

Milk Production and Composition, and Intake of Holstein Lactating Cows Fed Diets with Partial Substitution of Soybean Meal with Flaked Field Pea

Research Article

A. Pirzadeh Naeiny¹, M. Danesh Mesgaran^{1*}, A.R. Vakili¹ and H. Ebrahimi¹

¹ Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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*Correspondence E-mail: danesh@um.ac.ir

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ABSTRACT

The objective of this study was to investigate the effect of a partial substitution of soybean meal with field pea (*Pisum sativum*) in Holstein lactating dairy cow diets on dry matter intake, milk yield and composition, blood metabolites, rumen ammonia-N concentration and pH. Eighteen lactating Holstein cows were randomly assigned into three groups based on parity, days in milk, and milk yield. Flaked field peas replaced soybean meal (SBM) at the levels of 0.0, 33 and 66% (P₀, P₃₃ and P₆₆, respectively) and were fed to the animals for 33 days. Dry matter intake, milk yield, milk fat and protein content, blood metabolites, rumen ammonia-N concentration and pH were not significantly (P<0.05) affected by the diets. The results of this study demonstrated that field pea could safely replace soybean meal up to 66% in the diet of high-producing dairy cows.

KEY WORDS dairy cow, field pea, intake, milk composition, milk yield.

INTRODUCTION

The protein sources commonly used in concentrate feeds for dairy cows are few and include mainly soybean meal (SBM) and canola meal. Therefore, the need for protein sources alternative to soybean meal arises, which can be partially or totally substituted in the diets of dairy cows and other farm animals. Among the protein sources alternative to soybean meal, pea has been successfully used in diets for dairy cows. Pea is a high quality and relatively cheap source of protein, used in monogastric and ruminant nutrition (Moschini *et al.* 2005b; Masoero *et al.* 2006). Field pea (*Pisum sativum*) has a relatively high crude protein (CP) content and a significant amount of energy in the form of starch (NRC, 2001; Beyer *et al.* 2003), which makes it a unique feed; it can be substituted for higher-priced protein and energy commodities like SBM in dairy cow diets. The pea protein seems to have a higher rumen degradability

than SBM (78% vs. 65%), (Michalet-Doreau and Cerneau, 1991; Aufrere *et al.* 1994; Masoero *et al.* 2005). Similar values were obtained by Khorasani *et al.* (2001), (84.5 vs. 67.2%), observing a higher degradation rate (11.4 vs. 9.6% h⁻¹) and more soluble protein fractions (59.3 vs. 20.9%). In addition, leguminous seeds are rich in phenolic compounds (Duenas *et al.* 2004), which have strong antioxidant activities and may contribute to the milk flavour and enhance the health benefits of milk (Besle *et al.* 2005; Moon *et al.* 2006). Petit *et al.* (1997) reported no effect of inclusion of 20% peas in the diet (replacing corn grain and SBM) on dry matter intake, milk yield and composition. Other reports (Khorasani *et al.* 2001; Masoero *et al.* 2006) also found no effect of feeding peas on milk production or composition, or perhaps the results were influenced by the differences in dry matter (DM) and CP intake (Froidmont and Bartiaux-Thill, 2004). The various responses in milk production when pea is used in dairy cow diets might be influenced by

the applied processing methodology. Heat treatment has been widely used to reduce ruminal CP degradability of protein supplements, such as, canola (Moshtaghi Nia and Ingalls, 1992; McAllister *et al.* 1993) and soybean meal (Demjanec *et al.* 1995). However, studies on feeding steam flaked field peas to dairy cows are very limited. Heat treatments have the desirable effect of reducing protein degradability, resulting in increased rumen by-pass protein that aids nitrogen utilization in the intestine (Rémond *et al.* 1997). The rumen by-pass protein can be doubled by wet heat processing between 105 and 136 °C for 10-15 minutes (Poncet *et al.* 2003). However, heat treatments can also increase the starch degradability in the rumen, which is not desirable (Corbett, 1997). Thus, more research is needed on the effect of inclusion of steam flaked field peas in the diets of lactating dairy cows on feed intake, milk yield and composition and blood metabolite parameters. The objective of this study was to determine the effect of replacing soybean meal with steam flaked pea on feed intake, milk yield and composition, and blood metabolite parameters including glucose, albumin, cholesterol, triglycerides in lactating dairy Holstein cows.

