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Field bean inclusion in the diet of early-lactation dairy cows: Effects on performance and nutrient utilization

D. J. Johnston,[1,2]* K. Theodoridou,[2] A. W. Gordon,[3] T. Yan,[1] W. C. McRoberts,[3] and C. P. Ferris[1]
[1]Agri-Food and Biosciences Institute, Hillsborough, Co. Down, BT26 6DR, United Kingdom
[2]Queens University Belfast, Co. Antrim, BT7 1NN, United Kingdom
[3]Agri-Food and Biosciences Institute, Belfast, Co. Antrim, BT9 5PX, United Kingdom

ABSTRACT

The European livestock sector has a significant deficit of high-quality protein feed ingredients. Consequently there is interest in using locally grown protein grain crops to partially or completely replace imported protein feeds in dairy cow rations. Field bean (FB; *Vicia faba*) has been identified as a locally grown crop with significant potential. The current study was designed to examine the effects of FB on cow performance and nutrient utilization in the diet of early-lactation dairy cows, including high levels of FB (up to 8.4 kg/cow per day). The experiment used 72 dairy cows in a 3-treatment continuous design (from calving until wk 20 of lactation). All cows were given ad libitum access to a mixed ration comprising grass silage and concentrates [45:55 on a dry matter (DM) basis]. Concentrates offered contained either 0, 349, or 698 g of FB/kg of concentrate (treatments FB0, FB-Low, and FB-High, respectively), with FB completely replacing soybean meal, rapeseed meal, maize gluten, and wheat in the concentrate for the FB-High treatment. Following completion of the 20-wk experiment, ration digestibility, nutrient utilization, and methane (CH_4) production were measured using 4 cows from each treatment. Neither silage DM intake, total DM intake, nor milk yield were affected by treatment. Cows on FB0 had a higher milk fat content than those on FB-High, and cows on FB0 and FB-Low had higher milk protein contents than did those on FB-High. Field bean inclusion increased the degree of saturation of milk fat produced. Milk fat yield, milk protein yield, and milk fat plus protein yield were higher with FB0 than with either FB-Low or FB-High. Treatment had no effect on the digestibility of DM, organic matter, nitrogen (N), gross energy, or neutral detergent fiber, whereas digestibility of acid detergent fiber was higher with FB0 than with FB-High. Neither the efficiency of gross energy or N utilization, nor any of the CH_4 production parameters examined, were affected by treatment. Similarly, none of the fertility or health parameters examined were affected by treatment. The reduction in milk fat observed may have been due to the higher starch content of the FB-High diet, and the reduction in milk protein may have been due to a deficit of methionine in the diet. It is likely that these issues could be overcome by changes in ration formulation, thus allowing FB to be included at the higher range without loss in performance.
Key words: dairy cattle, field bean (*Vicia faba*), milk production, nutrient utilization, methane production

INTRODUCTION

The increasing milk yield potential of dairy cows in many European countries has led to a requirement for more nutrient-dense diets. Increased nutrient density has often been achieved through the adoption of higher-concentrate feed levels, and this in turn has increased the demand for high-quality protein ingredients. However, European agriculture has a significant deficit of high-quality protein feedstuffs (Watson et al., 2017). This is a particular problem in the United Kingdom, driving considerable interest in increased use of locally grown protein ingredients (Wilkins and Jones, 2000). One protein crop of particular interest, especially in the cooler, wetter regions of the United Kingdom, is the field bean (**FB**; *Vicia faba*).

Although FB have a moderate CP content (280 g/kg of DM), they have a relatively high starch content (400 g/kg of DM; Ewing, 1997) and, as such, might appear to be ideal in ruminant diets. However, the inclusion of FB in ruminant diets is normally limited due to the perceived risk associated with antinutritional substances, including trypsin inhibitors, tannins, lectins, and protease inhibitors, which can reduce DM intake and animal performance (Dvořák et al., 2006). In addition, FB contain several phytoestrogens, some of which are known to have negative effects on reproductive performance (Zdunczyk et al., 2005), which has

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*Corresponding author: djohnston35@qub.ac.uk

also contributed to the limited inclusion of FB in dairy cow diets.

The use of FB in dairy cow diets has been examined in several studies, with the findings sometimes conflicting. For example, although neither DM intake nor milk yield were affected by FB inclusion in studies by Ingalls and McKirdy (1974) and Johnston et al. (2019), Ramin et al. (2017) observed a reduction in milk yield with FB inclusion. In contrast, Puhakka et al. (2016) found both DM intake and milk yield to be reduced when rapeseed meal was replaced by FB.

