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Single nucleotide polymorphism C994g of the cytochrome P450 gene possess pleiotropic effects in *Bos taurus*, L.

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ABSTRACT The chronic consumption of mycotoxin-contaminated forage tends to the weight loss of young cattle, decreasing the reproductive success of adult animals, milk yield, and tolerance to high temperatures. The aim of this paper was to study the effects of the SNP C994G in the *CYP3A28* gene on the productive and reproductive characteristics of dairy and beef breeds. Cows were measured by body weight dynamics, exterior, reproduction, milk production traits, bone mineral density, bulls were analyzed estimating their progeny traits. Dairy cows received dietary betaine supplement with the measurement of homocysteine levels. Molecular genetic was performed by PCR-RFLP method. The effects of SNP C994G were shown in relation to the udder size, the constitution and birth weight of Abredin-Angus cows, as well as the fat and protein content of Ukrainian Red-and-White Dairy cows milk. The higher sensitivity of the GG genotype to less homocysteine level after the addition of betaine was shown. Given the identified correspondence, pleiotropic effects of *CYP3A28* gene can be concluded. A microscopic analysis of forage supported the findings in relation to greater efficiency of the CC genotype under conditions of low mycotoxins contamination, to which *CYP3A28* is selective.

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Introduction

Cytochrome P450 (CYP) is a hemoprotein that plays a key role in drug and xenobiotic metabolism in mammals (Estabrook et al. 1963; Hüttemann et al. 2011). Klingenberg described the CYP family in 1954 because of studies on metabolism of steroid hormones in liver (Klingenberg 1958). These enzymes catalyze redox reactions and electron transport in liver as well as steroids and fatty acids hydroxylation in the adrenal cortex. There are 57 genes encoding 18 families of CYPs (Nebert et al. 2013); most of them encode CYP2, CYP3 and CYP4 (Nelson et al. 2014). The prevalence of these genes in the genome is likely to be associated with increased sensitivity to environmental factors, such as diet, chemical inducers and xenobiotics.

Our study of CYP genes in cattle was caused by their

participation in the metabolism of fungi mycotoxins often-contaminating vegetative forage. *Bos taurus*, L. has following well-studied polymorphic variants in CYP genes: 1A, 2B, 2C, 2D14, 2E1, 2J, 3A28 and 4A (Grasso et al. 2005). Among the *CYP3A* gene in cattle, there are four known isoforms: *CYP3A28*, *CYP3A38* (*CYP3A5*) and *CYP3A48* (Zancanella et al. 2010). Localized on chromosome 25, *CYP3A28* gene (NCBI Reference Sequence: NC_037352.1) carries only two known SNPs: C994G Genbank: Y10214.1 (Larson et al. 2009; Sales et al. 2012; Zancanella et al., 2014; Rosenkrans and Ezell 2015).

CYP3A28 gene in *Bos taurus*, L. is known to be associated with the metabolism of mycotoxins produced by micromycetes parasitizing on feed from storage. The detoxification rate of mycotoxins in animals depends on SNP C994G genotype of *CYP3A28* gene, therefore associated with ovulatory cycle, pregnancy and lactation processes,

level of prolactin decreased due to mycotoxins neutralization (Sales et al. 2012). Specific targets of CYP3A28 are ergovalin, ergovalinin and indole mycotoxins (lolitrem B) of diterpene type alkaloids. These mycotoxins are produced by *Claviceps* spp., *Balansia* spp. and *Epichloe* spp., as well as *Neotyphodium coenophialum*, that predominantly infect cereals, such as *Lolium arundinaceum* [Schreb.] and *Lolium perenne*, L. Moreover, St. John's worth (*Hypericum perforatum*) was proposed as one of the plant inducers of cytochrome activity (Markowitz et al. 2003).

The chronic consumption of mycotoxin-contaminated forage tends to the weight loss of young cattle, decreasing the reproductive success of adult animals, milk yield, and tolerance to high temperatures (Porter and Thompson 1992; Cline et al. 2016). Subsequent changes include the gangrenous ulcers appearance, shortness of breath and a heart rate turbulence (Stowe et al. 2013). Adipose tissue necrosis and muscle weakness effects on animal carcasses quality. Thus, identification of genetic alterations in the activity of key enzymes of mycotoxins biotransformation pathway allows to compensate losses from mycotoxin poisoning in part of the population.

Therefore, the aim of the paper was to study the effects of the SNP C994G in the CYP3A28 gene on the productive and reproductive characteristics of dairy and beef breeds.

Materials and methods

Animals, experimental design and diets

Animal Care and Use Committee of the National University of Life and Environmental Sciences of Ukraine approved the animal procedures used in this research. The study was conducted in beef and dairy animal groups. The beef animals included 52 cows, 281 calves and 6 bulls of Aberdeen-Angus breed from Kharkiv region farm, Eastern Ukraine. The dairy cows group comprised 35 cows of the Ukrainian Red-and-White Dairy breed from farm in Cherkasy region, Central Ukraine and 12 bulls of Ukrainian Red-and-White Dairy breed (3), Holstein (6) and Brown-Swiss (3). Used for dairy herds bull's seed and the productivity of their 334334 daughters were assessed in different Ukrainian farms in 2014-2018.

