Effect of ß-lactoglobulin Locus Polymorphism on Milk Related Traits in Romanian Spotted

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Abstract
The main aim of the study was to assess the influence of ß-lactoglobulin (*LGB)* genotype on milk related traits in Romanian Spotted (R.S.) breed. Altogether 254 cattle were genotyped for in order to establish the share of *A* and *B* allele in *LGB* locus using PCR-RFLP assay. The most prevalent was *A* allele (0.662) compared to *B* allele (0.338). Comparable frequencies (P>0.084) were recorded for *AA* (0.434) and *AB* (0.455) genotypes. The *BB* heterozygous recorded a lower frequency (0.111) compared with others (P≤0.001). For the *LGB* polymorphism, no significant differences (P>0.05) were observed according to milk production. The higher milk production was associated to *AB* (6094.31±103.22 kg) compared to *AA* (5912.22±91.7 kg, P>0.53) and *BB* (5977.7±81.12 kg, P>0.71) genotypes. The higher fat percentage (4.26±0.02%) was recorded for *BB* genotype, compared to *AA* genotype (4.19±0.02%, (P≤0.019). A significantly increased protein percentage was associated with *AB* genotype (3.43±0.03%) compared with *AA* (3.28±0.02%, P≤0.027). No significant difference (P>0.66) was recorded compared to *BB* genotype (3.42±0.01%) related to this trait. The results obtained encourage including marker assisted-selection and use the genotyped sires for genes with economic values in the future breeding scheme of Romanian Spotted breed.

Keywords: *LGB* polymorphism, milk related traits, Romanian Spotted

1. Introduction

The polymorphism of *LGB* locus was initially discovered in 1955 by Aschaffenburg and Drewry [1]. The studies conducted over the years found eleven genetic forms (*A, B, C, D, E, F, G, H, I, J, W*), among the most common variants, are *A* and *B* in all breeds (*Bos taurus, Bos indicus* and *Bos grunniens)* [2]. The share of different proteins variants is not uniform among the cattle breeds. Thus, the *A* variant is frequently meet in Zebu, *B* variant in Holstein Friesian and Brown [3, 4], *C* variant in Jersey [5]. The *D* variant was reported in Simmental with a lower frequency of 0.04 [6]. The whey proteins comprise approximately 20% of the total milk proteins and the *LGB* comprise 60% from the total of whey proteins [7]. Many surveys were conducted in order to determine the influence of *LGB* polymorphism on milk production, chemical composition and processing properties [8]. The obtained results are inconsistent highlighting significant differences in the expression of *LGB* polymorphism on milk production traits [9 - 11]. Most of the studies have been focused on dairy cattle breeds, dual purpose breeds being somewhat ignored due to the relative lower milk productive level. The large share of Romanian Spotted among the others breeds from Romania require more studies regarding both the alleles and genotypes frequency and also, more precise studies regarding its influences on milk production traits. In this respect, the consumers have a high contribution regarding the demand of high biological value products. Currently, there is a lack of knowledge with respect to *LGB* polymorphism among Romanian Spotted [12, 13] and there are very few outcomes regarding

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correlations between genetic polymorphism, milk yield, chemical composition and processing properties [12, 14]. Also, there are a very few studies regarding the correlation between *LGB* locus polymorphism and reproductive indices or disease resistance, particularly mastitis [15]. Therefore, the importance of indigenous breeds as gene reservoirs is recognized worldwide and requesting more precise outcomes [16] also more precise knowledge in order to improve the breeds genetic values with no negative effects on animal welfare.

In current research we focused on a) determining the genotypes and alleles frequency of *LGB* locus in Romanian Spotted dual-purpose cattle breed and b) characterization of the different genotypes from *LGB* locus in terms of milk production and milk chemical composition.

