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Original article

Suitability of lupin and pea seeds as a substitute for soybean meal in high-producing dairy cow feed

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Abstract – Two experiments were conducted to investigate the substitution of soybean meal by coarsely ground lupin and/or pea seeds in high-producing dairy cow feed. In experiment 1 (Exp. 1), four Holstein dairy cows (35.9 ± 2.2 $L \cdot d^{-1}$ milk) were put on a control diet consisting of 50% maize silage, 11% grass silage and 36% concentrates on a dry-matter (DM) basis. Soybean meal was partially replaced (75%) by lupin, pea and a mixture (1/1, DM) of lupin and pea on a DM basis, following a 4 × 4 Latin square design. Milk production was lower with pea seeds, intermediate with a lupin/pea seed mixture and higher with lupin and soybean meal diets. Milk fat percentage increased with the lupin diet, which induced a lower proportion of medium-chain fatty acids and a higher proportion of long-chain fatty acids in the milk compared to the pea diet. In experiment 2 (Exp. 2), six Holstein dairy cows (36.0 ± 4.9 $L \cdot d^{-1}$ milk) were put on a control diet consisting of 47% maize silage, 7% grass silage and 42% concentrates on a DM basis. Soybean meal was totally replaced by lupin or a lupin/pea mixture (1/1, N) on a nitrogen basis, following a duplicated Latin square design (6 animals × 3 diets). Standard milk production did not differ with the dietary protein source but milk fat percentage was reduced with the lupin diet. This effect, not observed in Exp. 1, was probably related to the lipid content of the lupin diet. Nitrogen efficiency was not modified by the protein source. The results also showed that a high ingestion of lupin seeds induced a lower milk ω6/ω3 ratio and $C_{18:2}$ content. In conclusion, coarsely ground lupin seeds can efficiently replace soybean meal in high-producing dairy cow feed.

dairy cow / soybean meal / lupin / pea / milk

Résumé – Les graines de lupin et de pois en tant qu'alternative au tourteau de soja dans l'alimentation des vaches laitières hautes productrices. Deux expériences ont été menées pour étudier la substitution du tourteau de soja par du lupin et/ou du pois grossièrement moulu dans l'alimentation de la vache laitière haute productrice. Dans l'expérience 1, quatre vaches laitières Holstein (35,9 ± 2,2 $L \cdot j^{-1}$ de lait) ont reçu un régime témoin contenant 50 % d'ensilage de maïs, 11 % d'ensilage d'herbe et 36 % de concentré sur base de la matière sèche (MS). Le tourteau de soja a été partiellement remplacé (75 %) par du lupin, du pois et un mélange (1/1, MS) de lupin et de pois sur base de la MS, selon un dispositif expérimental en carré latin 4 × 4. La production laitière a été inférieure avec le pois, intermédiaire avec le mélange lupin/pois et supérieure avec les régimes lupin

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et tourteau de soja. Le taux butyreux du lait a augmenté avec le régime lupin, qui a induit une plus faible proportion d'acides gras à chaîne moyenne et une plus grande proportion d'acides gras à longue chaîne comparativement au régime pois. Dans l'expérience 2, six vaches laitière Holstein (36,0 ± 4,9 L·j^{-1} de lait) ont reçu un régime témoin contenant 47 % d'ensilage de maïs, 7 % d'ensilage d'herbe et 42 % de concentré sur base de la MS. Le tourteau de soja a été entièrement remplacé par du lupin ou un mélange lupin/pois (1/1, N) sur une base azotée, selon un dispositif expérimental en carré latin dupliqué (6 animaux × 3 régimes). La production de lait standard n'a pas différé avec la source de protéines alimentaires mais le taux butyreux du lait a été réduit avec le régime lupin. Cet effet, non observé dans l'expérience 1, était probablement lié à la teneur élevée en matières grasses du régime lupin. L'efficacité de l'azote n'était pas modifiée par la source de protéines. Les résultats ont également montré qu'une ingestion élevée de lupin induit un plus faible rapport ω6/ω3 et moins de $C_{18:2}$ dans le lait. En conclusion, le lupin grossièrement moulu semble pouvoir remplacer efficacement le tourteau de soja chez la vache laitière haute productrice.

vache laitière / tourteau de soja / lupin / pois / lait

1. INTRODUCTION

Soybean meal is the main exogenous protein source used in cattle feeding. The interdiction to use meat and bone meal following the bovine spongiform encephalopathy crisis has increased this undeniable fact. The high proportion of genetically modified crops and the difficulty of ensuring the complete traceability of animal products are the major problems of using soybean meal. The development of autochthon protein crops is a solution to improve the valorisation of products and forage grown on the farm and to insure a better traceability of livestock feedstuffs. Moreover, most protein crops are legumes and are therefore very interesting for crop rotation and, overall, for sustainability [23].

