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Association study between g.16024A>G polymorphism of the FASN gene and milk production of Holstein cattle

Asociačná štúdia g.16024A>G polymorfizmu génu FASN s produkciou mlieka holštajnského dobytka

Martina MILUCHOVÁ[1], Michal GÁBOR[1] (✉), Juraj CANDRÁK[1], Danka ŠŤASTNÁ[2], Juraj GAŠPER[1]

[1] Institute of Nutrition and Genomics, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

[2] Institute of Animal Husbandry, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

✉ Corresponding author: michal.gabor@uniag.sk

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ABSTRACT

Fatty acid synthase regulates the *de novo* biosynthesis of long chained fatty acids and is considered to be a potential candidate gene for fat content and fatty acids composition in milk. The objective of the study was to estimate the impact of FASN-16024 polymorphism on milk production in Holstein cows. This study determined the genetic structure of the analysed population of 1050 Holstein cows to possible relationships between genetic variants of *FASN-16024* and average breeding values for traits of milk production. PCR-RFLP was used to identify genetic polymorphism for *FASN-16024*. The following genotype distributions were observed: *AA* (2.38%), *AG* (30%), and *GG* (67.62%). The frequencies of alleles *A* and *G* were 17.38% and 82.62%, respectively. The positive effect of the *FASN* gene polymorphism on the yield of milk, protein, and fat in the milk of Holstein cows was observed. No effect of individual genotypes of *FASN-16024* gene polymorphism on fat and protein percentage was observed. The analysis of *FASN* gene polymorphism showed that the *AA* genotype significantly increased the average breeding value of the yield of milk ($P<0.05$), compared to the *GG* genotype. At the same time, the genotypes *AA* and *AG* increase the breeding value for the yield of fat ($P<0.05$) and yield of protein ($P<0.05$) in kilograms compared to the *GG* genotype. Therefore, the *FASN* gene may be used as a potential genetic marker for milk production in Holstein cattle with an emphasis on further research.

Keywords: Holstein cattle, milk production, genetic structure, *FASN*

ABSTRAKT

Syntáza mastných kyselín reguluje *de novo* biosyntézu mastných kyselín s dlhým reťazcom a považuje sa za potenciálny kandidátsky gén pre obsah tuku a zloženie mastných kyselín v mlieku. Cieľom štúdie bolo odhadnúť vplyv polymorfizmu *FASN-16024* na produkciu mlieka u holštajnských kráv. V tejto štúdii bola stanovená genetická štruktúra analyzovanej populácie 1050 holštajnských kráv a možné vzťahy medzi genetickými variantmi *FASN-16024* a priemernými plemennými hodnotami pre znaky produkcie mlieka. Na identifikáciu genetického polymorfizmu *FASN-16024* bola použitá PCR-RFLP. Boli pozorované nasledujúce genotypy: *AA* (2,38 %), *AG* (30 %) a *GG* (67,62 %). Frekvencie alel A a *G* boli 17,38 % a 82,62 %. Bol pozorovaný pozitívny vplyv polymorfizmu génu *FASN* na úžitkovosť mlieka, bielkovín a tuku v mlieku holštajnských kráv. Nebol pozorovaný žiadny vplyv jednotlivých genotypov polymorfizmu génu *FASN-16024* na percento tuku a bielkovín. Analýza polymorfizmu génu *FASN* ukázala, že genotyp *AA* výrazne zvyšuje priemernú plemennú hodnotu pre množstvo mlieka ($P<0,05$) v porovnaní s genotypom *GG*. Genotypy *AA* a *AG* zároveň zvyšujú plemennú hodnotu pre množstvo tuku ($P<0,05$) a množstvo bielkovín ($P<0,05$) v kilogramoch v porovnaní s genotypom *GG*. Preto môže byť gén *FASN* použitý ako potenciálny genetický marker na produkciu mlieka u holštajnského dobytka s dôrazom na ďalší výskum.

