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Pea (*Pisum sativum*) in dairy cow diet: effect on milk production and quality

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ABSTRACT

The use of alternative plant protein in place of soybean meal protein in diets for farmed animals aims to reduce the extra-EU soybean import and partially substitute GMO in the food chain. Among the possible alternatives, the heat-processed (flaked) pea appears interesting in dairy cow diets. Two consecutive experiments were carried out to test flaked peas as a partial substitute for soybean meal in the diet of Reggiana breed dairy cows producing milk for Parmigiano-Reggiano cheese-making. In both experiments a "Control" concentrate (8.3% soybean meal) was compared to a "Pea" concentrate (5% soybean meal and 15% flaked peas). Forages fed to animals included mixed grass hay and alfalfa hay in experiment 1, and hay (mixed grass and alfalfa) plus mixed grass in experiment 2. Milk yield and quality, and the characteristics of grab faecal samples, examined to get some empirical indicators of digestibility, were similar between feeding groups. Compositional changes (crude protein and solubility) in forages used as common base in the diets of both experiments had a slight effect on milk and plasma urea contents. There was a tendency for a higher milk urea content in the "Pea" group (32.3 *vs* 30.1mg/dl in experiment 1, P<0.1; 30.2 *vs* 28.0mg/dl in experiment 2, P<0.1). The plasma urea content was different only in experiment 2 (4.9 *vs* 5.6mmol/l, respectively for "Control" and "Pea" groups; P<0.05). The inclusion of the heat-processed pea within the allowed limit of the Parmigiano-Reggiano Consortium for diet formulation could represent a feasible opportunity for a partial substitution of soybean meal.

Key words: Pea, Dairy cow, Milk quality, Parmigiano-Reggiano cheese.

RIASSUNTO

INCLUSIONE DEL PISELLO PROTEICO (*PISUM SATIVUM*) NELLE DIETE PER BOVINE IN LATTAZIONE: EFFETTI SULLA PRODUZIONE E SULLA QUALITÀ DEL LATTE

La ricerca scientifica ha mostrato negli ultimi anni un crescente interesse verso l'utilizzazione di fonti proteiche alternative alla soia nell'alimentazione degli animali in produzione zootecnica, col duplice intento di svincolarsi, almeno in parte, da un'onerosa voce di importazione da Paesi extra-UE, e di ridurre l'impiego *di materie prime OGM. Tra le diverse fonti vegetali potenzialmente utilizzabili, il pisello proteico appare un alimento interessante nelle diete destinate alle lattifere, in quanto apporta sia una buona quantità di proteina che di amido. In due prove sperimentali successive il pisello proteico in forma fioccata è stato introdotto a parziale sostituzione della farina di estrazione di soia nella dieta di bovine di razza Reggiana, il cui latte è destinato alla trasformazione in formaggio Parmigiano-Reggiano. In entrambe le prove una dieta "Controllo" (8,3% f.e. soia) è stata confrontata con una dieta "Pisello" (5% f.e. soia, 15% pisello fioccato). La base foraggiera era rappresentata da solo fieno (prato polifita e medica) nella prova 1, da fieno polifita, fieno di medica ed erba di prato polifita nella prova 2. La produzione e la qualità del latte, nonché le caratteristiche di campioni fecali, esaminati per ottenere alcuni indicatori empirici di digeribilità, sono risultate simili tra i due gruppi sperimentali. Alcune variazioni nella composizione dei foraggi utilizzati come base comune per le due diete (proteina greggia e solubile) hanno indotto leggere differenze sul contenuto in urea del latte e del plasma. Il contenuto di urea nel latte è stato leggermente superiore per le bovine del gruppo "Pisello" (32,3* vs *30,1mg/dl nella prova 1, P<0,1; 30,2* vs *28,0mg/dl nella prova 2, P<0,1) e, limitatamente alla seconda prova, il livello ematico di urea è stato più elevato nelle bovine dello stesso gruppo (5,6* vs *4,9mmol/l; P<0,05). I risultati ottenuti consentono di affermare che il pisello proteico può essere utilizzato nella dieta delle lattifere ai livelli massimi consentiti dal Regolamento di Produzione del Parmigiano-Reggiano, in parziale sostituzione alla farina di estrazione di soia.*

Parole chiave: *Pisello proteico, Vacca da latte, Qualità del latte, Formaggio Parmigiano-Reggiano.*

Introduction

The protein sources commonly used in concentrate feeds for dairy cows are few and include mainly soybean meal (SBM), sunflower meal, canola meal and full-fat soybean. The need for alternative protein sources to soybean meal, partially or totally substituted in diets of dairy cows and other farmed animals is looming and it has two main reasons: a partial limit to SBM imports from extra-EU Countries which represents a negative line item on the commercial balance sheet; secondly to prevent the presence of GMO in the food chain (Mordenti and De Castro, 2005; Formigoni *et al.*, 2007)

