Archiv Tierzucht 55 (2012) 2, 132-139, ISSN 0003-9438

Milk quality, manufacturing properties and blood biochemical profile from dairy cows fed peas (*Pisum sativum* L.) as dietary protein supplement

Vincenzo Tufarelli[1], Shabana Naz[2], Rifat Ullah Khan[3], Domenico Mazzei[1] and Vito Laudadio[1]

[1]Department of Animal Production, Faculty of Veterinary Medicine, University of Bari »Aldo Moro«, Valenzano, Italy, [2]Department of Zoology and Fisheries, GC University, Faisalabad, Pakistan, [3]Department of Animal Health, KPK Agricultural University, Peshawar, Pakistan

Abstract

Pea (*Pisum sativum* L.), a protein-rich legume seed well adapted to many climatic areas and widely used for feed and food, was fed to Italian Friesian breed cows for 16 weeks to assess its effects on milk yield and production, renneting properties and metabolic responses. Cows within each group were assigned to two isonitrogenous and isoenergetic concentrates based on corn plus soybean meal or peas. Individual milk samples were collected from two consecutive milkings, composited, and then analysed for fat, protein, casein and lactose contents and somatic cells count as well as blood and milk urea and milk technological characteristics. Cow blood samples were taken and plasma were analysed for metabolites, biological enzymes, β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA). Peas supplementation had no effects on metabolic blood profile as well as on milk composition traits and clotting aptitude. Milk and blood from cows fed peas indicated a reduction ($P<0.05$) of their urea concentrations compared to those fed soybean meal. Our findings indicate that peas can replace soybean meal as protein source in diet of dairy cows without unfavourable effects on milk quality and cheesemaking properties.

Keywords: cow, milk quality and yield, renneting properties, blood profile, pea

Introduction

The development of locally grown protein crops may be a solution to improve the valorisation of products and to assure a better traceability of feedstuffs (Froidmont & Bartiaux-Thill 2004).

Among alternative protein sources to soybean, peas have been successfully used in diets for dairy cows especially in American and Mediterranean countries (Petit *et al.* 1997, Vander Pol *et al.* 2008, Tufarelli *et al.* 2012). Peas (*Pisum sativum* L.) are grown primarily for human consumption; however, surplus grain or grain that does not meet human food grade specifications is available to be used as a livestock feed (Loe *et al.* 2004). Field pea is a legume high in crude protein (CP; 217 g/kg), highly rumen degradable (780 g/kg of CP; NRC 2001), the starch content is 540 g/kg (McLean *et al.* 1974) and the net energy for lactation (NE_L) is 1.81 MCal/kg (NRC 2001). Previous trials have focused on using field pea as a protein source in diets for non-ruminants (Laudadio & Tufarelli 2010) and dairy cattle (Vander Pol *et al.* 2008).

Field pea has been shown to be a valuable substitute for soybean meal (Khorasani *et al.* 2001) and soybean meal and other proteinic meal combinations (Petit *et al.* 1997) in diets fed to lactating dairy cows. Moreover, pea was reported to yield much herbage with 7.5 t/ha fresh matter with 240 g/kg dry matter (DM) (Devkota *et al.* 1993, Hayashi *et al.* 2007). Therefore, it could be used as an important forage legume to enhance feed values for dairy ruminants. To date, there are few works concerning the inclusion of field peas in dairy cow rations.

Therefore, since peas have a good potential to substitute the conventional grain ingredients, such as soybean meal and/or corn in dairy cow rations, it is necessary to assess the inclusion of pea to provide a satisfactory feeding strategy for dairy cow farmers comparing the nutritional value of pea-based concentrates to conventional diets, and to evaluate their effects on milk characteristics and metabolic profile in dairy cows.

