

Running title: Pea in organic dairy diets

**Extruded pea (*Pisum sativum*) as alternative to soybean protein for dairy cows  
feeding in organic Alpine farms**

**Giulio Cozzi, Aziza Boukha, Barbara Contiero and Flaviana Gottardo**

Dipartimento di Scienze Animali. Università di Padova, Italy

*Corresponding author.* Prof. Giulio Cozzi. Dipartimento di Scienze Animali. Università di Padova.  
Viale dell'Università 16 -35020 Legnaro (PD) Italy - Tel.: +39 049 827 2662; fax +39 049 827 2669 –  
Email: [giulio.cozzi@unipd.it](mailto:giulio.cozzi@unipd.it)

ABSTRACT

The study evaluated the use of extruded pea as an alternative to soybean in the protein feeding of dairy cattle raised in organic Alpine farms. The research was carried out in a commercial organic dairy farm located in the Province of Trento (Northern Italy) and it considered two separate periods of cow's lactation: early and late lactation. According to the traditional management practice of alpine dairy herds with the seasonal calving of the cows in early winter, the former period was carried out during the cold season when cows were housed indoors, while the latter period started after the transfer of the entire herd to an alpine pasture for the summer grazing. In both periods, 16 cows of Rendena breed were equally assigned to 2 experimental groups. The dietary forage (meadow hay in early lactation or pasture in late lactation) was supplemented to one group of cows with a Control concentrate in which soybean expeller, sunflower expeller and wheat bran were the main protein feeds. Soybean proteins were replaced by extruded peas in the Soy-free concentrate given to the other group of cows. The daily amount of concentrate was adjusted to the individual milk yield on a weekly basis adopting ratios of

0.360 and 0.125 kg of DM per kg of milk in early and late lactation periods respectively. Cows receiving Soy-free concentrate showed a higher milk yield than the Control cows in both lactation periods (18.7 vs 17.5 kg/d in early lactation and 9.3 vs 8.6 kg/d on pasture respectively). Milk fat and protein were not affected by the diet at any stage of lactation, while a higher concentration of milk urea was observed in milk samples taken from Soy-free cows in both periods of the study. This result could have been promoted by the higher soluble fraction of extruded pea proteins in comparison to that of soybean expeller. Cows feeding behaviour was monitored only in the early lactation period and despite of the different amount of concentrate consumed by the two groups of cows (7.0 vs 6.6 kg/cow/d for Soy-free and Control respectively), their total time spent eating and ruminating was not affected by the diet. Based on these findings, extruded peas can be considered a valuable alternative to soybean in the protein feeding of cattle raised for organic milk production in the Alpine region.

Key words: Extruded pea, Protein sources, Organic milk production, Alpine farms.

## RIASSUNTO

### IL PISELLO ESTRUSO (*PISUM SATIVUM*) COME ALTERNATIVE ALLA PROTEINA DELLA SOIA NEL RAZIONAMENTO DI BOVINE DA LATTE IN ALLEVAMENTO BIOLOGICO ALPINO

Lo studio ha valutato l'utilizzo del pisello estruso in alternativa alla soia quale principale fonte proteica di bovine da latte allevate in aziende di montagna secondo metodo biologico. La ricerca, realizzata in una azienda biologica della Provincia di Trento, ha considerato due diverse fasi della lattazione: l'inizio

e la fine. Secondo il tradizionale calendario gestionale delle aziende da latte alpine che prevede la stagionalizzazione dei partì all'inizio dell'inverno, la prima fase della ricerca è stata realizzata nella stalla aziendale nel periodo vernino-primaverile, mentre la seconda ha avuto inizio dopo il trasferimento della mandria in una malga per l'alpeggio estivo. In entrambi i periodi, 16 bovine di razza Rendena sono state equamente ridistribuite in 2 gruppi sperimentali. La dieta somministrata ad un gruppo di bovine ha integrato la frazione foraggera (fieno di prato in apertura di lattazione e l'erba del pascolo in alpeggio) con un mangime pellettato (Controllo) in cui pannello di soia, pannello di girasole e crusca di frumento rappresentavano le principali fonti proteiche. Il secondo gruppo ha invece ricevuto, in sostituzione del mangime Controllo, un pellet isoproteico (Soy-free) in cui il pannello di soia è stato sostituito con pisello estruso. La quantità giornaliera dei due concentrati è stata modulata settimanalmente in funzione della produzione di latte di ciascuna bovina adottando un rapporto pari a 0.360 kg di sostanza secca di mangime/kg di latte nel periodo di inizio lattazione e di 0.125 kg di sostanza secca/kg di latte nella fase di fine lattazione. Rispetto alla tesi Controllo, le bovine della tesi Soy-free hanno evidenziato una positiva risposta produttiva in entrambe le fasi della sperimentazione (18.7 vs 17.5 kg/d di latte nella fase di inizio lattazione e 9.3 vs 8.6 kg/d in malga). Il contenuto di grasso e di proteina del latte non hanno risentito di un effetto dieta in entrambe le fasi della lattazione, mentre i campioni di latte della tesi Soy-free hanno evidenziato una più elevata concentrazione di urea. Questo risultato è stato probabilmente causato dalla più elevata solubilità della frazione proteica del pisello estruso rispetto al pannello di soia. Il comportamento alimentare delle vacche è stato monitorato solo nella fase di inizio lattazione in quanto al pascolo non era possibile registrare in modo attendibile l'attività giornaliera di ciascuna bovina. Nonostante la diversa quantità di mangime consumata dalle bovine delle due tesi in questa fase (7.0 vs 6.6 kg/capo/d rispettivamente per Soy-free e Controllo), il tempo medio totale dedicato all'attività di ingestione e alla ruminazione non ha risentito di un

