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A REVIEW OF LATVIAN BLUE (LZ) COWS FROM THE LIST OF ANIMAL GENETIC RESOURCES IN LATVIA

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Latvian Blue (LZ) is a native breed of Latvian dairy cattle (*Bos taurus*) that is one of a most endangered breed of Animal Genetic Resources (AnGR) in Latvia and therefore play an important role in the start of conservation processes of five breeds of Latvian AnGR from 1995, and thus for starting to analyze the composition and conservation status of this breed, as well as to provide suggestions for updating list of protected and endangered breeds. An important role of Latvian AnGR research and conservation work updating very important role are practically organized initiatives of different countries and different research projects in the Baltics in the past 20 years. The aim of this work was to analyze the composition of milk protein polymorphisms (alpfa S1-, beta - and kappa – casein, alfa – lactalbumin and beta – lactoglobulin) of LZ breed, and also to analyze the possible effects of these different milk protein varieties on milk productivity of LZ breed.

Key words: Latvian Blue, Animal Genetic Resources, milk protein polymorphism.

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INTRODUCTION

Since the 1960s, the Food and Agriculture Organization of the United Nations (FAO) has worked on genetic resources for food and agriculture. But it focused on plant genetic resources, but since 1990 it has started to work in the area of animal genetic resources for food and agriculture (AnGR). Under the guidance of the CGRFA, the first International Technical Conference on Animal Genetic Resources for Food and Agriculture took place in Switzerland in 2007, comprised a two pronged approach aimed at achieving both the technical and the policy outcomes requested by the CGRFA - it is a permanent intergovernmental forum, which has developed international agreements, voluntary undertakings and codes of conduct to promote and to facilitate the management, access to and benefit – sharing genetic resources. 2010 had been declared the International Year of Biodiversity by the United Nations. 2010 also marks three years since the adoption of the Global Plan of Action for Animal Genetic Resources – the first internationally agreed framework for the management of animal genetic resources (AnGR). In 2011, as requested by CGRFA, all countries prepared progress reports on their national activities in implementing the Global Plan of Action, that FAO could prepare a synthesis report for consideration by the FAO Commission. Conservation of genetic diversity between and within domestic animal breeds is insurance for the future to establish and to manage the gene bank collections, to research genetics and cryobiology, to advice on genetics management of small and threatened populations and to work with monitoring and documentation (FAO 2010).

Latvian Blue or Latvian Blue cow, also called the cow of Lyvonia, the sea cow or cow of the moon is one of the bovine (*Bos primigenius taurus*) primitive varieties which originate in the Kourland region of Latvia, its abbreviation is LZ, and it is one of the Latvian genetic resources (Grīslis 2006).

Latvian dairy cattle breeding started Professor, Dr. agr. Paulis Lejiņš (the first President of the Latvian Academy of Sciences, 1946 - 1951; Latvijas Enciklopēdija 2007) in 1910, as a Head of Semmigalia's the Luste Manor Farm of education and experimentation (Friedrichlust), where had started to breed Latvian Brown cows (Lejiņš 1910). Dr. Paulis Lejiņš wrote in his 1st publication "Remarks for pastorials" in 1909 how to keep and look after dairy cows, also he about experience of farmers who kept Latvian Blue cattle by saying: "Latvians of Courland farmers from father fathers have inherited good, durable, productive and modest cattle. In recent years, improving care, they even proved highly productive, with a particularly high fat content in milk. Therefore strongly oppose all efforts to eradicate the cattle. Work hard to make every effort to cultivate these same cattle" (Griņevičs & Grosvalds 2011, Stradiņš 2013).

Dr. P. Lejiņš with collegues collected from different regions of Latvia and started to manage breeding of five Latvian native breeds of dairy cattle – Latvian Blue, Latvian Red, Latvian Red and White, Latvian Black and White, and Latvian Brown in the Farm of education and experimentation "Rāmava" of Faculty of Agriculture of Latvia University from 1922, and after his initiative all five varieties was introduced in the herd – book (Grīslis 2006, Griņevičs & Grosvalds 2011, Stradiņš 2013).

The first purebred bull of Latvian Blue breed that entered Latvian dairy herd – book of Volume IV in 1925, was a bull Lurich LZ 1, which is owned by Ruben P. in the Vestiena (Apšukalns) region of Latvia. But from 1927 there had left only three native breeds of dairy cows for breeding and selection continuation - Latvian Red and White, Latvian Black and White, and Latvian Brown. Latvian Blue had left for crossing with these breeds, mostly with Latvian Brown. Although the LZ cow homeland and the main holding is always considered Kourland, but as stated in the scriptures, "the last Latvian blue purebred herd was holding in Cesis district, Veselava's Parish (Apiņkalns farm, owner Lapiņš), and renounced from the pure Latvian Blue of livestock production, starting to use cross - breeding with other breeds in 1935 (Grīslis 2006, Griņevičs & Grosvalds 2011). Latvian scientist of agriculture A. Cālitis has investigated deeper about why few latvian cattle breeds - Latvian Latvian Red and White, Latvian Black and White were no longer bred, and as authors mentioned these varieties have disappeared (Grīslis 2006).