Materials and methods

Animals and diets

The trial was conducted in a dairy farm located in Bari Province in the Apulia region in Southern Italy, for 18 weeks and involved 24 multiparous Italian Friesian breed cows in the early stage of lactation. The study included a 2 week adaptation period to the diet, followed by 16 weeks of feeding the two diets. Cows (620±23 kg body weight, 2.8±0.3 in body condition score and 22±4 days in milk at the beginning of the trial) were cared in accordance with animal welfare standard requirements.

Animals were individually housed in pens during the adaptation period and in tie-stalls during the experimental period. Each stall had a separate manger for hay feeding and was equipped with a watering outlet. Animals had free access to water during the feeding trial. Health status of cows was checked throughout the experimental period and no cases of clinical mastitis were reported. Cows (n=12 per treatment) were randomly allocated to two dietary treatments according to calving date, parity (2nd or 3rd) and their initial milk yield and composition.

The dietary treatments were as follows:

- control concentrate, containing soybean meal as the main protein source (150 g/kg)
- experimental concentrate, containing pea seeds as the main protein source (345 g/kg) in total substitution of soybean meal (Table 1).

Both concentrates contained 170 g/kg of CP on DM basis (Table 2). The pea seeds utilized in the ration formulation were organically and locally grown (*Pisum sativum* L. cv. Spirale) containing 251 g/kg CP (on DM basis) and estimated 7.57 MJ/kg of net energy for lactation (NE_L), and they were processed and coarsely ground at a commercial feedstuff factory.

The diet fed to the soybean meal group was made of *ad libitum* oat hay supplemented with soybean meal-concentrate, whereas the pea seeds group replaced the control with pea seeds-concentrate. Hay and concentrates were supplied in three meals daily (6.00, 12.00 and 18.00). The concentrate levels were adjusted on a weekly basis on cows' milk yield. The amount of concentrate was always totally eaten by cows, and no refusals were recorded during the whole feeding period. Individual hay intake was measured by decreasing the daily dose offered to each cow by the residue recovered in the manger. The diets fed in this

trial were formulated according to NRC (2001) to provide similar amounts of NE_L and CP. The energy values for lactation (UFL) of the hay and concentrates were also estimated Jarrige (1989).

Table 1
Ingredient composition of experimental concentrates fed to dairy cows

Item	Dietary treatment	
	Soybean meal diet	Pea seeds diet
Ingredient, g/kg as-fed		
Ground corn	345.0	125.0
Durum wheat bran	249.0	244.0
Soybean meal, 44 % CP	150.0	-
Peas[1]	-	375.0
Sunflower meal, 28 % CP	75.0	75.0
Dehydrated beet pulp	75.0	75.0
Corn gluten feed	50.0	50.0
Calcium carbonate	24.0	24.0
Dicalcium phosphate	10.0	10.0
Sodium chloride	5.5	5.5
Sodium bicarbonate	5.5	5.5
Vitamin-Mineral premix[2]	5.0	5.0
Magnesium oxide	3.5	3.5
Yeast	2.5	2.5

[1]Pea seeds obtained from locally and organically grown *Pisum sativum* L. cv. Spirale; [2]Supplied per kg of diet: vitamin A 40 000 IU; vitamin D3 4 000 IU; vitamin E 60 mg; vitamin B1 10 mg; vitamin B3 500 mg; choline chloride 250 mg; vitamin B12 0.03 mg; Co 1.25 mg; Fe 100 mg; I 5 mg; Mn 100 mg; Cu 20 mg; Se 0.25 mg; Zn 215 mg.

