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Peas may be a candidate crop for integrating silvoarable systems and dairy buffalo farming in southern Italy

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Abstract Integration of trees with agricultural crops and livestock systems may offer advantages in term of productivity, economic return, and sustainability. In order to identify a candidate crop for Mediterranean silvoarable systems to be used in livestock farming as a protein source locally produced, a study was undertaken to evaluate the effect of feeding peas (*Pisum sativum* L.) as main protein source on milk yield and in vivo digestibility of primiparous buffaloes. Two almost isonitrogenous concentrates (on average, crude protein 240 g/kg dry matter) were formulated to contain, as fed basis, either 350 g/kg of soybean cake (SoyC) or 450 g/kg of extruded peas (PeaC) as the main protein source. Twenty primiparous buffaloes were blocked by age and body weight into two dietary treatments (Soyand Pea) from 10 to 100 day in milk. All cows were fed in the barn a total mixed ration containing 3 kg of SoyC and in the milking parlour they were individually supplemented by either 3 kg of SoyC or PeaC according to the group assignment. The substitution rate of soybean protein by pea protein was approximately 30%. Milk yield was not affected by the dietary use of extruded peas, as well as, milk fat and protein percentages, and clotting properties. Moreover, in vivo digestibility did not differ between the two dietary groups. Results support the partial substitution of soybean cake with extruded peas in diets for lactating buffaloes.

Keywords Field peas · Mediterranean silvoarable systems · Organic livestock farming · Primiparous buffaloes · Milk traits · Digestibility

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Introduction

The objective of modern agroforestry systems is a profitable agricultural use until the trees are harvested (Chalmin and Mastel 2011). From this perspective, integration of trees with agricultural crops and livestock systems may offer advantages combining productivity and economic return, sustainability and adaptability to climate change (Jose 2009; Nerlich et al. 2012). The design of intercropping systems has been traditionally focused on selection and management of tree species, with little attention given to the crop components (Nair 1998). Indeed, the integration of trees with crops should be designed to optimize the use of growth resources (i.e. water, light, micro and macro nutrients) (Jose et al. 2004; Thevathasan et al. 2004) especially with regard to nitrogen (N) that means the primary nutrient limiting plant production (Gardner et al. 2017). Legumes play a critical role in natural, agricultural, and agroforestry ecosystems due their ability to fix atmospheric N in symbiosis (McNeill and Fillery 2008; Isaac et al. 2012). In the Mediterranean region, scarce are the tree species of commercial interest able to fix atmospheric N, so that soil N supply of silvoarable systems may be improved by the combination of leguminous crops with non-nitrogen-fixing trees (Mahieu et al. 2016; Querné et al. 2017). Legume pulse crops have also played an important role in livestock feeding for centuries, since they are rich sources of protein (Osman et al. 2012; Martin et al. 2017). Currently, about 70% of plant-based protein concentrates fed to livestock in Europe is imported from extra-EU Countries, mainly as soybean and its derivatives (Watson et al. 2017), thereby potentially raising problems of sustainability, trade distortions, scarcity and price volatility of soybean on the global market (Bureau and Swinnen 2018).

In Campania, a region of southern Italy, the typical lowland silvoarable systems based on fruit trees intercropped with cereals and pulses have suffered a sharp decline until recent decades (Eichhorn et al. 2006). Currently, only in the internal hilly areas remain fragments of traditional orchards (mainly fruit and olive trees) planted at wide spacing (Regione Campania 2013). Profitability of these systems could be enhanced through intercropping and integration of crop farming with livestock production (Sereke et al. 2015; Daoui and Fatemi 2014), in particular with the dairy buffalo enterprises that are well-established in the coastal plains of Campania Region (Masucci et al. 2016; Uzun et al. 2018a).

Pea (*Pisum sativum* L.) is a legume crop of interest for Mediterranean area due to extreme flexibility as rotational crop, potential of winter sowing, quickly growth capability and early ripening, which allow escape from high temperature and drought stress (Annicchiarico and Iannucci 2007; Annicchiarico 2008). Pea seeds can be used as protein source for ruminant diets due to the high levels of both protein and and starch, which make them a unique dual-purpose feed (Borreani et al. 2007; Vander Pol et al. 2008). Therefore, the use of peas from silvoarable Mediterranean systems may be an alternative of interest from environmental and economic point of view. This study aimed to shed light on the influence of partial replacement of soybean with peas in diets for primiparous buffaloes on milk yield and quality under ecofriendly farming system.