The second remark is acquiring widespread interest since an increasing percentage of consumers clearly state a refusal towards the presence of GMO feeds, both in their own diet and in the diet of the animals which produce milk, meat, etc… for their table. This tendency becomes significantly stronger when "typical/traditional foods" are considered. For instance, the Consortium of the Parmigiano-Reggiano (PR) cheese recently stated (Molfino, 2007) that it would adhere to a campaign promoting the use of GMO-free foods in Italy and Europe, and encourage the PR producers to use GMO-free concentrates in animal diets. As a consequence, an interest in studying the possibility of growing and using GMO-free crops has spread in recent years in the PR producing area.

Among the possible protein sources, lupins, peas and faba beans were successfully used in ruminants and non ruminants (Burel *et al.*, 2000; Bonomi, 2005; Moschini *et al.*, 2005; Masoero *et al.*, 2006; Vandoni *et al.*, 2007), however, the first of these is not included in the list of allowed feeds for PR.

The pea (*Pisum sativum*) has lower protein and higher starch content than SBM, and is similar to barley for starch rumen fermentability (Masoero *et al.*, 1997, 2006); thus, it may be considered as an interesting "dual purpose" feed for its protein and energy contents. In particular, when compared to SBM, the protein is richer in lysine although lower in methionine, and the use along with corn meal should avoid an unbalance of amino acids (Masoero *et al.*, 2006).

Rumen degradability and soluble fractions are higher in pea protein compared to the SBM protein (Corbett *et al.*, 1995; Khorasani *et al.*, 2001; Schroeder, 2002; Masoero *et al.*, 2005). As with other legumes, the pea contains some anti-nutritional factors and, although some studies have reported no detrimental effects in the use of crude peas (Corbett *et al.*, 1995; Pasquini *et al.*, 2003; Formigoni *et al.*, 2007), the heat-based processing treatments seem advisable leading to a lowering of protease inhibitors and other contents constituting anti-nutritional factors, and to an increase in the protein escaping the rumen fermentation (Focant *et al.*, 1990; Walhain *et al.*, 1992; Masoero *et al.*, 2005).

Extrusion and expansion applied to peas increased the insoluble protein fraction and reduced the amount of protein degraded in the rumen, whereas the extrusion itself increased the rumen and the *in vitro* starch degradability (Petit *et al.*, 1997; Masoero *et al.*, 2005). Thus, heat processed peas should provide both a source of rumen degradable carbohydrates and a good amount of rumen undegradable proteins, thus meeting the protein need at the duodenum.

There are works (Hoden *et al.*, 1992; Petit *et al.*, 1997; Corbett *et al.*, 2005; Masoero *et al.*, 2006) showing the feasibility of using peas as a partial or whole substitute for SBM, without negative consequences on the performance of lactating cows. Contributions about the use of flaked peas are limited (Battini *et al.*, 2003), and the technique seems to have no effect on pea protein degradability and starch gelatinization (Focant *et al.*, 1990). An investigation into the implementation of flaked peas in PR producing cows therefore seems interesting.

The aim of the present research was to study the feasibility of introducing the flaked peas into the diet of Reggiana breed cows. This ancient breed represents a small and very efficient reality within the area of the PR cheese; the quality of its milk is recognized as superior for cheese-making due to a higher content of casein, in particular the B type k-casein (CVPARR, 1999). The dietary rules for the Reggiana are even stricter compared to other PR producing cows as the National Association of Reggiana Cattle Breeders (ANaBoRaRe, 2008) does not allow the use of the GMO feeds. The Association itself is thus particularly interested in studies promoting the growing and the usage of GMO-free feeds.

Material and methods

Animals and diets

Two consecutive experiments were carried out under farm conditions in a medium size Reggiana breed dairy farm (80 cows in milking) located in a plain area in Northern Italy. The milk produced is transformed into PR cheese.

The forages used in both experiments were self-produced and green grass was used for about eight months a year according to the Rules of Production of the breed (ANaBoRaRe, 2008).

Experiment 1 started in late winter (February) and it was characterised for having the forage component made only of hay (70% mixed grass and 30% alfalfa), whereas in experiment 2, carried out in spring (April-June), part of the mixed grass hay (about 50%) was substituted by green forage.

The cows were fed forages *ad libitum* and concentrate feeds by means of computer-controlled self-feeders (BouMatic, Madison, Wisconsin, USA). The daily amount of concentrate intake was recorded individually. Since cows were kept in the same fenced area it was not possible to monitor the individual forage dry matter intake.

