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## Original article

# Effects of the level and source of dietary protein on intake and milk yield in dairy cows

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**Abstract** — The objectives of this trial were to determine the effect of dietary protein content and the effect of the source of protein supplements on dry matter intake (DMI) and milk yield in dairy cows fed ad libitum. In addition, the interaction of protein supplement with the level of milk yield was studied to test the hypothesis that the DMI response to protein supplement depends on the nutrient requirements of the cows. Two groups of six lactating cows differing in milk yield ( $45 \text{ kg} \cdot \text{d}^{-1}$  and  $38 \text{ kg} \cdot \text{d}^{-1}$  pre-treatment) were assigned to a double  $3 \times 3$  Latin square design over periods of 3 weeks. The 3 treatments consisted of 3 complete diets (60% maize silage and 40% concentrate) with different protein sources and levels: low-protein supplement (LP) (5% soybean meal + 1.2% of urea in the diet), high-protein supplement containing soybean meal (HP-SB) (19% soybean meal) and high-protein supplement containing formaldehyde-treated soybean meal (HP-FTSB) (12% formaldehyde-treated soybean meal + 1.2% of urea in the diet). HP treatments significantly increased ( $P < 0.05$ ) DMI ( $+1.2 \text{ kg} \cdot \text{d}^{-1}$ ), milk yield ( $+1.8 \text{ kg} \cdot \text{d}^{-1}$ ), 4% FCM ( $+2.5 \text{ kg} \cdot \text{d}^{-1}$ ) and fat content ( $+1.9 \text{ g} \cdot \text{kg}^{-1}$ ) compared to the LP treatment. These improvements were not related to the source of protein used or the level of milk yield. Compared to LP treatment, the HP-SB treatment increased milk protein content ( $+1.4 \text{ g} \cdot \text{kg}^{-1}$ ) ( $P < 0.05$ ), whereas the HP-FTSB treatment had no significant effect. Intake and milk yield were increased by higher protein content in the diets independently of the source of protein used or the level of milk yield.

**dairy cow / intake / protein / milk yield**

**Résumé** — Effet du traitement de tannage des protéines au formol sur l'ingestion et la production des vaches laitières. Cet essai a pour objectif d'étudier l'effet de la teneur et de la nature des protéines de la ration sur les quantités ingérées et la production laitière chez les vaches laitières alimentées à volonté. De plus, le dispositif expérimental utilisé a permis d'étudier l'interaction entre le supplément protéique et le niveau de production afin de savoir si la réponse d'ingestion à un supplément de protéines était fonction des besoins nutritionnels des vaches. Deux groupes de six vaches laitières constitués selon le niveau de production ( $45 \text{ kg} \cdot \text{j}^{-1}$  et  $38 \text{ kg} \cdot \text{j}^{-1}$ ) ont été répartis dans un schéma en double carré latin  $3 \times 3$  avec des périodes de 3 semaines. Les traitements consistaient en : (1) un régime témoin négatif (LP) (5 % tourteaux de soja + 1,2 % d'urée dans la ration), (2) un régime tourteaux de soja (HP-SB) (19 % de tourteaux de soja) et (3) un régime tourteaux de soja tanné (HP-FTSB) (12 % de

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tourteaux tannés + 1,2 % urée dans la ration). L'accroissement de la teneur de la ration en protéines alimentaires peu dégradables dans le rumen par rapport au régime témoin entraîne des augmentations significatives ( $P < 0,05$ ) des quantités ingérées ( $+1,2 \text{ kg} \cdot \text{j}^{-1}$ ), de la production laitière ( $+1,8 \text{ kg} \cdot \text{j}^{-1}$ ), des quantités de lait standard ( $+2,5 \text{ kg} \cdot \text{j}^{-1}$ ) et du taux butyreux ( $+1,9 \text{ g} \cdot \text{kg}^{-1}$ ). Comparée au régime témoin (LP), l'utilisation des tourteaux de soja normaux améliore le taux protéique du lait ( $+1,4 \text{ g} \cdot \text{kg}^{-1}$ ) contrairement aux tourteaux tannés. Les quantités ingérées et la production laitière sont augmentées chez la vache laitière par la supplémentation de protéines dans le régime indépendamment de la nature du supplément protéique et du niveau de production.

