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Polymorphism of the *Kappa Casein* gene and milk production in cows resulting from crossbreeding with holstein or montbeliarde in the peri-urban area of Bamako

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ABSTRACT

The objective of this contribution is to study the polymorphism of the *kappa casein* gene in crossbred cows in the peri-urban area of Bamako. Fifty (50) cows, including 22 Montbeliarde crosses and 28 Holstein crosses, were subjected to descriptive analysis comparing average milk production and milk fat and protein percentages and genetic analysis of the kappa-casein gene and its interaction with the crossbred types or breed. The results showed 2 types of crosses for each breed, ½ and ¾ exotic blood. The average daily milk production was 8.2±0.46 kg/d for the Holstein crosses and 7.2±0.73 kg/d for the Montbeliarde crosses with a significant difference. No significant differences were observed for the fat and protein content between Holstein crosses (3.56 ± 0.14% and 3.68±0.02%) and Montbeliarde crosses (3.79 ± 0.10% and 3.74±0.02%). Genetic analysis revealed three genotypes of *kappa casein* (AA, AB and BB). The AA genotype (0.5) was the most frequent followed by AB (0.41) and BB (0.09). A close link was observed between milk production and the polymorphisms of the *kappa casein* gene. Individuals with the AB genotype had the highest daily milk production followed by individuals with the AA genotype and those with the BB genotype with respective mean values of 8.6±0.64 kg/d; 7.3±0.56 kg/d and 6.6±1.58 kg/d. Individuals of the BB genotype presented a higher fat content (4.0±0.08%) than that of the other genotypes. The mean milk protein content varied little among the genotypes. These results will contribute to the strengthening of knowledge on the genetic effects of *kappa casein* polymorphisms on milk production performance and milk composition of crossbred cows in Mali.
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Keywords: Cows, dairy performance, milk fat, milk protein, *kappa casein* polymorphisms, Montbeliarde, Holstein, Mali.

INTRODUCTION

Dairy production plays a social and economic role in Mali, but dairy cows' performances in the country are low (Coulibaly et al., 2005). Therefore, Mali has made significant efforts to improve dairy cows' performances, in particular by creating the Station du Sahel at Niono in the region of

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Ségou where open-nucleus selection schemes are conducted to genetically improve the milk production performances and productivity of Maure and Fulani zebu cattle breeds (Tamboura et al., 1982). Since 2015, a program to improve the zootechnical performance of indigenous cattle breeds through artificial insemination, in particular for milk production, has been running (CNIA, 2020). Cows from this program entered production, materialized by a significant increase in the level of milk production (Kassa et al., 2016) in the peri-urban area of the district of Bamako (CNIA, 2020).

Any genetic improvement program requires a good knowledge of genetic resources. Unlike exotic breeds from developed countries, little data exists on the genetic characteristics of indigenous cattle breeds in Mali (Konaté et al., 2018). Several genes are associated with high milk production and quality (Patel et al., 2007; Margawati et al., 2017) of which several genes produce approximately 80% of the total milk protein content; in cattle all of them are located on chromosome 6 (Rijnkels, 2002; Tailford et al., 2003; Sebastiani et al., 2020). Kappa-casein belongs to the casein gene family and represents 8 to 15% of the caseins contained in cattle milk ((Patel, 2015). It occurs in several allelic forms, the most common of which are the A and B alleles (Farrell et al., 2004; Shahlla et al, 2014). The different combinations of these two alleles are associated with both milk quantity and quality (Aleandri et al., 1990; Alipanah et al., 2005; Shahlla et al., 2014; Tahira et al., 2014). The *Kappa casein* gene, like other caseins, can be used in marker-assisted selection schemes (Rijnkels, 2002; Sebastiani et al., 2020).

The present study aims to evaluate the effects of genetic polymorphisms of the kappa-casein gene and their interactions with different genotypes on daily milk yield and composition of cows resulting from artificial insemination of local zebu cattle with Montbeliarde and Holstein semen in the per-urban area of Bamako.

MATERIALS AND METHODS

Sampling site

The study area extends over a radius of 100 km around the city of Bamako, covering the rural communes of Baguinéda, Sanankoroba and Nafadji in the circle of Kati in the Koulikoro region. These rural communes benefit from a rather humid Sudanese tropical climate characterized by a rainy season from June to October and a dry season from November to May with a cold dry season from October to February and a hot dry season from March to May. They are between isohyets 800 and 1100 mm, with an average annual rainfall of 900 mm (Derbal, 1954). Moreover, these areas are practically covered by a wooded savannah thus ensuring the grazing of animals.