Kľúčové slová: holštajnský dobytok, produkcia mlieka, genetická štruktúra, *FASN*

INTRODUCTION

Good nutrition and access to an adequate healthy diet are essential for the development of the child, body maintenance, and protection against infectious and non-communicable diseases in adulthood (Muehlhoff et al., 2013). The nutritional quality of milk depends on breed, genetic variation within the breed (Stoop et al., 2009; Marchitelli et al., 2013), diet (Rolinec et al., 2018 a,b), environment (Šťastná and Šťastný, 2016), and health (Šťastná and Šťastný, 2015).

Milk fat content and composition are one of the most important components influencing the nutritional and technological quality of dairy products (Chilliard et al., 2003). Milk fat is low in of polyunsaturated fatty acids (PUFA) and high in saturated fatty acids (SFA). As a result, fatty acid composition (FA) is considered an important economic trait, and improving milk FA composition, especially increasing unsaturated FA, is important (Mannen, 2011). The composition of milk fatty acid is a heritable trait with heritabilities ranging from 0.31 to 0.73 (Inoue et al., 2008). Recent studies have suggested that genetic improvement of the milk nutritional quality based on the fatty acid profile is possible (Abe et al., 2009; Conte et al., 2010; Matsumoto et al., 2012; Mauric et al., 2019).

Fatty acid synthase (*FASN*) regulates the *de novo* biosynthesis of FA with a long chain and is considered to be a potential candidate gene for fat content and FA composition in meat and milk (Ciecierska et al., 2013; Kala et al., 2016; Li et al., 2016; Mauric et al., 2017). The bovine *FASN* gene is located on BTA19 (19q22) (ROY et al., 2006) and is 19,770 bp long and consists of 42 exons and 41 introns (KALE et al., 2021). The *FASN-16024G>A* SNP, which Roy et al. (2006) identified as *16009A>G*, is caused by an *A/G* substitution in exon 34 of the bovine *FASN* gene. The *A/G* results in a change of the amino acid Thr for Ala in a region that has enol reductase and keto-acyl reductase activity. Matsumoto et al. (2012) presented that of 13 identified SNPs at *FASN*, two non-synonymous SNPs were found on exon 34 with potential linkage to lactation traits. The *T/C* substitution at position 5863 was predicted to result in an amino acid substitution from tryptophan to arginine (*W1955R*) and the *A/G* at position 5848 resulted in a threonine to alanine substitution (*T1950A*). Abe et al. (2009) and Matsumoto et al. (2012) found that the *T1950A* genotypes correspond with the *W1955R genotypes*, so they suggesting that these SNPs are fully linked in Holstein and Japanese Black cattle. Other significant *FASN* polymorphisms associated with milk production include g.17924A>G (Schennik et al., 2009; Oztabak et al., 2014), g.8805C>T, g.13126C>T, g.15532A>C (Abe et al., 2009), g.15603A>G, g.17860C>T (De Souza et al., 2012), g. 16039T>C, g. 18440G>A (Oztabak et al., 2014), g.13965C>T, g.18663T>C (Alim et al., 2014).

Previous studies have revealed that *FASN g.16024G>A* had significant effects on FA composition (Abe et al., 2009; Schennink et al., 2009; Matsuhashi et al., 2011; Matsumoto et al., 2012; Mauric et al., 2019) and can provide useful information in terms of predicting the FA composition of dairy products, or that they can be used as genetic markers to improve milk quality. However, despite the accumulation of data, the link between the *FASN* gene and economic traits, such as milk, fat, and protein yield expressed in kilograms, and the percentage of milk fat and protein, still remains elucidated.

The aim of this report is therefore to evaluate the impact of *FASN-16024* genetic polymorphism on the milk production traits of Holstein cows.

MATERIALS AND METHODS

Animals

A total of 1050 Holstein cows from two farms were used for the present study. Based on the Slovak production index used in the Holstein cow population in the Slovak Republic, we selected three equally numerous groups of animals on each farm. The Slovak production index consists of estimated breeding values of milk, protein, and fat in kilograms. The first group of cows consisted of genetically high-quality animals (animals with an index value higher than the mean plus one standard deviation). The second group formed of genetically average animals

(animals with an index value between plus and minus one standard deviation from the mean) and the third group were genetically low-quality individuals (animals with an index value lower than the mean minus one standard deviation). Three qualitative groups were applied due to the sufficient and expected occurrence of multiple genotypes.