MATERIALS AND METHODS

Steam flaking, animals and diets

The steam-flaked peas were processed at atmospheric pressure. The steaming time was ~30 min, while the temperature of the seeds on removal from the steamer was 100 °C; the peas were flaked with a rolling pressure of 30 kg cm⁻². The trial was conducted in the dairy farm of the Animal Science Department of Ferdowsi University of Mashhad for 33 days and involved 18 Holstein lactating dairy cows. The study included a five-day adaptation period to the diet, followed by 28 days of feeding the three diets to the animals. The animals were individually housed in tie-stalls during the experimental period. Each stall was equipped with a watering outlet for free access to water during the feeding trial. The health status of the cows was checked throughout the experimental period.

The cows (n=6 per treatment) were randomly allocated to three dietary treatments according to parity, days in milk, milk yield and body weight. The dietary treatments were a control diet (0.0% flaked pea, P_{0.0}), containing soybean meal as the main protein source and experimental diets containing flaked pea that replaced SBM at the levels of 33% (P₃₃) and 66% (P₆₆), respectively (Table 1). The diets were fed as *ad libitum* as total mixed ration (TMR) of alfalfa hay and corn silage and concentrate (NRC, 2001). The experimental diets were given to cows twice daily at 09:00 a.m. and 18:00 p.m. The individual intake was measured daily by the residue recovered in the manger. Feed residuals were collected daily.

The cows were milked three times daily (8.00 a.m., 15.00 and 22.00 p.m.) using pipeline milking machines. Milk yield was recorded daily for the duration of the study. Milk samples were taken every other day from day 23 of the experiment. The individual milk samples, consisting of proportional volumes of morning, afternoon and night milk, were taken after cleaning and disinfection of the teats and discharging the first streams of foremilk. The samples were collected in 200 mL sterile glass containers at fortnightly intervals through the lactation period and taken to the laboratory under refrigeration.

Cows behaviour, blood and rumen fluid sampling

The feeding behaviour of the cows was monitored on day 32 of the experiment. The observation sessions were carried out by a team of trained persons. The cows were observed for 24 h starting at the time of feed delivery in the morning. Feeding related activities (eating and rumination) were recorded every 10 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 10-min interval. The eating and ruminating times per kg of dietary DM intake were calculated by dividing the total time spent performing each activity by the DM intake. The chewing activity was calculated as the sum of the total time spent eating and ruminating. The rumen fluid was sampled on day 33 of the experiment. The samples (100 mL) of rumen fluid were taken 4.30 h after the morning feeding. The ruminal fluid was strained through two layers of cheesecloth. The pH was measured before freezing (-20 °C) for subsequent ammonia analyses. Blood samples were taken from the caudal vein twice from all cows: on the first day of adaptation and on the 31st day of the experiment prior to the morning feeding. Blood samples of cows were collected in evacuated glass tubes and allowed to coagulate. The samples were then transported to the laboratory within two hours of collection. The serum was separated by centrifuge at 3000 × g for 15 minutes and stored at -20 °C until the next analysis. The 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 nitrogen (BUN) and triglycerides content

Chemical analyses

The samples were dried in a forced-air oven (56 °C), ground to pass a 2-mm screen and analysed for the total N (Kjeldahl method, Kjeltac 2300 Autoanalyzer, Foss Tecator AB, Hogans, Sweden), neutral detergent and acid detergent fibre ([neutral detergent fiber (NDF) and acid detergent fiber (ADF)], Van Soest *et al.* 1991), ether extract (AOAC, 2000) and ash (AOAC, 2000) content.

Table 1 Ingredient and chemical composition of the experimental diets

Item	Treatments ¹		
	P _{0.0}	P ₃₃	P ₆₆
Ingredients (% of DM)			
Corn silage	4.37	4.37	4.37
Alfalfa hay	6.71	6.71	6.71
Corn grain	4.14	4.14	4.14
Ground barley grain	4.14	4.14	4.14
Rapeseed meal	1.29	1.29	1.29
Beet pulp	0.45	0.45	0.45
Fish meal	0.72	0.72	0.72
Wheat bran	2.23	1.49	0.74
Soybean meal	2.16	1.44	0.72
Flaked peas ²	0.00	1.44	2.88
Sodium bicarbonate	0.09	0.09	0.09
Sodium carbonate	0.06	0.06	0.06
Sodium bantionate	0.20	0.20	0.20
Dicalcium phosphate	0.05	0.05	0.05
Mineral-vitamin mix ³	0.20	0.20	0.20
Chemical composition			
Dry matter (%)	61.5	62.2	62.8
Crude protein (% of DM)	17.2	17.2	17.1
Fat (% of DM)	3.1	3.3	3.5
Neutral detergent fiber (NDF) (% of DM)	32.0	32.2	32.6
Acid-detergent fiber (ADF) (% of DM)	16.7	16.9	17.3
Ash (% of DM)	4.0	3.5	3.0

¹ Percentage of soybean meal (SBM) replaced by pea in the concentrate: P_{0.0}: control group; P₃₃: field peas replaced SBM at the level of 33% and P₆₆: field peas replaced SBM at the level of 66%.

