 = SCC <math>200 \times 10^3 \text{ cells.ml}^{-1}</math>, G2 = SCC between

**Keywords:** ewe, milk, somatic cell counts

### 1. Introduction

A serious health and economic problem in dairy sheep is mastitis, which causes economic and breeding losses. SCC in the milk of individual dairy sheep can be used as an indicator of husbandry and milking hygiene, the welfare of animals, but especially the udder health and the presence of subclinical mastitis. In dairy sheep the mastitis leads to decrease in milk production and its quality, increase presence of pathogens inappropriate for consumption of dairy products and increase cost for treatment (Riggio and Portolano, 2015). The prevalence of subclinical forms of ewes mastitis ranges from 5 to 50% (Bergonier et al., 2003; Contreras et al., 2007; Olechnowicz and Jaskowski, 2014). From the preliminary results obtained in our dairy practice the incidence of subclinical mastitis ranged from 10–43% (Tančín et al., 2017b). In last mentioned work the criterion for subclinical mastitis was based on SCC (somatic cell count) over

The physiological values of SCC in sheep's milk are still under discussion. Pengov (2001) determined a limit value for physiological SCC in sheep milk of

The aim of study was to describe the frequency of distribution of ewes on the basis of their individual SCC per test day in selected SCC groups during whole lactation. Additionally if high level of somatic cells per lactation could affect somatic cells during following lactation.

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## 2 Materials and methods

This study was realized by the experimental herd of the NPPC Research Institute for Animal Production Nitra, Slovakia, during 2016 and 2017. Experimental ewes were in their first to third lactations of Slovak dairy sheep (SD) and Lacaune (LC) breeds. Machine milking was performed two times a day in milking parlour 1\*16. During each milking the ewes received in parlour 0.1 kg concentrate per head. The milk yield recording and milk sampling were performed once a month during morning milking as a part of milk recording services. Samples of milk were taken in 2016 from April to September and in 2017 from April to August.

### Laboratory analysis

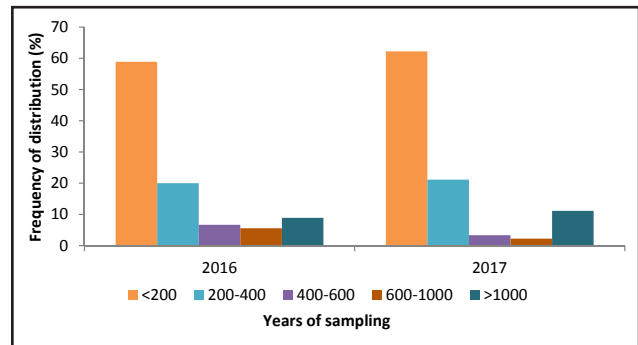
Milk samples from each udder were transported to the certificated Central laboratory of Breeding services of the Slovak Republic (Plemenárske služby š.p. Bratislava) for milk analysis.

### Categories of somatic cell count (SCC)

For evaluation only ewes with 4 and more sampling during lactation within both 2016 and 2017 were included into study. Thus minimum eight observations were available per animal. A total of 771 milk samples from 73 SD ewes and 17 LC ewes were individually collected. On the basis of individual SCC from milk recording the ewes were divided into the five SCC groups: G1 = SCC <200 × 10<sup>3</sup> cells.ml<sup>-1</sup>, G2 = SCC between 200–400 × 10<sup>3</sup> cells.ml<sup>-1</sup>, G3 = SCC between 400–600 × 10<sup>3</sup> cells.ml<sup>-1</sup>, G4 = SCC between 600–1,000 × 10<sup>3</sup> cells.ml<sup>-1</sup> and G5 = SCC >1,000 × 10<sup>3</sup> cells.ml<sup>-1</sup> to evaluate the distribution of individual ewes into SCC groups in different months and years of study. Additionally animals were individually divided into above mentioned SCC groups on the basis of their SCS per lactation (somatic cell score) calculated as a mean from transformed individual SCC data into SCS obtained during milk recording throughout lactation. SCS was calculated according formula  $SCS = \text{LOG}_2(\text{SCC}/100)/0.693147 + 3$ . Thus distribution of ewes on the basis of SCS into SCC groups was done by conversion of linear scores to somatic cell counts. Mathematical analysis was done by Microsoft Excel program.

## 3 Results and discussion

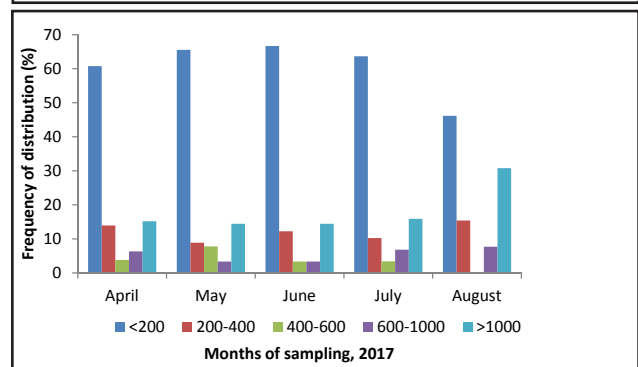
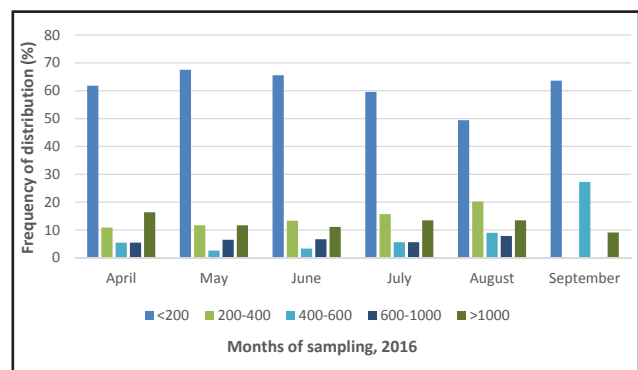
On the basis of SCS throughout lactation the most ewes were observed in first two SCC groups (below 400 × 10<sup>3</sup> cells.ml<sup>-1</sup>) in 2016 and 2017 (78.89% and 83.33%, respectively) (Figure 1). In 2016 13 animals (8SD, 5LC) were in SCC groups over >600 × 10<sup>3</sup> cells.ml<sup>-1</sup>, however in next lactation only 6 of them did not improve SCC in next lactation (2017) during dry period, where 5 of them belonged to Lacaune breed. Based on these results, it would be possible for farmers to select such animals for



**Figure 1** Frequency of ewes in SCC groups (× 10<sup>3</sup> cells.ml<sup>-1</sup>) based on SCS per lactation in 2016 and 2017

culling from the herds on the SCC data. In 2017 12 animals were in above mentioned SCC groups (4SD, 8LC).

Data shown in figure 2 presents frequency of distribution of animals in different months of milk recording in 2016 and 2017, respectively. In both years the most of the ewes were in SCC group below 400 × 10<sup>3</sup> cells.ml<sup>-1</sup> indicating a good individual udder health of studied ewes. On the other side the percentage of ewes in both last SCC groups (mainly over 1,000 × 10<sup>3</sup> cells.ml<sup>-1</sup>) is relatively low and thus could be considered as health problem of udder. Our data contribute to the findings of Leitner et al. (2008) and Arias et al. (2012) related to physiological level of SCC in ewes.



**Figure 2** Frequency of ewes in SCC groups (× 10<sup>3</sup> cells.ml<sup>-1</sup>) based on individual SCC in different months of sampling and year

In our previous studies, Idriss et al. (2015) concluded that 78% of the samples were below  $600 \times 10^3$  cells.ml<sup>-1</sup>. In the sample group, up to  $100 \times 10^3$  cells.ml<sup>-1</sup>, the largest percentage of Tsigai and Improved Valachian (Idriss et al., 2015) were found out. Vršková et al. (2015) found that 76% of Tsigai had SCC below  $300 \times 10^3$  cells.ml<sup>-1</sup>. Tančin et al. (2017a) in their study found that 82.03% of the milk samples were below the  $400 \times 10^3$  cells.ml<sup>-1</sup>, 71.79% of the milk samples were below the  $200 \times 10^3$  cells.ml<sup>-1</sup> and only 8.89% of the samples were above  $1,000 \times 10^3$  cells.ml<sup>-1</sup> with the possible effect of breed and farm.

#### 4 Conclusion

The results our study indicated that the most of the animals were in SCC group below  $400 \times 10^3$  cells.ml<sup>-1</sup>. In some ewes the high SCC during whole previous lactation negatively influenced SCC in following lactation which could be used in culling program. Possible physiological level of SCC could be taken into account but more data in dairy practice should be evaluated. Thus regular milk recording should include also SCC analysis.

#### Acknowledgements

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## Fatty acid composition of Baranjski kulen from two diverse production systems

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The aim of the present study was to compare the fatty acid composition of dry fermented sausage made from Crna slavonska pig and modern hybrids reared in diverse production systems. The study was performed on Baranjski kulen, a traditional PGI (Protected Geographical Indication) labelled sausage. Baranjski kulen produced from pigs included in the study differed in their fatty acid profile, with kulen made from Crna slavonska pigs having a higher content of MUFA, lower content of PUFA and a more favourable PUFA/SFA ratio. The results of the present study demonstrate that meat originating from breeds raised in specific production system affects the fatty acid composition as one of the major determinants of nutritional product quality.

**Keywords:** autochthonous breed, fatty acids, nutritional quality, pigs

### 1 Introduction

Today there is a growing interest in the consumption of traditional meat products made from local pig breeds, as these products are considered to be of higher eating and nutritional quality than those made from conventional pig breeds and their hybrids. The fatty acid composition of pig muscle and fat tissue, being one of the most important nutritional quality traits, is influenced by several factors including fatness, body weight, age, gender, diet as well as the genetic background (Karolyi et al., 2007; Kasprzyk et al., 2015; Nevrlka et al., 2017). Crna slavonska (CS) pig is an autochthonous fatty-lean type of pig, with fat/meat ratio at *longissimus lumborum* cut being around 1 when raised outdoor (Djurkin Kušec et al., 2017) and with better meat quality traits (colour, final pH, water holding capacity) than PIC hybrids (Komlenić et al., 2018), making it particularly suitable for the production of traditional meat products. However, the investigations on its meat fatty acid composition are scarce and most certainly they have never been performed on traditional meat products.

Therefore, the aim of the study was to compare the fatty acid profile of Baranjski kulen as a traditional meat

product, made from CS pigs and modern pig hybrids as two genotypes raised in diverse production systems.

### 2 Materials and methods

The study was performed on traditional PGI labelled Croatian sausage Baranjski kulen (BK) produced from CS pigs and PIC hybrids. The pigs (16 animals per group) were raised according to the production system optimized for a particular breed, i.e. Crna slavonska pigs outdoor (on pasture) and PIC hybrids indoor (intensive production system). After the end of fattening period, pigs were slaughtered in a commercial slaughterhouse. At 24 hours *post mortem* ham and shoulder were sectioned from the carcasses and used for production of BK.

BK were produced according to recipe and technology described in details in the Specification (HR/PGI/0005/01207). From each production system, six parallel samples were analyzed for fatty acid profile.

The fat was extracted using Soxhlet method, in which the samples are digested with acid hydrolysis and the fats are then extracted with petroleum ether using a Soxtherm 2000 automated device (Gerhardt, Munich, Germany). The extracted lipids were converted into fatty

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acid methyl esters (FAMES) for gas chromatography (GC) analysis according to ISO 12966-2:2011. The procedure of preparing the samples for GC analysis and determination of fatty acid composition is described in detail by Medić et al. (2018). The determination of fatty acid composition was performed using GC/MS 5975C device (Agilent Technologies, Palo Alto, CA, USA) equipped with flame ionization detector and split/splitless injector. A TriPlus auto-sampler (Thermo Scientific, Austin, TX, USA) was used for the injection.

The obtained data were analyzed using ANOVA procedure of Statistica ver. 10.0 Software (StatSoft Inc. 1984–2011, USA). Least square (LS) means were computed and compared using the Tukey's honest significant difference (HSD) test, where  $P < 0.05$  was classified as significant difference and  $P < 0.1$  as a tendency.

### 3 Results and discussion

The profile of fatty acids in BK produced from pigs reared in two production systems is presented in Table 1.

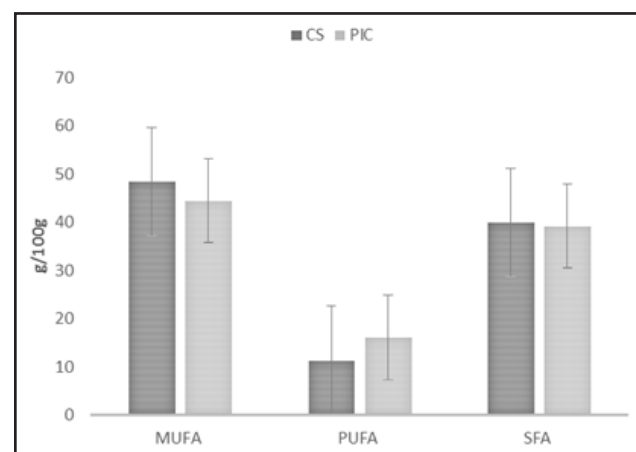
**Table 1** Fatty acid profile (LS means) of pork with regard to the production system

Fatty acid	Production system		SEM
	CS	PIC	
C 6:0	0.053	0.054	0.007
C 10:0	0.050 <sup>b</sup>	0.058 <sup>a</sup>	0.002
C 12:0	0.100 <sup>a</sup>	0.076 <sup>b</sup>	0.005
C 14:0	1.408 <sup>a</sup>	1.260 <sup>b</sup>	0.012
C 15:0	0.070 <sup>a</sup>	0.034 <sup>b</sup>	0.002
C 16:0	25.065 <sup>a</sup>	24.292 <sup>b</sup>	0.147
C 16:1	3.040 <sup>a</sup>	2.262 <sup>b</sup>	0.018
C 17:0	0.378 <sup>a</sup>	0.216 <sup>b</sup>	0.002
C 17:1	0.353 <sup>a</sup>	0.186 <sup>b</sup>	0.002
C 18:0	12.655 <sup>B</sup>	12.968 <sup>A</sup>	0.100
C 18:1	45.183 <sup>a</sup>	42.090 <sup>b</sup>	0.100
C 18:2	10.445 <sup>b</sup>	14.920 <sup>a</sup>	0.252
C 18:3	0.425 <sup>b</sup>	0.694 <sup>a</sup>	0.024
C 19:0	0.038 <sup>a</sup>	0.024 <sup>b</sup>	0.002
C 20:0	0.243 <sup>a</sup>	0.214 <sup>b</sup>	0.002
C 20:4	0.508	0.542	0.035
*P/S	0.284 <sup>b</sup>	0.411 <sup>a</sup>	0.011
Total fat g.100 g <sup>-1</sup>	29.175 <sup>a</sup>	18.780 <sup>b</sup>	0.443

<sup>a, b</sup> different letters in the same row show statistically significant difference ( $P < 0.05$ ); <sup>A, B</sup>  $P < 0.1$ ; \*CS-Crna slavovska; PIC-Pig Improvement Company; \*P/S ratio of polyunsaturated and saturated fatty acids; SEM-standard error of the mean

Regardless of the production system used for production of BK, the monounsaturated oleic acid (C18:1) was the most abundant fatty acid. Its content was significantly higher ( $P < 0.05$ ) in sausages produced from CS pigs than in ones made from PIC hybrids. It is well known that oleic acid, also found in olive oil at similar content as in pork or pork products, has many health benefits, such as reducing the blood cholesterol, and is therefore particularly interesting for human health. Interestingly, BK produced from PIC hybrids had significantly more linoleic (C18:2) fatty acid than ones produced from CS pigs, while the content of palmitoleic acid (C16:1) was higher in BK made from CS pig. Recent studies have shown that this naturally occurring omega-7 monounsaturated fatty acid acts through activation of AMPK and enhances insulin production and secretion, increases fat breakdown, and has a significant anti-inflammatory properties (Morse, 2015). BK made from CS pig had a higher content of palmitic acid (C16:0) and myristic acid (C14:0), while the content of the stearic acid (C18:0) was around 3% in both products. Although it is generally accepted that saturated fatty acids have a negative impact on human health through increase of the risk for coronary heart disease, this opinion is being recently seriously questioned by researchers.

One of the most important parameters used for assessment of nutritional quality of the food lipid fractions is P/S ratio and is generally accepted that it should not exceed 0.4. In the case of BK made from CS pigs this ratio was below the threshold, and was significantly lower ( $P < 0.05$ ) than in BK made from PIC pigs. Interestingly, the P/S ratio was in those sausages somewhat higher than 0.4. The results of the present study are in agreement



**Figure 1** The differences between Baranjski kulen made from CS and PIC pigs in total MUFA, PUFA and SFA

CS-CSpig; PIC-Pig Improvement Company; MUFA-monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; SFA-saturated fatty acids; <sup>a, b</sup>  $P < 0.05$ ; <sup>A, B</sup>  $P < 0.1$

with those reported by Parunović et al. (2017) for kulen made from Mangalitsa, Moravka and Swedish Landrace pig breeds.

BK made from CS pigs had in total higher MUFA content and lower PUFA content, while in SFA a tendency can be observed (Figure 1). The similar levels of fatty acids were also found by others in the meat of traditional pig breeds, such as Pulawska pig (Kasprzyk et al., 2015) or in the traditional sausages made from Mangalitsa and Moravka breeds (Parunović et al., 2017).

#### 4 Conclusions

The results of the present study demonstrate that meat originating from breeds raised in their specific production system affects the fatty acid profile of the fermented sausages, such as BK. Kulen made from CS pigs raised outdoors has reasonably more healthful fatty acid composition than one made from modern pig hybrids raised in indoor intensive production system. These results should encourage the pig producers on sustainable breeding of CS pigs.

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## Characteristics and possible utilisation of Busha population in different Balkan countries

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Busha cattle are indigenous breed in many Balkan countries. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like Busha. In the past several decades, as a result of uncontrolled crossing of this cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed. Since Busha is bred in many Balkan countries, the aim of this study was to define main productive, reproductive and exterior traits of Busha cattle in the following countries: Bosnia and Hercegovina, Croatia, Macedonia and Serbia. Also, number of animals and possibility of future production system was analysed. Analysed data indicate that highest milk production potential and lowest age at first mating has Serbian Busha (till 2,000 kg in lactation, at 18 months). Regarding the exterior traits, smallest frame was observed in Bosnian and Macedonian Busha. The population decrease in the past several decades was determined in all analysed countries. The conservation programs in Croatia and Serbia resulted in certain increase of Busha population, but aiming successful preservation of the breed, economically effective production systems should be put in practice. The branding of Busha's products could result in necessary added value. Taking into account similarity between all Busha breeds as well as population sizes, branding should be organized on regional level.

**Keywords:** Busha breed, Balkan region, characteristics, preservation

### 1 Introduction

Busha cattle are indigenous breed in many Balkan countries. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like Busha (Bunevski et al., 2017). Busha, has been bred for centuries in Balkan Peninsula and belongs to a group of primitive short horned cattle (*Bos brachyceros* Europaeus). Busha was dominant and most important breed in almost all Balkan countries until 50s and 60s of the XX century but today in lowland areas with intensive farming it is replaced with more productive and specialized cattle breeds. Busha is officially classified as triple purpose breed (for meat, milk and work) but considering its low productive capabilities it is more similar to some primitive working breeds. Today, these cattle are no longer used for work but because of absence of systematic cattle improvement program

these animals have retained their poor beef and dairy production capability. It could be said that the Busha's genome is very elastic since this breed in unfavourable conditions easily achieves better milk production and bigger body weight (Bunevski et al., 2017). In the past several decades, as a result of uncontrolled crossing of this cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed. Since Busha is bred in many Balkan countries, the aim of this study was to define main productive, reproductive and exterior traits of Busha cattle in the following countries: Bosnia and Hercegovina, Croatia, Macedonia and Serbia. Also, number of animals and possibility of future production system was analysed.