Genomic DNA was extracted from hair root samples using a commercial NucleoSpin Tissue column kit (Macherey-Nagel).

PCR-RFLP analysis and genotyping of FASN

The PCR-RFLP method described by Abe et al. (2009) was used to genotype the *FASN g.16024A>G* gene polymorphism. The amplification of 353 bp fragment of marker *FASN g.16024A>G* was done using a forward primer (5´-CTACCAAGCCAGGCAGGTC-3´) and a reverse primer (5´-GCCATTGTACTTGGGCTTGT-3´). The reaction mixture in the total volume of 20 µl contained 2 µl template DNA, 1 U MyTaq HS DNA polymerase (Bioline), 1X MyTaq reaction buffer, and 0.4 pM of each primer. PCR cycling condition with gradient thermocycler C1000 TouchTM (Biorad) included 95 °C for 3 minutes followed by 35 cycles of 95 °C for 5 seconds, 60 °C for 20 seconds, and 72 °C for 30 seconds. The reaction was terminated with a final elongation step at 72 °C for 10 minutes. The genotyping of the samples was finalized by restriction splicing. The amplified 353 bp PCR products were cleaved using 10 U restriction endonuclease *Hhal* (Thermo Scientific). Fragments of restriction digestion were separated on 2.5% agarose gel (Serva) with GelRedTM intercalating dye (Biotium) in 1×SB buffer (Brody and Kern, 2004) at 180 V for 15 minutes. The visualization and record of results restriction fragments describing the presents of specific alleles were done using UV light and a documentary system Olympus C-7070.

Statistical analysis

Based on the molecular genetic analyses, the genotypic structure of the studied population was established for *FASN g.16024A>G* gene polymorphism and allelic frequencies were calculated. A statistical significance of the differences between observed and expected genotype frequencies was verified using Chi-square statistic. The efficiency of allele occurrence was assessed using the following parameters: expected heterozygosity (He_{exp}), observed heterozygosity (He_{obs}), expected homozygosity (E), effective number of alleles (ENA), polymorphism information content (PIC), and level of realization of possible variability (V%).

Experimental heterozygosity (He exp) (Nei 1973)

$$He_{exp} = 1 - \sum (p^2 + q^2)$$

Polymorphism information content (PIC) (Botstein et al., 1980)

$$PIC = 1 - \sum \left(p^2 + q^2\right) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2 \right)$$

Coefficient of homozygosity (C_a) (Crow and Kimura, 1970)

$$C_a = \sum p_i^2$$

Effective number of alleles (ENA) (Crow and Kimura, 1970)

$$ENA = \frac{1}{p^2 + q^2}$$

Level of possible variability realization (V%) (Crow and Kimura, 1970)

$$V = \frac{1 - Ca}{1 - \frac{1}{N}} \times 100$$

In the association study, five breeding values of the cows (milk, protein, and fat yield expressed in kilograms, and percentage of protein and fat in milk) were used as analyzed traits. Breeding values were used from the official Slovak national Test-Day-Animal model. Environmental effects in the genetic evaluation model: Herd-Test-Day-Lactation, Breed groups, Age of calving, Season of calving, and Parity groups (fixed effects or fixed regressions curves effects). Three lactations were available in all analyzes and estimates. Genotyping was intended only for cows. The results of molecular genetic analysis were used to confirm the relationship between *FASN-16024* gene polymorphism and production traits.

Statistical analysis was used to describe the impact of *FASN-16024* genotypes on the average breeding values of kilograms of milk, protein, and fat yield, as well as the percentage of protein and fat in milk. The influence of

individual genotypes of the *FASN-16024* gene on the variability of average breeding values was evaluated using a two-way analysis of variance:

$$y = \mu + G_i + S_j + e_{ij}$$

where y is the breeding value (kg milk, kg protein, kg fat, % protein, % fat), μ is the overall mean, G_i is the fixed effect corresponding to the genotype, i = 1, 2, 3, S_j is the fixed effect of the herd, j = 1, 2 and e_{ij} is any random residual.

