Original Paper

Silages from legume-cereal mixtures as a factor of dairy cow milk quality

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Currently, the prices of dairy cows feed with protein concentrates are rising and the climate change is also manifesting itself with a growing drought in animal production. These are the reasons why there is an increasing interest in growing silage from legume-cereal mixtures (L-C-M) and in increasing their share in feed rations for dairy cows. Furthermore, the L-C-Ms improve the soil fertility during the crop rotation. The effect of these feeds on milk quality was evaluated by adding L-C-M silages into feeding rations. Czech Fleckvieh and Holstein breed dairy cows (8 herds; feeding periods without and with L-C-M silages) were included in the evaluation for 3 years. There were used 126 L-C-M (experimental) and 463 control (without L-C-M) bulk tank milk samples in this dairy analysis. Lactose content, solids non fat content and total count of mesophilic bacteria in milk of L-C-M group was increased as compared to control (zero hypothesis probability varied from $P \leq 0.05$ to $P \leq 0.001$). On the contrary, as new knowledge, milk freezing point depression and somatic cell counts were decreased in L-C-M group as compared to control ($P \leq 0.001$ and $P \leq 0.05$). Other milk indicators such as contents of fat, crude protein, milk urea and thermostability ($P > 0.05$) were not affected by evaluated factor. The residues of inhibitory substances in bulk tank milk in the L-C-M group were not indicated, but one case was in the control group. Feeding of dairy cows with an increased proportion of L-C-M silage in the roughage component a part of the feeding ration is a safe way to replace part of the protein concentrates in animal feeding, regarding milk quality.

Keywords: breed, protein, milk freezing point depression, milk thermostability, hygienic milk indicators

1 Introduction

Feeding of silages from legume-cereal mixtures (L-C-M) expands considerably in the diet of cattle and especially dairy cows in the Czech Republic (CR). Currently, the prices of dairy cows feed with protein concentrates are rising and the climate change is also manifesting itself with a growing drought in animal production. These are the reasons why there is an increasing interest in growing silage from legume-cereal mixtures (L-C-M) and in increasing their share in feed rations for dairy cows. Furthermore, the L-C-Ms improve soil fertility during the crop rotation.

The world's population should behave ecologically and take the climate change into account. Also it is necessary to take the resources rarefaction like phosphorus and water, and losses of fertile lands into account (Bedoussac et al., 2015). There are more options in agriculture. The use of biologically fixed nitrogen (N_2) by agricultural plants should be increased (Jensen and Hauggaard-Nielsen, 2003; Hauggaard-Nielsen et al., 2003, 2008, 2009). As known, the cultivation of legumes helps enrich the soil with nitrogen; hence, cultivated with cereals in rotation or in association, they contribute to higher fertility in soils (Magrini et al., 2019). Enhancing the crop diversity is a crucial factor for sustainable agro-ecology. Legumes, as a main source of biological nitrogen, are also able to

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reduce synthetic nitrogen fertilizers use and associated fossil energy consumption (Lüscher et al., 2014; Bedoussac et al., 2015). This can support organic farming and the sustainability of this humane activity. This way, possible advantages of eco-functional intensification in organic farming can be reached. This could be obtained by intercropping cereal and grain legume species sown and harvested together (Bedoussac et al., 2014, 2015).

Yigezu et al. (2018) mentioned the favorable economic effects of alternating cultivation of legumes and cereals in arid areas (Morocco). Prins and de Wit (2006) supported the intercropping of cereals and grain legumes as this showed the positive potential of organic farming in many ways. Also Huňady and Hochman (2014) support this concept with their opinion: intercropping of grain legumes and cereals is effective in organic farming. Good basis for increase and stabilisation of yields and sustaining plant health exists. On agriculture farms pea-cereal mixtures are an effective crop, harvested for green fodder as well as for feed concentrates, in dairy cows feeding. Other authors (Salcedo, 2007; Stoddard et al., 2009; Ksiezak and Straniak, 2009) also confirmed the importance of legume cultivation and L-C-M silage production in the world agriculture and animal production for biological fixation of atmospheric nitrogen in the soil, increasing the soil fertility, interrupting cycles of cereal diseases, the possibility of improving the protein value of feed and increasing nitrogen matter intake in dairy cows and possible food improving.