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### **Bosnian Busha**

Busha is, relative to modern European breeds of cattle, a small animal. The productivity of Bosnian Busha cow is generally modest and it is considered to be a beef – diary – draft type cattle. By the colour of its coat is mostly considered to be one coloured as streaked, black, tan, cream white, dark grey or red. The streaked variants are rare. Over the head, neck, and body they have typical 1–2 cm wide, parallel dark or black stripes. The black colour is even less frequent. It is actually brownish – black with lighter stripes across the back and over the muzzle. Horns and hoofs are dark grey as it the udder which sometimes may be brownish – red. The characteristics of Bosnian Busha are presented in Table 1.

### **Croatian Busha**

Busha is, regarding the frame, the smallest Croatian original breeds. The main exterior measures, productivity and reproduction characteristics is shown in Table 2. This breed is mainly bred in the area of Lika and Dalmatia. The program of conservation started in 2003, and since 2007 Busha is included in the national breeding program (CAA, 2017). In year 2017 the effective population size (Ne) in was 241.5 (65 bulls and 852 females), which characterized this breed as potentially endangered (II).

### **Macedonian Busha**

In the mountain rural regions of Macedonia, the dominant type of cattle are the crosses of the Busha breed. In Macedonia Busha was officially classified as triple purpose breed (for meat, milk and work) but considering its low productive capabilities it is more similar to some primitive working breeds. According to colour Busha breed is classified in following strains: Black, Brown, Red, Gray and Tiger that also differ in their productive, reproductive and morphological traits (Bunevski et al., 2017). The average daily milk production in grey strain was 3.8 kg, and in brown strain was 4.1 kg, with 3.89 i.e. 4.01% of fats, 3.7% i.e. 3.62% proteins and dry unfatted matters 9.49% i.e. 9.62% in milk from brown i.e. brown strain of Busha cows. According to the measurement of some morphological traits of adult Busha cows, in grey strain the wither height was 107 cm, in black strain 105, and in brown strain also 105 cm, with the similar values for the traits back height and rump height in cows. The length of head was 38 cm i.e. 37 in grey i.e. black and brown strain, and length of horns 16 i.e. 15 cm in grey and black i.e. brown strain of cows. The average body mass of new born calves was 15 kg i.e. 14 kg in grey and brown i.e. black strain. The average body weight at first mating was 125 kg in male and 150 kg of female Busha cattle, and the average age at first calving was 28 months.

**Table 1** Bosnian Busha – Characteristics

Main exterior measure		Productivity		Reproduction	
Wither high (cm)	90–112	Lactation (days)	240	Sexual maturity (month)	16
Hip height (cm)	117	Milk yield (l)	800–1,200	Age of first mating (month)	22–25
Body length (cm)	116–132	Fat content (%)	4–6	Fertility (calves/year)	1
Body weight (kg)		Protein content (%)		Breeding time (years)	10–13
Bulls	300	Meat (kg)		Lifetime (years)	20
Cows	150–250	Dressing percentage (%)	52–55		
Calves (at birth)	15				
Chest girth (cm)	146				

Source: Adilović and Andrijanić, 2005

**Table 2** Croatian Busha – Characteristics

Main exterior measure		Productivity		Reproduction	
Wither high (cm)	100–115	Lactation (days)	240	Sexual maturity (month)	15–16
Body weight (kg)		Milk yield (l)	750	Age of first mating (month)	24
bulls	300	Fat content (%)	4–6	Fertility (calves/year)	1
cows	250			Breeding time (years)	10–12
calves (at birth)	15			Lifetime (years)	20

Source: CCA, 2017

**Table 3** Cattle breed distribution according the official data in the Republic of Macedonia (AVF, 2015)

Breed	2008		2010		2012		2014	
	No.	%	No.	%	No.	%	No.	%
Busha cattle	29535	12.1	27242	7.8	20363	10.1	12064	5.6
Crosses (Busha × other breeds)	89707	36.8	104961	43.6	113720	38.9	98958	46.3
All cattle	243667	100.0	269443	100.0	261073	100.0	213747	100.0

**Table 4** Serbian Busha – Characteristics (Institute for Animal Husbandry (2018)

Main exterior measure		Productivity		Reproduction	
Wither high (cm)	90–120	Lactation (days)	240	Sexual maturity (month)	15–16
Body weight (kg)		Milk yield (l)	1,000–2,000	Age of first mating (month)	18–24
Bulls	300	Fat content (%)	4–6	Fertility (calves/year)	1
Cows	150–250			Breeding time (years)	10–15
Calves (at birth)	15–20			Lifetime (years)	20

During the last few years, there are certain negative trends in population size of Busha cattle (Table 3). These trends are mainly due to the decreasing of rural population in hill-mountain regions and small interest of young people to rear indigenous breeds like Busha cattle. Also, in the past several decades, as a result of uncontrolled crossing of this cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed (Bunevski et al., 2017).

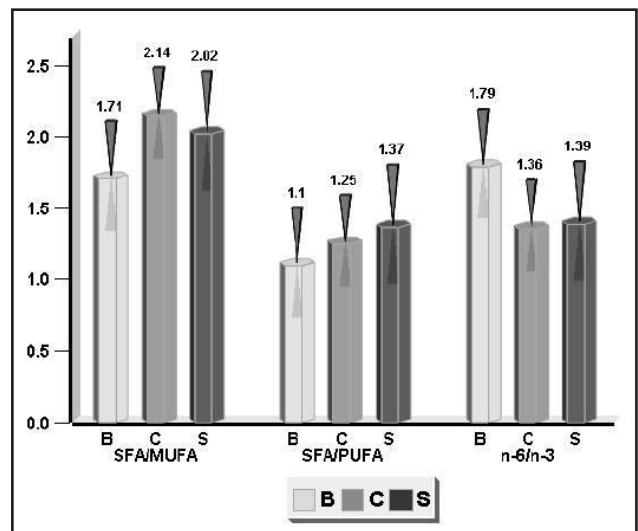
### Serbian Busha

Busha is low-productive three purpose breed that is evolutionarily adapted to unfavorable conditions of breeding and is characterized by exceptionally modest requirements in terms of feeding, care and breeding. This is late maturing, relatively small breed (Table 4). Regarding to colour, in Serbia, Busha is classified in following strains: Gray, Red, and Tiger.

The conservation program of Busha breed as a genetic resource started in year 1993 with an estimated population size of 1,000 to 100,000 animals. Currently, the size of the entire Busha population is about 1,500 animals, of which 847 are breeding animals. The effective population size ( $N_e$ ) from 1999 almost continuously increased and in 2017 amounted 78.11 (Institute for Animal Husbandry (2018)).

### Fatty acid profile in milk of Busha

The analysis of fatty acid profile in milk of Busha, Cika and Simmental cattle (Škrčić et al., 2008) showed that Busha's milk contained significantly lower content of SFA, higher content of MUFA and PUFA, lower SFA/MUFA and SFA/



**Figure 1** SFA/MUFA, SFA/PUFA (\*10–1) and n-6/n-3 PUFA ratios according to breeds B – Busha; C – Cika; S – Simmental (means – histograms with values and standard errors as triangles)  
 Source: Škrčić et al., 2008

PUFA, but higher content of n-6 PUFA and higher n-6/n-3 PUFA ratio in regard to Cika's and Simmental's milk (Figure 1). The significant differences in fatty acid profile between Busha's and other analysed breeds (Cika and Simmental) milk could be basis for branding of Busha dairy products.

### 4 Conclusions

Analysed data indicate that highest milk production potential and lowest age at first mating has Serbian Busha (till 2000 kg in lactation, at 18 months). Regarding the exterior traits, smallest frame was observed in Bosnian



and Macedonian Busha. The population decrease in the past several decades was determined in all analysed countries. The conservation programs in Croatia and Serbia resulted in certain increase of Busha population, but aiming successful preservation of the breed, economically effective production systems should be put in practice. The branding of Busha's products could result in necessary added value. Taking into account similarity between all Busha breeds as well as population sizes, branding should be organized on regional level.

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## The Lipizzan breed history in the Republic of Croatia

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The Lipizzan breed presents an indispensable part of numerous cultural and folklore events, equestrian games, folklore festivals and horse shows in Croatia. Also this is the third most populous breed of horses in Croatia and have status of Croatian protected breed. The aim of this paper was to show the history of breeding of Lipizzan horses and their introduction to Croatia. The origin of Lipizzan breed is connected with the year 1580 when Charles II, Archduke of Austria bought the village of Lipica near Sežana in Slovenia and founded a horse breed there. The Lipizzan were initially used for the protocol needs of the Vienna Court, and later as a working aid. Due to its exceptional beauty and wide usage, this breed began to spread through the Austro-Hungarian Monarchy. Today, the Lipizzan breed, consists of 7 lines of stallions and 18 mare family lines. Throughout history, the breeding goal of the Lipizzan was harmonized depending on the country where it was grown, depending on the time and needs of the source of labour in agriculture and transport, but also depending on the culture of living in a particular region. More than 300 years of Lipizzan breeding in Croatia resulted in noble Baroque horse acclimated for Croatian conditions and deeply correlated with Croatian people and tradition.

**Keywords:** breeding, Croatia, horses, Lipizzan breeds

### 1 Introduction

The Lipizzan breed, is the third most populous breed of horses in the Republic of Croatia (HPA, 2018) probably due to the fact that this breed become part of the tradition and life of the Slavonic and an indispensable part of numerous cultural and folklore events, equestrian games, folklore festivals and horse shows (Lončar et al., 2016). In accordance to Baban et al. (2006) Lipizzan have an expressive head with the occasional rams nose (a trait of the African inheritance), a highly set neck, relatively low withers, a fairly long and powerful back, which runs into a muscular crupper, pronounced bodily proportions and simple profiled limbs with strong well forms joints and elegant hooves; the tail and the main are thick and fine haired – absolutely the baroque horse. The horse's size is between 155 and 158 cm. He has a springy gait with a high knee action and is particularly nimble. Furthermore, the Lipizzan matures late, but also lives to a ripe old age of often between 28 and 32 years. As for the colouring the white horse is dominant – a brown Lipizzan is a rarity, which for tradition's sake however can always be seen at the Spanish Riding School. The foals are born dark or black brown and acquire their white

apparel only further on between the age of 4 and 10 years. The breeding goals of Lipizzan breed is to raise quality animals for team sport and riding at both Stud farm and private breeding. Since this breed is one of the most popular horse breed in Croatia and have status of Croatian protected breed the aim of this paper was to show the history of breeding of Lipizzan horses and their introduction to Croatia.

### *The origin and usage of Lipizzan breed*

The Hapsburg family controlled both Spain and Austria when the art of classical riding revived in Europe during the Renaissance. There was a need for light, fast horses for use in the military and the riding school. The Spanish horse, produced during Moorish rule by crossing Berber and Arab stallions with Iberian mares, was considered the most suitable mount because of its exceptional sturdiness, beauty, and intelligence. In 1562, Maximilian II brought the Spanish horse to Austria and founded the court stud at Kladub. His brother Archduke Charles established a similar private imperial stud farm with Spanish stock in 1580 at Lippiza (nowadays: Lipizza [Italian], or Lipica [Slovenian]). He selected that area because it was the

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site of the former Roman city Aquileia in which ancient Romans breed excellent horses and where, even after the Roman era, fast and durable karst horses were continued to be raised on the Slovenian karst areas (Benčević et al., 1965). Here, the origin of Lipizzan breed occurred. Lipizzan horses are believed to be named after the town Lipica (Župan, 2004). Today in Europe the breed is called Lipizzan or, in America, Lipizzan.

The Lipizzan were initially used for the protocol needs of the Vienna Court, and later as a working aid (Lončar et al., 2016). According to Steinhausz, the stud farm in Lipica was created by interbreeding of the karst horses with those of Spanish, Kladrup, Italian, Danish, German, and most frequently of Arabic origin (Steinhausz 1924). It is believed that the interbreeding of different breeds of horses with karst horses, is due to Archduke Charles' wish to ennoble the karst horses. This is additionally confirmed by the fact that apart from the Arab breeds, no other breed has managed to stay present in the Lipizzan (Lončar et al., 2016). Due to its exceptional beauty and wide usage, this breed began to spread through the Austro-Hungarian Monarchy, and as such through Croatia (Čačić, 2011).

## 2 Materials and discussion

An Imperial herd of horses was formed on the Lipizza Estate in 1580 from 9 stallions imported from Spain and 24 Karst Mares. Of all the sires used during the 18<sup>th</sup> and 19<sup>th</sup> century, only six of these horses were accepted to found the family lines of the Lipizzan as known today:

- **CONVERSANO**, black, a Neapolitan (1767). Conversano's have Arab blood, strong ram-like heads short backs, broad hocks and dignified movements;
- **FAVORY**, dun, a Bohemian origin (1779), transferred from Kladrub. The Arab influence is noticeable in the Favory's by their lighter build but the soft curve of their nose still calls to mind their Spanish ancestry;
- **MAESTOSO**, white (not grey), a crossbred of Neapolitan sire and a Spanish dam (b. 1819), transferred from Kladrub. Maestoso's are powerful horses with a long back, extremely muscular cruppers and heavy heads;
- **NEAPOLITANO**, bay (brown), from another Neapolitan sire (1790). Neapolitans retain their original tall, rangier appearance and they have graceful movements and high action;
- **PLUTO**, grey, Danish stud (1765). Pluto's, their ancestors from Spain and Denmark, are sturdy horses with a rectangular build, ram-like heads and a high set neck;

- **SIGLAVY**, grey, an Arabian (1810). The Siglavy's typify the Arab Lipizzaner with aristocratic heads, a slender neck, high withers and a relatively short back.

In addition to the stallions, there are 18 mare family lines. Every stallion has two names, the sire's name and the dam's name. This explains the name such as Pluto Theodorosta.

Today, the Lipizzan breed, consists of 7 lines of stallions, two of which are of Kladrup origin (Maestoso and Favory), two of Neapolitan origin (Neapolitan and Conversano), one Arabic (Sigla), one Danish (Pluto), one English-Arabic (Sirdar), 1 Mezőhegyes-Fogaras (Incitato), and one Croatian-Slavonian line (Tulipan) (Steinhausz, 1924).

The tradition of breeding of these horses is also reflected today through the work and activity of 9 European stud farms. These are: Lipica (Slovenia), Đakovo (Croatia), Lipik (Croatia), Piber (Austria), Monterotondo (Italy), Beclean (Romania), Fagaras – Simbata de Jos (Romania), Topolčianky (Slovakia), and Szilvásvárad (Hungary). The Vučijak stud farm (Bosnia and Herzegovina), which in 2010 received the status of the national stud farm, may also be added to the list. In each of these, there are also national elements to horse breeding. Their bearers are various private breeders whose aim is to cultivate the tradition of the Lipizzan breeding.

The breeding of the Lipizzan on the territory of Croatia is linked to the year 1700 and the Count of Janković, who at Terezovac in the vicinity of Suhopolje, first bred Neapolitan and Spanish horses. When Lipizzan arrived to his estate, that breed also became present in that area (Horvath, 1996). Later on, the development of the Lipizzan breed in Croatia was related to the Đakovo Stud Farm which was originally owned by the Đakovo Diocese. In fact, the first arrival of Lipizzans in the territory of Đakovo is connected to the beginning of 1806, when the imperial stud farm from Lipice had to withdraw away from Napoleon's military campaigns (Steinhausz, 1924). After the imperial stud farm left from Đakovo a year later, i.e. in 1807, some of these horses remained in Đakovo.

The one-year stay of Lipizzan in Đakovo influenced the awareness of their value and further breeding. Napoleonic Wars, in addition to causing damage to buildings in Lipica, led to the destruction of breeding documentation of that valuable breed (Horvath 1996). Bishop Josip Juraj Strossmayer, who financed the purchase of a number of Lipizzaner horses by selling oak forests, made a significant contribution to the cultivation of Lipizzaner horses in the territory of Đakovo, which after 1856 systematically began with organized breeding of that breed in the area (Župan, 2004). Along with Strossmayer's help and love



**Figure 1** Ivandvor is the second location of the State Stud Farm Đakovo where the stud herd of mares with their foals are situated  
Photo: M. Gregić

for Lipizzans, the stud farm made significant advances in the number and quality of horses, which is attested by awards at various exhibitions. Besides Lipizzans, Arab stallions were sometimes used for breeding at the stud farm. It is mentioned that in 1898, there were 56 mares, 4 stallions, and 109 foals and colts (Ilančić, 1975).

Today, in Croatia, Lipizzan breeding is predominately oriented to Slavonia as a rich region that has inherited this exceptionally valuable breed of horse (Čačić, 2011). Although, with the development of mechanization, Lipizzans are no longer used to work the fields, this breed of horses has always been considered the breed of the future. Due to their character traits and exceptional learning abilities, Lipizzans have created an indestructible link between man and horse (Lončar et al., 2016).

The popularity of a Lipizzan with the Croatian man, is illustrated by the fact that it is the third most populous breed of horses in the Republic of Croatia (HPA, 2018). The reason for this lies in the fact that the Lipizzan has slowly become part of the tradition and life of the Slavonic man for several centuries, thus becoming an indispensable part of numerous cultural and folklore events, equestrian games, folklore festivals and horse shows (Lončar et al., 2016). The organic horse breeding in the Republic of Croatia has two most important natural resources: Lipizzan and pasture (Gregić et al., 2013.). East Croatia has unused potential of originally protected horses breeds (Gregić et al., 2018.).

### 3 Conclusions

Throughout history, the breeding goal of the Lipizzan was harmonized depending on the country where it was grown, depending on the time and needs of the source of labour in agriculture and transport, but also depending on the culture of living in a particular region. More than 300 years of Lipizzan breeding in Croatia resulted in noble Baroque horse acclimated for Croatian conditions and deeply correlated with Croatian people and tradition.

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## Reproduction performances, growth and slaughter traits analysis of rabbit of Nitra breed

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The aim of the research was to analyse the Rabbit of Nitra in terms of its reproduction and production performance. All rabbits used for research were raised within a home environment (4 breeding sources). The research was focused on reproduction parameters (the number of live born kits, the number of weaned kits), growth parameters – growth performance (1<sup>st</sup> day, 21<sup>st</sup> day, 42<sup>nd</sup> day to 119<sup>th</sup> day), slaughter parameters (dressing out percentage, the weight of individual parts of carcass body). Detected live weight in adult males weight was 5,291.48 ±546.39 g. In adult females average live weight was 4,623.10 ±458.41 g. The weight of live born pups of rabbits on day 1 was 57.84 ±1.08 g, weight on 21<sup>st</sup> day 310.09 ±7.21 g, weight on 42<sup>nd</sup> day from 1,034.26 ±50.70 g up to 1,128.13 ±30.78 g, 77<sup>th</sup> day from 2,126.48 ±85.49 g up to 2,243.70 ±47.07 g and 91<sup>th</sup> day from 2,379.29 ±31.22 g up to 2,653.53 ±37.86 g. An average dressing out percentage was 62.47±0.23%.

**Keywords:** dressing performance, growth performance, meat production performance, rabbits

### 1 Introduction

The Rabbit of Nitra is the 3<sup>rd</sup> Slovak national breed. The goal was to create a white rabbit for traditional home-farming and for intense-farming also. Three basic breeds were used in the process of breeding. In the first step were used Argente de Champagne and Himalayan rabbits. By coincidence, at that time, the Californian rabbits were imported into the territory of Slovakia and were involved in process of breeding as a third breed. In an attempt to characterize the two rabbit breeds (Rabbit of Nitra – Ni and Zobor Rabbit – Zo) concerning the shift on the genetic level Vašíčková et al. (2016) studied microsatellite and genetic markers associated with coat color. This authors described, that overall population size of Ni and Zo rabbit breeds is very low. Stability of these two breeds is constantly eroded by cross-breeding efforts. The aim of the research was to analyse (selected utility properties) the Slovak national breed – Rabbit of Nitra.

### 2 Materials and methods

The data for the analysis were obtained (data was collected between 2016 and 2018) from four home-farming holdings (fancy breeders) of the Rabbit of Nitra.

