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Original article

Effect of duodenal perfusion of protein on the intake of dairy cows with or without incomplete milking

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Abstract — The protein content of the diet modifies feed intake in lactating dairy cows, but the mechanisms are unclear. The objective of this trial was to determine whether a duodenal perfusion of protein, with or without the expected increase of milk production, plays a role in the feed intake response. Treatments were (C): control perfusion (500 g·d⁻¹ of glucose + 145 g·d⁻¹ of urea); (P): duodenal perfusion of soy-protein isolate (500 g·d⁻¹); and (Pim): the same treatment as (P) but with milk yield limited to the control by incomplete milking. Six rumen and duodenal-cannulated cows producing 35 kg·d⁻¹ of milk were assigned in a double 3 × 3 Latin square design with periods of 4 weeks and inter-periods of 2 weeks. The intake, number and size of the meals, intake duration, chewing activities, digestibility (DM, OM, CP, NDF and ADF), molar proportions of volatile fatty acids and ammonia were not significantly affected by protein perfusions (P and Pim) ($P > 0.05$). Duodenal protein perfusion (P) significantly increased ($P < 0.05$) milk yield (+ 4.7 kg·d⁻¹), protein production (+ 160 g·d⁻¹) and protein content of milk (+ 1.8 g·kg⁻¹) in comparison to the control perfusion (T). With incomplete milking (Pim), the milk production was the same as in the control but protein content increased (+ 1.9 g·kg⁻¹) and fat content decreased (-3.2 g·kg⁻¹). Incomplete milking did not reduce intake, suggesting that mechanisms controlling intake and milk production were partly dissociated.

dairy cow / intake / protein / incomplete milking

Résumé — Effet d'un supplément duodéal de protéines sur l'ingestion des vaches laitières avec ou sans traite incomplète. L'équilibre protéique d'un régime alimentaire constitue un moyen important de réguler l'appétit des vaches laitières, mais les mécanismes sont encore mal connus. L'objectif de cet essai est de voir si l'apport direct de protéines dans le duodénum permet d'accroître les quantités ingérées et si l'augmentation de production laitière attendue est à l'origine de la réponse sur l'ingestion. Trois traitements ont été réalisés : (C) une perfusion duodénale témoin (500 g·j⁻¹ de glucose + 145 g·j⁻¹ d'urée), (P) une perfusion duodénale d'isolats de protéines de soja (500 g·j⁻¹) et (Pim) le même traitement que (P) mais en limitant la production de lait à celle du témoin grâce à une traite incomplète. Six vaches laitières fistulées du rumen et du duodénum, produisant en moyenne 35 kg de lait par jour, ont été réparties dans un schéma en double carré latin 3 × 3 avec des périodes

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de 4 semaines et des interpériodes de 2 semaines. Les quantités ingérées, la cinétique d'ingestion, la vitesse d'ingestion, le nombre de repas, la digestibilité des MS, MO, MAT et les paramètres biochimiques du rumen n'ont pas été affectés significativement par l'apport de protéines (P, Pim) ($P > 0,05$). La production laitière, le taux protéique et les matières protéiques du lait du traitement P ont augmenté significativement ($P < 0,05$) par rapport au traitement témoin de 4,7 kg ; 1,8 g·kg⁻¹ et 160 g·j⁻¹ respectivement. Par contre, avec les traites incomplètes, la production de lait a été proche de celle du témoin, mais le taux protéique a été accru de 1,9 g·kg⁻¹ et le taux butyreux a diminué de 3,2 g·kg⁻¹. La réduction de la production par la traite incomplète n'ayant pas modifié l'ingestion, ceci suggère que les mécanismes régulant la production laitière et les quantités ingérées sont en partie dissociés.

vache laitière / ingestion / protéine / traite incomplète

1. INTRODUCTION

The levels and sources of protein offered to dairy cows in early lactation could affect both milk production [1, 7, 23, 28] and feed intake. The analysis of the literature shows an increase of DMI by an average of 0.4 kg per 1 percentage point when dietary CP (expressed as % DM) increases from 12.5% to 15.7%. Above 16% CP (% DM), DMI still increases, but at a rate reduced by half [22]. The effect of dietary proteins on feed intake could be explained either by their effect on microbial fermentation and digestion or by the effect of the amino acid supply on whole animal metabolism and yield [22].