significativo effetto dieta. Sulla base dei risultati emersi dalla ricerca, è possibile affermare che il pisello estruso può rappresentare una valida alternativa alla proteina della soia nel razionamento di bovine da latte allevate secondo metodo biologico in ambiente alpino.

Parole chiave: Pisello estruso, Fonti proteiche, Produzione di latte biologico, Aziende alpine.

## **Introduction**

From January 2008 on, milk production under organic conditions should be entirely based on organically grown feedstuffs and, according to the European legislation in force (EU Council Directive, 2007), most of these feeds should derive from locally grown crops. These nutritional constraints are particularly difficult to be accomplished by the organic dairy farms located in the Alpine region, where there are no agronomic solutions alternative to forage production from natural meadows and pasture grazing (Cozzi and Bizzotto, 2004). Therefore, in order to meet the nutrient requirements of the lactating cows, local forages must be supplemented with organic energy and protein sources bought from the feed market. Maize and barley are the main energy sources included in these diets while most of the proteins come from soybean and sunflower seeds. Nowadays though, the use of soybean products is jeopardized by their increasing risk to be genetically modified (Froidmont and Bartiaux-Thill, 2004). Recent data about the global status of biotech crops indicate that, in the year 2006, 64% of the 91 million hectares of soybean planted globally are transgenic (James, 2006). Among protein sources alternative to soybeans, rapeseed products have been successfully used in organic diets for dairy cattle in Northern Europe (Khalili *et al.*, 2002; Johansson and Nadeau, 2006). Extruded pea (*Pisum sativum*) could represent another interesting alternative as recently suggested by Masoero *et al.* (2006) in a study with conventional dairy diets. Raw field peas have a lower protein content than

soybeans and pea protein has a higher rumen degradability in comparison to soybean meal (NRC, 2001). However, the high temperatures of the extrusion process have shown to increase the insoluble fraction of the pea protein, thus reducing the amount of protein being degraded into the rumen with no changes on in vitro digestibility of the feed (Masoero et al., 2005); the content of anti-nutritional factors is reduced as well. From an energy point of view, peas have a starch content of 44% on DM basis (INRA, 2002), which makes them an interesting ingredient for lactating diets based on meadow hay or pasture in which there is a need for supplementing energy sources rich in readily rumen fermentable carbohydrates. Therefore, the present study aimed at evaluating the effects on milk yield and composition induced by the substitution of soybean protein with extruded peas in organic diets fed during early lactation and summer grazing in an Alpine dairy farm.

## **Material and methods**

### *Farm location and management*

The study was carried out at the organic dairy farm “Antica Rendena” located about 800 m above the sea level in the town of Giustino in the Rendena Valley of the Trentino-Alto Adige, Italy. The cattle herd is made by 30 lactating cows and 20 young stocks of Rendena breed, a local dual purpose breed (Rendena, 2008). According to a traditional farming system still widely applied in the Alpine region, cows are kept indoor in a closed barn during autumn, winter and spring, while they are transferred to an alpine pasture located at high altitude (1600 m a.s.l.) in early summer. All cows have seasonal calving within the period October-January in order to face the summer grazing in a late phase of lactation.

### *Feeding treatments*

The study compared two organic isonitrogenous pelleted concentrates with different protein composition. Soybean expeller, sunflower expeller and wheat bran were the main protein feeds of the Control concentrate, while maize and barley meals were its main energy sources (Table 1). As shown in the same table, soybean proteins were replaced by extruded peas in the Soy-free concentrate which included also a minimum amount of maize gluten meal to balance the rumen degradable protein content (Table 2). To balance the different starch and protein content between extruded pea and soybean expeller in the corresponding experimental pellets, the inclusion of the pea lowered the amount of the other starch sources such as maize and barley, while it increased the content of sunflower expeller (Table 1). Moreover, according to NRC (2001), extruded peas protein has a higher readily degradable fraction (15.7 vs 8.7%) and a faster degradation rate of the fraction degradable at measurable rate (13.1 vs 2.4%/h) than soybean expeller protein. For this reason, aiming at balancing the rumen degradable protein content of the pellets extruded peas were combined with a small amount of maize gluten meal a protein source highly resistant to microbial degradation (Cozzi *et al.*, 1993).