The age of Aberdeen Angus cows was from 1.5 to 13 years and they had up to 8-10 calves. Calves were conceived by natural mating, most calves were born in the spring months from March to May (91%). During the summer period from May to October, the animals were on the pasture; the ration contained grass and legumes. In winter, animals were kept loosely under sheds and fed diets represented hay, corn silage and grain mixture.

Selected from herd, group of dairy cows had milk production – 6514 L per annum, milk fat – 3.65% and

milk protein – 3.20% concentration on the average. 35 cows were selected shortly after calving, from 1 to 7 lactation. Animals in each group had the same light/dark schedule, humidity and ambient temperature. In the study of dairy cows, the ambient temperature at August-September in the daytime was 22-34 °C outside and 18-26 °C – in the cows' room. Cows were milked between 6 and 8 a.m., and from 5 to 7 p.m. daily. Part of the time animals spent in the walking areas. The base total mixed ration (TMR) was alfalfa haylage containing corn or other silage. Part of soybean meal was 3-5% of the grain mixture. Dairy cows received dietary betaine supplement (betaine-hydrochloride, Beta-Key; ORFFA, Werkendam, Netherlands). The betaine dose was fed at 40 g/d once a day. The supplement was mixed with grain mixture. Cows were fed individually. A few days we spent to adapt betaine group to the new diet.

Animal phenotype assessment

The beef cows and calves were analyzed to assess the body weight dynamics and the constitution. General health traits of dairy cows included: body weight, bone mineral density, reproduction traits – insemination number, days open and milk production traits – milk yield, fat and protein content (Database Orsek, Kiev, Ukraine). Genotyped bulls were estimated by their progeny traits: beef bulls by the calf number and their growth rate, bulls of dairy breeds by the daughter's dairy productivity and yield deviations – milk yield, fat and protein content. Data on the milk production of bull's daughters were obtained from the resource (<http://www.animalbreedingcenter.org.ua/catalog>).

The assessment of the animal exterior trait was carried out according the score system for 100-point scale: body composition (15 pts), musculature (10 pts), head and neck (5 pts), chest (10 pts), withers, back and coupling (15 pts), rump (10 pts), hind quarter (10 pts), udder (15 pts) and extremities (10 pts). Animals that scored a total number of points over 80 were classified as elite.

Bone mineral density (BMD) of dairy cows was estimated using the ultrasonic densitometer Sunlight Omni 7000 (Sunlight Medical, Tel-Aviv, Israel). The control point of taking bone densitometry measurements was the middle third of the 12th pair of rib-bone of animals.

Dairy cow's blood plasma homocysteine level was analyzed before and after consuming the dietary supplement. Blood samples from cows were collected by jugular or caudal venipuncture under aseptic conditions into vacuum test tubes containing the coagulation activator (SiO) Venosafe (Terumo, Leuven, Belgium). Total homocysteine in plasma was measured by a Bovine Homocysteine (Hcy) ELISA Kit (MyBioSource, San Diego, USA) using Sunrise plate reader (Tecan, Männedorf, Switzerland).

Table 1. Alleles frequency, genotypes distribution and Hardy-Weinberg equilibrium by SNP C994G and study group.

Study groups	n	Genotypes by SNP C994G			Alleles by SNP C994G		HWE, <i>p</i>
		CC	CG	GG	C	G	
Aberdeen-Angus cows	52	36	33	31	0.53	0.47	<0.05
Aberdeen-Angus bulls	6	66.7	33.3	0	0.83	0.17	<0.05
Ukrainian Red-and-White Dairy cows	35	51.4	34.3	14.3	0.69	0.31	>0.05
Dairy bulls	12	25	33	42	0.58	0.42	>0.05

Notes: HWE, *p* – Hardy-Weinberg disequilibrium at the level of *p*.

Cytochrome P4503A28 genotyping

Blood samples from cows and bulls were collected by jugular or caudal venipuncture under aseptic conditions and placed into test tubes containing anhydrous ethylenediaminetetraacetic acid salt (EDTA) Venosafe (Terumo, Leuven, Belgium). Blood samples were stored at -20 °C until assayed. Sperm samples of dairy bulls were obtained as semen from official sellers of genetic resources in Ukraine.

DNA for genotyping at SNP CYP3A28, C994G, was extracted from 100 µL of blood or sperm samples using NeoPrep DNA kit (Neogene, Kyiv, Ukraine). Molecular genetic analysis was performed by PCR-RFLP using previously described methods (Rosenkrans and Ezell 2015).