The animals were stabled in outdoor wooden warrens with a compact floor and straw. Composition of the feed dose: hay, complete feed mixture + barley (50 : 50%) and water. We monitored growth parameters, reproduction parameters and slaughter parameters. Weighing was done on scales with an accuracy of 1 g. The rabbits were slaughtered at a relatively old age (91–119 days) and high body weight (2,785–3,612 g) because this is typical for small studs (home-farming). The carcass was processed according to the methodology reported by Blasco-Ouhayoun (1996). For statistical analyses the computer program Microsoft Excel has been used.

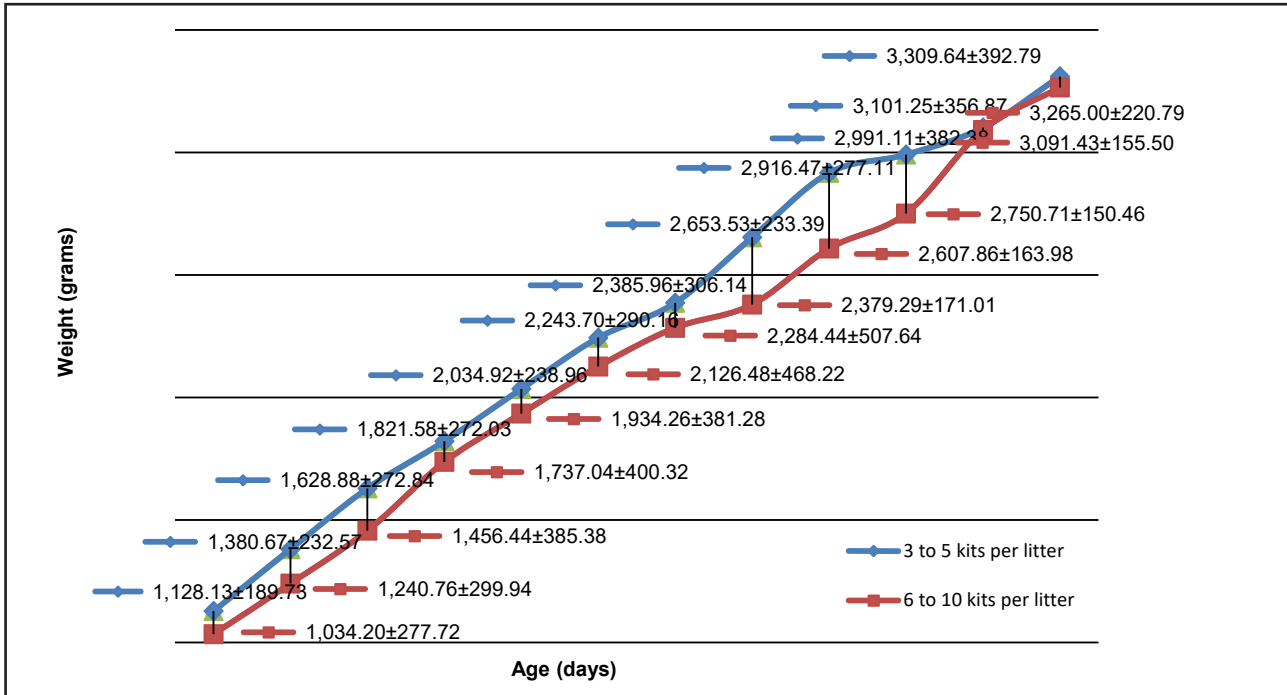
### 3 Results and discussion

Weight of the Rabbit of Nitra 1<sup>st</sup> day was 57.84 ±1.08 g and 21<sup>st</sup> day after birth 310.09 ±7.21 g.

Number of live born kits in litter (41 litters) was 6.88 ±0.28. Number of dead born kits in litter was 0.09 ±0.06. Number of weaned kits in litter was 5.98 ±0.29. Topczewska et al. (2013) showed the effect of breed on reproductive performance. These authors found the litter size 6.27 kits in the Californian breed and 8.49 in Popielno white. Kits weight at 35 day of age ranged from 603.21g (Alaskan)

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**Figure 1** Growth performance (litter size 3–5 and 6–10 kits) n = 32

to 736.10g (Giant Chinchilla). Average live weight in adult males (31 pcs) over 12 months of age was 5,291.48 ± 546.39 g (3,740 to 5,862 g). In adult females (38 pcs) over 10 months of age, average live weight was 4,623.10 ± 458.41 g (3,820 g to 5,352 g).

Dalle Zotte (2002) described perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. The quality of the rabbit carcass defines (Dalle Zotte, 2000). All these features should reach a certain level to accomplish the economic aim, especially in broiler rabbit farming, but also in some minor breeding conditions (Bianospino et al., 2004). Mach et al. (2006) mention for broiler rabbits a dressing out

percentage 58%. Jenisová (2013) came in broiler rabbits from home-farming holdings slaughtered between 2,470–3,425 g to dressing out percentage 54.89–55.18%. Dressing out percentage 57.03–60.81% detected Šmehýl (2010). The closest values to our recognized Dokoupilová et al. (2006), an average dressing out percentage 61.81% with genotype includes 75% of traditional breeds and 25% of broiler rabbits. Skřivanová et al. 2000 found out an average dressing out percentage from 60.9% for Zika rabbits to 62.4% for HY2000 rabbits. Bízková and Tůmová (2009) detected an average dressing out percentage for medium breeds of rabbits 59.50%. They also detected thighs percentage from the carcass body 31.30%. Back

**Table 1** Slaughter parameters of the Rabbit of Nitra

Indicator	n	$\bar{x}$	s	$s_x$	v
Live weight (g)	56	3051.25	429.30	52.45	0.14
Weight of skin (g)	32	538.10	63.79	7.79	0.12
Weight of gastrointestinal tract (g)	32	497.05	64.91	7.93	0.13
Weight of intestines (g)	32	126.55	23.69	2.89	0.19
Weight of head (g)	32	167.35	17.56	2.15	0.10
Weight of back (g)	32	444.25	85.97	10.50	0.19
Weight of thorax (g)	32	396.60	88.17	10.77	0.22
Weight of shoulder blades (g)	32	232.75	29.01	3.54	0.12
Weight of thighs (g)	32	542.80	99.97	12.21	0.18
Dressing out percentage (%)	56	62.47	1.92	0.23	0.03

percentage from the carcass body they recognized the value 19.5%. Tůmová and Hrstka (2013) detected in Czech white rabbit breed dressing out percentage with head 60.18% and thighs percentage from the carcass body 33.42%. Volek et al. (2013) recognized in Czech white rabbit breed dressing out percentage 59.80%. Šmehýl (2017) figured out the live weigh in 1<sup>st</sup> day after birth 56.92–93.23 g and live weight in 21<sup>st</sup> day after birth 325.50–552.20 g. In our research of the Rabbit of Nitra were these values lower in both cases. Šmehýl (2010) shows the number of weaned kits 4.10 to 7.93 pcs and the average mortality values until weaning 8.90–32.20%. Our average number of weaned kits was 5.98 pcs and we recorded weaning mortality 13.79%, which is a relatively low value. The authors Tůmová et al. (2013) described selected performance characteristics of Czech local breeds and to compare these breeds with a commercial hybrid. Czech White breeds grew non-significantly faster than Hyplus. The highest daily weight gain was observed in Czech white. Slaughter characteristics mostly correlated with live weight; the highest dressing out percentage was in the small breed Czech Gold (62.0%) and the lowest in the Hyplus rabbit (57.0%). Volek et al. (2013) said in analysis of Czech white breed live weight at 42 days of age – 882 g, 63 days of age – 1,715 g, 70 days of age – 2,018 g a 91 days of age – 2,704 g. Compared to Czech white rabbit breed has the Rabbit of Nitra higher growth performance to 70 days of age and from this age is the intensity compared to that breed considerably lower. These authors Tůmová et al. (2011) described that litter size of medium breeds (Czech white and Moravian white of brown eye) was 6.83 (Czech white) and 5.72 (Moravian white of brown eye) kits, weaned of kits per litter 6.46 (Czech white) and 5.22 (Moravian white of brown eye) and mortality till weaning 5.38% (Czech white) and 8.32% (Moravian white of brown eye). In this work the mortality till weaning was 13.08%. Tůmová et al. (2011) reported growth of national breed rabbit. Czech white day 42–889 g, day 77–2,265 g, day 91–2,747 g. Moravian white of brown eye day 42–873 g, day 77–1,868 g, day 91–2,210 g. Zawiślak et al. (2015) showed mean body weights in New Zealand White (2,422–2,456 g) and Blanc de Termonde rabbits (2,471–2,364 g) on the 90<sup>th</sup> day of fattening (feed type – farm made). These results are in agreement with findings of Lukefahr et al. (1983), Mach (1992) or Bolet et al. (2004). Bolet et al. (2004) in a study of fertility of the European Rabbit Genetic Resources stated that higher number of weaned kits was in medium size breeds in comparison with giant or small breeds. Roberts, Lukefahr (1992) or Bolet et al. (2004) describe that litter size of medium breeds is between 6 and 7.3.

## 4 Conclusions

Results of the study show the data of Rabbit of Nitra national breed. The preliminary data of fertility and growth revealed that Rabbit of Nitra is breed which may be a source of traits suitable for meat production, especially for traditional rabbit breeds (home-farming). On the other hand, there is the need for further studies of all production characteristics Slovak national rabbit breeds.

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## The impact of genetic and non-genetic factors on somatic cell count as a monitor of udder health in Slovak Simmental dairy cows

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The aim of study was to evaluate the impact of genetic and non-genetic factors on somatic cells count in Slovak Simmental dairy cows in period 2009 to 2013. We observed subsequent results in dairy cows of 55,822 Slovak Simmental cattle. Data were analysed using the SAS and linear model with fixed effects of herd, years and months controls, sire, breeding type. The results of somatic cells count during the years 2009 and 2013 were as follows: the highest number of samples was analysed during 2010 ( $n = 288,215$ ), where the average count was  $560.48 \times 10^3 \cdot \text{ml}^{-1}$ . The lowest average value of somatic cells count was of amount of  $535.93 \times 10^3 \cdot \text{ml}^{-1}$  ( $n = 280,732$ ) in 2009. The linear model represents coefficient determination  $R^2 = 0.038296\%$  ( $P < 0.001$ ) for SCC with all fixed effects. According to the analyses by the effects on SCC the highest effect was the effect of herd  $R^2 = 0.021625$ , then effect of sire  $R^2 = 0.015075$ . These effects were highly statistically significant ( $P < 0.001$ ). Correlation coefficients among milk in kg, fat, protein in % with somatic cells count were  $r = -0.12918$ ,  $r = 0.04166$  and  $r = 0.11423$ . These coefficients were highly statistically significant ( $P < 0.001$ ).

**Keywords:** dairy cows, milk production, Slovak Simmental cattle, somatic cells count and coefficient of determination

### 1 Introduction

The somatic cells count (SCC) of milk is widely used to monitor udder health and the milk quality (Sharif, Muhammad, 2008; Jadhav et al., 2016). The composition of milk from dairy cows is of major interest to milk producers, processors and consumers because of its health related issues and also market demand. It directly affects the economy of milk production as well as economic condition of these dairy farmers (Boro et al., 2016). Milk composition can be affected by a wide array of factors: breed, season, age, stage of lactation and diet of the animal (Savič et al., 2017; Tančin et al., 2018). Factors affecting on somatic cells count shows in publications others authors as Cerón-Muñoz et al., 2002; Souza et al., 2005; Rhone et al., 2008; Oudah, 2009; Saravanan et al., 2015 and Alhussien, Dang, 2018. The aim of study was to evaluate the impact of genetics and non-genetics factors on somatic cells count in Slovak Simmental dairy cows.

### 2 Materials and methods

The material for evaluation traits in population of dairy cows Slovak Simmental cattle between 2009 and 2013

years were received from of Breeding Service of Slovak republic (B.S. SR, S.E., 2014). We observed subsequent results of 55,822 in dairy cows (1,131,509 control samples) Slovak Simmental cattle: milk in kg (DMY), fat in % (DFC), protein in % (DPC) and somatic cells count (SCC). We according to divided dairy cows a breed-type  $S_0$  – cows with genetic proportion of pure Slovak Simmental blood (into 87.5%),  $S_1$  – cows with genetic proportion of pure Slovak Simmental blood (from 75% to 87.4%),  $S_2$  – cows with genetic proportion of pure Slovak Simmental blood (from 50% to 74.9%). To determine the effect of SCC, it was divided into 4 groups according to the SCC values: I. – up to 100,000  $\text{SCC cm}^{-3}$ ; II. – 101–400  $\text{SCC cm}^{-3}$ ; III. – 401–500  $\text{SCC cm}^{-3}$ ; IV. – 501–1 million  $\text{SCC cm}^{-3}$  and V.  $> 1$  million  $\text{SCC cm}^{-3}$ . The basic statistical analysis of milk production traits and SCC were performed using the Statistical Analysis System (SAS) version 9.3 (TS1M2) Enterprise Guide 5.1. (SAS, 2011). For the actual computation a linear model with fixed effects was used:

$$y_{ijklm} = \mu + H_i + YS_j + b_k + c_l + e_{ijklm}$$

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where:

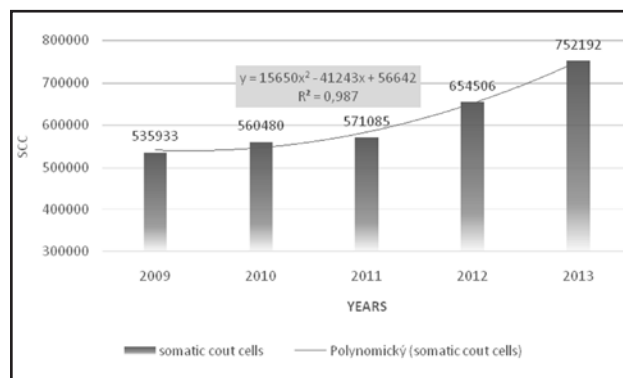
- $\mu$  – mean value
- $H_i$  – effect of herd
- $YS_j$  – effect of years and month of controls
- $b_k$  – sire
- $c_l$  – breeding type
- $e_{ijklm}$  – residual error

### 3 Results and discussion

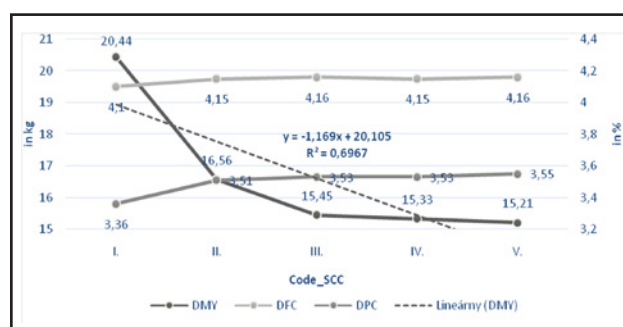
The basic traits of milk production and somatic cells count (SCC) in evaluated population of dairy cows Slovak Simmental cattle are presented in Table 1.

Figure 1 shows results of SCC by year's evaluation and trends of rising SCC which related with negative correlation among traits of milk production (DMY, DFC and DPC) and SCC. The correlation coefficients among DMY, DFC and DPC with SCC were  $r = -0.12918$ ,  $r = 0.04166$  and  $r = 0.11423$ . These coefficients were highly statistically significant  $P < 0.001$  (Table 2). These results are correspondence with Japertienè et al., 2016. De Freitas et al., 2017 shows correlation coefficients among DMY, DFC and DPC with SCC ( $r = -0.18116$ ,  $r = 0.09046$  and  $r = 0.08100$ ).

The results of milk traits in control samples by code of SCC were divided into 5 groups and are presented in Figure



**Figure 1** Estimation of SCC in Slovak Simmental dairy cows by years of control samples



**Figure 2** Statistical characteristics of DMY, DFC and DPC in dairy cows of Slovak Simmental by code of SCC

**Table 1** Statistical characteristic of DMY, DFC, DPC and SCC in dairy cows of Slovak Simmental

Traits	<i>n</i>	$\bar{x} \pm SD$	CV	Mode	Median
DMY (kg)	55 822	17.68 ± 8.08	44.91	16.60	17.0
DFC (%)		4.10 ± 0.86	20.98	3.87	4.04
DPC (%)		3.46 ± 0.38	10.85	3.40	3.47
SCC ( $\times 10^3 \cdot ml^{-1}$ )		594.75 ± 1620.55	272.48	31.0	156.0

*n* – number of observation,  $\bar{x}$  – average, *SD* – standard deviation, *CV* – coefficient of variation, *Mode* – value that appears most often in a set of data, *Median* – value separating the higher half of a data

**Table 2** Relation between SCC and traits of milk production (DMY, DFC and DPC)

Traits	DMY	DFC	DPC
SCC	-0.12918 <sup>+++</sup>	0.04166 <sup>+++</sup>	0.11423 <sup>+++</sup>

<sup>+++</sup>  $P < 0.001$

**Table 3** Factors affecting SCC in Slovak Simmental dairy cows

Sources of variability	<i>DF</i>	Mean Square	<i>F</i> -Value	<i>R</i> -Square
				somatic cells count (SCC)
Herd	489	131,407,525.5	51.12 <sup>+++</sup>	0.021625
Years-month	59	152,738,015	58.33 <sup>+++</sup>	0.003033
Sire	907	49,390,629.528	19.08 <sup>+++</sup>	0.015075
Breeding_type	2	765,211,084.45	291.53 <sup>+++</sup>	0.000515

*DF* – grades of freedom, *R*-Square – coefficient of determination ( $R^2$ ), <sup>+++</sup>  $P < 0.001$



2. The average of DMY, DFC and DPC are 15.21 to 20.44, 4.10 to 4.16 and 3.36 to 3.55 by code of SCC. These results are similar with conclusions of Jattawa et al., 2012 and de Freitas et al., 2017, where values perceptual of milk traits by codes of SCC have rising tendency.

Using the linear model we have found out the coefficient determination  $R^2 = 0.038296\%$  ( $P < 0.001$ ) for SCC with all fixed effects. The analyses by the effect on SCC showed the highest effect of the effect of herd  $R^2 = 0.021625$ , followed by the effect of sire  $R^2 = 0.015075$ . These effects were highly statistically significant  $P < 0.001$  (Table 3). These results are similar with results of Cerón-Muñoz et al., 2002; Souza et al., 2005; Rhone et al., 2008; Saravanan et al., 2015; Boro et al., 2016 and Savić et al., 2017.

#### 4 Conclusions

The results confirm that the effect of herd on somatic cells count was higher  $R^2 = 0.021625\%$ , than the effect of sire  $R^2 = 0.015075\%$ . These effects were significant ( $P < 0.001$ ). For comparison, the correlation among evaluated somatic cells count (SCC) and traits of milk production (milk in kg, fat and proteins in %) were lower and negative  $r = -0.12918$ ,  $r = 0.04166$  and  $r = 0.11423$ . These results were statistical high significant ( $P < 0.0001$ ).

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## Finding ‘the long-lost’ Croatian Lipizzan mare families

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Lipizzan horse breed was founded in 1580 in Lipica and introduced to Croatia in 1800 by earl Andrija Jankovich-Bésán. Earl Jankovich founded stallion bloodline Tulip and mare families Czirka, Ercel, Traviata, Margit, Manczi and 502 Moszgo Perla. Herd books showed that South African Lipizzan population is based on Czirka and Ercel mare families that are originally Croatian thus provided potential genetic enrichment of Croatian Lipizzan gene pool. In this study a 648 bp mitochondrial DNA fragment from 50 South African Republic Lipizzan horses was analysed and 249 sequences from Čačić doctoral thesis was retrieved. Mitochondrial DNA analysis of South African Lipizzan horses and their comparison with Croatian Lipizzan horses present that South African Lipizzan horses have five unique haplotypes but still maintain connection with Croatian Lipizzan by sharing a haplotype. Future analysis with high throughput genetic marker such as SNP or WGS will surely provide interesting results.