Among the protein crops that could replace soybean meal, lupin has the highest protein content (35–40% crude protein, CP), pea (23% CP) is the most produced, while production and CP content of the faba bean are intermediate (30% CP). The current interest in lupin stems from recent scientific advances for this crop, especially the genetic selection of low alkaloid varieties, high protein cultivars and the development of winter cultivars, which offer more stable yields for the producer [14].

Genetic selection has greatly improved the production potential of Holstein dairy cows but has resulted in a lower nitrogen (N) utilisation efficiency. To satisfy their protein requirement, dairy cow feed calls for new technologies and specific formulations. Poncet et al. [30] synthesised the influence of technological treatments on the nutritional value of pulses. Grinding [25], roasting [39] and extrusion [7] of lupin seeds all have beneficial effects for ruminants. Coarse grinding has the advantage of being cheap and easy for the producer and of limiting ruminal degradability compared to a finer grinding [33]. In addition, ruminal degradation and undegraded protein supply of coarsely ground pulses are often biased in feeding tables where values are related to smaller screen size grinding [10] and estimated from the in situ technique, which underestimates the nutritive value of lupin seed crude protein [31].

Compared to the pea, lupin seed contains more lipids (9.8 vs. 1.3%), more fibres (21.4 vs. 13.3% NDF) and less starch (7.9 vs. 50.5%), and is therefore a high quality feedstuff for ruminant feeding, avoiding acidosis and supplying high amounts of net energy. The aim of this study was to investigate the influence of the partial or total substitution of soybean meal by coarsely ground lupin and/or pea seeds, on a dry-matter (DM) or N basis, respectively, on the amount and quality of the milk, the efficiency of N utilisation and the live weight evolution of the animals.

Table I. Composition of the offered diets (Exp. 1).

	Dietary protein source			
Ingredients (kg·d^{-1} DM)	Soybean meal	Lupin	Pea	Lupin/Pea
Maïze silage	11.50	11.50	11.50	11.50
Grass silage, 65% DM	2.50	2.50	2.50	2.50
Concentrate				
Production concentrate[1]	4.04	4.04	4.04	4.04
Corrector concentrate[2]	1.01	1.01	1.01	1.01
Soybean meal, 44% CP	3.18	–	–	–
Ground lupin	–	3.18	–	1.59
Ground pea	–	–	3.18	1.59
Minerals and vitamins[3]	0.20	0.20	0.20	0.20
Alfalfa + Cr_2O_3	0.40	0.40	0.40	0.40
Total DM	22.83	22.83	22.83	22.83
Chemical composition (g·kg^{-1} DM)				
Organic matter	917	923	910	910
Crude protein	184	167	147	156
Crude fibre	167	173	165	167
VEM[4]	954	974	942	951
DVE[5]	102	87	83	84
OEB[6]	15	13	3	8
Forage/concentrate ratio (%)	61	61	61	61

[1] Lactopro 40 (40% CP), Quartès, Deinze, Belgium (70% of soybean meal).
[2] Noten Eco 20 (20% CP), Huys, Brugge, Belgium (17% soybean meal).
[3] Combi Maïs, SCAR, Herve, Belgium. Composition (%): Ca 19, P 5, Na 7, Mg 9; (mg·kg^{-1}): I 30, Co 20, Se 10, Cu 850, Mn 650, Zn 1300; (IU·kg^{-1}): Vit A 300000, Vit D3 60000.
According to the Dutch system [36, 37]: [4] Net Energy, [5] Digestible protein in the small intestine, [6] Degradable N / Fermentable energy balance in the rumen.

2. MATERIALS AND METHODS

2.1. Experimental protocols

2.1.1. Experiment 1

Four Holstein dairy cows (57 ± 15 days of lactation at the beginning of the trial) were individually penned and were put on four diets in a 4 × 4 Latin square design (Tab. I) at an intake level of 22.8 kg DM·d^{-1}. The soybean meal in the control diet was partially replaced (75%) by lupin (*Lupinus albus* var. Arès), pea (*Pisum sativum*) or a lupin/pea mixture (1/1, DM) on a DM basis. The pulses were coarsely ground through a 0.95 cm screen before distribution. Fresh water was available at all times. Feed was fed as a total mixed ration provided twice a day, in equal amounts, just after milking at 06h30 and 16h30. After 15 days of adaptation, the four experimental periods were composed of 7 days of diet adaptation and 7 days of milk and faeces sampling. Individual milk production was monitored daily. For each cow, the milk sample (100 mL) from the evening milking was cooled (4 °C) and mixed (1/1) with milk collected the next morning. A few mL were frozen (–18 °C) for urea

Table II. Composition of the offered diets (Exp. 2).