In ruminant production, forage legumes as a plant group present some specific advantages and disadvantages (Phelan et al., 2015). In case legumes are compared to grasses or cereals, the main advantages are in general low reliance on fertilizer nitrogen inputs, high voluntary intake and animal production when feed supply is non-limiting and high protein content. On the other hand, the main disadvantages of forage legumes are in general lower persistence as compared to grass under grazing, high risk of livestock bloat and difficulty to conserve as silage or hay. The last dry years could have been a reason for turning to this type of feeding in dairy cows. Possible technological troubles in L-C-M green matter harvesting as concequence of rainy weather and subsequent soil pollution in silages can deteriorate preservability (silageability) and contaminate silage with unwanted microorganisms (Andersen and Jensen, 1987, cit. Kratochvíl, 1991) and their spores (bacilli). These can penetrate subsequently into milk and worsen its quality (Murphy et al., 2016). For these reasons, the technological question of the possibility of increased feeding influence by L-C-M silages in dairy cow herds on the raw milk quality is increasing (Lobacz et al., 2016) in practice now.

Milk thermostability (lactoprotein thermostability; TES) is a technological property. The TES shows the resistance of milk proteins to their possible heat coagulation respectively to their thermal denaturation and TES may be significantly endangered by a lactoprotein quality decrease. Therefore, TES can be affected by dairy cow nutrition which is a crucial factor. Cows metabolic disorders, such as subclinical rumen acidosis, could also reduce the technological quality of milk by reducing the content and quality of protein (so called low protein syndrome; Illek, 1995). Chramostová et al. (2014) stated the thermostability as an important indicator in assessment of the raw milk quality. This is valid particularly in terms of the heat load to which milk is exposed during its processing. In general, at raw milk processing the good thermostability in the production of durable products is required (Singh, 2004). It means at processing products with long shelf live such as condensed and sterilized UHT milk (Patrovský and Gajdůšek, 1988). Milk TES is also an economic indicator. The TES in dairy is always related to technologies which lead to products with higher added value. This TES test in technology is simple, nevertheless labor-intensive and lengthy. That is the reason why the data files for milk TES investigations are of small scale in terms of sample number. This is usually in tens of samples as maximum (Chramostová et al., 2014). This fact underlines the uniqueness of the database used in this work ($n = 535$).

From these reasons the paper goal was to evaluate the effects of L-C-M silage feeding in dairy cows (Czech Fleckvieh and Holstein as main milked cattle breeds in the CR) on their raw milk quality and TES, which was carried out on a larger data set by modifying the roughage and concentrate portions in feeding rations.

2 Material and methods

2.1 Animals and milk samples

In this survey, 8 dairy cow herds with Czech Fleckvieh and Holstein dairy cow breed (4 and 4) were included in the 3-year observation. Dairy cows were milked regularly twice a day. These dairy cows were kept in stables with free (5) and binding (3) housing. The animals were fed by roughage feeding rations with L-C-M (≥20% in the dry matter of the ration) or without L-C-M (NO-L-C-M) silages according to real randomly and irregularly spaced periods in the relevant herds. There was applied grazing in some herds in the summer feed season. The cow herds were kept at altitude of 341 ±47 m. According to the sampling periods, the average annual rainfall sums were 589 ±122 (L-C-M) and 598 ±124 mm (NO-L-C-M) and were comparable to the groups. This way according to the technical design of sampling on average 292 ±170

(L-C-M) and 250 ±161 (NO-L-C-M) animals were included in the groups during the whole experiment. The milking was performed in the milking parlor (5) and into the pipeline (3). The milk yield in kg per day in dairy cows was on average 29.02 ±6.22 (L-C-M) and 25.79 ±7.28 (NO-L-C-M) and thus was comparable although it was slightly higher in L-C-M. During the 3-year experimental period there were collected 589 bulk tank milk samples in total two-weekly to monthly intervals: 126 in L-C-M; 463 in NO-L-C-M feeding regime.

2.2 Model feeding rations of dairy cows in a pilot study case

A feeding model of experimental and control cows was developed to assess the effect of L-C-M on the quality of raw cow's milk in the field observation. L-C-M silages based on the majority of peas and barley or peas and triticale were included in the feed ration characteristics. A detail description of the dairy cow feeding model has been introduced in our previous work (Hanuš et al., 2018; Table 2).