Table 2
Chemical composition (% on DM basis) of diets fed to dairy cows

	Dietary treatment		
	Soybean meal	Pea seeds diet	Oat hay
Chemical composition, %			
Dry matter	88.65	89.02	89.87
Crude protein	16.97	16.32	6.35
Ether extract	2.67	2.52	2.04
Crude fibre	7.46	7.79	29.77
Ash	8.15	8.07	9.29
aNDF	21.13	21.87	53.44
ADF	9.22	9.69	31.85
Lignin(sa)	2.37	2.67	3.32
Starch	29.88	29.58	-
NFC	51.08	48.78	28.05
Acid insoluble ash	0.89	1.01	1.09
Ca	1.38	1.38	0.38
P	0.70	0.71	0.15
Na	0.43	0.42	0.02
NE_L, Mcal/kg DM	1.39	1.38	0.53
Milk FU, *n*/kg DM	0.89	0.88	0.51

NFC: 100–(% aNDF+% crude protein+% ether extract+% ash), NE_L estimated according to NRC (2001), Milk FU: Feed Unit for Lactation, estimated according to INRA (1989)

Cows' bodyweights were recorded at the beginning and at the end of experimental period. The body condition score of cows was measured by the same observer according to Edmonson *et al.* (1989), and cows were scored at the beginning and subsequently at the end of the feeding trial. Cows were milked twice daily (6.00 and 18.00) using pipeline milking machines.

Sampling and chemical analyses

Hay, concentrates and refusals samples were daily collected, whereas concentrate feeds were sampled weekly. Each sample was ground in a hammer mill with a 1 mm pore size screen and analysed in triplicate for their content in DM (65 °C in a forced-air oven, dried to a constant weight), ash, CP (N × 6.25), crude fibre (CF), ether extract (EE), Ca, P and Na according to the procedures described by the AOAC (2000). Neutral detergent fibre (aNDF, using heat-resistant α-amylase without sodium sulphite) and acid detergent fibre (ADF) were analysed according to Van Soest *et al.* (1991) and was corrected for residual acid insoluble ash (AIA). Lignin (sa) were analysed according to Mertens (2002). Composite samples (hay and concentrates) were analysed for AIA as reported by Van Keulen and Young (1977). Starch was measured after acid hydrolysis and polarimetric detection according to Holm *et al.* (1986). Non-fibre carbohydrate (NFC) content was calculated by difference as NFC=1 000−(aNDF g/kg+crude protein g/kg+ether extract g/kg+ash g/kg).

Blood samples were taken every 2 weeks, prior to morning feeding via coccygeal venepuncture and immediately chilled. Plasma samples, obtained after centrifugation at 4 000 rpm for 20 min, were stored at −20 °C until the analysis. Stored samples were determined using commercial kits (Sentinel Chemical, Milano, Italy) and an automatic spectrophotometer (Cobas FARA 2, Roche Diagnostic, Basel, Switzerland) for total protein, albumin, glucose, total cholesterol, blood urea (BU), triglycerides, nonesterified fatty acids (NEFA), calcium, inorganic phosphorus and β-hydroxybutyrate (BHBA). Globulin content was calculated as the difference between total protein and albumin. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by commercial kits produced by Bio-Merieux (Marcy-l'Etoile, France) and Boehringer Mannheim (Maylan, France), following the methodology suggested by the producers.

The daily milk yield was recorded by means of graduated measuring cylinders attached to individual milking units. Individual milk samples, consisting of proportional volumes of morning and evening milk, were taken after cleaning and disinfection of teats and discharging the first streams of foremilk. Samples were collected in 200 ml sterile plastic containers at fortnightly intervals through the lactation period and taken to our laboratory under refrigeration.

The following measurements were made biweekly: protein (N × 6.38) according to AOAC (2000), fat and lactose content, using an infrared spectrophotometer Milko Scan 133B (Foss, Hillerød, Denmark); casein content (AOAC 2000); somatic cell count (SCC), using a Fossomatic 90 cell counter (Foss, Hillerød, Denmark). Milk production was standardised and corrected to 4 % of fat (FCM) according to NRC (2001). Milk urea (MU) was determined by the urease method-kits (Boehringer). Milk pH was measured using a pH meter with a temperature probe (Expandable Ion Analyzer model EA940 and electrode Ross model 81-02, Research Inc., Boston, MA, USA).