These studies have demonstrated that in some circumstances FB can be included in dairy cow diets at levels between 4.7 and 5.0 kg/d (Ingalls and McKirdy, 1974; Johnston et al., 2019) with no adverse effects on DM intake and milk production, but higher inclusion levels do not appear to have been examined previously. Given the high starch content of FB, which suggests that it could replace both conventional protein and energy components of the diet, the effects of higher FB inclusion levels need to be examined. In addition, as highlighted by Tufarelli et al. (2012), information on the effects of FB inclusion in the diet of dairy cows in early lactation, the period that encompasses the breeding season, is limited at present. This is important, given concerns about the presence of estrogenic compounds in FB, although there is no evidence that dairy cow fertility is reduced when FB are offered. Consequently, the current study was designed to examine the effects on cow performance, nutrient utilization efficiency, and fertility when early-lactation dairy cows were offered diets containing higher levels of FB than examined previously. We hypothesized that these parameters would be unaffected by these higher levels of FB inclusion.

MATERIALS AND METHODS

This experiment was conducted at the Agri-Food and Biosciences Institute, Hillsborough, Northern Ireland (**NI**). All experimental procedures were conducted under an experimental license granted by the Department of Health, Social Services and Public Safety for NI, in accordance with the Animals (Scientific Procedures) Act 1986.

Animals and Housing

This continuous-design 20-wk experiment involved 72 Holstein-Friesian dairy cows, 33 primiparous and 39 multiparous; mean parity 2.07 (SD 1.19). During the 3-wk period before calving, cows had ad libitum access to grass silage, while a dry-cow mineral and vitamin mix, along with calcined magnesite (Trouw Nutrition, Cheshire, UK), were mixed with the silage to achieve a target intake of 100 g/cow per day and 50 g/cow per day, respectively. Cows were moved to a straw-bedded maternity pen approximately 24 to 48 h before calving, based on behavioral observations. Following calving (normally within 24 h), cows were transferred to an experimental free-stall barn with solid concrete floors, where they had access to cubicles fitted with rubber mats, which were bedded with sawdust twice daily. The floor was scraped every 3 h, using an automated slurry scraper system.

Treatments

The variety of FB (*Vicia faba* var. Fuego) used in the study was described by Flores et al. (2013) as having "colored flower, high standing ability, high yield potential, big seeds." Colored-flower varieties of FB have long been known to have higher tannin contents than white-flowered varieties (Newton and Hill, 1983), with Abdulla (2017) recently recording the tannin content of the Fuego variety as 6.1 mg/g of DM. This variety was chosen for use in this study because it has been successfully grown by local arable and livestock farmers in NI over several years. The study by Flores et al. (2013) also demonstrated that this variety could be successfully grown over wide areas of Europe, with this variety having been evaluated previously in a dairy cow study in Lithuania (Kudlinskienė et al., 2018). The beans offered within this study were grown in NI. The crop was sown during spring 2016, harvested on September 28, 2016, and then dried at 80°C for approximately 2 h to achieve a moisture content of approximately 14%.

Cows were randomly allocated to 1 of 3 treatments at calving (FB0, FB-Low, FB-High), with primiparous and multiparous cows allocated separately. A check was made to ensure that each treatment group remained balanced for parity, BW, and BCS at drying-off, and, in the case of multiparous cows, for previous lactation 305-d milk yield and milk fat and protein contents.

Treatments differed in concentrate type offered (ingredient composition in Table 1). The concentrate offered with FB0 contained no FB, whereas concentrates offered with FB-Low and FB-High contained 349 and 698 g of FB per kilogram (fresh basis), respectively. The experimental concentrates (in the form of a meal) were mixed with grass silage (forage:concentrate ratio of 45:55 on a DM basis), together with 320 g/cow per day of straw and 50 g/cow per day of a rumen buffer (Acid Buf, Celtic Sea Minerals, Cork, Ireland; buffering capacity approximately 2.5× that of sodium bicarbonate), and offered in the form of a mixed ration. The grass silage component of the diet was produced from a primary growth (approximately 75% of cow feeding days) and a primary regrowth (approximately 25% of

