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# The nature of preserved forage changes butter organoleptic properties

Catherine HURTAUD\*, Luc DELABY, Jean-Louis PEYRAUD

INRA, Agrocampus Rennes, UMR1080, Production du Lait, 35590 Saint-Gilles, France

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**Abstract** – Two trials were conducted to study the effect of offering dairy cows maize silage or hay from mixed sward (white clover and ryegrass) in trial 1, or from permanent sward in trial 2, on milk yield and composition and on butter properties. In trial 1, 24 Holstein cows were arranged in a changeover design during two 6-week periods. They were offered either maize silage with soyabean meal or hay plus maize grain. In trial 2, 31 Holstein cows were used in a continuous design. They were offered either maize silage with soyabean meal or hay plus cereal concentrate. With both hay diets, the amounts of concentrate were low and equal even if the energy content of the hay in trial 2 was lower. Therefore, compared with maize diets, energy levels with hay diets were slightly lower in trial 1 (–2.5 UFL) and greatly lower in trial 2 (–7.3 UFL). Offering hay significantly decreased daily dry matter intake (–3.1 kg·d<sup>–1</sup>), milk yield (–4.3 kg·d<sup>–1</sup>), protein content (–2.3 g·kg<sup>–1</sup>), and protein and fat yields (respectively, –179 and –199 g·d<sup>–1</sup>). Milk fat content was unaffected by hay in trial 1, but decreased in trial 2 (–3.0 g·kg<sup>–1</sup>). Compared with maize silage, offering hay to dairy cows resulted in a reduced proportion of saturated fatty acids and an increased proportion of mono- and polyunsaturated fatty acids in the milk fat (C18:1, C18:2 and C18:3). There was no significant difference in butter-making parameters between hay and maize silage. Nevertheless, the butter produced had a softer texture when cows were offered hay. The butter produced with hay was judged to be softer by trained panellists, with less odour in both trials. In trial 2, hay decreased churning time and significantly decreased butter dry matter content. Yellow colour intensity was higher with hay in trial 1 and with maize silage in trial 2. The hay diet produced butters with a more rancid flavour.

**forage / milk / butter / dairy cow / sensory property / texture / colour / flavour**

**摘要 – 自然贮藏的饲草对奶油感官品质的影响。**分别设计了两组饲草喂饲奶牛, 第一组喂饲青贮玉米或来源于混合草场的干草(白苜蓿和黑麦草), 第二组为来源于永久草场的干草, 对比研究了两组的产奶量、乳成分和奶油的性质。在第一组中, 将 24 头荷斯坦奶牛分成两小组, 前后 6 周两小组的饲料完全互换, 饲料是青贮玉米加豆粕或者干草加玉米粒。在第二组中, 31 头荷斯坦奶牛用来进行连续试验, 提供的饲料是青贮玉米加上豆粕或者干草加谷类浓缩饲料。在两种干草饲料中, 第二组干草饲料的能量较低, 因此, 与玉米相比, 第一组干草饲料的能量略低 (–2.5 UFL), 而第二组干草饲料的能量则非常低 (–7.3 UFL)。喂饲干草饲料的奶牛每日摄入的干物质质量非常低 (–3.1 kg·d<sup>–1</sup>), 产奶量 (–4.3 kg·d<sup>–1</sup>), 乳蛋白质含量 (–2.3 g·kg<sup>–1</sup>), 蛋白 (–179 g·d<sup>–1</sup>) 和脂肪的产量 (–199 g·d<sup>–1</sup>) 都降低。在第一组中, 干草饲料对乳脂肪含量没有影响, 但是在第二组中乳脂肪含量则显著下降 (–3.0 g·kg<sup>–1</sup>)。与青贮玉米相比, 喂饲干草的奶牛的乳脂肪中饱和脂肪酸的比例降低, 单不饱和及多不饱和脂肪酸 (C18:1, C18:2, C18:3) 的比例增加。在干草和青贮玉米饲料喂饲的奶牛之间, 奶油的加工参数性没有显著性差异。然而, 喂饲干草饲料的奶牛, 加工出奶油的质地较软, 感官评定也证明了这种奶油的质地较软且味道较淡。用第二组奶牛生产出的牛奶加工奶油时需要搅打的时间短,

\* Corresponding author (通讯作者): Catherine.Hurtaud@rennes.inra.fr

并且奶油中干物质的含量显著降低。在第一组中喂饲干草的和第二组中喂饲青贮玉米的奶油呈黄色, 喂饲干草组的奶油有较重的酸败味。

草料 / 乳 / 奶油 / 奶牛 / 感官特性 / 质地 / 色泽 / 风味

**Résumé – La nature du fourrage conservé modifie les propriétés organoleptiques du beurre.** Deux essais ont été menés pour étudier l'effet de l'ensilage de maïs ou du foin, issu de prairie mixte (trèfle blanc et ray-grass) dans l'essai 1 ou de prairie permanente dans l'essai 2, sur la production laitière, la composition du lait et les propriétés du beurre. Dans l'essai 1, 24 vaches laitières Holstein ont été conduites selon un schéma expérimental en inversion avec 2 périodes de 6 semaines. Elles recevaient soit de l'ensilage de maïs avec du tourteau de soja, soit du foin avec du maïs grain. Dans l'essai 2, 31 vaches laitières Holstein ont été conduites selon un schéma expérimental en continu. Elles étaient alimentées soit avec de l'ensilage de maïs et du tourteau de soja, soit avec du foin et un concentré énergétique à base de céréales. Avec les régimes foin, les quantités de concentré étaient faibles et équivalentes même si le foin de l'essai 2 était moins riche en énergie. En conséquence, par rapport au régime ensilage de maïs, les apports énergétiques ont été un peu plus faibles avec le foin dans l'essai 1 ( $-2.5$  UFL) et beaucoup plus faibles dans l'essai 2 ( $-7.3$  UFL). Le foin a entraîné une diminution significative des quantités ingérées ( $-3.1$  kg·j<sup>-1</sup>), de la production laitière ( $-4.3$  kg·j<sup>-1</sup>), de la production de matières grasses et de protéines et du taux protéique ( $-2.3$  g·kg<sup>-1</sup>). Dans l'essai 1, le taux butyreux n'a pas été modifié, mais a fortement chuté dans l'essai 2 ( $-3.0$  g·kg<sup>-1</sup>). Le profil en acides gras des laits a été modifié par le foin, surtout dans l'essai 2. Le foin a entraîné une diminution des acides gras saturés et une augmentation des acides gras mono- et poly-insaturés, en particulier du C18:1, C18:2 et C18:3. En conséquence, le beurre était moins dur avec le foin. Il n'y a pas eu de différence significative entre le foin et l'ensilage de maïs sur les paramètres de fabrication du beurre. Le beurre produit avec le régime foin a été jugé moins ferme et moins odorant dans les 2 essais, mais avec une flaveur rance plus prononcée. L'intensité de couleur jaune a été plus élevée pour le foin dans l'essai 1 et pour le maïs dans l'essai 2.