### *Experimental design*

The research investigated two separate periods of cows lactation: early and late lactation. The early lactation phase considered a group of 16 multiparous cows which calved from early November 2006 to the end of January 2007. Cows were allocated to two dietary treatments according to calving date, parity, mature equivalent and their initial milk yield measured at day 11 post partum (Table 3). The two experimental groups of 8 cows each were housed face to face in a tied stalls barn. Each stall had a separate manger for hay feeding and was equipped with a waterer and an automatic feeder for concentrate administration. The diet offered to the former group of cows was made of *ad libitum* meadow hay supplemented with the Control pellet. The second group of cows replaced the Control

concentrate with the Soy-free pellet. Regardless of the treatment, each cow received the daily amount of hay in two meals at h 06:30 and 17:00 while the concentrate was offered in five separate meals at h 07:00; 09:00; 14:00; 18:00 and 20:00. The daily amount of concentrate supplied to each cow was adjusted to its milk yield on a weekly basis adopting a ratio of 0.360 kg of concentrate DM per kg of milk. This amount of concentrate was always fully eaten by each cow and no concentrate refusals were recorded during the entire experimental phase which lasted 21 weeks.

The second period of the study considered the summer grazing season, when cows in late lactation were transferred to an alpine pasture of 100 hectares located at 1600 m a.s.l. (malga Bandalors in Giustino, Trento, TN). As in the previous period, 16 lactating cows (10 of which used also in the early period of the study) were selected for the feeding trial which started on early June and lasted 15 weeks. Cows were allocated to 2 balanced groups according to their parity, mature equivalent, days in milk and initial milk yield (Table 3). Both groups of cows freely grazed the same pasture plots and herbage was supplemented with 0.125 kg DM of one of the two concentrates per kg of milk. The daily amount of concentrate offered to each cow was provided at the two milkings at h 06:00 and 17:00 and it was always completely consumed by all the cows.

### *Experimental measures*

Individual milk yield and the intake of concentrate were recorded at least once a week during the two experimental periods. These daily values were pooled per experimental week and then the average data per cow per week was calculated. Individual intake of hay was measured only in the early lactation period by decreasing the daily dose offered to each cow by the residue recovered in the manger. Consistent with the previous variables, also the individual daily data of forage intake were

pooled and averaged per experimental week. The late lactation period of the study was carried out on pasture and under these feeding conditions it was not possible to measure individual forage intake.

Individual milk samples were collected at week 2, 6 and 10 of both periods of the study. Samples were preserved with Azidiol (150 µl) and stored at 4°C during the transfer to chemical laboratory of Trentingrana Concast at Gardolo (TN). Milk samples were analysed by Milko-scan (Foss Electric, Hillorød, Denmark) for fat, protein and urea content, and somatic cell count according to the International Dairy Federation standards.

Cows' body condition score (BCS) was recorded by a trained technician of the Rendena breed at the initial and final day of each period adapting to the five point scale system (from 1 = emaciated to 5 = obese) proposed for Holstein cows by Edmonson *et al.* (1989).

Monthly samples of the two experimental concentrates were collected in both periods along with samples of hay used during the early lactation period of the study. All these samples were analysed for dry matter (DM), crude protein, ether extract, ash and starch according to AOAC procedures (1990). Neutral detergent fibre (NDF) analysis was performed according to Van Soest *et al.*, (1991).

Rumen degradable protein content of the two concentrates was estimated using reference values proposed by NRC (2001) for each feed ingredient. A similar procedure was adopted to estimate their energy density using the Unité Fouragère Lait (UFL) values of INRA (2002). The UFL value of meadow hay was estimated by using the reference value for a roughage with similar chemical composition.

### *Cows behaviour*



Cows feeding behaviour was monitored only during the early lactation period when cows were kept in the tied stalls barn. Two observation sessions were carried out at wk 15 and 20 of the experimental period by a team of trained personnel. Cows were observed for 24 h starting at the time of hay delivery in the morning. Cows posture (standing or lying) and feeding related activities (eating and rumination) were recorded every 5 minutes using a scan-sampling technique (Martin and Bateson, 1993), and according to Maekawa *et al.* (2002), each activity was assumed to persist for the entire 5-min interval. Eating and ruminating times per kg of dietary DM intake were calculated by dividing the total time spent performing each activity by DM intake. The chewing activity was calculated as sum of total time spent eating and ruminating.