Microscopic analysis of forage

The forage was analyzed in two farms containing the Aberdeen-Angus and Ukrainian Dairy Red-and-White breeds. The samples of feeds from storages used during the winter-spring season were tested to determine the species and quantitative composition of potential mycotoxin-producers (Bilay and Kurbatskaya 1990). Microscopic fungi were isolated by sowing them on a nutrient medium in Petri dishes – on Chapek's agar or wort agar (Pharmaktiv, Kyiv Ukraine), as well as in wet chambers with Van Iterson medium. Forages were analyzed in two farms: alfalfa hay and corn silage that was consumed by beef animals and corn silage, vetch hay, milled grain mixture (wheat, maize, barley), that was consumed by dairy cows.

Statistical analysis

Statistical analysis was performed with checking the distribution of quantitative dates for compliance to the law of normal distribution using the Shapiro-Wilk and Kolmogorov-Smirnov methods. Statistical hypotheses were tested by criteria *t* and χ^2 . Analysis of variance was used for multiple comparisons. To assess the effect on calf growth, GLM was considered, which included as genotype variables of the father, sex of the calf, interaction genotype *sex and month of calving. Pearson correlation

coefficients were calculated between dependent variables. The statistical significance was established at the level of $p < 0.05$. All calculations were performed in the Statistica 13.0 (2015, Dell™).

Results

The alleles frequency and genotypes distribution by SNP C994G in each group studied are given in Table 1. For breeding herd creation, an assessment of animals exterior was carried out (Table 2). The cows with different genotypes by SNP C994G of *CYP3A28* gene have differences in head size, withers, back and coupling, udder, and the total score of exterior assessment. The largest udder was observed in CC-cows.

The most of commercial herds are assessed for body weight dynamics up to 2 years of age, whereas in our study on a breeding herd (Aberdeen-Angus), cows were evaluated before they were aged 5 years old (Table 3).

Table 2. Aberdeen-Angus cows exterior assessment by SNP C994G (Mean ± SEM).

Parameter	Genotypes by SNP C994G		
	CC	CG	GG
<i>n</i>	19	17	16
Body composition	12.2 ± 0.2	11.8 ± 0.2	11.9 ± 0.2
Musculature	8.3 ± 0.1	7.9 ± 0.3	8.2 ± 0.1
Head and neck	4.1 ± 0.1*	3.9 ± 0.1*	4.1 ± 0.1*
Chest	8.0 ± 0.1	7.8 ± 0.2	8.1 ± 0.2
Withers, back and coupling	12.2 ± 0.2*	11.7 ± 0.2	11.4 ± 0.3*
Rump	8.1 ± 0.1	8.0 ± 0.1	8.0 ± 0.1
Hind quarter	8.6 ± 0.1	8.4 ± 0.1	8.4 ± 0.1
Udder	12.7 ± 0.1*	12.1 ± 0.2*	12.3 ± 0.2
Extremities	8.0 ± 0.0	8.0 ± 0.0	7.9 ± 0.1
Total score	82.3 ± 0.7*	79.5 ± 0.7*	80.3 ± 1.0

Notes: *n* – cow number; * – significant at $p < 0.05$ level; SEM – standard error of mean.

Table 3. Aberdeen-Angus cows body weight dynamics by SNP C994G (Mean±SEM).

Parameter	Genotypes by SNP C994G		
	CC	CG	GG
<i>n</i>	19	17	16
Birth weight, kg	31.7 ± 0.9*	31.1 ± 1.5	29.0 ± 0.8*
Average daily gain, g	773 ± 20	768 ± 20	773 ± 23
Weight at the age of 8 month, kg	217.5 ± 5.6	210.9 ± 6.6	207.9 ± 4.4
Weight at the age of 12 month, kg	282.8 ± 7.8	272.3 ± 5.1	278.8 ± 5.6
Weight at the age of 15 month, kg	329.4 ± 6.5	317.1 ± 6.5	324.5 ± 5.2
Weight at the age of 18 month, kg	375.1 ± 8.1	358.6 ± 6.3	368.3 ± 6.3
Weight at the age of 2 years, kg	428.8 ± 8.9	407.4 ± 7.9	407.7 ± 5.0
Weight at the age of 3 years, kg	462.9 ± 11.7	440.5 ± 12.0	437.1 ± 6.2
Weight at the age of 4 years, kg	500.7 ± 11.5	484.1 ± 14.8	472.1 ± 6.7
Weight at the age of 5 years, kg	570.9 ± 17.8	595.4 ± 30.7	529.3 ± 16.9

Notes: *n* – cow number; * – significant at $p < 0.05$ level; SEM – standard error of mean.

Regardless the age, cows body weight corresponded to the pattern $CC \geq CG \geq GG$, but the inter-group difference at any age did not exceed 5%.

Growth dynamics data of 261 calves produced by six Aberdeen-Angus bulls were analyzed (Table 4 and Table 5). The sex ratio was close to 1:1 (130:151). However, heifers and bulls body weight were analyzed separately, taking into account the difference in body weight between these groups in almost every age from 5 to 10% ($p < 0.01$).