fourrage / lait / beurre / vache laitière / propriété sensorielle / texture / couleur / flaveur

## 1. INTRODUCTION

The diet offered to dairy cows can have a significant impact on the fatty acid composition of milk fat [4, 5]. In particular, conserved grass induces an increase in unsaturated fatty acids in milk fat compared with maize silage. This modification of the fatty acid composition, in particular the increase in the C18:1/C16:0 ratio, could affect the organoleptic properties of butter, and as already demonstrated by the Arilait study [2], spreadability of the butter was improved, but the risk of rancidity or oxidation was greater due to an increase in the concentration of unsaturated fatty acids.

There are limited data on impact of forage on the organoleptic characteristics of butter in lowlands. Houssin et al. [15–18] demonstrated that butter properties, colour and texture in particular, were moderately

modified by grass or hay silage, in comparison with maize silage.

The interest in hay for cheese-making is partly linked to the absence of butyric spores that may otherwise be present in silage-type forages. Comparisons between cocksfoot-based hays or hay harvested at various elevations in Auvergne or in the Alps showed that some sensory characteristics of Saint-Nectaire-type cheeses differed between forages, although these effects were relatively small [32] and were probably linked to the floristic composition of the hay.

The aim of this study was therefore to characterise the effect of hay on milk yield, milk composition and on the sensory properties of butter and to compare it with that of maize silage. Two trials were conducted with that objective. The first study compared maize silage with good

quality hay made from ryegrass and white clover swards. The second trial compared maize silage with hay from natural lowland swards, with a wider botanical species variety.

## 2. MATERIALS AND METHODS

### 2.1. Experimental diets

Two types of hay (HA1 and HA2) were compared with two maize silages (MS1 and MS2) during two successive trials in the winters of 2001 and 2002. The objective was to provide minimal levels of energy concentrate to the dairy cows, so as to maximise the specific effects of the forages. In both trials, the quantity of forage offered to dairy cows was equivalent to 95% of the ad libitum forage intake during two weeks of experimental diet adaptation.

In trial 1, hay from a reseeded sward was composed of a mixture of ryegrass (27%) and white clover (73%) harvested in July, under good weather conditions and after 56-day regrowth. The sward was reseeded in October 1999 with 30 kg·ha<sup>-1</sup> Ohio ryegrass and 5 kg·ha<sup>-1</sup> of Alice white clover.

In trial 2, hay, from a multispecies complex sward, managed without nitrogen supplementation, was composed of a mixture of hybrid and English ryegrass, incarnate clover, red clover and white clover. Advantages were present. The hay was harvested in late July 2001 after 63 days' regrowth (2nd cycle) following a period of wet weather. The sward had been reseeded in Spring 2000 with 10 kg·ha<sup>-1</sup> "Gladiator" hybrid ryegrass, 10 kg·ha<sup>-1</sup> "Modanta" English ryegrass, 5 kg·ha<sup>-1</sup> "Dipper" red clover, 5 kg·ha<sup>-1</sup> "Dawn" incarnate clover, 1 kg·ha<sup>-1</sup> "Donna" white clover and 1 kg·ha<sup>-1</sup> "Hareng" white clover.

In trial 1, care was taken in diet formulation to ensure that both dietary regimens provided the same amounts of digested

starch in the intestine, by taking account of the bypass starch contents in all diet components. Therefore, the hay diet (HA1) was supplemented with 4 kg maize grain (95% maize and 5% molasses) and 150 g mineral compound 12-12-5 (% of P - % of Ca - % of Mg). The maize diet (MS1) was supplemented with 3 kg soyabean meal and 300 g mineral compound 5-25-5 (% of P - % of Ca - % of Mg).

In trial 2, the hay diet (HA2) was supplemented with energy concentrate (4.4 kg) (21.5% wheat, 22% barley, 17% wheat bran, 20% sugarbeet pulp, 15% rapeseed meal, 2% molasses, 0.5% CaCO<sub>3</sub>, 1% NaCl and 1% NaHCO<sub>3</sub>, DM basis) and 150 g mineral compound 12-12-5 (% of P - % of Ca - % of Mg). The maize diet (MS2) was supplemented with 2.6 kg soyabean meal and 300 g mineral compound 5-25-5 (% of P - % of Ca - % of Mg).

The composition and nutritional values of the forages and energy concentrates are shown in Table I.

### 2.2. Animals and experimental design

Trial 1 was conducted according to a changeover experimental design over two 6-week periods with 24 dairy cows divided into 2 groups of 12 based on similarity in lactation number, lactation stage, milk yield, fat and protein yields and contents, liveweight, body condition score and DM intake. Milk yield at the beginning of the trial was on average 28.8 ± 3.4 kg·d<sup>-1</sup>, fat content was 42.4 ± 5.0 g·kg<sup>-1</sup>, protein content was 32.5 ± 2.0 g·kg<sup>-1</sup>, liveweight was 618 ± 52 kg and mean lactation stage was 106 ± 17 d.

