

Impact of Bacterial and Somatic Cells Content on Quality Fresh Milk in Small-Scale Dairy Farms in Kosovo

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Received August 1st, 2013; revised September 1st, 2013; accepted September 8th, 2013

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ABSTRACT

The basic goal of this research was to determine the impact of the presence of bacterial (CFU) and somatic cells count content (SCC) in quality of fresh milk in some small cattle farms in Kosovo. The survey was based on existing standards for milk quality in Kosovo placed under administrative guidance MA-no. 20/2006. The study was based on fresh milk analysis of 150 farms performed during the period September-December 2012, which was obtained in 9 different localities (collection points) of the Kosovo. Our study reveals that CFU and SCC in fresh milk were significantly affected by a number of factors, as: sampling period (repetition), locality, breed, and time of sampling (evening or/and morning). According to the results for CFU and SCC, there were big differences between the farms (milk collection points) included in the study ($P < 0.0403$) and ($P < 0.0293$). The results show that small size breed like Busha and its crosses tend to be less exposed to SCC/mL in milk (72.840) and (293.592), compared to Black Holstein (613.462), Simmental (521.519) and Brown Swiss (418.44). Milk produced in evening tended to be of better quality (259.854 CFU/mL) compared to the one from morning milking (576.689 CFU/mL). Fresh milk quality analyzed in the third repetition was better for about 33.3% compared with the repetition first. For CFU and SCC, the analyses show that about 74.7% and 64.7% of milk produced belongs to extra quality, while lower quality of milk of category three is 12.0% and 23.3%, respectively. Considering that about 85% of milk produced in Kosovo comes from small-scale dairy farms, the current study sets out that small-scale milk production system cannot be neglected by interest parties in dairy sector and needs permanent following up and improvement.

Keywords: Fresh Milk; Bacteria Content; Somatic Cell; Milk Quality Standards

1. Introduction

It is considered that about 90% of dairy farms in Kosovo produce milk in small-scale farms which produce milk to cover their own needs and rarely trade [1]. Milk produced on a small scale dairy farms can easily get contaminated by bacteria due to poor hygienic conditions maintained at “on farm” levels or due to inadequate handling, storage and transport conditions. Even there have been some noticeable improvement in dairy sector in Kosovo, still about 85% of milk produced is consumed directly by the rural households (self subsistence farming) or sold in the so called “Green market”, where there is no official control of raw milk quality at all [2]. This paper gives a general introduction regarding fresh milk quality in Kosovo in small scale dairy system, an actual over-

view of literature, describes the material and methods, presents the results and the discussion and draws first conclusions. Milk is a complex biological fluid that has an important place in human nutrition no matter if produced on commercial or small-scale farms. Milk is often described as a complete food because it contains adequate amounts of protein, fats, sugars, vitamins and minerals [3]. However, its nutritional composition at the same time provides a very suitable environment for the growth and development of many microorganisms, especially *Lactobacillus*, *Streptococcus*, *Staphylococcus* and *Micrococcus* sp. In this way it is difficult to avoid its contamination by microorganisms. Therefore, the content of bacteria in milk is a key feature that determines its quality [4,5]. Contamination of milk with bacteria can come from different sources such as air, equipment used for milking and storage of milk, food, soil, feces and

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animal health [5-7]. The quality of fresh milk in Kosovo [2] still remains quite low in comparison with existing standards and needs an immediate improvement by applying best practices advanced and appropriate intervention in infrastructure (e.g., milking equipment and adequate storage of milk, general hygiene on the farm, storage temperature, etc.). The bacteria content in milk is a reliable indicator of milk hygiene, showing a sick udder, non-hygienic manipulation (*i.e.*, unfavorable temperature during storage, storage device, etc.). In this regard, [8] in their work to carefully describe the steps necessary in order to minimize contamination of fresh milk (*i.e.*, cleaning hands, teats, nipples before milking and immediately after use, the milk containers for storage and transport of milk [9]). Depending on these factors, the total number of microorganisms in fresh milk can be from a few thousand to over 10 million [10]. Fresh milk obtained in a hygienic way usually contains less than 5000 organisms per mL of milk. On the other hand, we must be careful when interpreting the results with a low presence of bacteria in milk, because the milk may be present and other pathogens as causes of Brucellosis and Tuberculosis [11]. High somatic cell counts (SCC) present in milk are the main indicators of mammary gland infection caused by microorganisms. Normally, in milk from a healthy mammary gland, the SCC is lower than 100,000 cells/mL, and this includes epithelial cells and leukocytes (*Polymorphonuclear neutrophils*, *lymphocytes* and other *macrophages* cells) [12]. While bacterial infection can cause it to increase to above 1,000,000 SCC/mL [13]. High presence of SCC in milk has a negative influence on the fresh milk quality, like shorter shelf time, less sensory content, undesirable organoleptic characteristics, negative effect on milk product fermentation and chemical and physical properties [14,15]. The negative effect of SCC presence is also related to subclinical mastitis which is always related to low milk production, changes to milk consistency (density), reduced possibility of adequate milk processing, low protein and high risk for milk hygiene which may even contain pathogenic organisms [7].