	Dietary protein source		
Ingredients (kg·d^{-1} DM)	Soybean meal	Lupin	Lupin/Pea
Maïze silage	10.13	10.13	10.13
Grass silage, 65% DM	1.50	1.50	1.50
Concentrate			
Soybean meal, 48% CP	3.95	–	–
Ground lupin	–	6.14	3.07
Ground pea	–	–	4.13
Linseed meal, expeller	1.08	1.08	1.08
Coconut meal	1.50	1.50	1.50
Flattened wheat	2.50	2.50	2.50
Minerals and vitamins[1]	0.20	0.20	0.20
Chalk	0.16	0.16	0.16
Alfalfa + Cr_2O_3	0.40	0.40	0.40
Total DM	21.42	23.61	24.67
Chemical composition (g·kg^{-1} DM)			
Organic matter	928	936	939
Crude protein	203	184	176
Crude fibre	144	155	144
VEM[2]	998	1043	1037
DVE[3]	102	85	85
OEB[4]	28	36	31
Forage/concentrate ratio (%)	54	49	47

[1] Runergeen C15, Nutreco Belgium NV, Gent, Belgium. Composition (%): Ca 15, P 7, Na 4, Mg 3; (mg·kg^{-1}): I 60, Co 60, Se 20, Cu 600, Mn 2500, Zn 3500, Fe 2850, Vit E 400; (UI·kg^{-1}) Vit A 500000, Vit D3 100000.
According to the Dutch system [36, 37]: [2] Net Energy, [3] Digestible protein in the small intestine, [4] Degradable N / Fermentable energy balance in the rumen.

analysis and the rest was cooled with potassium bichromate (1 mg·mL^{-1}) before protein and fat analysis on daily individual milk samples. The amount of the last milk sample of each period was doubled and 100 mL were used to analyse the milk fatty acid pattern. Faeces were collected daily, mixed and sampled (400 g) before drying (60 °C, 48 h). Chromic sesquioxide (35 g·d^{-1}) incorporated in alfalfa pellets was used as an indigestible DM marker to determine faeces amounts. The animals were weighed on the first and last 3 days of each period. Refusals were collected daily before the morning feeding.

2.1.2. Experiment 2

Six Holstein dairy cows (78 ± 39 days of lactation at the beginning of the trial), individually penned, were put on three diets in a duplicated Latin square design (Tab. II). The soybean meal was totally replaced by lupin (*Lupinus albus* var. Arès) and a lupin/pea mixture (1/1, N) on a N basis. Table III presents the composition of the protein sources. The intake level reached respectively 21.4, 23.6 and 24.7 kg DM·d^{-1} for the soybean meal, lupin and lupin/pea diets in order to supply a similar amount of CP.

Table III. Composition of the protein sources.

	Soybean meal	Lupin seed	Pea seed
Organic matter (% DM)	92.09	96.04	96.77
Crude protein (% DM)	54.62	35.10	24.20
Crude fibre (% DM)	7.71	11.92	9.04
Ether extract (% DM)	1.30	9.81	1.32
Starch (% DM)	3.91	7.93	50.48
Fatty acids (% total fatty acids)			
$C_{10:0}$	5.07	0.54	0.18
$C_{10:1}$	2.84	0.29	0.65
$C_{12:0}$	0	0	0
$C_{14:0}$	1.10	0.29	0.45
$C_{16:0}$	19.31	10.09	14.39
$C_{18:0}$	4.27	1.84	3.06
$C_{18:1}$	11.53	57.19	32.20
$C_{18:2}$	46.85	21.14	45.37
$C_{18:3}$	9.03	8.49	3.50
$C_{20:0}$	0	0.05	0
$C_{22:0}$	0	0	0

Lupin and pea were ground (0.95 cm) before distribution. The sampling and experimental periods were similar to those in the previous experiment.

2.1.3. Laboratory analyses

After grinding (1 mm), DM, organic matter (OM), crude fibre [4], N (Dumas method, [1]) and feeding value according to the Dutch system (VEM, DVE and OEB - near infrared spectrometry) analyses were conducted daily on maize silage, grass silage and refusals. These analyses were done weekly for the other dietary ingredients. Ether extract was also analysed on an aliquot of each feedstuff [3]. The granulometry of the protein sources was determined using an 8-tray sieve (1 to 8 mm, Retsch, Haan, Germany).