Intake improvements with metabolisable protein supply to dairy cows are, however, matter of debate and the mechanisms have not yet been demonstrated. Some authors have not observed any change [11, 16, 17, 31], while others have recorded significant decreases [6, 15] in intake. On the contrary, significant increases in DMI have been shown. They are proportional to the levels of incorporation of rumen undegradable protein into the ration [3, 25, 28]. Improvement of milk yield observed in such trials could explain the positive DMI response observed [4]. The objectives of this trial were to determine (1) the effect of improved protein availability via duodenal infusions of soya proteins on the appetite

of high producing dairy cows, without modification of ruminal digestion and (2) if the possible positive effect on appetite is specific to an improved protein availability or if it depends on an increase of milk production.

2. MATERIALS AND METHODS

2.1. Treatments, experimental design and animals

Six multiparous Holstein cows were assigned to three treatments: (i) control (C) consisting of duodenal perfusion of glucose (500 g·d⁻¹) and urea (145 g·d⁻¹); (ii) duodenal perfusion of soy-protein isolate (P) (500 g·d⁻¹), and (iii) the same duodenal perfusion as (P), but with incomplete milking (Pim) in order to adjust milk yield to the control one. The levels of glucose and urea were calculated in order to supply similar amounts of nitrogen and energy in the control and protein perfusion groups. The cows were fitted with ruminal and duodenal cannulas and weighed on the average 592 kg. At the beginning of the experiment, the cows were in their 10th to 15th weeks of lactation and produced an average 35 kg milk·d⁻¹. The experimental design was a double 3 × 3 Latin square equilibrated for residual effects. Each experimental period lasted four weeks with inter-periods of two weeks.

The protein perfusion consisted of partially hydrolysed isolates of soya protein

enriched with methionine, lecithin and zinc sulphate (Nurish 1500, International Protein Technologies). Protein infusions were prepared daily by diluting 500 g of soya protein in 10 L of water at approximately 30 °C. The mean pump flow used in this trial was 435 mL·h⁻¹ for approximately 23 hours a day. The remaining hour was used for maintenance of the technical equipment and preparation of infusion solutions. The control infusion was prepared in the same way.

The cows were milked twice daily at 07.00 and 17.00. Each milking was stopped in the (Pim) treatment group when the amount of milk collected reached the preplanned value. This value was calculated to produce similar milk amounts in the control and (Pim) treatment groups. At the beginning of each period, the previous week was considered as a reference for this period. Then a persistency of 97% per week was used to calculate the daily milk amounts of each (Pim) experimental period. This value was split between morning and evening milkings according to the proportion of complete milking.

Cows were fed a total mixed diet twice daily at 07.30 and 17.30. Ad libitum conditions were respected with 10% refusals. The diet consisted of a complete ration containing maize silage (60%), dehydrated maize (14%) and lupin concentrate (25%). The total mixed ration was formulated to allow 6.94 MJ of NE_L, 421 g of NDF, 209 g of ADF, 125 g of CP, 76 g of protein truly digested in the small intestine (PDI) according to ruminal available energy, and 75 g of PDI according to fermentable N per kilogram of DMI.

2.2. Experimental procedure

The first two weeks of each experimental period were allowed for the adaptation to the treatments while data from the last two weeks were used for analyses. Samples of maize silage and refusals were taken and

weighed daily to determine DM content and calculate DMI. The dry samples were grouped according to the cow during the week of digestibility measurements in order to determine their chemical composition (OM, CP, NDF, ADF and ADL). Similarly, the samples of concentrates were made up to be used for the same chemical analyses as well as for the measurement of *in sacco* degradability. All these measurements allowed the calculation of the nutritive values of feeds.