Only cows between 10 and 250 days in milk were considered for entering the experiments.

Table 1. Ingredients (%) of the experimental concentrates.

	Control	Pea
Corn meal	35.0	35.0
Wheat bran	18.9	16.2
Wheat flour shorts	15.0	3.0
Flaked peas	-	15.0
Corn germ meal	10.0	10.0
Soybean meal	8.3	5.0
Beet pulp dehy	4.5	7.5
Sugar cane molasses	3.0	3.0
Calcium carbonate	3.0	3.0
Sodium chloride	0.6	0.6
Sodium bicarbonate	0.5	0.5
Mineral and vitamin supplement[1]	0.5	0.5
Magnesium oxide	0.4	0.4
Dicalcium phosphate	0.3	0.3

[1] *Composition (per kg): Vit.A U 50,000; Vit.D_3 U 5000; Vit.E mg 150; Vit.B_1 mg 3; Vit.PP mg 500; Vit.H mg 2; Mn mg 150; Fe mg 100; Zn mg 250; Cu mg 15; I mg 5; Co mg 1; Se mg 1.*

Two concentrates were used in both experiments (Table 1): a standard concentrate being used on the farm (Control) and an experimental concentrate (Pea) in which a part of the soybean meal and of wheat by-products (bran and flour shorts) were substituted with 15% of steam-flaked peas (*Pisum sativum*); the loss of fibre induced by these changes in comparison with the Control diet was balanced by raising the amount of dried sugar-beet pulp. The percentage of peas included was the maximum allowed by the Rules for PR cheese production, thus only a partial replacement of soybean meal was possible in order to maintain the equal protein content in the two concentrates. The pea flakes were obtained by steam-cooking (95-100°C, 20% moisture) the pea seeds for 45-50 minutes; they were then rolled (1mm, 70kg/L density) and dried to 11% moisture content (Consorzio Agrario Provinciale, Reggio Emilia, Italy). Then, flakes were ground (1,5mm screen) before being included in the experimental concentrate. Both concentrates were pelletted (6 mm diameter and 125kg/L density) for optimal use in the self-feeders.

Animals fed the Pea concentrate were adapted to the treatment diet by mixing the Control and Pea concentrate (50% w/w) for 10 days before starting the experiments.

In experiment 1 sixty cows were used and divided in two homogeneous groups for average daily milk yield (21.6±6.3 and 22.0±6.6kg/d), days in milk (130.2±72.0 and 130.9±60.7), parities (3.1±1.6 and 3.0±2.0) and average milk protein content (3.46±0.27 and 3.43±0.39%), respectively, for the Control and Pea treatment. The experiment lasted 26 days and milk yield and concentrate intake were individually recorded on a daily basis. Two sub-groups of 15 cows each, homogeneous for average daily milk yield (26.7±3.7 and 27.1±4.3kg/d), days in milk (99.5±66.4 and 103.4±46.1), parities (3.7±1.3 and 3.5±1.4), and average milk protein content (3.33±0.17% and 3.24±0.23%), respectively, for the Control and Pea treatment, were sampled for milk composition (day 0, 12, 19 and 26 of the experiment), faecal indexes (day 0, 12 and 26 of the experiment) and blood urea (day 0 and 26 of the experiment).

In experiment 2 forty-four cows were allotted to two homogeneous groups for average daily milk yield (25.5±6.6 and 25.3±6.6 kg/d), days in milk (119.6±61.7 and 125.4±61.7), parities (2.8±1.3 and 2.9±1.6) and average milk protein content (3.44±0.34 and 3.30±0.31), respectively, for the Control and Pea treatment. Cows were adapted for 36 days to the introduction of herbage in the diet. The experiment lasted 48 days and milk yield and concentrate intake were individually recorded on a daily basis. Two sub-groups of 15 cows each, homogeneous for average daily milk yield (25.1±5.6 and 24.9±6.2kg/d), days in milk (130.9±54.9 and 131.1±60.9), parities (2.9±1.2 and 3.0±1.5), and average milk protein content (3.49±0.37 and 3.28±0.29%), respectively, for the Control and Pea treatment, were sampled for milk composition (day 0, 8, 27 and 48 of the experiment), faecal indexes (day 8, 27 and 48 of the experiment) and blood urea (day 8 and 48 of the experiment).

Samples collection and analytical procedures

Concentrate feeds and forages were collected at the beginning and at the end of each experiment, dried in a ventilated oven at 65°C for 48h, ground with a 1mm sieve (Thomas-Wiley Laboratory Mill, model 4, Arthur H. Thomas Co., Philadelphia, PA), then analysed for dry matter, crude and soluble protein, starch (polarimetric method), crude fibre, ether extract, ash, neutral detergent fibre, acid detergent fibre and lignin contents (Martillotti *et al.*, 1987; Licitra *et al.*, 1996).