#### *Statistical analyses*

Experimental data were analysed using SAS (1990) package. Descriptive statistics for parity, mature equivalent, days in milk and initial milk yield reported in Table 3 were obtained with the proc MEANS procedure. Milk yield was analysed using a mixed model including the fixed effects of diet (Control *vs* Soy-free), period (Early lactation *vs* Summer grazing), time (21 wks), diet x period and diet x time interactions and the initial milk yield as covariate. Cow was included in the model as random effect. The intake of concentrates was processed according to a general linear model including the effects of diet, period, time, diet x period and diet x time. Milk constituents were analysed with a mixed model including the fixed effects of diet, period, week of sampling, diet x period and diet x week of sampling with cow as random effect. A log-transformation was performed for somatic cell count prior to analyses. A general linear model was used to process initial and final BCS, including the effects of period, diet, and their interaction. A simple model considering the effects of diet and observation session was used for feeding behaviour data.

Results were considered statistically significant for  $P < 0.05$ . In case of the mixed models we considered the model with a smaller Akaike Information Criterion index which measures the goodness of the fit.

## **Results and discussion**

### *Milk yield and body condition*

Regardless of the period of the study, cows receiving the Soy-free concentrate showed a higher milk yield than the Control ones (Table 4). The result observed in early lactation was consistent with previous findings by Corbett *et al.* (1995) and Petit *et al.* (1997) who observed an increase in 4% fat corrected milk by feeding a conventional pea-based concentrate to dairy cows as an alternative to soybean meal and rapeseed meal. Recently, Masoero *et al.* (2006) reported a significant increase in milk yield (35.5 vs 34.4 kg/d;  $P < 0.05$ ) replacing soybean meal with extruded peas in a conventional total mixed ration fed to Holstein cows in mid lactation (initial days in milk =  $140 \pm 25$ ).

In the present study, the positive production response of the cows receiving the Soy-free concentrate arose both from a higher intake of concentrate and from a greater energy mobilization from body tissues reserves (Table 4). The need of the Soy-free cows to mobilize more energy from body reserves could originate from a less efficient utilization of the starch from pea in comparison to that from cereal sources. Support to this hypothesis comes from Khorasani *et al.* (2001) who reported a linear increase in the concentration of rumen acetate and butyrate by substituting peas for barley and soybean meal in a diet fed to Holstein cows. Regarding BCS, a proper management of the dairy herd should try to prevent the loss observed for Soy-free treatment in late lactation period when cows must restore their energy reserves for subsequent lactation (Ferguson and Otto, 1989).

### *Milk composition*

Corbett *et al.* (1995) speculated that the lower rumen fermentation rate of the non-structural carbohydrates of peas compared to other starch sources such as barley should promote a more stable rumen pH and an increased acetate:propionate ratio leading to a higher milk fat. Our results on milk fat percentage and yield do not support this hypothesis and they are consistent with the findings of Petit *et al.* (1997), Khorasani *et al.* (2001), Masoero *et al.* (2006) and Volpelli *et al.* (2009).

Milk protein percentage and yield were not affected by diet at any stage of lactation (Table 5) and this result is in agreement with all the previous researches in which pea proteins substituted soybean proteins in diets for lactating dairy cows (Corbett *et al.*, 1995; Petit *et al.*, 1997; Khorasani *et al.*, 2001; Masoero *et al.*, 2006; Volpelli *et al.*, 2009). A higher concentration of milk urea was observed in milk samples taken from Soy-free cows in both periods of the study. Based on the reference values for the different protein fractions of dairy feedstuffs (NRC, 2001), we hypothesize that this result was probably promoted by the higher soluble fraction of extruded peas protein in comparison to soybean expeller. Recently Volpelli *et al.* (2009) reported a similar result for milk urea by partially substituting soybean meal with flaked peas in conventional diets fed to Reggiana breed cows. Petit *et al.* (1997) observed a positive trend for milk urea N when extruded peas replaced soybean meal in a conventional diet fed to Holstein cows in early lactation, while Khorasani *et al.* (2001) observed a linear increase of ruminal ammonia-N as pea progressively replaced soybean protein. No dietary effects were observed for lactose and somatic cell counts.

As expected, the period of lactation had a significant effect on both milk yield and composition (Tables 4 and 5). However, the drop in milk yield and quality observed in the late lactation period can not be explained only by the advanced days in milk of the cows (Schultz *et al.*, 1990). Grazing on a steep alpine pasture certainly increased cows maintenance requirements lowering the available

nutrients for milk production. In addition, consistent with what has been frequently reported for dairy herds grazing on alpine pastures during the summer (Agabriel *et al.*, 1997), cows of both dietary treatments increased milk somatic cells count (Table 5). Prolonged and forced walking by lactating cows has shown to induce an increase in somatic cells in both uninfected and previously infected udders which is often associated to higher pH and lower milk lactose (Coulon *et al.*, 1998). Pasture elevation range and the steepness of the grazing plots may exacerbate the magnitude of this phenomenon (Lamarche *et al.*, 2000).

