

PAPER

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

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Abstract

The use of alternative plant proteins in place of the soybean meal protein in diets for producing animals aims to reduce the extra-EU soybean import and partially substitute the GMO in the food chain. Among possible alternatives, the heat-processed legume grains seem interesting for dairy cow diets. Two consecutive experiments were carried out to evaluate flaked pea and faba beans as substitute for soybean meal in diets for Reggiana breed dairy cows producing milk for Parmigiano-Reggiano cheese-making. In both experiments a C concentrate (110 g/kg soybean meal, no pea and faba beans) was compared to a PF concentrate (150 g/kg flaked pea, 100 g/kg flaked faba beans, no soybean meal). Forages fed to animals were hay (mixed grass and alfalfa) in experiment 1 and hay plus mixed grass in experiment 2. Concentrate intake, milk yield and milk quality (rennet coagulation traits included) were similar between feeding groups. Parameters on the grab faecal samples, as empirical indicators of digestibility, had a smaller ($P < 0.01$) amount of residual concentrate in the PF group compared to the C group (2.4 vs 3.1 and 2.3 vs 2.8%, respectively for PF and C in experiment 1 and 2). Some blood indicators of nitrogen metabolism (protein, albumin, urea) were similar between the feeding groups.

The inclusion of pea and faba beans, within the allowed limit of the Parmigiano-Reggiano Consortium for diet formulation, could represent a feasible opportunity for a total substitution of soybean meal.

Introduction

Soybean meal (SBM) is the most common protein source included in concentrate feeds for dairy cows in Italy, and in other European Countries as well. The need for alternative protein sources to SBM, 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 (Ellis, 2004); secondly to prevent the presence of GMO in the food chain (Wilkins and Jones, 2000). The latter remark is acquiring widespread interest thanks to the increasing preference of consumers toward GMO-free food/feeds, both in their own diet and in the diet of producing animals (milk, meat, etc.) The production of *typical/traditional* and/or PDO (Protected Denomination of Origin) foods, where quality and traceability are keywords, are particularly implicated. Within the area of the Parmigiano-Reggiano (PR) cheese, the ancient Reggiana breeding, known for producing high-quality milk for cheese-making, must comply with dietary rules that are even stricter compared to other PR producing herds: the National Association of Reggiana Cattle Breeders (ANaBoRaRe, 2011) does not allow the use of the GMO feeds. Thus, the Association itself and the PR Consortium are particularly interested in researches promoting the home growing and the usage of GMO-free feeds.

Among the possible protein sources, peas and faba beans were successfully used in dairy cow feeding (Corbett *et al.*, 1995; Petit *et al.*, 1997; Masoero *et al.*, 2006; Martini *et al.*, 2008). These crops offer some agronomical advantages in comparison with soy: greater adaptability, lower chemical and nutritive demands (Ellis, 2004). The pea (*Pisum sativum*) and the faba (*Vicia faba*) beans have lower protein and higher starch content than SBM, and may be considered as interesting dual purpose feeds for protein and energy contents. In particular, the protein is rich in lysine although low in methionine, and the use along with corn meal could avoid an unbalance of amino acids (Link *et al.*, 2007).

The rumen degradability and the soluble

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fractions (albumins and globulins) of the protein are higher in the grain legumes compared to the SBM (Corbett *et al.*, 1995; Khorasani *et al.*, 2001; Schroeder, 2002; Masoero *et al.*, 2005): thus, grain legumes are more suitable as supplements to low protein-forages, or they should be heat-processed (Wilkins and Jones, 2000). The grain legumes contain some anti-nutritional factors; although some works report no detrimental effects in the use of raw peas (Corbett *et al.*, 1995; Khorasani *et al.*, 2001), the heat-based processing treatments - such as extrusion, expansion and flaking - seem advisable to lower the protease inhibitors and other anti-nutritional factors, and to increase the protein fraction escaping the rumen degradation, therefore meeting the animal protein need at the duodenum (Focant *et al.*, 1990; Van der Poel *et al.*, 1991; Walhain *et al.*, 1992; Masoero *et al.*, 2005). Goelema *et al.* (1998, 1999) carried out interesting researches on effects of different processing methods and anti-nutritional factors.

