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IMPACT OF FEEDING OF LEGUME-CEREAL MIXTURE SILAGES ON DAIRY COW MILK THERMOSTABILITY AND QUALITY

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Abstract

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Current changes in climate (increasing drought) and the rise in prices of protein concentrate feed are the reasons for the increasing interest in growing and production and increasing silages from legume-cereal mixtures (LCM) in rations for dairy cows. Another reason is to improve the soil fertility using LCM in crops rotation. The impact of feeding of dairy cows (Czech Fleckvieh and Holstein, 50 herds) on milk quality was monitored (for 3 years) when modifying the roughage feeding rations by additions of LCM silages. Bulk milk samples were tested (n = 641 (15 herds) LCM as experiment and 2,428 (35) as control). LCM was higher in crude protein, lactose, solids non-fat and total microorganisms (from $P \leq 0.05$ to $P \leq 0.001$). The lower was LCM in the milk freezing point and the urea content ($P < 0.05$ and $P < 0.001$). The fat content and the somatic cell count were insignificantly different ($P > 0.05$). There was a significant effect of LCM on milk thermostability (18.85 < 20.9 minutes, $P < 0.05$). The residues of inhibitory substances in milk were not recorded in the LCM group. The LCM application is a risk-free way of replacement of roughage component part of dairy cow feeding rations with regard to raw milk quality.

Keywords: dairy cow, Czech Fleckvieh, Holstein, fat, proteins, lactose, urea, somatic cell count, count of coli-form bacteria

INTRODUCTION

In 2015, the area of cultivation of legume-cereal mixtures (LCM) grew by 31%. Feeding of silage from LCM is markedly expanding in cattle nutrition in the Czech Republic (CR). The average dry matter content of LCM silages on the total dry matter of these dairy cow feeding rations can be estimated at 15 ± 5%. The climate changes, such as increasing droughts, as well as rising prices of protein feed concentrates, contribute to this increase. Another reason for cultivation of LCM is the possibility of improving soil fertility by subsidizing of atmospheric nitrogen during crop rotation. Stoddard *et al.* (2009) reported that legumes are important in world agriculture by providing biologically fixed nitrogen, interrupting the cycle of cereal diseases and contributing to food and feed. Ksiezak and Straniak (2009) evaluated the use of LCM for silages in organic farming. Viscous mixtures were characterized by a higher protein value. Salcedo (2007) reported in a long-term assessment of silage that the highest nitrogen intake in dairy cows was observed in legume-based silages. In comparison to grass or legume monocultures, grass + legume mixtures have particular advantages such as more balanced feeding values, increased resource use efficiency and increased herbage production. However, maintaining the optimum legume contents (40–60% of herbage dry matter) to achieve these benefits remains a major challenge on farms (Phelan *et al.*, 2015). Technical problems in LCM harvesting (rainy weather) and subsequent possible soil pollution of silage can aggravate its microbiological quality, most often by spore microorganisms (bacilli). They can penetrate milk from the stable environment and deteriorate its quality (Andersen and Jensen, 1987, cit. Kratochvíl, 1991). Therefore, the need for studying the effect of increased feeding of LCM silages in dairy cows on the quality of raw milk in practice increases.

The thermostability (TES) of milk (proteins) is a property that can be aggravated by a decrease in its quality. Good TES of raw milk is important in the production of durable products (condensed milk and sterilized UHT, Singh, 2004). The TES in dairy is always related to technologies that lead to products with higher added value. TES is an important parameter in assessing the raw milk quality, particularly in terms of the heat gains that milk is exposed during its processing (Chramostová *et al.*, 2014). This technology test is simple, yet labor-intensive and unpleasant and lengthy. Therefore, the TES data sets for study are of small scale, in number of tens of samples (Chramostová *et al.*, 2014). That is also reason why the data file used here is exceptional for its frequency.

Szterk *et al.* (2017) were interested in the experimental estimation of impact of maize silages on raw milk and its quality because of practical importance of roughage portion in cow feeding ration. Therefore, the aim of this study was to evaluate the impact of feeding of dairy cows on the TES and raw milk quality on a larger data set by modifying the roughage rations by supplements of LCM silages.