**Keywords:** Croatian Lipizzan horse, mitochondrial DNA analysis, shared haplotypes, South African Lipizzan horse

### 1 Introduction

Lipizzan horse breed is composed of eight stallion bloodlines and 63 mare families (Lipizzan International Federation, 2001) of which one stallion bloodline (Tulip) and 16 mare families (Czirka, Ercel, Traviata, Margit, Manczi, 502 Moszgo Perla, Rendes, Hamad-Flora, Eljen-Odaliska, Miss Wood, Fruska, Mima/Nana, Alka, Karolina, Munja, Rebeca-Thais) have Croatian origin (Čačić, 2008). Today only six mare families are represented in Croatian breeding. Lipizzan horses were first introduced to Croatia in 1800 by earl Andrija Jankovich-Bésán (Steinhausz, 1924). He founded stallion bloodline named Tulip and six mare families Czirka, Ercel, Traviata, Margit, Manczi and 502 Moszgo Perla. Mare families Czirka and Ercel are today present only in South African Republic (SAR) Lipizzan population (Čačić, 2011). Following the political situation in Croatia and Europe in 1929 earl Elemer Jankovich-Bésán migrated to Hungary where he stayed until 1944 then moved to Bavaria (Jankovich-Bésán, 2010), in 1947 moved to England and in 1948 finally to South African Republic (Dalglish, 2011). Along with his family he took 14 Lipizzan horses (nine mares with three

foals belonging to Czirka and Ercel mare family and two 2-year old stallions) that he transported to the Mooi River farm in SAR. Those 14 Lipizzan horses were nucleus that provided today South African Lipizzan population. Investigating breeding records of Lipizzan South African Center it is identified that present SAR Lipizzan population is composed of Czirka and Ercel mare families and that these two Croatian mare families were foundation of Lipizzan breeding in SAR since 1948 until today (Lipizzan South African Center, Breeding books). Since mare families Czirka and Ercel were transported from Croatia to SAR they disappeared not only from Croatian Lipizzan breeding lines but from the Europe breeding. These Croatian mare families represent valuable gene pool that will enrich Lipizzan genetic variability. The aim of this study was to characterize maternal genetic structure of SAR Lipizzan population as well as to compare them with Croatian Lipizzan population.

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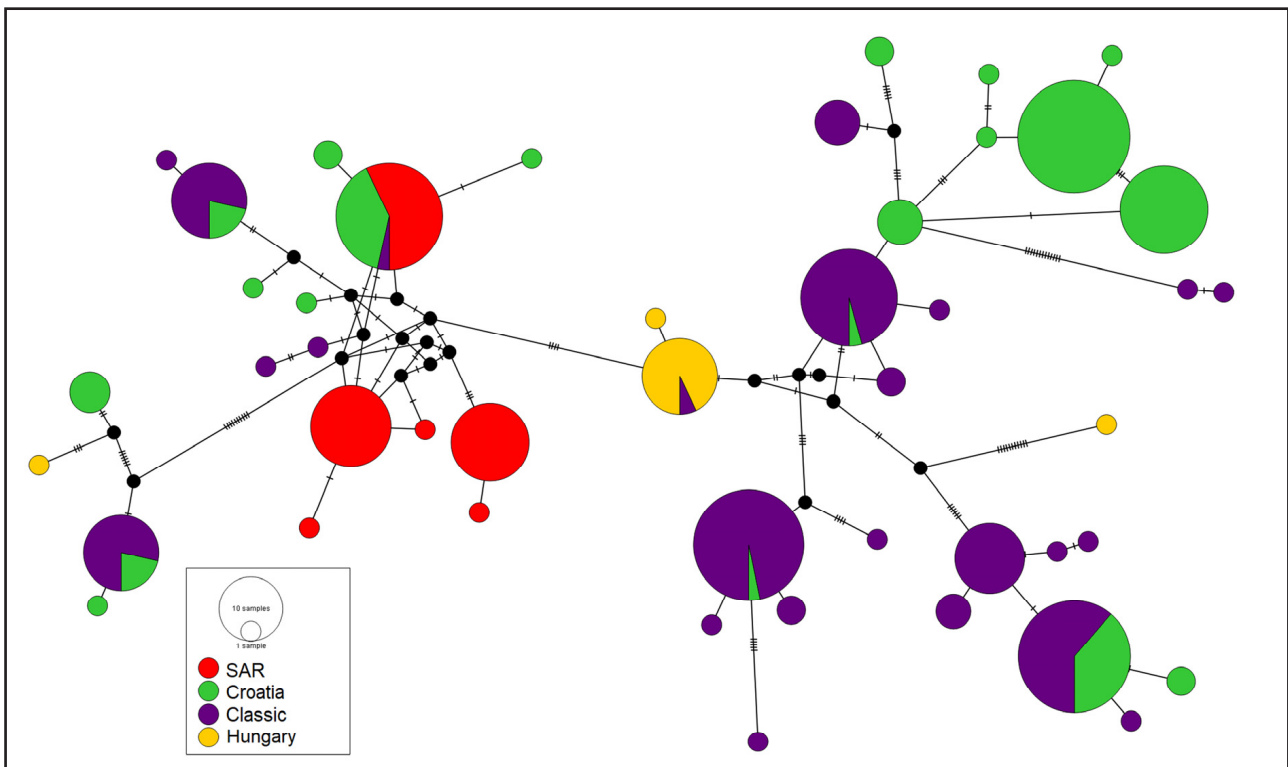
## 2 Materials and methods

DNA of a 68 Lipizzan horse hair samples from South African Republic were extracted with Qiagen Blood and tissue kit (Qiagen, Germany) according to the manufacturer instruction. Sequencing was performed following Aberle et al. (2007). Thus, a 1280 bp mitochondrial DNA D-loop fragment was amplified using forward (5'-AAC GTT TCC TCC CAA GGA CT-3') and reverse (5'-GCA TTT TCA GTG CCT TG CTT-3') primers. The polymerase chain reaction was performed in 20 µl reaction mix containing approximately 50 ng of total DNA, 0.2 µM of each forward and reverse primer and Master Mix. The PCR was carried out in a Mastercycler (Eppendorf, Germany) and consisted of an initial denaturation step at 95 °C for 8 min followed by 38 cycles at 95 °C for 1 min, annealing at 62 °C for 1 min, and elongation at 62 °C for 31 min with a final elongation step of 7 min at 72 °C. PCR products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega, USA) following the manufacturer's recommendations. DNA sequencing was performed from the PCR product on an ABI 3130 DNA automated sequencer (Applied Biosystems, USA) using the ABI Prism Big Dye Terminator 3.1 Sequencing Kit (Applied Biosystems, USA). For a more accurate comparison, total of 249 Croatian Lipizzan horse sequences from doctoral thesis of Čačić (2011), were added for this study. All sequences were aligned with referent sequence X79547 (Xu and Arnason, 1994) using Clustal omega (McWilliam

et al., 2013) and analyses were performed based on the 648 bp truncated fragment. DnaSP v5.10 (Librado and Rozas, 2010) was used to determine unique haplotypes. The haplotype network was performed using PopArt 1.7 (Bandelt et al., 1999).

## 3 Results and discussion

The analysis was performed on the 648 bp long mtDNA control region fragment. During process of DNA extraction, PCR and sequencing we had to discard 18 samples due to the low sample quality. On final data set of 299 Lipizzan horse sequences, 50 sequenced in this study and 249 retrieved from the Čačić (2011) doctoral thesis, 46 different haplotypes and 79 polymorphic sites were found. Sequences retrieved from Čačić doctoral thesis are all Croatian Lipizzan horses with different family origin (classical, Croatian or Hungarian) according to Studbook of the Origins of the Lipizzaner Breed (Spanish Riding school, 2010). SAR Lipizzan horses clustered in six haplotypes, five unique and one shared with horses from Croatian and classical Lipizzan families. Detailed characterization of all 46 observed haplotypes is presented in Figure 1. The D-loop region sequenced in this study was highly polymorphic, showing haplotype diversity (Hd) of 0.938 (sd 0.004) and nucleotide diversity (Nd) of 0,01617 (sd 0,00044). A median-joining network of the Lipizzan haplotypes is showed in Figure 2. As it is concluded from the median-joining network two horse



**Figure 2** Median-joining network of 46 Lipizzan haplotypes. Little dashes represent the number of mutations

populations are related, even sharing one haplotype. Considering the small size of the Lipizzan population, inclusion of new haplotypes will greatly enrich breed's maternal genetic diversity. This is the first genetic analysis of SAR Lipizzan population and it provides us insight into results that will be obtained with future analysis of whole genome.

#### 4 Conclusions

Old historical records saying that South African Lipizzan horses originate from Croatian breeding lines are confirmed by reviewing the SAR Lipizzan herd books. Croatian mare families Czirka and Ercel are foundation of current SAR Lipizzan breeding. As a first genetic analysis of maternal families, it is visible that SAR population is close to Croatian Lipizzan population. Introduction of the long-lost Croatian mare families will have great contribution to the maternal genetic diversity of Lipizzan population. Surely the future analysis on the whole genome using SNP chip or WGS will give us better understanding of the connection between two populations and will allow us to compare the SAR Lipizzan population with the other European and world Lipizzan populations.

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## The impact of the humic acid and phytobiotics on performance and carcass parameters of broiler chickens

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The aim of this experiment was to determine the influence of humic substances, and combination humic substances and selected phytobiotics on production and carcass parameters of broiler chickens. In experiment from total 200 one-day-old ROSS 308 chickens were randomized into four groups ( $n = 50$ ). The control group was fed with basal diet (BD) without any additives. Group of chickens marked as treatment 1 (T1) was fed a BD containing 2% of humic acid, the group marked as treatment 2 (T2) was fed a BD containing 78% of humic acids, 18% of garlic powder (*Allium sativum* L.), 1% of milled dried leaves of wormwood (*Artemisia absinthium*), 1% of milled dried leaves of thyme (*Thymus vulgaris*), 1% of milled dried leaves of oregano (*Origanum vulgare*) and 1% of milled dried leaves of bogbean (*Menyanthes trifoliata*), together 2 kg.100 kg<sup>-1</sup> complete feed mixture (BD). In the group marked as treatment T3 were chicken fed with BD containing industrially produced coccidiostats. Experiment lasted 42 days. At the end of the experiment was average body weight (values in the order of the groups: 1,808.03 ±212.39; 1,981.75 ±203.32; 1,895.59 ±178.75 and 1,955.31 ±237.16 g ±SD) significantly higher ( $P < 0.05$ ) in all treatment in compare to control group. In T2 was thigh part (29.27 ±1.50; 29.07 ±3.35; 30.45 ±2.15 and 29.49 ±2.34 mean ±SD) significantly higher ( $P < 0.05$ ) compared to control group. Carcass weight (values in the order of the groups: 1357.18 ±95.8; 1486.38 ±156.7; 1369.69 ±118.0 and 1440.68 ±132.1 g ±SD) and carcass yield (74.35 ±1.33; 76.10 ±1.97; 74.03 ±1.35 and 73.45 ±1.82 mean ±SD) were the highest in treatment T1 with humic acid addition ( $P > 0.05$ ).

**Keywords:** *Allium sativum* L., *Artemisia absinthium*, broiler chicken, carcass parameters, humic acid, *Menyanthes trifoliata*, *Origanum vulgare*, performance parameters, *Thymus vulgaris*

### 1 Introduction

A substantial growth in poultry industry has been observed mainly due to exploitation of various modern growth promoting strategies and appropriate disease preventive and control measures (Kuldeep Dhama et al., 2014). Antimicrobial growth promoters have made a tremendous contribution to profitability in intensive husbandry, but as a consequence of the increasing concern about the potential for antibiotic resistant strains of bacteria, the European Commission decided to ban all commonly used feed antibiotics. (Hassan et al., 2010). So, there is the need to find alternatives to the use of antibiotics. Humic acids are the most active substances with antioxidant effect. Humic acids have a positive impact on meat quality, increasing weight gains and improve the immune system of broiler chickens (Nagaraju et al., 2014). Spices and herbs, generally used for their flavouring characteristics, can be added to

meat products in various forms: whole, ground, or as isolates from their extracts (Diaz-Sanchez et al., 2015). Garlic helps to improve feed palatability, feed intake and feed efficiency, it has antioxidant effect (Khan et al., 2010). Oregano is known by several notable beneficial effects on animal growth performance, feed efficiency, production traits and product quality, as well as on modulation of immune system, intestinal architecture and bacterial microbiota (Giannenas et al., 2018). Thyme is aromatic plant possess stimulant properties. Thyme is used in poultry nutrition in the form of herbal feed additive as it is known that its contents, such as thymol and carvacrol, have a positive impact on broiler performance and feed utilization, which in turn results in enhanced economic profits (Alipour et al., 2015). The results of study of Bertella et al. (2018) suggest that the essential oil of *Artemisia* can be a source of natural antibacterial agents with potential pharmacological

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applications, the essential oil of this species was known for its therapeutic disinfectant, anthelmintic and antispasmodic virtues. *Menyanthes trifoliata* (bogbean) is a very valuable medicinal plant. Bogbean is generally used in conjunction with other medicinal herbs. The leaf of the bogbean is a source of valuable herbal material. Bogbean contains iridoid glycosides, flavonol glycosides, phenolic acids, coumarins, triterpenoids, and very small amounts of alkaloids. The iridoids are strongly bitter and stimulate digestive secretions and appetite (Bacler-Žbikowska, 2012).

Coccidiosis in chickens is a parasitic disease with great economic significance, which has been controlled successfully for decades using mainly anticoccidial products. It is caused by coccidian protozoans of the genus *Eimeria*. However, large-scale and long-term use of anticoccidial drugs has led to the worldwide development of resistance against all these drugs (Peek and Landman, 2011). Increasing concerns about parasite resistance, consumer health, and environmental safety of the commercial drugs warrant efforts to search for novel agents with anti-*Eimeria* activity. Currently, it appears to be promising to identify safe combinations of low-cost natural products with high anti-*Eimeria* efficacy for a potential use as feed supplementation in animal farming (Wunderlich, et al., 2014).

The aim of this study was to determine the effect of humic acid and the combination humic acid with phytobiotics on production parameters and carcass characteristic of broiler chicken of hybrid Ross 308. Our goal was verify possibility of replacement of chemical coccidiostats in the feed mixture of chicken by the substances on the natural basis, as well.

## 2 Material and methods

### *Animals, diets and treatments*

In experiment from total 200 one-day-old ROSS 308 meat hybrid chicken were randomized into four groups. Each treatment group contained 50 birds. Chickens in individual groups were stabled on deep litter, with a maximum occupation of the breeding areas 33 kg·m<sup>2</sup>. During the fattening period, the light regimen based on 23 h of light and 1 h of dark was used. The temperature at the beginning of the experiment was 31–33 °C and week fell by 2 °C to 20–22 °C. The temperature was maintained using electronic hen-like devices providing radiant heat. The fattening lasted 42 days.

The starter diet was used from 1 to 21 days of age, the grower diet was used from 22 to 35 days of age and a finisher diet was used from 36 to 42 days of age. In connection with include of supplement were feed mixtures in powdery form. Anticoccidial drugs

were not included in the feed mixtures for first and second treatment group. In the feed mixture for a third treatment group were incorporated anticoccidial drugs (coccidiostats). Feed and water were supplied *ad libitum*. Composition of complete feed mixtures (Biofeed a.s., Kollárovo, Slovakia) is presented in Table 1.

In control group we used complete feed mixture without any additives. Group of chickens marked as treatment 1 (T1) was fed a diet containing 2% of humic acid, 2 kg per 100 kg complete feed mixture (basal diet – BD) (Vetservis s.r.o., Nitra, Slovakia); the group marked as treatment 2 (T2) was fed a BD containing 78% of humic acids; 18% of garlic powder (*Allium sativum* L.); 1% of milled dried leaves of wormwood (*Artemisia absinthium*); 1% of milled dried leaves of thyme (*Thyme vulgaris*); 1% of milled dried leaves of oregano (*Origanum vulgare*) and 1% of milled dried leaves of bogbean (*Menyanthes trifoliata* L.) together 2 kg per 100 kg complete feed mixture (BD) (Vetservis s.r.o. Nitra, Slovakia). In the group marked as treatment T3 were chicken fed with BD containing industrially produced coccidiostats. In the starter diet was used coccidiostats Dicluzaril, in the grower diet was used coccidiostats Salinomycinát sodný. Additives were mixed into the feed by the manufacturer of feed mixture used in the experiment.

### *Monitored performance and carcass parameters*

Performance parameters as body weight (g), feed intake (kg) and feed conversion (kg) were recorded weekly. At the end of the experiment 10 chickens from each group were slaughtered. In the laboratory of the Department of Poultry Science and Small Farm Animals in Slovak University of Agriculture in Nitra analysis of samples of chickens was realized. We focused on the carcass weight (g), weight edible offal (g) and carcass yield (%).

### *Statistical analysis*

All data were analysed by analysis of variance using the general linear model procedure of the software program SAS (Statistical Analysis System). Differences between the indicators were tested using one-way analysis of variance by Duncan's test. Significance was considered at  $P < 0.05$ .

## 3 Results and discussion

The objective of the present study is to investigate the effect of humic acid with various herbal additives to body weight, feed consumption, feed conversion and carcass parameters – carcass weight, percentage of breast part, percentage of thigh part, weight edible offal and carcass yield of broiler chickens. The effect of humic acid, combination of humic acid with phytobiotics and coccidiostats on body weight is presented in Table 2.

**Table 1** Composition of starter, grower and finisher complete feed mixture

Ingredient	Feed mixture		
	Starter	Grower	Finisher
Wheat (%)	35.00	35.00	36.82
Maize (%)	35.00	40.00	37.00
Soybean meal (%)	21.30	18.70	20.00
Fish meal 71% (%)	3.80	2.00	0.00
Limestone (%)	1.00	1.05	1.10
Monocalcium phosphate (%)	1.00	0.70	1.00
Salt (%)	0.10	0.15	0.20
Lysine (%)	0.05	0.07	0.29
Methionine (%)	0.15	0.22	0.29
Premix (%)	0.50	0.50	0.50
Chemical composition			
Metabolic energy (MJ)	12.01	12.03	12.37
Crude protein (g)	210.76	190.42	170.58
Crude fiber (g)	30.18	29.93	30.54
Crude ash (g)	24.24	19.93	38.49
Lysine (g)	11.30	9.89	9.95
Methionine(g)	4.96	5.21	5.46
Ca (g)	8.15	7.27	7.37
P (g)	6.75	5.70	6.00

Broiler chickens fed a diets containing 2% of humic acid (T1) showed significantly ( $P < 0.05$ ) higher body weight at the age of 35 and 42 days compared to the control group (C). The group of chickens fed a diets containing humic acid and phytobiotics (T2) showed significantly ( $P < 0.05$ ) higher body weight at the age of 21; 28; 35

and 42 days compared to control group (C). The group with coccidiostats supplement showed significantly ( $P < 0.05$ ) higher values at the age of 28; 35 and 42 days. Body weight in all the treatment groups was significantly higher compared to control group ( $P < 0.05$ ). The highest body weight on the 42. day of fattening was recorded in the first experimental group of chickens fed a diets containing only humic acids but the different treatments were not statistically significant. Our results are in agreement with Taklimi et al. (2012) or Lala et al. (2017) who likewise recorded significantly increased weight gain by adding of humic acid. Milošević et al. (2013) reported that supplementation of 1.5% and 3.0% of garlic powder had significant positive effect of body mass ( $P < 0.05$ ). Increased body weight in the experimental group with garlic supplement was also observed in experiment Ramiah et al. (2014) with 0.5% garlic powder addition. Issa and Omar (2012) recorded in this parameter no significant influence of 0.2% and 0.4% garlic complement. Study conducted by Toghyani et al. (2010) reported that broilers receiving 5 g.kg<sup>-1</sup> thyme had a significantly higher body weight at day 42 of age, while a report published by Kamali Sangani et al. (2014) claimed the opposite by demonstrating that no significant effect was recorded. In the experiment Hafeez et al. (2016) were body weight of birds at day 42 and overall body weight gain from day 1 to day 42 higher in treatment by carvacrol, thymol, and limonene than birds in control treatment.