2.3 Treatment and analyses of bulk tank milk samples

The bulk tank milk samples were treated by the preservative with bronopol (0.03%) excluding these which were determined for microbiological analyses and stored in a refrigerator after sampling. Sample transport was carried out under cold conditions (<8 °C) in thermobox to an accredited dairy laboratory (Accredited Milk Laboratory Buštěhrad, Czech-Moravia Breeders Corporation a.s.). The milk analyses were performed under conditions which are prescribed by standard CSN EN ISO/IEC 17025. The milk indicators were determined as it follows in Table 1. Further also the energy (ketosis, in terms of animal health) milk coefficients fat/crude protein and fat/lactose, which are monitored also for individual cows, were determined by calculation, according to Siebert and Pallauf (2010), van Knegsel et al. (2010), Manzenreiter et al. (2013) and Hanuš et al. (2013).

A detailed description of milk analyse procedures, methods and instruments used is given in a previous work (Hanuš et al., 2018) and in Table 2. Milk analyzers were calibrated and checked regularly for result repeatability and reliability, according to standard operating procedures of accredited laboratory (CSN EN ISO/IEC 17025). For TES value determination the raw milk samples without preservation were used. The TES time was determined in the laboratory of Bohemilk Opočno dairy plant. 2.5 ml of milk was used in thick-walled glass tube for analysis.

2.4 Statistical evaluation of results

For mentioned milk indicators (Table 1) the main statistic prameters were calculated as follows: – mean values (arithmetic mean (*x*), median (*m*), geometric mean (*xg*)); – variability as standard deviation (sd) and variation coefficient (*vx* in %). Because of previous regular absence of normal frequency (Ali and Shook, 1980; Janů et al.,

Table 1 Used milk indicators with their units in L-C-M/NO-L-C-M group comparison in alphabetical order

Milk indicator	Abbreviation	Purpose of control	Unit
Count of coli-form bacteria	COLIB	milk hygiene	$CFU.ml^{-1}$
Crude protein (total N × 6.38) content	CEP	milk composition	%
Fat content	FT	milk composition	%
Fat/crude protein	FT/CEP	ketosis indicator	–
Fat/lactose monohydrate	FT/LE	ketosis indicator	–
Lactose monohydrate	LE	milk composition	%
Milk freezing point	MFPD	technological milk property	°C
Milk thermostability	TES	technological milk property	minute
Residues of inhibitory substances	RES	for possible occurrence of residues of antibiotic drugs and also for interference potential of possible phytoactive substances, milk quality	case
Solids non-fat	SNF	milk composition	%
Somatic cell count	SCC	dairy cow udder health	10^3 ml^{-1}
Total count of mesophilic bacteria	TCMB	milk hygiene	10^3 CFU ml^{-1}
Total solids content	TOT	milk composition	%
Urea content	UA	dairy cow nutrition	mg 100 ml^{-1}

colony forming unit = CFU; % = weight percentage (g 100 g^{-1})

Table 2 Used analytical procedures for milk indicators in alphabetical order

Abbreviation	Procedure	Note
COLIB	plate cultivation method (VRBL agar, 37 ±1 °C, abbreviated cultivation period 24 – 48 hours)	
CEP	CombiFoss FT + (Foss, Hilleröd, Denmark)	MIR-FT infrared spectroscopy (in mid range with interferometer and Fourier´s transformation), total N × 6.38
FT	look CEP	
FT/CEP	look CEP	calculation
FT/LE	look CEP	calculation
LE	look CEP	monohydrate
MFPD		MIR-FT, combined with electrical conductivity value
TES	time up to milk protein visible flocculation	under heat conditions at 135 °C in oil bath
RES	microbiological (*Geobacillus stearothermophilus*) inhibition assay (growth at 65 °C) with pH indicator Eclipse 50 (ZEU-INMUNOTEC, Spain)	mostly as residues of antibiotic drugs
SNF	look CEP	
SCC	look CEP	by flow cytometry with ethidium bromide staining
TCMB	IBC FC (Bentley Instruments, Chaska, Minnesota, USA)	by flow cytometry
TOT	look CEP	
UA	look CEP	

2007 a; Hanuš et al., 2011) for data distribution in values of some milk indicators (for instance SCC, TCMB, COLIB, TES) these were subjected to a logarithmic transformation ($\log_{10}$) for subsequent geometric mean calculations and also to application of reliable relevant statistic testing by parametric *t*-test. The classic unpaired *t*-test was used at testing of differences between value means of milk indicators for L-C-M and NO-L-C-M using MS Excel (Microsoft, Redmond, Washington, USA).