Some works already suggested the possibili-

ty of using pea or/and faba beans as partial or whole substitutes of SBM in diets for dairy cows, with similar productive results (Hoden *et al.*, 1992; Corbett *et al.*, 1995; Petit *et al.*, 1997; Masoero *et al.*, 2006; Mordenti *et al.*, 2007; Martini *et al.*, 2008). In our previous researches (Volpelli *et al.*, 2009, 2010) pea and faba flakes were used separately as partial substitutes of SBM in Reggiana dairy cows' diet. The aim of the present research was to study the effect of the complete substitution of SBM with peas and faba beans on milk production and quality, concentrate intake and on some blood and faecal traits.

Materials and methods

Animals and diets

Two consecutive experiments were carried out at 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 was transformed into PR cheese.

The experiments differed by the forage component: hay (70% mixed grass and 30% alfalfa) for experiment 1 which lasted 12 weeks (December 2008-March 2009) and both green forage (about 50%) and hay (35% mixed grass and 15% alfalfa) for experiment 2 which lasted 12 weeks (September-November 2009). The cows were kept in one pen and fed on forages *ad libitum* and concentrate feeds by means of computer-controlled self-feeders (BouMatic, Madison, Wisconsin, USA). The daily intake of concentrate was recorded individually. Only cows between 15 and 220 days in milk were considered in both experiments. Two concentrates were used in both trials (Table 1): a standard concentrate being in use on the farm (C) and an experimental concentrate (PF), in which the dehulled SBM was replaced by the maximum amounts of steam-flaked pea and faba beans allowed by the Rules for PR cheese-making: 150 and 100 g/kg, respectively. With the aim to obtain similar nutritive composition in the two concentrates, PF contained, in comparison with C: less corn meal (230 vs 400 g/kg), in order to provide equal starch; less beet pulp (60 vs 70 g/kg), to partially balance the fibrous provision from pea and faba; more corn gluten feed (100 vs 60 g/kg) in order to provide equal protein.

The flakes were obtained by steam-cooking (95-100°C, 20% moisture) the seeds for 30 (faba) or 45 min (pea), then seeds were rolled (1 mm, 70 kg/L density) and dried at 150°C to a 11% moisture content (Consorzio Agrario

Provinciale, Reggio Emilia, Italy). At the end the flakes as a whole were added in the experimental concentrate. Animals fed on the PF concentrate were adapted to the treatment diet by mixing the C and the PF concentrate (50% w/w) for 7 days before starting the experiments.

In experiment 1, thirty-eight cows were used and divided in two homogeneous groups for average daily milk yield (C and PF: 24.1±6.0 and 24.3±4.5 kg/d), days in milk (85.3±45.8 and 84.2±42.7), parities (4.7±1.2 and 4.2±1.3) and milk protein content (32.9±3.0 and 33.4±4.0 g/kg). The experiment lasted 84 days and individual milk yield was recorded daily.

Two sub-groups of 15 cows (assumed as congruous also in previous researches, Volpelli *et al.*, 2009, 2010) were selected, homogeneous for average daily milk yield (C and PF: 25.2±6.2 and 25.1±4.3 kg/d), days in milk (72.3±30.3 and 71.1±29.1), parities (4.5±1.1 and 4.4±1.4), and milk protein content (32.6±3.1 and 32.0±3.0 g/kg); these cows were sampled for milk composition, blood and faecal indexes at day 0, 28, 56 and 84 on trial.

In experiment 2, twenty-eight cows were allotted to two treatment groups homogeneous for average daily milk yield (C and PF: 23.2±4.7 and 23.2±4.5 kg/d), days in milk (139.4±67.2 and 150.4±58.0), parities (3.9±2.5 and 3.2±1.9) and milk protein content (33.9±2.7 and 33.5±2.5 g/kg). The experiment lasted 84 days and individual milk yield was recorded daily. All cows were sampled for milk composition, blood and faecal indexes at day 0, 28, 56 and 84 on trial.

An empirical estimate of concentrate efficiency was calculated, in both experiments on cow and daily base, by dividing the amount of milk dry matter produced to the amount of concentrate dry matter intake.

Samples collection and analytical procedures

Feeds

Concentrate feeds and forages samples were collected at the beginning and at the end of each experiment, dried in a ventilated oven at 65°C for 48 h and ground through a 1 mm sieve (Thomas-Wiley Laboratory Mill, model 4, Arthur H. Thomas Co., Philadelphia, PA, USA). Samples were assayed in duplicate according to the AOAC (1990) for dry matter, crude protein, crude lipids and ash content by methods 930.15, 975.06, 954.02 and 942.05, respectively. Soluble protein was analysed according to Licitra *et al.* (1996). Starch content was measured after acid hydrolysis and polarimetric detection (Martillotti *et al.*, 1987).

The neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) content (Van Soest *et al.*, 1991) were analyzed using the Ankom F57 filter bags in an Ankom200 fibre analyzer (Ankom Technology, Macedon, NY, USA). For NDF analysis, samples were treated with an α -amylase (Sigma A-3306, Sigma-Aldrich® Co., Milan, Italy), and the neutral detergent solution contained sodium sulfite (Carlo Erba 483257, Carlo Erba® Reagenti SpA, Rodano, MI, Italy), and NDF and ADF residues were corrected for residual ash.

Milk

Two individual milk samples were obtained by a proportional pooling of the morning and the evening milkings. Then, the 1st sample was analysed for fat, protein, lactose, casein and urea contents (infrared analysis, Milkoscan Model FT120 Foss Electric, Denmark) whereas the 2nd sample was analysed for the rennet coagulation characteristics (thromboelastographic method; Formawin 32, Foss Electric), according to the regulation adopted by the PR Cheese Consortium (Salvadori del Prato, 1998), and expressed as: clotting time (r), curd firming time (k_{20}) and curd firmness measured 30 min after rennet addition (a30). Then, an index describing the aptitude of milk for cheese-making (A=optimal; B, C, D=good/suitable; E= poor; F=not suitable) was calculated (Rossi and Vecchia, 1994).

Table 1. Ingredients of the experimental concentrates.

	C	PF
Corn meal, g/kg	400	230
Wheat bran, g/kg	180	180
Soybean meal, dehulled, g/kg	110	-
Wheat flour shorts, g/kg	100	100
Flaked peas, g/kg	-	150
Flaked faba beans, g/kg	-	100
Beet pulp dehy, g/kg	70	60
Corn gluten feed, g/kg	60	100
Sugar cane molasses, g/kg	30	30
Calcium carbonate, g/kg	20	20
Sodium chloride, g/kg	8	8
Sodium bicarbonate, g/kg	7	7
Dicalcium phosphate, g/kg	3	3
Magnesium oxide, g/kg	4	4
Mineral and vitamin supplement ^o , g/kg	8	8

C, 110 g/kg soybean meal, no pea and faba beans; PF, 150 g/kg flaked pea, 100 g/kg flaked faba beans, no soybean meal.
^oComposition (per kg): vitamin A, 50,000 U; vitamin D₃, 5000 U; vitamin E, mg 150; vitamin B₁, mg 3; vitamin PP, mg 500; vitamin H, mg 2; Mn, mg 150; Fe, mg 100; Zn, mg 250; Cu, mg 15; I, mg 5; Co, mg 1; Se, mg 1.

Faecal samples

Faecal samples were obtained directly from the rectum and the faecal score was immediately evaluated using the following scale (Skidmore *et al.*, 1996): 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 Dell'Orto and Savoini (2005). Each faecal sample was put in a sieve (1.5 mm mesh) and washed with running water until output water was clear. Then, residue was uniformly spread on 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. A blind analysis of the residual was carried out by a trained operator with a visual estimate of the concentrate presence (%).

Blood samples

Blood samples were obtained from the caudal vein and collected into Li-Heparinized (15 U/mL of blood) evacuated collection tubes (Venoject, Terumo Europe, Leuven, Belgium). Then samples were centrifuged at 2500 g for 15 min and plasma was collected and frozen stored at -20°C before analyses of total protein, albumin and urea contents (Beckman Coulter, SYNCHRON CX 5 Delta® automatic analyser) by using the kits supplied by Beckman Coulter.

Statistical analyses

Data from both experiments and measured over time (*i.e.*, milk yield; milk, blood and faecal traits) were analyzed as repeated measurements in a completely randomized design using the Mixed procedure of SAS (2001). The experimental unit was the cow. The statistical model included fixed effects of diet (treatment - TRT), time of measurement and the diet x time of measurement interaction, with cow as random effect. Data measured over time were considered from day 7 onward and the value at day 0 was used as covariate. Each variable analyzed was subjected to three covariance structures: being toeplitz, compound symmetry and unstructured. Using the Akaike information criterion and the Schwarz Bayesian criterion, the compound symmetry was the covariance structure that best fitted 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 jth subject assigned to treatment i

μ = overall mean

α_i = fixed effect of treatment i (i = C, PF)

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

Data were tested for normality with the Shapiro-Wilk test; the variable curd firming time was log-normal transformed before statistical analysis.