cow feeding days) of perennial ryegrass (*Lolium perenne*)–based swards. The rations were prepared and offered daily at approximately 0900 h (at proportionally 1.07 of the previous day's intake), with uneaten ration removed the following day at approximately 0800 h. Rations were prepared using a feeder wagon (Vari-Cut 12, Redrock, Armagh, NI). The total silage requirement for all 3 treatments was initially mixed for approximately 4 min and then deposited on a clean covered concrete yard. Silage for each individual treatment was then removed from this "pile" and returned to the feeder wagon. The appropriate quantities of concentrate, straw, and buffer were added to the mix, and mixing continued for another 6 min. The rations were then transferred from the wagon to a series of feedboxes mounted on weigh scales, with cows accessing feed in these boxes via an electronic identification system, enabling individual cow intakes to be recorded daily (Controlling and Recording Feed Intake, BioControl, Rakkestad, Norway). Cows had free access to fresh water at all times. In addition, all cows were offered 1.0 kg per day of soy hulls via an in-parlor feeder during milking (0.5 kg at each milking). Cows remained on the experiment until d 140 of lactation.

Table 1. Ingredient composition (g/kg) of concentrates offered during the experiment

Ingredient	Treatment		
	FB0	FB-Low	FB-High
Field beans (FB)	0	349	698
Soybean meal	170	85	0
Rapeseed meal	150	75	0
Maize gluten feed	74	37	0
Maize meal	245	178	110
Wheat	174	87	0
Soy hulls (toasted)	140	139	140
Molaferm[1]	25	25	25
Calcined magnesite	6	6	6
Limestone ($CaCO_3$)	7	6	5
Dicalcium phosphate	0	5	10
Salt	6	4	2
Mineral and vitamin premix[2]	4	4	4

[1]United Molasses, Belfast, UK.

[2]Devenish Nutrition, Belfast, UK. Contains a minimum of (per kg) vitamin A retinyl acetate (2,500,000 IU), vitamin D_3 cholecalciferol (500,000 IU), vitamin E alpha tocopherol (18,750 mg), vitamin B_{12} (7,500 μg/μg), biotin (500 mg), iodine from calcium iodate anhydrous (1,875 mg), selenium from sodium selenite (150 mg), selenium from organic form of selenized yeast inactivated (25 mg), cobalt from coated granulated cobalt carbonate (40 mg), manganese from manganese(II) oxide (12,500 mg), copper from copper(II) sulfate pentahydrate (5,625 mg), copper from copper(II) chelate of amino acid hydrate (1,875 mg), zinc from zinc oxide (18,750 mg), and zinc from zinc chelate of amino acid hydrate (6,250 mg).

Cow Measurements

During the experiment cows were milked twice daily (between 0500 and 0700 h, and between 1500 and 1700 h) using a 50-point rotary milking parlor (BouMatic, Madison, WI), with milk yields recorded automatically at each milking and a total daily milk yield for each cow for each 24-h period calculated. Milk samples were taken during 2 consecutive milkings each week, a preservative tablet was added (Broad Spectrum Microtabs II, D and F Control Systems, Advanced Instruments Inc., Norwood, MA), and samples were stored at 4°C until analyzed. Samples were analyzed for fat, protein, and lactose using an infrared milk analyzer (Milkoscan model FT+; Foss Electric, Hillerod, Denmark), and a weighted concentration of each constituent was determined for the 24-h sampling period. A single milk sample was collected for progesterone analysis from each cow on 2 occasions each week (Monday and Thursday p.m.) until 60 d postpartum. Samples were preserved and stored as above, for a maximum of 3 wk, with progesterone concentrations subsequently determined using a competitive ELISA kit (Ridgeway Science Ltd., Gloucestershire, UK). In addition, at 10 and 20 wk post-calving (±1 wk) a milk sample was taken in proportion to yield during 2 successive milkings (a.m. and p.m.), the 2 samples bulked, and the bulked sample frozen at −20°C. These samples were subsequently analyzed for milk FA, as follows: milk fat was extracted from 1.0 mL of homogenized milk using a chloroform methanol extraction method (Bligh and Dyer, 1959), and FAME prepared. The FA composition was determined using GLC, with an aliquot (1.0 μL) of the FAME extract injected onto a CP Sil88 capillary column (100 m × 0.25-mm internal diameter × 0.2-μm film thickness) in a, Agilent 7800 gas chromatograph (both from Agilent Technologies, Santa Clara, CA), equipped with a temperature programmable injector operated in the split mode and a flame ionization detector. The oven was initially held at 50°C for 4 min, then increased at 8°C/min to 110°C, then at 5°C/min to 170°C (hold time 10 min), and finally at 2°C/min to 225°C (hold time 30 min). Fatty acids were identified by their retention time with reference to commercially available FA standards (37 Supelco FAME mix) and individual standards for those not in the mix (Sigma-Aldrich Co. Ltd., Gillingham, UK), and were quantified using C13 FAME as an internal standard.