**vaches laitières / ingestion / protéines alimentaires / production laitière**

## 1. INTRODUCTION

In ruminants, increasing the protein content of the diet enhances DMI, milk yield and body weight gain [3, 20], but the responses vary greatly according to the type and level of protein supplements. The supplementation of diets with non-degradable protein improves milk yield in dairy cows [1, 7, 13], but DMI responses vary among the different trials [11, 14, 26]. On the one hand, heat-treated or formaldehyde-treated protein supplements are the usual source of PDIA (dietary protein truly digested in the small intestine) [24] used in dairy production, but heat-treatment of proteins may decrease their digestibility in the small intestine [6]. On the other hand, formaldehyde can affect microorganism activities and consequently alter digestion [16].

Whether rations are offered to dairy cows with formaldehyde-treated or non-treated protein supplements, improvements in milk yield are the same and a large amount of protein supplement is economised [23]. Nevertheless, few trials have been conducted with cows fed ad libitum in order to compare different protein sources allowing the same contribution of PDIE (protein truly digested in the small intestine allowed by energy) and PDIN (protein truly digested in the small intestine allowed by nitrogen) [24] in the rations, and to verify the consequences of formaldehyde treatment on intake. Some authors have suggested that the response of

dairy cows to protein supply could be influenced by their potential yield, but these authors were interested only in parity and did not compare the responses of multiparous cows according to their milk yield [12].

The objectives of this trial were (1) to verify that enhancement of protein digestible in the intestine (PDI) improves DMI, (2) to see if the formaldehyde treatment of soybean meal affects DMI at similar levels of PDI supply, and (3) to verify whether the DMI response to protein supplements differs with the milk yield in dairy cows.

## 2. MATERIALS AND METHODS

### 2.1. Experimental procedure

Three complete diets with different protein supplements were compared in two groups of high and medium milk-yielding cows using a split-plot experimental design. Twelve multiparous cows at the beginning of lactation (58 days postpartum) were divided into two groups according to milk yield ( $44.8 \pm 5 \text{ kg} \cdot \text{d}^{-1}$  and  $37.7 \pm 2 \text{ kg} \cdot \text{d}^{-1}$  for groups 1 and 2, respectively) and body weight ( $643 \pm 60 \text{ kg}$  and  $614 \pm 65 \text{ kg}$  for groups 1 and 2, respectively) during the pre-experimental week. Cows in each group were assigned successively to 3 experimental diets according to a double  $3 \times 3$  Latin Square with 3-week periods and balanced for residual effects. Cows received the same

diet containing maize silage (60%) and concentrate (40%) which contained one of the three protein supplements: (1) low-protein treatment (LP), 5% soybean meal + 1.2% urea in the diet; (2) high-protein treatment with soybean meal (HP-SB), 19% soybean meal in the diet; (3) high-protein supplement with formaldehyde-treated soybean meal (HP-FTSB), 12% formaldehyde-treated soybean meal + 1.2% urea in the diet. The total mixed diet (Tab. I) for ad libitum intake was fed to the cows twice daily after each milking.

Milk yield and DMI were recorded daily and cow body weight was recorded weekly.

Daily and weekly samples were collected for maize silage and concentrate, respectively, to estimate their DM composition. A representative sample of each feed was prepared during each period and analysed in order to determine its chemical composition and nutritive value. Milk samples from three consecutive daily milkings were collected every week and analysed for fat and protein contents. During the 3rd week of each experimental period, 10-ml blood samples were drawn off from the tail vessels before the morning meal and were analysed for urea, glucose, NEFA, BHBA, plasma proteins and insulin.