The coagulation milk properties were weekly determined evaluating the three renneting parameters according to Zannoni and Annibaldi (1981) by Formagraph apparatus (Foss Italia, Padova, Italy): rennet clotting time (r), as the time from rennet addition to the beginning of coagulation; curd firming time (k_{20}), as the time from coagulation until reaching the curd firmness corresponding to an amplitude of 20 mm on the Formagraph trace; and curd firmness (a_{30}), as the amplitude of the trace 30 min after the rennet addition. Measurements were performed in triplicate and at milk natural pH.

Statistical analysis

Experimental design was a randomised block design, with 12 replicates per treatment. Milk yield and composition parameters were analysed as repeated measures with diet as a non-repeated factor using GLM procedure of SAS 8.2 (SAS Institute Inc., Cary, NC, USA). Sources of variation included cows and dietary treatment. Cows within treatment were fitted as term of error.

Data were presented as means of each treatment and standard errors of the means. When significant effect was found, means were compared using Student's *t*-test. Significant differences were set at $P<0.05$ and a trend at $P<0.10$.

Results and discussion

The two concentrates had similar concentrations of CP, crude fibre, NE_L and NDF, but the pea diet had slightly lower NFC with similar starch content, which reflects the difference in starch content between corn grain and pea (Table 2). The peas utilized in the present trial contained 251 g/kg crude protein (% DM basis) and an estimated 7.57 MJ NE_L/kg DM. Replacing the soybean meal by pea in the concentrate-diet did not influence body condition score change of dairy cows ($P=0.32$) as well as body weight during the whole trial ($P=0.44$). The DM intake and daily milk yield produced by dairy cows were not different ($P=0.39$ and 0.27, respectively) between the two dietary groups (Table 3). Further, the 4 % fat-corrected milk yield, and the percentages and yields of the milk components were also not influenced by the supplementation of peas in the ration.

The comparison between our results and previous trials must be done with caution because of earlier studies conducted on cows were done using a combination of other leguminous grains such as faba bean, pea and lupin (Petit *et al.* 1997, Froidmont & Bartiaux-Thill 2004, Vander Pol *et al.* 2008, Cozzi *et al.* 2010). To our knowledge, the present trial is one of the few studies in the literature that describes on the effect of the total replacement of soybean meal with peas on milk characteristics of dairy cows. Consequently, data on the influence of peas on the production of cows in the early-phase of lactation are limited.

In a recent study conducted by Vander Pol *et al.* (2008), when peas were included in diets for dairy cows the milk fat content was not affected, but conversely to our results milk protein concentration and yield were increased by the pea. However, according to our data, Petit *et al.* (1997) reported no difference in milk parameters in cows fed raw or extruded peas. Corbett *et al.* (1995) found that isonitrogenous and isoenergetic substitution of soybean meal, and canola meal with leguminous grain in pelleted supplement resulted in decreased milk yield at level of 10 % in dairy cows during the mid-phase of lactation. In agreement

with the present trial, Vander Pol *et al.* (2008) substituted 15 % of dietary DM (steam-rolled corn grain and soybean meal) with peas and found no influence of diet on DM intake and milk productive or qualitative parameters as well as milk urea content. On the contrary, in our study the blood and milk urea (Tables 3 and 4, respectively) levels in cows appeared to be decreased by the replacement of soybean with pea in concentrates (P=0.04 and P=0.06, respectively). Froidmont and Bartiaux-Thill (2004) found that dietary leguminous grains reduce the level of milk urea in dairy cows due to the low DM and protein intakes.