Latvian Blue Cow is a very rare breed, which is actually located on the verge of extinction. The 1st and 2nd World War and the mechanisation of agriculture caused a rapid fall in LZ breed numbers. Although the UN (the United Nations) Food and Agriculture parts of the financial support measures have been taken for the protection of varieties, LZ breed is still numerically very weak. Latvian Blue breed is used mainly in dairy production. Conservation work of Animal Genetic Resources in Latvia began directly with the measures to the preservation of Latvian Blue at 1995 (Grīslis & Šimkevica, 2014). According to the Latvian University of Agriculture research data in 2000, in Latvian herds was listed only 18 Latvian Blue cows (Grīslis 2006). Already for Latvian Blue cow was set up the Breed Conservation Association "Blue Cow" on 23 August 2000, and its mission is to preserve a rare LZ breed and improvement of LZ population.

According to the State Herd Register of 2010 data are grown in Latvian Blue cows – 1240 individuals, but from data of 2014 – 1288 individuals. Most of LZ cows located around Talsi, also in regions of Aizpute and Kuldiga, small amounts of LZ cows have found throughout all the Latvian territory (Grīslis &Šimkevica 2013, 2014).

In some sources have a hypothesis that Latvian Blue cows came along with the Indo - European immigrants before 4000 years, possibly LZ ancestors were horns ever inhabited the grasslands, and were also found in Latvian territory. The first half of the 17th century in Poland died last known horn. They are likely to have offspring gray steppe cattle as well as modern Hungarian steppe cows (Ciani et al. 2010).

But in EU have researches about Taurus and we found interesting the importance of Taurus that it were ancestor in the Latvian Blue breed origins. "Taurus" cattle - Maronesa, Maremmana (Fig.1), Sayaguesa, Pajuna, Podolica (Fig.2) and crossbreeds of these: breeds that are most closely related to the extinct known aurochs (Van Vuure 2002, 2005). In the Taurus Project of LIFE Nature program of Europe Comission (Bunzel – Drüke 2005) was deeply analyzed population of *Bos Primigenius,* and it shows that some of species have the similar genetic characteristics with our local LZ breed, example, massive grey Maremmana Primitive cattle, one of Europe's oldest breeds (*Bos primigenius*; classed as a Tipo Genetico Autoctona e Antica ("ancient and autochthonous genotype") in Italy was used for cross – breeding to improve Hungarian Grey or Steppe cattle, also Latvian Blue breed have some genetic similarities with Maremmana Primitive, Chianina and Hungarian grey or steppe cows. Some sources maintain that the Maremmana breed descended from the *Bos Taurus macrocerus* of which the archeological evidence is preserved, with a later admixture of Podolic cattle brought into the Italian peninsula from the East (Bigi & Zanon 2008), but some researchers suggest that the Maremmana is a direct descendant of those Asiatic grey cattle. But have also hypothesis that ancestor of Maremmana Primitive cattle could be aurochs (*Bos primigenius*), one of the wild ancestor of domestic cattle (Ciani et al. 2010).

The Maremmana is grey; but males are darker than females. The muzzle, hooves, switch and lower part of the scrotum are black; the skin is black, but some depigmentation at natural openings is tolerated. How is looking Latvian Blue? Proof of this assumption could be that blue cows have dark skin, which is south of cows sign in the dark pigment protects the skin from sunburn. As well as the southern varieties are bright, gray, gray, yellow, and brown or white hair (Grīslis 2006).

Fig. 1 Maremmana Primitive cattle (from Bunzel – Drüke 2005).

As in other breeds of Podolic origin, calves are born wheat - coloured and become grey at about three months old (Bigi and Zanon, 2008), and also LZ calves are born colored wheat – colored or light – grey,but most of LZ become grey at tree months old too (Grīslis 2006).

Latvian Blue historically was found near the sea – sides of Latvia (Grīslis, 2006) and also have the reports that Maremmana Primitive cattle dislocated mostly around the seaside of Italy, and Maremmana "Primitive" breed of the climate of bio – territory (agro – forestry – ecosystem) breeding also is in reality made a set of micro - climates, which vary in relation to the altitude and exposure, according to the proximity to the sea or reservoirs, for example, in coastal areas of Tuscany. Wild bovines eat longer and more coarse grasses than the horses do (Ciani et al. 2010).

In another version of the blue cows have arisen from the local bluish - colored trumpets subspecies of wild coastal grazing pastures for more than 2,000 years, or they were imported from Scandinavia, and in Norway today can find blue - gray cows. Compared to other varieties, Latvian Blue breed is more grown himself, using this breed's sires, however, as demonstrated by recent genetic studies of this variety turns out to be quite a close relationship with the Latvian Brown breed. This is evidenced by the Latvian Brown breed breeding history in Latvian Brown has been created by crossing local cows with imported red cattle breeding varieties. If in general studies confirmed Latvian Brown breed genetic similarity with red cows of the Baltic States, the research perfected surprises on the blue cows; because it turned out that they are contrary to previous hypotheses about Latvian Blue breed a passing relationship to Lithuanian Gray variety. This hypothesis was based on the fact that the Latvian Blue breed in building widely used in breeding Gaujars Lietuvietis 85002 whose father is Lithuanian Gray variety breeding Erli LJ4303 (Grīslis & Šimkevica 2013).

Fig. 2 Podolica cow_Taurus (from http://upload.wikimedia.org/wikipedia/commons/d/d7/Podolica_cow_Tauros.JPG, by Henri Kerkdijk – Otten; viewed 23.04.2015).