### *Cows behaviour*

Cows behaviour was monitored only in the early period of the study, since it was impossible to record their daily feeding activity during the summer grazing. Despite of the different amount of concentrate consumed by the two groups of cows in early lactation (Table 4), their total time spent eating and ruminating was not affected by the diet (Table 6). Even the partition of these feeding related activities across the 24 hours was not different between dietary treatments (Figure 1). On average, cows spent 18.7 min to consume one kilogram of DM and this time budget was longer than that of Holstein cows (14.6 min/kg DM) receiving *ad libitum* a 60:40 forage:concentrate ratio total mixed ration (Maekawa *et al.*, 2002). Since both studies reported a similar cows DM intake of about 18 kg/d, it is likely that the prolonged ingestive behaviour observed in the present research was promoted by the long fiber of the forage portion which was not chopped prior feeding. Still comparing our data with the results of Maekawa *et al.* (2002), it is interesting to notice that in the present study the prolonged eating time was balanced by a shorter rumination activity resulting in a similar total chewing time per day. Gottardo *et al.* (1999) studied the partition of the daily DM intake by Holstein dairy cows fed a total mixed ration in a free stall system. Despite the *ad libitum* feeding regimen and the free access to the

manger, they recorded two clear peaks of intake: the first right after the morning distribution of the diet and the second in the afternoon after milking. Compared to this pattern, the use of automatic feeders for concentrate administration in the present study altered cows feeding behaviour especially by prolonging the eating activity in the late afternoon (Figure 1).

As supposed for ruminant herbivores (Phillips, 1993), cows performed rumination activity mainly lying down both during day-light hours and overnight (Figure 1). Soy-free cows showed a reduced lying down time than Control cows (Table 6). However, it was difficult to hypothesize a robust nutritional explanation for this outcome as well as for the significant effect of observation day. Both results seemed more related to an abnormal behaviour of single cows of the two experimental groups.

## **Conclusions**

Extruded pea has shown to be a valuable alternative to soybean protein in the nutrition of dairy cattle in organic farms located in the alpine region. The inclusion of the peas had a positive productive response both in early lactation and during the summer grazing period. Milk fat and protein were not affected by the different protein source of the diet while in order to prevent the observed increase of milk urea it is advisable to combine the administration of extruded peas with energy sources readily fermentable in the rumen. Observations on cows feeding behaviour, carried out during the early lactation period, showed no dietary effect on total time spent eating and ruminating.

*Part of this work has been previously presented and published in the Proceedings of the 6<sup>th</sup> National Meeting of the Italian Association of Organic and Biodynamic Animal Production hold in Arezzo (Italy) on May 23<sup>rd</sup> 2008.*

*The authors are grateful to the personnel of organic dairy farm “Antica Rendena” for the precious technical support to the study. A special thank to dr. Dario Tonietto of the National Breeders Association of Rendena Cattle for the logistic support and to dr. Iller Landini of the Progeo Mangimi for the skilled cooperation in the feed supplements formulation.*

---

Research financed with a grant of the interregional project “Efficienza, Qualità e Innovazione nella Zootecnia Biologica (E.QU.I.ZOO.BIO.)”.

---

## **References**

- Agabriel, C., Coulon, J.B., Sibra, C., Journal, C., Hauwuy, A. 1997. Facteurs de variation del la numeration cellulaire du lait en exploration. *Ann. Zootech.* 46:13-19.
- AOAC, 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Corbett, R.R., Okine, E.K., Goonewardene, L.A., 1995. Effects of feeding peas to high-producing dairy cows. *J. Dairy Sci.* 75:625-629.
- Coulon, J.B., Pradel, P., Cochard, T., Poutrel, B., 1998. Effect of extreme walking conditions for dairy cows on milk yield, chemical composition, and somatic cell count. *J. Dairy Sci.* 81:994-1003.
- Cozzi, G., Bittante, G., Polan, C.E., 1993. Comparison of fibrous materials as modifiers of in situ ruminal degradation of corn gluten meal. *J. Dairy Sci.* 76:1106-1113.

- Cozzi, G., Bizzotto, M., 2004. Sustainability and environmental impact of the dairy production systems in mountain areas. *Acta Agriulturae Slovenica*, Suppl. 1: 21-28.
- Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72:68-78.
- European Council, 2007. Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. In: *Official Journal L189*:1-23
- Ferguson, J.D., Otto, K.A., 1989. Managing body condition score in cows. *Proc. Cornell Nutr. Conf. Feed Manuf. Conf.* pp.75-87.
- Froidmont, E., Bartiaux-Thill, N., 2004. Suitability of lupin and pea seeds as a substitute for soybean meal in high-producing dairy cow feed. *Anim. Res.* 53:475-487.
- Gottardo, F., Berzaghi, P., Burato, G.M., Andrighetto, I., 1999. The use of an automatic intake recording system to study the feeding behaviour of dairy cows housed in free stalls. pp. 434-436 in *Proc. 13th ASPA Congress, Piacenza, Italy.*
- INRA, 2002. Tables de composition et de valeur nutritive des matieres premières destinée aux animaux d'élevages. Institut National de la Recherche Agronomique, Paris, F.
- James, C., 2006. Global Status of Commercialized Biotech/GM Crops: 2006. Brief No. 3 International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY, USA.
- Johansson, B., Nadeau, E., 2006. Performance of dairy cows fed an entirely organic diet containing cold-pressed rapeseed cake. *Acta Agr. Scand. A-AN.* 56:128-136
- Khalili, H., Kuusela, E., Suvitie, M., Huhtanen, P., 2002. Effect of protein and energy supplements on milk production in organic farming. *Anim. Feed Sci. and Technol.* 98:103-119.

