The use of alternative plant proteins in place of the soybean meal protein in diets for farmed animals aims to reduce the extra-EU soybean import and partially substitute the GMO in the food chain. Among the possible alternatives, the heat-processed (flaked) faba beans appears interesting for dairy cow diet. Two consecutive experiments were carried out to test flaked faba beans 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 (12% soybean meal, no faba beans) was compared with a “Faba” concentrate (7.5% soybean meal and 10% flaked faba beans). Forages fed to animals were hay (mixed grass and alfalfa) plus mixed grass in experiment 1, hay only in experiment 2. Milk yield and quality and the characteristics of grab faecal samples as empirical indicators of digestibility, were similar between feeding groups. The milk urea content was slightly lower in the “Faba” group, particularly in experiment 2 (“Control” vs “Faba”: 34.6 vs 32.9 mg/dL in experiment 1, P<0.1; 27.4 vs 23.4 mg/dL in experiment 2, P<0.01); the plasma urea content in experiment 2 confirmed the trend observed in milk (3.9 vs 3.0 mmol/L, P<0.01). The inclusion of faba beans, within the allowed limit of the Parmigiano-Reggiano Consortium for diet formulation, could represent a feasible opportunity for a partial substitution of soybean meal.

### Introduction

The need for alternative protein sources to soybean meal (SBM) in domestic animal feeding has recently gained focus. The main reasons include the attempt to limit SBM import from extra-EU Countries, which represents a negative voice of the commercial balance; an effort to decrease costs of animal production and contemporarily reduce the loss of N compounds in the environment and the search to prevent the presence of GMO in the food chain (Wilkins and Jones, 2000; Mordenti and De Castro, 2005; Formigoni et al., 2007). Organic farming is obviously more concerned with the last remark (Martini et al., 2008; Ferruzzi et al., 2009), however the search for crops that are not obtained by genetic manipulation is acquiring interest also in non-organic farming, since a growing part of consumers clearly state a refusal towards the presence of GMO food, both in their own diet and in the diet of animals producing milk, meat, etc. for their table. The production of “typical/traditional” and/or PDO (Protected Denomination of Origin) foods, where quality and traceability are key-words, are particularly implicated: the several recent statements from the Consortium of the Parmigiano-Reggiano (PR) cheese towards the opportunity of using GMO free concentrates in cows diets are significant examples. Within the area of the PR cheese, the Reggiana breeding, known for producing high-quality milk for cheese-making (CVPARR, 1999), must comply with dietary rules that are even more strict compared to other PR producing herds: the National Association of Reggiana Cattle Breeders (AnABoRaRe, 2008) does not allow the use of the GMO feeds. Thus, the Association itself and the PR Consortium on the whole are particularly interested in researches promoting the increase and the usage of GMO-free feeds.

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), although the first is not included in the list of allowed feeds for PR. These crops offer some agronomical advantages in comparison with soy: greater adaptation ability, lower chemical and nutritive demands (Pistoia et al., 2004).

Before the twentieth century, there was a long tradition of faba bean (Vicia faba) cultivation in Europe for food or animal feed (Link et al., 2007). The recent concern about self-produced and non GM crops lead to a re-discovery of some grain legumes, including faba beans: in Italy, the cultivation is traditional in Central and Southern regions, but many mentions about this crop, dated from up to 5000 years ago, have been found also in the Po Valley (Piazza et al., 2006).

The faba bean has lower protein and higher starch contents than SBM and is similar to barley for starch rumen fermentability (Masoero et al., 1997, 2006): thus, it may be considered as interesting “dual purpose” feed for protein and energy contents. In particular, when compared with SBM, the protein is richer in lysine although lower in methionine and cysteine (Link et al., 2007) and its use along with corn meal should avoid amino acids unbalance in the diet (Masoero et al., 2006).