Materials and methods

Feeds, diets and animals

The study was carried out at an organic dairy buffalo farm located in Campania, a Region of Southern Italy (40°27 N, 15°01 E, 31 m a.s.l.) where the lactating buffaloes were fed a commercial concentrate (Bioforces 23) containing soybean cake (soyben concentrate—SoyC) as the main protein source (350 g/kg as fed basis). For experimental purposes, an alternative concentrate was formulated by substituting soybean cake by 450 g/kg of extruded peas (experimental concentrate—PeaC). Twenty pregnant primiparous buffaloes were blocked by age (on average, 31.5 $\pm$ 1.6 months) and body weight (BW, 585 $\pm$ 39 kg) and assigned to one of two dietary groups (Soy and Pea) from 10 d of lactation onwards. The experimental period covered the first 100 d of day in milk (DIM).

The two groups were housed together in a free stall barn with external paddock and cubicles (1.14 $\times$ 2.34 m) with rubber mats, and with access to water by troughs. Both groups were fed once a day (starting at 0730) for ad libitum intake (10% of expected orts) a total mixed ration (TMR) containing 3 kg of the SoyC. Twice a day, at each milking time (0400 and 1500), the cows were individually fed either 1.5 kg of SoyC or PeaC according to the dietary group.

Then, in the parlor were given about 650 g/d of crude protein (CP) i.e. the 30% of the daily intake; about 64% of CP from soybean were replaced by peas (Table 1).

Experimental measurements and sample collection procedure

Individual milk yield was measured daily, beginning at calving by a computerized system. Milk samples from each cow were collected every 2 weeks. At 10, 30, 60 and 90 d of DIM bulk milk were sampled from both groups to determine coagulation parameters and fatty acids composition. At 90 d, grab fecal samples were collected from each cow to determine in vivo digestibility according to the procedure described by Masucci et al. (2011). Along fecal sampling, DM intake was determined by the difference between TMR offered and refused, and samples of the TMR, orts, and feeds were collected.

Analytical methods

All analyses were completed at least in duplicate. The AOAC (2002) official methods were used to determine dry matter (DM), ash, CP, and ether extract (EE) contents of feeds and feces as described by procedures 930.15, 942.05, 976.05 and 954.02, respectively. The organic matter (OM) content was calculated as the difference between DM and ash contents, with ash determined by combustion at 550 °C overnight. Neutral detergent fibre (NDF) exclusive of residual ash were determined by methods of Van Soest et al. (1991), without the use of an amylase and sodium sulfite. Starch content was measured after acid hydrolysis and polarimetric detection by using a Polax-2 l polarimeter in 200 mm long observation tubes (Garcia and Wolf 1972). Soluble protein (SP) was determined according to Licitra et al. (1996). Feed and fecal samples were analyzed for acid insoluble ash by the 2 N hydrochloric acid procedure of Van Keulen and Young (1977). Individual milk samples were analyzed for fat, protein, lactose, non-fat solid (Milkoscan 605, Foss Electric, Sweden), and urea (CL 10, Eurochem, Italy). Coagulation parameters were determined by means of a Formagraph on 10 ml of milk, at 35 °C, with the addition of 0.2 ml of a rennet solution and a technical time of analysis of 30 min, according to the procedure described in Masucci et al. (2006). Gas-chromatographic analysis of milk fatty acids (FA) were performed by means of trans-esterification reaction according to the procedure described in Romano et al. (2010) and Romano et al. (2014).

Calculation and statistical analyses

Milk yield was standardized to 8.3% of fat and 4.1% of protein according to Campanile et al. (1998). The daily records of yield of each cow were averaged into weekly arithmetic means. Mozzarella cheese yield was calculated according Altiero et al. (1989). Statistical analysis was performed by means of SAS statistical software (1990). Data on milk yield and quality (i.e. fat, protein, lactose, pH, urea) underwent analysis of variance for repeated measures (mixed procedure) with the dietary treatment (Soy and Pea) as a non-repeated factor and time and diet $\times$ time as repeated factors. The cow variance was considered as random and utilized as the error term to test the main effect of the diet. Coagulation parameters and milk FA composition were analyzed by one-way analysis of variance (Soy and Pea) with sampling time as experimental unit. A t test was used to compare in vivo digestibility. Statistical significance was declared at $P < 0.05$.