The feeding behaviour was measured daily during each pre-experimental week and during the 3rd week of each period by mangers placed on sensors fitted with strain gauges and connected to a microcomputer according to the protocol described by Baumont [5]. Milk yields were recorded at each milking, and samples were collected three days per week to determine fat and protein contents. Another sample of 1 kg of milk was taken during the 3rd week of each period and analysed in order to determine the proportions of fatty acids. The animals were weighed after morning milkings the first day of each period as well as the first day of each inter-period. A condition score was attributed after each weighing.

The digestibility of the ration was measured during 5 days of the 4th week of each period. During this week each stall was equipped with a filter in order to separate faeces from urine which was not considered in the calculation of digestibility. The faeces of each cow were collected daily at 09.00, and were then homogenised and sampled (1% of the fresh weight). In the same way, samples of the refusals (1 kg) were taken. These various samples (refusals and faeces) were dried at 80 °C for 48 h, grouped by cow and by period, and ground through a 0.8 mm mesh screen.

During the same week, 9 rumen fluid samples were taken from the ventral sac every 2 hours from 07.30 to 23.30 and the last one at 05.30. Rumen fluid pH was determined immediately with a glass electrode, and samples were acidified and frozen until

analysis for volatile fatty acids and ammonia. Nine samples of jugular vein blood were collected every 2 hours as for the rumen fluid samples. Blood was collected in heparinised tubes for the analysis of plasma concentration of free amino acids and EDTA tubes were used to determine the plasma concentrations of insulin, NEFA, urea, albumin and proteins.

2.3. Chemical analyses

Food, refusals and faeces samples were analysed for OM, CP, NDF, ADF, and ADL according to the analytical methods of the AOAC [2]. VFA concentration and composition was determined by gas chromatography as described by Jouany [21]. Ammonia was determined using the method of Berthelot adapted to an auto-analyzer (Technicon). The protein and fat contents of milk were measured with an infrared analyser (Milkoscan, Foss Electric, Hillerd, Denmark). Butyl esters of milk fatty acids were determined by gas chromatography using a 25 m × 0.32 mm fused silica capillary column and helium as the carrier gas according to the method described by Mielke and Schingoethe [24]. Insulin concentration was determined by a radioimmunoassay as described by Faverdin et al. [14]. Blood was prepared and analysed for blood concentrations of glucose, NEFA, urea, and amino acids as described by Guinard et al. [18].

2.4. Statistical analyses

Data were analysed by the GLM procedure of SAS [26]. The intake and production data during the 3rd week of each period were subjected to covariate analysis using data of the 2nd week of each inter-period as the covariants. When the Fisher tests of treatments were significant ($P < 0.05$), the adjusted means were compared using the Student test. The experimental model was the following:

$$Y_{ijk} = \mu + T_i + P_j + V_k + b \cdot C_{ijk} + e_{ijk}$$

where Y_{ijk} = dependent variable for cow k receiving treatment i during the period j covariating to the pre-treatment effect ijk ; μ = mean; T_i = treatment effect; P_j = period effect; V_k = cow effect; $b \cdot C_{ijk}$ = pre-treatment effect; e_{ijk} = residual.

Data concerning blood parameters, rumen parameters, digestibility, milk fatty acids, and plasma amino acids were analysed using the following model:

$$Y_{ijk} = \mu + T_i + P_j + V_k + e_{ijk}$$

where Y_{ijk} = dependent variable for cow k receiving treatment i during period j ; μ = mean; T_i = treatment effect; P_j = period effect; V_k = cow effect; e_{ijk} = residual.

Data were excluded from the statistical analysis: one cow was excluded for hepatic insufficiency, and another cow was removed only during the 3rd period because of arthritis.

3. RESULTS

3.1. Intake and behaviour-related activities

No effect of protein treatments (P and Pim) on appetite was observed (Tab. I). The intake kinetics for one day and during the two hours following the first morning distribution were not significantly modified by treatments ($P > 0.05$). The number of meals (8.4 on average), the total duration of ingestion (392 min on average), the duration of the meal (48 min on average) and the rate of ingestion (47.7 g DM·min⁻¹ on average) were not affected by the treatments ($P > 0.05$). In the same way, no significant difference was recorded for the total duration of rumination (556 min on average), the number of ruminating periods (12.3 on average) nor the ruminating time per kg of DM (30 min on average) ($P > 0.05$).

