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Original article

Effects of level and type of energy source (volatile fatty acids or glucose) on milk yield, composition and coagulating properties in dairy cows

Catherine Hurtaud*, Henri Rulquin, Raymond Vérité

Station de recherches sur la vache laitière, Inra, 35590 Saint-Gilles, France

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Abstract – Four fistulated Holstein cows were arranged in a 4×4 Latin square design to study the effects of level and type of energy source on milk yield and composition. Treatments consisted of a basal diet fed alone (low energy treatment) or with 3.3 Mcal of net energy for lactation from extra nutrients perfused either into the rumen (either propionic acid or a mixture of volatile fatty acids) or into the duodenum (glucose). Increasing the energy input without changing the volatile fatty acid profile improved milk yield and slightly increased milk protein and fat yields. Compared with the isoenergetic mixture of volatile fatty acids, both propionic acid and glucose infusions significantly decreased fat content (-4.5 g/kg) and yields (respectively, -111 and -160 g/d), but affected fatty acid proportion and yield differently (more elongation process and less C18 with glucose because of the counterbalanced effects on milk yield (-1.3 kg/d) and protein content (1.5 g/kg). The coagulating properties of milk were directly linked to variations in protein, casein and mineral contents. In conclusion, propionic acid or glucose scarcely affected milk protein content, but induced a similar decrease in milk fat content probably through different metabolic pathways. © Inra/Elsevier, Paris

milk composition / milk coagulation / VFA infusion / propionic acid infusion / glucose infusion

Résumé – Effets du niveau et de la nature de l'énergie (AGV ou glucose) sur la production, la composition et l'aptitude à la coagulation du lait. Les effets du niveau et de la nature de l'énergie sur la production et la composition du lait ont été étudiés au cours d'un essai mené avec quatre vaches fistulées selon un schéma en carré latin 4×4 . Les vaches recevaient soit le régime de base seul (témoin négatif), soit supplémenté avec 3,3 Mcal d'énergie nette lait sous forme de nutriments perfusés dans le rumen (mélange d'acides gras volatils ou acide propionique) ou dans le duodénum (glucose). L'augmentation du niveau énergétique sans modification du profil

^{*} Correspondence and reprints

E-mail: hurtaud@st-gilles.rennes.inra.fr

fermentaire a entraîné une augmentation de la production laitière et des productions de matières grasses et de protéines. En comparaison avec le mélange isoénergétique d'acides gras volatils, les infusions d'acide propionique et de glucose ont provoqué une chute importante du taux buty-reux (-4.5 g/kg) et des productions de matières grasses (respectivement -111 et -160 g/j), mais ont affecté de manière différente la composition en acides gras du lait (quantité plus importante des acides gras moyens et moins d'acides gras en C18 avec le glucose). La production de protéines a été légèrement augmentée par l'infusion d'acide propionique, mais non par l'infusion de glucose à cause de l'effet opposé sur le taux protéique (+ 1,5 g/kg) et la production laitière (-1,3 kg/j). L'aptitude du lait à la coagulation reflétait les modifications de composition en protéines, en sensible la synthèse des protéines du lait mais ont entraîné une diminution similaire du taux butyreux probablement par des voies métaboliques différentes. © Inra/Elsevier, Paris

composition du lait / coagulation du lait / AGV / acide propionique / glucose

1. INTRODUCTION

Adjustments to the diets of dairy cows can be one of the quickest ways to improve milk quality, especially the protein content and cheese-making suitability required by the processing industry. According to Coulon and Rémond [7]. using concentrates to increase the energy level of the diet also provides an increase in milk protein content (0.3 g/kg for each Mcal of NE₁) in multiparous cows in early lactation. Several mechanisms may be involved because concentrate supplementation may increase 1) ATP necessary for protein synthesis, 2) glucose supply (ruminal propionic fermentation or by-pass starch) that spares glucogenic AA [40], or 3) AA supply that is induced by the greater bacterial synthesis associated with the increase in rumen-fermented OM [17].

Increased AA caused by greater bacterial synthesis (item 3) can be avoided by infusing energy as VFA into the rumen [5, 28, 33, 34] or glucose into the duodenum [6, 29]. Unfortunately, in most of these experiments, the effects of level (item 1) and type of energy (item 2) were confounded (see review of Rulquin and Hurtaud [36]). Indeed, except for Holter et al. [13], Hurtaud et al. [14] with propionic acid (C3) infusion and for Vik-Mo et al.

[41], Clark et al. [6], Ørskov et al. [29] and Lemosquet et al. [24] with glucose infusion, comparisons of treatments were not isoenergetic because, when VFA and glucose were infused, the total energy supply (diet and infusion) was increased. The effect on milk protein yield varies in relation to the type and level of additional energy supplied. Milk protein yield often increased with infusion of C3 except in isoenergetic situations [10, 13, 14] and with glucose infusion except for Clark et al. [6] and Oldick et al. [27]. In most cases, energy supplementation, either in the form of C3 or glucose, is followed by a decrease in butter fat content [4, 5, 6, 10, 11, 13, 14, 33, 34, 42]. However, only one comparison [10] has been made between glucose and C3 intravenous infusions. In this experiment, C3 and glucose infusions had no effect on milk protein synthesis. The effects of C3 and glucose have often been confounded in glucogenic theories (sparing effect of amino acids). In fact, the mechanisms by which C3 and glucose function in reducing milk fat synthesis and in regulating protein secretion are quite unknown even if the effect of the two nutrients seemed to be the same on fat content.