- Khorasani, G.R., Okine, E.K., Corbett, R.R., Kennelly, J.J., 2001. Nutritive value of peas for lactating dairy cattle. *J. Dairy Sci.* 81:541-551.
- Lamarche, A., Martin, B., Hauwuy, A., Coulon, J.B., Poutrel, B., 2000. Evolution of milk somatic cell count of cows grazing an alpine pasture according to the infection of udder by pathogens. *Ann. Zootech.* 49:45-54.
- Maekawa, M., Beuchemin, K.A., Christensen, D.A., 2002. Effect of concentrate level and feeding management on chewing activities, saliva production and ruminal pH of lactating dairy cattle. *J. Dairy Sci.* 85:1165-1175.
- Martin, P., Bateson, P., 1993. *Measuring behaviour*. Cambridge University Press, Cambridge, UK.
- 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., Rossi, F., 2005. Effect of estrusion, expansion and toasting on the nutritional value of peas, faba beans and lupins. *Ital. J. Anim. Sci.* 4:177-189.
- National Research Council, 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academy Press, Washington, DC, USA.
- 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.
- Phillips, C.J.C., 1993. *Cattle behaviour*. Farming Press Books, Ipswich, UK.
- Rendena, 2008. <http://eng.agraria.org/cattle/rendena.htm>
- SAS, 1990. *SAS/STAT® User's Guide: Statistics*. Version 6. SAS Inst. Inc., Cary, NC, USA.
- Schultz, M.M., Hansen, L.B., Steuernagel, G.R., Kuck, A.L., 1990. Variation of milk, fat, protein and somatic cells for dairy cattle. *J. Dairy Sci.* 73:484-493.



- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Method for dietary fiber, neutral detergent fiber and non starch 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.

TABLE 1. Feed composition of the two experimental organic concentrates (g/kg as fed)

|   | Control | Soy-free |
|---|---------|----------|
| <i>Feed ingredients:</i>                    |         |          |
| Maize meal                                  | 290     | 150      |
| Wheat bran                                  | 250     | 250      |
| Barley meal                                 | 195     | 154      |
| Soybean expeller                            | 160     | --       |
| Sunflower expeller                          | 55      | 95       |
| Extruded peas                               | --      | 272      |
| Maize gluten meal                           | --      | 29       |
| Calcium carbonate                           | 26      | 26       |
| Sodium bicarbonate                          | 8       | 8        |
| Sodium chloride                             | 7       | 7        |
| Dicalcium phosphate                         | 3       | 3        |
| Trace minerals-vitamins premix <sup>1</sup> | 6       | 6        |

<sup>1</sup>Contained per kilogram of concentrate: Fe, 93 mg; Cu, 10 mg; Zn, 108 mg; Mn, 216 mg; Co, 1.2 mg; I, 0.4 mg; Se, 0.4 mg. 18000 IU of vitamin A; 2400 IU of vitamin D3; 18 mg of vitamin E.

TABLE 2. Chemical composition of the experimental organic concentrates and of the meadow hay fed in early lactation period (mean  $\pm$  sd values).

|                             |                 | Concentrates   |                | Meadow hay        |
|-----------------------------|-----------------|----------------|----------------|-------------------|
|                             |                 | Control        | Soy-free       |                   |
| <i>Chemical composition</i> |                 |                |                |                   |
| Dry matter                  | %               | 89.9 $\pm$ 1.2 | 89.5 $\pm$ 1.5 | 91.4 $\pm$ 0.9    |
| Ash                         | % DM            | 8.8 $\pm$ 0.4  | 8.8 $\pm$ 0.5  | 6.9 $\pm$ 1.3     |
| Crude protein               | “               | 19.1 $\pm$ 0.4 | 19.1 $\pm$ 0.3 | 6.2 $\pm$ 0.6     |
| Ether extract               | “               | 4.3 $\pm$ 0.8  | 2.8 $\pm$ 0.6  | 1.1 $\pm$ 0.1     |
| NDF                         | “               | 20.8 $\pm$ 1.2 | 22.0 $\pm$ 0.9 | 62.2 $\pm$ 4.4    |
| Starch                      | “               | 37.8 $\pm$ 0.5 | 40.3 $\pm$ 0.7 | n.d. <sup>1</sup> |
| RDP <sup>2</sup>            | % Crude protein | 63.1           | 63.2           | --                |
| UFL <sup>3</sup>            | /kg DM          | 1.09           | 1.06           | 0.54              |

<sup>1</sup>n.d.: not determined.