The values of feed intake and feed conversion ratio of broiler chicken both treatment and control group were comparable ( $P > 0.05$ ) in our experiment. Average feed conversion ratio in the order of the groups: 1.77; 1.75; 1.77 and 1.67 kg. In contrast, Šamudovská and Demeterová (2010) or Lala et al. (2017) introduce that feed consumption of broiler chickens supplemented with humic acids improved feed conversion ratio. In the

**Table 2** The impact of humic acid, humic acid with phytobiotics and coccidiostats supplement on body weight of broiler chickens of hybrid Ross 308 (g)

Age/day	Group			
	C	T1	T2	T3
1.	40.78	40.50	39.54	40.08
7.	145.90 ± 14.59	147.85 ± 17.25	139.38 ± 18.53	143.70 ± 14.84
14.	341.29 ± 36.81	331.90 ± 47.46	334.67 ± 34.65	347.65 ± 45.10
21.	602.78 ± 55.56	600.45 ± 79.62	628.58 ± 59.64*	624.20 ± 82.35
28.	987.38 ± 113.02	1009.92 ± 125.86	1052.45 ± 89.04*	1040.11 ± 117.56*
35.	1380.80 ± 160.52	1480.23 ± 176.07*	1477.20 ± 122.04*	1461.55 ± 174.29*
42.	1808.03 ± 212.39	1981.75 ± 203.32*	1895.59 ± 178.75*	1955.31 ± 237.16*

C – BD (complete feed mixture), T1 – BD + humic acid, T2 – BD + humic acids + garlic (*Allium sativum* L.) + wormwood (*Artemisia absinthium*) + thyme (*Thyme vulgaris* L.) + oregano (*Origanum vulgare* L.) + bogbean (*Menyanthes trifoliata* L.); T3 – BD + coccidiostats; Values are Means ± SD; n = 50; Distinct superscript within row = significant difference ( $P < 0.05$ )

**Table 3** The impact of humic acid, humic acid with phytobiotics and coccidiostats supplement on carcass parameters of broiler chickens Ross 308

Parameter	Group			
	C	T1	T2	T3
Carcass weight (g)	1,357.18 ±95.8	1,486.38 ±156.7	1,369.69 ±118.0	1,440.68 ±132.1
Breast part (%)	32.37 ±1.65	32.35 ±1.60	31.95 ±1.03	31.37 ±1.59
Thigh part (%)	29.27 ±1.50	29.07 ±2.35	30.45 ±2.15*	29.49 ±2.34
Weight of liver (g)	45.61 ±6.12	43.25 ±9.46	43.13 ±6.83	45.75 ±7.02
Weight of heart (g)	9.73 ±1.23	10.56 ±1.55	9.66 ±1.80	10.22 ±0.94
Weight of gizzard (g)	34.55 ±4.02	33.61 ±7.96	32.55 ±4.62	33.28 ±5.39
Carcass yield (%)	74.35 ±1.32	76.10 ±1.97	74.03 ±1.35	73.45 ±1.82

C – BD (complete feed mixture), T1 – BD + humic acid, T2 – BD + humic acids + garlic (*Allium sativum* L.) + wormwood (*Artemisia absinthium*) + thyme (*Thyme vulgaris* L.) + oregano (*Origanum vulgare* L.) + bogbean (*Menyanthes trifoliata* L.); T3 – BD + coccidiostats; Values are Means±SD; n = 10; Distinct superscript within row = significant difference ( $P < 0.05$ )

experiment of Samanthi et al. (2015) was the highest ( $P < 0.05$ ) feed intake observed in birds fed with zero garlic level. The highest weight gain and lowest feed conversion ratio were observed in birds fed with 1 kg.ton<sup>-1</sup> of garlic ( $P < 0.05$ ). Similarly, Stanačev et al. (2011) with addition of 2% garlic and Mansoub et al. (2011) with addition of 1 g.kg<sup>-1</sup> garlic powder and 1 g.kg<sup>-1</sup> thyme powder to the feed mixture reported increased body weight and better feed conversion ratio.

The body weight, feed consumption, feed conversion ratio or carcass weight were not statistically significant influenced in the experiment of Pourmahmoud et al. (2013) or Haselmeyer et al. (2014) by adding different doses of thyme. Authors Seddiek et al. (2011) found out improvement the weight and feed conversion ratio using of *Artemisia herba-alba* extracts (0.4 g.kg<sup>-1</sup> body weight) in drinking water.

The effect of humic acid, their combination with phytobiotics and coccidiostats on carcass parameters present Table 3. Significant improvement in thigh part ( $P < 0.05$ ) we recorded as a result of combination of humic acid and phytobiotics supplementation in our experiment. In the others carcass parameters we found out any significant differences. Carcass weight (1,357.18 ±95.8; 1,486.38 ±156.7; 1,369.69 ±118.0 and 1,440.68 ±132.1 g ±SD) was the highest in treatment T1 with humic acid addition ( $P > 0.05$ ), however with statistically non-significant difference in compare with control group. Similarly, carcass yield (values in the order of the groups: 74.35 ±1.33; 76.10 ±1.97; 74.03 ±1.35 and 73.45 ±1.82 mean ±SD) was non-significantly the highest ( $P > 0.05$ ) in treatment T1 with humic acid supplement. A similar increase of carcass yield after administration of humic acid was found Marcinčáková et al. (2015). In the experiment of Ozturk et al. (2012) humic acid addition did not significantly affect carcass parameters. In the

experiment of Ceylan et al. (2003) feed conversion ratio in period from 4 to 6 weeks was significantly improved by supplement of humic acid, probiotic and prebiotic. Mortality and carcass yield were not influenced by experiment. At the termination of 42-day-trial with supplementation of humic acid no significant differences were observed in dressing percentage, breast-meat yield, abdominal fat pad, relative weights of liver, heart, gizzard, spleen and bursa among different treatments in the experiment Nagaraju et al. (2014). The results of the studies of Slyranda Baltini Aji et al. (2011) with adding of 50 and 100 mg dose of garlic and Zamora et al. (2017) with 0.4 g.kg<sup>-1</sup> oregano supplement showed that additives did not affect the carcass yield of the birds.

#### 4 Conclusions

Based on the obtained results, it can be concluded that the humic acid, combination humic acid and phytobiotics as well as coccidiostats supplement has positive effect on production parameters of broiler chicken. Broiler chicken fed a diets containing 2% of humic acid (T1), humic acid and phytobiotics and coccidiostats showed significantly ( $P < 0.05$ ) higher body weight compared to the control group. The group of chicken fed a diets containing of humic acid and phytobiotics (T2) showed significantly ( $P < 0.05$ ) higher percentage of thigh part in comparison to the control group. In feed conversion ratio, carcass weight, weight of heart, liver, gizzard and carcass yield of broiler chicken were not observed statistically significant difference ( $P > 0.05$ ) in compare with the control group. According to the results, humic acid, eventually their combination thyme can be used as a good alternative for commercial antibiotic growth promoters.



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## Non-genetic factors affecting somatic cell count in milk of dairy goat populations in Croatia and Slovenia

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Somatic cell count (SCC) is a useful indicator of intramammary infection of cow's udders and a standard of quality and hygiene of cow's milk in many countries. The main non-inflammatory factors influencing SCC in goat milk are intrinsic depending directly on the animal and extrinsic where some of them are routinely recorded along milk recording. The objective of this study was to determine the sources of non-genetic variation for SCC in Alpine (ALP) and Saanen (SAN) dairy goat populations based on Croatian (CRO) and Slovenian (SVN) milk recording data. For that purpose, test day records (327,404 in total) were used. They were collected over the period from 2005 to 2017 in Alpine (ALP) and Saanen (SAN) goat populations in Croatia (CRO) and Slovenia (SVN). The majority of records (310,371) represent CRO data, while SVN data included 17,033 records. Data have been obtained from the Central database of Croatian Agricultural Agency and Slovenian Central Database for Small Ruminants, which are collected according to the ICAR standards. Data were analysed using MIXED procedure in SAS/STAT, based on the REML method. Results showed significant effect of the population, parity, litter size, year and month of kidding and interaction between them as well as the effect of the stage of lactation nested within the population on the SCC in milk of studied goat populations.

**Keywords:** Alpine goat, Croatia, dairy goat, logSCC, Saanen goat, Slovenia, somatic cell count

### 1 Introduction

Alpine and Saanen goats are widely used populations among dairy breeds in both countries. The breeds are well adapted to the environment, since they originate from similar conditions in the Switzerland. They are seasonally fertile and kiddings occurred once per year, in the springtime. According to the Annual report of Croatian Agricultural Agency (CAA, 2016) around 4,500 animals of Alpine goat (CRO ALP) and around 700 animals of Saanen goat (CRO SAN) have been encompassed by the breeding work. In Slovenia (2017), there were around 1,100 animals of Slovenian Alpine goat (SVN ALP) and around 700 animals of Slovenian Saanen goat (SVN SAN) (Savšek et al., 2018). Milk recording is performed according to ICAR guidelines (ICAR, 2018) using regular alternate AT4 scheme (morning/evening system) in both countries. The milk of all mammals contains different types of somatic cells (SC), which are blood-borne SC and epithelial one. Somatic cells are present in healthy mammary glands, but regarding mammary inflammation, there is an increased influx of

blood leukocytes. The presence of leukocytes in milk results in increased somatic cell count (SCC) values. On the other hand, the non-infectious factors can influence on the SCC as well. Epithelial cells in milk result from desquamation of the epithelium of alveoli and ducts of the mammary gland that is mainly physiological, due to regeneration of the normal epithelia. Besides the presence of SC, there are also cytoplasmic particles in the milk that originated from the distal alveolar mammary secretory cells. These formations are very abundant when milk secretion is apocrine, as in the case of goats, and very few or virtually absent, when the discharge is merocrine, as in cattle (Jimenez-Granado et al., 2014).

SCC is a useful indicator of intramammary infection of cow's udders and a standard of quality and hygiene of cow's milk in many countries. However, three characteristics distinguish goat milk from sheep or cow milk: higher values of SCC, cytoplasmic particles and polymorphonuclear neutrophils. In the absence

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of mastitis, SCC in goat milk varies between  $270 \times 10^3$  and  $2,000 \times 10^3$  SC/ml what confirmed that SCC in goat milk is not highly correlated to intramammary infection (Jimenez-Granado et al., 2014). The main non-inflammatory factors influencing SCC in goat milk are intrinsic – depending directly on the animal (fraction of milking, time between milking, milking frequency, daily variations, stage of lactation, number of lactation, prolificacy, breed, production level, heat) and extrinsic (type of milking, feed, stress, seasonality, farming system, facilities) (Jimenez-Granado et al., 2014). Some of them are routinely recorded along milk recording (Jimenez-Granado et al., 2014).

The objective of this study was to determine the sources of non-genetic variation for SCC in Alpine (ALP) and Saanen (SAN) dairy goat populations based on Croatian (CRO) and Slovenian (SVN) milk recording data.

## 2 Materials and methods

Test day records of Alpine (ALP) and Saanen (SAN) dairy goat populations from two countries, Croatia (CRO) and Slovenia (SVN) collected in the period from 2005 to 2017, were used for the joint analysis. Croatian data were taken from the Central database of the Croatian Agricultural Agency, while Slovenian data were provided by the Central Database for Small Ruminants, which is maintained by the Department of Animal Science, Biotechnical Faculty, University of Ljubljana. Test-day records were included in the analysis while SCC was

the analysed trait. These records were collected in accordance to the ICAR guidelines (ICAR, 2018) using regular alternate AT4 scheme (morning/evening system), while goats were machine milked twice a day. The raw data contained 497,372 test-day records. CRO data represented the majority of the records containing 329,022 records of the CRO ALP and 35,576 of the CRO SAN population. The number of test-day records was considerably lower in the SVN ALP (91,468) and SVN SAN (37,306) populations. The reason was that in Croatia the costs of the SCC analysis is covered by the state, while in Slovenia breeders need to pay analysis by themselves. In the further analysis, goat breeds from both countries were considered as four different populations.

Prior to the statistical analysis, some of the test-day records were excluded or modified. The following was excluded: records collected after sixth parity, records collected before 5<sup>th</sup> and after 305<sup>th</sup> days in milk, and records where SCC was unknown value. All multiple litters were considered as the same class (2+). The season of kidding was defined as a month of kidding. Seasons with less than 30 records were joined to the previous or the next season to improve the data structure. After pruning of the original data set, a total of 327,404 records obtained from 23,923 does were used in the statistical analysis. In the preliminary study, the normality of SCC was tested and logarithmic transformation for SCC (logSCC) was performed. Descriptive statistics for the analysed traits are presented in Table 1.

**Table 1** Descriptive statistics for the milk production traits

Breed (Country)	Trait	N	Mean	SD	Min	Max
All populations together	DMY (kg)	327,404	2.34	1.06	0.30	6.00
	SCC (log)	327,404	9.13	1.96	1.00	15.00
	DIM (days)	327,404	148.88	69.93	5.00	305.00
ALP (Croatia)	DMY (kg)	279,286	2.32	1.05	0.30	6.00
	SCC (log)	279,286	9.14	1.96	3.00	15.00
	DIM (days)	279,286	150.00	69.94	5.00	305.00
SAN (Croatia)	DMY (kg)	31,085	2.66	1.13	0.31	5.99
	SCC (log)	31,085	9.14	1.97	3.00	14.87
	DIM (days)	31,085	148.47	68.68	5.00	305.00
ALP (Slovenia)	DMY (kg)	8,720	1.98	1.05	0.30	6.00
	SCC (log)	8,720	9.43	1.96	1.00	13.65
	DIM (days)	8,720	127.47	61.47	5.00	288.00
SAN (Slovenia)	DMY (kg)	8,313	1.97	0.85	0.30	6.00
	SCC (log)	8,313	8.51	1.81	1.00	13.29
	DIM (days)	8,313	135.30	76.99	5.00	305.00

DMY – daily milk yield, SCC – somatic cell count, DIM – days in milk, ALP – Alpine goat, SAN – Saanen goat, N – number, SD – standard deviation, Min – minimum, Max – maximum

The average logSCC was 9.13 and daily milk yield 2.34 kg.day<sup>-1</sup> (Table 1) for all populations. There was no strong deviation of any population for the logSCC. The average lactation length (days in milk) was 148.88 days.

The following model present the best fit for logSCC and is shown in scalar notation (1):

$$y_{ijklmno} = \mu + B_i + \sum_{p=1}^4 b_{pi} t_p + P_j + L_k + Y_l + M_m + YM_{lm} + e_{ijklmno} \quad (1)$$

Effects of population ( $B_i$ ), parity ( $P_j$ ), litter size ( $L_k$ ), year of kidding ( $Y_l$ ), month of kidding ( $M_m$ ), and interaction between the year and month of kidding ( $YM_{lm}$ ) were considered as fixed class effects. Days in milk ( $t_{ijklmno}$ ) was fitted as a covariate and modelled using the Ali-Schaeffer lactation curve (Ali and Schaeffer, 1987) nested within the population. A transformation of the days in milk (Eq. 2) was done with the constant of 305 as follows:

$$t_1 = \frac{t_{ijklmno}}{305}, \quad t_2 = \left( \frac{t_{ijklmno}}{305} \right)^2, \quad (2)$$

$$t_3 = \ln \left( \frac{305}{t_{ijklmno}} \right), \quad t_4 = \left( \ln \left( \frac{305}{t_{ijklmno}} \right) \right)^2$$

Flock effect nested within the population ( $f_{in}$ ) was included in the model as a random effect. Analyses were performed using the proc MIXED procedure in the SAS/STAT statistical package (SAS Inst. Inc., 2011) that is based on the restricted maximum likelihood method (REML). Least squares means of logSCC were computed for each of the significant fixed effects.

### 3 Results and discussion

The present study showed that all effects included in the model were significant ( $P < 0.001$ ). The random effect of the flock accounted for a relatively large part of the phenotypic variance for the SCC (Table 2).

The effect of the breed significantly affected SCC in milk. The lowest logSCC was found in the milk of SVN ALP goat (8.81 ± 0.19) and the highest logSCC in the milk of CRO SAN goat (9.48 ± 0.05) (Table 3). Rupp et al. (2011) found expected lower values of logSCC in the milk of first kidding does of Alpine (5.09 ± 1.36) and Saanen (5.32 ± 1.19) goats. Jimenez-Granado et al. (2014) explained breed variability with the difference in the health status, milk yield and the management used.

Litter size significantly affected SCC in milk, with the lowest logSCC (9.11 ± 0.06) in the milk of does with single kid and the highest logSCC (9.22 ± 0.06) in the milk of does with twins or more kids (Table 3). Jimenez-Granado et al. (2014) explained the difference with worse health

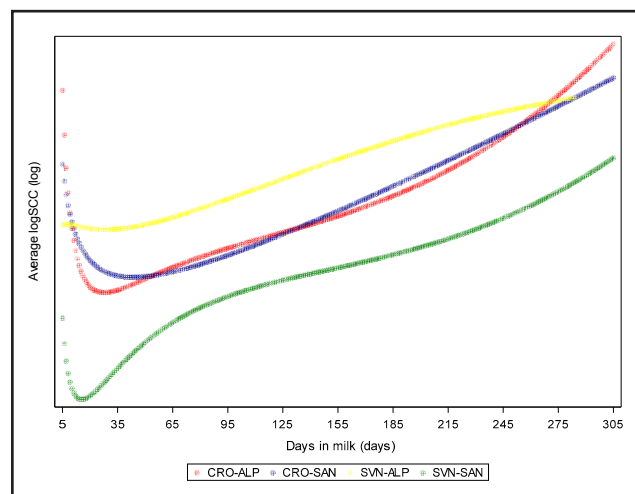
status of the udder in mothers who breastfeed two kids compared to those who only nurse one.

**Table 2** Significance ( $P$ -values) of the effects fitted in the model and variance component estimation for somatic cells count (SCC)

Trait	$P$ -values
$B_i$	<0.001
$P_j$	<0.001
$L_k$	<0.001
$Y_l$	<0.001
$M_m$	<0.001
$YM_{lm}$	<0.001
$b_{1i}$	<0.001
$b_{2i}$	<0.001
$b_{3i}$	<0.001
$b_{4i}$	<0.001
Variance estimation	
$\sigma_{fp}^2$	0.4542 ± 0.039
$\sigma_e^2$	3.2481 ± 0.008

$B_i$  – population,  $P_j$  – parity,  $L_k$  – litter size,  $Y_l$  – year of kidding,  $M_m$  – month of kidding,  $YM_{lm}$  – interaction between year and month of kidding,  $b_{1i}$ ,  $b_{2i}$ ,  $b_{3i}$ , and  $b_{4i}$  – parameters of lactation curve nested within the population,  $\sigma_{fp}^2$  – variance of flock nested within the population,  $\sigma_e^2$  – residual variance

Parity significantly affected SCC in milk, with the lowest logSCC (8.59 ± 0.07) in the milk of does from the first parity and the highest logSCC (9.64 ± 0.07) in the milk of does from the sixth parity (Table 3). The influence of the parity on the SCC seems to depend on the health status of the udder, what could be attributed to a longer



**Figure 1** Least squares means for SCC across days in milk CRO ALP – Croatian Alpine goat, CRO SAN – Croatian Saanen goat, SVN ALP – Slovenian Alpine goat, SVN SAN – Slovenian Saanen goat

exposure of the older animals to pathogens compared to younger one (Jimenez-Granado et al., 2014).