Table I. Effects of protein infusion in the duodenum or incomplete milking on responses of feed intake and lactational performance of dairy cows.

	C ¹	P ²	Pim ³	R.S.D. ⁴
DM intake (kg·d ⁻¹)	18.3	18.6	18.3	0.74
Milk yield (kg·d ⁻¹)	27.8 ^a	32.1 ^b	26.7 ^a	0.98
4% FCM yield (kg·d ⁻¹)	25.5 ^a	28.7 ^b	24.6 ^a	1.18
Fat yield (g·d ⁻¹)	941	1061	908	87
True protein production (g·d ⁻¹)	787 ^a	965 ^b	819 ^a	26
Milk composition				
Fat (g·kg ⁻¹)	34.1	32.9	32.1	4.15
True protein (g·kg ⁻¹)	28.3 ^a	30.1 ^b	30.1 ^b	0.68
Nutrient balance				
NEL (UFL·d ⁻¹) ⁵	1.2	0.4	1.7	1.1
PDI (g·d ⁻¹)	-255.9 ^a	-100.3 ^b	100.0 ^c	47.9

¹ Control; ² 500 g·d⁻¹ of soya protein; ³ 500 g·d⁻¹ of soya protein with an incomplete milking; ⁴ Residual standard deviation; ⁵ Estimated using the equations of Vermorel [29] and expressed according to the french feed unit systems, 1 UFL = 7.11 MJ of NEL.

^{a,b,c} Within rows, means with no common superscript differ ($P < 0.05$).

3.2. Milk production

Protein perfusion (P) significantly increased ($P < 0.05$) milk production, 4% fat corrected milk production (FCM), protein content, and protein yield (Tab. I). Fat production and fat content of milk were not significantly modified by the treatments ($P > 0.05$). With the (Pim) treatment, milk production did not differ from the control, but the 4% FCM yield was significantly decreased because of the low fat content ($P > 0.05$). On the contrary, protein content was significantly higher with incomplete milking than with the control. Whatever the mode of milking, the proportions of palmitic acid (C₁₆) were significantly decreased by protein perfusion, whereas the fatty acids with shorter (C₁₀, C₁₂) and longer chains (C_{18:0}; C_{18:2}) were increased (Tab. II).

3.3. Ruminal fermentation and digestibility

Ruminal pH, total VFA concentration (109 mmol·L⁻¹ on average), as well as the

relative proportions of major and minor VFA, were not affected ($P > 0.05$) by the different treatments. The acetate:propionate ratio (2.4 on average) and the ruminal ammonia concentration (106 mg·L⁻¹ on average) were not affected ($P > 0.05$). No significant difference was observed for the digestibilities of DM (72.4% on average), OM (73.1% on average), CP (66.6% on average), NDF (50.6% on average) and ADF (48.5% on average).

3.4. Blood parameters

The plasma concentrations of glucose, NEFA, insulin, protein and albumin were not modified significantly by the various treatments ($P > 0.05$) (Tab. III), but the blood urea concentration was lower ($P > 0.05$) in the cows receiving the protein perfusions (Tab. III). Plasma concentrations of most of the essential amino acids were significantly higher ($P < 0.05$) in the cows receiving protein treatments (Tab. IV). This was also true for alanine and ornithine. Plasma concentrations of glutamine, glycine, and

Table II. Effect of protein infusion in the duodenum or incomplete milking on the composition of milk fatty acids.