2. Materials and methods

The study was carried out at the Research and Development Station for Bovine Arad-Romania on 225 Romanian Spotted dual-purpose cows reared under loose system. All cows were included in the Official Performance Recording Scheme through which the milk production and chemical composition data were registered. The research activities were performed in accordance with the European Union's Directive for animal experimentation (Directive 2010/63/EU) and were approved by the Scientific Council at the Research and Development Station for Bovine Arad (No.50/29.10.2015). For the genotyping of animals, the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used [17, 18]. Milk production traits were recorded by Official Performance Recording Scheme, statistically analyses being expressed as MEAN ± SEM. Comparisons between milk productions traits associated with the three genotypes were carried out using ANOVA protocol with "cow genotypes" as a categorical factor. Differences between the three genotypes were statistically tested using the Shapiro-Wilks test [19]. Decisions about the acceptance or rejection of statistical hypothesis have been made at the 0.05 level of significance. All the statistical inferences were carried out using Statistica software v.13.0 [20].

3. Results and discussion

In the current study, the *LGB* locus has been genotyped using the PCR-RFLP method. Alleles and genotypes frequency are presented in Table 1.

Table 1. Polymorphism of *LGB* locus for the studied Romanian Spotted cows herd

Locus	Genotype	Frequency	Allele (frequency)
LGB	*AA*	0.434^{a}	p_A-0.662^{a}
	AB	0.455^{a}	q_B-0.338^{b}
	BB	0.111^{b}	

Different superscript per column differ significantly at $P \leq 0.05$, within the same locus

A significant difference ($P \leq 0.001$) was recorded between *A* (0.662) and *B* (0.338) alleles. Consistent results were obtained also by Famula T.R. and Medrano J.F. (1994) on Holstein Friesian, which recorded significant differences between *A* (0.94) and *B* (0.06) alleles frequencies [21]. Contradictory results were obtained by Grădinaru A. *et al.*, in a study conducted in 2013 on Romanian Spotted cows, which found closer values related to *A* (0.525) and *B* (0.451) allele's frequencies [14] and Tsiaras A.M. *et al.* (2015) in Holstein, which recorded 0.52 and 0.48 for *A* and *B* allele, respectively [22]. Also, closer values regarding *A* (0.543) and *B* (0.449) alleles frequencies in Simmental were reported in a study conducted in 2010 by Bonfatti *et al.* [23]. Low frequencies for *A* allele were found in milk specialized breed by Grădinaru A. *et al.* (2013) in Montbeliarde (0.306 and 0.694 for *A* and *B* allele), also by Strzalkowska N. *et al.* (2002) in Polish Black and White, 0.37 for *A* and 0.63 for *B* allele, respectively [14, 24].

Closer values were recorded for the *AA* (0.434) and *AB* (0.455) genotypes ($P \geq 0.084$) in the current study. Lower frequency was associated with *BB* genotype (0.111, $P \leq 0.001$) compared to others. Early studies conducted on local breeds highlighted lower frequencies for *AA* genotype in Romanian Brown (0.12) and Romanian Simmental (0.1) compared to 0.44 and 0.7 for *AB* or 0.44 and 0.2 for *BB* genotype, respectively [12, 25]. In *LGB* locus, most studies recorded inconsistent results, reporting lower frequency of *AA* genotype. Thus, Strzalkowska N. *et al.* (2002) found in Polish Black and White cows a frequency of 0.14, also Tsiaras A.M. *et al.* (2005) in Holstein Friesian recorded a frequency of 0.28 [24, 22]. These results are consistent with those recorded by Ilie D. *et al.* (2008, 2009) in local Romanian Brown and Romanian Simmental breeds [25, 12]. Lower frequencies for the *AA* genotype were recorded by Cury R.A. *et al.* (2005) for Simmental (0.133) and Angus (0.025) crossbreed [26]. Contradictory results regarding a higher frequency of *AA* (0.43) genotype were provided by Yahya M. *et al.* (2013), compared to others two genotypes [27].

The milk production traits (milk, fat percentage and protein percentage) were assessed according to the three genotypes associated with *LGB* locus. Data recorded are presented in Table 2.