The maximum temperatures are recorded in April and May (44°C) and the minimum in December and January (12 to 14°C). The vegetation is of the (wet) wooded savannah type. The herbaceous and ligneous species encountered belong to the Andropogonaceae, Pinaceae, Eragrosteae and Oryzeae (Derbal, 1954).

Herd management

The herds were managed in a semi-intensive way through the exploitation of natural pastures during the twelve months of the year and supplemented by crop residues and concentrate feeds. Sanitary monitoring was carried out by animal health agents as needed in the farms. It consisted of antibiotic therapy operations, treatment against internal and external parasites, blood tests (against trypanosomosis) and vaccinations (against contagious bovine peri pneumonia, anthrax and symptomatic anthrax, bovine pasteurolosis and foot-and-mouth disease). The mode of reproduction of the animals was ensured essentially by artificial insemination. Only Montbeliarde and Holstein crossbred cows were included in the study (Table 1).

Control and collection of milk samples

The study was carried out with 50 crossbred cows in four (04) farms in the peri-urban area of Bamako. The farming system is generally of the semi-intensive type. It is

practiced by livestock owners residing for the most part in Bamako. It is within this breeding system that artificial insemination is often practiced with the semen of European breeds. Farms generally oriented towards dairy production are located in the immediate vicinity of the city of Bamako, in a radius called the central zone (going up to 25 km and more around Bamako). The average size of these farms is generally 1.5 to 10 ha consisting of a barn for housing animals and agricultural space for growing corn and other crops such as fodder crops. The main legal status of farms is the land title. The herds are made up of an average of 30 dairy cows. The genetic structure is characterized by the predominance of improved cows with 80% of the total number (exotic crosses against only 20% of local breeds. The livestock buildings are more or less adapted to the conditions of the area. They are built in the majority of permanent cases. All farms have at least one borehole equipped with a drinking trough Herd management is entrusted to one or more salaried shepherds. Milking is mostly manual, but more and more mechanical milking machines appear in our farms.

Each farm was visited once a month over a period of six months. The daily milk production (kg/d) was assessed, meaning that the production of the evening and that of the following morning was assessed. When collecting milk, the share suckled by the calf was not taken into account. After milking of each cow, the milk was decanted into a bucket and weighed by a scale with a maximum capacity of 25 kg, and a readability of 100g.

A recording sheet was drawn up containing the following information: the ID of the cow, the genotype of the cow, the rank of lactation (1-3 ranks), the stage of lactation (1-6 months) and the milk production of the day (in kg). Over the 6-month study period, 22% of the animals were monitored in 6 months, 14% were monitored in 5 months, 30% were monitored in 4 months and 34% in 3 months.

A 45 ml sample of the milk was collected in a 50 ml conical type, labelled, stored in a cooler under ice and transported to the Animal Nutrition Laboratory of the Regional Center for Agronomic Research of Sotuba for analyzes of physico-chemical parameters (milk fat protein percentage), using the Milko-Tester apparatus (Grappin and Jeunet, 1979). Table 1 gives an overview of the composition of the herds visited.

Collection of blood samples

Four (4) ml of blood were taken from each cow having undergone milk recording, at the level of the jugular vein in EDTA tubes. Blood samples were labeled according to the ID of the cow, kept under ice in a cooler and transported for molecular analysis to the Research Laboratory in Microbiology and Microbial Biotechnology (LaboREM-Biotech) of the Faculty of Sciences and Techniques of the University of Sciences, Techniques and Technologies of Bamako.

- ***Extraction of genomic DNA***

Genomic DNA was extracted from whole blood samples using the Promega ReliaPrepTM Blood gDNA Miniprep System Extraction Kit (add company name). The concentration of the extracted DNA was determined using the EPPENDORF BioPhotometer®6131 spectrophotometer (Konaté D, Traoré D, Dao S, Fané R, Ouattara O, Diop R Babana A H, 2018). Then, the DNA was diluted in the nuclease free water solution at 20 ng/µl and stored at 4°C for the molecular analysis.