Indexes describing the aptitude of milk for cheese-making were grouped into three classes (optimal+good, poor, not suitable), analyzed by chi-square test (Pilla, 1985) and expressed as percentage of each class on total. Significant differences were accepted if $P \leq 0.05$.

Results and discussion

The chemical composition of concentrates used in both experiments is reported in Table 2. The crude protein content of faba flakes was, as expected, higher than pea content (259 and

206 g/kg DM), while the soluble protein fraction was similar between the two flakes (466 and 479 g/kg total protein). In our previous researches (Volpelli *et al.*, 2009, 2010) faba flakes had values of crude protein (253 g/kg) and soluble protein (468 g/kg total protein) similar to the present findings, whereas pea flakes was analogous for crude protein (215 g/kg) but had higher solubility (581 g/kg).

The soluble protein fractions of the two flakes were intermediate between the values found by Masoero *et al.* (2005) for crude meal (pea: 745; faba: 711 g/kg) and extruded beans (pea: 200; faba: 194 g/kg) and probably due to the physical treatment, less intensive for flaking compared to extrusion. The total protein solubility was similar between the two experimental concentrates (232 and 251 g/kg total protein for C and PF, respectively), as well as it was for the starch content (386 and 389 g/kg), whilst the presence of the two flakes produced an increase of fibrous fractions in PF. The chemical composition of the forages had limited variations from the 1st to the 2nd experiment (Table 3). No health problems that could be attributed to the diet being fed were observed in animals in either experiment.

Among faecal parameters (Table 4), faecal scores and undigested fractions appeared sim-

Table 2. Chemical composition of the experimental concentrates.

	Flaked pea	Flaked faba	C	PF
Dry matter, g/kg	930	924	908	910
Crude protein, g/kg DM	206	259	155	154
Soluble protein, g/kg total protein	479	466	232	251
Crude lipids, g/kg DM	15	20	23	23
NDF, g/kg DM	93	137	195	232
ADF, g/kg DM	73	127	78	95
ADL, g/kg DM	17	18	17	23
Starch, g/kg DM	431	449	386	389

C, 110 g/kg soybean meal, no pea and faba beans; PF, 150 g/kg flaked pea, 100 g/kg flaked faba beans, no soybean meal; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

Table 3. Chemical composition of the forages.

	Mixed grass hay		Alfalfa hay		Green grass
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 2
Dry matter, g/kg	924	845	897	885	168
Crude protein, g/kg DM	89	112	169	186	192
Soluble protein, g/kg total protein	226	210	320	270	340
Crude lipids, g/kg DM	18	15	16	14	18
NDF, g/kg DM	581	507	583	443	520
ADF, g/kg DM	368	377	392	347	337
ADL, g/kg DM	45	52	83	80	50

NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

ilar between groups of cows, whereas a significant difference was observed, in both experiments, in the percentage of residual concentrate, which was estimated lower by a trained operator, acting blind, in the faeces of PF cows: these seem to be positive results, although these empirical parameters are not sufficient to prove a similar, or even better, digestibility of the two concentrates.

Blood proteins values were comparable between the experimental groups, whereas a slight but significant ($P < 0.05$) increase of blood urea was observed in cows fed pea and faba (C vs PF: 4.54 vs 4.95 mmol/L in experiment 1; 4.04 vs 4.38 mmol/L in experiment 2).

The increase was only for the last sampling of both experiments (day 84), and in the 1st experiment, but not in the 2nd, this was confirmed by a significant ($P < 0.05$) treatment x time interaction. The differences were anyway negligible from a physiological point of view, and all values were within the normal range for adult bovines (1.7 to 7.5 mmol/L; Rosenberger, 1993). In our previous researches, the use of 150 g/kg flaked pea (Volpelli et al., 2009) increased both blood and milk urea, as a likely consequence of an increase of the ammonia in the rumen due to a lack of effect of steam-flaking on pea protein degradability; on the contrary, a trend towards a decrease of

milk and blood urea was observed when only flaked faba (100 g/kg) was used (Volpelli et al., 2010). The sum of the two amounts of flakes probably produced, in the present research, a slight increase of ammonia in the rumen, which reflected into a blood urea increase, with no effects on milk urea concentration (Tables 5 and 6).