The father's genotype effect was maximally manifested on the weight of calves at birth: regardless of sex, calves of CG-bulls weighed on average 0.7 kg or 3% more than calves of CC-bulls. Furthermore, the calf growth rate is controlled by their own genes, the mother's genes determining the quality of lactation, and other external factors (Fedota et al. 2018). The results of the GLM model evaluation (Table 6) indicate that the factors "calving month" and "gender" have influenced on the calf weight at different ages than the father's genotype by *CYP3A28* gene.

The analysis of SNP C994G effects was carried out in

dairy cows (Table 7). The body weight of CC-cows was 30-50 kg higher than that of cows with other genotypes ($p = 0.028$).

BMD is a parameter that represents an integrated health assessment (Fedota et al. 2017). The relationship between any allele or genotype for SNP C9944G in our study has not been established, although GG-cows possessed the highest BMD.

The effect of SNP C9944G on reproductive performance has not been previously described in the literature. In general, the duration of the open days in CC cows was shorter by 30-50 days than in cows with other genotypes. CG-cows required an average of 0.4 more inseminations before fertilization.

As a result of the correlation analysis, it was found that allele C was associated with an increased milk yield by 300-320 kg per year, $r = 0.415$ ($p = 0.1$), with an decreasing in the fat and protein content by 0.13% and 0.05%, $r = 0.30$ and $r = 0.34$ ($p = 0.09$ and $p = 0.05$). In general, in the presence of the G allele and the CG genotype in our

Table 4. Body weight dynamics of calves produced by six Aberdeen-Angus bulls (Mean±SEM).

Bull	Genotype by SNP C994G	Birth weight, kg		Average daily gain, g	
		Heifers	Bulls	Heifers	Bulls
1	CC	27.2 ± 0.4 (27)*	29.1 ± 0.2 (29)*	805.9 ± 34.2 (17)*	797.9 ± 11.6 (9)*
2	CC	27.2 ± 0.5 (19)*	29.1 ± 0.3 (24)*	802.9 ± 31.1 (10)	804.2 ± 26.7 (9)
3	CC	26.8 ± 0.5 (19)*	29.5 ± 0.5 (28)*	777.6 ± 25.5 (7)	826.2 ± 18.7 (10)
4	CC	26.5 ± 0.5 (20)*	28.6 ± 0.4 (16)*	760.3 ± 19.3 (6)	761.9 ± 0 (2)
5	CG	27.2 ± 0.5 (21)*	29.7 ± 0.7 (19)*	808.7 ± 31.4 (11)	791.1 ± 16.5 (8)
6	CG	28.0 ± 0.5 (24)*	29.8 ± 0.3 (35)*	791.3 ± 32.9 (23)*	823.5 ± 20.2 (18)*

Notes: *n* – cow number; * – significant at $p < 0.01$ level; SEM – standard error of mean.

Table 5. Body weight dynamics of calves by SNP C994G (Mean ± SEM).

	Father genotype by SNP C994G			
	CC		CG	
Heifers	Mean ± SEM	n	Mean ± SEM	n
Birth weight, kg	26.9 ± 0.2*	85	27.6 ± 0.3*	45
Average daily gain, g	793.3 ± 17.1	30	796.9 ± 24.2	34
Weight at the age of 8 month, kg	205.2 ± 3.8	30	202.9 ± 4.1	24
Weight at the age of 12 month, kg	256.7 ± 7.9	29	258.2 ± 8.1	22
Weight at the age of 15 month, kg	302.9 ± 10.3	17	300.9 ± 10.9	11
Weight at the age of 18 month, kg	336.4 ± 8.6	14	352.5 ± 11.0	10
Bulls				
Birth weight, kg	29.1 ± 0.2**	97	29.8 ± 0.3**	54
Average daily gain, g	806.8 ± 10.8	30	813.6 ± 15.0	26
Weight at the age of 8 month, kg	209.0 ± 3.2	20	211.1 ± 5.2	18
Weight at the age of 12 month, kg	257.0 ± 5.9	20	266.7 ± 8.5	18
Weight at the age of 15 month, kg	342.0 ± 11.2	5	339.4 ± 7.7	9
Weight at the age of 18 month, kg	410.0 ± 23.6	3	417.5 ± 10.4	6

Notes: n – calves number; differences are significant at level * – $p = 0.08$ and ** – $p = 0.04$; SEM – standard error of mean

study, the quality of cow’s milk was higher, but the yield was slightly lower than with other genotypes.

High reproductive success can be achieved in the long term perspective by breeding, and in the short term by modifying the diet of animals. Betaine is a dietary supplement used as a donor of methyl groups and helps maintain folate metabolism. Its lipotropic effect is associated with the transfer of the methyl group from homocysteine to S-adenosylmethionine. Therefore, we believed that it is important to determine the level of homocysteine in the blood plasma of cows: these changes are a marker of diseases of the cardiovascular system, complications of pregnancy, inflammatory bowel diseases, subacute acidosis, etc. (Doolin et al. 2002; Forges et al. 2007).