The main objective of the research was twofold: firstly, to study the impact of bacterial load (CFU/mL) and somatic cell content (SCC/mL) in the quality of fresh milk in small cattle farms based on existing standards for fresh quality milk; secondly, to draw valuable recommendations for the overall dairy industry and food safety standards in Kosovo.

2. Material and Methods

2.1. Data Registration, Handling and Sample Analysis

In this study we included the 150 small-scale dairy farms,

in the three repetitions (one per month) all around Kosovo. The analyzes of CFU and SCC derived from raw milk samples using the standard method-A4 according to the International Committee for Animal Registration (ICAR), covering the period from September-December 2012. The daily test milk sample (evening and morning) amounted to 40 - 50 mL of milk which was mixed and pooled together in a sterile bottle using a calibrated syringe when cows were milked by hand. Azidol chemical was used for sample preservation. For the two specimens (evening and morning) and for transport to the laboratory, milk samples were placed in a portable cooler at 4°C. In general, registration cards were used for cow data recording with: identification number, breed, sample number, lactation period, farm name, birth date, calving date, amount of milk produced, and some other information relevant to this study. The incomplete data were omitted from the dataset. CFU and SCC content on fresh milk were analyzed at the Agency of Food and Veterinary laboratory using "Bactoscan" and "FossomaticMinor" equipment, respectively. The results gained were compared according to the [16] used to define milk quality standards and raw milk category (**Table 1**).

2.2. Characteristics of Small-Scale Dairy Farms in Kosovo

According to Ministry of Agriculture Forestry and Rural Development (MAFRD) data it is estimated that in Kosovo there are over 83,000 livestock farms, most of which are small size (about 2.2 milking cows and 1.5 Ha of arable land), mainly subsistent and semi subsistent farms. Typical farm feature is a high level of land fragmentation. Dairy products constitute a main source of food and a high share of production still serves subsistence purposes. The small-scale dairy farms have poor hygienic and zoo-management conditions. Commercial farms and some of the semi commercial farms have more advanced managerial condition. The largest part of the dairy sector is cow production, followed by small ruminant (sheep and goats). Barn facilities are mostly old, without any proper management plan that in most cases does not meet hygienic parameters, due to lack of ventilation and cleanliness. Generally, cows are milked manually and there is an evidence of lack of proper milk storage facilities, which makes difficult to keep the milk uncontaminated by bacteria.

Table 1. Milk quality standard in Kosovo (CFU/mL and SCC/mL in milk).

Standard for fresh milk	Extra Class	Class I	Class II	Class III
CFU/mL	<80,000	<100,000	<200,000	<500,000
SCC/mL	<300,000	<400,000	<500,000	<600,000

2.3. Statistical Analyses

The data were analyzed using JMP-starter packet a business unit of SAS program, [17] by PROC-GLM procedure (General Linear Model). The Duncan test was used [18] to see the effect of different variables in CFU and SCC.