This experiment was designed to assess the specific effects of energy supplemen-

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tation and type of energy on the synthesis of milk components, on the mechanisms of their syntheses (as reflected by blood metabolites), and on the cheesemaking suitability of the milk. A treatment that was deficient in energy was compared with three other treatments that provided equal amounts of energy in the form of either a mixture of VFA or C3 infused into the rumen or of glucose infused into the duodenum.

2. MATERIALS AND METHODS

2.1. Treatment and experimental design

The low energy treatment (basal diet alone) provided 88 % of the energy requirements. The other treatments provided 100 % of energy requirements and included the same basal diet plus either a ruminal infusion of C3 (13.4 mol/day, 266 kcal NE₁/mol; [16]), or a ruminal infusion of a mixture of VFA (17 mol/day as 64 % acetic acid, 21 % propionic acid, and 15 % butyric acid, 210 kcal NE₁/mol; [16]), or finally a duodenal infusion of glucose (1 295 g/day, 2.75 Mcal NE₁/kg; [2]). The effect of level and type of energy was studied according to a 4 × 4 Latin square design over 14-day periods (1 week of adaptation and 1 week of measure).

2.2. Cows and feeding

The test was conducted in four Holstein cows (mean BW, 630 ± 61 kg) yielding 30 ± 8 kg/d of milk at approximately 2 months after calving. The cows were individually fed on a restricted basal diet (60 % corn silage, 10 % hay, 17.5 % energy concentrate and 12.5 % oil meal; DM basis) (table I) enriched with urea (70 g/d) and sodium caseinate (250 g/d). The diet provided 100 % of the animal protein requirements [17]. All feeds were mixed and administered in two meals at 0800 and 1700 hours. The solutions were infused continuously by peristaltic pumps for 24 h. A special device prevented direct contact between the ruminal wall and VFA. Each cow was continuously perfused with three separate solutions: VFA mixture or C3 and buffer (or water plus salts) in the rumen, glucose (or water) in the duodenum (table II).

2.3. Measurements and sampling

The amounts of feed offered and refused were weighed daily. Feed DM content was determined daily for corn silage and weekly for concentrate. At each milking, milk yield was recorded, and fat and protein contents were determined by infrared analysis (Milkoscar; Foss Electric, HillerØd, Denmark) for 14 separate milkings every week.

Jugular blood was sampled once each period (day 11 or 12) at 1 h before the morning meal

Ingredient		СР	ОМ	Crude fiber	ADF	NDF	Energy	PDI ¹
	(% of diet)			(%)			(Mcal/kg)	(g/kg)
Corn silage	60.0	7.8	96.5	17.7	20.6	37.1	1.56	60
Hay (ryegrass)	10.0	12.2	91.2	28.8	34.1	58.8	1.38	94
Concentrate ²	17.5	13.5	90.8	11.8	15.6	29.6	1.65	82
Oil meal ³	12.5	48.7	93.0	8.2	10.9	18.7	1.78	346
Total diet	100.0	14.3	94.5	16.6	19.9	35.7	1.58	103

Table I. Ingredient and chemical composition of the basal diet and its ingredients.

¹ Protein truly digested in the small intestine [17]; ² contains 77.4 % energy concentrate (25 % fine wheat bran, 30 % sugar beet pulp, 25 % barley, 10 % dehydrated alfalfa, 5 % molasses, 1 % fat, and 4 % mineral salts), 2.1 % urea, 5.1 % of a mixture of mineral salts and vitamins (Coopérative des Agriculteurs de Bretagne, Landerneau, France), 10.3 % NaHCO₃ and 5.1 % sodium caseinate; ³ formaldehyde treated oil meal from soybeans (80 %) and rapeseeds (20 %).

	<u> </u>			Infusion	
Site of infusion		Basal treatment	VFA	C3 ¹	Glucose
	(vol. of water)	а. I			
Rumen	50 L	_2	VFA ³	C3 ⁴	_2
Rumen	10 L	salts ⁵	buffer ⁶	buffer ⁶	salts ⁵
Duodenum	10 L	_2	_2	_2	glucose ⁷

Table II. Nature and composition of infusions in the rumen and duodenum.

 1 C3 = Propionic acid; 2 pure water; 3 17 mol/d of VFA as 64 % acetic acid, 21 % propionic acid and 15 % butyric acid; 4 13.4 mol/d of pure C3; 5 275 g of NaCl and 205 g of KCl; 6 395 g of NaHCO₃ and 205 g of KHCO₃; 7 1295 g/d of pure glucose.

for acetate, glucose, β -hydroxybutyrate, NEFA, urea, and AA for each cow with heparinized or EDTA syringes (Sarstedt, Nümbrecht, Germany). Preparation and analysis of blood samples were the same as those described by Guinard et al. [12].