The milk fat and milk protein contents were analysed using the gravimetric method of Röse-Gottlieb [19] and the Kjeldhal method [18], respectively. Milk urea concentration was determined by differential pH-metry (EFA-Hamilton, Bonaduz, Switzerland). The fatty acid pattern was measured after fat extraction [20] and the preparation of methyl ester of fatty acids [21] by gas chromatography [11, 22] using a chromatograph (Fison 8000, Fison instruments, Goetingen, Germany) equipped with an FID detector and a silica capillary column CP-Sil 88 [100 m × 0.25 mm (id)] preceded by a methyl deactivated non polar pre-column [30 m × 0.53 mm (id)]. A standard solution was analysed to determine the response factor of each fatty acid and $C_{9:0}$ was used as the internal standard. A certified control sample was analysed every six injections in order to verify the analysis stability.

Chromic sesquioxide was analysed in alfalfa pellets [12]. After freeze-drying and grinding (1 mm), DM, OM, crude fibre [4], N (Dumas method, [1]) and chromic sesquioxide [12] were analysed in the faeces.

2.2. Statistics

In both experiments, statistical analyses were conducted using the general linear procedure of Minitab [26]. Three effects (diet, animal and period) were considered for the milk fatty acid pattern and the average weight gain of the animals; four effects (diet, animal, period and sampling day in the periods)

Table IV. Ingestion and faecal apparent digestibility of nutrients (Exp. 1).

	Dietary protein source					
	Soybean meal	Lupin	Pea	Lupin/Pea	SEM[4]	*P*
Ingestion						
DM (kg·d^{-1})	22.12^{a}	21.94^{ab}	21.38^{b}	22.12^{a}	0.08	0.005
OM (kg·d^{-1})	20.32^{a}	20.18^{ab}	19.72^{b}	20.45^{a}	0.08	0.009
CP (kg·d^{-1})	4.03^{a}	3.73^{b}	3.29^{c}	3.48^{d}	0.01	0.001
Crude fibre (kg·d^{-1})	3.64^{a}	3.81^{b}	3.64^{a}	3.75^{ab}	0.05	0.001
VEM[1] (VEM·d^{-1})	21149^{a}	21319^{a}	20347^{b}	21365^{a}	79.67	0.001
DVE[2] (g·d^{-1})	2248^{a}	1910^{b}	1809^{c}	1891^{b}	6.37	0.001
OEB[3] (g·d^{-1})	301^{a}	291^{a}	90^{b}	153^{c}	24.69	0.001
Digestibility						
DM (%)	64.7^{a}	68.6^{b}	66.1^{a}	65.2^{a}	0.289	0.001
OM (%)	69.2^{a}	72.6^{b}	70.1^{a}	69.4^{a}	0.266	0.001
CP (%)	68.5^{a}	69.8^{a}	64.3^{b}	64.8^{b}	0.271	0.001
Crude fibre (%)	50.9^{a}	57.9^{b}	55.2^{bc}	51.8^{ac}	0.475	0.001

[a, b, c] Within a row, means lacking a common superscript letter differ ($P < 0.05$).
According to the Dutch system [36, 37]: [1] Net Energy, [2] Digestible protein in the small intestine, [3] Degradable N / Fermentable energy balance in the rumen.
[4] Standard error of the mean.

were analysed for daily measurements such as ingestion, nutrient digestibility, milk production and milk composition. Least squares means were used to compensate for some missing data (oestrus, mastitis) and differences between means were compared using the Tukey test.

3. RESULTS AND DISCUSSION

For both experiments, the granulometry of protein sources, estimated by the median diameter of particles, reached 1.2, 2.1 and 1.7 mm for soybean meal, lupin and pea seeds, respectively.

3.1. Experiment 1

Despite providing iso-DM diets, DM and OM ingestions decreased with the pea diet (Tab. IV). According to Allen [2], DM intake could increase from 0.18 to 0.84 kg·d^{-1} per percentage unit increase in diet CP content. Compared to the control, pea diet ingestion was reduced by 0.20 kg·d^{-1} per percentage unit decrease in CP content. This effect was not observed with lupin-based diets, which were as well ingested as the soybean meal diet. This observation did not confirm the results of Guillaume et al. [13], who compared iso-N diets and mentioned a lower DM intake of lupin compared to soybean meal. CP and crude fibre ingestions differed greatly and reflected the composition of dietary protein sources. Likewise, ingestions of net energy (VEM), digestible protein in the small intestine (DVE) and rumen degradable N/ fermentable energy ratio (OEB) were influenced by the feeding value of each protein source and the lower ingestion of the pea diet. Faecal apparent digestibility of DM and OM was higher for the lupin diet. CP digestibility increased with the lupin and soybean meal diets, while crude fibre was better digested with the lupin and pea diets.