The statistical analysis using the GLM (General Linear Model) procedures and the LSM (Least Squares Means) adjustment for multiple comparisons was done by SAS Enterprise Guide version 9.3 (SAS Inc., 2011). A probability of less than 0.05 was considered significant (P<0.05).

RESULTS

SNP identification and genotyping

The *FASN-16024* polymorphism of Holstein cows detected in PCR-RFLP fragments is shown in Figure 1.

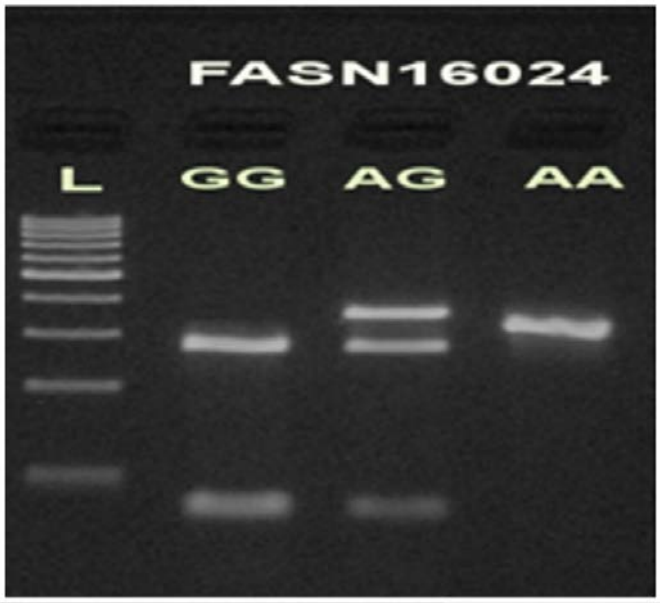

Figure 1. Illustration of *FASN-16024* genotypes on the agarose gel

L – 100 bp ladder (Thermo Scientific BioScience); genotype *GG* (262 bp, 91 bp); genotype *AG* (353 bp, 262 bp, 91 bp); genotype *AA* (353 bp)

There were all three *FASN-16024* genotypes found in this study. In a tested population of Holstein cows kept in Slovakia, we determined a superiority of the *GG* genotype (67.62%), followed by heterozygote genotype *AG* (30%), and the lowest level was observed in the *AA* genotype (2.38%). The results show a significant predominance of the *G* allele (82.62%) over the *A* allele (17.38%). The allele and genotype frequencies of Holstein cows for *FASN-16024* are presented in Table 1.

The genetic equilibrium of the analyzed population was assessed by χ^2-test. In the population included in the study, the differences in genotype frequencies for marker *FASN-16024* were not significant. The efficiency of the *FASN-16024* alleles in the tested population is shown in Table 2.

In our study, a higher increase in homozygosity (0.7128) was observed for the marker *FASN-16024*, due to the high proportion of homozygous *GG* genotype (67.62%) in the tested population of Holstein cattle. This resulted in a reduction in the level of realization of the possible variability (28.86%), which also corresponds to the alleles effectiveness in the population (1.4029). The decreased value of effectiveness of alleles (ENA), shows that the effect of *A* and *G* alleles is not balanced. The according to the PIC classification (PIC value <0.25 – low polymorphism; PIC value between 0.25 and 0.50 – intermediate polymorphism; and PIC value >0.50 – high polymorphism), the studied locus (PIC value 0.2460) possessed low genetic diversity.