Milk samples for days of collection were obtained by proportional pooling by cow of the morning and evening milkings. Then, samples were analysed for fat, protein, lactose, casein and urea contents (infrared analysis, Milkoscan Model FT120 Foss Electric, Denmark).

A further sample from each cow was taken from both morning and evening milkings during experiment 2; all the samples belonging to the same group were then mixed, since the paucity of financial support for this research did not permit the analysis of individual samples. The two resulting pooled samples were analysed for the rennet coagulation characteristics (tromboelastographic method; Formawin 32, Foss Electric, Denmark) according to the regulation adopted by the PR Cheese Consortium (Salvadori del Prato, 1998) and were expressed as clotting time (r), curd firming (k_{20}) and curd firmness measured 30 min after rennet addition (a_{30}). From these values an index was calculated which describes the aptitude of milk to cheese-making: A=optimal; B=good; E=poor, and intermediate indexes (Rossi and Vecchia, 1994).

Faecal samples were taken directly from the rectum, and the faecal score was immediately evaluated using the following scale (Masoero *et al.*, 2006): 1=very liquid faeces; 2=faeces are runny and do not form a nice pile; 3=porridge-like consistency; 4=moderate thickening of the faeces; 5=firm faecal balls.

Faecal samples were also evaluated according to the method proposed by Mancin *et al.* (2004) and Dell'Orto and Savoini (2005). Each faecal sample was put in a sieve (1.5mm mesh), weighed, washed with running water until output water was clear, and weighed again. An empirical index of undigested residue (%) was calculated. The residue was then uniformly spread on a white paper, and evaluated on the basis of the amount of "Undigested Fraction" by means of a score ranging from 1=small particles of very ground forages (optimal) to 5=large incidence of very coarse materials. The analysis of the residual was completed by the visual evaluation of the incidence of concentrate (%).

Blood samples were obtained from the caudal vein and collected into Li-Heparinized (15U/ml of blood) evacuated collection tubes (Venoject, Terumo Europe, Leuven, Belgium), then centrifugated at 2500g for 15 minutes. Then, plasma was separated and frozen stored at –20°C before analyses of total protein and urea contents (Beckman Coulter "SYNCHRON CX 5 Delta®" automatic analyser) by using the kit supplied by Beckman Coulter. Total Protein (TP) reagent was used to measure the total protein concentration by a timed-endpoint biuret method (Hiller *et al.*, 1948). Urea reagent was used to determine the urea concentration by means of an UV-based kinetic-enzymatic method (urease) (Talke and Schubert, 1965; Tiffany *et al.*, 1972).

Statistical methods

Response variables from both experiments that were measured over time (i.e., milk yield, milk fat, protein and lactose contents, milk casein, milk urea, undigested fraction, faecal score, blood protein and urea contents) were subjected to ANOVA using the repeated statement in the mixed procedure of SAS (2001) in a completely randomized design where the experimental unit was cow. In both experiments the statistical model included fixed effects of diet, time of measurement and the diet x time of measurement interaction with cow as the random variable. Each variable analysed was subjected to three covariance structures: being toeplizt, compound symmetry and unstructured. Using the Akaike information criterion and the Schwarz Bayesian criterion, the compound symmetry was the covariance structure that best fit the model.

The statistical general model in both experiments was as follows:

$Y_{ijk}=\mu+\alpha_i+b_{ij}+\gamma_k+(\alpha\gamma)_{ik}+\varepsilon_{ijk}$

Where:

Y_{ijk}=the dependent variable at time k on the j^{th} subject assigned to treatment i

μ=overall mean

α_i =fixed effect of treatment i (i=Control, Pea)

b_{ij}=random effect for subject j assigned to treatment i

γ_k=fixed effect of time

$(\alpha\gamma)_{ik}$=fixed effect of treatment x time interaction

ε_{ijk}=residual error with covariance matrix

Significance was declared at P<0.05 and a trend at 0.05<P<0.1.

Results and discussion

The chemical composition of concentrates used in both experiments is reported in Table 2. The flaked pea had a crude protein content of 21.5% and a soluble protein fraction of 58.1%, higher than the 20 to 30% range values found in peas processed by extrusion, expansion or toasting (Remond *et al.*, 2003; Masoero *et al.*, 2005). The solubility data observed in our experiment are probably due to a less intensive physical treatment (95-100°C for 45-50min) compared to expansion (130°C and 30atm) or toasting (130-140°C for 20min).