In trial 2, cows received two diets over three 5-week periods: period 1: maize silage; period 2: hay; period 3: maize silage. Thirty-one dairy cows were included in this trial. Milk yield at the beginning of the trial was 30.8 ± 5.7 kg·d<sup>-1</sup>

**Table I.** Chemical composition of feeds in both trials.

	Maize silage		Hay <sup>1</sup>		Soyabean meal		Energy concentrate <sup>2</sup>	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
	MS1	MS2	HA1	HA2				
DM <sup>3</sup> (%)	32.7	35.6	85.0	92.5	88.0	88.2	86.0	88.2
OM <sup>4</sup> (g·kg <sup>-1</sup> DM)	966	963	888	919	934	931	980	930
Crude protein (g·kg <sup>-1</sup> DM)	70	60	154	111	485	500	99	166
Crude fibre (g·kg <sup>-1</sup> DM)	195	219	282	398	71	74	23	101
NDF <sup>5</sup> (g·kg <sup>-1</sup> DM)	407	450	782	679	151	143	127	281
ADF <sup>6</sup> (g·kg <sup>-1</sup> DM)	235	246	329	444	86	82	31	128
UFL <sup>7</sup> (kg <sup>-1</sup> DM)	0.90	0.88	0.77	0.62	1.19	1.19	1.24	1.05
LFU <sup>8</sup> (kg <sup>-1</sup> DM)	1.05	1.02	1.07	1.17	/	/	/	/
PDIE <sup>9</sup> (g·kg <sup>-1</sup> DM)	67	66	89	72	236	236	124	107
PDIN <sup>10</sup> (g·kg <sup>-1</sup> DM)	43	38	96	69	343	343	83	108

<sup>1</sup>Trial 1: 73% white clover, 27% ryegrass, harvested on 17/07/2000 after 56-day regrowth; trial 2: mixture of hybrid and English ryegrass, incarnate clover, red clover and white clover.

<sup>2</sup>Trial 1: 95% maize, 5% molasses; trial 2: 22% barley, 21.5% wheat, 20% beet pulp, 17% wheat bran, 15% rapeseed meal, 2% molasses, 0.5% CaCO<sub>3</sub>, 1% NaHCO<sub>3</sub>, 1% NaCl.

<sup>3</sup>DM = Dry matter.

<sup>4</sup>OM = Organic matter.

<sup>5</sup>NDF = Neutral-detergent fibre.

<sup>6</sup>ADF = Acid-detergent fibre.

<sup>7</sup>UFL = Feed unit for lactation.

<sup>8</sup>LFU = Fill unit for lactating dairy cows.

<sup>9</sup>PDIE = Protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-fermented organic matter [20].

<sup>10</sup>PDIN = Protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-degraded organic matter [20].

on average, fat content was  $40.0 \pm 4.5$  g·kg<sup>-1</sup>, protein content was  $29.2 \pm 1.9$  g·kg<sup>-1</sup>, liveweight was  $584 \pm 69$  kg and mean lactation stage was  $72 \pm 21$  d.

## 2.3. Measurements

### 2.3.1. Animal performances and milk measurements

In trial 1, individual cow feed intakes were recorded individually on a daily basis. During trial 2, the cows were not fed individually, but in batches. The amounts of feed given and refused were then weighed daily and globally for all the cows. The cows were milked twice daily and milk yield was measured at each milking. Fat

and protein contents were measured on three days each week (6 milkings) during the first four (trial 1) or three (trial 2) weeks of each period and four days per week (8 milkings) during the last two weeks of each period (both trials). Fat and protein contents were measured with an infrared analyser (Milkoscan, Foss Electric, Hillerød, Denmark).

In trial 1, the 24 cows' individual milk was sampled once per period at the morning milking. In trial 2, a mixture of individual milk samples from two milkings (evening and morning) from nine cows representative of the 31 cows (same milk yield, average fat and protein contents) was collected once in every period. Total nitrogen content, non-protein

nitrogen matter (NPN), non-casein nitrogen (NCN), casein and urea were assessed according to the methods described by Hurtaud et al. [19]. Total and soluble calcium were analysed by atomic spectrophotometry absorption on milk yield and milk ultrafiltrate, respectively. Milk fatty acid composition was analysed by gas chromatography after extraction [1].

### **2.3.2. Butter manufacture and physicochemical properties**

Butter was manufactured twice during each experimental period. Milk from two consecutive milkings of 12 cows in each treatment group in each period was used in trial 1. The milk from four consecutive milkings of the 31 cows was used in trial 2. In trial 1, butter from each of the two consecutive milkings was made on the Tuesday (hay treatment) and Wednesday (maize treatment) of the last two weeks of each period (2 butter-makings per period) and in trial 2, on each Tuesday of the final two weeks of each period (2 butter-makings per period). Milk was stored at 4 °C until processing. It was subsequently heated to 50 °C with a hot-plate exchanger, then skimmed with a MM1254 cream separator (Wesfalia, Chateau-Thierry, France). The cream was pasteurised with a hot-plate exchanger (80 °C for 20 s) and standardised around 400 g·kg<sup>-1</sup> of fat. After pasteurisation, cream was cooled as rapidly as possible at 4 °C. Butter was made as described by Couvreur et al. [7].

Physical measurements were made on the butter 14 days after manufacture. The colour of the butter was measured on three samples (four measurements per sample) with a MINOLTA chromameter [24]. Butter samples were tested for resistance to penetration using a universal testing machine (Instron, model 4501, Norwood, USA) on 3 samples of butter conserved at 4 °C for 15 d and on 3 samples conserved

at 12 °C during the preceding 20 h [7]. Butter pH and dry matter content (by recording the mass lost by a sample of butter of 5±1 g during drying in an oven at 102 ± 2 °C for 15 h) were also measured.

Butter sensory attributes were evaluated by the sensory analysis laboratory panel of ENILIA of Surgères (Charente-Maritime, France) at a tasting temperature of 14 ± 1 °C, as recommended by standard FIL 99C [12]. This panel comprised 10 trained panellists who were trained according to ISO 8586 standard recommendations [21]. In single sessions, each panel member was requested to evaluate odour (total intensity, rancid, cream, milk, grass, hay and hazelnut), flavour (total intensity, rancid, acidity, bitterness, cream, milk, grass, hazelnut and metal), firmness, and melting in the mouth, giving a score between 0 and 10 (the more intense the criteria was, the greater the score) on a continuous rating scale.