² Flaked peas contained (DM %): dry matter: 95.7; crude protein: 25.9; Fat: 6; NDF: 35.4; ADF: 6.58 and ash: 2.1.

³ Composition of mineral and vitamin mix per kg diet: Ca: 196.0 g; P: 96.0 g; Mg: 19.0 g; Fe: 3.0 g; Na: 71.0 g; Cu: 0.3 g; Mn: 2.0 g; Zn: 3.0 g; Co: 0.1 g; I: 0.1 g; Se: 0.01 g and vitamin A: 500000 IU; vitamin D: 100000 IU and vitamin E: 100 IU.

Statistical analysis

The response variables of the experiment that were measured over time (dry matter intake (DMI), milk yield and milk composition) were analysed as a completely randomized design using the proc mixed procedure of SAS (2001). The means were compared using the LSMEANS/DIFF with treatments as a fixed term and the cows nested within treatments as a random effect and the day of sampling as a repeated measure. Other data were analysed by SAS (2001) using the general linear model procedure as a completely randomized design with the cows as replicates. The statistical differences were determined using Duncan's multiple range tests at $P < 0.05$.

RESULTS AND DISCUSSION

Dry matter intake, milk yield and milk composition

The effect of substituting pea for soybean meal did not influence the dry matter intake, milk yield and milk composition (Table 2). Cows in P₀, P₃₃ and P₆₆, had an average dry matter intake of 26.7, 26.7 and 26.6 kg/d, respectively, indicating that the partial replacement of soybean meal with flaked field pea had no adverse effect on the feed intake of cows. It also suggests that increasing the level of peas in the diet does not influence palatability as previously reported (Khorasani *et al.* 2001).

Corbett *et al.* (1994) reported that milk yield in late-lactation cows was not influenced by substituting peas for a combined soybean meal/corn meal supplement. These results are also in agreement with Petit *et al.* (1997) and Ward *et al.* (1989).

Corbett *et al.* (1994) and Petit *et al.* (1997) speculated that early lactating cows fed with pea-based concentrates had a higher 4% fat corrected milk (FCM) yield and milk fat content. Hoden *et al.* (1992) found similar milk yield, milk fat and protein content after the inclusion of peas in the diet of lactating cows, although the highest producing cows showed a decrease in milk and fat production when fed with peas. However, the replacement of soybean protein with pea protein did not influence the milk fat percentage in this study. No differences were reported in mid late-lactating cows fed on diets with increasing levels of pea (Khorasani *et al.* 2001).

Petit *et al.* (1997) reported no changes in milk composition associated with increasing the level of peas in the diet. No differences were observed in milk protein content in the current study. Khorasani *et al.* (2001) reported a quadratic response of the milk protein content in mid late-lactating cows when the SBM protein was replaced by the pea protein. Only a tendency towards higher milk protein content was observed by Petit *et al.* (1997) when replacing the SBM with extruded peas.

The author’s conclusions indicated a higher nitrogen digestibility, which could have increased the amount of nitrogen available for milk protein synthesis.

Cows behaviour

The total time spent by the cows eating and ruminating was 848, 806 and 784 minutes in P₀, P₃₃ and P₆₆, respectively. There were no significant differences between the treatments (Table 3).

Gottardo *et al.* (1999) evaluated the partition of the daily DM intake by Holstein dairy cows that were fed a total mixed ration in a free stall system. Despite the *ad libitum* feeding regimen and the free access to the 13 mangers, 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. Cozzi *et al.* (2010) reported the use of extruded pea as an alternative to soybean in the protein feeding of dairy cattle raised in organic Alpine farms. The feeding behaviour of the cows was monitored only in the early lactation period. Despite 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.

Ruminal responses and blood metabolites

Ruminal pH and ammonia concentration (Table 4) were not different among the treatments. Vander Pol *et al.* (2009) reported that the inclusion of field pea in dairy cow ration had no effect on ruminal pH. In another research, Soto-Navarro *et al.* (2004) reported minimal changes in the pH with the increased levels of field peas in medium-concentrate diets.