The rumen degradability and the soluble fractions (albumins and globulins) of the protein are higher in the grain legumes compared with 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). Grain legumes...
also contain some anti-nutritional factors; although some works report 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 to lower the protease inhibitors and other anti-nutritional factors and to increase the protein fraction escaping the rumen degradation (Focant et al., 1990; Walhain et al., 1992; Masoero et al., 2005). Faba beans, in particular, show a high content of tannins, particularly in the coats, whilst the trypsin inhibitor activity is low in comparison with many other legumes (Link et al., 2007). Dehulling and processing like flaking or extrusion were effective in lowering the tannin content of faba beans (Van der Poel et al., 1991; Pistoia et al., 2004; Ferruzzi et al., 2009).

Extrusion and expansion applied to peas and faba beans increased the insoluble protein fraction and reduced the amount of protein degraded inside the rumen, whereas the extrusion itself increased the rumen and the in vitro starch degradability (Petit et al., 1997; Goelema et al., 1998, 1999; Masoero et al., 2005). Thus, heat processed grain legumes should provide both a source of rumen degradable carbohydrates and a good amount of rumen undegradable proteins, therefore meeting the animal protein need at the duodenum.

There are few works about the use of faba beans in dairy cow feeding and are often connected with the use of both faba beans and peas (Pasquini et al., 2003; Olivetti et al., 2006; Formigoni et al., 2007; Mordenti et al., 2007; Martini et al., 2008), with productive results comparable with SBM. High levels of raw faba beans were used by Liponi et al. (2009) in sheep, without negative effects on the palatability and digestibility of the diets.

Contributes about the use of flakes are even more lacking and confined only to peas (Battini et al., 2003), where flaking seems to have no effect on protein degradability and starch gelatinization (Focant et al., 1990). An investigation on the implement of flaked faba beans in PR producing cows seems therefore interesting.

The aim of the present research was to study the effect of partially replacing SBM with faba beans in Reggiana dairy cows’ diet on milk production and quality, concentrate intake and on some blood and faecal traits. Because of the limit to the inclusion of faba beans (10%) imposed by the Rules for PR cheese production, only a partial replacement of SBM was possible.

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

|                  | Control  | Faba     |
|------------------|----------|----------|
| Corn meal        | 36.0     | 32.5     |
| Wheat bran       | 17.0     | 17.0     |
| Wheat flour shorts | 15.0   | 15.0     |
| Flaked faba beans | -       | 10.0     |
| Soybean meal, dehulled | 12.0 | 7.5      |
| Corn gluten feed | 6.0      | 6.0      |
| Beet pulp dely   | 5.0      | 3.0      |
| Sugar cane molasses | 3.0   | 3.0      |
| Calcium carbonate | 3.0     | 3.0      |
| Sodium chloride  | 0.8      | 0.8      |
| Sodium bicarbonate | 0.7   | 0.7      |
| Dicalcium phosphate | 0.5   | 0.5      |
| Magnesium oxide  | 0.4      | 0.4      |
| Mineral and vitamin supplement* | 0.6 | 0.6     |

*Composition (per kg): Vit.A U 50,000; Vit.D3 U 5000; Vit.E mg 150; Vit.B1 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.

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

|                  | Flaked faba | Control Experiment 1 | Control Experiment 2 | Faba Experiment 1 | Faba Experiment 2 |
|------------------|-------------|----------------------|----------------------|------------------|------------------|
| Dry matter, %    | 92.1        | 91.3                 | 90.7                 | 89.6             | 90.3             |
| Crude protein    | 25.3        | 16.2                 | 15.8                 | 16.9             | 16.2             |
| Soluble protein, % total | 46.8   | 29.2                 | 26.8                 | 25.4             | 26.6             |
| Crude lipids     | 1.1         | 2.4                  | 1.9                  | 1.6              | 1.9              |
| Crude fibre      | 7.9         | 5.4                  | 5.4                  | 5.4              | 5.3              |
| NDF              | 13.2        | 21.6                 | 21.3                 | 18.0             | 18.1             |
| ADF              | 10.9        | 8.5                  | 8.0                  | 8.0              | 8.0              |
| ADL              | 1.3         | 2.1                  | 2.2                  | 1.6              | 1.7              |
| Starch           | 44.1        | 38.7                 | 38.4                 | 37.1             | 37.4             |
Materials and methods

Animals and diets

Two consecutive experiments were carried out in farm condition 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 lasted 8 weeks (October-December) and its forage component was consisting of green forage (about 50%) and hay (35% mixed grass and 15% alfalfa), whereas only hay was used in experiment 2 (9 weeks, January-March).