The inheritance of Latvian Blue cow, as its name indicates, is a blue - gray color. They can be lighter and darker blue, the summer period, some quite light fade. The legs have a darker, and around the eyes have a blue "glasses". Sometimes they have a lighter head, face and temporal part of nasal mirror is dark gray or black. Horns white with black tips, it is characterized by a crescent shape, which, moreover, the night light shines so the cows got the name of the moon cow. It is smaller than Latvian brown. Its height is 133 crosses cm, weight from 350 kg. LZ cow has a very calm and serene nature. They are also very conservative and resistant to disease. They never are suffering from leukemia, and inflammation of the udder is very rare (Grīslis 2006).

Reproduction of Latvian Blue cows is at the very critical situation because it is threatened by the forecasted increase in inbred populations, which represents approximately 2.8% of generation. Scientists doubt that such a level of inbred

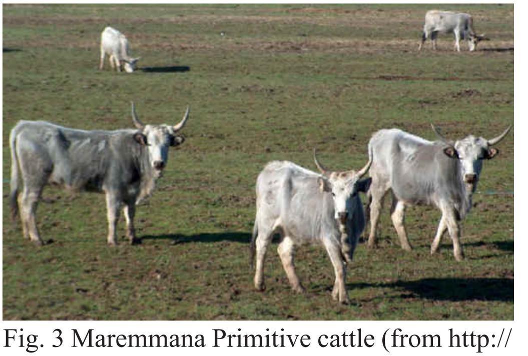

Fig. 3 Maremmana Primitive cattle (from http://eng.agraria.org/cattle/maremmana.htm).

populations Latvian Blue existence is threatened. One of the most viable options to reduce the increase in the inbred population numerically weak as Latvian Blue is to prevent excessively high disproportion between the male and female number of individuals. It is known, that the control of the family size can contribute to the effective population size increases. If all families are created for each of one male and one female specimen, family size dispersion becomes zero, and the effective population size of approximately doubling. Then sex ratio would be expensive to achieve, but to incorporate the use of some 20-25 young bulls might be realistic task. To avoid inbred, young bulls be used to obtain the "blood filler" method. There are plans to get young bulls from the excellent bull mothers of Latvian Brown breed using LZ breeding bulls (Grīslis &Šimkevica 2013, 2014).

Although, the absolute milk yield of Latvian Blue cow is lower, however, compared to the mass of a body, it is not so small. As a LZ breeder V. Šteinbergs says: "Blue cows are small, about 350 kg in weight, and Holstein cows - 600 kg. If the LZ in summer gives 22 liters per day, but Holstein cows only two liters more, then do we can say that last one is more productive cow?" Latvian Blue cow is relatively easy childbirth. They do not like when a person is present at the time of calving. To the possibility of delaying the time and brought it to the owner is not present, since Latvian blue is petite and calf is less than other cows''. Economic benefits to the community affect many local varieties of the agricultural animals' population size, also a population of LZ breed. The Latvian Blue breed is an ancient of the Latvian established milk breed (Grīslis & Šimkevica 2014).

More than 95% of ruminants' milk proteins are coded by 6 structural genes: two main whey proteins (α - lactalbumin and β - lactoglobulin) and 4 caseins (α_{S1}- and α_{S2} - caseins, β - casein and κ - casein) (Martin et al., 2002). In revisions of milk protein nomenclature indicates the 9 α_{s1}-casein, 4 α_{s2} - casein, 14 β - casein, 16 κ - casein, 13 β - lactoglobulin, and 4 α - lactalbumin variants within the cattle species. Originally the ancestral alleles of caseins were α_{s1} - casein C, α_{s2} _ casein A, β – casein A2, κ – casein A, but from whey proteins, genes of the common ancestor were α – lactalbumin B and β – lactoglobulin B, and those still predominant in *Bos indicus* while very important shifts have occurred in *Bos Taurus* (Farrell et al. 2004, Caroli et al. 2009, b, Hristov et al. 2012, Martin et al. 2013).

In 21st century the gene assisted selection (GAS) will help us to analyze and to implement new standards also for LZ dairy livestock breeding to improve the productivity of cows, average milk yield, protein quantity, protein content of the total amount and quality in dairy farming of Latvia. Molecular genetics research in animal production began only in recent years, with Molecular Genetics Research Laboratory's development in Latvia University of Agriculture.

The aim of this work was to analyze the composition of milk protein polymorphisms (alpfa S1-, beta - and kappa – casein, alfa – lactalbumin and beta – lactoglobulin) of LZ breed, and also to analyze the possible effects of these different milk protein varieties on milk productivity of LZ breed.

MATERIAL AND METHODS

The analyzed group of the dairy cows' breeds raised in Latvia was formed from the 719 cattle biological material, including 179 samples of Latvian Blue breed. The blood of cows was taken from the jugular vein and samples of sperm were got from the Sigulda Station of Breeding and Artificial Insemination (Sigulda CMAS) of Latvia. The research has done in the Laboratory of Molecular Genetic Researches of Faculty of Agriculture of LUA (Latvia University of Agriculture) in Jelgava (Latvia).

DNA extraction was done from cows' blood and bulls' sperm samples. In the work were used two kits of genomic DNA extraction: Genomic DNA Purification Kit #K512 (Fermentas, Vilnius, Lithuania) and PUREGENE® DNA Isolation Kit (QIAgene, USA).