### **2.4. Statistical analyses**

Data were analysed according to the GLM procedure of SAS [28]. In trial 1, the statistical model used for analysing milk and butter composition parameters was as follows:  $Y_{ijk} = \mu + p_i + v_j + c_k + e_{ijk}$  where  $\mu$  was the mean,  $p_i$  the period effect,  $v_j$  the cow effect (or batch for butter),  $c_k$  was the treatment effect and  $e_{ijk}$  was the residual error.

In trial 2, data were analysed according to the MIXED procedure of SAS [28]. The model tested the effect of period, and orthogonal contrasts were used to analyse the effects of periods 1 (Per1) and 3 (Per3) (maize silage diet) versus period 2 (Per2) (hay diet).

In both trials, sensory assessment scores were analysed according to the CATMOD procedure of SAS [28]. The model only tested the treatment effect.



**Table II.** Effect of maize silage (MS) and hay (HA) on DM intake and energy and protein balances.

	Trial 1				Trial 2				
	MS1	HA1	RSD <sup>1</sup>	P-value	Per1 MS2	Per2 HA2	Per3 MS2	RSD <sup>1</sup>	P-value
DMI (kg·d <sup>-1</sup> )	19.2	17.8	1.28	0.001	19.7	14.4	19.2	/	/
Forage (kg DM·d <sup>-1</sup> )	15.8	13.9	1.21	< 0.001	17.0	10.3	16.0	/	/
Concentrate (kg DM·d <sup>-1</sup> )	3.4	3.9	0.11	< 0.001	2.7	4.2	3.1	/	/
Energy (UFL·d <sup>-1</sup> )	17.9	15.4	1.01	< 0.001	17.5	10.1	17.3	/	/
PDIE (g·d <sup>-1</sup> )	1790	1696	115	0.010	1755	1186	1796	/	/
PDIN (g·d <sup>-1</sup> )	1743	1634	127	0.007	1563	1159	1681	/	/
Energy balance (UFL·d <sup>-1</sup> )	1.8	0.4	0.96	< 0.001	0.8	-2.6	2.7	/	/
PDI balance (g·d <sup>-1</sup> )	191	289	114	0.007	-47	17	280	/	/

<sup>1</sup>RSD = Residual standard deviation.

**3. RESULTS**

**3.1. Dairy cow performance and milk composition parameters**

The quality of maize silage used in both trials was similar, as expected (Tab. I). In contrast, the hays were very different. In trial 2, the energy and nitrogen values of the hay were clearly lower (0.62 vs. 0.77 UFL and 72 vs. 89 g·kg<sup>-1</sup> PDIE, and 69 vs. 96 g·kg<sup>-1</sup> PDIN, respectively) than in trial 1 and its fill value (LFU) was higher (1.17 vs. 1.07) (Tab. I).

In both trials, forage intake and total intake were markedly reduced with the hay diet. This reduction was more marked in trial 2 (-1.4 kg·d<sup>-1</sup>, *P* = 0.001 in trial 1; -4.9 kg·d<sup>-1</sup>, non-statistically tested, in trial 2) (Tab. II). Overall, concentrate intake amounts were low: 21% of total intake amounts on average. In trial 1, energy and PDI balances were positive for both diets, but energy balance was clearly lower (respectively, 0.4 vs. 1.8 UFL·d<sup>-1</sup>, *P* < 0.001) and PDI balance was higher with the hay diet (respectively, 191 vs. 289 g·d<sup>-1</sup> of PDI, *P* = 0.007). In trial 2, energy and PDI balances were positive with the maize silage diet, although with the hay diet, the energy balance was highly negative (-4.4 UFL compared with MS2 average diet) and the

PDI balance was low (-106 g·d<sup>-1</sup> of PDI compared with MS2 average diet). Energy and PDI balances were not or were little different between the two trials with the maize silage diet (for energy balance, 1.8 vs. 1.8 UFL·d<sup>-1</sup> and for PDI balance, 191 vs. 123 g·d<sup>-1</sup> of PDI). In contrast, they were numerically much lower in trial 2 than in trial 1 with the hay diet (for energy balance, 0.4 vs. -2.6 UFL·d<sup>-1</sup> and for PDI balance, 289 vs. 17 g·d<sup>-1</sup> of PDI) (Tab. II).

In both trials, cows offered the hay diet had a significantly reduced milk yield (-3.2 kg·d<sup>-1</sup>, *P* < 0.001 and -5.5 kg·d<sup>-1</sup>, *P* < 0.001 for trials 1 and 2, respectively), protein content (-1.5 g·kg<sup>-1</sup>, *P* < 0.001 and -3.1 g·kg<sup>-1</sup>, *P* < 0.001) and fat and protein production. In trial 1, fat content was not significantly modified by the hay diet, whereas in trial 2, fat content dropped sharply (-3.0 g·kg<sup>-1</sup>, *P* < 0.001). In trial 1, the cows' liveweight was not affected by treatments, whereas it was significantly decreased by the hay diet in trial 2 (-13 kg, *P* < 0.001) (Tab. III).

Offering hay-based diets to dairy cows resulted in a reduction in milk total nitrogen contents in both trials (Tab. IV). This decrease was essentially linked to that in casein content in trial 1 and to that in casein and soluble protein contents in trial 2.

**Table III.** Effect of maize silage (MS) and hay (HA) on milk production and milk composition.

	Trial 1				Trial 2				
	MS1	HA1	RSD <sup>1</sup>	P-value	Per1 MS2	Per2 HA2	Per3 MS2	RSD <sup>1</sup>	P-value
Milk (kg·d <sup>-1</sup> )	23.2	20.0	1.10	< 0.001	27.2	19.1	22.1	1.39	< 0.001
Fat-corrected milk (kg·d <sup>-1</sup> )	23.6	20.6	1.18	< 0.001	27.3	18.1	22.0	1.41	< 0.001
Fat content (g·kg <sup>-1</sup> )	41.4	42.5	2.42	0.155	40.3	36.8	40.1	1.65	< 0.001
Protein content (g·kg <sup>-1</sup> )	31.8	30.3	1.07	< 0.001	28.9	25.7	29.3	0.74	< 0.001
Fat yield (g·d <sup>-1</sup> )	957	842	58.5	< 0.001	1093	697	881	63.1	< 0.001
Protein yield (g·d <sup>-1</sup> )	737	602	31.3	< 0.001	784	488	647	45.4	< 0.001
Liveweight (kg)	611	617	12.5	0.161	579	570	590	9.5	< 0.001

<sup>1</sup>RSD = Residual standard deviation.**Table IV.** Effect of maize silage (MS) and hay (HA) on milk composition.