This experimental design allowed L-C-M (experiment) versus NO-L-C-M (control) testing on the same localities under comparable conditions because of equilibrium conditions the same herd in the NO-L-C-M sampling period created the reference values for experimental results in the L-C-M sampling period. Thus, the interference effect of any uncontrollable factors on the results was eliminated as much as possible.

3 Results and discussion

3.1 Properties and composition of milk under L-C-M and NO-L-C-M feeding conditions

The mean values and variability parameters of components and properties of milk under the mentioned L-C-M and NO-L-C-M group feeding conditions in this pilot case study are included in Table 3 and 4. According to the expectation, lower values of variability (from LE 1.1 to UA 17.7%) were found in milk components (fat, crude protein, lactose, total solids, solids non-fat and urea content) and higher (from SCC 41.7 to TCMB 314.4%) in microbiological (hygiene) indicators (total count of mesophilic bacteria, coli-form bacteria count, somatic cell count). From the overall view of the average values and corresponding variability of all milk indicators, these varied in the normal range of relevant reference values for both dairy cow breeds under usual dairy technology conditions (Janů et al., 2007a, b; Hanuš et al., 2007, 2011) in the Czech Republic. Further, average values of log TCMB and log COLIB were lower then these which reported Godič-Torkar and Golc-Teger (2008; Slovenie) and comparable to these which reported Pytlewski et al. (2012; Poland).

The TES means as an important technological property were 19.58 and 19.53 (L-C-M and NO-L-C-M, xg 18 and 18; Table 4) minutes with variability of 40.8 and 40.6%. These TES values are on average similar, however, in variability much higher as compared to the results of other work in the CR (Chramostová et al., 2014). Further, in this analyse, there was 1 finding of RES (NO-L-C-M), ie 0.17% and it was most likely (95%) of antibiotic occurrence. This is a value comparable to the average European quality values. Nevertheless, this suggests that, under the given conditions, there is practically no risk of degradation of milk quality by L-C-M silage feeding, either in the form of an apparent inhibition production in relation to some potent bioactive agents of the phytoinhibitor or phytoestrone type.

3.2 Differences in milk composition and properties between evaluated groups

Under these mentioned dairy technological (feeding and environment of dairy cow herds (L-C-M and NO-L-C-M group)) conditions, a statistically significant effect (Table 3 and 4) was shown on: TCMB, where L-C-M values were higher (*P* ≤0.05 and *P* ≤0.01); COLIB, where L-C-M values were lower (*P* >0.05 and *P* ≤0.05); SCC, where L-C-M figures were slightly lower (*P* ≤0.05 and *P* ≤0.05); lactose, where L-C-M figures were slightly higher (*P* ≤0.001); SNF (Figure 1), where L-C-M values were higher (*P* ≤0.001); MFP, where L-C-M figures were slightly lower (*P* ≤0.001); TOT, where L-C-M values were higher (*P* ≤0.001). The main milk components, including milk freezing point, were only slightly affected, mostly in favor of L-C-M, partly by lactose effect and this increase is in fact practically not essential.

More papers were very interested in the experiment assessment of effects of maize silages on raw milk composition and properties as roughage portion has practical importance in cow feeding ration Szterk et al. (2017a, b). Pozdíšek and Huňady (2020) mentioned an increase in the concentrations of nitrogenous substances in mixed stands of cereals (wheat, barley, oats) with peas in comparison with the concentrations of nitrogenous substances in the corresponding monocultures (the weighted average values), which can be assessed as a positive manifestation of the interaction effect of legume-cereal mixtures, including their silages. Therefore, it is clear that an estimation of possible impacts of silage feeding on raw milk quality is important from dairying point of view. The dairy cow milk yield between the 70th and the 150th day of lactation, crude protein and fat content in milk were not different, regarding the type of fed silage, as it was stated by Urbański and Brzóska (1996) at silage evaluation including L-C-M. In this context, an increase of milk yield of dairy cows, when legumes were