**Table 3** Least squares means (LSM) ± standard errors (SE) of logSCC across fixed effect classes

Trait	logSCC (LSM ±SE)
Population	
CRO ALP	9.47 ±0.10
CRO SAN	9.48 ±0.05
SVN ALP	8.81 ±0.19
SVN SAN	8.89 ±0.14
Litter size	
1	9.11 ±0.06
2	9.22 ±0.06
Parity	
1	8.59 ±0.07
2	8.86 ±0.07
3	9.11 ±0.07
4	9.30 ±0.07
5	9.50 ±0.07
6	9.64 ±0.07

CRO ALP – Croatian Alpine goat, CRO SAN – Croatian Saanen goat, SVN ALP – Slovenian Alpine goat, SVN SAN – Slovenian Saanen goat

Days in milk were modelled using Ali-Schaeffer lactation curve nested within the population (Figure 1). The highest logSCC across days in milk was found in the milk of SVN ALP goat followed by the CRO ALP and SAN goat. The lowest logSCC across days in milk was found in milk of SVN SAN goat (Figure 1). Several authors reviewed in Jimenez-Granado et al. (2014) have explained the increase in SCC during the lactation due to a dilution effect and decreasing milk production according to days in milk. Consequently, SCC was higher at the end of lactation.

## 4 Conclusions

This study for the first time compared SCC in the milk of Slovenian goat breeds with those of goat breeds from a neighboring country. Analysis of variance showed the significant effect of population, parity, litter size, year of kidding, month of kidding and the interaction between year and month of kidding on the SCC in milk what was in accordance to the literature investigated goats breeds in several countries. Taking all above into consideration it is necessary to study in details factors contributing to elevations in SCC, since subclinical mastitis is not detected visually and requires an indirect method, such as the SCC, to detect goat udder health.

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## Comparative study of productive performance and carcass parameters of Oravka, Amrock and their reciprocal crossbred chickens

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The objective of this study was to compare the pure chicken breeds Oravka (OR;  $n = 50$ ), Amrock (AM;  $n = 50$ ) and their cross Oravka  $\times$  Amrock (ORAM;  $n = 50$ ) and Amrock  $\times$  Oravka (AMOR;  $n = 50$ ) for productive and carcass parameters. The birds were maintained on a deep litter system for a period of 20 weeks. We recorded that crossbred ORAM and AMOR chickens performed better than the average of parental genotypes for body weight and body weight gain in brooding and growing period. The poor ( $P < 0.05$ ) feed conversion was observed in AM chickens and better feed conversion was recorded in OR and both crossbred chickens. The crossbred chickens had lowest ( $P < 0.05$ ) mortality than pure bred AM chickens. The carcass parts, giblets and abdominal fat percentages had non-significant ( $P > 0.05$ ) difference among pure and crossbred chickens. The highest carcass yield was observed in ORAM (62.53%) followed by AMOR (62.48%), AM (62.41%) and OR (62.39%) chickens.

**Keywords:** body conformation, body weight, chicken, crossbreeding, feed utility

### 1 Introduction

Dual purpose chicken genotypes are increasingly popular in some regions of Eastern and Middle Europe and Asia, where they play an important social role among farmers and have a positive impact on maintaining rural society and traditional form of agriculture as well as gratify certain local traditions (Almasi et al., 2012).

Cross breeding can be carried out as two-way, three-way or four-way crosses, back crosses or rotational crosses. This system also maximizes the expression of heterosis, or hybrid vigour in the cross, normally reflected in improved fitness characteristics (Hoffmann, 2005).

A good combining ability resulting from a choice of the best performing crossbred could lead to the production of birds that will be better in growth rate, efficiency of feed conversion and reproductive traits, without sacrificing adaptation to the local environment, thereby resulting in reduced costs of production (Adebambo et al., 2011; Khawaja et al., 2016).

The aim of present study was designed to develop a rural breed resulting in reciprocal crossing between native

Oravka and Amrock breeds with better body weight and feed conversion efficiency.

### 2 Materials and methods

A total of 200 unsexed day-old-chicks of Oravka (OR,  $n = 50$ ), Amrock (AM,  $n = 50$ ) and their reciprocal crossbred (OR male  $\times$  AM female: ORAM,  $n = 50$ ; and Amrock male  $\times$  Oravka female: AMOR,  $n = 50$ ). All of the chicks were reared under standard temperatures 33 °C at chick level for 1 week, followed by a reduction of 2 °C/week until the temperature reached 19 °C at 6 week of age. The birds were maintained in floor pens on deep litter system for a period of 20 weeks.

Chickens were *ad libitum* fed standard feed mixtures (208.95 g crude protein, 11.76 MJ metabolizable energy, 8.46 g calcium, 5.72 g available phosphorus up to 8 weeks of age and 162.94 g crude protein, 12.01 MJ metabolizable energy, 8.96 g calcium, 5.30 g available phosphorus up to 20 weeks of age).

The growth performance data (initial body weight, final body weight, and feed conversion) were recorded at 8 and 20 weeks of age. Feed conversion was calculated

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as the ratio of grams of feed to grams of weight gain. Mortality was also recorded over period.

At the age of 20 weeks, 10 representative birds from each replicate were slaughtered to obtain their carcass parameters. The breast, thighs, back, wings, giblets and abdominal fat were collected and weighed individually and their percentages in relation to live body weight were calculated. The results obtained were used to calculate carcass yield.

The statistical analyses were conducted using JASP 0.8.6 software (JASP, 2018). Significant difference was used at 0.05 probability level and differences among groups were tested using the Duncan's Multiple Range Test (Duncan, 1955).

### 3 Results and discussion

The growth performance and mortality of Oravka, Amrock and crossbred chickens during growing phase is shown in Table 1. The average day-old weight was highest in OR (34.26 g), intermediate in ORAM (34.11 g) and AMOR (33.98 g), lowest in AM (33.89 g). In brooding and growing period, we that found both crossbred ORAM and AMOR chickens recorded better than the average of parental genotypes for body weight and body weight gain. The poorest ( $P < 0.05$ ) feed conversion was observed in OR chickens and the best feed conversion was recorded in ORAM crossbred chickens. The results showed that both ORAM and AMOR crossbred chickens had lower ( $P < 0.05$ ) mortality than pure AM chickens.

The carcass parts, giblets and abdominal fat percentages had a non-significant ( $P > 0.05$ ) difference between pure and crossbred chickens (Table 2). We found no significant difference ( $P > 0.05$ ) in carcass yield between all crossbred chickens (Table 2). Numerically, the highest carcass yield was found in ORAM (62.53%) followed by AMOR (62.48), AM (62.41%), and OR (62.39%) chickens.

In present experiment, crossbred animals performed better than the average of parental genotypes for body weight, body weight gain and feed efficiency. Heterosis was found in body weight and body weight gains, as reported by Khawaja et al. (2016). In contrast, Sharaf et al. (2006) recorded that crossbreeding did not improved body weight at sexual maturity. The results of our experiment are partially in line with the findings of Nawar et al. (2004), Iraqi et al. (2005) and Besbes (2009), who found that crossbreeding improved chick viability.

Breed differentiation showed no significant ( $P > 0.05$ ) difference in body composition of crossbred chickens at age of 20 weeks. The results agreed with the work of Khawaja et al. (2016) for first generation of newly evolved hybridized pure chicken and their crossbred parents. According to the literature, the mean yield for slow-growing chickens ranged between 13.4 and 26% for breast, between 24.6 and 37.4% for thighs (Janocha et al., 2003; Sengül et al., 2003).

Accordingly with Khawaja et al. et al. (2014), we found out no significant ( $P > 0.05$ ) effect of crossbreeding on carcass yield. Although, carcass yield is affected by a number of

**Table 1** Comparative productive performance of Oravka, Amrock and reciprocal crossbred chickens during brooding and growing period

Parameter	Age (weeks)	Breeds			
		OR	AM	ORAM	AMOR
Day old weight (g.bird <sup>-1</sup> )	–	34.26 ±3.86	33.89 ±3.72	34.11 ±3.87	33.98 ±3.49
Body weight (g)	8	641.23 ±143.08	652.98 ±149.97	651.69 ±148.47	648.57 ±146.81
	20	1,844.21 ±249.78	1,889.76 ±255.88	1,871.88 ±251.64	1,862.45 ±252.62
Body weight gain (g)	0–8	606.97 ±3.29	619.09 ±3.24	617.58 ±3.31	614.59 ±3.37
	8–20	1,202.98 ±4.47	1,236.78 ±4.52	1,220.19 ±4.28	1,213.88 ±4.42
	0–20	1,809.95 ±4.88	1,855.87 ±5.02	1,837.77 ±4.91	1,828.47 ±4.86
Feed conversion	0–8	6.42	6.39	6.22 <sup>a</sup>	6.25 <sup>b</sup>
	8–20	3.78	3.89	3.73	3.74
	0–20	4.37	4.31	4.24	4.26
Mortality (%)	0–8	2.00	4.00	2.00	2.00
	8–20	2.00	2.00	2.00	2.00
	0–20	4.00 <sup>a</sup>	6.00	4.00 <sup>b</sup>	4.00 <sup>c</sup>

<sup>a-c</sup> Means with different letters differ significantly ( $P < 0.05$ ); OR – Oravka, AM – Amrock, ORAM, OR male × AM female; AMOR, AM male × OR female. Data are expressed as mean ± standard deviation

**Table 2** Comparative carcass parameters of Oravka, Amrock and reciprocal crossbred chickens

Parameter	Age (weeks)	Breeds			
		OR	AM	ORAM	AMOR
Breast (%)	20	16.69 ±0.51	16.84 ±0.49	16.92 ±0.51	16.89 ±0.48
Thighs (%)	20	23.21 ±0.79	23.28 ±0.77	23.48 ±0.82	23.34 ±0.76
Back (%)	20	14.74 ±0.55	14.89 ±0.59	14.98 ±0.58	14.81 ±0.56
Wings (%)	20	6.29 ±0.38	6.33 ±0.36	6.34 ±0.33	6.32 ±0.36
Giblets (%)	20	4.86 ±0.14	4.79 ±0.12	4.91 ±0.17	4.84 ±0.15
Abdominal fat (%)	20	0.31 ±0.09	0.28 ±0.11	0.33 ±0.07	0.32 ±0.12
Carcass yield (%)	20	62.39 ±0.37	62.41 ±0.39	62.53 ±0.32	62.48 ±0.36

OR – Oravka, AM – Amrock, ORAM, OR male × AM female, AMOR, AM male × OR female. Data are expressed as mean ±standard deviation

factors including genetic, slaughtering conditions, feed, and live weight (Havenstein et al., 2003; Brickett et al., 2007).

#### 4 Conclusions

In conclusion, crossbred chickens gained better body weight than Oravka and moderate than Amrock chickens with partially lower mortality. The carcass parameters had no significant difference between pure and crossbred chickens. The crossbred chickens of ORAM showed better performance in all traits than crossbred chickens of AMOR.

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## The use of new technologies in horse selection

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Most successful selection strategies are not in use in horse selection. This study describes the use of current strategies of selection in horse breeding and tries to answer why well known, scientifically proven selection strategies are still not implemented. In the era of genomic selection (GS) in animal breeding, decision making in horse selection, even classical breeding values (BV), are not fully taken advantage of. Breeding organizations (BO) which implement BVs as selection criteria in their breeding programs show great success in genetic gain. However, a horse's achievements and the popularity of its relatives still play an important role in selection level. Genomic based selection tools in horse breeding have limitations in quantitative traits since it is difficult to establish reference population. Mendelian traits have been studied for several years, especially those related to horse health and functional longevity (long sport or race careers), yet there are still only a small number of validated mendelian traits offered for horses. The important benefit of GS is the prevention of related mating based on genomic data, in addition to pedigree data. The specialty in horse breeding is the use of cloning. From the point of view of genetic variability, cloning is useful in enabling geldings or non-genetically infertile animals to carry genes to next generations. In sport and race BOs, breeding stallions from foreign BOs are frequently used. In such cases a need for comparable BVs exists. These factors were the motivation behind the establishment of Interstallion 20 years ago. Due to difficulties in BO collaboration, Interstallion has not been as successful as planned. On the whole, it is expected that the sector of horse selection will need to change considerably in the future.

**Keywords:** breeding value, cloning selection, genomic selection, inbreeding, strategies

### 1 Introduction

Horse selection is not much younger than horse domestication. Breeders most commonly use foals for the next generation of breeding mares and stallions if they show positive traits such as: health, fast growth, easy handling, temperament attuned to the intended use, etc.

The variety of possibility of horse use is one of the largest in any domestic animal species. From domestication through the Industrial revolution, horses have been used mainly for food, transport, work in agriculture and forestry, work in the military and for other different kinds of work. More recently, the main role of horses in developed countries has been changed and their previous roles have become much less important. Today, the most economically important industries involving horses are those of racing and sport. However, most people involved in the horse industry are leisure riders. A growing use of horses can also be found in tourism, equine assisted activities and therapies (EAAP), and the niche use for meat and milk production. Additionally, in the last few

decades, there has been an interest in returning to an almost forgotten use of horses in agriculture and forestry (Liljenstolpe, 2009).

For a long time, breeders for selection have made decisions based purely on phenotypic observations. These points of interest have mostly been conformation traits. In the middle of the 20<sup>th</sup> century, the first study on how to estimate the additive genetic effect, known as BV, was done by Henderson (1953). From a scientific point of view BV is the most powerful tool for efficient selection. In comparison with phenotypic value, which is the measurement of the animal itself, BV describes the expected value of offspring (Koenen et al., 2004).

The first use of BV estimation was done for Islandic horses (Árnason, 1984) after finding 30 years of theoretical basis. At the time, BV estimation was used in dairy cattle, where recording different economically important traits was widely in use. In a short period of time, BVs in dairy cattle have become the main selection criteria and bull semen has been priced according to their BVs. Consequently, the

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knowledge and importance of BV for successful selection has spread from science to practice. In the horse sector, this has not happened yet. Breeders do not trust BV and horse prices are still tailored predominantly according to phenotypic measurements than BVs. Several examples have shown that the use of BV in horse breeding programs have had equal success as in cattle. Both BOs, as well as some breeders within BOs, who use BVs in their breeding decisions have had great success; examples include the Islandic horse, Swedish warmblood, and Royall Dutch sport horse (KWPN) (Koenen, 2005).

The aim of this study is to describe the use of current strategies of selection in horse breeding and to find answers to why well known, scientifically based selection strategies, routinely practiced in cattle selection, are not in use in horse selection.

### ***Selection strategies in horse breeding***

It is well known that the most successful breeding programs in dairy cattle cannot be reproduced in horses. The main reasons are: significantly smaller populations, longer generation intervals, more traits – some of which are difficult to measure – and the strong effect of preferential treatment. On the other hand, pedigree data in horses is more complete and most traits are measured for both genders. These facts need to be taken into consideration in selection programs for horse populations (Koenen et al., 2004).

### ***Classical selection***

Today, classical selection involves the use of BVs as selection criteria, which are estimated based on phenotypical records and pedigree information.

Most sport BO publish BVs for economically important traits. Important BV for trotters do the same. However, the making of selection decisions, based on a horse's performance and that of its relatives (their phenotype) still plays an important role. A unique situation in selection can be seen in thoroughbreds. The only selection criteria there is phenotype. A good example is one of the best thoroughbred stallions in the southern hemisphere, Savabell born in 2001. He has excellent performance and is a long standing carrier stallion, having produced more than 1,000 offspring. None of his offspring have reached or surpassed his results but at the age of 17 years his mating price is still growing (Waikato, 2018). There is general agreement that there is no longer any genetic progress in thoroughbreds. On the other hand, there are several strategies on how to breed good race horses with inbreeding. The most well-known is the so-called Rasmussen Factor, developed by Leon Rasmussen, which occurs when there is a duplication of a female ancestor between an individual's sire and dam, within five generations or closer (FMITCHELL07, 2009).

Conversely, selection decisions based on BVs have proven to be successful in sport horses. After the KWPN BO separated the selection goals for dressage and show jumping horses, at the beginning of the 21<sup>st</sup> century, it became the first BO in the World Breeding Federation of Sport Horses (WBFSH) rankings which was classified as the best BO in dressage and show jumping in 2009 and remained the best BO for both disciplines for three years. To date, no other BO has reached such success (WBFSH, 2018).

In several other BO which are not mainly selected for sport (local breeds) BVs are also estimated mainly for conformation traits. Of course, in these breeds BVs are even less reliable since populations are smaller than in sport horses and data recording is most often less consistent. In smaller populations prevention of related mating is most important. For these purposes breeders mostly check the pedigree information and do not use stallions with a common ancestor of the broodmare in at least the first three generations.

The fact, in horse breeding, is that the value of a horse is generated mostly based on their and their relatives' results, as well as their conformation regardless of the fact that BVs are scientifically and practically better criteria for selection decision making.

### ***Genomic selection***

The main advantage of GS, according to classical selection, is the reduction of the generation interval. Although the reliability of BVs based on genomic data (GBV) are lower, the expected genetic gain is higher because reduction of the generation interval is so high (Eggen, 2012). Those of us who know that the generation interval in horses is much longer than in dairy cattle, where GS has been in routine use, with great success, during the last decade, also have high expectations for horse selection (Reed, 2018). However, a lot must be done to gain enough quality reference population for quantitative traits. For this, there are not many strategies. A single horse BO is not large enough to set up its own reference population. The only option seems to be to merge the data between BO with similar breeding goals. This idea is quite old and resulted in the founding of the Interstallion organization 20 years ago (Thorén Hellsten, 2008). Unfortunately, it has yet been unable to compound a common data set for international BV evaluation. In the Brown Swiss cattle population this problem was solved with the successful Intergenomics project, the results of which have been in routine use since 2012 (Jorjani et al., 2012).

Other options for GS are more realistic for implementation in horse selection schemes. Reducing gene frequency for genetic disorders, increasing the frequency for desired mendelian traits; and prevention of related matings



according to functional inbreeding which is based on genomic data (Mark, 2013).

Mendelian traits have been studied intensively for several years. There is huge interest within BO to include mendelian traits which are related to horse longevity, into breeding programs. The work investment with young horses is quite high, especially in sport and race horses, so a payoff with a long sport and race career is desirable (Mark, 2013). Nevertheless, there is still a lack of reliable results from genomic associated studies in comparison with other species. The OMIA database has only 57 mendelian trait entries in horses, but 312 in dogs and 246 in cattle (OMIA, 2018). The reason is probably well known in horse breeding – lack of collaboration; in this case a lack of data exchange.

The prevention of related mating is very important for all horse breeds. In larger populations the reason for inbreeding is too often the use of popular stallions and, in indigenous breeds, the size of the population. Traditionally, breeders check the common ancestors between their mare and potential stallions or the relationship coefficient based on pedigree data and the inbreeding coefficient of each breeding animal. Pedigree based inbreeding and relationship coefficients are not as reliable as genomic based coefficients, while pedigree information is never complete and mistakes are common. Genome based relationship coefficients express relationships between animals based on the degree of gene function equality. This is the reason that management of inbreeding in a population is much more under control with the use of genomic data which motivates BO to implement such methods in their breeding programs (Reed, 2018).

### ***Cloning***

Generally, cloning is not known as a useful tool in animal selection. In populations under selection programs we have a positive genetic trend towards economically important traits. Younger animals are better, on average, and there is no sense in duplicating (cloning) the old genomes. However, in sport horses the situation is somewhat different. Behavior in most types of competitions is affected by gender. Rankings of horses in many disciplines show that most top horses are geldings, especially in steeple-chase, endurance, eventing, and dressage.

With cloning we get a breeding animal out of a gelding and this is a good cost benefit for owners. For an attractive stallion (a clone of the gelding) the estimated added value would be more than two million US dollars. This amount of money can be reached in just five years if we assume 200 services per year and the cost of service at \$ 2000. This economical potential motivated science and industry to introduce cloning into routine practice.

Just two years after the first equine was cloned in 2003, the CRYOZOOTECH company cloned the castrated endurance champion Pieraz (Reis et al., 2012). Cruising, one of the most successful Irish sport horses, was cloned in 2011 which resulted in two colts born in 2012: Cruising Arish (ISH) and Cruising Encore (ISH), whose frozen semen is available in the US (Shelbourne Farm, 2018). In the same period, the most famous KWPN dressage stallion Jazz was cloned twice. The service of his clone Broere Jazz was available in the 2018 mating season in the Netherlands at a cost of 2,850 € (Broere Jazz, 2018).