In our study, homocysteine levels were measured before and after betaine intake (Table 8). Prior to the study, parameters of cows with different genotypes were

comparable ($p = 0.60$). At the conclusion of the study, some difference between the genotypes was noted ($p = 0.09$). Only GG-cows showed a decrease in the homocysteine level by 24% relative to the initial level ($p = 0.032$). The effect of betaine on other productive and reproductive indicators has also been shown earlier (Fedota et al. 2017).

The productivity of daughters produced by bulls of dairy breeds was analyzed and presented in Table 9. By the average number of daughters, bulls in different groups are comparable. The maximum milk and fat yield were observed in CC-bulls daughters. On the other hand, the highest fat and protein content in milk was observed in daughters of GG-bulls. The maximum daughter yield deviation for almost all parameters of daughter’s productivity was observed in GG-bulls.

The ability to compare our findings is limited by the lack of publications that examine the effect of the father’s

Table 6. Effects of factors affecting the Aberdeen-Angus calves body weight dynamics

	Factor effects			
	Father’s genotype	Sex	Father’s genotype x sex	Month of birth
Birth weight, kg	-0.10	-0.54**	-0.01	0.18**
Average daily gain, g	0.05	-0.31**	0.02	-0.12
Weight at the age of 8 month, kg	0.01	-0.36**	0.10	-0.13
Weight at the age of 12 month, kg	-0.17	-0.29*	-0.01	-0.04
Weight at the age of 15 month, kg	-0.09	-0.70**	-0.13	0.01
Weight at the age of 18 month, kg	-0.12	-0.76**	-0.06	0.06

Note: ** – significant at $p < 0.01$ level

Table 7. The characteristics of Ukrainian Red-and-White Dairy cows by SNP C994G.

Cows characteristics	Trait SNP C994G		
	CC	CG	GG
Genotype	CC	CG	GG
n	5	12	18
Body weight, kg	561.4 ± 7.8*	508.2 ± 4.0	533.1 ± 10.4
BMD, m/s	3448.5 ± 119.5	3334.8 ± 20.9	3470.8 ± 95.9
Insemination number before fertilization	1.4 ± 0.4	1.8 ± 0.4	1.4 ± 0.2
Days open	111.8 ± 9.6	159.5 ± 17.0	139.6 ± 31.3
Maximum yield per lactation, kg	6760.0 ± 296.6	6427.5 ± 104.6	6644.2 ± 121.8
Lactation: Fat content, %	3.60 ± 0.03*	3.76 ± 0.02*	3.73 ± 0.02*
Lactation: Protein content, %	3.25 ± 0.02*	3.30 ± 0.01*	3.31 ± 0.01*

Notes: n – cow number; * – significant at $p < 0.05$ level; SEM – standard error of mean

Table 8. Homocysteine level before and after betaine supplement consumption by SNP C994G.

Homocysteine level	Genotypes by SNP C994G		
	CC	CG	GG
Homocysteine, mmol/l (before betaine supplement)	6.78 ± 1.92	5.07 ± 0.30	5.86 ± 1.32
Homocysteine, mmol/l (after betaine supplement)	6.79 ± 1.27	5.65 ± 0.37	4.70 ± 0.35

genotype on the productivity of daughters for SNP C994G. When drawing the conclusion on the effectiveness of a particular genotype, it is necessary to take into account the results of the forage analysis. The results of forage microscopic analysis are given in Tables 10 and 11.

A relatively high CFU of *Cladosporium* spp. and toxigenic micromycetes *Aspergillus fumigatus* founded in forage of beef animals and a relatively high spore amount of *Trichothecium roseum*, *Fusarium oxysporum*, *Cladosporium* spp. and toxigenic micromycetes *Aspergillus flavus* was found in forage of dairy cows. In the same time, we have not detected *Claviceps* species, being producers of ergotoin.

Discussion

In natural populations, deviation from the Hardy-Weinberg equilibrium supports disruptive selection leading to the stratification of populations into subpopulations. The observed Hardy-Weinberg disequilibrium in Aberdeen-

Table 9. Daughters' productivity characteristics produced by dairy bulls depending on the genotype by SNP C994G.

Trait	Genotype by SNP C994G			p
	CC	CG	GG	
Bulls' breeding value by national Ukrainian scale (pts)	545.7 ± 245.0	589.0 ± 88.8	1037.8 ± 59.6	0.267
Daughters number, n	116.7 ± 54.2	100.8 ± 29.8	116.6 ± 36.4	0.778
Daughter milk performance traits				
Milk yield (kg)	9202.0 ± 1369.0	7178.0 ± 1937.0	6872.6 ± 514.7	0.239
Fat yield (kg)	342.0 ± 45.0	282.0 ± 86.0	288.4 ± 29.5	0.611
Protein yield (kg)	286.5 ± 45.5	323.0	264.0 ± 18.4	0.541
Fat content (%)	3.73 ± 0.06	3.89 ± 0.15	4.18 ± 0.28	0.559
Protein content (%)	3.11 ± 0.03	3.54	3.65 ± 0.25	0.432
Daughters' yield deviation				
Milk yield (kg)	498.3 ± 256.8	556.0 ± 153.3	818.0 ± 111.6	0.446
Fat yield (kg)	23.3 ± 5.2	22.0 ± 8.9	39.2 ± 1.8	0.03
Protein yield (kg)	16.3 ± 6.9	19.7 ± 2.2	29.3 ± 3.4	0.417
Fat content (%)	0.06 ± 0.12	0.02 ± 0.05	0.09 ± 0.03	0.155
Protein content (%)	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.354

Table 10. The results of microscopic analysis of forage consumed by Aberdeen-Angus cows.