At a study of association of milk productivity indicators of dairy cows reared in Latvia with the genotypes of milk protein genes (α_{S1} - casein, β - casein, κ - casein, α - lactalbumin and β – lactoglobulin) were selected 153 dairy cows of Latvian Blue breed with data at State agency "Agricultural Data Centre" of the Ministry of Agriculture of the Republic of Latvia about productivity in at least one of three lactations. Calving years were from 1993 to 2012, from 1994 to 2012 and from 1996 to 2012, respectively for 1st, 2nd and 3rd lactation cows. For analyse was used parametrical data about milk amount or yield (kg), protein and fat amount (kg), and contents (%). As more important indicator was considered yield quantity which depends from the protein and fat quantities (in kilograms). Then, in order of importance is the protein and fat content as percentages which may depend on the protein and fat quantities in kilograms, respectively.

In this study was selected the genetic method of restriction enzyme site polymorphisms (RESP) and most often analysed allele of milk proteins, respectively, for CSN1S1 allele B and C, for CSN2 – A1 and A2, but for CSN3, LAA and LGB alleles A and B. Respectively, in casein genes: C*SN1S1* c.15707A > G, which leading to the amino acid change Glu $_{192(207)}$ Gly or protein variation change B to C; *CSN2* – c.4451A > C (Pro $_{67(82)}$ His; A1 and A2 variations); for *CSN3* were selected two SNPs: c.11625C > T and c.11661A > C, which leading to Thr $_{136\ (157)}$ Ile and Asp$_{148(169)}$Ala or A variation change to B variation. For whey proteins, respectively, for *LAA* was selected SNP, which is located in no-translated region or c.15A > G, in which case it is considered that changes A variations of the B, but for LGB c.3106T > C, which leading to the amino acid change Val$_{118\ (134)}$ Ala or protein variation change A to B.

Numbers and frequencies of alleles and genotypes for an entire population were estimated by direct counting from results of molecular genetic analysis, but for the group of each breed were calculated by dividing samples. Difference of apportionment of allele and/or genotypes between cattle's of entire population and Latvian Blue breed were define by using Pirson χ^2 test to determinate difference between groups and Cramer's V test to determinate association or correlation between indicators. Expected heterozygote indexes were estimated for both alleles at the locus or polymorphism. Deviations from the Hardy - Weinberg equilibrium (Falconer et al. 1996) were tested by the Chi Square (χ^2) test at the confidence $p_\chi < 0.05$ (Liepa, 1974). Calculations were made by the Microsoft Office Excel 2007 standard package assistance, but the computer program package TFPGA (Tools for Population Genetic Analyses,Version 1.3) was used as a population genetic basis of the accuracy of testing (Miller 1997).

Statistically significant association for each milk protein variation with indicators of milk quantity and quality was assessed by analysing: (1) difference between average value of parametric indicators in each group of non - parametric indicators (genotype or haplotype), using one ANOVA test at the confidence $p_\chi < 0.05$ (Liepa 1974); (2) association or correlation between indicators, using eta (η) coefficient test. Association strength was defined by value of η coefficient. For analysis have used statistical programs: IBM SPSS Statistics version 21.0 (IBM Corp. Released 2012) and PAST (PAlaentological Statistics, ver. 1.63; Hammer et al. 2001).

RESULTS AND DISCUSSION

For research genes of bovine protein genes have used dairy cattle from the individual dairy cattle herds whose owners breed the native breed of Latvian Genetic Resources - Latvian Blue dairy cows around all regions of Latvia.

DNA samples of 719 dairy cows from different herds in Latvia, including 179 cows of Latvian Blue, analysed on polymorphisms of five milk proteins by studying alleles and genotypes of each SNPs of each gene. Therefore we get view about Latvian cows' population and about Latvian historical breed – Latvian Blue.

For CSN1S1 gene, the most frequently occurring allele (table 1) is B (0.96) and genotype – BB (0.93) in Latvian population, but in LZ group incidence of rear allele and genotype of heterozygote is a little bigger (0,05 and 0,10, respectively), but not statistically significant. For this locus wasn't found homozygote genotype of rear allele.

In the research results of other scientists of different countries, we found out that the results of the research of Estonia (EE) are similar to ours. For Estonian Native breed cows' (n = 118) α_{s1} - CN allele B is with frequency 0.92, but allele C – 0.08 (Lien et al., 1999). Also, Lithuanian (LT) researchers have similar results: for dairy cows' population of LT (n = 427) frequency of allele B is 0.95 (Pečiulaitiene, 2005).

In case of CSN2 the common allele for dairy cows population (table 1) is allele A1 with frequency 0.67 and genotypes A1A1 (0.42), and A1A2 (0.49), despite the fact that in literature the preferable for cows' populations and common allele more often is A2 (Reichelt et al. 1991, Cade et al. 2000, McLachlan 2001, Cardak 2005, A2 Corporation 2006, Cieslinska et al. 2007, Pečiulaitiene et al. 2007). For the Latvian Blue breed the frequency for common allele is 0.76, and 0.56 - for common genotype or homozygote genotype of common allele. We can see an expressing difference for apportionment of alleles ($p=4.87x10^{-4}$) and genotypes ($p = 9.98x10^{-4}$) of LZ from whole population. But the incidence of positive ancestral allele A2 (Reichelt et al. 1991, Cade et al. 2000, McLachlan 2001, Cardak 2005, A2 Corporation 2006, Cieslinska et al. 2007, Pečiulaitiene et al. 2007) have very low frequency also in Latvian historical LZ breed. Looking at the population's balance or deflection from Hardy - Weinberg at the given locus shown that there is statistically significant difference ($p=2.72x10^{-3}$) between expected and obtained heterozygote frequencies. Similar results we got for LZ breed where $p= 4.12x10^{-2}$. The frequency of genotype A2A2 is very low. These results can be a sign on the low milk level of LZ breed compering to Holstein cows (Cardak 2005, A2 Corporation 2006, Cieslinska et al. 2007).