During the time of cloning of these two famous stallions, a heated debate about cloning ethics within stakeholders of the horse industry took place (FEI, 2012). For race horses, thoroughbreds, and trotters cloning is not allowed, but the World Breeding Federation for Sport Horses allowed clones to be registered in 2012. From that time on, cloning of top sport horses has become quite popular. Not only the cloning of geldings but also stallions and mares to prolong the longevity of their genomes – for reproductive purposes.

On the other hand, the possibilities of cloning are also an issue for indigenous breeds, especially when a breeding animal becomes infertile and it is important from the genetic pool of a particular breed. For now, cloning in such cases is not practiced and breeding programs of indigenous breeds are still quite conventional since the cost benefit of the clone cannot yet cover the cloning costs.

### ***International comparison of breeding values***

In sport horses especially, where breeding stallions from foreign BO are widely in use, breeders need to compare BVs between different scales. Interstallion was founded in 1998 for this very purpose (Thorén Hellsten, 2008). It was a logical decision since Interbull, in 1994, published the 1<sup>st</sup> routine evaluation of international BVs for dairy bulls and in 1996 become the official reference laboratory for BV evaluation in the EU. Interbull BVs have been widely used in national breeding programs. More than 60% of the progeny of tested bulls in Brown Swiss and Holstein populations have foreign sire (Philipsson, 2009).

In twenty years, Interstallion has not succeeded in collecting enough data from different BO to make an official international evaluation for sport horses. The main reason being that BO for sport horses tend not to share data. Considerable progress has been made with Interstallion's guidelines for standardization of publishing BV. Currently, their main objectives are to harmonize linear scoring systems for type traits between BO (Stock et al., 2015; Stock et al., 2018).

## 4 Conclusions

Well known and effective selection strategies and technologies in animal selection are in use in dairy cattle populations. It would be expected that such methods would also be used in the horse, especially sport and race horse populations.

In terms of GS on quantitative traits it is fair to say that it is much more difficult to produce a reference population in horses than in dairy cattle and we need time for simulation studies to be introduced and validated in practice. On the other hand, use of genomic tools to prevent genetic disorders and select for other mendelian traits in horse selection programs, seems to be in the near future. More so than in cattle selection. The reason being investment in the training of sport or race horses based to their functional longevity.

Selection of quantitative traits is still traditionally based on a horse's performance and its relatives' achievements. Herein lies an opportunity for BO, individual breeders, or companies to choose horses with imperfect results and no famous relatives but with high BVs, based on selection knowledge. Such horses are not as expensive but can produce very good offspring.

In long term selection, prevention of related mating will play an important role. The horse population is small and famous stallions are used too often, especially with new techniques (artificial insemination, cloning). To reduce the increase of inbreeding in populations, mating decisions based on genomic data will be more useful than pedigree-based ones. Cloning can play an important role in cases where a mare or stallion is not related to the population but is infertile due to non-genetic causes (too old, castrated, other fertility problems) and has no offspring.

In general, knowledge-based selection decisions need to be used more in the future in order to effectively deal with the challenges that await us in horse populations.

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## Genetic diversity in five Czech native horse breeds assessed using microsatellite markers

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The aim of the present study was to analyse the genetic diversity of the endangered horse breeds kept in the Czech Republic. A set of 13 microsatellites was used for genotyping 349 Silesian Norikers, 397 Norikers, 552 Czech-Moravian Belgian horses, 271 Old Kladrubers (175 greys, 95 blacks) and 241 Hucul horses. The proportion of obtained heterozygosity indicates no major loss of genetic diversity within analyzed breeds. The Wright's  $F_{ST}$  and genetic distances indicated genetic segregation of both colour varieties of the Old Kladruber breed and small genetic distances between draft horse breeds. Moreover, the membership probability outputs showed that the frequencies of alleles varied across the three main regions. First region is represented by draft horse breeds, second region is represented by Old Kladruber horse and the last is represented by Hucul breed. The study provides data and information utilizable in the management of conservation programs in order to reduce inbreeding and to minimize loss of genetic variability.

**Keywords:** admixture, endangered breeds, horse, loss of genetic diversity

### 1 Introduction

Genetic diversity studies in domestic animals focus on evaluating genetic variation within and across breeds mainly for conservation purposes. The evaluation of genetic diversity between livestock breeds is an important prerequisite for developing effective and meaningful breed conservation programs. An effective management of farm animal resources requires comprehensive knowledge of the breeds' characteristics including data on population size and structure and within and between breed genetic diversity. In animal breeding, the knowledge of genetic characterization and genetic structure is the first step in breed conservation and may have implications for future breeding strategies and management plans. The analysis of the genetic structure of a population can be carried out using genealogical or molecular information. In the case of missing or incomplete pedigree, it would be better to use molecular information to characterize a population; moreover, the molecular information indicates the additional relatedness between animals

appearing as founders in the pedigree (Delgado et al. 2014). The aim of this study was to analysis of genetic diversity within and between five endangered Czech horse breeds.

### 2 Materials and methods

#### Data

In the present study 1809 individuals from five endangered Czech horse breeds were used: 349 Silesian Norikers (SN), 397 Norikers (N), 552 Czech-Moravian Belgians (CMB), 270 Old Kladruber horses (175 greys – OKg, 95 blacks OKb) and 240 Huculs (H). Data were provided by the Association of Horse Breeder Unions as an umbrella organization of individual breeders. The oldest individual was born in 1978, whereas the youngest animals were born in 2016. The total set of 13 microsatellite markers (AHT4, AHT5, ASB2, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, and VHL20) recommended for parentage testing by the International Society for Animal Genetics (ISAG) and Equine Genetics Standing Committee was used for the analysis.

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### Genetic diversity

Genetic variability within populations was characterized as allele frequency, mean number of alleles, observed heterozygosity ( $H_o$ ) and genetic diversity, which is often called expected heterozygosity ( $H_e$ ) (Weir 1996). Testing of the Hardy-Weinberg equilibrium and the Wright's fixation indices:  $F_{IS}$  – reduction in heterozygosity of an individual due to non-random mating within its subpopulation,  $F_{ST}$  – reduction in heterozygosity of subpopulation due to random genetic drift (fixation index), and  $F_{IT}$  – reduction in heterozygosity of an individual due to non-random mating and population subdivision relative to total population (overall inbreeding coefficient) were evaluated using the GenAEx program (Peakal and Smouse, 2012). The analysis of molecular variance was done using the PEGAS package (Paradis, 2010). Genetic differences among individuals and between populations were evaluated by Nei's distances ( $D_A$ ) (Nei et al., 1983), which assume differences caused by mutations and genetic drift. These indices provide more reliable results specifically for microsatellite data.

### Population structure and genetic relationship

Subsequently, to determine genetic structure and to infer genetic admixtures, a discriminant analysis of principal components (DAPC) implemented in the Adegenet R package (Jombart and Ahmed 2011) was used for microsatellite data. The DAPC approach proposes an optimum distribution of individuals into predefined groups in relation to the discriminant function of principal components. An optimum number of clusters was defined by the  $K$ -averaging algorithm that makes

use of the Bayesian information criterion. In addition, the DAPC was used to assign individuals and to obtain the membership probability which presents the overall genetic background of an individual. A trade-off between the power of discriminant analysis and overfitting of the given analysis was assessed by the  $\alpha$ -score (Jombart and Ahmed 2011).

## 3 Results and discussion

### Genetic diversity

Each of the analyzed loci appeared as polymorphic and their alleles were present in or shared by all studied populations. The total number of alleles, average number of alleles on 13 microsatellite markers across all breeds and overall information about differences and total statistics are shown in Table 1. Statistically significant deviations from the Hardy-Weinberg equilibrium were found in all microsatellite loci. The parameters of observe heterozygosity ( $H_o$ ), genetic diversity ( $H_e$ ), allelic richness ( $N_A$ ), effective number of alleles ( $N_e$ ) and Wright's  $F_{IS}$  are present in Tables 2. The higher values of  $N_A$  and  $N_e$  were estimated for  $H$  breed.  $H_o$  and the  $F_{IS}$  index, indicate a sufficient proportion of heterozygosity across all breeds except  $H$  and  $OKb$ .

### Genetic structure

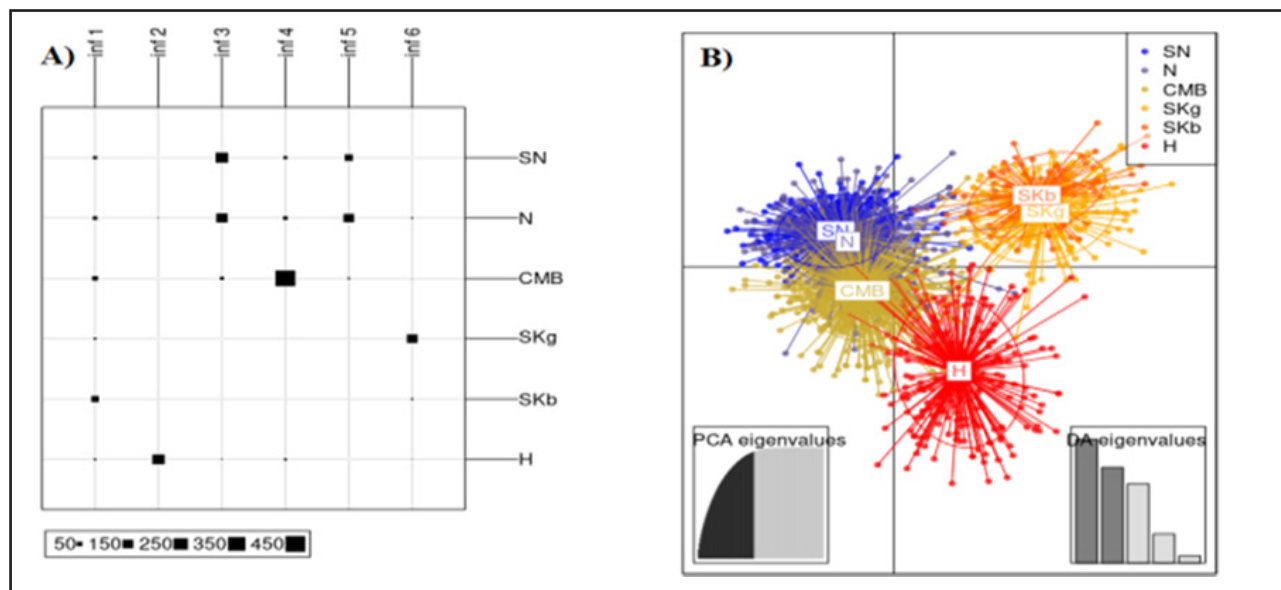
Genetic differences between populations were tested by the pairwise  $F_{ST}$  coefficients and Nei's genetic distances ( $D_A$ ) (Table 3). The largest distance was determined between  $OKb$  and  $H$  breeds ( $F_{ST} = 0.064$ ) and the smallest between  $SN$  and  $N$  ( $F_{ST} = 0.004$ ). To infer the population

**Table 1** Characteristics of 13 microsatellite loci analyzed in five horse populations

	$N$	$N_A$	$N_e$	$H_o$	$H_e$	$F_{IS}$	$F_{IT}$	$F_{ST}$
VHL20	1,808	13	5.232	0.827	0.803	-0.030	0.042	0.069
HTG4	1,806	10	3.553	0.709	0.711	0.002	0.092	0.090
AHT4	1,808	20	5.147	0.802	0.803	0.000	0.044	0.044
HMS7	1,789	12	3.469	0.637	0.671	0.051	0.157	0.112
HTG6	1,804	12	1.946	0.483	0.474	-0.018	0.011	0.029
AHT5	1,797	14	4.233	0.776	0.761	-0.020	0.036	0.055
HMS6	1,807	11	3.076	0.699	0.673	-0.039	0.008	0.045
ASB2	1,801	19	4.361	0.771	0.758	-0.017	0.053	0.069
HTG10	1,772	14	3.522	0.669	0.701	0.045	0.168	0.128
HTG7	1,790	11	2.856	0.617	0.621	0.006	0.104	0.099
HMS3	1,741	16	3.485	0.694	0.694	0.000	0.046	0.047
HMS2	1,800	15	3.706	0.734	0.719	-0.021	0.057	0.077
HMS1	1,801	8	2.414	0.554	0.569	0.026	0.100	0.076

$N$  – number of successfully genotyped individuals,  $N_A$  – number of alleles,  $N_e$  – effective number of alleles,  $H_o$  – observed heterozygosity,  $H_e$  – genetic diversity and  $F_{IT}$ ,  $n = 1,806$





**Figure 1** The Bayesian information criterion (BIC) statistic results referring to differentiation between inferred and original clusters (A) and the genetic clusters determined using discriminant analysis of principal components (B)

genetic structure, DAPC was applied to genotyping data. The distribution of individuals according to the Bayesian information criterion (BIC) analysis showed that inferred clusters do not correspond to actual groups (Figure 1a). Based on the  $\alpha$ -score (Jombart and Collins 2015), 32 PCA axes were left in DAPC. The four discriminate functions obtained correspond to 93% of variance. The first discriminate function clearly detected two major genetic clusters corresponding to draft horses and both colour

variants of Old Kladruber horse. Using the first and the second discriminate function, a very close relationship was determined within two groups of breeds – draft horses and colour variety of Old Kladruber horse. Hucul breed is clearly separated from other analyzed breeds (Figure 1b), as expected.

Genetic variability, determined by means of microsatellite markers in endangered Czech horse breeds was analysed

**Table 2** Genetic diversity across five horse populations

	MNA	$N_e$	$H_o$	$H_e$	$F_{IS}$ (CI 95%)
SN	7.308 ±0.485	3.387 ±0.266	0.691 ±0.028	0.680 ±0.028	-0.017 (-0.041–0.007)
N	7.154 ±0.421	3.702 ±0.253	0.718 ±0.021	0.714 ±0.020	-0.006 (-0.024–0.012)
CMB	7.615 ±0.561	3.537 ±0.326	0.680 ±0.038	0.678 ±0.038	-0.004 (-0.026–0.018)
OKg	7.000 ±0.320	3.477 ±0.367	0.674 ±0.042	0.669 ±0.038	-0.004 (-0.024–0.016)
OKb	5.615 ±0.368	3.285 ±0.300	0.652 ±0.044	0.654 ±0.040	0.005 (-0.040–0.050)
H	11.846 ±0.823	4.303 ±0.410	0.726 ±0.027	0.739 ±0.026	0.016 (-0.009–0.041)

Mean number of alleles (MNA), effective number of alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), genetic diversity ( $H_e$ ) and Wright's  $F_{IS}$  index with confidence intervals (95%)

**Table 3** Wright's  $F_{ST}$  (above the diagonal) and Nei's minimum genetic distance (below the diagonal) per pair of breeds

	SN	N	CMB	OKg	OKb	H
SN		0.004	0.036	0.053	0.061	0.044
N	0.018		0.031	0.049	0.055	0.037
CMB	0.167	0.150		0.047	0.058	0.044
OKg	0.265	0.252	0.234		0.043	0.049
OKb	0.311	0.294	0.299	0.191		0.064
H	0.257	0.230	0.244	0.271	0.361	



in this study. All studied microsatellite markers showed high variability, and all markers were deviated from the Hardy-Weinberg equilibrium. Generally, the genetic diversity of microsatellite loci can be affected by many factors, including genetic drift, impact of selective breeding, effect of individual stallions and random effects. Estimated parameters of genetic diversity exhibited non-significant differences between draft horse breeds and between two colour variants of Old Kladruber horse, which fully corresponds to their breeding history.

Quite surprising is a fact that genetic differences estimated between draft horse breeds was smaller than between two colour variants of Old Kladruber horse. Genetic diversities within the studied breeds reached similar values to those obtained in the Old Kladruber horse by Kasarda et al. (2016). The determined values of genetic diversity were lower than e.g., those in other European endangered population – Polish Konik (Szwaczkowski et al. 2016). Observed heterozygosity and the coefficient of inbreeding measured by Wright's FIS index, indicate a sufficient level of heterozygosity in the studied populations except H and OKb breeds. The moderate  $F_{ST}$  value and the value in a genetic distance matrix ( $D_A$ ) show a good genetic distance between the group of draft horses, both colour variants of Old Kladruber horse and Hucul breed. The H as original mountain horse breed with small body frame was never used in regeneration process of draft horses or in OKH breed.

#### 4 Conclusions

This study gives an insight into the genetic structure and diversity of endangered breeds kept in the Czech Republic. The obtained results suggest a low level of differentiation as well as a high gene flow between draft horse breeds and colour variant of the OKH. The H breed is clearly separated from others. The low  $F_{ST}$  values between SN and N breeds can be explained by the commonly used crossing between these breeds. The results of this study should be applied to the conservation of gene resources of horses in the Czech Republic.

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## Analysis of foot and claw diseases/disorders in Czech Holstein cows

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Foot and claw diseases/disorders from 24 545 lactations of 10 340 Holstein cows were recorded on 7 farms in the Czech Republic from 1999 to 2018. Three groups of foot and claw disorders were defined: skin diseases (SD), including digital and interdigital dermatitis and interdigital phlegmon; disorders of the claw horn (CH) including ulcers, white line disease, horn fissures, and double sole as well as overall claw disease (OCD) comprising all the recorded disorders. Between 1<sup>st</sup> and 305<sup>th</sup> days of lactation OCD was recorded in 52.56% of all observed lactations. For SD and CH, the respective values were 28.61% and 27.15%. For the purposes of analyses, foot and claw disorders were defined as 0/1 occurrence per lactation. Genetic parameters for analyzed traits were estimated using linear animal models which included the random additive genetic effect of animal (A) and the permanent environmental effect of cow (PE). Fixed effects for claw diseases were parity, farm, year and season of calving, and age at calving in classes. The estimated heritability for OCD was 13.84%, whereas that for CH 12.64% and for SD was 9.83%, for ulcers (U) 8.73% and for dermatitis digitalis and interdigitalis (DD) was 9.97%, respectively. Genetic correlation between SD and CH was 17.66%, while between SD and DD 98.4% and between CH and U 92.62%. The work was supported by the project QJ1510144 and the institutional support MZE-RO0718 of the Ministry of Agriculture of the Czech Republic.

**Keywords:** cattle, foot and claw disorders, genetic parameters, health traits, udder

### 1 Introduction

Claw and foot diseases/disorders are among the most important health traits in dairy cattle with noticeable negative impact on farm profitability and production efficiency, animal welfare, food safety and quality (van der Waaij et al., 2005, Egger-Danner et al., 2013). Claw and foot diseases/disorders cause impairing the milk production, reproduction and longevity of cow. Decreasing of the incidence of them can be achieved by improvement in management practices and possibly by genetic selection. Genetic selection depends on sufficient genetic variability that is manifested by particular claw disease/disorder as was showed in many published scientific papers e.g. Buch et al. (2011), Chapinal et al., (2013) or Pérez-Cabal & Charfeddine (2015). The direct economic importance of claw diseases as breeding objectives were observed by authors Krupová et al. (2016).

The aim of our study was to analyze the frequencies of claw and foot diseases/ disorders recorded in the Czech Republic including estimation of genetic parameters for the analyzed traits.