Results and discussion

Feeds and diets

Ingredients and chemical composition of the protein sources, the concentrates and the diets are in Table 1. The two protein feeds largely differed. According with the existing literature (Masoero et al. 2006; Hejdysz et al. 2017; Omosebi et al. 2018), the extruded peas showed, compared to the soybean cake, lower CP and EE values and higher SP and starch contents. By contrast, SoyC and PeaC were almost isonitrogenous and had similar energy and starch contents. Only SP and EE remained slightly different between the two concentrates, but they were almost identical in the corresponding diets. Both rations were based on maize and alfalfa, forages largely used in dairy buffalo farming (Uzun et al. 2018b), indicating that there is no need to change forage crops in organic system.

Table 1 Chemical composition (g/kg dry matter unless otherwise stated) of the protein sources, the concentrates and the rations fed to the primiparous buffaloes

	Protein source		Concentrate		Diet	
	Soybeancake[a]	Extruded peas[b]	Soybean[c]	Pea[d]	Soybean	Pea
Ingredients						
Maize silage					22	22
Alfalfa silage					4.5	4.5
Alfalfa hay					1.5	1.5
Wheat straw					0.5	0.5
Soy concentrate[a]					6.0[e]	3.0[f]
Pea concentrate[b]					–	3.0[g]
Chemical composition						
Dry matter g/kg	925	860	892	909	530	530
Crude protein,	423	206	239	245	113	114
Soluble protein	54	100	42	76	34	39
Ether extract	100	15	75	63	28	27
Starch	31	485	219	214	149	149
NDF	156	145	242	244	481	481
NEL MJ/kg dry matter	8.18	7.18	7.68	7.39	5.55	5.47

[a]Protein source included in the Soybean Concentrate

[b]Protein source included in the Pea Concentrate

[c]Concentrate containing extrudes soybean cake as the main protein source based on: soybean cake (35%), maize meal, dehydrated maize plant, faba bean, alfalfa dehydrated meal, wheat bran, barley meal, maize gluten, sodium bicarbonate, calcium carbonate, dicalcium phosphate, sodium chloride

[d]Concentrate containing extrudes pea as the main protein source based on: peas (45%), maize meal, dehydrated maize plant, maize gluten faba bean, alfalfa dehydrated meal; wheat bran, barley meal, soybean cake (3%), sodium bicarbonate, calcium carbonate, dicalcium phosphate, sodium chloride

[e]3 kg were given with the total mixed ration, 3 kg were given in the milking parlor

[f]Given with the total mixed ration

[g]Given in the milking parlor

Milk yield and quality

Milk yield and composition of the Soy and Pea groups are presented in Table 2. The interaction diet × time was not significant for any parameters indicating that there was no effect of the protein source along the lactation. By contrast, the effect of time was always significant, except for pH, reflecting the modifications of milk composition as the lactation progressed.

The isonitrogenous substitution of PeaC for SoyC in the diet did not substantially affect milk yield. In contrast to what happens in dairy cattle, in lactating buffaloes an unbalanced diet fed in the early lactation stage does not result in weight loss, but in yield drop (Zicarelli 1997). So, this result could indirectly indicate the suitability of peas in feeding primiparous buffalo cows.

In regard to milk quality, no differences were observed between groups for fat, protein and lactose contents, whose values fall in the normal ranges for primiparous buffaloes bred in Italy (Bartocci et al. 2006). Moreover, the milk urea of Pea group was comparable to that of Soy group, although the higher solubility of protein of peas would have affected it. These results confirm those observed in our previous trial carried out on pluriparous cows (Di Francia et al. 2009), but also indicate suitability of peas in primiparous cows wherein additional growth requirements can influence severity of a negative energy balance (Morales Piñeyrúa et al. 2018).