Milk fatty acid (g·100 g ⁻¹ of total fatty acids)	C ¹	P ²	Pim ³	R.S.D. ⁴
C _{4:0}	2.7	2.8	2.8	0.26
C _{6:0}	2.0	2.1	2.1	0.17
C _{8:0}	1.3	1.4	1.4	0.11
C _{10:0}	3.1 ^a	3.5 ^b	3.4 ^{ab}	0.19
C _{12:0}	4.0 ^a	4.4 ^b	4.4 ^b	0.22
C _{14:0}	13.1	13.4	13.1	0.45
C _{15:0}	1.3	1.2	1.1	0.27
C _{16:0}	33.4 ^a	29.5 ^b	28.7 ^b	1.84
C _{16:1}	1.8 ^a	1.4 ^b	1.5 ^b	0.14
C _{17:0}	0.6	0.5	0.5	0.08
C _{18:0}	9.4 ^a	11.3 ^b	10.9 ^{ab}	0.85
C _{18:1}	18.2	18.2	20.3	1.21
C _{18:2}	2.0 ^a	2.5 ^b	2.8 ^b	0.24
Sum C _{4 to 12}	13.2	14.2	14.0	0.85
Sum C ₁₈	34.7 ^a	37.8 ^b	39.0 ^b	1.33

¹ Control; ² 500 g·d⁻¹ of soya protein; ³ 500 g·d⁻¹ of soya protein with an incomplete milking; ⁴ Residual standard deviation.

^{a,b,c} Within rows, means with no common superscript differ ($P < 0.05$).

Table III. Effect of protein infusions in the duodenum or incomplete milking on some blood parameters.

	C ¹	P ²	Pim ³	R.S.D. ⁴
Urea (g·L ⁻¹)	0.34 ^a	0.25 ^b	0.28 ^b	0.03
Glucose (g·L ⁻¹)	0.64	0.61	0.64	0.02
NEFA (mol·L ⁻¹)	97.1	102.2	99.9	8.4
Protein (g·L ⁻¹)	84	93	92	3.9
Albumin (g·L ⁻¹)	42	42	43	0.8
Insulin (ng·L ⁻¹)	1.02	0.96	1.06	0.17

¹ Control; ² 500 g·d⁻¹ of soya protein; ³ 500 g·d⁻¹ of soya protein with an incomplete milking; ⁴ Residual standard deviation.

^{a,b,c} Within rows, means with no common superscript differ ($P < 0.05$).

serine were significantly higher when cows received the control perfusion ($P < 0.05$). Only the plasma concentrations of threonine, glutamic acid, and tyrosine showed no significant variation ($P > 0.05$) (Tab. IV).

4. DISCUSSION

The objective of this trial was to test the impact of duodenal protein supplementation

on the intake of dairy cows and to examine the relationship between an increased intake and milk production. The assumption of a relationship between appetite and metabolisable protein supplementation was not confirmed in this trial. As a consequence, the role of milk yield in the increase of DMI associated to a higher amino acid supply could not be elucidated. This absence of any effect is discussed further, below. A relative independence was, however, observed

Table IV. Effect of protein infusions in the duodenum or incomplete milking on the composition of plasma amino acids.

	C ¹	P ²	Pim ³	R.S.D. ⁴
Essential amino acids (mg·100 mL ⁻¹)				
Arg	0.74 ^a	0.90 ^b	1.07 ^c	0.034
His	0.16 ^a	0.48 ^b	0.57 ^b	0.103
Ileu	0.68 ^a	1.07 ^b	1.24 ^c	0.045
Leu	0.64 ^a	0.98 ^b	1.20 ^c	0.108
Lys	0.49 ^a	0.67 ^b	0.82 ^c	0.049
Met	0.23 ^a	0.50 ^b	0.55 ^b	0.047
Phe	0.49 ^a	0.52 ^b	0.63 ^c	0.043
Thr	0.86	0.90	0.84	0.070
Val	1.17 ^a	1.81 ^b	1.96 ^c	0.069
Non essential amino acids (mg·100 mL ⁻¹)				
Ala	1.41 ^a	1.64 ^b	1.70 ^b	0.098
Cit	1.34	1.26	1.39	0.092
Gln	3.22 ^a	2.70 ^b	2.82 ^{ab}	0.235
Glu	0.48	0.47	0.56	0.062
Gly	3.09 ^a	2.80 ^b	2.33 ^c	0.161
Orn	0.35 ^a	0.46 ^b	0.55 ^c	0.025
Ser	1.15 ^a	0.85 ^b	0.76 ^c	0.042
Tyr	0.79	0.70	0.79	0.081

¹ Control; ² 500 g·d⁻¹ of soya protein; ³ 500 g·d⁻¹ of soya protein with an incomplete milking; ⁴ Residual standard deviation.