With a lower supply of dietary proteins (Tab. IV), the lupin diet enabled a similar milk production (Tab. V) as the control diet. The coarse grinding of the lupin seeds, and the chemical specificity of lupin proteins [5],

Table V. Dietary protein source effects on production factors, nitrogen efficiency and live weight variation of animals (Exp. 1).

	Dietary protein source					
	Soybean meal	Lupin	Pea	Lupin/Pea	SEM[3]	*P*
Milk production factors						
Production ($L \cdot d^{-1}$)	33.5^{a}	33.5^{a}	30.6^{b}	32.2^{c}	0.14	0.001
Standard production[1] ($L \cdot d^{-1}$)	28.9^{a}	29.7^{a}	25.9^{b}	27.2^{c}	0.17	0.001
Fat (%)	3.00^{a}	3.23^{b}	2.85^{a}	2.80^{a}	0.04	0.002
Protein (%)	3.07	3.04	3.03	3.09	0.01	0.275
Fat ($g \cdot d^{-1}$)	991^{ab}	1064^{b}	867^{a}	902^{ac}	12.87	0.001
Protein ($g \cdot d^{-1}$)	1020^{a}	1008^{a}	921^{b}	988^{a}	4.45	0.001
Urea ($mg \cdot L^{-1}$)	394^{a}	355^{b}	281^{c}	317^{d}	3.41	0.001
N efficiency[2] (%)	25.39^{a}	27.02^{b}	27.98^{bc}	28.45^{c}	0.14	0.001
Live weight variation (kg)	+5.5	+6.2	+7.8	+4.8	1.38	0.949

[a, b, c, d] Within a row, means lacking a common superscript letter differ ($P < 0.05$).
[1] Standard milk production (L) = [0.337 + (0.116 × % Fat) + (0.06 × % Protein)] × Production (L).
[2] Dietary N efficiency (%) = [Milk protein ($g \cdot d^{-1}$) / Protein ingested ($g \cdot d^{-1}$)] × 100.
[3] Standard error of the mean.

probably contributed to optimise the intestinal dietary protein supply by the lupin diet. The pea diet induced a reduction in milk production, while the lupin/pea mixture was intermediate. These results suggest that, under our experimental conditions, the protein content of pea was too low to replace 75% of soybean meal. According to Khorasani et al. [24], pea protein could indeed replace all soybean meal protein in iso-N diets without reducing milk production. Another solution for using pea as an alternative to soybean meal consists in its extrusion, which raises its nitrogenous value to a level comparable to that of the soybean meal [29].

As suggested by Brunschwig and Lamy [8], soybean meal substitution by lupin seeds increased milk fat percentage. The high fibre content of lupin could lead to an increase of acetate liberation in the rumen, being a precursor of milk fat. The supplemental dietary fat supplied by lupin seeds could also influence milk fat content. Milk protein percentage was not influenced by the dietary protein source. Milk urea concentration was highly correlated to protein ingestion ($r^2 = 0.993$). The low protein supply by the pea diet was confirmed by a lower milk urea content. Nitrogen efficiency decreased with protein intake and animal weight was not influenced by the diets (Tab. V).

The lupin diet tended to reduce the proportion of short-chain fatty acids in the milk, especially $C_{16:0}$, and to increase long-chain fatty acids compared to the other diets (Tab. VI). Differences were significant with the pea diet. The lupin effect on the milk fatty acid pattern had been previously investigated by Robinson and McNiven [32]. These authors reported that high uptake of long-chain fatty acids inhibits the de novo synthesis of short-chain fatty acids by mammary tissue [28]. The increase in $C_{18:0}$ is consistent with the fatty acid pattern of lupin for which $C_{18:1}$ is predominant (Tab. III), in contrast to other oilseeds containing more $C_{18:2}$. As suggested by Robinson and McNiven [32], the coarse grinding of lupin seeds results in a larger particle size that might protect lupin fat from ruminal hydrogenation. The effect of lupin on milk fat composition should be beneficial for human health

Table VI. Dietary protein source effect on milk fatty acid pattern (% of total fatty acids), (Exp. 1).