Table 2 Milk yield and quality (least square mean) of buffaloes fed the concentrates based on soybean or peas

	Soy group	Pea group	SE	*P*
Milk yield, kg/d	9.85	9.97	0.17	NS
Fat %	8.3	8.3	0.5	NS
Protein %	4.4	4.3	0.073	NS
Lactose %	4.9	4.9	0.05	NS
Urea, ml/dl	38.5	40.2	0.55	NS
pH	6.70	6.64	0.037	NS
Mozzarella Cheese yield %	24.73	24.38	0.48	NS
Rennet clotting time, min	18.06	18.38	1.25	NS
Curd firming time 20 mm, min	1.97	1.68	0.10	NS
Curd firmness 30 min, mm	41.90	41.10	2.68	NS

NS Not significant $P > 0.05$

The studies investigating the effects of the substitution of soybean with peas in dairy cow diets are inconsistent (Khorasani et al. 2001; Froidmont and Bartiaux-Thill 2004; Masoero et al. 2006). Differences in ration composition, level of peas inclusion and technological treatments of the legume grains are most likely the reasons of these conflicting results. In this study, the similar milk yield and milk urea content of the two groups indicate that protein fraction repartition of both diets was suitable to meet amino acid needs of the buffaloes. Additionally, the extrusion of peas has most likely reduced protein degradability contributing so to improve nitrogen availability in the rumen (Walhain et al. 1992). Thus, overall, we can safely assume that the higher protein solubility of peas, if properly balanced in the ration, does not necessarily lead to nitrogen losses from the rumen.

Both mozzarella cheese yield and milk clotting properties showed similar values between the diets - due to the fact that milk protein and fat contents were almost identical (De Marchi et al. 2008). This result is of importance as buffalo milk is almost exclusively used to produce mozzarella cheese.

The milk FA composition did not differed among Soy and Pea groups (Table 3). It is well established that dietary inclusion of fresh herbage as well as free oils or oil-rich feeds can strongly affect FA composition of milk fat (Varricchio et al. 2007; Esposito et al. 2014). The EE percentages of extruded peas and soybean cake were very different and this fact would have to modify the milk FA composition. However, the two concentrates had a similar fat content and probably the rate of substitution of peas for soybean was below the threshold to change the FA profile.

In vivo digestibility

The effects of dietary inclusion of pea on apparent total tract digestibility are depicted in Table 4. Although the digestibility coefficients of Soy group

Table 3 Fatty acid composition (least square mean) of milk fat of buffaloes fed the concentrates based on soybean or peas

Fatty acid% weight	Soy group	Pea group	SE	*P*
Butyric	2.4	2.7	0.27	NS
Caprinic	2.21	2.24	0.19	NS
Capronic	1.4	1.46	0.21	NS
Myristic	11.6	11.2	0.26	NS
Palmitic	33.56	32.15	0.42	NS
Stearic	10.2	11.7	0.33	NS
Oleic	22.4	23.0	0.41	NS
Linoleic	2.49	2.66	0.087	NS
Linolenic	0.99	1.01	0.035	NS
Conjugated linoleic acids (CLA)	0.94	0.89	0.04	NS
Others	11.8	10.9	0.33	NS

NS Not significant $P > 0.05$

Table 4 In vivo digestibility coefficients (least square mean) of buffaloes fed the concentrates based on soybean or peas

	Soy group	Pea group	SE	*P*
Organic matter	0.631	0.622	0.063	NS
Crude protein	0.590	0.572	0.100	NS
NDF	0.474	0.459	0.123	NS

NS Not significant $P > 0.05$

were numerically higher than Pea group, the differences were not significant. Again, these results agree with our previous report carried out on pluriparous buffaloes (Di Francia et al. 2009). In dairy cattle, dietary use of peas on digestibility can have negative (Khorasani et al. 2001; Vander Pol et al. 2008), positive (Froidmont and Bartiaux-Thill 2004; Vanhatalo et al. 2004) or no effects (Vander Pol et al. 2009). Diet characteristics, inclusion level and the protein feeds replaced by peas are the factors can explain these contrasting results.

Conclusions

Our results indicate that peas may safely replace soybean cake in a properly balanced ration for primiparous buffaloes at the replacement rate of 30% of protein supply. At this substitution rate, in agreement with previous results on pluriparous cows, no effects on milk yield, or milk composition in terms of macro-components, clotting proprieties and FA composition of fat were observed. Then, due to lack of effect even on vulnerable primiparous cows, peas may be a protein source of great interest in both organic and conventional buffalo breeding. We conclude that pea may be a candidate crop for Mediterranean silvoarable systems to be used in buffalo farming as a protein source locally produced. Finally, the global problems of sustainability and trade distortions related to soybean are further incentives for peas use.

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