Individual samples of ruminal liquid were obtained on day 12 of each period just before the morning feeding and at 1, 2, 3, 6 and 9 h after the morning feeding. A preservative was added to each sample, and the samples were mixed to obtain a daily pool. The VFA were determined by gas chromatography [18].

2.4. Milk analysis – coagulation and syneresis measurements

On day 9, 2 L of milk were taken from each cow at the morning milking and preserved with sodium G penicillin (2 000 IU/L). A subsample was reserved to determine milk fatty acid proportions by gas chromatography. Milk samples were immediately skimmed and stored at 4 °C for chemical analysis and coagulation studies. Total solids, lactose, true protein nitrogen, casein, casein fractions, soluble proteins, and total and soluble calcium were measured in the skimmed milk. After restoration of the chemical balance (1 h at 35 °C), rennet coagulation properties were measured with a Formagraph, either at the initial pH or at pH 6.5 (standardized with lactic acid) [44]. Laboratory curd yields were also measured by centrifugation. Sampling and analyses were performed as described by Hurtaud et al. [14].

2.5. Statistical analyses

An ANOVA was performed for the Latin square using SAS [37]. The model included cow, period and treatment as factors of variability. The significance threshold was set at P < 0.05 unless otherwise noted. The effect of the level of energy was tested between basal treatment and VFA mixture infusion. The effect of the type of energy was tested by comparing C3 or glucose infusions with VFA mixture infusion.

3. RESULTS

3.1. Effect of energy level

Regardless of the treatment, the cows ate the whole diet offered. Therefore, total NE_L input was similar for the VFA mixture, C3, and glucose treatments and was lower by 12 % for the basal treatment (*table IV*).

The VFA mixture treatment (versus the basal treatment) had no significant effect on the ruminal VFA composition (*table III*). Increasing the energy level by infusing VFA significantly increased milk, fat and protein yields by 0.9 kg, 42 g/d and 37 g/d, respectively (*table IV*). The fat and protein contents of milk did not vary, but SNF significantly increased (*table V*). The casein content, the proportions of diffe-

	Basal		Infusion			
VFA	treatment	VFA	C31	Glucose	RMSE ²	<i>P</i> <
Concentration (mmol/L)	82.0	89.7	84.5	83.1	4.9	0.225
Composition (mol/100 me	ol)					
Acetic	64.5 ^a	63.1 ^a	56.6 ^b	63.8 ^a	1.5	0.001
Propionic	19.9 ^a	20.9 ^a	29.8 ^b	20.3 ^a	1.4	0.001
Isobutyric	0.8	0.8	0.9	0.8	0.1	0.665
Butyric	10.7 ^a	11.6 ^a	9.0 ^b	10.9 ^a	0.6	0.003
Isovaleric	1.9	1.7	1.9	2.0	0.2	0.294
Valeric	1.5	1.4	1.5	1.6	0.1	0.189
Caproic	0.6 ^a	0.5 ^a	0.3 ^b	0.7ª	0.1	0.011

Table III. Effect of nature and level of source of energy on ruminal digestive paramaters.

a,b Means within rows with no common superscripts differ (P < 0.05); ¹C3 = propionic acid; ² root mean square error.

Table IV. Effect of level and nature of source of energy on feed intake, nutrient balance, milk yield and composition.

	Basal		Infusio	n		
	treatment	VFA	C3 ¹	Glucose	RMSE ²	<i>P</i> <
DMI ³ (kg/d) Energy intake (Mcal NE _L) Energy balance ⁴ (Mcal NE _L)	16.6 25.6 ^b -2.6 ^c	16.4 28.9 ^a 0.1 ^b	16.6 29.0 ^a 1.2 ^a	16.5 29.0 ^a 2.2 ^a	0.4 0.7 0.6	0.981 0.001 0.001
CP intake (g) Protein intake (g PDI ⁵)	2 799 1 690	2 787 1 681	2788 1682	2 778 1 674	35 27	0.870 0.876
Protein balance ³ (g PDI ⁵)	87	20	-20	12	42	0.056
Milk (kg/d) 4 % FCM (kg/d) Fat	26.7 ^b 24.7 ^b	27.6 ^a 25.7 ^a 978 ^a	28.1 ^a 24.3 ^b 867 ^c	26.3 ^b 22.8 ^c	0.4 0.4 23	0.003 0.001
g/kg	35.8ª	36.1ª	31.5 ^b	31.8 ^b	1.0	0.001
True protein g/d g/kg	762 ^c 28.9 ^b	799 ^b 29.2 ^b	825ª 29.6 ^{ab}	799 ^b 30.7 ^a	14 0.7	0.004 0.032

 1 C3 = propionic acid; 2 root mean square error; 3 without infusion; all the other results are with infusions; 4 difference between intake and requirement [17]; 5 protein digested in the small intestine [17].

a, b, c, d Means within rows with no common superscripts differ (P < 0.05).

rent types of case (α -, β - and κ -case in) and the Ca content and distribution (colloidal and soluble Ca) of the skimmed milk were unchanged (table V). The proportion of fatty acids changed with the increase in energy level. The proportions of $C_{18:1}$, and $C_{18:0}$ significantly decreased, whereas that of $C_{16:0}$ significantly increased (table VI).