^{a,b,c} Within rows, means with no common superscript differ ($P < 0.05$).

between dairy production and intake regulation.

The lack of response of feed intake does not originate from the inefficiency of protein perfusion. The protein perfusion improved the supply of amino acids, as shown by the increased plasma concentrations of most of the essential amino acids (Tab. IV). These concentrations were higher with (Pim) treatment but also with complete milking (P) in spite of a higher protein output by the mammary gland. Thus, the quantity of proteins perfused was sufficient to generate an improvement in recorded dairy performances, particularly in milk yield (+ 4.3 kg·d⁻¹) and protein content (+ 1.8 g·kg⁻¹). These results agreed with most previous trials with protein perfusions [8, 10, 12, 30, 32] and confirmed that the protein balance of the control was overdrawn as shown by the PDI balance (Tab. I).

The control treatment itself might have stimulated the appetite as much as the protein perfusions, but this seems unlikely since no significant increase of intake was observed with glucose infusions in most other trials [10, 12, 30, 32]. Moreover, the amounts of glucose used in our trial were relatively low. Concerning urea, few data exist on duodenal perfusions, but it is possible to imagine that it could act by improving rumen recycling. This assumption was not confirmed, considering that there was no significant improvement of digestive parameters, therefore allowing an increase of intake. On the contrary, the variation of ruminal ammonia concentrations during the day in our trial should not have limited ruminal digestion and consequently voluntary intake. The levels of ruminal ammonia concentration did not go down below 50 mg·L⁻¹ which was considered to be the required concentration for an optimum

ruminal digestion [27]. The uremia of the control treatment was higher than with the protein treatment (Pim), in spite of a similar nitrogen input and protein production (Tabs. I and III). This observation could be explained by higher body protein accretion with the (Pim) treatment due to the duodenal perfusion of protein.

The low intake of the diet used in this trial (Tab. I) may have inhibited an increase of feed intake by the perfused protein. The incorporation of white lupin (0.02% of alkaloids) in the concentrate as the only source of protein in the ration could have been a cause of the low intake [17, 19]. In our trial, DMI decreased before the use of lupin concentrate and the infinitesimal alkaloid levels remaining after processing may not have significantly modified food intake in ruminants [20]. In this trial, other factors probably limited the intake, even in the presence of a protein supplement: mainly the use of dehydrated whole-plant maize as a concentrate. Such a diet is characterised by its slow ruminal digestion [33] and, consequently, a long and increasing retention time in the rumen that could generate the low levels of intake observed.

Protein supplementation would also appear to stimulate appetite by mobilising body reserves that are necessary in order to cover the recorded dairy production using protein perfusion (P). This lipomobilisation could generate signals to increase the intake and, consequently, restore the mobilised reserves [13]. In the present trial, the energy balance calculated for each treatment was always positive (Tab. I). The low plasma concentration of the NEFA (Tab. III), even before meals, shows that the milk yield improvements so obtained did not induce any substantial lipomobilisation. The slight significant increase observed between treatments in long-chain fatty acid levels (C_{18}) in milk (Tab. II) seemed to be independent of the energy balance, when comparing the plasma NEFA concentration of the two protein treatments (Tab. III). Appetite stimulation, and consequently any long-term

restoration of lipid reserves, could have been masked by the short duration of the experimental periods. However, this seems unlikely because in some short-duration trials (11 d on average) with protein perfusion, dairy production and DMI were increased [9, 10]. Therefore, in spite of a substantial improvement in dairy production with protein treatment (P), the energy balance did not go into deficit, which may partly explain the absence of intake response.

5. CONCLUSION

This trial showed that a direct protein supplementation into the duodenum increases the milk yield and protein content, but does not stimulate the appetite, which is probably limited by other factors. The type of diet used probably accounted for the low feed intake. The reduction in milk yield brought about by incomplete milking does not generate a decrease of intake. This result indicates that the appetite of dairy cows does not seem to be dependent on the energy needs of the mammary gland, principally when the energy balance is positive.

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