3. Results

The number of milk samples per farm, the least square means (LSM), standard error (SE), and the standard deviations (SD) for the presence of bacterial load and somatic cell count in fresh milk are shown in **Table 2**. According to the results for CFU and SCC there were large differences between the farms (milk collection points) included in the study ($P < 0.0403$) and ($P < 0.0293$), respectively. In general, based on the existing standards from 9 collection points included in this study, only 2 of

them for CFU produce extra quality milk, about 80.000 CFU/milk (CPIII and CPIV), followed by CPVII and CPVIII ranging from 105.701 - 106.752. The lowest quality fresh milk was produced by CPV (1080.130), CPI (639.784), CPVI (486.561), CPII (441.622) and CPIX (379.854), respectively. For SCC, there were CPVIII and CPI that produced extra milk quality ranging from 152.587 - 280.899 SCC/mL. In other tree collection points CPIII, CPVI, CPIX, CPIV and CPV produced ranged from 315.225 - 481.733 SCC/mL, covering class I and II of current standards. CPII and CPVIII, produced lowest quality of milk with about 929.438 and 1022.904 SCC/mL, respectively.

The square means, standard error and the analysis of the effect of the breed, sampling time, and sampling repetition variables on CFU and SCC are shown in **Tables 3-5**. For breed variable, the observed differences were clearly significant for SCC ($P < 0.0112$), while CFU did not show

Table 2. Effect of collection place (CP) on bacterial and somatic cell contamination in fresh milk (CFU/mL and SCC/mL).

CP	N	CFU/mL		SCC/mL	
		X \pm SE	SD	X \pm SE	SD
CP I	12	639.784 \pm 34.31 ^{ab}	103.93	280.899 \pm 16.79 ^{ab}	50.36
CP II	21	441.622 \pm 18.31 ^{ab}	84.03	929.438 \pm 26.60 ^a	114.20
CP III	9	80.000 \pm 41.89 ^{ab}	80.00	315.225 \pm 12.09 ^{ab}	48.27
CP IV	14	80.000 \pm 33.26 ^b	80.00	443.431 \pm 21.78 ^{ab}	75.99
CP V	15	1080.130 \pm 32.92 ^a	250.49	481.733 \pm 29.67 ^{ab}	96.96
CP VI	9	486.561 \pm 31269 ^{ab}	93.08	341.113 \pm 16.67 ^{ab}	32.02
CP VII	10	105.701 \pm 14.32 ^{ab}	45.28	1022.904 \pm 40.99 ^a	134.54
CP VIII	12	106.752 \pm 14.10 ^b	48.85	152.587 \pm 58.89 ^b	20.00
CP IX	48	379.854 \pm 212.07 ^{ab}	146.23	422.851 \pm 19.61 ^b	75.39
Variance Analyses	Df = 8	Pr > F < 0.0403		Pr > F < 0.0293	

Number of samples (N); Least square means (LSM); Standard error (SE); Standard deviation (SD); Analyses of variance (Pr > F), Degree of freedom (Df), Levels not connected by same letter are significantly different.

Table 3. Effect of breed on bacterial and somatic cell contamination in fresh milk (CFU/mL and SCC/mL).

Breed	N	CFU/mL		SCC/mL	
		X \pm SE	SD	X \pm SE	SD
Brown Swiss	9	84.111 \pm 4.11 ^a	12.33	418.444 \pm 27.16 ^{ab}	68.47
Busha	25	248.400 \pm 86.17 ^a	40.86	72.840 \pm 73.69 ^b	68.47
Holstein	13	85.846 \pm 5.44 ^a	19.63	613.462 \pm 28.28 ^a	103.37
Busha Crosses	76	496.539 \pm 17.50 ^a	157.41	293.592 \pm 64.75 ^b	54.52
Simmental	27	462.407 \pm 28.88 ^a	123.20	521.519 \pm 14.90 ^{ab}	78.13
Variance Analyses	Df = 4	Pr > F < 0.6880		Pr > F < 0.0112	

Number of samples (N); Least square means (LSM); Standard error (SE); Standard deviation (SD); Analyses of variance (Pr > F), Degree of freedom (Df), Levels not connected by same letter are significantly different.

Table 4. Effect of sampling time on bacterial and somatic cell contamination in fresh milk (CFU/mL and SCC/mL).

Time of Sampling	N	CFU/mL		SCC/mL	
		X ± SE	SD	X ± SE	SD
Evening	89	259.854 ± 10.53 ^b	97.82	562.501 ± 135.54 ^a	108.59
Morning	61	576.689 ± 20.21 ^a	153.72	444.304 ± 72.46 ^a	63.60
Variance Analyses	Df = 1	Pr > F < 0.0012		Pr > F < 0.4073	

Number of samples (N); Least square means (LSM); Standard error (SE); Standard deviation (SD); Analyses of variance (Pr > F), Degree of freedom (Df), Levels not connected by same letter are significantly different.