**Table I.** Composition of diets and feeding values of the experimental complete diets.

	LP <sup>1</sup>	HP-SB <sup>2</sup>	HP-FTSB <sup>3</sup>
	(%) Diet		
Maize silage	60	60	60
Concentrate <sup>1</sup>	32.3	19.5	25.3
SBM	5	19	0
FT-SBM	0	0	12
Urea	1.2	0	1.2
Mineral and vitamin premix	1.5	1.5	1.5
	(%) DM		
DM <sup>a</sup>	51.0	50.6	51.5
OM <sup>b</sup>	92.6	93.7	92.6
CP <sup>c</sup>	14.1	16.5	17.0
NDF <sup>d</sup>	38.0	36.3	37.8
ADF <sup>e</sup>	18.5	18.0	18.4
ADL <sup>f</sup>	1.8	1.7	1.8
NE <sub>L</sub> <sup>g</sup> (UFL·kg <sup>-1</sup> of DM)	0.93	0.96	0.94
PDIA <sup>h</sup> (g·kg <sup>-1</sup> of DM)	30	51	60
PDIN <sup>h</sup> (g·kg <sup>-1</sup> of DM)	87	111	115
PDIE <sup>h</sup> (g·kg <sup>-1</sup> of DM)	83	105	111

<sup>1</sup> Low protein; <sup>2</sup> soybean meal; <sup>3</sup> formaldehyde-treated soybean.

<sup>a</sup> Dry matter; <sup>b</sup> organic matter; <sup>c</sup> crude protein; <sup>d</sup> neutral detergent fibre; <sup>e</sup> acid detergent fibre; <sup>f</sup> acid detergent lignin; <sup>g</sup> estimated using the equations of Vermorel [22] and expressed according to the French feed unit system, 1 UFL = 7.11 MJ of NE<sub>L</sub>; <sup>h</sup> dietary protein truly digested in the small intestine (PDIA), protein truly digested in the small intestine allowed by N (PDIN) or energy (PDIE) and fermented in the rumen, and estimated using the equations of Vérité and Peyraud [24] according to the French feed unit system; <sup>i</sup> contains: 20% barley, 21.5% wheat, 37% beet pulp, 17% wheat bran, 2% animal fat, 2% molasses, 0.5% calcium bicarbonate, 1% sodium bicarbonate and 1% sodium chloride.

## 2.2. Chemical analyses

Chemical analyses were performed on dry feed samples that had been ground using a 0.8-mm mill. DM, OM, ADF and CP contents were analysed according to the AOAC methods [2], and the NDF content as described by Van Soest et al. [21] (procedure A). Protein and fat contents of milk were determined by infrared analysis (Milkoscan; Foss Electric, Hillerød, Denmark) [2]. Blood samples were prepared and analysed for urea, glucose and NEFA as described by Guinard et al. [10]. Insulin, BHBA and plasma proteins were determined as described by Faverdin et al. [9].

## 2.3. Statistical analysis

Data were analysed statistically using the GLM procedure of SAS [19]. The statistical model of variance analysis was the following:

$$Y_{ijkl} = \mu + MY_i + C_j(MY_i) + P_k + T_l + \text{pre}T_m + T_l \times MY_i + e_{ijkl}$$

$Y_{ijkl}$  = experimental variable;  $\mu$  = mean;  $MY_i$  = pre-treatment milk yield effect (high or medium);  $C_j$  = cow effect;  $P_k$  = period effect;  $T_l$  = treatment effect;  $\text{pre}T_m$  = previous treatment effect;  $T_l \times MY_i$  = interaction between treatment effect and milk yield effect;  $e_{ijkl}$  = residual. The milk yield effect was tested with the error  $C_j(MY_i)$ .

Four orthogonal contrasts were made in order to test the effect of some treatments on the experimental variables: (1) the effect of dietary protein content (PDIA = LP vs. HP); (2) the effect of formaldehyde treatment (FT = HP-SB vs. HP-FTSB) compared to normal soybean meal; (3) the interaction between the pre-treatment milk yield and the dietary protein content (PDIA  $\times$  MY); and (4) the interaction between the formaldehyde treatment and the pre-treatment milk yield (FT  $\times$  MY). Statistical difference was determined at  $P < 0.05$ .

## 3. RESULTS

No significant interaction was found between the level of pre-treatment milk yield of dairy cows and the treatments, so the contrasts (3) and (4) are not presented in the tables ( $P > 0.05$ ).