Plasma acetate, glucose, and β-hydroxybutyrate contents (table VII) were slightly higher for cows infused with VFA mix-

	Basal		Infusion			
	treatment	VFA	C3 ¹	Glucose	RMSE ²	<i>P</i> <
Casein (g/kg)	23.4 ^b	23.6 ^{ab}	24.4 ^{ab}	25.4ª	0.7	0.024
Soluble proteins (g/kg)	5.5 ^b	5.6 ^b	5.8 ^{ab}	6.1 ^a	0.2	0.038
NPN (g/kg)	0.21	0.20	0.20	0.20	0.08	0.448
Colloidal Ca (mg/kg)	823	779	831	855	32	0.068
Lactose (g/kg)	45.5	47.3	48.1	45.1	3.6	0.635

Table V. Effect of level and nature of source of energy on skim milk composition.

^{a, b} Within rows, means with no common superscripts differ (P < 0.05); ¹C3 = propionic acid; ² root mean square error.

	Basal	4-1-1-10-1-man	Infusion			
	treatment	VFA	C31	Glucose	RMSE ²	<i>P</i> <
Fatty acids (%)						
C _{4:0}	2.52 ^a	2.43 ^{ab}	2.08 ^b	2.20 ^{ab}	0.15	0.023
C _{6:0}	1.83 ^a	1.80 ^a	1.57 ^b	1.77 ^a	0.07	0.005
C _{8:0}	1.21 ^b	1.17 ^b	1.06 ^c	1.26 ^a	0.04	0.002
C _{10:0}	3.14 ^b	3.20 ^b	3.30 ^b	3.83 ^a	0.15	0.002
C _{12:0}	3.97 ^b	4.14 ^b	4.18 ^b	5.28 ^a	0.18	0.001
C _{14:1}	1.47 ^b	1.35 ^b	1.34 ^b	1.84 ^a	0.20	0.037
C _{14:0}	13.0 ^{bc}	13.6 ^b	12.8 ^c	14.4 ^a	0.41	0.005
iso-C ₁₅	0.23	0.22	0.20	0.19	0.05	0.602
C _{15:1}	0.54	0.48	0.48	0.46	0.10	0.721
C _{15:0}	1.27 ^b	1.32 ^b	2.22 ^a	1.55 ^b	0.18	0.001
C _{16:1}	1.78	1.88	1.89	2.04	0.16	0.266
C _{16:0}	33.9 ^c	37.7 ^{ab}	36.3 ^b	38.5ª	0.91	0.002
iso-C _{17:0}	0.31	0.28	0.28	0.22	0.06	0.318
C _{17:1}	0.81	0.70	0.77	0.66	0.09	0.169
C _{17:0}	0.53	0.57	0.76	0.61	0.10	0.060
C _{18:2}	2.37	2.18	2.19	2.14	0.16	0.273
C _{18:1}	20.2 ^a	17.7 ^b	19.1 ^a	15.7°	0.65	0.001
C _{18:0}	10.9 ^a	9.3 ^b	9.5 ^b	7.3°	0.58	0.001
Fatty acids (g/d)						
$C_{4:0}$ to $C_{12:0}$	102 ^b	107 ^a	91 ^c	101 ^b	2.9	0.001
$C_{14:1} + C_{14:0}$	116 ^b	125 ^a	105 ^c	114 ^b	3.3	0.001
$C_{16:1} + C_{16:0}$	287 ^b	333 ^a	285 ^b	286 ^b	9.8	0.001
$C_{18:2} + C_{18:1} + C_{18:0}$	269 ^a	245 ^b	239 ^b	176 ^c	13.7	0.001

Table VI. Effect of level and nature of source of energy on milk fatty acids proportion.

^{a,b,c} Within rows, means with no common superscripts differ (P < 0.05); ¹C3 = propionic acid; ² root mean square error.

	Basal	<u> </u>	Infusion			
	treatment	VFA	C3 ¹	Glucose	RMSE ²	<i>P</i> <
Glucose (mg/100 mL)	69.9	74.2	73.4	72.5	3.0	0.281
NEFA (µmol/L)	138	85	94	82	58	0.532
Acetate (mg/100 mL)	3.8 ^b	4.4 ^a	3.0 ^c	2.9 ^c	0.2	0.001
β -hydroxybutyrate (mg/10	0 mL)3.9 ^a	4.3 ^a	2.4 ^b	2.7 ^b	0.3	0.001
Urea (mg/100 mL)	22.2	20.8	20.6	18.8	2.9	0.467

Table VII. Effect of level and nature of source of energy on blood parameters.