Table 3
Effect of diets containing soybean meal or field bean on metabolic blood profile of dairy cows

Item	Dietary treatment			
	Soybean meal	Pea seeds diet	SEM	*P*-value
Total protein, g/dL	7.6	7.7	0.05	0.45
Albumin, g/dL	3.4	3.5	0.02	0.36
Globulin, g/dL	4.4	4.1	0.07	0.47
Calcium, mg/dL	8.6	8.4	0.09	0.11
Phosphorus, mg/dL	5.6	5.8	0.13	0.51
Total cholesterol, mg/dL	236.9	219.9	4.01	0.08
Triglycerides, mg/dL	6.3	5.9	0.15	0.14
Blood urea, mg/dL	35.2	32.3	0.44	0.04
Glucose, mg/dL	48.7	46.4	0.25	0.19
AST, IU/L	86.2	87.7	0.33	0.08
ALT, IU/L	42.4	38.5	0.69	0.07
NEFA, mmol/L	0.3	0.3	0.02	0.36
BHBA, µmol/L	548.8	575.1	6.78	0.09

AST: aspartate aminotransferase, ALT: alanine aminotransferase, NEFA: nonesterified fatty acids, BHBA: β-hydroxybutyrate

In our study, the blood and milk urea concentrations recorded in cows fed experimental diet were reduced, almost certainly due to a decreased degradability of pea protein leading to a decreased ammonia level in the rumen and urea level in milk and blood, as also reported in a recent study of Volpelli *et al.* (2009).

No significant difference occurred in blood metabolites, electrolytes, or enzymes between control soybean and experimental pea diets (Table 3).

Milk pH was almost similar in the two dietary treatments, as well as for the milk clotting properties (Table 4). With regard to milk technological properties, a slight, but not significant, decrease of a_{30} parameter (curd firmness) was reported in the milk produced by cows fed pea-diet. These data could be related to the lower somatic cell count and pH levels, as previously reported by Park *et al.* (2007) in sheep and goat milk. The lack of influence of dietary treatment on milk clotting proprieties in relation to cheese-making might in part be due to the similar milk protein percentage of samples on which the renneting measurements were conducted, as also reported by Masucci *et al.* (2006). The present results confirm the suitability of the milk from pea-fed cows for cheese-making.

In conclusion, this study confirms that field pea can be used in dairy cows' diets as no reduction of productive traits was reported. In conclusion, substituting conventional soybean meal with alternative high-protein peas resulted in similar performances and milk technological properties of dairy cows.

Table 4
Effect of diets on DM intake, body weight and body condtion score changes, milk yield and composition, and milk rheological properties of dairy cows

Item	Dietary treatment			
	Soybean meal diet	Pea seeds diet	SEM	*P*-value
DMI, kg/d	23.5	23.9	0.39	0.39
Body weigth change, kg/d	0.16	0.19	0.06	0.44
Body condition score change	0.5	0.7	0.02	0.32
Yield (mean for 14 week of lactation)				
Milk, kg/d	27.0	26.9	0.37	0.27
4 % FCM, kg/d[1]	25.5	25.6	0.21	0.18
Fat, kg/d	0.98	0.99	0.07	0.11
Protein, kg/d	0.85	0.87	0.05	0.15
Milk composition				
Fat, %	3.62	3.69	0.09	0.13
Protein, %	3.17	3.25	0.11	0.21
Casein, %	2.43	2.51	0.07	0.09
Lactose, %	5.01	5.06	0.04	0.28
pH	6.69	6.73	0.01	0.43
Milk Urea, mg/dL	25.2	24.8	0.51	0.06
SCC, cells/mL × 10^3	342	311	104	0.47
Rheological properties				
r, min	12.44	12.63	0.15	0.31
k_{20}, min	2.59	2.82	0.09	0.19
a_{30}, mm	47.81	46.45	1.78	0.12

[1]4 % FCM: fat-corrected milk calculated according to NRC (2001), r: rennet clotting time, k_{20}: curd firming time, a_{30}: curd firmness

References

AOAC (2000) Official Methods of Analysis of the Association of Official Analytical Chemists, 17th ed., AOAC Intl, Gaithersburg, USA

Corbett RR, Okine EK, Goonewardene LA (1995) Effects of feeding peas to high-producing dairy cows. Can J Anim Sci 75, 625-629

Cozzi G, Boukha A, Contiero B, Gottardo F (2010) Extruded pea (Pisum sativum) as alternative to soybean protein for dairy cows feeding in organic Alpine farms. Ital J Anim Sci 9, 194-199