### 2 Materials and methods

Foot and claw diseases/disorders were recorded on 7 farms in the Czech Republic from 1999 to 2018. Dataset included 24 545 lactations of 10 340 Holstein cows comprising 35 717 records of foot and claw disorders. Three groups of foot and claw diseases/disorders were defined: skin diseases (SD), including digital and interdigital dermatitis and interdigital phlegmon; disorders of the claw horn (CH) including ulcers, white line disease, horn fissures, and double sole; and overall claw disease (OCD) comprising all the recorded disorders. For analyses, two particular diseases: a digital and interdigital dermatitis (DD) and an ulcer (U) were used beside SD, CH and OCD. The occurrence of disorders was examined in three parts of lactation: 1<sup>st</sup> to 90<sup>th</sup> days of lactation, 1<sup>st</sup> to 305<sup>th</sup> days of lactation and 200<sup>th</sup> to 450<sup>th</sup> days of lactation. For the purposes of estimation of genetic parameters, foot and claw diseases/disorders were defined as 0/1 occurrence per lactation. The following linear animal model was used to estimate genetic parameters for foot and claw diseases/disorders traits and CM traits:

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$$y_{ijklmno} = \text{parity}_i + \text{herd}_j + \text{year}_k + \text{season}_l + \text{age}_m + pe_n + a_o + e_{ijklmno}$$

where:

- $y_{ijklmno}$  – the analysed trait: SD, CH, OCD, DD, U  
 $\text{parity}_i$  – the effect of parity class  $i$  (5 levels, first, second, third, fourth, five and higher parity)  
 $\text{herd}_j$  – the effect of herd  $j$  (7 levels)  
 $\text{year}_k$  – the effect of calving year  $k$  (20 levels)  
 $\text{season}_l$  – the effect of calving season (4 levels, January – March; April – June; July – September; October – December)  
 $\text{age}_m$  – the effect of age at calving (13 levels)  
 $pe_n$  – the random permanent environmental effect on cow traits across  $n$  parity  
 $a_o$  – the random additive genetic effect of cow  $o$   
 $e_{ijklmno}$  – the random residual effect

The pedigree file contained 26 356 records. Data were analyzed using the DMU package (Madsen and Jensen, 2010) or VCE 6.0 program (Groeneveld et al., 2008). Genetic correlations between traits were estimated by bivariate models.

### 3 Results and discussion

The occurrence of foot and claw disorders in parity differed according particular disorder. Infectious diseases as SD and DD were more frequent in the lower parities in comparison to claw horn disorders. There was lower incidence of DD in higher parities. Number of records for claw horn disorders increased with parity especially number of ulcers.

When the records according days in milk are considered the highest incidence was found closely after calving and also at the end of lactation. Later is probably connected with treatment of claws before dry period of cow.

Lactation incidence rate for OCD (see Table 1) was much higher than those of SD and CH (see Table 1). Cows suffered more than one disorder per lactation, SD and CH combined in the course of lactation. The lactation incidence rate for SD and DD was higher in the first three months of lactation in comparison with the end of lactation. For CH and U, the ratios were opposite.

The relationship between incidence of foot and claw disorders in particular part of lactation is shown in Table 2.

**Table 1** Lactation incidence rate (%)

Days in milk	SD	CH	OCD	U	DD
1 <sup>st</sup> to 90 <sup>th</sup>	15.89	12.65	28.28	7.22	12.65
1 <sup>st</sup> to 305 <sup>th</sup>	28.61	27.15	52.56	18.33	24.46
200 <sup>th</sup> to 450 <sup>th</sup>	11.67	14.92	27.37	10.14	10.69

SD – skin disease, CH – disorders of the claw horn, OCD – overall claw disease, U – ulcers, DD – digital and interdigital dermatitis

**Table 2** Pearson's phenotypic correlations between parts of lactation (%)

Trait	1 <sup>st</sup> to 90 <sup>th</sup> × 200 <sup>th</sup> to 450 <sup>th</sup>	1 <sup>st</sup> to 90 <sup>th</sup> × 1 <sup>st</sup> to 305 <sup>th</sup>	200 <sup>th</sup> to 450 <sup>th</sup> × 1 <sup>st</sup> to 305 <sup>th</sup>
Skin disease	6.72	68.67	38.47
Disorders of the claw horn	9.97	60.21	45.06
Overall claw disease	3.34	59.65	35.45
Ulcers	12.06	58.88	49.52
Digital – interdigital dermatitis	5.32	65.99	39.73

**Table 3** Variances and heritability of foot and claw disorder traits in 1<sup>st</sup> to 305<sup>th</sup> days in milk

Trait	Additive variance	Variance of permanent environment	Heritability
Skin disease	0.018	0.005	9.83%
Disorders of the claw horn	0.022	0.012	12.64%
Overall claw disease	0.030	0.006	13.84%
Ulcers	0.011	0.011	8.73%
Digital – interdigital dermatitis	0.015	0.006	8.97%

It emerged that there is only low relationship between the incidence in the first part of lactation and the incidence of claw disorders at the end of lactation. The relationship between other parts lactation is intermediate or strong.

The heritabilities of foot and claw disorders (Table 3) were between 8.73% U and 13.84% OCD. Additive variance was higher than variance of permanent environment for the all disorders except for U.

The SD and CH disorders showed low genetic correlations (see Table 4). The Permanent environmental correlations between SD and CH were lower then genetic one. Both estimated correlations between SD and DD or between CH and U strong.

**Table 4** Genetic and permanent environmental correlations between foot and claw disorders

Traits	Genetic	Permanent environmental
SD × CH	17.66	5.80
SD × DD	98.40	97.62
CH × U	92.62	91.47

SD – skin disease, CH – disorders of the claw horn, OCD – overall claw disease, U – ulcers, DD – digital and interdigital dermatitis

Compare to recent studies that have reported prevalence of 40% to 70% of cow with foot and claw disease/disorder (Sogstad et al. 2005, Buch et al., 2011; Chapinal et al., 2013, van der Spek et al. 2013) the incidence reported in presented study is on average. In preceding analysis, Krpálková et al. (2016) also found the frequency of claw diseases/disorders over 50%.

The foot and claw diseases traits increased with parity with higher incidence of disease at the beginning of lactation and at the end of the lactation and during the dry period. Krpálková et al. (2016) point out that better care and management combined with more controls of legs leads to higher recording of diseases.

The reported estimates of heritability are in line with those reported in the literature, which range from 1% to 17% (van der Waaij et al., 2005, Buch et al., 2011; Chapinal et al., 2013, Pérez-Cabal & Charfeddine 2015).

#### 4 Conclusions

There is evidence that susceptibility to presented foot and claw disease/disorders is heritable. Presented study is the first step on the way to the national genetic evaluation of the foot and claw diseases/disorders in dairy cattle.

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## Nutritional value of hybrid *Rumex patientia* L. × *Rumex tianschanicus* A. Los (Rumex OK 2) in different periods

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The aim of this study was to determine the nutritive value of hybrid *Rumex patientia* L. × *Rumex tianschanicus* A. Los (Rumex OK 2). Rumex OK 2 can be considered as a technical or energetic plant for renewable production of biomass or as a feed as source of nutrients for animals. In this study two harvestings were planned, first cut was realised in June (growth from March to June) and second cut was realised in November (growth from July to November). Samples of Rumex OK 2 plants were collected in the 20<sup>th</sup> day of the month in March to June and in September to November. Concentration of nutrients were detected according to Regulation no. 2145/2004-100. Fresh Rumex OK 2 samples from March 7.42%, April 8.71% and September 4.89% had very low concentration of dry matter (DM). On the other hand samples from March, April, September and October had high concentration of crude protein in range from 31.42 to 24.54% of DM. From start of growth in spring to time of first cut in June increased both concentration of dry matter from 7.42 to 56.62% and concentration of crude fibre from 14.86 to 47.38% of DM. Concentration of fat in Rumex OK 2 is low and similar to that of maize plant or alfalfa, whereas concentration of nitrogen free extract in Rumex OK 2 plant is similar only to alfalfa. Results of this article bring compact view over nutritional characteristic of Rumex OK 2, which can be according to gained results about nutritional value used as a source of nutrients in animal nutrition, or as a source of renewable biomass for bioenergy production.

**Keywords:** Rumex OK 2, months, nutrients, fiber complex

### 1 Introduction

Production of agricultural biomass as a renewable energy is very important part of sustainable development of countryside. It is planned to replace a part of agricultural land in intensive farming with agricultural land used for production of technical or energetic plants. Ušťák (2007) as well as Rakhmetov (2018) consider hybrid of *Rumex patientia* L. × *Rumex tianschanicus* A. Los (Rumex OK 2) as one of most perspective energetic plant in climatic condition of V4 countries. Gross energy concentration in Rumex OK 2 plant as well as in silage made from wilted plants were published in our previous publications (Rolinec et al., 2018a and 2018b). Ignore the content of oxalic acid, Rumex OK 2 can be utilised as well as a source of nutrients for ruminants. Unique results of experiment with Rumex OK 2 were published by Petříková (2012). She claimed that Rumex OK 2 can be used for green feeding,

as a pasture or in form of silage, and she concluded, that feeding of Rumex OK 2 to dairy cows positively affected the production and composition of milk (Petříková, 2012). Ušťák (2007) characterised Rumex OK 2 as plant with 4 to 6 stalks and with leaves in clump. Average height of Rumex OK 2 is 235 centimetres and leaves in clump are high 45 to 60 centimetres in average. Weight of 1000 seeds is 3.02 (2.8 to 3.3) grams. Dry matter ideal for production of biogas or for feed producing is according Ušťák (2007) from 18 to 22%. Harvesting by this dry matter can provide 3 cuts per year with average production of 30 to 50 tons of biomass per hectare. For better know about nutritional parameters of hybrid *Rumex patientia* L. × *Rumex tianschanicus* A. Los (Rumex OK 2) we aimed to determine the concentration of basic nutrients of this plant in selected months.

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## 2 Material and methods

This experiment was realized with fresh plant of hybrid *Rumex patientia* L. × *Rumex tianschanicus* A.Los (Rumex OK 2). Rumex OK 2 plants were grown in experimental fields under Institute of Biodiversity Conservation and Biosafety (SUA in Nitra). It was planned with two cuts. First after mature of seeds in June and second in late autumn. After first cut, Rumex OK 2 starts with growth after rains in summer months. Samples of fresh matter were collected always around 20<sup>th</sup> day of the month. Samples were collected during autumn months of the year 2017 (September, October and November) and spring months of the year 2018 (March, April, May) and in June 2018. In each sampling time six samples were analysed. Sample of fresh plant collected in October was not analysed because of mistake in predrying. Samples of fresh plant material were cut, the theoretical length of cut was 1 cm and then predried in a hot air oven at 60 °C for two days. After predrying samples were homogenized by laboratory mill. The size of particles after homogenisation was less than 1 mm. The concentration of nutrients were detected according to Regulation no. 2145/2004-100. Concentration of dry matter was determined by the gravimetric method, crude protein by the Kjeldahl's method, fat: extraction by light petroleum, crude fiber: gravimetrically as the residue remaining after extraction in acid and alkali reagent, acid detergent fiber (ADF): gravimetrically as the residue remaining after extraction in acid detergent solution, neutral detergent fiber (NDF): gravimetrically as the residue remaining after extraction in neutral detergent solution, acid detergent lignin (ADL): gravimetrically as the residue remaining upon ignition after 72% H<sub>2</sub>SO<sub>4</sub> treatment, ash: ashing with the use of a muffle furnace by 550 °C. Cellulose (CEL) and hemicellulose (HE) were calculated, CEL = ADF - ADL; HE = NDF - ADF. Content of nitrogen free extract (NFE)

and organic matter (OM) were calculated NFE = dry matter - crude protein - crude fiber - fat - ash; OM = dry matter - ash (Juráček et al., 2011). Results were statistical analyzed in statistic program IBM SPSS v. 20.0. Differences of means between months within nutrient were tested by Tukey HSD test. *P* < 0.05 was considered as significant.

## 3 Results and discussion

The concentration of dry matter of Rumex OK 2 increased from 7.42% in March to 56.62% in June. Depends on weather condition and dry matter concentration, the harvesting of Rumex OK 2 for direct combustion is the best at the end of June or during July (Rolinec et al., 2018b), when is the concentration of dry matter the highest. With enough water in soil starts growth of Rumex OK 2 in few weeks after first harvesting. In this case produced Rumex OK 2 in autumn months second crop, however autumn crop is characteristic with very low concentration of dry matter (Table 1). As published Hejduk and Doležal (2004) genus *Rumex* L. is generally known to have very low content of dry matter. Derrick et al. (1993) determined DM content of *Rumex* on value 11.4% and Bockholt and Kannewurf (2001) published DM content in May from 13.0% to 27.0%.

The concentration not only of dry matter but also of other nutrients is affected by fertilisation (Hric et al., 2018; Kovár et al., 2017). According to concentration of crude protein and crude fiber is nutritional quality of Rumex OK 2 in March and April very good (Table 1). Similar statement published also Hejduk and Doležal (2004). Then in May and June came to high decrease in crude protein and high increase in crude fiber concentration, which means decrease of the nutritional value. Interesting was the highest concentration of crude protein in September, at the start of growth of second crop. Crude protein content of Rumex OK 2 plant in early growth stages (Table 1) is

**Table 1** Nutritional characteristic of Rumex OK 2 (whole fresh plant)

Month	DM	CP	CFa	CFi	NFE	OM	Ash
	g.kg <sup>-1</sup>	g.kg <sup>-1</sup> of DM					
March 2018	74.2 <sup>ab</sup>	261.7 <sup>a</sup>	22.3 <sup>ac</sup>	148.6 <sup>a</sup>	553.8 <sup>ac</sup>	986.4 <sup>a</sup>	13.6 <sup>a</sup>
April 2018	87.1 <sup>a</sup>	245.4 <sup>a</sup>	16.4 <sup>ab</sup>	218.6 <sup>a</sup>	481.6 <sup>ab</sup>	962.0 <sup>a</sup>	38.0 <sup>a</sup>
May 2018	169.1 <sup>c</sup>	100.9 <sup>b</sup>	11.6 <sup>b</sup>	384.9 <sup>b</sup>	438.2 <sup>b</sup>	935.4 <sup>ab</sup>	64.6 <sup>a</sup>
June 2018	566.2 <sup>d</sup>	52.2 <sup>b</sup>	8.9 <sup>b</sup>	473.8 <sup>b</sup>	416.8 <sup>bc</sup>	951.7 <sup>ab</sup>	48.3 <sup>ab</sup>
September 2017	48.9 <sup>b</sup>	314.2 <sup>a</sup>	25.3 <sup>ac</sup>	152.5 <sup>a</sup>	363.5 <sup>c</sup>	855.5 <sup>c</sup>	144.5 <sup>b</sup>
November 2017	77.0 <sup>ab</sup>	302.3 <sup>a</sup>	26.9 <sup>c</sup>	115.0 <sup>a</sup>	444.5 <sup>bc</sup>	888.7 <sup>bc</sup>	111.3 <sup>b</sup>
SEM	47.2	26.4	1.9	35.1	16.2	12.5	12.5
P	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

DM – dry matter; CP – crude protein; CFa – crude fat; CFi – crude fiber; NFE – nitrogen free extract; OM – organic matter; SEM – standard error of the mean; P – effect of sampling time on nutrient content in that column at *P* value; <sup>ab</sup>... – means within a column bearing different superscript differ significantly at *P* < 0.05

**Table 2** Fiber characteristic of Rumex OK 2 (whole fresh plant)

Month	ADF	NDF	ADL	CEL	HE
	g.kg <sup>-1</sup> of DM				
March 2018	172 <sup>a</sup>	216 <sup>a</sup>	18.4 <sup>a</sup>	153 <sup>ab</sup>	43.8
April 2018	267 <sup>a</sup>	337 <sup>ab</sup>	54.5 <sup>ab</sup>	212 <sup>b</sup>	70.3
May 2018	452 <sup>b</sup>	511 <sup>bc</sup>	115 <sup>bc</sup>	337 <sup>c</sup>	59.6
June 2018	593 <sup>b</sup>	665 <sup>c</sup>	199 <sup>c</sup>	394 <sup>c</sup>	72.8
September 2017	196 <sup>a</sup>	258 <sup>a</sup>	46.4 <sup>ab</sup>	149 <sup>ab</sup>	62.1
November 2017	133 <sup>a</sup>	170 <sup>a</sup>	21.5 <sup>a</sup>	111 <sup>a</sup>	37.1
SEM	43.6	46.7	17.2	27.0	5.0
P	≤0.001	≤0.001	≤0.001	≤0.001	>0.05

ADF – acid detergent fiber; NDF – neutral detergent fiber; ADL – acid detergent lignin; CEL – cellulose; HE – hemicellulose; DM – dry matter; n.a. – not analysed SEM – standard error of the mean; P – effect of sampling time on nutrient content in that column at P value; ab... – means within a column bearing different superscript differ significantly at  $P < 0.05$

like that of alfalfa before flowering, which is 240 g.kg<sup>-1</sup> of DM (Gálik et al., 2016). Young plants, mainly the leaves of Rumex OK 2 offers feed and pasture with high content of crude protein. Questionable is the pasture intake by animals. Derrick et al. (1993) published, that by sheep is intake of fresh feed from *Rumex obtusifolius* L. worse than that of wilted or dried feed. Hejduk and Doležal (2004) claimed that mowed *Rumex obtusifolius* L. is grazed by beef cattle, whereas no mow *Rumex obtusifolius* L. go unnoticed. On the other hand Petříková (2012) published results where dairy cows were fed during summer with grass silage (2/3) together with pasture on Rumex OK 2 (1/3) had mote fat and protein in milk compared to dairy cows in control group fed without Rumex OK 2. The intake of Rumex OK 2 by animals neither in form of pasture or silage need further research as well as its effect on performance of animals. Concentration of ash in Rumex OK 2 samples from April to June (Table 1) is similar to that of maize plant 57 to 84 g.kg<sup>-1</sup> of DM however samples from autumn months contains similar concentration of ash like alfalfa 129 g.kg<sup>-1</sup> of DM (Třináctý et al., 2013). Concentration of fat in all Rumex OK 2 samples was compared to maize and alfalfa on lower values (Gálik et al., 2016; Juráček et al., 2012). Concentration of nitrogen free extract was in Rumex OK 2 samples similar as in alfalfa (Gálik et al., 2016) but lower than in maize (Juráček et al., 2012).

The fiber complex, structural polysaccharides, is in forages an important component and source of energy for ruminants. Fiber complex form 40 to 60% of the dry matter of forages. The content of ADF and NDF are used for the quantitative representation of components of fiber complex (Šimko et al., 2010). Crude fiber content of Rumex OK 2 varied and depends on growth phase and sampling time (Table 1). For comparison, average content of crude fiber is in alfalfa 278 g.kg<sup>-1</sup> of DM and

in maize 225 g.kg<sup>-1</sup> of DM (Gálik et al., 2016; Petrikovič et al., 2000). Determined values of fiber complex of Rumex OK 2 samples are shown in Table 2. The development of concentration of ADF, NDF, ADL, CEL and HE (Table 2) is similar to crude fiber development (Table 1), the lowest values were detected at start of growth and then increased. ADF, NDF and HE concentration of alfalfa in first cut are in range from 245.6 to 339; from 372.8 to 468.4 and from 127.2 to 129.4 g.kg<sup>-1</sup> of DM respectively (Třináctý et al., 2013). Silage maize contains following concentrations of ADF 189.2 to 237.5 g.kg<sup>-1</sup> of DM, NDF 367.7 to 465.1 g.kg<sup>-1</sup> of DM and ADL 16.3 to 26.5 g.kg<sup>-1</sup> of DM (Juráček et al., 2012). All crops are typical for its concentration of fiber and its components. The concentration of fiber components develop similar, the older crop means the higher concentration of fiber components (Table 2). This support also results of this study. Significant ( $P < 0.001$ ) effect of sampling time on DM content as well as on content of all nutrients and fiber components (except HE) was detected (Table 1 and 2). Differences of average means of basic nutrients and fiber components between months are shown and marked with superior character in Table 1 and 2. Changes of nutrients as well as fiber components concentration of Rumex OK 2 are caused due to aging effect of plant.

#### 4 Conclusions

Results of this article bring compact view over nutritional characteristic of Rumex OK 2. Nutritional value of Rumex OK 2 was compared to that of fresh maize or alfalfa plants. From this results is clear, that young Rumex OK 2 contains in March, April and then after first harvesting in September and November very low dry matter content but with interesting content of crude protein. Concentration of crude fat in Rumex OK 2 is similar to that in maize. Concentration of crude protein in early growth

stage of Rumex OK 2 is similar to that of alfalfa. For better understanding of protein quality, analyze of the amino acids is needed. Anyway, Rumex OK 2 can be used as a source of nutrients in animal nutrition, or as a source of renewable biomass for bioenergy production.

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