MATERIALS AND METHODS

Conditions of dairy cow rearing and bulk milk samples

During the 3 years, 50 herds of dairy cows of Czech Fleckvieh and Holstein breed (36 and 11 and 3 herds of both breeds) which were milked twice a day were included in this survey. Dairy cows were housed in free stables (36) and in binding cowsheds (14). The milking was carried out in the milking parlor (36) in free stables and into pipeline in binding cowsheads (14). The total number of animals covered and monitored was 8,928 (heads). The number of animals in the herds ranged from 4 to 630 (an average of 186 ± 164 dairy cows). Animals were fed with roughage feeding rations with LCM silages or without LCM silages (NOLCM) and the herds with feeding of LCM silages for at least 7 months of the year were included in the LCM group. The grazing was applied in the summer feed season in some herds. During the 3-year experimental period 3,069 bulk milk samples were taken at two-weekly or monthly intervals. Additional characteristics of experimental dairy herds are included in Tab. I.

Model dairy cow feeding rations in pilot case study

A model characteristic of feeding rations was developed to assess possible influencing of milk quality of dairy cows in which LCM silage (mostly pea and barley or pea and triticale) was included into feeding rations as compared to similar non-LCM feeding rations (NOLCM). The average, characteristic feeding rations which are adjusted to the level of cows' milk yield according to the lactation phase are in Tab. II.

The frame, total, average composition of the feeding rations (of course, these were fed with volume modifications according to lactation phase and milk yield) was supplemented with

I: *The main and additional group characteristics of herds of dairy cows*

Characteristic/Group	LCM	NOLCM
Number of herds in feeding regime	15	35
Average number of dairy cows per herd	196 ± 211	183 ± 149
Average altitude (m)	367 ± 71	343 ± 67
Mean of total amount of water precipitation (mm)	526 ± 118	562 ± 149
Average day delivery of milk to processing into dairy plant (kg)	3,940	4,591
Mean milk yield during standard lactation (305 days/kg)	5,688	7,005
Mean cow milk yield (kg/day)	18.65 ± 8.03	22.97 ± 7.95
Number of analyzed bulk milk samples	641	2,428

LCM roughage feeding with legume-cereal mixture silage; NOLCM roughage feeding without legume-cereal mixture silage.

the consumption of forage cereal concentrates according to the feed tables for the given milk yield OK (cereal concentrate = wheat, calcium carbonate, sodium chloride and sodium hydrogen carbonate 93, 4, 2 and 1%), BK (protein concentrate = rape seed extract, calprosan and urea 85, 12 and 3%) and Corngold plus vitamin and mineral supplements (Unicum and Glycomel). The quality of the applied complete compound feeding rations in the observation can be considered as moderate overall and especially with regard to the roughage feeding portion. Feeding of animals was mostly carried out in the form of total mixed ration (TMR) using a mobile feeding mixer.

Analysis of bulk milk samples

The samples were preserved with bronopol (0.03%) and stored in a refrigerator. Then the samples were transported in cold conditions (< 8 °C) to an accredited dairy laboratory (LRM Buštěhrad, ČMSCH a.s.) and analyzed. Values for components (contents) and milk properties were determined: fat (F); crude protein (CP, total N × 6.38); lactose monohydrate (L); total solids (TS); solids non-fat (SNF); urea (U); milk freezing point (MFP); somatic cell count (SCC); total count of mesophilic microorganisms (TCM); count of coli-form bacteria (COLI); milk thermostability (TES); residues of inhibitory substances (RIS, for possible occurrence of antibiotica (drugs) residues and also for possible interference of potential phytoactive substances). In addition, energy (ketose) milk (cow) coefficients F/CP and F/L (Steen *et al.*, 1996; van Knegsel *et al.*, 2010; Hanuš *et al.*, 2013; Manzenreiter *et al.*, 2013) were calculated.

Milk analyzes were performed according to relevant methods with calibrated and controlled analytical techniques according to standard operating procedures. The milk components (F, CP, L, TS, SNF, U) and properties (MFP) were determined by the indirect method of MIR-FT infrared spectroscopy (with interferometer and Fourier's transformation, in case of MFP with electrical conductivity measurement) CombiFoss FT+ (Foss Electric, Hilleröd, Denmark). The SCC was determined by flow cytometry on the same device. TCM was also determined by flow cytometry using IBC FC (Bentley Instruments, Chaska, Minnesota, USA). The COLI count was determined by plate cultivation method (VRBL agar, 37 ± 1 °C, abbreviated cultivation period 24–48 hours). The RIS (+/–) were determined by a microbiological (*Geobacillus stearothermophilus*) inhibition assay (growth at 65 °C) with pH indicator Eclipse 50 (ZEU-INMUNOTEC, Spain). The TES was determined in minutes in non-preserved milk. The time was determined up to visual denaturation (flocculation) of milk proteins when heated in an oil bath at 135 °C. The procedure was carried out with 2.5 ml of milk in a relevant thick-walled glass tube in the Bohemilk Opočno laboratory.