Frequency of preferable allele A2 of *CNS2* in the breed of Estonian Native cows (Värv et al., 2009) is significantly higher (0.60) than in Latvian Blue breed cows (0.24). We can conclude, than in Estonian Native breed frequency of benevolent allele A2 of β – CN is two times higher.

In study of third casein protein CSN3 we found, that in population common allele is A with frequency 0.92 and common homozygote genotype AA – 0.86 (table 1). Common allele and genotype in Latvian Blue breed are the same, but the frequencies are statistically significant difference: 0.72 for allele ($p = 1.86 x10^{-12}$) and for genotype – 0.54 ($p = 5.80 x10^{-10}$). From literature is known that cows' with genotype BB of κ – casein distinguished by better dairy technological features, the higher cheese outcome (Martin et al., 2002), but in our population this genotype and, respectively, allele B are very rear (BB = 0.03 and B = 0.08). There is pronounced predominance of allele A in whole analyzed population, as demonstrated by analyse of Hardy - Weinberg equation, in which was recognized statistically significant difference between expected and observed heterozygote genotypes ($p_{\chi} = 1.19 x10^{-3}$). But in LZ group such deviation from the equilibrium was not found.

Our data about very high occurrence of κ – casein allele A in dairy cows of Latvian population comport with the research data in Lithuania and Estonia (Pečiulaitiene 2005; Pečiulaitiene et al., 2007; Värv et al., 2009). Estonian researchers found higher frequency of allele B in Estonian Native breed cows (0.24) and in Estonian Red breed cows (0.37). Despite the low level of allele B, Estonian scientists found that all parameters of milk coagulation were better to cows with genotype BB of *CSN3* (Kübarsepp et al., 2005). In Lithuanian population bvof dairy cows the frequency of allele A is 0.74 and of allele B – 0.22 (Pečiulaitiene et al. 2007).

Second group of milk proteins are whey proteins, where for both, LAA and LGB, common allele is B (0.94 and 0.78, respectively) and common genotype is BB (0.88 and 0.60, respectively) in whole examined population of Latvian dairy

Table 1. Apportionment of alleles and/or genotype of cows raised in Latvia and Latvian Blue breed

Group	Positive samples	Apportionment of alleles (frequency)		Difference of alleles		Apportionment of genotypes (frequency)			Difference of genotype		Hardy - Weinberg equilibrium	
		1st allele	2nd allele	p value	Cramer's V	Homozyg. of 1st allele	Hetero-zygote	Homozyg. of 2nd allele	p value	Cramer's V	Expected hetero-zygote	Difference (p value)
CSN1S1 (alleles B and C)												
Latvian population	675	96.30	3.70	0.34	0.02	92.59	7.41	0.00	0.33	0.03	0.07	0.32
Latvian Blue breed	154	95.13	4.87			90.26	9.74	0.00			0.09	0.53
CSN2 (alleles A1 and A2)												
Latvian population	716	66.83	33.17	4.87×10^{-4}	0.08	42.18	49.30	8.52	9.98×10^{-4}	0.12	0.44	2.72×10^{-3}
Latvian Blue breed	178	76.40	23.60			55.62	41.57	2.81			0.36	4.12×10^{-2}
CSN3 (alleles A and B)												
Latvian population	487	91.79	8.21	1.86×10^{-12}	0.21	86.45	10.68	2.87	5.80×10^{-10}	0.27	0.15	1.19×10^{-10}
Latvian Blue breed	65	71.54	28.46			53.85	35.38	10.77			0.41	0.29
LAA (alleles A and B)												
Latvian population	603	6.14	93.86	0.32	0.03	0.17	11.94	87.89	0.46	0.05	0.12	0.37
Latvian Blue breed	142	7.75	92.25			0.00	15.49	84.51			0.14	0.32
LGB (alleles A and B)												
Latvian population	649	21.88	78.12	0.47	0.02	3.39	36.98	59.63	0.48	0.04	0.34	$\mathbf{3.73 \times 10^{-2}}$
Latvian Blue breed	138	19.93	80.07			1.45	36.96	61.59			0.32	0.06

In bold – statistically significant p value cows. Nevertheless, for Latvian Blue dairy cows the frequencies are 0.92 and 0.80, respectively for common allele, and 0.85 and 0.62, respectively for common genotype.

From the data of different researches (Bell et al. 1981, Formaggini et al. 1999) about LAA gene we can conclude that variation B is typical or ancestral for *Bos Taurus*, *Bos Indicus, Bos (Poephagus) Grunniens*, therefore variation A isn't widespread in *Bos Taurus.* Hypothetically, we can conclude that our data confirm the researchers' indicated results about low frequency of variation A in different breeds and origins.

Compering our results of LGB gene with other researches' publicized data ((Ikonen et al. 1996, Erhardt et al. 1997, Pečiulaitiene 2005, Kübarsepp et al. 2005, Zaton - Dobrowolska et al. 2006), we conclude that they show a slightly lower frequency of allele B, researching polymorphisms of whey milk protein LGB in different cows' breeds at national and/or special herds. Only one breed with higher frequency of allele B compering to Latvian population, and it is Lithuanian Red with frequency 0.92 (Pečiulaitiene 2005).