^{a,b,c} Within rows, means with no common superscripts differ (P < 0.05); ¹C3 = propionic acid; ² root mean square error.

ture, but only the increase in acetate was significant. Urea and total AA contents were unaffected, but the Glu and Gln concentrations were significantly increased (*table VIII*).

3.2. Effect of energy type

For cows receiving infusions of VFA mixture, C3 or glucose, the amount of consumed diet was the same. So the differences registered between the three treatments were only imputed to the nature of the energy infused.

As expected, the C3 treatment (versus the VFA mixture treatment) significantly increased ruminal C3 (29.8 % versus 20.9 %) at the expense of acetic acid (56.6 % versus 63.1 %), butyric acid (9.0 % versus 11.6 %), and caproic acid (0.3 % versus 0.5 %). The glucose treatment did not affect the VFA profile (*table III*).

Relative to the VFA mixture treatment, the C3 treatment significantly increased the protein yield (26 g/d), although both milk yield and protein content were scarcely changed. In contrast, the glucose treatment did not alter protein yield, because milk yield decreased (1.3 kg/d), and protein content increased (1.5 g/kg), both significantly. Proportions of different types of casein (α -, β - and κ -casein) did not change for cows infused with either C3 or glucose. Soluble proteins significantly increased for cows infused with glucose (0.5 g/kg). Colloidal calcium tended to increase for cows infused with either treatment (*table* V). Both C3 and glucose treatments significantly decreased the fat content (close to 4.5 g/kg) and yield (111 g, C3 treatment; 160 g, glucose treatment) (*table IV*).

Although fat content was similarly decreased, the secretion of individual fatty acids (g per d) was affected differently by the C3 or glucose treatments, except for C4:0, which was largely decreased for cows infused with either treatment (figure 1 and table VI). For the even-numbered fatty acids, the C3 treatment (versus VFA treatment) depressed the secretion of C6:0, C8:0, C_{14} , and C_{16} (g/d). Conversely, with the glucose treatment (versus VFA treatment), the large decrease observed in C4:0 secretion was gradually counterbalanced by increases of other short and medium fatty acids. Consequently, the differences between VFA mixture and glucose infusions decreased as the chains of fatty acids increased and even for C12:0, glucose infusion induced an increase compared to VFA infusion. A reverse trend occurred for C₁₈ so that its secretion was largely depressed in contrast to what occurred following the C3 infusion, which had no effect on C_{18} yield (figure 1). The odd-numbered fatty acids (mainly C_{15}) were significantly increased following the C3 treatment but not for the glucose treatment.

	Basal		Infusion			<u>, 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</u>
	treatment	VFA	C3 ¹	Glucose	RMSE ²	<i>P</i> <
EAA^3 (mg/100 mL)		d				
Lys	1.37	1.26	1.04	1.09	0.27	0.386
His	0.85	0.88	0.82	0.84	0.11	0.886
Arg	1.65	1.49	1.28	1.36	0.29	0.369
Thr	1.14	1.21	1.15	1.20	0.20	0.949
Val	3.01	2.75	2.19	2.13	0.41	0.057
Met	0.31	0.31	0.31	0.31	0.05	0.998
Ile	1.42	1.39	1.03	1.03	0.20	0.058
Leu	1.71	1.58	1.16	1.18	0.28	0.076
Phe	0.90	0.92	0.79	0.85	0.14	0.588
Total	12.4	11.8	9.8	10.0	1.82	0.216
NEAA ⁴ (mg/100 mL)						
Asp	0.06	0.06	0.10	0.05	0.06	0.695
Asn	0.75	0.83	0.75	0.83	0.12	0.724
Ser	1.31	1.25	1.17	1.28	0.20	0.793
Glu	0.49 ^b	0.61 ^a	0.51 ^b	0.51 ^b	0.04	0.026
Gln	2.16 ^b	2.64 ^a	2.84 ^a	2.73 ^a	0.24	0.027
Gly	3.66	3.51	3.31	4.17	0.58	0.287
Ala	2.20	2.29	1.88	1.84	0.28	0.149
Tyr	0.71	0.71	0.65	0.72	0.14	0.869
Cys	0.61	0.60	0.65	0.62	0.08	0.867
Tau	0.59	0.43	0.51	0.43	0.13	0.434
Orn	0.85	0.74	0.65	0.74	0.14	0.345
Cit	1.43	1.16	1.18	1.26	0.21	0.348
Total	12.7	12.2	11.4	12.4	1.64	0.704
Total AA	25.0	24.0	21.1	22.4	3.37	0.443

Table VIII. Effect of level and nature of source of energy on blood plasma AA.

^{a,b} Within rows, means with no common superscripts differ (P < 0.05); ¹C3 = propionic acid; ² root mean square error; ³ essential amino acids; ⁴ non-essential amino acids.

The C3 and glucose treatments had the same effects on energy and plasma nitrogen metabolites. Relative to the VFA mixture treatment, C3 and glucose treatments did not affect the NEFA and glucose contents, but both significantly decreased the β -hydroxybutyrate and acetate contents (*table VII*). The C3 and glucose treatments had no effect on plasma urea and tended to decrease total essential AA in plasma. Contents of branched AA tended to decrease for cows infused with either C3 or glucose (respectively, 1.3 and 1.4 mg/100 mL; P < 0.063). Among the nonessential AA, the concentration of Glu

decreased, and that of Ala tended to decrease (P < 0.149) (*table VIII*).