Statistic data treatment and evaluation

Mean values (arithmetic mean (x), median (m)), variability in the form of standard deviation (sd) and variation coefficient (vx in %) were calculated for mentioned milk indicators. The indicators (such as SCC, TCM, COLI, TES) with usual absence of normal data frequency distribution (Reneau, 1986; Janů *et al.*, 2007 a; Hanuš *et al.*, 2009) were transformed in logarithmic way ($\log_{10}$) to following determination of geometric means (xg) and for possibility of reliable statistic testing with parametric t-test. The differences between milk value means for LCM and NOLCM were tested by the classic t-test (MS Excel, Microsoft, Redmond, Washington, USA).

RESULTS AND DISCUSSION

Composition and properties of LCM and NOLCM milk

The parameters of variability and average values of milk components and properties under

II: *Model, average, characteristic feeding rations (kg) adjusted to the level of cows' milk yield*

In original dry matter	LCM 30 l of milk	LCM 15 l of milk	NOLCM 30 l of milk	NOLCM 15 l of milk
Corn silage	15	–	14	–
Grass silage	7	14	17	19
Triticale silage, dry m. 39%	6	9	7	9
Peas silage, 27% dry m.	10	4	–	–
Meadow hay	0.5	1.5	0.5	1.5
OK 2/17	4.9	3.4	4.8	3.4
BK 7/16 Calpr.	2.9	0.7	3.3	0.7
Meadow growth, 18% dry m.	20	20	22	22
Unicum	–	0.1	–	0.1
Glycomel	0.4	–	0.4	–
CORNGOLD	2	–	2.7	–

III: *The test results of impacts of feeding by LCM silages (versus NOLCM) on dairy cow raw milk thermostability and quality*

Group	IND PAR	F %	CP %	L %	TS %	SNF %	U $mg.100ml^{-1}$	MFP °C	SCC $10^3.ml^{-1}$	log SCC	TCM $10^3CFU.ml^{-1}$	log TCM	COLI $CFU.ml^{-1}$	log COLI	TES minute	log TES	F/CP	F/L
LCM	n	635	635	635	635	635	303	635	635	635	641	641	641	641	558	558	635	635
	x	3.92	3.44	4.91	12.88	8.96	25.76	-.52655	247	2.3331	88.5	1.5961	25.5	0.6132	18.9	1.2344	1.14	0.8
	sd	0.48	0.21	0.12	0.5	0.24	8.2	.004831	130	0.2506	254.4	0.4402	46.3	0.8348	8.0	0.1923	0.15	0.11
	vx	12.3	6.1	2.4	3.8	2.7	31.8	0.9	52.7		287.3		182.0		42.6		13.4	13.3
	xg								215		39.0		4.0		17.0			
	m	3.93	3.44	4.92	12.85	8.96	25.0	-.527	225	2.3522	34.0	1.5315	1.0	0	17.0	1.2304	1.15	0.8
	min.	1.75	2.87	4.56	10.54	8.27	9.0	-.544	6	0.7782	1	0	1	0	3.0	0.4771	0.46	0.34
	max.	6.58	4.57	5.32	15.27	10.19	54.0	-.509	1,032	3.0137	2,999	3.477	151	2.179	40.0	1.6021	2.18	1.39
NOLCM	n	2,358	2,380	2,380	2,358	2,380	1,501	2,380	2,378	2,378	2,428	2,428	2,428	2,428	2,076	2,076	2,358	2,358
	x	3.89	3.39	4.88	12.76	8.88	24.24	-.52591	241	2.328	56.3	1.4579	28.5	0.6805	20.9	1.2842	1.15	0.8
	sd	0.35	0.19	0.12	0.45	0.22	6.14	.006583	134	0.2255	171.7	0.4123	48.0	0.8585	8.1	0.1826	0.1	0.08
	vx	9.0	5.5	2.4	3.5	2.4	25.3	1.3	55.4		305.0		168.8		38.9		8.4	10.0
	xg								213		29.0		5.0		19.0			
	m	3.85	3.4	4.89	12.75	8.9	25.0	-.526	228	2.3579	26.0	1.415	1.0	0	21.0	1.3222	1.13	0.79
	min.	2.32	2.64	4.09	10.6	8.0	9.0	-.603	15	1.1761	5	0.699	1	0	4.0	0.6021	0.78	0.48
	max.	6.35	4.24	5.19	15.42	9.85	50.0	-.482	1,950	3.29	2,999	3.477	169	2.2279	47.0	1.6721	2.04	1.48
Diff.	t	1.76	5.88	5.74	5.83	8.09	3.69	2.29	1.02	0.49	3.77	7.44	1.41	1.77	5.3	5.65	2.01	0
	P	ns	***	***	***	***	***	*	ns	ns	***	***	ns	ns	***	***	*	ns