### 3.1. Intake and milk yield

HP treatments brought about a significant increase in DMI (+1.2 kg·d<sup>-1</sup>) ( $P < 0.05$ ), milk yield (+1.8 kg·d<sup>-1</sup>), 4% FCM yield (+2.5 kg·d<sup>-1</sup>), protein content (+0.8 g·kg<sup>-1</sup>), fat content (+1.9 g·kg<sup>-1</sup>), protein yield (+82 g·d<sup>-1</sup>) and fat yield (+138 g·d<sup>-1</sup>) (Tab. II) compared to LP treatment (Tab. II). However, the HP-FTSB treatment of soybean did not affect intake ( $P > 0.05$ ) (Tab. II) but significantly decreased ( $P < 0.05$ ) the protein content of milk compared to HP-SB treatment. On the other hand, the high-producing dairy cows ate more than medium-producing cows receiving LP treatment (+2.3 kg·d<sup>-1</sup>) ( $P < 0.05$ ), but DMI increased similarly with protein supplements in both groups of cows. Protein and energy balances did not differ in both groups ( $P > 0.05$ ).

### 3.2. Blood plasma analysis

HP treatments significantly increased ( $P < 0.05$ ) the plasma concentrations of urea (+13.6 mg·dL<sup>-1</sup>) and protein (+0.3 g·dL<sup>-1</sup>), but no significant effect was observed in plasma concentrations of insulin, glucose or BHBA (Tab. III). Compared to HP-SB treatment, plasma concentrations of NEFA were significantly higher in cows fed the HP-FTSB diet ( $P < 0.05$ ) (Tab. III).

## 4. DISCUSSION

In this trial, the significant increases observed in DMI were dependent on improvements in the PDIE content of diets; such results are consistent with other

**Table II.** Effects of soybean meal or formaldehyde-treated soybean protein supplementation on intake, milk yield and milk composition of dairy cows.

	LP <sup>1</sup>	HP-SB <sup>2</sup>	HP-FTSB <sup>3</sup>	RSD <sup>4</sup>	PDIA <sup>5</sup>	FT <sup>6</sup>
DMI (kg·d <sup>-1</sup> )	23.2 <sup>a</sup>	24.5 <sup>b</sup>	24.2 <sup>b</sup>	1.0	0.007	0.467
Milk yield (kg·d <sup>-1</sup> )	37.2 <sup>a</sup>	38.7 <sup>b</sup>	39.4 <sup>b</sup>	1.1	0.001	0.178
4% FCM yield (kg·d <sup>-1</sup> )	35.0 <sup>a</sup>	37.5 <sup>b</sup>	37.5 <sup>b</sup>	1.3	0.001	0.990
Total fat (g·d <sup>-1</sup> )	1331 <sup>a</sup>	1468 <sup>b</sup>	1471 <sup>b</sup>	67	0.001	0.912
Total protein (g·d <sup>-1</sup> )	102 <sup>a</sup>	1124 <sup>b</sup>	1095 <sup>b</sup>	32	0.001	0.059
Milk composition						
Fat (g·kg <sup>-1</sup> )	36.1 <sup>a</sup>	38.2 <sup>b</sup>	37.7 <sup>ab</sup>	2.0	0.035	0.542
True protein (g·kg <sup>-1</sup> )	27.8 <sup>a</sup>	29.2 <sup>b</sup>	28.0 <sup>a</sup>	0.5	0.001	0.001
Nutrient balance						
NE <sub>L</sub> (UFL·d <sup>-1</sup> )	-0.4	0.3	-0.4	0.96	0.432	0.105
PDI (g·d <sup>-1</sup> )	-71 <sup>a</sup>	413 <sup>b</sup>	580 <sup>c</sup>	107	0.001	0.004

<sup>1</sup> Low protein; <sup>2</sup> soybean meal; <sup>3</sup> formaldehyde-treated soybean; <sup>4</sup> residual standard deviation.

Contrasts: <sup>5</sup> low dietary protein vs. high dietary protein; <sup>6</sup> formaldehyde-treated supplement vs. non-treated supplement.