Tables 5 and 6 report the concentrate intake, milk yield and composition of cows fed SBM or peas and faba beans in experiment 1 and 2, respectively. All registered traits had similar values between the two feeding groups. Also the calculated values concentrate efficiency (milk yield DM/concentrate DM intake; C and

Table 4. Faecal and blood parameters as influenced by the different diets fed to animals.^o

	Treatment		SEM	P		
	C	PF		TRT	Time	TRTxTime
Experiment 1						
Faecal score	2.4	2.4	0.061	0.792	0.002	0.277
Undigested fraction	2.4	2.3	0.052	0.488	0.549	0.392
Residual concentrate, %	3.1	2.4	0.126	0.001	0.582	0.706
Blood total protein, g/L	76.05	74.64	0.892	0.297	0.001	0.177
Blood albumin, g/L	35.53	36.04	0.476	0.459	<0.001	0.673
Blood urea, mmol/L	4.54	4.95	0.139	0.049	<0.001	0.026
Experiment 2						
Faecal score	2.3	2.4	0.047	0.482	0.633	0.053
Undigested fraction	2.5	2.4	0.070	0.564	0.030	0.774
Residual concentrate, %	2.8	2.3	0.107	0.005	<0.001	0.091
Blood total protein, g/L	73.21	71.76	0.870	0.261	<0.001	0.079
Blood albumin, g/L	35.53	35.79	0.477	0.711	<0.001	0.158
Blood urea, mmol/L	4.04	4.38	0.098	0.027	<0.001	0.210

^oSampled cows: n=15 per group in experiment 1; n=14 per group in experiment 2. C, 110 g/kg soybean meal, no pea and faba beans; PF, 150 g/kg flaked pea, 100 g/kg flaked faba beans, no soybean meal.

Table 5. Experiment 1: concentrate intake, milk yield and milk composition as influenced by the different diets fed to animals.^o

	Treatment		SEM	P		
	C	PF		TRT	Time	TRTxTime
Concentrate intake/cow, kg/d	8.6	8.1	0.194	0.064	<0.001	<0.001
Milk yield, kg/d	22.1	21.8	0.568	0.731	<0.001	0.010
Concentrate efficiency [‡] , %	37.48	39.33	0.810	0.116	<0.001	<0.001
Milk composition						
Fat, g/kg	39.9	38.9	0.119	0.574	0.029	0.631
Protein, g/kg	34.7	35.0	0.051	0.698	<0.001	0.702
Lactose, g/kg	49.0	49.0	0.027	0.910	<0.001	0.868
Casein, g/kg	27.1	27.3	0.042	0.733	<0.001	0.567
Urea, mg/dl	25.46	27.48	0.812	0.093	<0.001	0.022
4% fat corrected milk, kg/d	21.8	21.2	0.677	0.067	<0.001	0.268
Fat yield, kg/d	0.96	0.84	0.044	0.069	<0.001	0.304
Protein yield, kg/d	0.80	0.77	0.030	0.581	<0.001	0.747
Casein yield, kg/d	0.62	0.60	0.023	0.581	<0.001	0.784

^oSampled cows: n=19 per group for concentrate intake/cow and for milk yield; n=15 for all the other variables. C, 110 g/kg soybean meal, no pea and faba beans; PF, 150 g/kg flaked pea, 100 g/kg flaked faba beans, no soybean meal. [‡]Concentrate efficiency = milk yield DM / concentrate DM intake.

Table 6. Experiment 2: concentrate intake, milk yield and milk composition as influenced by the different diets fed to animals.^o

	Treatment		SEM	P		
	C	PF		TRT	Time	TRTxTime
Concentrate intake/cow, kg/d	7.5	7.5	0.215	0.884	<0.001	0.655
Milk yield, kg/d	20.7	20.8	0.733	0.858	<0.001	0.429
Concentrate efficiency [‡] , %	41.31	40.85	1.106	0.775	<0.001	0.257
Milk composition						
Fat, g/kg	40.9	39.7	0.146	0.555	<0.001	0.054
Protein, g/kg	37.7	37.4	0.065	0.757	<0.001	0.287
Lactose, g/kg	47.1	47.3	0.037	0.714	0.011	0.633
Casein, g/kg	29.2	29.0	0.047	0.760	<0.001	0.269
Urea, mg/dL	22.48	22.29	0.773	0.864	<0.001	0.028
4% fat corrected milk, kg/d	19.7	19.8	0.695	0.869	<0.001	0.849
Fat yield, kg/d	0.78	0.77	0.029	0.742	<0.001	0.136
Protein yield, kg/d	0.73	0.73	0.023	0.966	<0.001	0.731
Casein yield, kg/d	0.57	0.57	0.018	0.994	<0.001	0.693