The analytical parameters of concentrates were rather constant throughout the experiments.

The chemical composition of the forages is reported in Table 3. The protein content

Table 2. Chemical composition of the experimental concentrates (% DM, unless otherwise stated).

	Flaked pea	Control		Pea	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
Dry matter (%)	97.7	91.0	92.5	91.0	92.0
Crude protein	21.5	15.7	15.4	15.5	15.8
Soluble protein (% total)	58.1	27.1	27.4	27.0	25.4
Crude lipids	1.4	3.8	3.5	3.8	3.6
Crude fibre	6.1	7.1	6.6	6.9	6.3
NDF	13.5	21.4	21.5	20.9	20.4
ADF	7.2	9.3	8.3	9.4	9.0
ADL	2.3	3.5	2.8	3.4	2.6
Starch	49.5	37.2	37.2	39.4	37.8

and solubility of mixed grass hay and alfalfa hay changed between experiments. On average, the crude protein was lower in experiment 2 (i.e. 13.8 *vs* 16.1 and 13.8 *vs* 15.5), whereas the protein solubility values were 21.3 *vs* 27.8 and 35.7 *vs* 32.8% of the total protein, for mixed grass hay and alfalfa hay, respectively.

No health problems that could be attributed to the diet being fed were observed in animals in both experiments.

No differences were observed for faecal parameters in both experiments (Tables 4 and 5) except for the residual indigested concentrate in experiment 2 which was lower in the Pea diet compared to the Con-

Table 3. Chemical composition of the forages (% DM, unless otherwise stated).

	Mixed grass hay				Alfalfa hay				Mixed grass	
	Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 2	
	start	end	start	end	start	end	start	end	start	end
Dry matter (%)	85.3	86.5	88.1	92.2	84.5	84.7	90.6	92.4	22.9	22.2
Crude protein	16.7	15.5	13.6	13.9	15.6	15.4	13.8	13.7	13.1	16.1
Soluble protein (% total)	30.7	25.5	19.9	22.7	35.6	30.0	33.7	37.6	37.2	27.2
Crude lipids	1.4	1.5	1.2	1.6	1.1	0.9	0.8	0.9	1.1	1.6
Crude fibre	27.2	33.0	28.6	27.9	37.4	48.3	32.3	37.8	26.5	25.5
NDF	49.3	51.3	53.5	56.2	49.9	49.9	48.0	50.5	40.8	44.8
ADF	35.4	35.6	33.2	33.7	42.2	38.9	36.5	42.5	30.2	29.8
ADL	7.5	6.7	4.0	5.4	12.1	10.7	7.8	7.6	4.6	5.6

Table 4. Experiment 1: faecal parameters as influenced by the different diets fed to animals.

		Diet		SEM	Significance
		Control (n=15)	Pea (n=15)		
Undigested residue	%	52.16	55.57	2.368	ns
Faecal score		2.25	2.28	0.086	ns
Undigested fraction		2.52	2.59	0.044	ns
Residual concentrate	%	1.89	1.99	0.165	ns

ns: not significant.

Table 5. Experiment 2: faecal parameters as influenced by the different diets fed to animals.

		Diet		SEM	Significance
		Control (n=15)	Pea (n=15)		
Undigested residue	%	52.17	53.70	1.967	ns
Faecal score		2.44	2.45	0.055	ns
Undigested fraction		2.53	2.45	0.079	ns
Residual concentrate	%	2.10	1.74	0.119	*

**P<0.05; ns: not significant.*

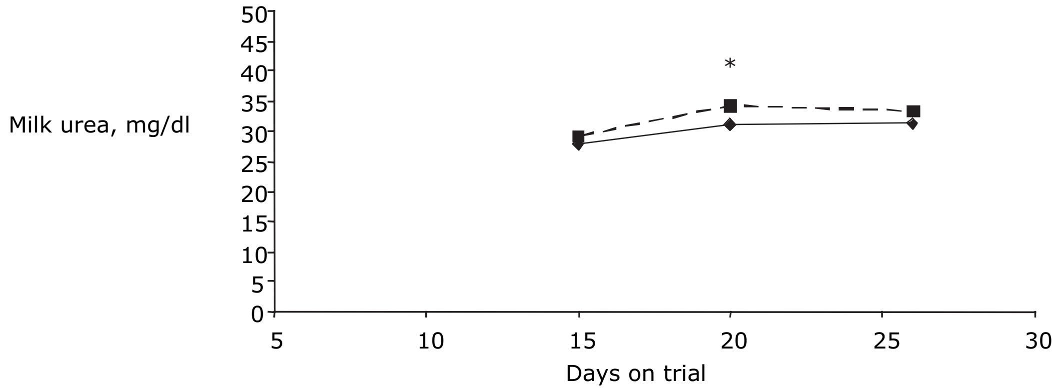
trol diet (Table 5). This might indicate, considering the empirical characteristic of the variable, a slight improvement (P<0.05) of the concentrate digestibility.