Fungal name	Specific mycotoxins	Lucerne hay		Corn silage	
		Wort agar	Czapek agar	Wort agar	Czapek agar
<i>Cladosporium</i> spp.	-	18.25 × 10 ⁴	10.00 × 10 ⁴	-	-
<i>Alternaria alternata</i>	Alternariol	1.50 × 10 ⁴	2.0 × 10 ⁴	-	-
<i>Aspergillus fumigatus</i>	Fumitremorgins A, B, C, gliotoxin, aspergill- lic and kojic acids, fumigatin, verruklogen, TR-2 fumitoxins AD	-	-	3.25 × 10 ⁴	1.25 × 10 ⁴
<i>Aspergillus</i> sp. (section <i>Aspergillus</i>)	-	1.20 × 10 ⁴	3.20 × 10 ⁴	-	0.75 × 10 ⁴
<i>Aspergillus proliferans</i>	-	3.00 × 10 ⁴	0.25 × 10 ⁴	-	-
<i>Mucor</i> spp.	-	0.25 × 10 ⁴	-	-	-
<i>Rhizopus microsporus</i>	-	-	0.25 × 10 ⁴	-	-
<i>Penicillium lanosum</i>	Citrinin	1.25 × 10 ⁴	0.75 × 10 ⁴	4.25 × 10 ⁴	2.25 × 10 ⁴
<i>Penicillium pallidum</i>	-	-	1.25 × 10 ⁴	-	-
<i>Rhodotorula rubra</i>	-	0.25 × 10 ⁴	-	-	0.25 × 10 ⁴
<i>Aureobazidium pullulans</i>	-	0.75 × 10 ⁴	-	-	-
Yeast-like fungi	-	1.75 × 10 ⁴	-	34.50 × 10 ⁴	19.25 × 10 ⁴
Total CFU	-	28.20 × 10 ⁴	17.70 × 10 ⁴	42.00 × 10 ⁴	23.75 × 10 ⁴

Angus bulls attributed to small sample size, whereas in case of Aberdeen-Angus cows it is likely be observed due to the instability of conditions important for realization of the studied SNP effect. Essentially, this refers to forages mycotoxins loading, which depends on the conditions of their storage. In dairy cows and bulls bred at another farm, the nature of forage mycotoxins contamination is likely to be less varying in long-term dynamics.

The effect of genotype on the udder's size probably related to the fact that mammary glands are known to be the main target organ of the prolactin. According to

literature data (Sales et al. 2012), the prolactin level in CC-animals depends on the mycotoxins contamination of forage. It was shown a lower prolactin level in animals consuming the mycotoxins-contaminated forage, and higher prolactin level otherwise. The involvement of prolactin in immune responses may also explain the larger body size. The advantage of CC-animals within studied group as for individual body parts, as for the overall score indicates that cows were likely received mycotoxins-free forage.

The observed higher body weight of CC-cows at birth

Table 11. The results of microscopic analysis of forage consumed by Ukrainian Dairy Red-and-White cows.

Fungal name	Specific mycotoxins	Forage		
		1	2	3
<i>Trichothecium roseum</i>	Trichothecin, scirpenes, trichothecolone	+	+	-
<i>Fusarium oxysporum</i>	Fuzarenon-X, T-2 toxin, neoslanol, zearalenone, moniliformin	-	-	+
<i>Cladosporium</i> spp.	-	+	-	+
<i>Alternaria alternata</i>	Alternariol	-	-	+
<i>Aspergillus glaucus</i>	-	-	-	+
<i>Aspergillus sydowi</i>	Sterigmatocystin	+	+	-
<i>Aspergillus flavus</i>	Kojic acid, aflatoxins B1, B2, G1, G2, sterigmatocystin, tremor- gen toxins, cyclopiazon acid	+	+	+
<i>Aspergillus candidus</i>	Candidusins	-	+	-
<i>Mucor</i> spp.	-	-	-	+
<i>Penicillium lanosum</i>	Citrinin	-	+	+
<i>Rhodotorula rubra</i>	-	+	-	-
<i>Aureobazidium pullulans</i>	-	-	-	+
Yeast-like fungi	-	+	+	-
Total	-	8.25×10 ⁴	10.50×10 ⁴	28.75×10 ⁴

by 0.6-2.7 kg or 2-8% ($p < 0.05$) can be explained by the increased CYP3A28 activity under the lack of necessity to metabolize xenobiotics. Our results are partially consistent with the recent study of Sales et al. (2016) in Holstein and Jersey animals: GG-cows who received contaminated feeds possessed lowest average daily gain during 84 days of lactation and body weight at the end of this period. In general, the negative effect of fescue toxicosis on the cattle growth is well-known (Gadberry et al. 2015).