For association analyse was divided samples of Latvian Blue breed with the data about indicators of milk quantity and quality in at least one of three lactations. For genotypes of all three casein genes: alpha$_{S1}$ – casein, beta - casein and kappa – casein**,** wasn't found any statistical significant association with milk amount or yield (kg), protein and fat amount (kg), and/or contents (%). Looking for the positive impact of genotypes for these genes, we found out that in case of CSN1S1 isn't possible to make any relation of genotypes, which would work in all lactations. In case of CSN2 gene was able to express the positive impact of genotypes in relation: A2A2 > A1A1 > A1A2 for amount indicators, A2A2 > A1A2 > A1A1 for the fat contents and A1A2 > A1A1 > A2A2 for protein contents. Overall, we can conclude that our results are similar to the data obtained by scientists of other countries (Ehrmann et al. 1997, Winkelman & Wickham, 1997, Kaminski et al. 2006), that in order to increase the productivity of dairy cows must use selection of the cows' population to increase the genotype A2A2 frequency of CSN2 gene. The positive impact of genotypes of CSN3 gene was able to express in relation: BB > AA > AB for all

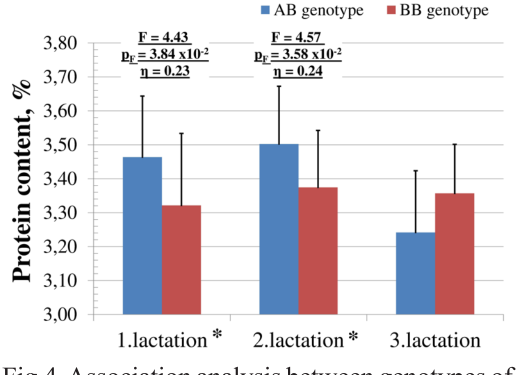

Fig.4. Association analysis between genotypes of A and B alleles of alpha – lactalbumin gene and protein content (%) at the first three lactations. *Bar with a straight edge points to signs of an average group size with a standard deviation; * - refers to the association with the performance at a particular lactation; F – index of ANOVA; p_F – statistical signification; η – index of correlation analyse.*

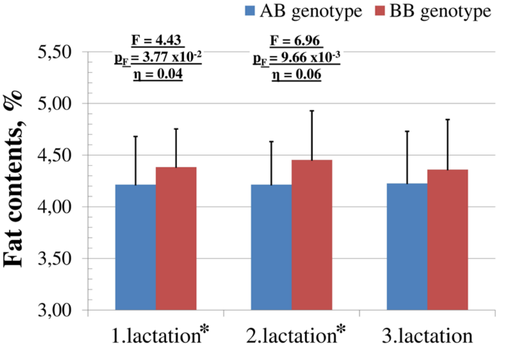

Fig. 5. Association analysis between genotypes of A and B alleles of β - lactoglobulin gene and fat (%) at the first three lactations. *Bar with a straight edge points to signs of an average group size with a standard deviation; * - refers to the association with the performance at a particular lactation; F – index of ANOVA; p_F – statistical signification; η – index of correlation analyse.*

indicators of amount in kilograms, but for content indicators wasn't able to make relation.

In the second group of proteins or whey proteins: alpha – lactalbumin and beta – lactoglobulin, was found statistically significant associations between genotypes and the same indicators of milk quantity and quality. The positive impact of genotypes for LAA gene can be expressed in relation: AB > BB. Genotype AB statistically significant promotes an increase of protein content (percentage) in milk of cows of Latvian Blue breed at first and second lactations (Fig.4.).

For cows with genotype AB showed statistically significant higher protein content in first and second lactation (+ 0.14 and + 0.13 %, respectively) that cows with genotype BB (p_F = 3.84 x10^{-2} and 3.58 x10^{-2}, respectively). At the correlation analyse have found very weak (η = 0.23 and 0.24, respectively) association between genotypes of *LAA* and protein content. The protein content level increase in the both genotype's groups at the third lactation, furthermore in group of AB genotype reduction is 0.26 %, but in BB genotype only 0.02 %. In the results a level of protein content for cows with AB genotype is lower than for cows with BB genotype. Parallely we found statistically significant association between protein amount and LAA genotypes at the first lactation (p_F = 2.26 x10^{-2}).

By the results, we think that forcing out of allele A from population in the result of the genetic drift and increasing frequency of allele B is because of the positive effect of allele B on cows' organisms, including indicators of milk quality and quantity. In case of LGB genotype the positive impact can be written as BB > AB. The genotype AA was found only in one dairy cow with lactation data and therefore we couldn't make average score of group and excludes from association analyses. Genotype BB statistically significant increases of the fat content (Fig.5) comparing to genotype AB. For cows of Latvian Blue breed with BB genotype in average is higher than 0.17 and 0.24 % fat content level in first and second lactation (p_F=3.77x10^{-2} and 9.66x10^{-3}). Predominance of genotype BB in the third lactation is only 0.13% ($p > 0.05$).

We also found statistically significant association between LGB genotypes and protein content in the first lactation. BB genotype increase the protein content for cows of Latvian Blue breed for about 0.08% (p_F = 3.66 x10^{-2}).