	Dietary protein source					
	Soybean meal	Lupin	Pea	Lupin/Pea	SEM[2]	*P*
$C_{4:0}$	3.77	4.05	4.28	3.83	0.091	0.286
$C_{6:0}$	2.88	2.51	2.75	2.76	0.038	0.063
$C_{8:0}$	1.59	1.29	1.52	1.57	0.033	0.068
$C_{10:0}$	3.39	2.55	3.14	3.26	0.105	0.111
$C_{12:0}$	3.96	2.97	3.65	3.85	0.126	0.109
$C_{14:0}$	11.68	10.36	12.06	12.23	0.231	0.097
$C_{16:0}$	31.40[ab]	28.17[b]	33.88[a]	32.41[ab]	0.509	0.035
$C_{16:1}$	1.28	1.23	1.41	1.20	0.034	0.249
$C_{18:0}$	9.89[ab]	11.86[a]	8.09[b]	9.50[b]	0.220	0.005
$C_{18:1}$	22.04	26.32	20.65	20.83	0.715	0.087
$C_{18:2}$	3.17	3.44	3.46	3.32	0.078	0.561
$C_{18:2\ c9t11}$	0.49	0.52	0.49	0.39	0.017	0.133
$C_{18:3}$	0.35	0.41	0.35	0.38	0.011	0.275
$C_{20:0}$	0.13[a]	0.32[b]	0.13[a]	0.22[c]	0.004	0.001
$C_{22:0}$	0.04[a]	0.15[b]	0.03[a]	0.11[c]	0.002	0.001
ω6/ω3[1]	6.05	5.55	6.40	5.77	0.128	0.206
∑ Saturated fatty acids	71.13	66.45	71.90	72.03	0.774	0.122
∑ Monounsaturated fatty acids	24.97	29.34	23.94	23.97	0.704	0.098
∑ Polyunsaturated fatty acids	4.01	4.29	4.25	4.10	0.102	0.750

[a, b, c] Within a row, means lacking a common superscript letter differ ($P < 0.05$).
[1] $C_{18:2\ c9c12}$ / $C_{18:3\ c9c12c15}$.
[2] Standard error of the mean.

because $C_{16:0}$, in contrast to $C_{18:0}$, increases the risks of cardiovascular diseases. No difference was observed for the ω6/ω3 ratio, nor for rumenic acid concentration representing the main CLA in the milk [34]. Milk monounsaturated fatty acid concentration tended to be higher with the lupin diet. According to Robinson and McNiven [32], the increased proportion of unsaturated fatty acids in milk from lupin-supplemented cows suggests that the resulting butter would be more spreadable at low temperatures. These authors also reported that the risks of oxidation of fat at temperatures above 20 °C are low because of low levels of $C_{18:2}$ and $C_{18:3}$ in milk from lupin-supplemented cows.

The results of the first trial confirmed that coarsely ground lupin seeds can replace the greater part of soybean meal in dairy cow feed without affecting milk production. Lupin is more expensive than soybean meal (262 vs. 209 euros·T^{-1}) and the choice of testing iso-DM diets was conditioned by economic constraints, implying that the higher cost of lupin should be compensated for by increasing the milk price. When lupin is grown by the breeder, the cost falls to 167 euros·T^{-1} and is therefore not an obstacle to its incorporation into dairy cow feed. Therefore, experiment 2 (Exp. 2) investigated the complete replacement of soybean meal by lupin and pea seeds on a N basis. High levels of lupin incorporation were used in order to evaluate the quality of each protein source.

3.2. Experiment 2

Despite previous analyses of feedstuff batches, CP ingestion was 6.5 and 4.4%

Table VII. Ingestion and faecal apparent digestibility of nutrients (Exp. 2).

	Dietary protein source				
	Soybean meal	Lupin	Lupin/Pea	SEM[4]	*P*
Ingestion					
DM ($kg \cdot d^{-1}$)	21.5^{a}	23.7^{b}	24.8^{c}	0.046	0.001
OM ($kg \cdot d^{-1}$)	20.0^{a}	22.2^{b}	23.3^{c}	0.040	0.001
CP ($kg \cdot d^{-1}$)	4.209^{a}	4.485^{b}	4.295^{c}	0.166	0.001
Crude fibre ($kg \cdot d^{-1}$)	3.123^{a}	3.653^{b}	3.535^{c}	0.012	0.001
VEM[1] ($VEM \cdot d^{-1}$)	21385^{a}	24782^{b}	25790^{c}	37.73	0.001
DVE[2] ($g \cdot d^{-1}$)	2156^{a}	2022^{b}	2111^{c}	2.706	0.001
OEB[3] ($g \cdot d^{-1}$)	566^{a}	869^{b}	783^{c}	2.217	0.001
Digestibility					
DM (%)	65.8^{a}	70.8^{b}	67.8^{c}	0.294	0.001
OM (%)	67.8^{a}	72.3^{b}	69.5^{a}	0.284	0.001
CP (%)	71.7^{a}	74.3^{b}	68.9^{c}	0.262	0.001
Crude fibre (%)	46.4^{a}	53.3^{b}	48.5^{a}	0.583	0.001