- ***DNA amplification with Kappa-casein gene***

DNA extracts were amplified with a *kappa casein* gene primer pair (Forward: 5'ATC ATT TAT GGC CAT TCC ACC AAA G 3'; and Reverse: 5'GGC CAT TTC GCC TTC TCT GTA ACA GA 3') of a 350 bp fragment according to (Patel et al., 2007). A 25 µl reaction mix was formed with 8.5 µl of pure water, 12.5 µl of Go Taq Green Master Mix (2X), 1 µl of forward primer (100 pmol), 1 µl of reverse primer (100 pmol) and 2 µl of genomic DNA. The mixture was distributed between the 8-Strip PCR tubes forming a negative control (without DNA). The tubes were introduced into the TECHNE-PRIME thermal cycler (company name) according to the following program: initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, hybridization at a temperature of

58°C for 1 minute, elongation at 72°C for 2 minutes, final elongation at 72°C for 10 minutes and final storage at 4°C. Denaturation, hybridization, and elongation constituted one cycle and were repeated 35 times.

- ***Electrophoresis of amplified products***

Ten (10) µl of each PCR product were loaded onto an ordinary 2% (g/ml) agarose gel (Konaté et al., 2021) and migrated for 1 hour 30 minutes at 80V. The gel was then photographed with the Gel Documentation System E-BOX VX2 device version 15.06 (Vilber Smart Imaging).

- ***Enzymatic digestion of PCR products***

All the samples showing the size band corresponding to 350 bp after visualization on an ordinary gel were digested with the Hinf I restriction endonuclease (Promega corporation R6201 kit). A reaction mixture of 10 µl was formed with 4.3 µl of pure water, 2 µl of buffer (10X), 05 µl of HinfI (10U/µl), 0.2 µl of BSA (150mg/ml) and 3 µl of amplification products. The mixture was distributed between PCR 8-Strip tubes and placed in the water bath for 2 hours at 37°C.

Data analysis

- ***Molecular analyses***

Allele and genotype frequencies of the *kappa casein* gene were calculated using the formulas below and expressed as percentage.

Frequency of AA genotype

f(AA)=NAA/N where NAA was the number of samples exhibiting the AA genotype and N the total number of samples amplified;

Frequency of AB genotype

f(AB)=NAB/N where NAB was the number of samples exhibiting the AB genotype and N the total number of samples amplified.

Frequency of BB genotype

f(BB)=NBB/N where NBB was the number of samples exhibiting the BB genotype and N the total number of samples amplified.

Frequency of the pA allele:

pA=[2(AA)+(AB)]/2N where AA was the number of samples exhibiting the AA genotype, AB the number of samples exhibiting the AB genotype and N the total number of samples amplified;

qB allele frequency

qB=[2(BB)+(AB)]/2N where BB was the number of samples presenting the BB genotype AB the number of samples presenting the AB genotype and N the total number of samples amplified.

- ***Statistical analyses of milk records***

Data were analyzed with a fixed linear model considering the classification factors of herd, and the three protein variant genotypes. The dependent variables were milk production, fat yield, protein yield, fat percentage, protein percentage.

The data collected were subjected to descriptive analysis and a comparison of means (ANOVA) using Prism 5 software and SPSS (IBM SPSS Statistic 23). Highlighting the links between the variation factors and milk production using ANOVA (for qualitative factors) and correlation (for quantitative factors). We did not perform correction tests on the data because they are homogeneous.

Table 1: Composition of the herd in the farms visited (number of cows).

Genetic type	Exotic blood level	
	½ blood	¾ blood
Holstein crosses	20	8
Montbeliarde crosses	14	8
Total cows	34	16

RESULTS

Daily milk production

The Holstein crosses had an average milk production higher than that of the Montbéliarde crosses, ie 8.2 ± 2.4 kg/d against 7.2 ± 3.4 kg/d. The difference is not statistically significant at the threshold of (P<0.05). Then the average milk production of ½ Holsteins is 8.0±2.1 kg/d lower than that of ¾ Holstein blood 8.3±2.6 kg/d. Here, the difference is not significant because we have the significance (0.67>0.05). Finally, the average values of ½ and ¾ Montbéliarde are respectively 7.12 and 7.17 kg/d. Again, the difference between the parameters is not significant at the threshold of (P<0.05).