Table 5. Effect of sampling repetition on bacterial and somatic cell contamination in fresh milk (CFU/mL and SCC/mL).

Sampling Repetition	N	CFU/mL		SCC/mL	
		X ± SE	StdDEV	X ± SE	StdDEV
Sampling I	50	971.480 ± 8.17 ^a	24.75	652.100 ± 18.46 ^a	97.061
Sampling II	50	111.260 ± 7.04 ^b	20.50	418.580 ± 14.41 ^a	80.031
Sampling III	50	83.360 ± 2.61 ^b	11.35	406.440 ± 10.73 ^a	75.674
Variance Analyses	Df = 2	Pr > F < 0.0002		Pr > F < 0.2709	

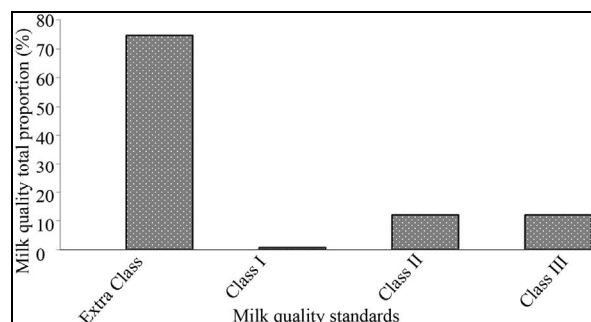
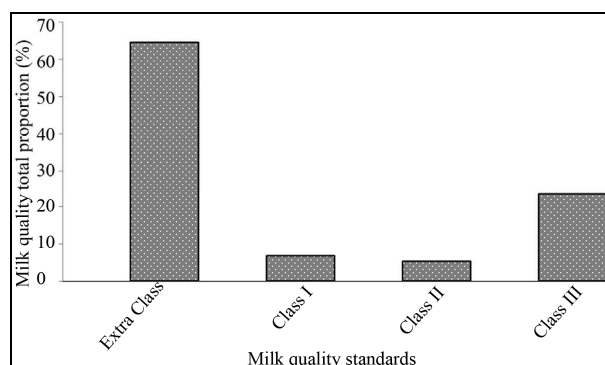
Number of samples (N); Least square means (LSM); Standard error (SE); Standard deviation (SD); Analyses of variance (Pr > F), Degree of freedom (Df), Levels not connected by same letter are significantly different.

any differences no matter which breed was managed ($P < 0.6880$). From analyzes made in **Table 3** the results show that high producing cattle breeds such as Black Holstein (613.462), Simmental (521.519) and Brown Swiss (418.44), tend to be more exposed to the higher presence of SCC/mL in milk. Small size breed Busha and its crosses tend to have lower SCC (72.840) and (293.592), which according to milk quality produced is in Class Extra.

The effect of sampling time (evening and morning) on CFU in fresh milk shows a higher presence of CFU in milk produced in the morning (576.689) compared to evening milk (259.854). The differences between two milking times were significant ($P < 0.0012$). For SCC, differences were not significant, ($P < 0.4073$). In both milking times, SCC presence ranged from 444.304 - 562.501 SCC/mL.

The effect of sampling repetition (once per month) on CFU in fresh milk shows a higher presence of CFU in milk sampled for the first time (971.480), compared to second and third time, (111.260) and (83.360), respectively. The differences between three repetitions were significant ($P < 0.0002$), favoring third one. For SCC, differences were not significant, ($P < 0.2709$). In three sampling repetitions, SCC presence ranged from 406.440 - 652.100 SCC/mL.

The results of analyses for the total proportion of fresh milk quality according to milk standard for CFU and SCC, according to raw milk quality standards in Kosovo are shown in the **Figures 1 and 2**.

**Figure 1. Total proportion of fresh milk quality according to milk standard for “bacterial content (CFU/mL)”.****Figure 2. Total proportion of fresh milk quality according to milk standard for “somatic cells count (SCC/mL)”.**

For CFU, the analyses show that about 74.7% of milk produced belongs to extra quality, followed by class II and III quality standard represented with about 12.0% for

both. Milk quality standard of class I was almost non significant, presented with about 0.7%.

For SCC, the analyses show that about 64.7% of milk produced belongs to extra quality, followed by class III quality standard represented with about 23.3%, and class I with about 6.7% and class II with about 5.3%, respectively.