$^{a, b, c}$ Within a row, means lacking a common superscript letter differ ($P < 0.05$).
According to the Dutch system [36, 37]: [1] Net Energy, [2] Digestible protein in the small intestine, [3] Degradable N / Fermentable energy balance in the rumen.
[4] Standard error of the mean.

higher with the lupin diet compared to the soybean meal and lupin/pea diets, respectively (Tab. VII). These differences came from some refusals and variations in feedstuff composition. Crude fibre, VEM, DVE and OEB ingestions reflected the intake level variation and the composition of each protein source. The soybean meal diet supplied more DVE (+ 6.5%) and less OEB than the lupin diet. Crude fibre, CP, DVE and OEB supplies in the lupin/pea diet were intermediate, while its net energy supply was higher owing to the increase in the intake level. As in experiment 1 (Exp. 1), faecal nutrient digestibility increased in the lupin diet, decreased in the soybean meal diet and was intermediate in the lupin/pea diet. However, the forage to concentrate ratio and DMI differed among diets. These factors are both negatively related to nutrient digestion [38] and, on the contrary to experiment 1, influenced digestibility measurements as well as the protein source. Similar nutrient digestibility in dairy cows receiving mixed diets with lupin or soybean meal as protein source were reported by Singh et al. [35] where the forage to concentrate ratio, DM and CP intake did not differ.

Daily milk production was higher with the lupin diet (Tab. VIII) and probably reflected the variation of CP intake. The difference among the diets disappeared for standardised milk production. According to May et al. [25], standardised milk production increased when 75% of soybean meal proteins were replaced by lupin proteins (iso-N diets), but not for substitution rates of 0, 25, 50 or 100%. In contrast to experiment 1, milk fat percentage was depressed with the lupin diet. This could be due to lower forage to concentrate ratios of lupin-based diets [38], inducing a modification of the rumen volatile fatty acid synthesis. However, milk fat depression was less pronounced with the lupin/pea diet. Another hypothesis concerns the effect of an excess of fat from lupin seeds on ruminal fermentations [6]. Dietary fat contents reached 3.5, 5.2 and 4.0% for the soybean meal, lupin and lupin/pea diets, respectively. The amount

Table VIII. Dietary protein source effects on production factors, nitrogen efficiency and live weight variation of animals (Exp. 2).

	Dietary protein source				
	Soybean meal	Lupin	Lupin/Pea	SEM[3]	*P*
Milk production factors					
Production ($L \cdot d^{-1}$)	34.2^{a}	35.7^{b}	34.7^{a}	0.15	0.001
Standard production[1] ($L \cdot d^{-1}$)	31.8	31.8	31.4	0.18	0.616
Fat (%)	3.52^{a}	3.21^{b}	3.34^{ab}	0.03	0.001
Protein (%)	3.18	3.16	3.18	0.01	0.602
Fat ($g \cdot d^{-1}$)	1186	1121	1127	11.89	0.077
Protein ($g \cdot d^{-1}$)	1081^{a}	1124^{b}	1100^{ab}	4.96	0.004
Urea ($mg \cdot L^{-1}$)	403^{ab}	416^{a}	372^{b}	5.07	0.004
N efficiency[2] (%)	25.74	25.05	25.57	0.12	0.062
Live weight variation (kg)	–15.3	+2.3	–10.04	3.59	0.230

[a, b] Within a row, means lacking a common superscript letter differ ($P < 0.05$).
[1] Standard milk production (L) = [0.337 + (0.116 × % Fat) + (0.06 × % Protein)] × Production (L).
[2] Dietary N efficiency (%) = [Milk protein ($g \cdot d^{-1}$) / Protein ingested ($g \cdot d^{-1}$)] × 100.
[3] Standard error of the mean.

of fat supplied by the lupin diet did not exceed a value that could result in disturbances in intake or milk production [2, 28]. According to Palmquist et al. [27], the fatty acid pattern of dietary fat also influences the milk fat content. Hansen and Knudsen [15, 16] showed that $C_{16:0}$ stimulates de novo synthesis of short-chain fatty acids by lactating tissue, but $C_{18:1}$ inhibits this synthesis. Seeds from Lupinus albus varieties are especially rich in $C_{18:1}$ and poor in $C_{16:0}$ (Tab. III). This specificity probably influenced milk fat content in experiment 2. Such an effect was not observed by May et al. [25] when 5.2 $kg \cdot d^{-1}$ of lupin seeds were incorporated into feed but total fat content of their diets was lower than 4% DM. As in experiment 1, milk protein percentage was not influenced by the dietary protein source. Milk urea concentration differed significantly among diets ($P < 0.05$), but differences were low because of similar CP intakes. Brunschwig et al. [9] considered that lupin seeds could replace soybean meal but induced an excess of degradable N. This excess was not confirmed in our experiment by milk urea concentration data. No difference was observed concerning N utilisation, suggesting that the protein source did not influence the quality of dietary proteins.