Tables 6 and 7 report the concentrate intake, milk yield and composition of experimental groups in experiment 1 and 2, respectively. The presence of the pea in the concentrate did not affect the concentrate intake suggesting no negative effects on palatability, in accord with Khorasani *et al.* (2001).

The milk yield and composition in both experiments were not affected by the use of peas in the diet. No differences were previously reported in milk yield in mid late-lactating cows fed diets with increasing levels of peas (Khorasani *et al.*, 2001), whereas early lactating cows fed pea-based concentrates had higher 4% FCM yield and higher milk fat content (Corbett *et al.*, 1995; Petit *et al.*, 1997). Also Hoden *et al.* (1992) found similar milk, fat and protein production after the introduction of peas in the diet of lactating cows, although the highest producing cows showed a decrease in milk and fat production when fed peas. Even though no differences between groups were observed in milk protein contents, Khorasani *et al.* (2001) reported a quadratic response of the milk protein content in mid late-lactating cows fed peas, whereas only a tendency toward a higher milk protein content was observed by Petit *et al.* (1997) in cows fed extruded peas.

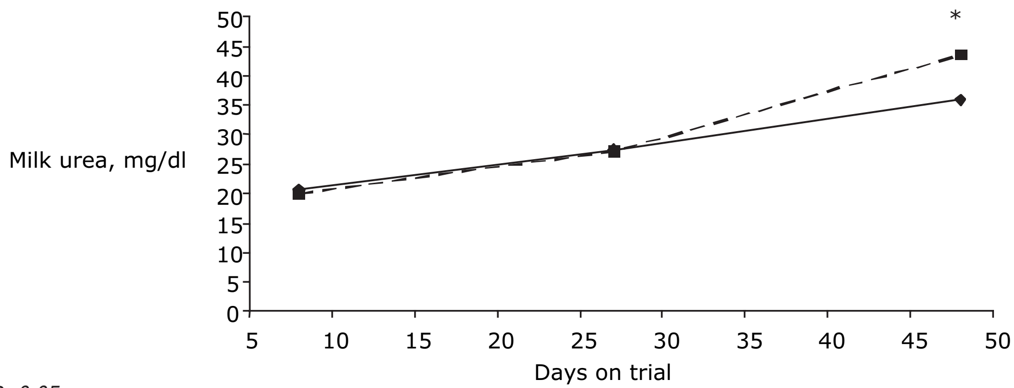
When feeding the Pea diet the milk

Figure 1. Experiment 1: urea content in milk from cows fed the Control (♦) or Pea (■) diet.



**P<0.05.*

Figure 2. Experiment 2: urea content in milk from cows fed the Control (♦) or Pea (■) diet.



**P<0.05.*

urea tended to increase by about 8% (32.3 *vs* 30.1mg/dl in experiment 1 and 30.2 *vs* 28.0mg/dl in experiment 2; P<0.1). As shown in Figure 1 and 2, the milk urea content was significantly higher (34.2 *vs* 31.0mg/dl; P<0.05) at the 20^{th} day in experiment 1 and at the 48^{th} day in experiment 2 (43.5 *vs* 35.9; P<0.05).

Even though the soluble protein fraction was rather constant in the concentrates (Table 2) the increase in milk urea of the pea group might be due to a higher degradability (not measured) of the protein fraction of processed pea. Khorasani *et al.* (2001) obtained a linear and cubic response of rumen ammonia-N to increasing levels of pea in the diet.

Table 6. Experiment 1: concentrate intake, milk yield, milk composition, blood protein and blood urea as influenced by the different diets fed to animals.

		Diet			
		Control (n=30)	Pea (n=30)	SEM	Significance
Concentrate intake/cow	kg/d	7.80	8.14	0.340	ns
Milk yield	"	21.52	20.92	0.457	ns
Milk composition:					
Fat	%	3.61	3.56	0.100	ns
Protein	"	3.37	3.27	0.051	ns
Lactose	"	4.98	4.92	0.043	ns
Casein	"	2.65	2.55	0.042	ns
Urea	mg/dl	30.05	32.29	0.849	†
Fat yield	kg/d	1.00	0.91	0.053	ns
Protein yield	"	0.92	0.84	0.039	ns
Casein yield	"	0.73	0.66	0.031	ns
Blood total protein	g/l	74.50	73.57	1.161	ns
Blood urea	mmol/l	4.55	4.83	0.167	ns

†P<0.10; ns: not significant.