<sup>2</sup>RDP: Rumen degradable protein calculated from NRC table values for each feed ingredient.

<sup>3</sup>UFL: Unité Fourragère Lait calculated from INRA table values for each feed ingredient.

TABLE 3. Parity, mature cow equivalent, days in milk and initial milk yield of Rendena cows at the beginning of the two periods of the study (mean  $\pm$  sd values).

|                    |      | Early lactation |                 | Late lactation |                |
|--------------------|------|-----------------|-----------------|----------------|----------------|
|                    |      | Control         | Soy-free        | Control        | Soy-free       |
| <i>Animals</i>     | n.   | 8               | 8               | 8              | 8              |
| Parity             | n.   | 3.0 $\pm$ 1.7   | 3.4 $\pm$ 1.9   | 3.5 $\pm$ 1.6  | 3.6 $\pm$ 3.2  |
| Mature equivalent  | kg   | 4226 $\pm$ 516  | 4151 $\pm$ 512  | 4027 $\pm$ 429 | 3958 $\pm$ 455 |
| Days in milk       | d    | 11 <sup>1</sup> | 11 <sup>1</sup> | 196 $\pm$ 50   | 191 $\pm$ 75   |
| Initial milk yield | kg/d | 16.0 $\pm$ 1.8  | 17.2 $\pm$ 2.4  | 16.9 $\pm$ 2.8 | 18.2 $\pm$ 2.9 |

<sup>1</sup>No sd value is reported since all the cows began the early lactation period at day 11 post-partum.

1

TABLE 4. Milk yield, feed intake and body condition score of Rendena cows fed the two experimental organic diets in early and late lactation.

|                       |          | Early lactation |            | Late lactation |          | Significance |        |             | RMSE  |
|-----------------------|----------|-----------------|------------|----------------|----------|--------------|--------|-------------|-------|
|                       |          | Control         | Soy-free   | Control        | Soy-free | Diet         | Period | Diet*Period |       |
| Milk yield            | kg/d     | 17.50           | 18.65      | 8.55           | 9.25     | **           | ***    | ns          | 1.94‡ |
| Feed intake:          |          |                 |            |                |          |              |        |             |       |
| Concentrate           | kg of DM | 6.61            | 6.98       | 1.40           | 1.61     | **           | ***    | ns          | 1.35  |
| Hay                   | “        | 11.0 ±0.2       | 10.9 ± 0.3 | --             | --       | --           |        | --          | --    |
| Body condition (BCS): |          |                 |            |                |          |              |        |             |       |
| Initial               | score    | 3.49            | 3.52       | 3.15           | 3.45     | *            | *      | ns          | 0.15  |
| Δ (BCS)               | score    | 0.03            | -0.03      | 0.05           | -0.25    | *            | ns     | ns          | 0.18  |

2 \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns: not significant.3 ‡Covariate effect of initial milk yield significant at  $P < 0.001$ .

4

1

TABLE 5. Milk composition of Rendena cows fed the two experimental diets in early and late lactation.

|                          |                     | Early lactation |          | Late lactation |          | Significance |        |             | RMSE |
|--------------------------|---------------------|-----------------|----------|----------------|----------|--------------|--------|-------------|------|
|                          |                     | Control         | Soy-free | Control        | Soy-free | Diet         | Period | Period*diet |      |
| <i>Milk composition:</i> |                     |                 |          |                |          |              |        |             |      |
| Fat                      | %                   | 3.37            | 3.38     | 3.39           | 3.13     | ns           | ns     | ns          | 0.65 |
| Fat yield                | kg/d                | 0.57            | 0.64     | 0.32           | 0.33     | ns           | ***    | ns          | 0.10 |
| Protein                  | %                   | 3.20            | 3.12     | 3.31           | 3.25     | ns           | *      | ns          | 0.24 |
| Protein yield            | kg/d                | 0.54            | 0.59     | 0.31           | 0.34     | ns           | ***    | ns          | 0.06 |
| Lactose                  | %                   | 4.90            | 5.00     | 4.84           | 4.87     | ns           | **     | ns          | 0.14 |
| Urea                     | mg/100ml            | 33.37           | 38.59    | 25.00          | 26.25    | *            | ***    | ns          | 0.60 |
| Somatic cell count       | 10 <sup>3</sup> /ml | 177             | 164      | 373            | 293      | ns           | ***    | ns          | 24   |

2 \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns: not significant.