3.3. Milk coagulation properties

The increase in energy had no effect on milk coagulation properties, except on curd firmness, which significantly increased at standardized pH with the VFA mixture treatment (*table IX*).

The source of energy had no effect on curd yields. At the initial pH, the C3 treatment did not change milk coagulation properties, but the glucose treatment signifi-



Figure 1. Responses in the individual fatty acid yields of milk to C3 (\Box) or glucose (\blacksquare) infusions as compared to VFA infusions (** : P < 0.01; * : P < 0.05; NS : P > 0.05).

	Basal		Infusion			
	treatment	VFA	C31	Glucose	RMSE ²	P <
Curd yield (%)						
Fresh	13.3	13.7	14.3	14.9	1.2	0.328
N	76.5	76.8	77.2	77.5	0.7	0.578
Dry	39.2	39.1	40.6	40.7	2.1	0.303
Coagulating properties						
At initial pH	6.65	6.67	6.66	6.63	0.02	0.117
Rennet clotting time, min	15.0 ^a	14.8 ^a	14.7 ^a	13.2 ^b	0.5	0.009
Rate of firming, min	7.5 ^a	7.2 ^a	6.7 ^a	5.2 ^b	0.6	0.006
Curd firmness, mm	33.4 ^b	36.5 ^{ab}	37.9 ^{ab}	43.5 ^a	3.6	0.036
At standardized pH ³						
Rennet clotting time, min	8.7	8.4	7.6	8.5	1.1	0.505
Rate of firming, min	4.3	4.1	3.6	3.3	0.5	0.070
Curd firmness, mm	47.7 ^c	50.4 ^b	53.6 ^a	55.1ª	1.8	0.004

Table IX. Effect of level and nature of source of energy on milk coagulation properties.

^{a,b,c} Within rows, means with no common superscripts differ (P < 0.05); ¹C3 = propionic acid; ² root mean square error; ³ pH = 6.5.

cantly decreased rennet clotting time and firming rate. At the standardized pH, both the C3 and glucose treatments increased curd firmness (*table IX*).

4. DISCUSSION

The VFA pattern and glucose availability were significantly modified by the C3 and glucose infusions, although these changes were not unusual when compared with some types of diets, either with high levels of concentrate [4] or with rapidly degradable starchy concentrates [30].

Under our experimental conditions, the ruminal VFA profiles observed did not indicate any modification in the fermentation products of the feed portion when extra VFA were infused. Similar observations have been made previously [35]. not only with regards to VFA but also for ruminal liquid turnover and duodenal AA flow. It can also be reasonably assumed that the absorption of the extra VFA was complete. The absorption of the infused glucose should also have been nearly complete. No disturbance was observed regarding the glucose absorption, and a 73-90 % absorption rate has been previously found with similar doses of glucose [22, 23]. Therefore, it can be reasonably assumed that glucogenic nutrients were increased by C3 or glucose infusions.

4.1. Protein synthesis

Increasing the energy level (from the basal treatment to VFA mixture treatment) or modifying ruminal fermentation products with the same total energy supply (from VFA mixture treatment to C3 treatment) had an almost identical effect on the milk protein synthesis (37 g for cows administered the VFA mixture treatment and 26 g for cows infused with C3). These slight increases in protein synthesis appeared to have different causes. The increase in energy level was reflected by an increase in milk yield and a small increase in protein content (0.1 g/kg for each extra)Mcal of energy). That increase in protein content, induced by the higher energy level, was slightly less than that induced by concentrates (0.15 to 0.3 g/kg for each extra Mcal of NE_I) [9, 32, 39] In our trial, when changing from the basal treatment to VFA mixture infusion, only the energy level was altered, without any effect on AA supply or on microbial synthesis. The increase in protein content could only be linked to a higher ATP availability for milk protein synthesis. This contrasts with the effects of energy concentrates that modify ATP and AA together. Moreover, our treatment periods were relatively short (14 days) compared to trials on energy concentrates (often at least 4 weeks) that could also explain the small effect of energy level on milk synthesis.

The slight increase (26 g/d) in protein synthesis recorded for the cows infused with C3, relative to that for cows infused with VFA mixture, was in the same range than previously recorded responses (from -56 to 72 g/d; table X gathering trials where intake was not dramatically decreased by energy treatments). In view of the results of table X, protein synthesis appeared not to be improved by increasing ruminal C3 level in isoenergetic situations (mean value, -30 g/d) ([5] (trial 1); [13, 14] and the result of this trial). The increased energy level due to C3 infusions seemed to have a slight positive effect on protein synthesis (mean value 34 g/d) ([5] (trial 2); [33, 34, 42]. This increase (8.3 g of protein/ Mcal NE_L added) is even lower than that obtained by increasing energy level with the VFA mixture in this trial (11.2 g of protein/ Mcal NE_{I} added).