Table 3 The effects of dairy cow feeding by L-C-M silages (against NO-L-C-M as control) on raw milk composition and properties

Group	IND	CEP	LE	TOT	FT	SNF	UA	SCC	log SCC	MFPD
	PAR	%	%	%	%	%	mg 100 ml^{-1}	10^3 ml^{-1}	–	°C
L-C-M	*n*	126	126	126	126	126	2	126	126	126
	x	3.43	5.02	12.9	3.84	9.06	29.0	204	2.2574	-.52806
	sd	0.19	0.06	0.43	0.3	0.17	1.41	85	0.2356	.003906
	vx	5.5	1.1	3.3	7.7	1.9	4.9	41.7		0.7
	xg							181		
	m	3.42	5.02	12.79	3.77	9.06	29.0	208	2.3181	-.529
	min.	2.98	4.83	12.12	3.33	8.52	28.0	38	1.5798	-.539
	max.	3.92	5.13	14.35	5.01	9.43	30.0	383	2.5835	-.514
NO-L-C-M	*n*	460	460	460	460	460	343	459	459	460
	x	3.42	4.92	12.74	3.8	8.94	26.93	227	2.3053	-.52603
	sd	0.16	0.1	0.37	0.26	0.19	4.77	112	0.2221	-.004729
	vx	4.6	2.0	2.9	6.8	2.1	17.7	49.2		0.9
	xg							202		
	m	3.44	4.92	12.76	3.79	8.96	27.0	213	2.3284	-.527
	min.	2.98	4.57	11.44	2.82	8.22	10.0	15	1.1761	-.558
	max.	3.8	5.17	13.82	4.93	9.42	42.0	1,124	3.0508	-.493
Diff.	*t*	0.6	10.87	4.11	1.49	6.38	0.61	2.16	2.11	4.42
	P	ns	***	***	ns	***	ns	*	*	***

Explanations in alphabetical order: dairy cow roughage feeding ration with legume-cereal mixture silage (experiment) – L-C-M; dairy cow roughage feeding ration with absence of L-C-M (control) – NO-L-C-M; arithmetic mean – *x*; difference between means (L-C-M – NO-L-C-M) – Diff; geometric mean – xg; indicator – IND; maximum – max.; median – *m*; minimum – min; parameter – PAR; probability of zero hypothesis (impact of factor) – *P*, *P* >0.05 – ns (no significant), *P* ≤0.05 = * (significant), *P* ≤0.01 = **, *P* ≤0.001 = ***; sample number – *n*; standard deviation – sd; value of *t*-test – *t*; variation coefficient – *vx*. Milk indicators: crude protein content – CEP; fat content – FT; milk freezing point depression – MFPD; monohydrate lactose concentration – LE; solids non-fat content – SNF; somatic cell count – SCC (after transformation – SCC $\log_{10}$); total solids content – TOT; urea content – UA

Table 4 The effects of dairy cow feeding by L-C-M silages (against NO-L-C-M as control) on raw milk quality and lactoprotein thermostability

Group	IND	COLIB	log COLIB	TCMB	log TCMB	FT/CEP	FT/LE	TES	log TES
	PAR	CFU ml^{-1}	–	10^3 CFU ml^{-1}	–	–	–	minute	–
L-C-M	*n*	119	119	119	119	126	126	123	123
	x	23.8	0.5223	65.0	1.5505	1.12	0.77	19.6	1.2548
	sd	46.9	0.8251	204.3	0.3358	0.06	0.07	8.0	0.1827
	vx	197.3		313.1		5.2	8.4	40.8	
	xg	3.0		36.0				18.0	
	m	1.0	0	34.0	1.5315	1.11	0.75	18.0	1.2553
	min.	1	0	11	1.0414	0.97	0.65	6.0	0.7782
	max.	151	2.179	2,086	3.3193	1.38	1.01	39.0	1.5911
NO-L-C-M	*n*	462	462	463	463	460	460	412	412
	x	32.1	0.7152	39.9	1.4391	1.11	0.77	19.5	1.2538
	sd	51.1	0.8902	47.3	0.3459	0.07	0.06	7.9	0.1821
	vx	159.1		118.7		5.9	7.4	40.6	
	xg	5.0		27.0				18.0	
	m	1.0	0	24.0	1.3802	1.1	0.77	18.0	1.2553
	min.	1	0	5	0.699	0.79	0.58	4.0	0.6021
	max.	151	2.179	422	2.6253	1.42	1.04	41.0	1.6128
Diff.	*t*	1.61	2.14	2.4	3.15	1.54	0	0.06	0.05
	P	ns	*	*	**	ns	ns	ns	ns