The results of analyses for the proportion of fresh milk quality according to milk standard for CFU and SCC, for three repetitions, according to raw milk quality standards in Kosovo are shown in the **Figures 3 and 4**. For CFU, the analyses show that from only about 50.7% of milk of extra quality produced in the first repetition, this quality was improved to about 90.0% in third one, respectively. While the third class standard dropped from 32.6% to almost non existing milk quality under this standard.

For SCC, the analyses show that from about 60.2% of milk of extra quality produced in the first repetition, this was improved to about 70.4% in third one, respectively. While the third class standard for SCC was dropped from 32.1% to 18.2% of milk quality produced under this standard. For both, SCC and CFU, quality standard of class I and II, was not much presented, ranging from 0.2% - 8.3%.

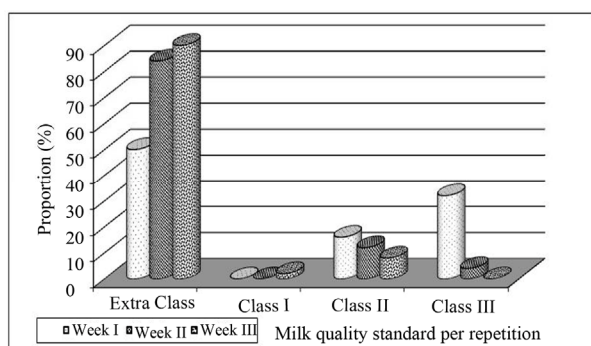


Figure 3. Proportion of fresh milk quality per repetition according to milk standard for “bacterial content (CFU/mL)”.

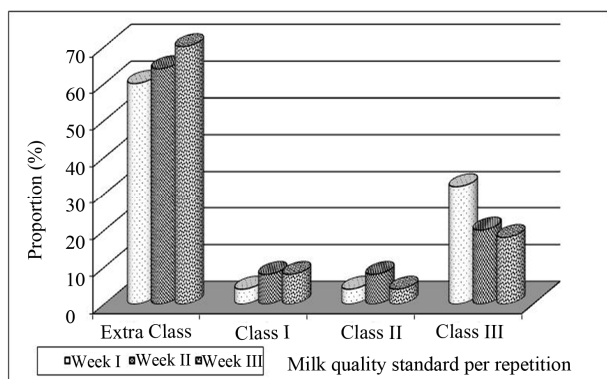


Figure 4. Proportion of fresh milk quality per repetition according to milk standard for “somatic cells count (SCC/mL)”.

The results of analyses for the proportion of fresh milk quality according to milk standard for CFU and SCC, for all milk collection points, according to raw milk quality standards in Kosovo are shown in the **Figures 5 and 6**. For CFU, the analyses show large variations between them ranging from only about 60.2% (CP II) to about 91.0% (CP VII) of milk of extra quality produced. Class I and class II, ranged from 0.1% - 10.5% and 0.2% - 29.2%, respectively. While milk produced under the milk quality standard of class III, ranged from 1.8% - 32.2%.

For SCC, the analyses show large variations between them ranging from only about 38.5% (CP II) to about 90.6% (CP VII) of milk of extra quality produced. Class I and class II, ranged from 0.0% - 22.2% and 0.1% - 11.8%, respectively. While milk produced under the milk quality standard of class III, ranged from 8.5% - 48.4%.

4. Discussion

It is estimated that some 40 - 50 percent of the Kosovar population, living on rural areas, mostly small-scale holders keep livestock for the purpose of producing milk for self consumption or sale (occasionally). The current study sets out that small-scale milk production system cannot be neglected by interest parties in dairy sector (government authorities, milk processors, consumers,

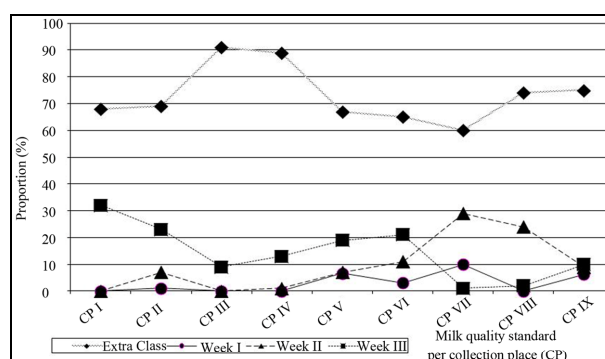


Figure 5. Proportion of fresh milk quality according to milk standard for “bacterial content (CFU/mL)”.