Table 2. Means (± SEM) for milk production traits according to *LGB* locus in Romanian Spotted dual-purpose cows herd

Genotype	Milk (kg)	Fat (%)	Protein (%)
AA	$5912.22{\pm}91.7^{a}$	$4.19{\pm}0.02^{a}$	$3.28{\pm}0.02^{a}$
AB	$6094.31{\pm}103.22^{a}$	$4.22{\pm}0.03^{a}$	$3.43{\pm}0.03^{b}$
BB	$5977.7{\pm}81.12^{a}$	$4.26{\pm}0.02^{b}$	$3.42{\pm}0.01^{b}$

Columns means with different superscript differ significantly at $P \leq 0.05$

The highest milk production was associated with *AB* genotyped cows (6094.31 ± 103.22 kg). The *BB* homozygous genotype allowed a milk production of 5977.7 ± 81.12 kg while the lowest production has been associated with *AA* genotype (5912.22 ± 91.7 kg). No significant differences (P > 0.05) between the three genotypes yields were recorded. Consistent results were reported by McLean D.M. *et al.* (1984) and also by Ojala M. *et al.* (1997) [28, 29]. For Romanian Simmental breed, the results provided by Gradinaru A. *et al.* (2014) did not record a significant influence of *LGB* polymorphism on milk yield [30]. Conversely, results were provided by Tsiaras A.M. *et al.* (2005) which associated the higher milk production to *AB* genotype, also detecting significant differences between the three genotypes [22]. Similar inconsistent results were reported by Aleandri R.L. *et al.* (1990) and Bovenhuis H. *et al.* (1992) for *AA* or Jairam B.T. and Nair P.G. (1983) for *BB* genotyped cows [8, 31, 32]. No significant effects of *LGB* polymorphism were obtained by Nina S. *et al.* (2002) which, in a hierarchy of influential factors on milk yield, provides the *LGB* genotype on the last place while the top places were taken by stage and lactation rank [24]. According to lactation rank, Meyer F. *et al.* (1990) reported that *AA* genotyped primiparous had lower milk yield than *AB* or *BB* genotyped multiparous cows [33]. Also, the joint of *LGB* and *CSN3* genetic polymorphism exerts significant effects on milk yield, chemical composition and coagulation traits of it. In a study conducted on Fleckvieh cows, Matejicek A. *et al.* (2008) recorded higher milk yield for *LGB AB* genotype joined with *CSN3 BB*, compared to others possible haplotypes [34].

The effect of *LGB* locus polymorphism on milk fat content was extensively discussed and results obtained worldwide were conversely. In the current study, the milk fat content showed an increased tendency related to *LGB* genotypes. The lower fat content was associated to *AA* genotyped cows (4.19% ± 0.02) and no significant differences were recorded related to milk fat content of *AB* genotype (4.22% ± 0.03, P > 0.2). The higher fat percentage was found in *BB* genotyped cows (4.26% ± 0.02) significantly increased compared to *AA* ($P \leq 0.019$) genotype. The extremes productive values associated with *LGB* homozygous genotypes highlighted the potential and importance of matching pairs to obtain the subsequent generation. Similar results were obtained by Ahmadi M. *et al.* (2008) in Holstein Friesians [35]. In most of the studies, no significant influence of *LGB* genotypes on milk fat content was recorded. Thus, no effect of *LGB* genotypes was recorded in the studies conducted on different purpose breeds by Strzalkowska N. *et al.* (2002), Kim S. (1994) or Karim K. *et al.* (2009) [24, 4, 36]. Also, on local Romanian Simmental breed, Gradinaru A. *et al.* (2013) found similar fat content for *AA*, *AB* and *BB* genotypes [14]. The positive effect of *AB* genotype was recorded by Sitkowska B. *et al.* (2013) on Holstein Friesian cows [37]. The literature provides surveys which recorded positives associations between *LGB BB* genotype and milk fat content. Thus, in Holstein dairy breed Hill J.P. (1993) found increased fat content for *BB* genotyped cows (+ 11%) compared to the *AA*, similar results reporting Dokso A. *et al.* (2011) also [38, 39]. As in the current study, the findings along Fleckvieh breed reported by Manga I. *et al.* (2008), showed an increased milk fat content in *LGB BB* genotyped cows [40]. A similar survey was conducted on eastern European local breed (Bulgarian Rhodopean) by Hristov P.I. *et al.* (2013) which found that *BB* genotype provided a higher fat percentage compared to others two genotypes [41]. The *LGB BB* genotype provided a higher milk fat content joined with both *CSN3 AA* and *AB* genotypes [34].