**Table III.** Effects of soybean meal or formaldehyde-treated soybean protein supplementation on some blood parameters.

	LP <sup>1</sup>	HP-SB <sup>2</sup>	HP-FTSB <sup>3</sup>	RSD <sup>4</sup>	PDIA <sup>5</sup>	FT <sup>6</sup>
Urea (g·L <sup>-1</sup> )	0.25 <sup>a</sup>	0.40 <sup>b</sup>	0.38 <sup>b</sup>	0.52	0.001	0.357
Glucose (g·L <sup>-1</sup> )	0.62	0.63	0.62	0.31	0.691	0.369
NEFA (mmol·L <sup>-1</sup> )	0.11 <sup>a</sup>	0.12 <sup>a</sup>	0.20 <sup>b</sup>	0.07	0.103	0.022
Protein (g·L <sup>-1</sup> )	84.7 <sup>a</sup>	88.0 <sup>b</sup>	87.9 <sup>b</sup>	2.7	0.009	0.973
Insulin (ng·L <sup>-1</sup> )	0.47	0.52	0.53	0.18	0.534	0.891
BHBA (mmol·L <sup>-1</sup> )	0.47	0.54	0.59	0.17	0.134	0.529

<sup>1</sup> Low protein; <sup>2</sup> soybean meal; <sup>3</sup> formaldehyde-treated soybean; <sup>4</sup> residual standard deviation.

Contrasts: <sup>5</sup> low dietary protein vs. high dietary protein; <sup>6</sup> formaldehyde-treated supplement vs. non-treated supplement.

findings [3, 18]. The formaldehyde treatment of soybean meal did not affect DMI response. In some trials [15, 23], DMI increased, but not significantly ( $P > 0.05$ ), when protein supplements were formaldehyde-treated (+0.9 kg·d<sup>-1</sup> on average). The overprotection of protein supplements with formaldehyde (> 0.3 g·100 g<sup>-1</sup> of CP) appears to decrease DMI, but not significantly, when such supplements are compared to non-treated supplements [18, 25].

This decrease may be due to the modifications in ruminal digestion caused by the low level of degradation of the formaldehyde-treated soybean, which normally decreases rumen ammonia and reduces apparent nitrogen digestibility [4]. In this trial, the lower degradability of FTSB was compensated by urea addition in order to maintain a sufficient level of degradable nitrogen in the rumen. Some previous works have shown that the nature of degradable nitrogen in the

rumen could slightly affect NDF digestibility [17] but does not significantly modify the digestibility of a maize silage-based diet. The increases in DMI in this trial are mainly due to a higher amino acid supply to the cows, independently of the type of supplement used.

The second objective of this work was to determine if the DMI response to protein supplement was related to the milk potential and nutrient requirements of the cows. In this trial, the lower milk yield of the medium group was associated with a lower DMI. Thus the energy and protein balances of the medium- and high-producing dairy cows were similar. The milk yield of the medium group was probably much more limited by nutrient availability than by mammary gland capacity. In this case, the similar DMI response of the two groups is logical. Nevertheless, the mechanisms involved in the DMI increase induced by protein supplements ( $P < 0.05$ ) (Tab. II) are largely unknown.

The significant increase in milk yield observed with HP treatments is due to the PDIE content of diets, and should not depend on the kind of protein source of the supplement ( $P < 0.05$ ) (Tab. II). The slightly higher improvement in milk yield observed in cows receiving HP-FTSB treatment compared to those fed a HP-SB supplement ( $+ 0.7 \text{ kg} \cdot \text{d}^{-1}$ ) could be explained by body reserve mobilization as reflected by energy balance ( $P < 0.05$ ) (Tab. II) and plasma concentration of NEFA ( $P < 0.05$ ) (Tab. III). Thus it should not be linked to the formaldehyde treatment or to a better use of amino acids by the mammary gland [6, 8]. However, the protein content of milk ( $+ 1.2 \text{ g} \cdot \text{kg}^{-1}$ ) is significantly higher in cows fed an HP-SB supplement ( $P < 0.05$ ) (Tab. II) than those fed an HP-FTSB treatment. Formaldehyde treatment of soybean meal could significantly reduce the amount of some amino acids absorbed from the small intestine and consequently affect their use by the mammary gland in cows that are fed such diets [5].

## 5. CONCLUSION

Intake, milk yield and other zootechnical parameters in dairy cows are seen to increase with the dietary protein content without any interaction with the milk yield. At the same level of PDIE and PDIN input into the diet, formaldehyde-treated or non-treated supplements have a similar and significant effect on DMI and milk yield compared to dairy cows fed a low-protein diet. However, the formaldehyde treatment significantly decreases the protein content of milk.

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