The response of milk protein yield (0 g/d) observed in this trial with glucose infusion is rather lower than responses generally observed in identical energetic

ReferencesForagePropiand trials(Mcal)1Hay and corn silage22Corn silage33Trial 1Grass silage1	pionate 1 NEL/d) 2.7	Control							
ReferencesForagePropiand trials(Mcal)1Hay and corn silage22Corn silage33Trial 1Grass silage1	pionate I NEL/d) 2.7		values			Fa	ţ	Prot	ein
1Hay and corn silage22Corn silage33Trial 1Grass silage1	2.7	Milk yield (kg/d)	Fat content (g/kg)	Energy supply (Mcal NEL/d)	Milk yield (kg/d)	Content (g/kg)	Yield (g/d)	Content (g/kg)	Yield (g/d)
2 Corn silage 3. 3 Trial 1 Grass silage 1.		21.0	43.0	0.5	- 2.0	- 5.0	- 180	1.2	- 43
3 Trial 1 Grass silage 1.	3.6	26.5	37.9	-1.0	- 1.7	- 5.1	- 190	- 0.4	- 56
	1.7	12.6	43.9	1.4	- 1.4	- 3.7	-103	0.0	- 45
Mean value 2.	2.7	20.0	41.6	0.3	- 1.7	-4.6	- 158	0.3	- 48
3 Trial 2 Grass silage 2.	2.3	17.8	44.7	3.7	0.9	- 6.2	- 76	0.1	31
4 Trial 1 Hay 3.	3.2	10.3	34.2	3.2	0.9	- 3.2	0	1.4	50
Trial 2 3.	3.7	14.5	38.7	3.9	- 0.6	- 2.9	- 59	2.6	28
Trial 3 5.	5.3	8.3	30.8	5.4	0.1	- 3.6	- 28	2.4	12
5 Trial 1 Hay 2.	2.3	16.3	37.8	2.4	- 1.1	- 2.1	-41	pu	pu
Trial 2 5.	5.6	17.4	33.8	5.6	-2.1	- 0.9	- 48	4.0	0
6 Trial 1 Hay 2.	2.6	9.7	49.8	2.7	1.3	- 9.0	- 55	0.2	45
Trial 2 2.	2.6	9.9	46.2	pu	1.2	- 4.0	- 18	3.0	72
Mean value 3.	3.4	13.0	39.5	4.3	0.1	- 4.0	- 41	2.0	34
This study Corn silage 3.	3.6	27.6	36.1	0.1	0.5	- 4.6	- 111	0.4	26

Table X. Effect of propionic acid infusions on milk yield, protein content and yield, fat content and yield in the literature.

situations (mean value, 18 g/d; table XI) except for Clark et al. [6] (-20 g/d). However, in high energetic situations [27, 43], protein yield decreased with glucose infusions. This decrease could be related to the negative effect on milk yield that seemed to be observed when glucose is infused with a corn based diet [24, 27] or with diets rich in concentrates [29, 43]. This trial does not bring any support to the hypothesis that increasing C3 or glucose supply could spare glucogenic AA and hence increase milk protein content. Indeed, all the infusions (VFA mixture, C3 and glucose) provided the same amounts of energy and PDI and they did not increase the level of glucogenic AA in plasma comparatively to basal treatment. In contrast, the amount of branched AA decreased as for Clark et al. [6], Whitelaw et al. [43] and Dhiman et al. [8] with glucose infusions. These AA were perhaps used preferentially for muscle synthesis [3] under hormonal control.

The infusion of C3 or glucose was presumably the cause of increased rate of glucose supply as for Judson and Leng [20] and Seal and Parker [38] with C3 infusion and as for Clark et al. [6] and Amaral et al. [1] for glucose infusion. The difference between these two energy nutrients could be linked to the very nature of each one and to their infusion sites (rumen or intestine). Indeed, by comparing intravenous infusions of glucose and C3, Fisher and Elliot [10] found no significant and no different effect of these nutrients on milk protein content. However, this lack of difference in the intravenous comparison could be related to the same infusion sites and to short periods of infusion.

4.2. Fat synthesis

Decreasing the energy level, switching from acetic to propionic fermentation and increasing the duodenal glucose flux induced a decrease in fat synthesis. Under the basal treatment, cows were energy deficient and mobilized much body fat. The milk fat became rich in long-chain fatty acids at the expense of the short- and medium-chain fatty acids in response to the variations of their respective plasma precursors (acetate, β -hydroxybutyrate, NEFA). These modifications agree with that classically observed when cows are underfed [19].

Infusion of C3 or glucose induced similar decreases in fat content (-4.5 g/kg), but the decrease of fat yield, as in Fisher and Elliot [10], was more important for cows infused with glucose (-160 g versus -111 g for C3 infusion) because of the concomitant decrease in milk yield in our trial. Such decreases are similar to the published data for fat content but much greater for fat yield as summarized for C3 infusions (*table XI*) ([5] (trial 2); [6, 11, 24, 27, 29, 33, 34, 41-43]) except in isoenergetic situations with C3 infusions [5] (trial 2); [13, 14] (*table X*).