	Trial 1				Trial 2				
	MS1	HA1	RSD <sup>1</sup>	P-value	Per1 MS2	Per2 HA2	Per3 MS2	RSD <sup>1</sup>	P-value
Crude protein (g·kg <sup>-1</sup> )	33.6	32.4	1.52	0.010	30.6	28.8	31.5	0.66	< 0.001
Non-protein nitrogen (NPN) (g·kg <sup>-1</sup> )	1.55	1.36	0.12	< 0.001	1.22	1.47	1.39	0.26	0.132
Urea (mg·L <sup>-1</sup> )	239.0	184.3	45.4	< 0.001	120.6	217.4	148.7	77.7	0.018
True protein (g·kg <sup>-1</sup> )	32.1	31.0	1.48	0.022	29.4	27.3	30.1	0.68	< 0.001
Non-casein nitrogen (NCN) (g·kg <sup>-1</sup> )	7.71	7.50	0.53	0.177	6.74	6.68	7.36	0.61	0.158
Caseins (g·kg <sup>-1</sup> )	25.9	24.9	1.14	0.005	23.9	22.1	24.1	0.75	< 0.001
Soluble proteins (g·kg <sup>-1</sup> )	6.16	6.14	0.47	0.868	5.51	5.21	5.97	0.40	0.005
Ratio casein/protein (%)	80.8	80.3	0.86	0.036	81.2	80.9	80.0	1.44	0.653
Total Ca (mg·kg <sup>-1</sup> )	1235	1228	124.4	0.850	1117	966	1095	79.2	< 0.001
Soluble Ca (mg·kg <sup>-1</sup> )	282	288	26.4	0.400	307	241	279	40.0	0.006
Colloidal Ca (mg·kg <sup>-1</sup> )	953	940	117.9	0.702	810	725	816	43.3	< 0.001
Ratio colloidal Ca/casein (mg·g <sup>-1</sup> )	37.9	36.8	4.97	0.444	34.1	33.0	34.5	3.17	0.328

<sup>1</sup>RSD = Residual standard deviation.

Overall, the casein to protein ratio was not modified by diets in trial 2, whereas it was slightly but significantly reduced in trial 1 with the hay diet ( $-0.5$  units,  $P = 0.036$ ). In trial 1, the hay diet induced a significant decrease in non-protein nitrogen and urea (respectively,  $-0.19$  g·kg<sup>-1</sup>,  $P < 0.001$  and  $-54.7$  mg·L<sup>-1</sup>,  $P < 0.001$ ), whereas in trial 2, it only induced an increase in urea ( $82.8$  mg·L<sup>-1</sup>,  $P = 0.018$ ). The hay diet did not affect the total, soluble or colloidal calcium contents in trial 1, whereas it strongly

decreased them in trial 2. The colloidal calcium to casein ratio did not vary in both trials (Tab. IV).

Hay-based diets induced a significant decrease in the proportion of saturated fatty acids in favour of monounsaturated and polyunsaturated fatty acids (Tab. V). The effect was more clearly seen in trial 2: the unsaturated fatty acid contents increased by 8.8 units percent versus 1.8 in trial 1. Unsaturated fatty acids were almost 30% of total fatty acids with the hay diet in



**Table V.** Effect of maize silage (MS) and hay (HA) on milk fatty acid composition.

Fatty acids, %	Trial 1				Trial 2				
	MS1	HA1	RSD <sup>1</sup>	P-value	Per1	Per2	Per3	RSD <sup>1</sup>	P-value
	MS2	HA2	MS2						
C4:0	2.50	2.37	0.09	< 0.001	3.55	3.01	3.19	0.23	0.002
C5:0	0.019	0.013	0.006	0.002	0.020	0.011	0.017	0.006	0.012
C6:0	1.87	1.80	0.06	< 0.001	2.61	2.20	2.48	0.13	< 0.001
C7:0	0.023	0.014	0.007	< 0.001	0.005	0.004	0.020	0.011	0.082
C8:0	1.35	1.22	0.06	< 0.001	1.63	1.34	1.64	0.09	< 0.001
C9:0	0.042	0.027	0.009	< 0.001	0.034	0.018	0.042	0.008	0.002
C10:0	3.37	2.92	0.26	< 0.001	3.18	2.86	3.83	0.67	0.030
C11:0	0.087	0.050	0.020	< 0.001	0.058	0.037	0.079	0.012	< 0.001
C12:0	4.28	6.63	0.40	< 0.001	3.97	3.27	4.69	0.39	< 0.001
C13:0	0.136	0.097	0.021	< 0.001	0.103	0.086	0.119	0.01	< 0.001
C14:1	1.36	1.29	0.13	0.106	1.20	1.25	1.35	0.15	0.663
C14:0	13.3	12.5	0.45	< 0.001	12.7	11.9	13.2	0.93	0.014
IsoC15	0.28	0.31	0.02	< 0.001	0.22	0.33	0.22	0.02	< 0.001
C15:1	0.54	0.60	0.05	< 0.001	0.48	0.78	0.47	0.06	< 0.001
C15:0	1.29	1.47	0.12	< 0.001	1.01	1.39	1.05	0.10	< 0.001
C16:1	2.16	2.28	0.19	0.041	1.74	2.23	1.94	0.24	0.001
C16:0	40.0	40.3	1.75	0.481	40.8	34.0	38.5	2.68	< 0.001
IsoC17	0.32	0.38	0.03	< 0.001	0.30	0.46	0.31	0.043	< 0.001
C17:1	0.75	0.90	0.05	< 0.001	0.65	1.22	0.68	0.095	< 0.001
C17:0	0.53	0.73	0.03	< 0.001	0.47	0.80	0.46	0.057	< 0.001
C18:0	7.77	7.52	0.74	0.255	8.67	7.66	7.60	0.75	0.142
C18:1	16.0	17.3	1.50	0.006	14.9	22.7	16.4	2.91	< 0.001
C18:2	1.98	1.75	0.18	< 0.001	1.52	2.08	1.63	0.28	< 0.001
C18:3	0	0.46	0.07	< 0.001	0.13	0.31	0.10	0.053	< 0.001
Saturated fatty acids	77.2	75.4	1.73	0.002	79.3	69.4	77.4	3.21	< 0.001
Unsaturated fatty acids									
Monounsaturated	20.8	22.4	1.63	0.003	19.1	28.3	21.0	2.97	< 0.001
Polyunsaturated	1.98	2.21	0.17	< 0.001	1.64	2.39	1.73	0.28	< 0.001
Ratio C18:1/C18:0	2.10	2.32	0.19	< 0.001	1.75	2.99	2.18	0.29	< 0.001
Ratio C16:1/C16:0	0.054	0.057	0.006	0.095	0.042	0.066	0.050	0.008	0.003
Ratio C14:1/C14:0	0.102	0.104	0.009	0.460	0.095	0.105	0.104	0.011	0.259
Ratio C18:1/C16:0	0.40	0.44	0.06	0.050	0.37	0.68	0.44	0.120	< 0.001