CONCLUSIONS

We can conclude that an apportionment of alleles and /or genotypes of Latvian Blue dairy cows in three genes of milk protein (α_{S1} - casein or CSN1S1, α – lactalbumin or LAA and β – lactoglobulin or LGB) is similar to completely to the Latvian dairy cows' population, but in case of β - casein or CSN2 and κ - casein or CSN3 this apportionment is different. It could mean that for these genes have the same special importance in Latvian Blue breed or the same implication of a feature, which could be found in the researches of the future.

In association with milk productivity indicators we didn't found any statistically significant results in case of all three casein genes of milk protein, but there are significant association in case of the whey protein genes. The genotype AB of α - lactalbumin statistically significant increase protein content in the first two lactations, but the genotype BB of β – lactoglobulin have the statistically significant increase of fat content in the first two lactations and also have the incresase of protein content in the first lactation.

The molecular genetics researches need to continue in Latvian historical breed of dairy cows: Latvian Blue, and to make the same researches of second historical local breed – Latvian Brown. We can get significant results for the future selection of Latvian local breeds and to save Latvian animal genetic resources of endangered local breeds of cows for food and agriculture.

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EIROPAS SOCIĀLAIS FONDS

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REFERENCES

A2 Corporation. 2006. *Annual Report* [online] [viewed 26.05.2009]. Available: http://aboutthea2milkcompany.com

Bell K., Hopper K. E., McKenzie H. A. 1981. *Bovine* α – lactalbumin *C* and α_{S1} –, β –, κ – caseins of Bali (Banteng) Cattle, *Bos (Bibos) javanicus*. *Australian Journal of Biological Sciences*, 34. p. 149 – 159.

Bunzel - Drüke M. 2005. Projekt Taurus – En økologisk erstatning for uroksen. *Lohne: ABU 2004*. Århus, DK. Nepenthes. 1 – 24. www.nepenthes.dk; www.abu-naturschutz.de (Translated into Danish by Thomsen K.).

Cade R., Privette R., Fregly M. Rowland N., Sun Z., Zele V. 2000. Autism and schizophrenia: intestinal disorder. *Nutritional Neuroscience*. 3: 57 – 72.

Cardak A. D. 2005. Effects of genetic variants in milk protein on yield and composition of milk from Holstein - Friesian and Simmentaler cows. *South African Journal of Animal Science,* 35 (1): 41 – 47.

Caroli A.M., Chessa S., Erhardt G.J. 2009. Milk protein genetic variation in cattle: Impact on animal breeding and human nutrition. *Journal of Dairy Science*, 92: 5335 – 5352.

Ciani F., Giorgetti A., Sargentini C., West M., Matassino D. 2010. Bovino Maremmano ''Primitivo'' ecologia, origine, etologia e allevemento. *Taurus*, 21 (4): 21 – 34. (In Italian; abstract in English). Available: http://aspa.unitus.it/matassino/150 PDF/BOVINO MAREMMANO PRIMITIVO Taurus rev Ciani.pdf

Cieslinska A., Kaminski S., Kostyra E., Sienkiewicz – Szlapka E. 2007. Beta – casomorphin 7 in raw and hydrolysed milk derived from cows of alternative – casein genotypes. *Milchwissenschaft*, 62: 125 – 127.

Erhardt G., Prinzenberg E.M., Buchberger J., Krick – Saleck H., Krause I., Miller M. 1997. Bovine k – casein *G* detection, occurrence, molecular genetic characterization, genotyping and coagulation properties. Palmerston North, New Zealand. *Proceedings IDF "Milk Protein Polymorphism Seminar II",* International Dairy Federation, Bruxelles, Pp. 328 – 329.

Falconer D.S., Mackay T. F. C. 1996. *Introduction to Quantitative genetics*. Edition 4th, Longmans Green, Harlow. Essex, UK.

FAO. 2010. *International Journal of Animal Genetic Resource,*47: 157.

Farrell H. M., Jimenez - Flores R., Bleck G. T., Brown E.M., Butler J. E., Cramer L. K., Hicks C. L., Hollar C. M., Ng – Kwai - Hang K. F., Swaisgood H. E. 2004. Nomenclature of the Proteins of Cows' Milk – Sixth Revision. In: *Journal of Dairy Sciences*, 87: 1641 – 1674.

Formaggioni P., Summer A., Malacarne M., Mariani P. 1999. Milk protein polymorphism: Detection and diffusion of the genetic variants in *Bos* genus. *Annali della Facolta di Medicina Veterinaria Universita degli*

Studi di Parma, 19: 127 – 165 [online] [viewed 20.07.2012]. Available: http:// www.unipr.it/ arpa/ facvet/annali/1999/ formaggioni/formaggioni.htm

Griņēvičs I, Grosvalds I. latvijas govju šķirņu izveidošana un Latvijas Zilās govs izzušana. *LU 69.konference.* Stenda ziņojums. Rīga. Pp. 27. [online] [Viewed 20.04.2015]. Available: http://priede.bf.lu.lv/konf/apsek/zoo/2011/Grinevics_Grosvalds_Blue_Cow.pdf (In Latvian).

Grīslis Z. 2006. Zilās govis Vidzemē. Jelgava. *BŠSA "Zilā govs"*: 1- 36. (In Latvian).

Grīslis Z., Šimkevica D. 2013. Latvijas zilie buļļi. Jelgava, *BŠSA* "*Zilā govs*": 1 - 36. (In Latvian).