^oSampled cows: n=14. C, 110 g/kg soybean meal, no pea and faba beans; PF, 150 g/kg flaked pea, 100 g/kg flaked faba beans, no soybean meal. [‡]Concentrate efficiency = milk yield DM / concentrate DM intake.

Table 7. Rennet coagulation characteristics as influenced by the different diets fed to animals.^o

	Treatment		SEM	P		
	C	PF		TRT	Time	TRTxTime
Experiment 1						
Clotting time r, min	16.35	16.66	0.535	0.866	<0.001	0.644
Curd firming time k ₂₀ , Ln min	0.80	0.84	0.077	0.741	<0.001	0.899
Curd firmness a ₃₀ , mm	38.07	39.18	2.018	0.701	<0.001	0.894
Index of coagulation, %						
optimal+good	73	80	0.569 [‡]	-	-	-
poor	16	13				
not suitable	11	7				
Experiment 2						
Clotting time r, min	20.33	20.51	1.194	0.917	0.530	0.536
Curd firming time k ₂₀ , Ln min	0.83	1.15	0.105	0.054	0.495	0.244
Curd firmness a ₃₀ , mm	33.00	27.16	3.130	0.209	0.070	0.310
Index of coagulation, %						
optimal+good	52	47	1.902 [‡]	-	-	-
poor	35	29				
not suitable	13	24				

^oSampled cows: n=15 per group in experiment 1; n=14 per group in experiment 2. [‡]χ² value (P>0.05).

PF: 37.48 and 39.33% in the experiment 1, 41.31 and 40.85% in the experiment 2, respectively), FCM, fat-protein-casein yield did not differ between C and PF groups. Few works on these legumes in dairy cow feeding are reported in literature, and most of them concerns pea, whereas the use of faba beans is uncommon. A diet inclusion of 150 g/kg rolled peas, as partial substitute of SBM and corn grain, did not affect the dry matter intake, the milk yield, the milk protein and fat contents (Vander Pol *et al.*, 2008). No difference on milk yield and composition was also reported by Petit *et al.* (1997) in Holstein cows fed 202 g/kg pea (raw or extruded) versus SBM. Corbett *et al.* (1995)

found no effect of a diet with 250 g/kg field pea in mid- and late-lactating Holstein cows, whereas the milk yield was higher for cows in early lactation. Mordenti *et al.* (2007) reported a reduced dry matter intake and milk yield, and higher milk fat in Holstein dairy cows fed a diet with faba beans and peas (200 g/kg) in substitution of SBM.

Table 7 reports the rennet coagulation characteristics of the individual milk samples collected during the two experiments. No significant effect of the two different diets could be detected on r, k₂₀ and a₃₀ traits, which give a forecast of milk attitude to cheese-making, nor on the derived indexes of coagulation.

Conclusions

The substitution of a long-used feed such as SBM with flaked pea and faba beans in diets for Reggiana dairy cows did not induce negative effects on concentrate intake, milk yield and composition, and milk aptitude for cheese-making. Faecal and blood parameters were also unaffected, and the slight increase in blood urea observed in treated group was irregular and within the normal range.

When used within the allowed limit of the Parmigiano-Reggiano Consortium, the tested legumes represent an opportunity for substi-

tuting SBM in diet formulation. The results of this research, along with previous works, might represent a base of discussion for a possible increase of the maximum allowed level of inclusion of alternative protein sources in diets for cows within the Parmigiano-Reggiano Consortium.