<sup>1</sup>RSD = Residual standard deviation.

trial 2. The increase in monounsaturated fatty acids with the hay diet was linked to the increase in C15:1, C16:1, C17:1 and C18:1. The C18:1/C16:0 ratio was marginally increased by the hay diet in trial 1 (0.04 units,  $P = 0.05$ ) and more markedly in trial 2 (0.27 units,  $P < 0.001$ ). The hay diet increased the C18:1/C18:0 ratio in both trials and the C16:1/C16:0 ratio in trial 2. Polyunsaturated fatty acid content responded differently between trial 1

and trial 2. In trial 1, the hay diet decreased linoleic acid (C18:2), whereas in trial 2, it significantly increased the same acid ( $-0.23$ ,  $P < 0.001$  and  $+0.50$  unit percent,  $P < 0.001$ , respectively). The hay diet induced a significant increase in linolenic acid (C18:3) in both trials, with a greater effect in trial 1. Among the saturated fatty acids, the hay diet reduced the proportion of short-chain fatty acids (C4:0 to C10:0) in both trials. In trial 2, the reduction

**Table VI.** Effect of maize silage (MS) and hay (HA) on butter manufacture.

	Trial 1				Trial 2			
	MS1	HA1	RSD <sup>1</sup>	P-value	Per1	Per2	RSD <sup>1</sup>	P-value
	MS2	HA2						
Churning time (min)	17.8	20.5	2.12	0.400	15.5	6.0	1.11	0.010
Butter yield <sup>2</sup> (%)	48.4	43.0	4.45	0.425	42.1	40.2	1.35	0.306
Fat-corrected butter yield <sup>3</sup> (%)	96.7	94.8	1.06	0.309	98.8	93.1	2.44	0.145
Fat yield <sup>4</sup> (%)	98.5	96.6	0.35	0.113	99.7	94.6	2.54	0.184
pH after 2 weeks	4.85	4.85	0.02	0.918	4.92	5.01	0.24	0.727
MS after 2 weeks (%)	85.9	85.0	0.13	0.087	86.4	84.6	0.22	0.011

<sup>1</sup>RSD = Residual standard deviation.

<sup>2</sup>Butter weight / cream weight.

<sup>3</sup>Butter weight × butter fat content / cream weight × cream fat content.

<sup>4</sup>(Butter weight × butter fat content + buttermilk weight × buttermilk fat content) / cream weight × cream fat content.

also extended to medium-chain fatty acids, C12:0 and C16:0 in particular. The hay diets induced an increase in the proportion of long-chain odd fatty acids, such as C15:0 and C17:0 (Tab. V).

### 3.2. Butter manufacture and organoleptic characteristics of butter

Offering cows hay-based diets produced no significant effect on milk suitability for butter manufacture (Tab. VI). Butter yield, fat-corrected butter yield and fat yield were not modified in any of the trials. The hay diets did not affect butter pH and DM contents. Only churning time was reduced in trial 2 with the hay diet (−9.5 min) (Tab. VI).

In trial 1, butter texture properties, as measured at 4 °C, were not altered by the hay diet except for forces at 10 and 18 mm of penetration, which tended to increase at 4 °C (respectively, 0.022 kN,  $P = 0.065$  and, 0.04 kN,  $P = 0.090$ ). In trial 2, butter was much softer with the hay diet, regardless of the measurement temperature. At 5, 10 or 18 mm of penetration into butter, the forces were less than half those noted

with butter produced from cows offered the maize diet (Tab. VII).

The sensory analysis panel also found a different effect of the two hay diets on butter firmness in the mouth (Tab. VIII). In trial 1, the hay diet produced butters assessed as slightly less firm in the mouth (respectively, 6.04 vs. 6.79,  $P = 0.036$ ) than with the maize diet, but with no difference in the mouth melting rating. In trial 2, the hay diet produced butters that were also clearly less firm in the mouth but had a much higher mouth melting rating than with the maize diet (Tab. VIII). Maize butters had very similar physical properties across the two trials.

Dietary effects on the colour of butter also differed between trials. In trial 1, butter was more yellow with the hay diet, whereas in trial 2, butter from hay was not as yellow as butter from maize, which was much more coloured than the maize butter in trial 1. This difference in colour, which was measured by reflectance, was also found by the sensory analysis panel in trial 2 (Tabs. VII and VIII). However, in trial 1, the sensory analysis panel did not make any colour distinction between butters from either diet while in trial 2, butter from hay was assessed as significantly