IND indicator; PAR parameter; Diff. difference between means; n sample number; x arithmetic mean; sd standard deviation; vx variation coefficient (%); xg geometric mean; m median; min. minimum; max. maximum; F fat content; CP crude protein content; L monohydrate lactose concentration; TS total solids content; SNF solids non-fat content; U urea concentration; MFP milk freezing point; SCC somatic cell count; SCC log10; TCM total count of mesophilic microorganisms; TCM log10; COLI coli-form bacteria count; COLI log10; TES milk thermostability; TES log10; F/CP ketosis coefficient fat/crude protein; F/L ketosis coefficient fat/lactose; t t-test value; P probability (of impact) of zero hypothesis; ns (no significant) $P > 0.05$; * (significant) $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

the mentioned conditions of the pilot case study for the LCM and NOLCM group are shown in Tab. III. Lower values of variability (from L and SNF 2.4 to U 31.8%) were in milk components (F, CP, L, TS, SNF, U) and higher (from SCC 52.7 to TCM 305.0%) in hygienic indicators (TCM, COLI, SCC). The mean values and their variability of all milk indicators these were in the usual range of relevant reference values for both breeds under conditions in the CR (Janů *et al.*, 2007 a, b; Hanuš *et al.*, 2007, 2009).

TES showed the means 18.85 and 20.9 (LCM and NOLCM, xg 17 and 19) minutes and variability of 42.6 and 38.9%. In other work Chramostová *et al.* (2014) mentioned mean values which were similar to these but with markedly lower variability. In the study, there were indicated 2 findings (0.065%) of RIS (NOLCM). Here it means with the highest probability (95%) the occurrence of antibiotics. The value of 0.065% is comparable with the best European dairy countries in term of quality such as Austria, Switzerland and Bavaria and it is also now approximately on the level which is lower by one third as compared to the CR results. Under the above mentioned monitoring conditions, this indicates a zero risk of LCM silage feeding on possible milk quality deterioration in terms of inhibition. The results support a zero-level risk assessment for a possible false-positive inhibition from possible interference effects of certain bioactive substances such type as phytoinhibitors and phytoestrons.

Differences in milk composition and properties between LCM and NOLCM group

Without a hypothetical explanation, under the mentioned conditions in LCM (Tab. III) there were higher values for CP (Fig. 1), L, SNF and TCB (P from ≤ 0.05 to ≤ 0.001). The MFP and U mean was lower ($P < 0.05$ and $P < 0.001$). The F content and SCC was insignificantly different ($P > 0.05$). The main components including MFP were not markedly affected and basically in favor of LCM, in both TS and SNF it was partly also due to lactose. In the fact, this increase is not practically too severe.