Milk protein content was found significantly influenced by *LGB* genotypes. Higher protein percentage was associated with *AB* and *BB* genotyped cows, proved that *B* allele has a favourable effect in respect to this milk production trait. The homozygous *AA* genotype was associated with lowest protein percentage in milk (3.28% ± 0.02). Significant differences were calculated for milk protein content according to *AB* (3.43% ± 0.03, $P \leq 0.027$) and *BB* (3.42% ± 0.01, $P \leq 0.022$) genotypes. No significant differences were recorded between protein percentages related to genotypes containing *B* allele ($P > 0.66$). The favourable effect of *B* allele on milk protein content was reported by Tsiaras A.M. *et al.* (2005) even that calculated differences weren't statistically significant related to *LGB* genotypes [22]. Also, the higher protein content associated with *AB* genotype sustains our own results. Similar results that confirm the productive superiority of heterozygous *AB* genotype were also reported by Karim K. *et al.* (2009) in a study conducted on Iranian Najdi cattle, rearing in different environmental conditions compared to those breeds rearing in Europe [36]. Homozygous *BB* genotype was reported as favourable for milk protein content on dairy breeds as Jersey, Holstein Friesian or Brown along with several studies conducted by McLean D.M. *et al.* (1984) and Lum L.S. *et al.* (1997) [28, 42]. Conversely, results were reported in the literature, highlighted a higher protein percentage in *AA* Holstein Friesian and Jersey genotyped cows [8, 31, 43, 44]. No significant effect was reported between *LGB* genotypes related to milk protein content in Fleckvieh breed [14, 45]. Inconsistent results provided by literature were submitted according to several influential factors. Thus, in respect to findings reported by Sitkowska B. *et al.* (2013), the milk protein content was highly influenced by herd management and cow's *LGB* genotypes [37]. Also, protein percentage was influenced by joined *CSN3* and *LGB* genotypes, amenable to managerial and environmental influential factors [38]. Higher milk protein content was related to haplotypes between *LGB BB* and *CSN3 AB* or *AA* genotypes [34]. Genetic polymorphism of *LGB* locus was investigated in respect with cattle growth rate. Thus, the *AB* and *BB* genotyped cattle has a higher average daily gain, in dual purpose or meat breeds [26].

4. Conclusions

The current study offers preliminary results regarding the influence of *LGB* genotypes on milk production traits in local Romanian Spotted breed. Comparable frequencies were found between *AA* and *AB* genotypes. The homozygous *BB* genotype recorded the lowest frequency in *LGB* locus. Milk composition was influenced by *LGB* genetic variants. Higher milk production was associated with *AB* genotype. Milk fat content recorded higher value in *BB* genotype while higher protein percentage was performed by *AB* genotyped cows.

Numerous studies were conducted in order to establish the influence of *LGB* locus polymorphism on milk production traits. Anyway, is difficult to determine the best genotype for these traits, despite the fact that nowadays there is a lot of knowledge about the influences of different genotypes on productive parameters. In addition to *LGB* genotypes, many external factors can affect milk production and its chemical composition. Here we can include the managerial and environmental factors as well as factors dependent on animals like cow's parity, stage of lactation, welfare condition, udder health and others. Conflicting results can appear due to availability and accuracy of data recorded and analysis. Also, breeds or population size can affect the accuracy of outcomes. More studies are necessary due to contradictory outcomes from literature. Further, the cow's genotypes must be included in improving production scheme due to influences in milk production. In this respect, individual genotypes and influential share of others factors must be known. Importance of the current study emerges from the actual lack of known regarding local breeds genetic structure, particularly milk proteins genetic variants.

Current findings are applicable to a better knowledge of the local breed genetic structure in order to contribute to Romanian Spotted selection.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Agriculture and Rural Development, throughout the projects ADER 5.1.5. and 5.2.4.

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