**Table VII.** Effect of maize silage (MS) and hay (HA) on butter physical properties.

	Trial 1				Trial 2			
	MS1	HA1	RSD <sup>1</sup>	P-value	Per1 MS2	Per2 HA2	RSD <sup>1</sup>	P-value
Measurements at 4 °C								
Slope (N·mm <sup>-1</sup> )	30.42	32.17	4.10	0.581	32.61	18.37	4.94	0.102
Force at 5 mm (kN)	0.036	0.041	0.005	0.204	0.058	0.024	0.005	0.017
Force at 10 mm (kN)	0.126	0.148	0.013	0.065	0.185	0.080	0.02	0.031
Force at 18 mm (kN)	0.33	0.37	0.026	0.090	0.41	0.21	0.050	0.053
Measurements at 13 °C								
Slope (N·mm <sup>-1</sup> )	17.79	16.51	3.38	0.623	15.73	6.45	1.55	0.023
Force at 5 mm (kN)	0.019	0.020	0.006	0.655	0.023	0.009	0.002	0.025
Force at 10 mm (kN)	0.065	0.070	0.020	0.766	0.079	0.027	0.006	0.010
Force at 18 mm (kN)	0.187	0.183	0.042	0.882	0.194	0.070	0.019	0.018
Colour (chromameter)								
L (white index)	93.6	93.3	0.50	0.457	91.3	93.1	0.12	0.003
a (red index)	-2.96	-3.41	0.24	0.054	-3.02	-2.81	0.16	0.310
b (yellow index)	12.78	15.12	0.80	0.016	18.99	11.90	0.78	0.009

<sup>1</sup>RSD = Residual standard deviation.

less coloured than butter from maize; it should be noted that the intensity of yellow colour was generally low in both trials regardless of treatment.

Globally, the scores given by the sensory analysis panels for odour and flavour were not very high, the maximum average score hardly exceeding 5 (Tab. VIII). Hay or maize diets did not produce butters very different in taste. In trial 1, of the fifteen odour and flavour descriptors, only two descriptors were affected by diet: firmness and rancid flavour. In trial 2, eight descriptors were affected by diet: colour, firmness, melting, fat texture, total intensity of odour, odour of cream, odour of hazelnut and rancid flavour. Total intensity of odour tended to be or was stronger with maize butter in both trials (respectively, 0.56,  $P = 0.070$  and 1.75,  $P = 0.003$ ), while butter from the hay diet tended to have a more oxidised metal flavour (0.42,  $P = 0.108$  and 0.34,  $P = 0.102$ ). In both trials, the hay diet produced butters with a significantly stronger rancid flavour (0.33,  $P = 0.011$  and 0.58,  $P = 0.013$ ) although

the scores of this parameter were below 1. In trial 2, the magnitude of the differences was greater (Tab. VIII).

## 4. DISCUSSION

### 4.1. Effect of hay-based diets on animal performance and milk production

The marked effects of the hay diets on milk yield were characteristic of nutrient restriction. Indeed, the amounts of DM ingested with hay compared with maize silage were lower, resulting in a lower energy supply. In trial 2, the energy balance was even highly negative. Furthermore, to highlight the forage effect, a low level of concentrate supplementation was used in both trials (maximum 4 kg·d<sup>-1</sup> DM of concentrate). Low milk production with hay diets has already been reported by a number of authors [14, 16, 30] and is entirely consistent with the milk production and protein content response to

**Table VIII.** Effect of maize silage (MS) and hay (HA) on butter characteristics determined by sensory analysis.

	Trial 1			Trial 2		
	MS1	HA1	<i>P</i> -value	Per1 MS2	Per2 HA2	<i>P</i> -value
Sensory analysis (1 to 10)						
Colour	0.50	0.59	0.573	1.15	0.38	0.008
Odour						
Total intensity	4.87	4.31	0.070	5.20	3.45	0.003
Cream	3.52	2.99	0.131	3.58	2.06	0.007
Milk	1.70	1.38	0.203	1.33	0.82	0.135
Grass	0.51	0.36	0.344	0.42	0.41	0.941
Hay	0.24	0.22	0.834	0.27	0.08	0.189
Hazelnut	0.17	0.18	0.874	0.19	0	0.043
Rancid	0.37	0.59	0.204	0.26	0.33	0.671
Flavour						
Total intensity	3.71	3.48	0.398	3.52	2.70	0.096
Acid	0.53	0.37	0.256	0.23	0.31	0.600
Bitter	0.60	0.59	0.982	0.11	0.28	0.090
Cream	2.55	2.12	0.183	1.94	1.26	0.133
Milk	1.43	1.54	0.670	1.28	1.11	0.515
Hazelnut	0.11	0.22	0.187	0.07	0.03	0.391
Grass	0.15	0.17	0.797	0.12	0.07	0.459
Rancid	0.25	0.58	0.011	0.22	0.80	0.013
Metal	0.51	0.93	0.108	0.18	0.52	0.102
Texture in mouth						
Firmness	6.79	6.04	0.036	6.68	2.73	< 0.001
Melting	3.64	3.46	0.631	2.31	4.50	< 0.001
Fat	3.81	3.90	0.822	4.28	2.72	0.002

energy supplied by the diets [6]. Based on the findings of these authors, the difference in energy supply observed in the present trial ( $-7.3$  UFL) was likely to induce a decrease in milk yield of 5.6 to  $7.8 \text{ kg} \cdot \text{d}^{-1}$ . The decrease in intake with the hay diet can be explained by the fact that this type of forage has a greater fill value than maize silage. This was more evident in trial 2, where the fill value of hay was 1.17 (vs. 1.07 in trial 1), due to higher crude fibre content [10]. The very sharp decrease in hay intake in trial 2 may also be explained by a palatability problem with that hay. Indeed, the decrease in DM intake ( $6.2 \text{ kg} \cdot \text{d}^{-1}$  DM between MS2 and HA2) was sharper than the difference predicted from hay fill value ( $2 \text{ kg} \cdot \text{d}^{-1}$  DM).

As Couvreur et al. [8] showed that there was no significant interaction between the type of forage and the energy level of the diet, and that the effects of forage and energy restriction were cumulative, the effect of the hay diet in trial 2 could thus have been increased by this cumulative phenomenon, combining the type of forage with energy deficit. The sharp increase in certain milk fatty acids with the hay diet in trial 2, C18:2 and C18:1, may have in part resulted from the energy deficit as the fatty acids can originate from hay and from adipose tissue mobilisation. According to Chilliard et al. [4], adipose tissue triglycerides would contain a significant amount of linoleic acid that might have been used during lipid mobilisation.