The average values of the fat and protein content of the Montbéliard crosses were higher than those of the Holstein crosses (Table 2) i.e. 3.7±0.50% against 3.5±0.78% and 3.7±0.11% against 3.6±0.14%. The statistical difference between these values is not significant at the threshold of (P<0.05) (Table 2).

Kappa casein gene polymorphism

The genetic analysis of polymorphism of the *kappa casein* gene made it possible to identify alleles A (36 individuals) and B (14 individuals) with the genotypes AA (26 individuals), AB (20 individuals) and BB (4 individual) (Table 3). The A allele was observed in 72% of the 50 blood samples against 28% of the B allele. The AA genotype was most frequently observed, followed by AB and BB with respective genotypic frequencies of 52%, 40% and 8%. The same observations were made for Holstein and Montbeliarde crossbred cows (Table 3).

Daily milk production and milk composition according to kappa casein genotypes

The daily milk production according to *kappa casein* genotypes averaged 7.3±0.56 kg/d for the AA genotype, 6.6±1.60 kg/d for the BB genotype and 8.6±0.64 kg/d for the AB genotype (Table 4). The average fat content by genotype was calculated at 3.5±0.15% for individuals with genotype AA, 3.8±0.14% for individuals with AB genotype and 4.0±0.08% for those with BB genotype (Table 4). The average milk protein content was similar for individuals with genotype AA, genotype AB and genotype BB (Table 4). Holstein crossbred individuals with the BB genotype had a daily milk production of 8.7±2.9 kg/d, followed by the AB genotype individuals with 8.6±2.3 kg/d and the AA genotype with 7.9±2.6 kg/d. The difference is not statistically significant at the threshold of (P<0.05) between the three kappa casein genotypes.

On the other hand, for Montbeliarde crosses, individuals with the AB genotype presented the highest daily milk production that is 8.5±3.6 kg/d, followed by individuals with the AA genotype with 6.5±3.2 kg/d and BB genotype individuals with 4.5±2.1 kg/d.

Table 2: Mean values of milk production and milk fat and protein levels according to genotypes.

Genetic types	Daily milk production (kg/d)	T-Test	ddl	Sig.	Physico-chemical composition of milk							
					Fat content (%)	T-Test	ddl	Sig.	Protein content (%)	T-Test	ddl	Sig.
Holstein crosses	8.2± 2.4	-1.22	36.54	0.229	3.5± 0.78	1.259	46.40	0.214	3.6±0.14	1.52	47.81	0.13
Montbeliarde crosses	7.2±3.4				3.7±0.50				3.7±0.11			
1/2 Holstein	8.0±2.1	-0.42	25.92	0.67	3.6±1.00	0.417	18.18	0.681	3.6±1.14	-0.15	25.29	0.87

3/4 Holstein	8.3±2.6				3.5±0.55				3.6± 1.14			
1/2 Montbeliarde	7.12±3.08	-0.03	17.12	0.97	3.68±0.58	-0.75	12.20	0.46	3.79±0.12	1.24	13.43	0.23
3/4 Montbeliarde	7.17±3.72				3.68±0.46				3.72±0.11			

ddl: degree of freedom, F : test value, Sig. : significant

Table 3: Genotype and allelic frequencies of *kappa casein* polymorphisms in crossbred cows (n = 50).

Genetic type	n	Allele Frequency (%)		Frequency of genotypes (%)		
		A	B	AA	AB	BB
Montbeliarde crosses	22	70	30	50	41	9
Holstein crosses	28	73	27	54	39	7
Total	50	72	28	52	40	8

n: number of animals

Table 4: Daily milk production and physico-chemical milk composition according to *kappa casein* genotypes.

Production parameters	*Kappa casein* genotypes					
	AA (n=26)	AB (n=20)	BB (n=4)	ddl	F	Sig.
Daily milk production (kg/d)	7.2±2.8	8.5±2.8	6.5±3.1	49	1.44	0.245
Milk fat (%)	3.5±0.7	3.8±0.6	4.0±0.17	49	0.973	0.386
Milk protein (%)	3.7±0.1	3.7±0.1	3.7±0.09	49	0.782	0.463

n: number of animals, ddl: degree of freedom, F: test value, Sig.: significant

DISCUSSION

The study showed that Holstein crosses had a higher average daily milk production than Montbeliarde crosses. These results are consistent with those of other authors (Gbodjo et al., 2013; Sokouri et al., 2014; Toure et al., 2019) who also found that Holstein crosses were more productive than Montbeliarde crosses. But the average daily milk production of our study is higher than theirs. The differences observed can be explained by the difference in local cattle breeds, production conditions and especially the lactation number and stage of the cows. However, in the present study, the evaluation did not take these parameters into account.