It is known that CYP3A28 effect on growth processes can also be mediated through prolactin. The structure and function of prolactin and growth hormone genes are similar in that they possess common antigenic determinants and have growth-stimulating and lactogenic activity. Probably both, the hormones and the receptor family, to have been appeared because of gene duplications in early vertebrate evolution (Ocampo Daza and Larhammar 2018), and then evolved independently. Thus, the effect on growth processes, particularly on weight gain, can be mediated through regulation of prolactin levels in cattle. The prolactin concentration and the prolactin: cortisol ratio correlate with body weight and average daily gain (Rosenkrans and Ezell 2015).

Detected effect of father's genotype on the weight of beef calves at birth probably may be determined by the relationship between the cytochrome gene activities and generally observed in mammal's spermatogenesis: seasonal changes in male fertility (mass and size of the testicles and sperm count) in proteins correlate with cytochrome activity (Zhang et al. 2010). On the other hand, references in literature about the influence of the father's genotype of *CYP 3A28* gene (or its ortholog in human 3A28) on progeny have not been discovered.

The effect of the SNP C9944G genotype on the body weight of dairy cows could be attributed to participation in mycotoxins metabolism. Since animal mycotoxicosis leads to prolactin level decreasing, fescue mycotoxins possess dopamine-like activity inhibiting prolactin secretion in animals consuming contaminated forage. Regardless to the level of forage contamination, the lowest prolactin level in Angus-sired crossbred CC-steers was detected (Sales et al. 2012). However, based on the cortisol level, CC genotype was more effective in mycotoxins metabolizing than other genotypes. Given the positive correlation between the prolactin level, prolactin: cortisol ratio, body weight and the average daily gain (Rosenkrans and Ezell 2015), we assumed that observed effect is likely be conditioned by the consumption of contaminated forage. Despite the lack of statistical significance of the genotype's influence on milk characteristics, obtained results can be considered as economically important.

In the same time, Larson et al. (2009) revealed that GG-cows in condition of mycotoxin-contaminated forage

consuming had the least daily milk yield. However, the rates of milk yield for the entire lactation period under the uninfected forage nutrition were comparable with other genotypes. The noncontaminated forage consuming CC-cows had the highest protein content in milk, but consumption of contaminated feeds led to minimization of this parameter. Based on the analysis of the obtained results, it could be assumed that the identified effects are attributed to the consumption of mycotoxin-contaminated forage.

The hypothesis on the association between G allele and reproduction parameters is consistent with the results of other authors on low activity of G allele in metabolism of mycotoxins and involvement of prolactin in their utilization.

Reduction of prolactin concentration resulted in lactation decreasing, estrogen activity increasing, and stimulation of reproductive function, accordingly. Thus, GG genotype indirectly leads to increasing of bone mineral density due to both, the negative effect of lactation and prevention of mineral elements migration from bone tissue into milk, and in cause of stimulatory effect of estrogens (Ho-Pham et al. 2013; Cauley 2015).

Forages contamination by mycotoxins, especially by ergotoin, has a negative effect on the concentration of prolactin and associated with low lactation and growth rates of young animals. It is known that ergotoxin is metabolized by CYP3A28. In the absence of the targeted mycotoxins – ergotoxins, produced by *Claviceps* spp., the CC-genotype will be beneficial, as we presented in our study. Our results are comparable to the literature data (Larson et al. 2009) regarding the advantages of the CC genotype under the condition of non-contaminated forage consumption. Namely, consuming non-contaminated by ergotoxin forage CC-animals will have advantages compared to GG-genotype.

Conclusions

The effects of SNP C994G shown in relation to the size of the udder, the overall assessment of the constitution and birth weight of Abredin-Angus cows, as well as the yield, fat and protein content in the milk of Ukrainian Red-and-White Dairy cows. Moreover, higher sensitivity of the GG genotype to homocysteine level after the addition of betaine was shown. The microscopic analysis results of forage support the findings concerning relation to greater efficiency of the CC genotype under conditions of low mycotoxins contamination, to which *CYP3A28* gene is selective. Given the identified effects, we can conclude about pleiotropic effects of *CYP3A28* gene.