In contrast to the lupin diet, the live weight of the cows tended to decrease with the soybean meal and lupin/pea mixture diets. For the soybean meal diet, the energy supply was probably too low to satisfy the animals' requirements due to the lower DMI and higher forage to concentrate ratio. This was not the case for the lupin/pea diet, supplying a large part of energy by starch, and for the lupin diet, which was more rich in fat. Forage to concentrate ratios for these diets were more similar but the dietary energy form was different and could also explain a part of the weight variation observed.

As in experiment 1, lupin tended to reduce medium-chain fatty acids ($P < 0.1$) and to increase long-chain fatty acids ($P < 0.1$, Tab. IX). However, in contrast to experiment 1, more modifications in the milk fatty acid pattern appeared and were probably due to the high incorporation rate of lupin seeds and to its fatty acid composition. So, $C_{18:2}$, rumenic acid ($C_{18:2c9t11}$) and the ω6/ω3 ratio were significantly reduced in the

Table IX. Dietary protein source effect on milk fatty acid pattern (% of total fatty acids), (Exp. 2).

	Dietary protein source				
	Soybean meal	Lupin	Lupin/Pea	SEM[2]	*P*
$C_{4:0}$	3.60	3.56	3.37	0.055	0.296
$C_{6:0}$	2.30	2.36	2.50	0.030	0.101
$C_{8:0}$	1.42	1.45	1.65	0.030	0.052
$C_{10:0}$	3.25[a]	3.33[ab]	3.92[b]	0.082	0.038
$C_{12:0}$	5.01	5.01	5.70	0.120	0.106
$C_{14:0}$	13.40	13.11	13.98	0.142	0.134
$C_{16:0}$	33.06	30.23	32.16	0.480	0.143
$C_{16:1}$	1.55[a]	1.20[b]	1.36[ab]	0.035	0.024
$C_{18:0}$	8.68[a]	11.36[b]	8.68[a]	0.156	0.001
$C_{18:1}$	20.31	21.13	18.90	0.538	0.336
$C_{18:2}$	2.88[a]	2.26[b]	2.61[ab]	0.070	0.038
$C_{18:2\ c9t11}$	0.54[a]	0.22[b]	0.26[b]	0.013	0.001
$C_{18:3}$	0.43[a]	0.55[ab]	0.57[b]	0.018	0.043
$C_{20:0}$	0.08[a]	0.32[b]	0.17[c]	0.006	0.001
$C_{22:0}$	0.07[a]	0.17[b]	0.07[a]	0.008	0.006
ω6/ω3[1]	4.50[a]	3.38[b]	3.71[b]	0.085	0.006
Σ Saturated fatty acids	72.76	72.66	74.12	0.537	0.535
Σ Monounsaturated fatty acids	23.49	24.15	22.14	0.515	0.369
Σ Polyunsaturated fatty acids	3.75	3.20	3.73	0.075	0.056

a, b, c Within a row, means lacking a common superscript letter differ ($P < 0.05$).
1 $C_{18:2\ c9c12}$ / $C_{18:3\ c9c12c15}$.
2 Standard error of the mean.

lupin-based diets compared to the soybean meal diet. The high level of $C_{18:0}$ in the milk of lupin-supplemented cows was also observed in experiment 1 and is consistent with the ruminal hydrogenation of $C_{18:1}$, the main fatty acid in lupin lipids [32]. The reduction of $C_{18:2}$ with the lupin-based diet could be explained by the low level of this fatty acid in lupin lipids and the fact that part of the dietary fatty acids is transferred in the milk [32]. High lupin incorporation in dairy cow feed also led to a reduction of the ω6/ω3 ratio that is recommended for human health [17].

In conclusion, coarsely ground lupin seeds appeared suitable to replace 75% of soybean meal on a DM basis in high-producing dairy cow feed, whereas the protein content of the pea was too low. Lupin protein was used as efficiently as soybean meal protein. Total soybean meal substitution by lupin seeds on a N basis is therefore possible without any loss of milk production. However, lupin seed incorporation in dairy cow feeding should be limited to 6 $kg \cdot d^{-1}$ in order to avoid an excess of some dietary fatty acids with practical feeding. The high content of $C_{18:1}$ in lupin seed influenced the milk fatty acids pattern with, notably, a reduction of the ω6/ω3 ratio.

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