Grīslis Z., Šimkevica D. 2014. Latvijas Zilās atlase. *Biedrība Šķirnes saglabāšanas apvienība 'Zilā govs'*, Jelgava: 1 – 38. (In Latvian).

Hammer Ø, Harper D. A. T, Ryan P. D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4 (1): 9.

Hristov P., Teofanova D., Mehandzhiyski I., Zagorchev L., Radoslavov G. 2012. Application of milk proteins genetic polymorphisms for selection and breeding of dairy cows in Bulgaria. *INTECH*. ISBN 978 – 953 – 51 – 0766 – 8. Pp. 31 – 52.

IBM Corp. Released .2012. *IBM SPSS Statistics for Windows*. Version 21.0. Armonk, NY: IBM Corporation.

Ikonen T, Routtinen O, Erhardt g, Ojala M. 1996 Allele frequencies of the major milk proteins in the Finnish Ayrshire and detection of a new k – casein variant. *Animal Genetics*., 27:179 – 181.

Kaminski S., Rusc A., Cieslinska A. 2006. A note on frequency of *A1* and *A2* variants of bovine beta – casein locus in Polish Holstein bulls. *Journal of Animal and Feed Sciences,* 15: 195 – 198.

Kübarsepp I., Henno M., Viinalass H., Sabre D. 2005. Effect of k – casein and β – lactoglobulin genotypes on the milk rennet coagulation properties. *Agronomy Research*, 3: 55 – 64.

Latvijas Enciklopēdija (Encyclopedia of Latvia). 2007. Vol. 4. Rīga: *Valērija Belokoņa izdevniecība*: Pp. 86 – 87. (In Latvian).

Lejiņš P. 1910. No lustes. *Zemturis*. 50. 985 p.

Lien S., Kantanen J., Olsaker I., Holm L. E., Eythorsdottir E., Sandberg K., Dalsgard B., Addalsteisson S. 1999. Comparison of milk protein allele frequencies in Nordic cattle breeds. *Animal Genetics*, 30: 85 – 91.

Liepa I. (1974) Biometrija. *Zvaigzne*. Rīga. 336 lpp.

Martin P., Szymanowska M., Zwierzchowski L., Leroux Ch. 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. In: *Reproduction Nutrition Development,* 42: 433 - 459.

Martin P., Bianchi L., Cebo C., Miranda G. (2013) Genetic Polymorphism of Milk Proteins. *Advanced Dairy Chemistry – 1A: Proteins: Basic aspects*. 4th Edition. Pp. 463 - 514.

Martin P., Szymanowska M., Zwierzchowski L., Leroux Ch. 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reproduction Nutrition Development*, 42: 33 – 459.

McLachlan C. N. 2001. Beta – casein *A1*, ischaemic heart disease mortality, and other illnesses. *Medical Hypotheses*, 56.: 262 – 272.

Miller M. P. 1997. Tools for population genetic analyses (TFPGA) version 3.1: a Windows program for the analysis of allozyme and molecular population genetic data. [online] [Viewed 20.04.2012]. Available http://www.marksgenetcsoftware.net/tfpga.htm.

Pečiulaitiene, N. 2005. Genetic types of milk proteins prevalence and their relation with production traits in Lithuanian dairy cattle breeds. Summary of doctoral thesis. *Biomedical sciences, zootechny (13B)*. Kaunas. 21 pages.

Pečiulaitiene N., Miceikiene I., Mišeikiene R., Krasnopiorova N., Kriauziene J. 2007. Genetic factors influencing milk production traits in Lithuanian dairy cattle breeds. *Žemes ūkio mokslai*, 14 (1): 32 – 38.

Reichelt K. L., Krem J., Scott, H. 1990. Gluten, milk proteins and autism: dietary intervention effects on behaviour and peptide secretion. *Journal of Apllied Nutrition*, 42: 1 – 11.

Stradiņš J. 2013. Paulis Lejiņš – pirmais latvijas Zinātņu akadēmijas presidents, Kāds viņš bija? *Zinātnes Vēstnesis*. 11 (450), 12 (451). (In Latvian).

Van Vuure T. 2002. History, Morphology and Ecology of the Aurochs (*Bos primigenius*). [online] [Viewed 20.03.2015]. Available: http://members.chello.nl/~t.vanvuure/oeros/uk/lutra.pdf

Van Vuure T. 2005. Retracing the Aurochs - History, Morphology and Ecology of an extinct wild Ox. *Pensoft Publishers*: 1 – 424.

Värv S., Belousova A., Sild E., Viinalass H. 2009. Genetic diversity in milk proteins among Estonian dairy cattle. *Veterinarija I Zootechnika*, 48: 93 – 98.

Winkelman A. M., Wickham B. W. 1997. Associations between milk protein genetic variants and production traits in New Zealand dairy cattle. In: *Milk protein polymorphism. Proceedings of the IDF Seminar held in Palmerston North, New Zealand.* International Dairy Federation. p. 38 – 46.

Zaton – Dobrowolska M., Čitek J., Filistovicz A., Rehout V., Szulc T. 2006. An estimation of the genetic distance between Polish red and other red cattle breeds on the basis of selected milk protein *Loci. Electronic Journal of Polish Agricultural Universities (EJPAU).* Volume 9. Issue 2. # 10. p. 1 – 9. [online] [Viewed 03.03.2012]. Available: http://www.ejpau.media.pl/volume9/issue2/art–10.html

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