## **4.2. Effects of hay diets on milk butter-making capacity and butter sensory characteristics**

### **4.2.1. Butter-making capacity**

Except for a decrease in churning time in trial 2, the type of forage had little effect on the suitability of cream for butter-making. The decrease in churning time with the hay diet in trial 2 could be explained by a decrease in the fat melting point (theoretical melting point calculated from the melting point of each fatty acid: 39.0 vs. 43.0 °C with the maize diet) due to the increase in unsaturated fatty acids [27]. Indeed, it would appear that cream characterised by high contents of low melting-point triglyceride would churn faster than cream poor in these triglycerides [27]. The lack of a hay effect on churning time in trial 1 may be linked to the absence of a treatment effect on triglyceride composition and more specifically on triglyceride melting point (theoretical melting point calculated from the melting point of each fatty acid: 43.8 vs. 43.2 °C with the maize diet).

### **4.2.2. Butter texture properties**

The effect of hay diet on the texture properties of butter was moderate in trial 1 and more pronounced in trial 2 both in terms of physical measurements and sensory panel assessments. Cullinane et al. [9] related texture improvements to fat thermal change characteristics, in particular to the decrease in solid fat content in grass silage-based butter. Indeed, the spreadability index, C18:1/C16:0 ratio and more generally the unsaturated fatty acid contents were increased by the hay diet in trial 2 but remained unchanged in trial 1. These results were fully consistent with sensory analysis scores, as in trial 2 hay butters were judged to be less firm in the mouth and also more melting. These results were comparable with those of Houssin et al. [17] who

also obtained more spreadable butter with hay.

The changes in fatty acid composition with hay, particularly the increase in unsaturated fatty acids, were consistent with the literature data [5, 17]. These unsaturated fatty acids could come directly from the lipid in hay. Indeed, the presence of linolenic acid in milk could only have a dietary origin [5]. Linolenic acid concentration was often moderate in our trials and much lower than noted by Chilliard et al. [5] (0.38% on average in our trial vs. more than 1.3% otherwise with hay diets), perhaps because of the conditions of preservation of the forages. Unsaturated fatty acids in milk could also derive from adipose tissue mobilisation. Hay may also have induced an increase in udder desaturase activity. The increase in both the C16:1/C16:0 and C18:1/C18:0 ratios in trial 2 would indicate a more intense activity of  $\Delta 9$ -desaturase in the udder [13].

The effects of hay on butter texture properties were more significant in trial 2. This result was consistent with the effects on fatty acid composition.

The impact of the hay diets in the present study was relatively weak by comparison with that of grass silage [16] or fresh grass [7]. Providing increasing amounts of cut grass to supplement a maize-silage-based diet altered milk fatty acid composition (more unsaturated fatty acids) and clearly improved butter organoleptic properties (a reduced firmness, more colour) [7]. The effect of hay diets cannot be generalised, as shown by the differing responses across the two trials. Conversely, the effects of maize diets on butter characteristics appeared to be rather constant from one year to the other.

### **4.2.3. Butter colour**

Hay diets had little effect on butter colour. This finding is consistent

with that of Houssin et al. [16, 18] on butter and Camembert cheese, and of Nozière et al. [26] on milk. Offering hay diets to dairy cows induced less coloured products than grazing [31, 32] or grass silage [15]. According to Nozière et al. [26],  $\beta$ -carotene contents could account for 40% of milk colour variability. The rate of decrease in  $\beta$ -carotene content in preserved grass, and in the milk produced, depends on the light exposure time after cutting [23, 29].

In trial 2, offering a hay diet to dairy cows produced a less coloured butter than in trial 1. The two hays had been harvested under different weather conditions. In trial 1, hay was harvested under fair weather, whereas in trial 2, hay had been produced during rainy weather. The colour differences between the two butters from hay could be explained by a greater loss of pigments in trial 2, resulting from prolonged weather exposure and greater loss of carotenoid pigments [23, 29].

Cows offered the maize diet produced a milk that gave a more coloured butter in trial 2 than in trial 1 and more coloured than butter made with milk from cows offered the hay diet. This result cannot be a diet-related artefact as cows were given the same amount of soyabean meal in both trials. Also, it was not an artefact linked to the chromameter because the difference was also detected by the sensory analysis panel. This type of result has not been previously documented in the literature. According to Martin et al. [23], the quantities of  $\beta$ -carotene ingested with maize silage were extremely low. Weiss [33] specified that maize silage contains 1 to 4 mg·kg<sup>-1</sup> DM of  $\beta$ -carotene vs. 5 to 100 mg·kg<sup>-1</sup> DM in hay and grass silage. An explanation could be that the plant in our study was cropped at an earlier stage (more cobs) or that other pigments were present in the maize, which may have contributed to increasing the yellow colour.

#### 4.2.4. Butter flavour

Butters derived from hay diets in trial 1 exhibited sensory characteristics which were similar to those from maize diets. In contrast, differences in sensory properties between hay and maize butters in trial 2 were clearly more marked. This may have been due to a wider floristic diversity in the hay, as can be the case with highland cheeses when comparing graminaceae-based and seeded leguminaceae swards and natural grassland hay as forages in cheese production [32].

The lower total intensity of flavour with hay diets in trial 2 could be the consequence of lower butyric and hexanoic acid contents in cream, as they appear to be the most significant fatty acids which contribute to butter flavour, according to Molimard et al. [25]. The lower cream odour and flavour with hay diets could be linked to reduced cetones in butter [3]. The stronger rancid flavour with the hay diet could be due to butter fat hydrolysis associated with higher humidity of butter under that treatment [22]. It could also be due to the nature of the feed, as shown by Ferlay et al. [11]: lower lipolysis with a corn silage diet compared with ryegrass hay or mountain natural grassland hay.

## 5. CONCLUSION

Compared with maize silage, hay diets with a low level of concentrate supplementation significantly reduced the milk production performance of dairy cows. Furthermore, the effects of hay are variable and depend on its botanical composition, on harvesting conditions, on its production area (plain, highlands or mountain) and are not as important as those of pasture. Nevertheless, compared with maize silage, hay had a positive effect on the nutritional qualities of butter by reducing the saturated fatty acid content and

therefore increasing the proportion of unsaturated fatty acids, linolenic acid in particular.

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