References

- Bilay VI, Kurbatskaya ZA (1990) The determinant of toxin-forming micromycetes. Kiev: Sciences. Dumka.
- Cauley JA (2015) Estrogen and bone health in men and women. *Steroids* 99:11-15.
- Cline GF, Muth-Spurlock AM, Voelz BE, Lemley CO, Larson JE (2016) Evaluating blood perfusion of the corpus luteum in beef cows during fescue toxicosis. *J Anim Sci* 94:90-95.
- Doolin MT, Barbaux S, McDonnell M, Hoess K, Whitehead AS, Mitchell LE (2002) Maternal genetic effects, exerted by genes involved in homocysteine remethylation, influence the risk of spina bifida. *Amer J Human Genet* 71:1222-1226.
- Estabrook RW, Cooper DY, Rosental O (1963) The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex. *Biochem Zeitsch* 338:741-755.
- Fedota OM, Ruban SYu, Mitioglo LV, Tyzhnenko TV, Gontar YuV, Lysenko NG (2017) Effects of dietary betaine on productive traits and reproductive health of dairy cows. *J Vet Med Biotechnol Biosafety* 3:18-25.
- Fedota OM, Puzik NG, Ruban SYu, Kolisnyk AI (2018) Effect of cow genotype by SNPS *L127V/GH, F279Y, A257G/GHR* and birth date on calf growth performance of Aberdeen-Angus. *J Vet Med Biotechnol Biosafety* 4:29-36.
- Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL (2007) Impact of folate and homocysteine metabolism on human reproductive health. *Human Reprod Update* 3:225-238.
- Gadberry MS, Hawley J, Beck PA, Jennings JA, Kegley EB, Coffey KP (2015) A meta-analysis of research efforts aimed at reducing the impact of fescue toxicosis on cattle weight gain and feed intake. *J Anim Sci* 93:5496-5505.
- Grasso E, Longo V, Cocceani F, Giovanni Gervasi P (2005) Cytochrome P450 expression and catalytic activity in coronary arteries and liver of cattle. *Biochim Biophys Acta* 1722:116-123.
- Ho-Pham LT, Nguyen ND, Nguyen TV (2013) Quantification of the relative contribution of estrogen to bone mineral density in men and women. *BMC Musculoskeletal Disorders* 14:366.
- Hüttemann M, Pecina P, Rainbolt M, Sanderson TH, Kagan VE, Samavati L, Doan JW, Lee I (2011) The multiple functions of cytochrome c and their regulation in life and death decisions of the mammalian cell: From respiration to apoptosis. *Mitochondrion* 11:369-381.
- Klingenberg M (1958) Pigments of rat liver microsomes. *Archives Biochem Biophys* 75:376-386.
- Larson MJ, Sales M, Reiter S, Brown HJr, Brown M, Looper M, Rosenkrans CFJr (2009) Effects of Forage Type and CYP3A28 Genotype on Beef Cow Milk Traits. *Arkansas Anim Sci Depart Report Res Series* 574:18-21.
- Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y, Wang J-S, Chavin KD (2003) Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. *J Amer Medic Association* 290:1500-1504.
- Nebert DW, Wikvall K, Miller WL (2013) Human cytochromes P450 in health and disease. *Philos Trans Royal Soc (Ser B, Biol Sci)* 368:20120431.
- Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW (2014) Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes, and alternative-splice variants. *Pharmacogenetics* 14:1-18.
- Porter JK, Thompson FN (1992) Effects of fescue toxicosis on reproduction in livestock. *J Anim Sci* 70:1594-1603.
- Rosenkrans CFJr, Ezell NS (2015) Relationships among ergot alkaloids, cytochrome P450 activity, and beef steer growth. *Frontiers Chem* 3:16.
- Ocampo Daza D, Larhammar D (2018) Evolution of the growth hormone, prolactin, prolactin 2 and somatolactin family. *Genes Comparative Endocrinol* 264:94-112.
- Sales MA, Larson MJ, Reiter ST, Brown AHJr, Brown MA, Looper ML, Coffey KP, Rosenkrans CFJr (2012) Effects of bovine cytochrome P450 single-nucleotide polymorphism, forage type and body condition on production traits in cattle. *J Anim Physiol Anim Nutr* 96:545-553.
- Sales MA, Meyer LR, Rosenkrans CFJr (2016) Cytochrome P450 polymorphisms influence growth and immune function of dairy heifers grazing stockpiled endophyte-infected tall fescue. *J Anim Sci* 94:37.
- Stowe HM, Miller M, Burns MG, Calcaterra SM, Andrae JG, Aiken GE, Schrick FN, Cushing T, Bridges WC, Pratt SL (2013) Effects of fescue toxicosis on bull growth, semen characteristics, and breeding soundness evaluation. *J Anim Sci* 91:3686-3692.
- Zancanella V, Giantin M, Lopparelli RM, Patarnello T, Dacasto M, Negrisola E (2010) Proposed new nomenclature for *Bos taurus* cytochromes P450 involved in xenobiotic drug metabolism. *J Vet Pharm Therap* 33:528-536.
- Zancanella V, Giantin M, Dacasto M (2014) Absolute quantification and modulation of cytochrome P450 3A isoforms in cattle liver. *Vet J* 202:106-111.
- Zhang H, Sheng X, Hu X, Li X, Xu H, Zhang M, Li B, Xu M, Weng Q, Zhang Z, Taya K (2010) Seasonal changes in spermatogenesis and immunolocalization of cytochrome P450 17 α -hydroxylase/c17-20 lyase and cytochrome P450 aromatase in the wild male ground squirrel (*Citellus dauricus* Brandt). *J Reprod Develop* 56:297-302.