Body weight was recorded twice daily (immediately after each milking) using an automated weighbridge, and a mean weekly BW for each cow was determined. The BCS of each cow was estimated fortnightly according to Edmonson et al. (1989) by a trained technician. Fecal scores were assessed at 6 and 12 wk post-calving (±1 wk) using a scale of 1 to 5, as described by Ireland-Perry and Stallings (1993). Blood samples were collected from the tail of each cow before feeding, at 2,

4, 6, 8, 10, and 14 wk (±3 d) post-calving. Samples were collected into evacuated tubes (Becton Dickinson, Oxford, UK), which were coated with either a clot activator or fluoride oxalate. The blood samples were centrifuged (1,800 × *g* at 17°C for 30 min) to obtain serum (tubes with clot activator) or plasma (fluoride oxalate tubes), which were separated and stored at −20°C until analyzed. Plasma was analyzed for glucose concentrations, whereas serum was analyzed for BHB, nonesterified fatty acids (**NEFA**), and urea concentrations. Plasma glucose concentrations were determined using the hexokinase method (Roche Diagnostics Ltd., Burgess Hill, UK), and serum biochemistry analysis was carried out on a Randox Imola chemistry analyzer system, using Randox reagent kits (Randox Laboratories, Crumlin, UK).

A clinical examination of vaginal mucus was conducted at 2, 4, and 6 wk postpartum, as an indicator of immune status. The methodology and scoring system used was as described by Little et al. (2017), with the combined results expressed on a scale of 0 to 5. In the current study, scores were subsequently grouped into 3 categories for analysis (0, 1, ≥2).

Nutrient Utilization

On completion of the 20-wk feeding study, 4 cows from each treatment were selected for use in a nutrient-utilization study, with the cows selected from each treatment group balanced for daily milk yield and BW. Cows were transferred in pairs from the main experimental group into a digestibility unit, with pairs transferred at approximately 0900 h on Monday or Thursday of each week. The first pair transferred comprised cows from treatments FB0 and FB-Low; the second, cows from treatments FB-High and FB0; and the third, cows from FB-Low and FB-High. This pattern was repeated for the remaining 3 pairs. Cows were tied by the neck in individual stalls fitted with a rubber mat, and continued to be offered their experimental rations via a feedbox located at the front of each stall. Rations were offered ad libitum daily at 0900 h, at proportionally 1.07 of the previous d intake, and uneaten ration was removed the following day at 0800 h. Soy hulls were offered (0.5 kg at each milking) via a plastic feed bucket, which was placed within the feedboxes during milking (at 0630 and 1630 h). Cows had access to fresh water at all times via a drinker located within each stall.

Measurements of nutrient utilization commenced 24 h after cows were placed in the digestibility unit and comprised a 6-d feeding period, followed 48 h later by a 6-d total feces and urine collection period. Following collection of feces and urine on d 3 of the 6-d collection period, each pair of cows was transferred into 2 indirect open-circuit respiration chambers for measurement of CH_4 production, with each chamber used twice with each treatment, to remove any possible chamber effects. Cows remained in the chambers for 72 h, with measurements of CH_4 and CO_2 production recorded during the final 48-h period used in the subsequent analysis. The milking routine, milk sampling, milk analysis, feces and urine collection methodologies, sample management throughout the 6-d collection period, specifications of the respiration chambers, details of measurements of CH_4 production, laboratory analysis processes of all feed, milk, urine, and feces, have been described in our previous work (Johnston et al., 2019). In the present study, a mean CH_4 recovery rate of 98.5% was recorded.