Explanations in alphabetical order – milk indicators: coli-form bacteria count – COLIB (after transformation – COLIB $\log_{10}$); ketosis coefficient fat/crude protein – FT/CEP; ketosis coefficient fat/lactose – FT/LE; milk thermostability – TES (after transformation – TES $\log_{10}$); total count of mesophilic bacteria – TCMB (after transformation – TCMB $\log_{10}$)

included into fed silages, was also found (Emile et al., 2008).

In the similar research and discussion Salcedo (2007) stated the highest milk crude protein content (3.18%) for clover silages in the inclusion of L-C-M silages into research result evaluation. However, this was without a relationship of variants of silage to the fat content, protein content and urea concentration in milk. Futhermore in our previous study (Hanuš et al., 2016) there was observed a higher content of fat by 0.1 and 0.2%, lower crude protein and casein content by 0.1 and 0.2% and a slightly lower content of lactose in the L-C-M herd milk.

The both energy (ketosis) coefficients of dairy cows and milk (FT/CEP and FT/LE) are not significantly different (Table 4; P >0.05) between groups. Also the insignificant impact of L-C-M cow feeding on milk TES (Table 4; 19.58 and 19.53 minutes, P >0.05) was registered. Unlike the results of our previous work (Hanuš et al., 2018), the TES results have a very slightly opposite trend and the difference between L-C-M and NO-L-C-M group is insignificant (P >0.05; Table 4) for both the original and the transformed form of the results. Such a conflicting result is possible and could be explained by some uncontrolled interference effects of the field experiment and also by another way of deriving of reference (control) values against the experimental ones. As mentioned (Singh, 2004; Kailasapathy, 2008), pH is a very important factor with effect on milk TES among many other influences. On the other hand, Chládek and Čejna (2005) did not observe the influence of higher urea content in cow milk on the lactoprotein TES. The weak resistance against heat treatment of milk was characteristic for Polish Holstein-Friesian (mean was 120 s), the best for Simmental dairy cows (mean was 300 s) and stage of lactation had no influence on the milk TES (Litwińczuk et al., 2016). Milk which was obtained during the autumn/winter period had significantly higher heat stability of lactoproteins (P ≤0.01), with the most significant difference observed in the Simmental dairy cows (Barłowska et al., 2014).

Although significant, the impacts on milk hygiene (microbiologic indicators) do not show an essential practice difference in advantages of L-C-M or NO-L-C-M group. Completely hypothetically, under degraded technological and environmental (rain and mud on relevant field during harvesting in higher proportion)

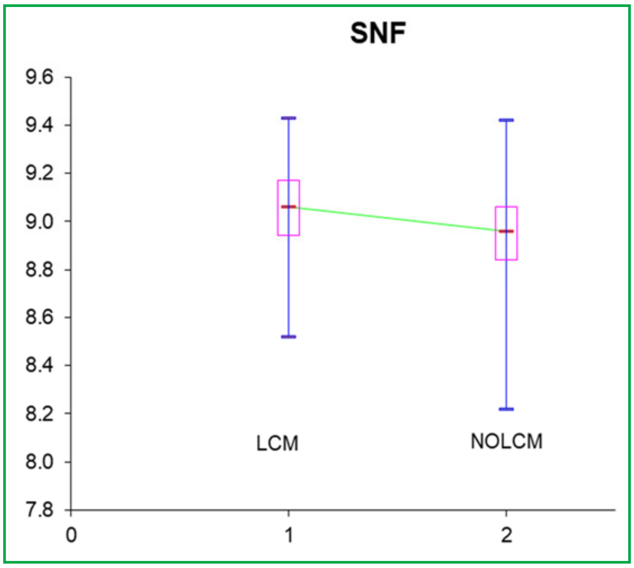

Figure 1 The impact of L-C-M (against NO-L-C-M as control) silages on content of solids non-fat (SNF; %) in bulk tank samples in raw cow's milk
(*P* <0.001) dairy cows roughage feeding ration with legume-cereal mixture silage – L-C-M (LCM; 1); dairy cows roughage feeding ration with absence of L-C-M = NO-L-C-M (NOLCM; 2)
The box graph scheme is as follows: the central short horizontal line – the median value; the box as tetragon – the top edge of 1st and 3rd quartile; the vertical line – difference between maximum an minimum value as relevant variation range