Devkota NR, Singh SB, Basnet KB, Tiwari S (1993) Identification of suitable forage grasses and legumes for supplying fodder throughout the year at IAAS livestock farm. In: Neupane FP (ed.) IAAS Research Report (1985-1991), Institute of Agriculture and Animal Science, Rampur, Nepal, 467-480

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G (1989) A body condition scoring chart for Holstein dairy cows. J Dairy Sci 72, 68-78

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

Hayashi Y, Devkota NR, Kumagai H (2007) Effects of field pea (*Pisum sativum* L.) hay feeding on dry matter intake and milk production of Murrah buffaloes (*Bubalus bubalis*) fed rice straw *ad libitum*. Anim Sci J 78, 151-158

Holm J, Björck I, Drews A, Asp NG (1986) A rapid method for the analysis of starch. Starch/Stärke 38, 224-226

Jarrige R (ed.) (1989) Ruminant Nutrition: Ruminant Nutrition Recommended Allowances and Feed Tables. INRA, Paris, France

Khorasani GR, Okine EK, Corbett RR, Kennelly JJ (2001) Nutritive value of peas for lactating dairy cattle. Can J Dairy Sci 81, 541-551

Laudadio V, Tufarelli V (2010) Growth performance and carcass and meat quality of broiler chickens fed diets containing micronized-dehulled peas (*Pisum sativum* cv. Spirale) as a substitute of soybean meal. Poult Sci 89, 1537-1543

Loe ER, Bauer ML, Lardy GP, Caton JS, Berg PT (2004) Field pea (*Pisum sativum*) inclusion in corn-based lamb finishing diets. Small Ruminant Res 53, 39-45

Masucci F, Di Francia A, Romano R, Maresca di Serracapriola MT, Lambiase G, Varricchio ML, Proto V (2006) Effect of Lupinus albus as protein supplement on yield, constituents, clotting properties and fatty acid composition in ewes' milk. Small Ruminant Res. 65, 251-259

Mertens DR (2002) Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J AOAC Int 85, 1217-1240

McLean LA, Sosulski FW, Youngs CG (1974) Effect of nitrogen and moisture on yield and protein in field peas. Can J Plant Sci 54, 301-305

NRC (2001) Nutrient Requirements of Dairy Cattle, 7th revised edn. National Academy Press, Washington, USA

Park YW, Juarez M, Ramos M, Haenlein GFW (2007) Physico-chemical characteristics of goat and sheep milk. Small Ruminant Res. 68, 88-113

Petit HV, Rioux R, Ouellet DR (1997) Milk production and intake of lactating cows fed raw or extruded peas. J Dairy Sci 80, 3377-3385

Tufarelli V, Khan RU, Laudadio V (2012) Evaluating the suitability of field beans as a substitute for soybean meal in early-lactating dairy cow: Production and metabolic responses. Anim Sci J 83, 136-140

Van Keulen J, Young BA (1977) Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J Anim Sci 44, 282-287

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74, 3583-3597

Vander Pol M, Hristov AN, Zaman S, Delano N (2008) Peas can replace soybean meal and corn grain in dairy cow diets. J Dairy Sci 91, 698-703

Volpelli A, Comellini M, Masoero F, Moschini M, Lo Fiego DP, Scipioni R (2009) Pea (*Pisum sativum*) in dairy cow diet: effect on milk production and quality. Ital J Anim Sci 8, 245-257

Zannoni M, Annibaldi S (1981) Standardization of the renneting ability of milk by Formagraph. Sci Tecn Lattiero-Cas 32, 79-94

Received 12 December 2011, accepted 27 January 2012.

Corresponding author:

Vincenzo Tufarelli
email: v.tufarelli@veterinaria.uniba.it

Department of Animal Production, University of Bari »Aldo Moro«, Strada Prov. le per Casamassima km 3, 70010 Valenzano (BA), Italy