Feed Analysis

Samples of grass silage were taken daily throughout the experiment and dried at 85°C for 24 h to determine oven DM (**ODM**) content. Subsamples of the dried milled silages were taken 3 times weekly and bulked for every 14 d, with the bulked sample analyzed for NDF, ADF, and ash concentrations. A fresh sample of grass silage was taken weekly and analyzed for concentrations of N, ammonia-N, lactic acid, acetic acid, ethanol, propanol, and gross energy (**GE**), and for pH, whereas ME content was predicted using near-infrared reflectance spectroscopy, as described by Park et al. (1998). A sample of each experimental concentrate offered (FB0, FB-Low, and FB-High) was taken weekly, bulked for each 2-wk period, and subsequently dried at 85°C for 24 h to determine ODM content. Additional samples of each experimental concentrate were taken weekly, bulked for each 2-wk period, dried at 60°C for 48 h, and analyzed for N, NDF, ADF, ash, GE, and starch concentrations. These latter samples were further bulked for each 4-wk period and analyzed using the procedure developed by Licitra et al. (1996) for ADIN and NDIN. Acid detergent insoluble protein (**ADIP**) and neutral detergent insoluble protein (**NDIP**) were calculated as ADIP = 6.25 × ADIN and NIDP = 6.25 × NDIN, respectively.

Straw and soy hulls offered were sampled weekly, the samples bulked every 4 wk, and the bulked samples dried at 85°C for 24 h to determine ODM content. Dried samples were subsequently analyzed for N, GE, ADF, NDF, and ash content. Laboratory analyses of all feed samples were undertaken as described previously by Purcell et al. (2016).

Statistical Analysis

Two primiparous cows from treatment FB-Low did not complete the study due to lameness and very low

milk yield, respectively. Mean weekly data for DMI, milk production, milk composition, milk constituent yields, and BW over the 20-wk experimental period were analyzed using REML repeated measures analysis (autoregressive order 1). The mixed model used included the following terms as fixed effects: Parity (1, 2, 3, >3) + Wk (1–20, as the repeated-measures time factor) + FB inclusion level (FB0, FB-Low, FB-High) + Wk × FB inclusion level, and cow was fitted as a random effect. Locomotion score and BCS data were analyzed using the same model, except that fortnightly data were used as the repeated-measures time factor. Milk FA and blood metabolites were analyzed using a similar model, except that for the former, sampling week (10 and 20 post-calving) was used as the repeated-measure time factor, whereas with the latter, sampling week (2, 4, 6, 8, 10, and 14-post calving) was used as the repeated-measures time factor. Continuous BW, BCS, and fertility data were analyzed by ANOVA, with parity included as a covariate. For each continuous variable, polynomial contrasts of order 1 were fitted to the treatment effects to test the data for linear effects associated with increasing FB level. Binomial fertility and health data were analyzed via generalized linear model regression analysis using the binomial distribution with a logit link function. The model included treatment as a term, with parity included as a covariate in the case of mastitis incidence. Mucus scores of 2 and 3 were combined into a single category (2–3), yielding categories of 0, 1, and 2–3 for analysis. These categorical data were analyzed using a generalized linear mixed model (ordinal logistic regression) with random effects (proportional odds model). The factorial arrangement of treatment (FB0, FB-Low, or FB-High) and sampling week (wk 6 and 12 for feces scores; wk 2, 4, and 6 for mucus scores) were fitted as fixed effects, and cow was fitted as a random effect. Significance was identified using chi-squared. Mean nutrient utilization data over the 6-d measurement period, and mean CH_4 production data over the 2-d measurement period, were analyzed using ANOVA. All data were analyzed using GenStat (Release 18.1; VSN International Ltd., Oxford, UK).

RESULTS

The silage offered had ODM, CP, and ME contents of 293 g/kg, 145 g/kg of DM, and 11.0 MJ/kg of DM, respectively (Table 2). The 3 experimental concentrates had similar CP contents (mean, 223 g/kg of DM), and the starch contents of FB0, FB-Low, and FB-High concentrates were 291, 313, and 338 g/kg of DM, respectively. However, the NDIP of the concentrates decreased, being 112, 98, and 90 g/kg of DM for FB0, FB-Low, and FB-High, respectively, and the respective values for ADIP were 28.5, 21.6, and 16.6. The straw offered had CP, ADF, NDF, and ash contents of 46 (SD ±11.2), 501 (±9.6), 866 (±20.7), and 57 (±13.2) g/kg of DM, and a GE content of 18.5 (±0.23) MJ/kg of DM. The respective values for the soy hulls offered were 111 (±4.3), 492 (±11.3), 685 (±29.4), 53 (±1.1) g/kg of DM, and 17.5 (±0.06) MJ/kg of DM, with a starch content of 13.9 (±7.84) g/kg of DM.