The cows were fed on 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 pen, it was not possible to monitor the individual intake of forage dry matter.

Only cows between 20 and 210 days in milk were considered in the experiments and some cows were present in both experiments.

Two concentrates were used in both trials (Table 1): a standard concentrate being in use on the farm (Control) and an experimental concentrate (Faba), in which a part of the dehulled soybean meal and of the corn meal were substituted by 10% of steam-flaked faba beans (Vicia faba). The percentage of faba beans 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.

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

| Forage          | Experiment 1 start | Experiment 1 end | Experiment 2 start | Experiment 2 end | Experiment 1 start | Experiment 1 end | Experiment 2 start | Experiment 2 end |
|-----------------|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|
| Dry matter, %   | 92.8               | 90.7              | 88.8               | 88.4              | 91.7               | 90.7              | 89.7               | 89.5              |
| Crude protein   | 9.1                | 11.2              | 9.7                | 8.2               | 14.7               | 14.7              | 15.2               | 14.3              |
| Soluble protein, % total | 18.2             | 22.4              | 24.4               | 20.1              | 28.4               | 29.2              | 30.3               | 32.7              |
| Crude lipids    | 1.0                | 1.4               | 1.1                | 2.7               | 1.2                | 1.4              | 1.8                | 1.7               |
| Crude fibre     | 28.1               | 27.3              | 31.2               | 25.7              | 29.3               | 26.6              | 33.4               | 29.7              |
| NDF             | 55.2               | 52.0              | 58.2               | 52.5              | 43.5               | 53.4              | 48.6               | 44.9              |
| ADF             | 36.8               | 33.7              | 40.5               | 36.8              | 36.9               | 37.6              | 42.0               | 37.6              |
| ADL             | 5.7                | 7.0               | 9.1                | 5.7               | 8.7                | 8.2              | 12.7               | 8.4               |

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

| Diet      | SEM | Significance |
|-----------|-----|--------------|
| Control   |     |              |
| Faba      |     |              |

| Parameter          | Control | Faba | SEM | Significance |
|--------------------|---------|------|-----|--------------|
| Undigested residue, % | 60.8    | 61.3 | 3.259 | ns           |
| Faecal score       | 2.6     | 2.7  | 0.066 | ns           |
| Undigested fraction | 2.5     | 2.4  | 0.067 | ns           |
| Residual concentrate, % | 2.8     | 2.2  | 0.192 | ns           |

ns: not significant.

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

| Diet      | SEM | Significance |
|-----------|-----|--------------|
| Control   |     |              |
| Faba      |     |              |

| Parameter          | Control | Faba | SEM | Significance |
|--------------------|---------|------|-----|--------------|
| Undigested residue, % | 56.9    | 58.8 | 2.629 | ns           |
| Faecal score       | 2.6     | 2.6  | 0.067 | ns           |
| Undigested fraction | 2.5     | 2.6  | 0.037 | ns           |
| Residual concentrate, % | 2.5     | 3.1  | 0.221 | ns           |

ns: not significant.

### 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      | SEM | Significance |
|-----------|-----|--------------|
| Control   |     |              |
| Faba      |     |              |

| Parameter          | Control | Faba |
|--------------------|---------|------|
| Concentrate intake/cow, kg/d | 7.87    | 7.81 |
| Milk yield, kg/d | 22.21   | 22.38 |
| Milk composition  |         |      |
| Fat, %            | 3.90    | 3.93 |
| Protein, %        | 3.47    | 3.39 |
| Lactose, %        | 4.95    | 4.94 |
| Casein, %         | 2.72    | 2.67 |
| Urea, mg/dL       | 34.58   | 32.93 |
| Fat yield, kg/d   | 0.86    | 0.88 |
| Protein yield, kg/d | 0.75     | 0.75 |
| Casein yield, kg/d | 0.59     | 0.59 |
| Blood total protein, g/L | 72.48   | 64.03 |
| Blood urea, mmol/L | 5.76    | 5.75 |