3

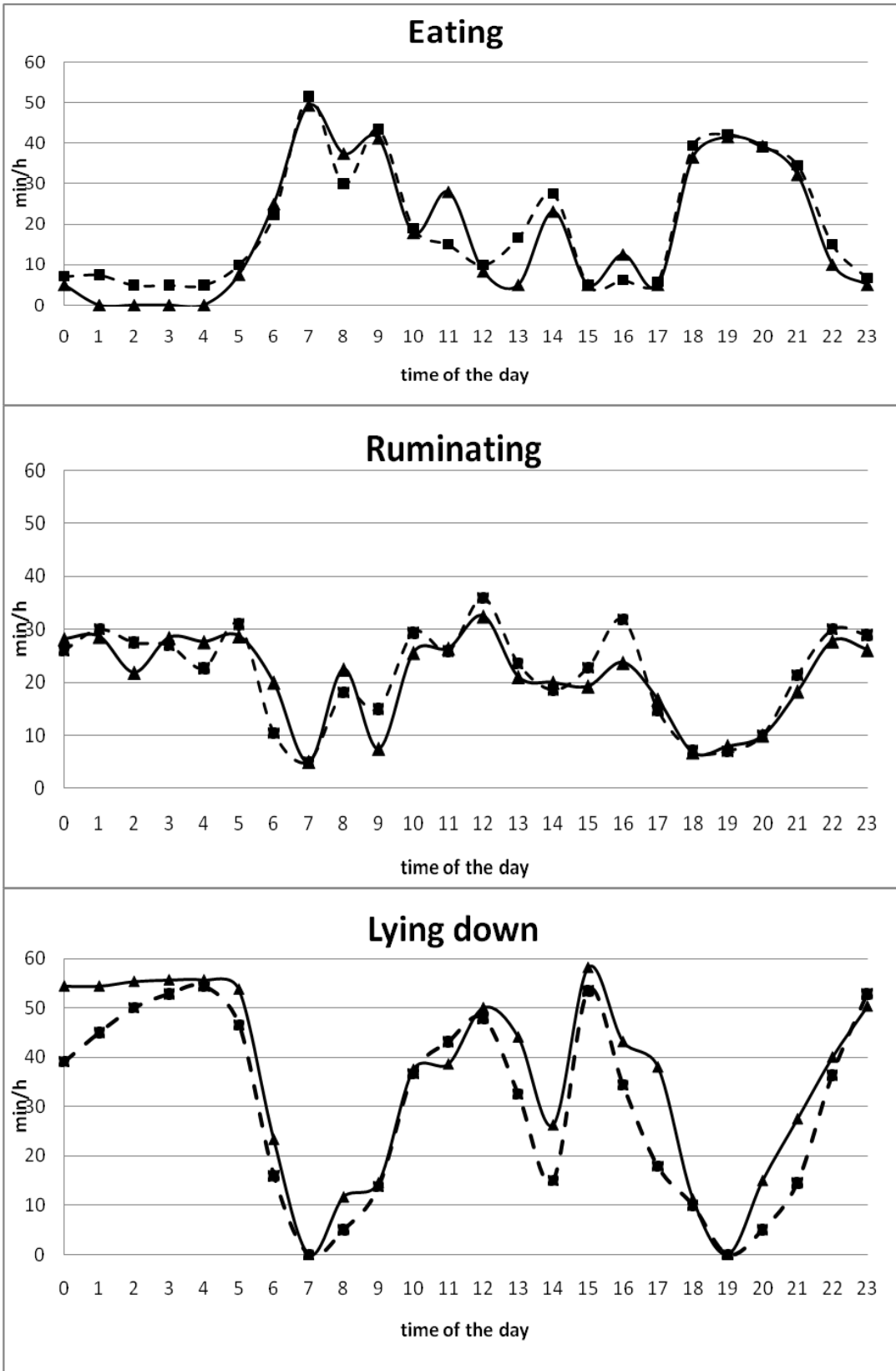
1

TABLE 6 . Feeding behaviour and lying down activity of the cows during the early lactation period.

|                      | Diet    |          | Significance |                 | RMSE |
|----------------------|---------|----------|--------------|-----------------|------|
|                      | Control | Soy-free | Diet         | Observation day |      |
| Eating:              |         |          |              |                 |      |
| Min/d                | 333     | 354      | ns           | ns              | 47   |
| Min/kg of DM         | 18.4    | 19.0     | ns           | ns              | 2.9  |
| Rumination:          |         |          |              |                 |      |
| Min/d                | 424     | 451      | ns           | ***             | 48   |
| Min/kg of DM         | 23.5    | 24.2     | ns           | ***             | 2.8  |
| Total chewing, min/d | 757     | 804      | ns           | ***             | 72   |
| Lying down, min/d    | 795     | 663      | **           | ns              | 103  |

2 \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns: not significant.

1 Figure 1: Eating, ruminating and lying down activities during the 24 hours in cows receiving  
 2 Soy\_free (—■—) and Control concentrate (—▲—) in the early lactation period.  
 3



6  
7