Table 7. Experiment 2: concentrate intake, milk yield, milk composition, blood protein and blood urea as influenced by the different diets fed to animals.

		Diet			
		Control (n=22)	Pea (n=22)	SEM	Significance
Concentrate intake/cow	kg/d	8.65	8.70	0.370	ns
Milk yield	"	24.37	24.24	0.380	ns
Milk composition:					
Fat	%	3.71	3.55	0.125	ns
Protein	"	3.49	3.33	0.073	ns
Lactose	"	4.92	4.86	0.038	ns
Casein	"	2.74	2.59	0.060	†
Urea	mg/dl	28.04	30.17	0.803	†
Fat yield	kg/d	0.87	0.83	0.059	ns
Protein yield	"	0.82	0.77	0.042	ns
Casein yield	"	0.64	0.60	0.033	ns
Blood total protein	g/l	67.99	70.96	1.177	†
Blood urea	mmol/l	4.91	5.57	0.167	*

*†P<0.10;*P<0.05; ns: not significant.*

Table 8. Experiment 2: rennet coagulation characteristics measured in pooled milk (average ±SD of three samples).

			Diet	
			Control	Pea
Clotting time "r"		min	18.01 ± 2.06	16.38 ± 3.12
Curd firming time "k_{20}"		"	2.20 ± 0.23	2.15 ± 0.15
Curd firmness "a_{30}"		mm	39.99 ± 8.43	37.81 ± 10.18
Index of coagulation:	day 8		B	B
	day 27		AE	AE
	day 48		A	A

The different plasma urea content observed only in experiment 2 (5.6 *vs* 4.9mmol/l; P<0.05; Table 7) might be due to the presence of the mixed grass which partially substituted the hay of the diet. Then, the higher protein content and higher solubility of the mixed grass (14.6% and 32.2%), compared to the mixed grass hay (13.7% and 21.3%), probably added to the highly degradable protein of peas, scarcely affected by flaking (see above: Focant *et al.*, 1990), and resulted in higher plasma urea content of the Pea group.

The plasma urea levels in our experiments were lower than previous reported data from cows fed raw or processed peas (Masoero *et al.*, 2006) obtained on more productive dairy cows fed 2.5kg/cow/day pea compared to the average 1.2kg/cow/day in the current experiments.

Table 8 reports the rennet coagulation characteristics of pooled milk collected during experiment 2. Although no statistical analysis could be performed on these data, it is interesting to note that the introduction of peas in the diet barely affected the milk coagulation trend, and the indexes were similar and optimal or good for both the pooled milks.

Conclusions

The inclusion of flaked peas in diets for Reggiana dairy cows did not produce negative effects on milk yield and composition.

When used within the allowed limit of the Parmigiano-Reggiano Consortium, the flaked peas could represent a feasible opportunity for a partial substitution of soybean meal in diet formulation.

These results could represent a base of discussion for a possible increase in the maximum level of pea inclusion currently authorised by the Parmigiano-Reggiano Consortium.

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REFERENCES

ANaBoRaRe (National Association of Reggiana cattle Breeders), 2008. Home page address: http://www.razzareggiana.it

Battini, F., Ligabue, M., Marmo, N., Vecchia, P., 2003. Pisello proteico nell'alimentazione delle bovine da latte. Inform. Agr. 59(1):27-30.

Bonomi, A., 2005. L'impiego dei semi di pisello espansi (*Pisum sativum* L.) nell'alimentazione dei suini produttori di carne da consumare fresca. Riv. Suinicolt. 46(4):189-193.

Burel, C., Boujard, T., Tulli, F., Kaushik, S.J., 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188:285-298.

Corbett, R.R., Okine, E.K., Goonewardene, L.A., 1995. Effects of feeding peas to high-producing dairy cows. Can. J. Dairy Sci. 75:625-629.

CVPARR (Consortium for enhancement of the old Reggiana Breed Products), 1999. L'oro delle vacche rosse. La Nuova Tipolito, Reggio Emilia, Italy.

Dell'Orto, V., Savoini, G., 2005. Alimentazione della vacca da latte. Edagricole, Bologna, Italy.

Focant, M., Van Hoecke, A., Vanbelle, M., 1990. The effect of two heat treatments (steam flaking and extrusion) on the digestion of *Pisum sativum* in the stomachs of heifers. Anim. Feed Sci. Tech. 28:303-313.

Formigoni, A., Fustini, M., Mordenti, A., Nocetti, M., Vecchia, P., 2007. Favino e pisello nella razione, buoni sostituti della soia. Inform. Agr. 63(17):40-44.