The ½ blood and ¾ blood crosses from Holstein respectively showed an average daily value higher than those obtained from the ½ blood and ¾ blood from Montbeliarde. However, these observed differences were not significant between the degrees of the improver breeds. The difference in production observed between Holstein crosses and Montbeliarde crosses could be explained by the difference in the improver breed. This also confirms the

results of (Sokouri et al., 2014). Indeed, crossbreeding between exotic and local dairy breeds gives progeny with average milk production higher than that of local breeds (Kassa et al., 2016).

No significant difference was observed between the mean fat content of Holstein and Montbeliarde crosses. Similarly, the ½ blood and ¾ blood Holstein showed similar levels to those of ½ and ¾ blood Montbeliarde. These values obtained are within the range of the fat content standard (33 to 47 g/l) in cows previously reported by Cayot (1998) and Pougheon (2001). The mean milk protein content did not show any significant difference between the Holstein and Montbeliarde crosses. These results are similar to those reported by other authors (Lindmark-Månsson et al., 2003) on milk samples taken from dairies in Sweden, i.e. 3.37% for protein content and 4.34% for fat content. Three genotypes (AA, BB and AB) of the *kappa casein* gene (*CSN3*) have been observed in the present study, with two alleles (A and B). The AA genotype was the most frequent. These results are similar to those found by other authors in local cattle breeds in Nigeria (Olanrewaju et al., 2020) and buffaloes in India (Patel et al., 2007) . In the present study, the frequency of the A allele was higher than that of the B allele. Similar results were reported for *Bos indicus* and *Bos taurus* breeds (Alipanah et al., 2008; Çardak, 2005; Olanrewaju et al., 2020). These animals show a predominance of the A allele of the *kappa casein* gene in the herds. On the contrary, a higher frequency of the B allele was reported by other authors (Zaglool et al., 2016), namely 64%.

The present study revealed an effect of the *kappa casein* polymorphisms on the average milk production, showing a genetic superiority of AB genotype, followed by the AA and BB genotypes.

On the contrary, mean fat content did not show any significant difference between the different *kappa casein* genotypes, although the average fat content of the BB genotype tended to be higher than that of the AB and AA genotypes. This result is similar to that reported on the average milk fat content of BB homozygotes (Doosti et al., 2017). Similarly, a study by (Patel et al., 2007) has shown that homozygote BB of bovine *kappa casein* is favorable to milk quality and milk-derived cheese quantity. On the contrary, the AB genotype was associated with a high total milk fat content (6. 1%) in buffalo milk (Tahira et al., 2014).

The milk protein content in the present study was constant across all *kappa casein* genotypes. This contradicts results obtained in a study of buffalo milk that showed that the AA and AB genotypes were associated with a high total milk protein content (Tahira et al., 2014), while other authors (Walawski et al., 1994) found a higher protein content in the milk of cows with AB and BB genotypes.

These results suggest that the selection for the BB genotype could increase milk fat in crossbred cows in Mali, as previously suggested by Doosti et al. (2011).

Conclusion

The evaluation of the daily milk production and milk composition of cows resulting from crossbreeding with Holstein and Montbeliarde cattle made it possible to highlight the level of milk production of crossbred cows in the peri-urban area of Bamako. Three genotypes (AA, AB and BB) of the *kappa casein* gene have been observed. Individuals with AB and AA genotypes showed higher daily milk production than

individuals with BB genotype. As for of the milk fat content, the BB genotype seemed to be more interesting compared to the other genotypes. The protein level remained constant across all genotypes. The results will contribute to the strengthening of knowledge on animal genetic resources in Mali and to the development of a strategy for improving milk production at the national level.

COMPETING INTERESTS

There is no conflict of interest between the authors.

AUTHORS' CONTRIBUTIONS

All the authors contributed to the realization of this work and to the preparation of the manuscript.

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