Effect of FASN-16024 genotype on milk production traits

In this study, a statistically significant difference was found between genotypes for average breeding values for the yield of milk, fat, and protein when the variability of the observed traits was evaluated as the polymorphism of the *FASN-16024* gene. It was found that the *AA* genotype significantly increased the average value of yield of milk (476.6 kg on average), compared to the *GG* genotype. At the same time, the genotypes *AA* and *AG* increase the breeding value for a yield of fat (13.3 kg and 5.1 kg, respectively) compared to the *GG* genotype. Similarly, the genotypes *AA* and *AG* increase the breeding value of protein yield in kilograms compared to the *GG* genotype, namely the genotype *AA* by 12.3 kg and the

Table 1. Allele and genotype frequencies of the *FASN-16024* in Holstein cows

Locus	Genotype frequencies			Allelic frequencies		χ^2	p
	AA	*AG*	*GG*	*A*	*G*		
FASN-16024	0.02	0.30	0.68	0.17	0.83	0.41	0.81

Table 2. Effectiveness of *FASN-16024* alleles in Holstein cattle population

Locus	Alleles	He_{obs}	He_{exp}	PIC	Ca	ENA	V%
FASN-16024	A; G	0.3000	0.2872	0.2460	0.7128	1.4029	28.86

Table 3. The association of *FASN-16024* genotypes with milk production traits of Holstein cows

Breeding values	Genotype (mean ± SE)			*P*
	AA (n = 25)	*AG* (n = 315)	*GG* (n = 710)	
BVM	1137.06^{a} ± 435	878.25^{ab} ± 592	766.26^{b} ± 538	*
BVF	22.82^{a} ± 8.93	16.35^{a} ± 15.23	11.57^{b} ± 15.568	*
BVF%	-0.27 ± 0.15	-0.23 ± 0.22	-0.24 ± 0.20	n.s.
BVP	29.89^{a} ± 9.07	23.06^{a} ± 15.45	18.39^{b} ± 14.16	*
BVP%	-0.08 ± 0.14	-0.06 ± 0.13	-0.09 ± 0.13	n.s.

BVM – breeding values for the yield of milk (kg); BVF – breeding values for the yield of fat (kg); BVF% – breeding values for the content of fat (%); BVP – breeding values for the yield of protein (kg); BVP% – breeding values for the content of protein (%); SE - standard error; p-values are for the significance of the genotype, we used Two-way ANOVA (genotype, herd); $^{a, b}$ – different superscripts within rows indicate statistically significant differences (*$P \leq 0.05$), we used Duncan Multiple Range Test; n.s. – non-significant.

genotype *AG* by 5 kg. In the case of average breeding values of the percentage of protein and fat in milk, no effect of individual genotypes of *FASN-16024* on their variability was observed (Table 3).

DISCUSSION

At the turn of the 19th and 20th centuries, dairy farmers were interested in increasing the production of milk, but a systematic selection strategy was not available (Miglior et al., 2017). In the process of selecting cattle based on their appearance (phenotype), the resulting efficacy of animal breeding is generally low (Yudin, Voevoda, 2015).

Nowadays, the widespread use of molecular genetics methods, makes it possible to identify genes responsible for economically useful traits and use them as selection markers in marker-assisted selection (MAS) breeding (Smaragdov, 2009; Yudin, Voevoda, 2015) which has helped advance in the dairy cattle industry (Miglior et al., 2017). Essentially, breeding programs are designed to identify better genotypes for various traits of economic interest based on information about the performance of animals and their relatives, as well as molecular information, so that their genes can be spread in the population (Cardoso et al., 2014). Milk composition and milk yield are considered to be the most economically important traits for dairy cows (Prata et al., 2015).

Fatty acid synthase (*FASN*) is an enzyme that regulates the *de novo* biosynthesis of long-chain FA and plays an essential role in determining fatty acid synthesis and the release of new fatty acids (Matsumoto et al., 2012; Li et al., 2016; Mauric et al., 2019). Roy et al. (2006) and Matsumoto et al. (2012) reported that allele *G* increases

the percentage of fat in milk. In contrast, Mauric et al. (2017) reported that *AG* had significantly higher fat content compared to genotype *GG*, which is in agreement with our finding. Čítek et al. (2021) reported that the *A* allele had significantly higher milk yield than the *G* allele, resulting in significantly higher fat and protein yield. However, they reported that protein content was slightly but significantly higher in homozygous *GG* cows. The effect of different genotypes of marker *FASN-16024* on the variability in the percentage of fat and percentage of protein in milk as reported by Roy et al. (2006), Matsumoto et al. (2012) and our results were not statistically significant. In other studies, the effect of the *FASN-16024* marker polymorphism on the variability of dairy production traits is not reported. Literature shows that genetic improvement of production traits in dairy cattle will bring clear economic and environmental benefits (Wall et al., 2010; Pryce, Bell, 2017; Amer et al., 2018; Matthews et al., 2019).