In some situations, in which cereal grains are used as supplements for forage-based diets, ruminal pH can be decreased (Sanson *et al.* 1990), however, the results are not universal, particularly when the grain inclusion is at low to moderate levels, as was the case in the current study. In addition to this, the supplementation of rumen degradable protein (RDP) resulted in lower ruminal pH (Bodine *et al.* 2000; Koester *et al.* 1996), presumably through increased ruminal fermentation when the RDP is supplemented to diets based on low-quality forage.

Khorasani *et al.* (2001) substituted SBM with peas at the levels of 0.0%, 33.3%, 66.7% and 100% of the concentrate portion. They obtained a linearly increased response of rumen ammonia-N as pea inclusion in the diets increased. The reported rumen ammonia-N when SBM was completely replaced by the pea was 159 mg/L.

Table 2 Dry matter intake, milk yield and milk composition of Holstein lactating cows fed with experimental diets

Items	Treatments ¹			SEM	Contrast P-value		Time
	P _{0.0}	P ₃₃	P ₆₆		Linear	Quadratic	
Dry matter intake (kg/d)	26.7	26.7	26.6	0.04	0.08	0.16	0.39
Milk yield (kg/d)	37.1	37.3	37.4	0.99	0.83	0.98	0.22
Milk composition							
Fat (%)	3.8	3.9	3.9	0.13	0.42	0.60	0.050
Protein (%)	3.3	3.4	3.4	0.16	0.32	0.70	0.060
Lactose (%)	5.7	5.9	5.8	0.10	0.63	0.12	0.080

¹ Percentage of soybean meal (SBM) replaced by pea in the concentrate: P0.0: control group; P33: field peas replaced SBM at the level of 33% and P66: field peas replaced SBM at the level of 66%. SEM: standard error of the means.

Table 3 Feeding behaviour and resting activity of the cows

Item	Treatment ¹			SEM	P-value
	P ₀	P ₃₃	P ₆₆		
Eating, min/d	416	368	352	14.4	0.17
Rumination, min/d	438	432	432	12.9	0.98
Total chewing, min/d	848	806	784	17.9	0.37
Resting, min/d	666	642	602	17.9	0.37

¹ Percentage of soybean meal (SBM) replaced by pea in the concentrate: P0.0: control group; P33: field peas replaced SBM at the level of 33% and P66: field peas replaced SBM at the level of 66%. SEM: standard error of the means.

Table 4 Ruminal pH and ammonia-N (mg/dL) concentration in rumen fluid collected four and a half hours from morning meal

Item	Treatments ¹			SEM	P-value
	P ₀	P ₃₃	P ₆₆		
Ruminal pH	6.34	6.32	6.31	0.02	0.81
Ammonia-N (mg/dL)	23.57	22.30	21.70	0.18	0.92

¹ Percentage of soybean meal (SBM) replaced by pea in the concentrate: P0.0: control group; P33: field peas replaced SBM at the level of 33% and P66: field peas replaced SBM at the level of 66%. SEM: standard error of the means.

Table 5 Blood parameters as influenced by different diets fed to animals

Item	Treatments ¹			SEM	P-value
	P ₀	P ₃₃	P ₆₆		
Albumin (g/L)	29.70	30.20	30.40	0.71	0.72
Glucose (mmol/L)	4.70	4.90	4.70	1.98	0.44
Cholesterol (mmol/L)	11.08	11.02	10.91	0.84	0.49
Triglycerides (mmol/L)	5.12	5.00	5.06	1.28	0.36
Blood urea nitrogen (mmol/L)	0.40	0.35	0.44	0.52	0.13

¹ Percentage of soybean meal (SBM) replaced by pea in the concentrate: P0.0: control group; P33: field peas replaced SBM at the level of 33% and P66: field peas replaced SBM at the level of 66%.

SEM: standard error of the means.

Blood parameters

No differences were observed among treatment groups on blood parameters (Table 5).

Petit *et al.* (1997) reported higher serum urea concentration in animals that fed with raw pea compared with SBM. The increase after feeding was greater for cows fed the SBM than for those fed with extruded peas, whereas no difference was reported between raw and extruded peas.

CONCLUSION

The inclusion of steam flaked peas in the diets for lactating dairy cows at a P₃₃ and P₆₆ level, replacing soybean meal, did not result in negative effects on dry matter intake, milk yield and composition, blood metabolite parameters (total protein, albumin, glucose, total cholesterol, blood urea nitrogen and triglycerides), ruminal pH and ammonia-N concentration. The observations carried out on the cows' feeding behaviour showed no dietary effect on the total time spent eating and ruminating. Therefore, regarding the appropriate price of steam flaked pea, it could represent a feasible opportunity for a partial substitution of soybean meal in diet formulation.

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