Milk has been seldom evaluated for LCM silage application in a roughage feeding ration for cows. Use of LCM silages in a dairy cow feeding ration as a feed alternative for economic reasons can slightly change the milk composition, improve it occasionally, but it certainly does not lead to a deterioration in the milk quality or the cow reproduction indicators (Hanuš *et al.*, 2016). At assessment of silages including LCM Urbański and Brzóska (1996) reported that the milk yield of cows between the 70th and the 150th lactation day, protein and fat in milk did not differ according to the type of fed silage. Kungurov *et al.* (1981) evaluated lactating cows with a basic feeding ration, where the cereal forage concentrates were partially replaced by silage from pea and oat. There was no significant difference between the groups in milk yield but the substitution group had lower solids in the milk. Emile *et al.* (2008) reported growth in milk yield in case of inclusion of legumes into silages. Laman *et al.* (2002) reported that the Use of silage from LCM allowed increased milk yield and reduced use of cereal concentrates. Salcedo (2007) mentioned the highest milk protein of 3.18% for clover silages in the inclusion of LCM silages into the monitoring but without the relationship of silage variants to fat, protein and urea in milk. The ketosis and energy coefficient of cows and milk F/L did not differ (Tab. III; 0.8 = 0.8, $P > 0.05$) and the similar F/CP coefficient was significantly but practically slightly lower for LCM group (Tab. III; $1.14 < 1.15$, $P < 0.001$).

In this evaluation, a significant effect of LCM silages on milk thermostability was registered (Tab. III; Fig. 2; $18.85 < 20.9$ for xg $17 < 19$ minutes, $P < 0.05$). One of the most important factors of thermostability is pH (Singh, 2004; Kailasapathy, 2008). Chládek and Čejna (2005) did not notice the effect of urea concentration increasing in cow‘s milk on the thermostability of lactoproteins. This result indicated the possibility of TES deterioration through a technological factor in the group of LCM silages.

Although they are significant for TCM (Tab. III; xg 39 LCM > 29 10^3CFU.ml^{-1} NOLCM, $P < 0.001$), nevertheless, the effects on hygienic indicators do not indicate a marked practical difference in favor of LCM or NOLCM. Theoretically, under deteriorated harvest conditions, the hygienic values of milk for LCM silages could be worse (Andersen and Jensen, 1987, cit. Kratochvíl, 1991). In this case it is so for TCM, but it is not so for COLI ($P > 0.05$). The effect on TCM and COLI is slightly opposite in terms of hygienic evaluation and at COLI against expectations. This is probably due to other interference influences, perhaps the influence of the season when LCM and NOLCM were fed. There was no effect of feeding of LCM silages on SCC (Tab. III; xg 215 LCM and 213 10^3.ml^{-1} NOLCM, $P > 0.05$). From the point of view of the possible interference effects mentioned for hygienic indicators this difference is not essential from a practical point of view.

CONCLUSION

Possible worsening of the milk indicators such as hygienic in the LCM is basically not marked except for the TES as technology indicator. However, for the most of milk indicators the improvements was noted especially for component indicators. Therefore, the application of LCM silages in feeding rations can be considered as a risk-free, neutral and practically adequate way how to replace a part of the roughage component (and also protein concentrates) of the feeding rations of dairy cows with regard to the raw milk quality under given monitoring conditions.

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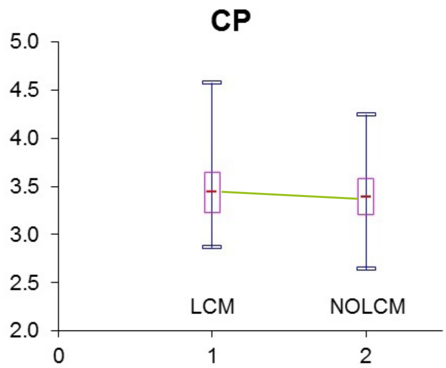
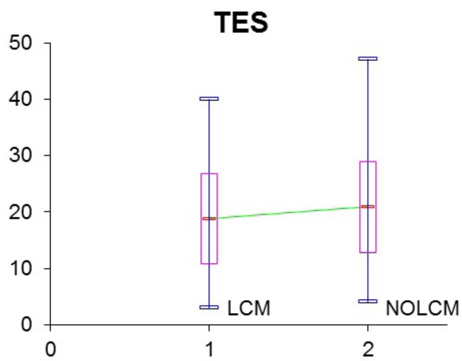
Appendix



1: *The influence of feeding by LCM (versus NOLCM) silages on crude protein content (CP; %) in raw cow's milk* (P < 0.001)

LCM roughage feeding with legume-cereal mixture silage; NOLCM roughage feeding without legume-cereal mixture silage. Construction of box graph: the file median (the central short horizontal line); the top edge of 1st and 3rd quartile (the tetragon); the variation range as difference between maximum an minimum (the vertical line).



2: *The influence of feeding by LCM (versus NOLCM) silages on thermostability (TES; minute) of raw cow's milk* (P < 0.001)

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