CONCLUSION

The *FASN* gene polymorphism *g.16024A>G*, in addition to regulating the *de novo* biosynthesis of long-chain FA, has a significant impact on the average breeding values for milk yield, fat, and protein in the milk of Holstein cows. If our findings were confirmed by other studies, *FASN* could be used as potential genetic marker not only for improving milk quality but also as a genetic marker for milk production in Holstein cattle.

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REFERENCES

Abe, T., Saburi, J., Hasebe, H., Nakagawa, T., Misumi, S., Nade, T., Nakajima, H., Shoji, N., Kobayashi, M., Kobayashi, E. (2009) Novel mutations of the *FASN* gene and their effect on fatty acid composition in Japanese Black beef. Biochemical Genetics, 47, 397-411. DOI: https://doi.org/10.1007/s10528-009-9235-5

Alim, M.A., Wang, P., Wu, X.P., Li, C., Cui, X.G., Zhang, S.L., Zhang, Q., Zhang, Y., Sun, D.X. (2014) Effect of *FASN* gene on milk yield and milk composition in the Chinese Holstein dairy population. Animal genetics, 45 (1), 111-113. DOI: https://doi.org/10.1111/age.12089

Amer, P.R., Hely, F.S., Quinton, C.D., Cromie, A.R. (2018) A methodology framework for weighting genetic traits that impact greenhouse gas emission intensities in selection indexes. Animal, 12, 5-11. DOI: https://doi.org/10.1017/S1751731117001549

Boltstein, D., White, R.L., Skolnik, M., Davis, R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphism. American Journal of Human Genetics, 32,314-331.

Brody, R.J., Kern, S.E. (2004) Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. Biotechniques, 36, 214-216. DOI: https://doi.org/10.2144/04362BM02

Cardoso, V.L., Pereira Lima, M.L., Nogueira, J.R., de Carneiro, R.L.R., Sesana, R.C., Oliveira, E.J., El Faro, L. (2014) Economic values for milk production and quality traits in south and southeast regions of Brazil. Revista Brasileira de Zootecnia, 43 (12), 636-642. DOI: https://doi.org/10.1590/S1516-35982014001200002

Ciecierska, D., Frost, A., Grzesiak, W., Proskura, W.S., Dybus, A., Olszewski, A. (2013) The influence of fatty acid synthase polymorphism on milk production traits in Polish Holstein-Friesian cattle. Journal of Animal and Plant Sciences, 23, 376-379.

Conte, G., Mele, M., Chessa, S., Castiglioni, B., Serra, A., Pagnacco, G., Secchiari, P. (2010) Diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, and sterol regulatory element binding protein 1 gene polymorphisms and milk fatty acid composition in Italian Brown cattle. Journal of Dairy Science, 93, 753-763. DOI: https://doi.org/10.3168/jds.2009-2581

Crow, J.F., Kimura, M. (1970) An introduction to population genetics theory. Harper and Row, New York, USA, 591 p.

Čítek, J., Brzáková, M., Hanusová, L., Hanuš, O., Večerek, L., Samková, E., Křížová, Z., Hoštičková, I., Kávová, T., Straková, K., Hasoňová, L. (2021) Gene polymorphisms influencing yield, composition and technological properties of milk from Czech Simmental and Holstein cows. Animal Bioscience, 34 (1), 2-11. DOI: https://doi.org/10.5713/ajas.19.0520

Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G. (2003) A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. Journal of Dairy Science, 86, 1751-1770. DOI: http://doi. org/10.3168/jds.S0022-0302(03)73761-8

de Souza, F.R.P., Chiquitelli, M.G., da Fonseca, L.F.S., Cardos, D.F., da Silva Fonseca, P.D., de Camargo, G.M.F., Gil, F.M.M., Boligon, A.A., Tonhati, H., Mercadante, M.E.Z., de Albuqrque, L.G. (2012) Associations of *FASN* gene polymorphisms with economical traits in Nellore cattle (Bos primigenius indicus). Molecular biology reports, 39 (12), 10097-10104. DOI: https://doi.org/10.1007/s11033-012-1883-6

Inoue, K., Shoji, N., Kobayashi, M. (2008) Genetic relations among fat melting point, fatty acid composition and carcass traits in Japanese Black cattle. Nihon Chikusan Gakkaiho, 79, 1-8.