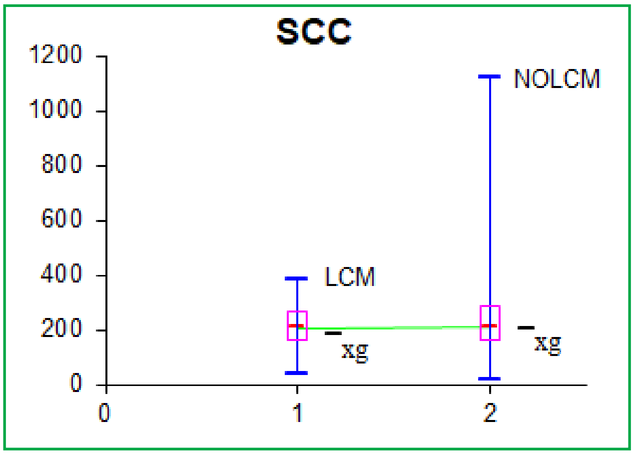

Figure 2 The impact of L-C-M (against NO-L-C-M as control) silages on somatic cell count (SCC, 10^3 ml^{-1}) in bulk tank samples in raw cow's milk
(*P* <0.05) dairy cows roughage feeding ration with legume-cereal mixture silage – L-C-M (LCM; 1); dairy cows roughage feeding ration with absence of L-C-M = NO-L-C-M (NOLCM; 2)
the box graph scheme is according to figure 1; geometric mean – *xg*

conditions at L-C-M harvesting, the milk hygiene indicators for L-C-M silages could be worse (Andersen and Jensen, 1987, cit. Kratochvíl, 1991). In this our evaluation this possible effect is observed at total count of mesophilic bacteria but this is almost missing at coli-form bacteria count (TCMB *P* <0.05, log TCMB P <0.01; COLIB *P* >0.05, log COLIB *P* <0.05; Table 4). In addition, these weaker effects are oriented in the opposite direction (for TCMB and for COLIB) in terms of the perception of the hygienic quality of raw milk. Further, for COLIB is this trend against theoretical expectations, where L-C-M values are lower as compared to NO-L-C-M figures. These facts are consistent with our former findings (Hanuš et al., 2018). There are possible more interference effects (for instance different technological and hygiene farm conditions and levels) for such phenomenon. These were mentioned for milk hygienic indicators in more details by Murphy et al. (2016). Therefore, this fact could likely be caused by other interference factors. Perhaps the season influence, when L-C-M and NO-L-C-M rations were used for feeding, could have such impact.

For the L-C-M group a significantly lower somatic cell count was recorded (Table 3; *xg* 181 <202 10^3 ml^{-1}; *P* <0.05) unlike our previous results (Hanuš et al., 2018). This finding could be very interesting from a new knowledge point of view but mentioned difference (Figure 2) may not be significant from a practical point of view, which is in accordance with our previous findings (Hanuš et al., 2018). The box graphs (Figure 1 and 2) show better representation of the data distribution characteristics as compared to the statistic characteristics in the tables (Table 3 and 4) including the inclusion of the selected important quartile limits and the positions of median value and geometric mean in relation to the relevant variability. For these mentioned interpretive advantages, these graphs were used for selected statistically significantly affected (L-C-M versus NO-L-C-M) milk indicators (SNF and SCC, Figure 1 and 2).

4 Conclusions

Possible worsening of milk quality indicators at L-C-M silage is not essential. Using of L-C-M silages in feeding rations for dairy cows with an increased proportion of L-C-M silage in the roughage component part of the feeding ration, to replace part of the protein concentrates, can be considered as a safe way of nutritional solution with regard to the milk quality.

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