Sampled cows: n=19 for concentrate intake/cow and for milk yield; n=15 for all other variables. *P<0.10; ns: not significant.
content in the two concentrates. Faba seeds were steam-cooked for 30 minutes (95-100°C; steam pressure 1.2 Bar), rolled (1 mm, 20 Bar) and dried at 150°C (Consorzio Agrario Provinciale, Reggio Emilia, Italy); faba flakes as a whole were added to the experimental concentrate.

Animals fed on the Faba concentrate were adapted to the treatment diet by mixing the Control and Faba 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 (Control and Faba: 25.2±4.8 and 25.3±6.6 kg/d), days in milk (89.4±57.2 and 89.6±51.9), parities (3.7±2.1 and 3.5±1.2) and milk protein content (3.46±0.29 and 3.32±0.24%). The experiment lasted 56 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 (26.1±4.1 and 25.9±5.9 kg/d), days in milk (69.7±39.3 and 66.8±34.0), parities (4.1±2.3 and 3.6±1.0), and milk protein content (3.28±0.26% and 3.22±0.26%), for the Control and Faba treatment respectively, were sampled for milk composition, blood protein and urea and faecal indexes (day 0, 26, 47 and 54 of trial).

In experiment 2, forty cows were allotted to two homogeneous groups for average daily milk yield (Control and Faba: 23.2±6.5 and 23.2±6.6 kg/d), days in milk (108.3±49.0 and 108.7±38.3), parities (3.7±1.4 and 3.7±2.2), and milk protein content (3.38±0.32 and 3.27±0.24). The experiment lasted 63 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 (24.3±6.9 and 24.2±7.0 kg/d), days in milk (91.3±33.1 and 91.7±30.0), parities (3.9±1.4 and 3.6±2.2), and milk protein content (3.27±0.26 and 3.21±0.24%), for the Control and Faba treatment respectively, were sampled for milk composition, blood protein and urea and faecal indexes (day 0, 21, 35 and 56 of trial).

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 48 hrs, ground with a 1mm sieve (Thomas-Wiley Laboratory Mill, model 4, Arthur H. Thomas Co., Philadelphia, PA, USA), 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).

Individual milk samples for collection day were obtained by proportional pooling of the morning and evening milkings. Then, samples were analysed for fat, protein, lactose, casein and urea contents (infrared analysis, Milkoscan Model FT120 Foss A/S, Denmark). A further sample from each cow was taken from both morning and evening milking; all samples belonging to the same group were then mixed and the two resulting pooled samples were analysed for the rennet coagulation characteristics (tromboelastographic method;
non-commercial use only

protein and urea contents) were subjected to concentration by means of an UV-based kinetic-biuret method (Hiller et al., 1972). Urea reagent was used to determine the urea content. Total Protein (CX 5 Delta) supplied by Beckman Coulter. 

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 Mancini et al. (2004) and Dell’Orto and Savoini (2005). Each faecal sample was put in a sieve (1.5 mm mesh), weighed, washed with running water until output water was clear and weighed again. An empirical index of the faecal residue was then calculated as percentage of the final weight upon the initial weight. The residue was then 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 coarse materials. The analysis of the residual was completed by the visual evaluation of the concentrate incidence (%).

Blood samples were obtained from the caudal vein and collected in Li-Heparinized (15 U/mL of blood) evacuated collection tubes (Venoject, Terumo Europe, Leuven, Belgium) and centrifuged at 2500 g for 15 minutes. Then, plasma was collected 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 measured over time (i.e., milk yield, milk fat, protein, 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 analyzed was subjected to three covariance structures: being toepzipl, 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: $Y_{ijk} = \mu + c_i + b_{ij} + \gamma_k + (ct)^{ik} + \epsilon_{ijk}$

Where:

- $Y_{ijk}$: the dependent variable at time $k$ on the $j$th subject assigned to treatment $i$
- $\mu$: overall mean
- $c_i$: fixed effect of treatment $i$ ($i =$ Control, Faba)
- $b_{ij}$: random effect for subject $j$ assigned to treatment $i$
- $\gamma_k$: fixed effect of time
- $(ct)^{ik}$: fixed effect of treatment $x$ time interaction
- $\epsilon_{ijk}$: 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 fava had a crude protein content of 25.3% and a soluble protein fraction of 46.8%; the latter was intermediate between the values found by Masoero et al. (2005) for crude meal and extruded beans (71.1 and 19.4%, respectively). The solubility data observed in our experiment are probably due to the physical treatment, less intensive for flaking in comparison with extrusion. The total solubility of protein was similar between concentrates (28 and 26% for Control and Faba, respectively), along with other analytical parameters (i.e., starch: 39 and 37%), with the exception of a lower NDF content in the Faba concentrate (22 and 18% for Control and Faba, respectively).

The chemical composition of the forages had limited variations throughout the two experiments (Table 3).

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

No differences were observed for faecal parameters in either experiment (Tables 4 and 5): this seems to be a positive result, although these empirical parameters are not sufficient to prove a similar digestibility of the two concentrates.

Tables 6 and 7 report the concentrate intake, milk yield and composition and measured blood parameters of groups in experiment 1 and 2, respectively. The faba beans did not affect the concentrate intake suggesting no negative effects on palatability. Also the milk yield and composition in both experiments were not affected by the usage of faba beans in the diet. It is difficult to compare these results with literature, since the use of faba beans in scientific trials for dairy cow feeding is unusual and peas are often contem- porarily used: for example, Mordenti et al. (2007) found that the substitution of SBM with faba beans and peas together reduced dry matter intake and milk yield in Holstein dairy cows.

A trend towards a decrease of milk and blood urea was observed in cows fed on the Faba concentrate: the difference from Control group was slight and restricted to milk in experiment 1 (Control vs Faba: 34.6 vs 32.9 mg/dL, $P<0.1$; Table 6) and significant in experiment 2 (milk: 27.4 vs 23.4 mg/dL, $P<0.01$), where blood data (3.9 vs 3.0 mmol/L, $P<0.01$) confirmed the decrease (Table 7 and Figure 1).

The milk and blood urea increase when feeding flaked pea (Volpelli et al., 2009), because of an increase of ammonia in the rumen due to a lack of effect of steam-flacking on pea protein degradability. In the current research, the milk and blood urea levels were numerically higher in animals fed on green forage and hay (experiment 1) compared to cows fed on hay only (experiment 2), as a feasible consequence of the protein level and grass solubility. Urea significantly decreased in milk and blood of the Faba group only in experiment 2; it may be argued that, whilst in experiment 1 the effect of green grass protein overpowered other effects, in experiment 2 the decreased degradability of fava beans protein due to steam-flacking could express its effect in the reduction of ammonia in the rumen and of urea in milk and blood.

The different effects observed with the flakes of the two legumes may be explained by a different response to heat processing, as reported by other researchers (Aguilera et al., 1992), although the physical form/particle size of flakes should also be considered.

Tables 8 and 9 report the rennet coagulation characteristics of pooled milk collected during the two experiments. Although no statistical analysis could be performed on these data, it seems interesting to note that the introduction
of faba beans in the diet barely affected the milk coagulation trend, which was always good for both the pooled milk.

Conclusions

The inclusion of flaked faba beans in diets for Reggiana dairy cows did not produce negative effects on milk yield and composition. The decrease of urea in blood and in milk, in particular in hay-based rations, appears as a positive result, which should be confirmed in cows with higher milk yield and concentrate intake.

When used within the allowed limit of the Parmigiano-Reggiano Consortium, the flaked faba beans represent an opportunity for a partial substitution of SBM in diet formulation. The use of pea and faba beans together in diet formulation, which we are currently testing, could allow the total substitution of SBM. The results of our and other researches could represent a base of discussion for a possible increase of the maximum allowed level of inclusion of some protein sources in diets for cows within the Parmigiano-Reggiano Consortium.

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