Kala, R., Samková, E., Čítek, J. (2016) Selected candidate genes affecting milk fatty acids. Acta Fytotechnica et Zootechnica, 19, 31-33. DOI: http://dx.doi.org/10.15414/afz.2016.19.si.31-33

Kale, D.S, Jaya Singh, Sathe, Y.B, Patil, D.V. (2021) *FASN* gene and its role in bovine milk production. International Journal of Biotech Trends and Technology, 11, 20-25. DOI: https://doi.org/10.14445/22490183/ijbtt-v11i1p604

Li, C., Sun, D., Zhang, S., Yang, S., Alim, M.A., Zhang, Q., Li, Y., Liu, L. (2016) Genetic effects of *FASN*, PPARGC1A, ABCG2 and IGF1 revealing the association with milk fatty acids in a Chinese Holstein cattle population based on a post genomewide association study. BMC Genetics, 17, 110. DOI: https://doi.org/10.1186/s12863-016-0418-x

Mannen, H. (2011) Identification and utilization of genes associated with beef qualities. Animal Science Journal, 82, 1-7. DOI: https://doi.org/10.1111/j.1740-0929.2010.00845.x

Marchitelli, C., Contarini, G., De Matteis, G., Crisà, A., Pariset, L., Scatà, M.C., Catillo, G., Napolitano, F., Moioli, B. (2013) Milk fatty acid variability: effect of some candidate genes involved in lipid synthesis. Journal of Dairy Research, 80, 165-173. DOI: https://doi.org/10.1017/S002202991300006X

Matsuhashi, T., Maruyama, S., Uemoto, Y., Kobayashi, N., Mannen, H., Abe, T., Sakaguchi, S., Kobayashi, E. (2011) Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle. Journal of Animal Science, 89, 12-22. DOI: https://doi.org/10.2527/jas.2010-3121

Matsumoto, H., Inada, S., Kobayashi, E., Abe, T., Hasebe, H., Sasazaki, S., Oyama, K., Mannen, H. (2012) Identification of SNPs in the *FASN* gene and their effect on fatty acid milk composition in Holstein cattle. Livestock Science, 144, 281-284. DOI: https://doi.org/10.1016/j.livsci.2011.12.003

Matthews, D., Kearney, J.F., Cromie, A.R., Hely, F.S., Amer, P.R. (2019) Genetic benefits of genomic selection breeding programmes considering foreign sire contributions. Genetics Selection Evolution, 51 (40), 1-11. DOI: https://doi.org/10.1186/s12711-019-0483-5

Mauric, M., Mašek, T., Ljoljic, D.B, Grbavac, J., Starčević, K. (2019) Effects of different variants of the *FASN* gene on production performance and milk fatty acid composition in Holstein × Simmental dairy cows. Veterinary Medicine, 64, 101-108. DOI: https://doi.org/10.17221/73/2018-VETMED

Mauric, M., Mašek, T., Benić, M., Špehar, M., Starčević, K. (2017) The effects of DGAT1, *FASN* and PRL genes on milk production and milk composition traits in Simmental and crossbred Holstein cattle. Indian Journal f Animal Science, 87, 859-863.

Miglior, F., Fleming, A., Malchiodi, F., Brito, L.F., Martin, P., Baes, CH.F. (2017) A 100-Year Review: Identification and genetic selectionof economically important traits in dairy cattle. Journal of Dairy Science, 100 (12), 10251-10271. DOI: https://doi.org/10.3168/jds.2017-12968

Muehlhoff, E., Bennett, A., McMahon, D. (2013) Milk and dairy product in human nutrition. Rome: FAO.