With glucose infusion, as opposed to VFA mixture infusion, and keeping in mind the unchanged supply of feed and microbial lipids, the differences in milk fatty acids patterns appear to indicate that: 1) the net contribution of body lipids to milk fat synthesis was greatly depressed perhaps because of lower lipolysis or greater lipogenesis [21] as indicated by a decrease in C18; 2) de novo synthesis was also depressed, but to a much lesser extent; 3) the elongation process was much more active as shown by the increase in intermediate chain length fatty acids $(C_{12:0})$ and the concomitant large decrease in the very short-chain fatty acids. This could also result partly from the lowered availability of precursors (acetate and β -hydroxybutyrate). The increased activity of the elongation process could also have affected positively the synthesis of C_{14} and C_{16} . However, because of their dual origin [26], and of the postulated decrease

			Contro	l values			Fa	t	Prot	ein
References and trials	Forage	Propionate (Mcal NEL/d)	Milk yield (kg/d)	Fat content (g/kg)	Energy supply (Mcal NEL/d)	Milk yield (kg/d)	Content (g/kg)	Yield (g/d)	Content (g/kg)	Yield (g/d)
1 Trial 1	Alfalfa hay	1.2	28.8	36.1	0.7	0.1	- 0.7	- 13	- 0.8	- 20
Trial 2		1.2	31.0	36.3	1.0	0.6	- 3.7	- 112	0.2	32
2 Trial 1	Hay and corn	0.8	15.5	40.6	0.1	0.9	- 3.5	-21	- 0.3	28
Trial 2	silage	1.3	23.3	28.5	0.1	0.6	- 2.7	- 47	0.8	37
3	Dehydrated corn	4.1	26.3	39.5	- 0.1	- 0.8	- 3.6	- 124	1.5	14
4	Barley straw	0.8	14.5	49.3	0.7	- 0.4	-2.0	L –	0.6	16
Mean value		1.6	23.2	38.4	0.4	0.2	- 2.7	- 54	0.3	18
5	Alfalfa hay	5.9	26.7	32.9	3.2	1.9	-3.1	- 137	- 1.4	26
6	Corn silage	2.7	31.0	30.0	2.6	- 0.8	- 1.0	- 30	0.0	- 20
7	Barley straw	2.2	13.0	45.9	2.4	- 0.7	- 3.8	- 85	0.7	6 -
Mean value		3.6	23.6	36.3	2.7	0.1	- 2.6	-84	- 0.2	ī
This study	Corn silage	3.6	27.6	36.1	0.1	- 1.4	- 4.3	- 160	1.5	0

Table XI. Effect of glucose infusions on milk yield, protein content and yield, fat content and yield in the literature.

Dietary energy and milk composition

of adipose tissue lipolysis or increase of lipogenesis, such an effect would be masked. With the C3 infusion, those changes appear to be intermediate. In particular, the net contribution of body lipids to milk fat was less affected than it had been with glucose infusion. Some of the energy supplied in the form of C3 might not have participated in hepatic neoglucogenesis and so would have been a limiting factor in short-chain fatty acid synthesis in the udder because of the reduction of energy under the form of glucose. Conversely, circulating C3 not captured in the liver might have been utilized in priority by the udder for synthesizing odd-numbered fatty acids, C_{15} in particular [14], because C_{17} has a dual origin [25]. Results from Hurtaud et al. [14] and Lemosquet et al. [24] give similar support to the above possibilities.

4.3. Milk coagulation properties

Level and nature of energy did not have a major effect on milk coagulation properties. At the initial pH, the significant effect of glucose infusion was only due to a slight decrease in milk pH [31]. At a standardized pH, the improvement in curd firmness with the change of nature of energy (C3 or glucose versus VFA mixture infusion) seemed to be a consequence of the increase in protein, casein and colloidal calcium [15, 31].

5. CONCLUSION

Changes in milk protein secretion or protein content with altered energy nutrient patterns in isoenergetic situations were not very large, nor consistent as they differed between C3 and glucose treatments. No support was found for the hypothesis that sparing glucogenic AA by providing glucogenic nutrients to the liver would result in an increased protein secretion. Both glucogenic nutrients had a similar adverse effect on fat contents but acted through different metabolic pathways. It was postulated that adipose tissue lipolysis or lipogenesis but also fatty acid elongation differed according to the energy nutrient patterns. Further information about hormonal control is necessary to complete the picture.

Concentrate manipulation (more concentrate or improved forage intake) increasing the energy supply, seems to be a better way to increase protein secretion. Experimentally, these conclusions seemed to be true for corn based diets with medium levels of concentrates which provided significant amounts of glucogenic nutrients.

Further work is required with other diets differing in starch content and degradability (as for instance grass silage). In order to manage the fat yield that is used at the basis of milk quota determination, it could also be of interest to explore the relationships between doses of glucogenic nutrients (as C3 or glucose) and secretion of fat in the milk of dairy cows.

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