References

- ANaBoRaRe, 2011. Available from: <http://www.razzareggiana.it>
- AOAC, 1990. Official Methods of Analysis. Vol. 1, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- 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.
- Dell'Orto, V., Savoini, G., 2005. Alimentazione della vacca da latte. 1st ed. Edagricole, Bologna, Italy.
- Ellis, T.H.N., 2004. Grain Legumes Integrated project: Promoting grain legumes for European livestock. Available from: <http://www.grainlegumes.com>
- 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.
- Goelema, J.O., Smits, A., Vaessen, L.M., Wemmers, A., 1999. Effect of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of borken peas, lupins and faba beans. *Anim. Feed Sci. Tech.* 78:109-126.
- Goelema, J.O., Spreeuwenberg, M.A.M., Hof, G., Van der Poel, A.F.B., Tamminga, S., 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim. Feed Sci. Tech.* 76:35-50.
- Hoden, A., Delaby, L., Marquis, B., 1992. Pois protéagineux comme concentré unique pour vaches laitières. *INRA Prod. Anim.* 5:37-42.
- 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.
- Link, W., Weber, H., Duc, G., 2007. Genetically increasing seed protein content and quality in faba bean. Available from: http://www.grainlegumes.com/aep/special_reports/seed_protein.
- Martillotti, F., Antongiovanni, M., Rizzi, L., Santi, E., Bittante, G., 1987. Metodi di analisi per la valutazione degli alimenti. Quaderni Metodologici n. 8. IPRA-CNR Ed., Roma, Italy.
- Martini, A., Migliorini, P., Lorenzini, G., Lotti, C., Rosi Bellière, S., Squilloni, S., Riccio, F., Giorgetti, A., Casini, M., 2008. Production of grain legume crops alternative to soya bean and their use in organic dairy production. Proc. 16th IFOAM Organic World Congr., Modena, Italy. Available from: www.ifoam.org/events/ifoam_conferences/owc/modules/pdf/OWC_ISOFAAR.pdf
- 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, expansion and toasting on the nutritional value of peas, faba beans and lupins. *Ital. J. Anim. Sci.* 4:177-189.
- Mordenti, A.L., Merendi, F., Fustini, M., Formigoni, A., 2007. Effects of different protein plants in cows diet on milk for Parmigiano Reggiano production. *Ital. J. Anim. Sci.* 6(Suppl.1):463-465.
- 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.
- Pilla, A.M., 1985. Metodologia Statistica e Programmazione degli Esperimenti. 1st ed. G. Missio Ed., Udine, Italy.
- Rosenberger, G., 1993. L'esame clinico del bovino. 3rd ed. Edagricole, Bologna, Italy.
- Rossi, A., Vecchia, P., 1994. La qualità del latte. *Agricoltura* 9 (Suppl.):1-5.
- Salvadori Del Prato, O., 1998. Trattato di tecnologia casearia, 1st ed. Edagricole, Bologna, Italy.
- SAS, 2001. SAS/STAT user's guide: statistics, ver. 9.1. SAS Inst. Inc., Cary, NC, USA.
- Schroeder, J.W., 2002. Field peas in dairy cattle diets. Available from: www.ag.ndsu.edu/pubs/ansci/livestoc/eb76-2.htm
- Skidmore, A.L., Brand, A., Sniffen, C.J., 1996. Monitoring milk production: defining pre-set targets and execution. In: A. Brand, J.P.T.M. Noordhuizen and Y.H. Schukken (eds.), Herd health and production management in dairy practice. Wageningen Press, Wageningen, The Netherlands, pp 223-262.
- Van der Poel, A.F.B., Gravendeel, S., Boer, H., 1991. Effect of different processing methods on tannin content and in vitro protein digestibility of faba bean (*Vicia faba* L.). *Anim. Feed Sci. Tech.* 33:49-58.
- Vander Pol, M., Hristov, A.N., Zaman, S., Delano, N., 2008. Peas can replace soybean meal and corn grain in dairy cow diets. *J. Dairy Sci.* 91:698-703.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Method for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Volpelli, L.A., Comellini, M., Masoero, F., Moschini, M., Lo Fiego, D.P., Scipioni, R., 2009. Pea (*Pisum sativum*) in dairy cow diet: effect on milk production and quality. *Ital. J. Anim. Sci.* 8:245-257.
- Volpelli, L.A., Comellini, M., Masoero, F., Moschini, M., Lo Fiego, D.P., Scipioni, R., 2010. Faba beans (*Vicia faba*) in dairy cow diet: effect on milk production and quality. *Ital. J. Anim. Sci.* 9:138-144.
- 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.
- Wilkins, R.J., Jones, R., 2000. Alternative home-grown protein sources for ruminants in the United Kingdom. *Anim. Feed Sci. Tech.* 85:23-32.