Nei, M. (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences USA, 70, 3321-3323. DOI: https://doi.org/10.1073/pnas.70.12.3321

Oztabak, K., Gursel F.E., Akis I., Ates, A., Yrdibi H., Turkay, G. (2014) *FASN* gene polymorphism in indigenous cattle breeds of Turkey. Folia Biologica (Krakow), 62 (1), 28-34. DOI: https://doi.org/10.3409/fb62_1.29

Prata, M.A., Faro, L.E., Moreira, H.L., Verneque, R.S., Vercesi Filho, A.E., Peixoto, M.G.C.D., Cardoso, V.L. (2015) Genetic parameters for milk production traits and breeding goals for Gir dairy cattle in Brazil. Genetics and Molecular Research, 14 (4), 12585-12594. DOI: https://doi.org/10.4238/2015.October.19.2

Pryce, J.E., Bell, M.J. (2017) The impact of genetic selection on greenhouse-gas emissions in Australian dairy cattle. Animal Production Science, 57, 1451-1456. DOI: https://doi.org/10.1071/AN16510

Rolinec, M., Bíro, D., Šimko, M., Juráček, M., Gálik, B., Ondrejáková, K., Hanušovský, O. (2018a) The effect of feeding change on nutrients and minerals composition of goat's milk. Journal of Central European Agriculture, 19, 877-882. DOI: https://doi.org/10.5513/JCEA01/19.4.2363

Rolinec, M., Bíro, D., Šimko, M., Juráček, M., Gálik B., Hanušovský, O., Ondrejáková, K. (2018b) Change of feeding affects fatty acids profile of goat's milk Journal of Central European Agriculture, 2018, 19, 883-889. DOI: https://doi.org/10.5513/JCEA01/19.4.2361

Roy, R., Ordovas, L., Zaragoza, P., Romero, A., Moreno, C., Altarriba, J., Rodellar, C. (2006) Association of polymorphisms in the bovine *FASN* gene with milk-fat content. Animal Genetics, 37, 215-218. DOI: https://doi.org/10.1111/j.1365-2052.2006.01434.x

SAS Institute Inc.: Administering SAS® Enterprise Guide® 5.1. Cary, NC: SAS Institute Inc, Version 9.3, 2011.

Schennink, A., Bovenhuis, H., Léon-Kloosterziel, K.M., Van Arendonk, J.A.M., Visker, M.H.P.W. (2009) Effect of polymorphisms in the *FASN*, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Animal Genetics, 40, 909-916. DOI: https://doi.org/10.1111/j.1365-2052.2009.01940.x

Smaragdov, M.G. (2009) Genomic selection as a possible accelerator of traditional selection. Russian Journal of Genetics, 45 (6), 633-636. DOI: https://doi.org/10.1134/S1022795409060015

Stoop, W.M., Schennink, A., Visker, M.H.P.W., Mullaart, E., Van Arendonk, J.A.M., Bovenhuis, H. (2009) Genome-wide scan for bovine milk-fat composition. I. quantitative trait loci for short- and medium-chain fatty acids. Journal Dairy Science, 92, 4664-4675. DOI: https://doi.org/10.3168/ jds.2008-1966

Šťastná, D., Šťastný, P. (2016) Effect of heat stress on reproduction and production in cow. SPU, Nitra, Slovakia, 71 p.

Šťastná, D., Šťastný, P. (2015) Microstructural changes of cows uterus in an infection caused by Haemophilus somnus. Journal of Central European Agriculture, 16, 269-288. DOI: http://dx.doi.org/10.5513/JCEA01/16.3.1620

Wall, E., Simm, G., Moran, D. (2010) Developing breeding schemes to assist mitigation of greenhouse gas emissions. Animal, 4, 366-376. DOI: https://doi.org/10.1017/S175173110999070X

Yudin, N.S., Voevoda, M.I. (2015) Molecular Genetic Markers of Economically Important Traits in Dairy Cattle. Russian Journal of Genetics, 51 (5), 506-517. DOI: https://doi.org/10.1134/S1022795415050087