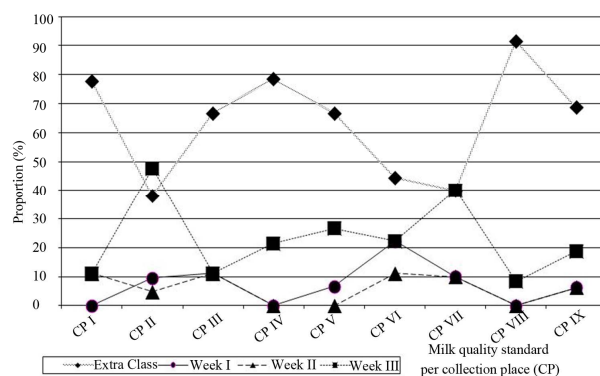


Figure 6. Proportion of fresh milk quality according to milk standard for somatic cells count (SCC/mL).

public health authorities, etc.). It can contribute significantly to the overall rural development and improve nutrition and food security, provide employment opportunities throughout the entire dairy chain, and create wealth in rural areas where the people live and maintain their livelihood. For CFU and SCC, based on the results of this study and others similar conducted in Kosovo [2], [13] showed that some dairy farms produce milk with lower quality compared to quality standards. Thus, there should be a continuous efforts made by all stake holders involved in a dairy sector to improve it. By improving milk quality, animal health, food safety, environmental conditions and considering their low production costs (low investments in “high tech facilities”, small-scale farms should be able to compete with large-scale, especially in the countries where intensive farming (large dairy farms) possibilities are limited. Considering that milk in the udder of healthy animals has very little bacteria, the results from our study show that milk sampled in a third repetition contained about 33.3% less bacteria compared with first sampling. It implies the need of implementation of proper farm hygienic practices to possibly stop sources of bacterial contamination (*i.e.*, milking premises, milker, air, skin of the animal and inflamed udder). Milk testing for a quality standards (CFU and SCC), should be a regular process in order to detect milk contamination, improve quality and profitability (better milk product processed, consumer trust, animal health, etc.) and avoid any pathogenic risk which nowadays is present still (brucellosis, tuberculoses, etc.). Currently, regarding bacterial contamination (CFU), the practices and conditions under which small-scale farms and their dairy chains operate make it difficult to produce high quality milk. For SCC, as in the [19], the results obtained that the amount of somatic cells (SCC) in fresh milk from the small-scale dairy farms is relatively acceptable according to the standard in the country. However, there are a lot of variation deriving from different farms (CP) indicating the large managerial divergence, breeds impact, farm infrastructure, and the present structure of animal health control in the country [1]. Increased SCC in the milk should not be underestimated as they are followed by the multi- dimensional negative effect related to animal health and farmer profitability. Previous study has shown that season trends influence the somatic cells count in USA and Canada [20,21]. While in our work we haven't seen any difference between months. This is probably because the sample analyses were during the autumn and temperature were almost the same in this time. In relation sheep with sheep in previous paper published by our group, we have seen that month influences the bacterial count [22]. Actually, current milk quality standards applied in Kosovo are lower compared to those in EU, therefore, Kosovo farmers should be aware that in

very near future they have to meet the EU milk quality standards in order to survive and compete.

Given the fact that small-scale dairy system is predominant in Kosovo, though with decreasing tendencies, still by improving milk quality set by national standards which will enable small-scale dairy producers to participate in the dairy market in a profitable manner depends not only on their own consumption needs, mainly determined by their production, quality, costs and safety dairy chains to which they belong. Therefore, recommendations for small-scale dairy development in Kosovo must include strategies to develop and increase fresh milk quality in order to reach quality standards in all segments of the dairy chain, increase milk production, improve milk processing, and consumption.

5. Acknowledgements

The authors acknowledge the support for the Ministry of Education Science and Technology of Republic of Kosovo for granting the study through the small grant project “Study of Autochthon Busha Cattle in Kosovo”. Special thanks to students, farmers and Kosovo Veterinary and Food Agency laboratory staff and others who contributed to this study.

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