Hiller, A., Plazin, J., Van Slyke, D.D., 1948. A study of conditions for Kjeldhal determination of nitrogen in proteins. Descriptions of methods with mercury as catalyst, and titrimetric and gasometric measurements of the ammonia formed. J. Biol. Chem. 176:1401-1420.

Hoden, A., Delaby, L., Marquis, B., 1992. Pois protéagineux comme concentré unique pour vaches laitières. INRA Prod. Anim. 5(1):37-42.

Khorasani, G.R., Okine, E.K., Corbett, R.R., Kennelly, J.J., 2001. Nutritive value of peas for lactating dairy cattle. Can. J. Anim. Sci. 81:541-551.

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Tech. 57:347-358.

Mancin, G., Savoini, G., Burlotto, V., Dell'Orto, V., 2004. La granulometria della miscelata influenza la produzione di latte. Inform. Agr. 60(41):38-43.

Martillotti, F., Antongiovanni, M., Rizzi, L., Santi, E., Bittante, G., 1987. Metodi di analisi per la valutazione degli alimenti di impiego zootecnico. Quaderni Metodologici n. 8. IPRA-CNR ed., Roma, Italy.

Masoero, F., Moschini, M., Fusconi, G., Piva, G., 2006. Raw, extruded and expanded pea (*Pisum sativum*) in dairy cows diets. Ital. J. Anim. Sci. 5:237-247.

Masoero, F., Pulimeno, A.M., Rossi, F., 2005. Effect of extrusion, espansion and toasting on the nutritional value of peas, faba beans and lupins. Ital. J. Anim. Sci. 4:177-189.

Masoero, F., Santi, M., Cabiddu, A., Moschini, M., 1997. Previsione del profilo fermentativo di miscele di alimenti per ruminanti. pp 25-26 in Proc. 12th Nat. Congr. ASPA, Pisa, Italy.

Molfino, M., 2007. Il Parmigiano Reggiano va all'estero con il certificato. L'Allevatore Magazine 63(19):18-19.

Mordenti, A., De Castro, P., 2005. Mangimi GM e produzioni tipiche, una convivenza forzata. Inform. Agr. 61(14):47-50.

Moschini, M., Masoero, F., Prandini, A., Fusconi, G., Morlacchini, M., Piva G., 2005. Raw Pea (*Pisum sativum*), raw Faba bean (*Vicia faba* var. *minor*) and raw Lupin (*Lupinus albus* var. multitalia) as alternative protein sources in broiler diets. Ital. J. Anim. Sci. 4:59-69.

Pasquini, M., Mattii, S., Trombetta, M.F., Tavoletti, S., 2003. On-farm feeding trial to test partial substitution of soybean with faba bean and field pea in dairy cows. pp 241-248 in Proc. 38th Int. Symp. of Zootechny. Milk and Research, Lodi, Italy.

Petit, H.V., Rioux, R., Ouellet, D.R., 1997. Milk production and intake of lactating cows fed raw or extruded peas. J. Dairy Sci. 80:3377-3385.

Remond, D., Le Guen, M.P., Poncet, C., 2003. Degradation in the rumen and nutritional value of lupin (*Lupinus albus* L.) seed proteins effect of extrusion. Anim. Feed Sci. Tech. 105:55-70.

Rossi, A., Vecchia, P., 1994. La qualità del latte. Agricoltura 22 (9), Monographic supplement.

Salvadori Del Prato, O., 1998. Trattato di tecnologia casearia. Edagricole, Bologna, Italy.

SAS, 2001. SAS/STAT user's guide: statistics, version 9.1. Statistical Analysis Systems, Institute Inc., Cary, NC, USA.

Schroeder, J.W., 2002. Field peas in dairy cattle diets. Proc. Conf. on Feeding field peas to livestock. Home page address: www.ag.ndsu.edu/pubs/ansci/livestoc/eb76-2.htm.

Talke, H., Schubert, G.E., 1965. Enzymatic urea determination in the blood and serum in the Warburg Optical Test. Klin. Wochenschr. 43:174-175.

Tiffany, T.O., Jansen, J.M., Burtis, C.A., Overton, J.B., Scott, C.D., 1972. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC Fast Analyzer. Clin. Chem. 18:829-840.

Vandoni, S.L., Vercelli, G., Borgo, G., Vitali, G., Gagliardi, R., Innocenti, M., Bassini, A., Sgoifo Rossi, C.A., 2007. Il pisello proteico come fonte di fibra. L'Allevatore Magazine 63(20):40-47.

Walhain, P., Foucart, M., Théwis, A., 1992. Influence of extrusion on ruminal and intestinal disappearance in sacco of pea (*Pisum sativum*) proteins and starch. Anim. Feed Sci. Tech. 38:43-55.