Neither total DMI nor milk yield were affected by treatment ($P > 0.05$; Table 3). Silage DMI was estimated as 9.7, 9.6, and 9.8 kg/d for FB0, FB-Low, and FB-High, respectively (based on ration formulation), and the respective values for concentrates were 11.8, 11.7, and 12.2 kg/d. Cows on FB0 had higher milk fat content than those on FB-High ($P = 0.031$), whereas cows on FB0 and FB-Low had higher milk protein content than those on FB-High ($P < 0.001$). Milk lactose content was not affected by treatment ($P > 0.05$). Milk fat yield ($P < 0.001$), milk protein yield ($P = 0.035$), and milk fat plus protein yield ($P = 0.007$) were higher with FB0 than with either FB-Low or FB-High. All of these DMI and milk production parameters varied with time post-calving ($P < 0.001$; see Figure 1 for DMI and Figure 2 for milk yield), but we found no treatment × time interaction for any of these parameters. Milk fat, protein, and lactose concentrations showed linear decrease with FB inclusion ($P = 0.012$, $P < 0.001$, and $P = 0.046$, respectively), as did milk fat yield ($P = 0.002$), milk protein yield ($P = 0.020$), and milk fat plus protein yield ($P = 0.006$).

The total concentration of SFA in milk fat was lower with FB0 than with either FB-Low or FB-High ($P = 0.028$), but the reverse was true for the total concentration of MUFA ($P = 0.016$; Table 3). Treatment had no effect on the total PUFA content of milk fat ($P > 0.05$). Concentrations of C18:0 ($P = 0.002$), C18:1 *cis*-9 ($P = 0.022$), and C18:2 *cis*-9,*trans*-11 ($P < 0.001$) decreased with increasing FB inclusion; however, concentrations of C16:0 ($P < 0.001$) and C18:3n-3 ($P < 0.006$) increased. Concentrations of C14:0 and C18:2 were not affected by treatment ($P > 0.05$). Although total PUFA concentrations ($P < 0.001$) and concentrations of C16:0 ($P = 0.002$), C18:0 ($P < 0.001$), C18:2 ($P < 0.001$), and C18:3n-3 ($P < 0.001$) differed between the 2 sampling periods (time) with the exception of the latter ($P < 0.001$), we found no treatment × time interactions for any of the FA recorded ($P > 0.05$). Total MUFA concentrations ($P = 0.016$) and concentrations of C18:0 ($P < 0.001$), C18:1 *cis*-9 ($P = 0.020$), and CLA ($P < 0.001$) showed a linear decrease with FB inclusion, but concentrations of C16:0 ($P < 0.001$) and C18:3n-3 ($P = 0.002$) showed the reverse trend.

Although average BCS was unaffected by treatment ($P < 0.05$), end-of-study BCS tended to increase with

Table 2. Chemical composition of the grass silage and experimental concentrates (FB0, FB-Low, and FB-High)[1] offered during the experiment

Item	Grass silage	SD	Concentrate					
			FB0	SD	FB-Low	SD	FB-High	SD
Oven DM (g/kg)	293	61.5	892	5.2	889	5.5	882	10.4
Volatile-corrected oven DM (g/kg)	311	61.8						
CP (g/kg of DM)	145	22.1	224	5.8	222	10.1	223	9.2
Neutral detergent insoluble protein (g/kg of DM)			112	15.7	98	12.4	90	18.8
Acid detergent insoluble protein (g/kg of DM)			28.5	2.83	21.6	2.76	17.6	1.06
Ash (g/kg of DM)	87	6.1	68	5.7	64	4.6	59	4.0
ADF (g/kg of DM)	294	23.6	159	21.9	161	29.0	164	24.0
NDF (g/kg of DM)	480	42.0	279	42.5	267	41.5	266	31.9
Gross energy (MJ/kg of DM)	18.9	1.47	18.3	0.12	18.1	0.13	18.0	0.10
Starch (g/kg of DM)			291	25.3	313	38.8	338	27.2
Ammonia N (g/kg of total N)	76	8.0						
pH	3.87	0.216						
Lactic acid (g/kg of DM)	114	56.8						
Acetic acid (g/kg of DM)	23	10.6						
Ethanol (g/kg of DM)	14	10.2						
Propanol (g/kg of DM)	0.9	0.94						
Metabolizable energy (MJ/kg of DM)	11.0	0.55	12.7[2]		12.5[2]		12.3[2]	
Calcium[2] (g/kg of DM)			6.9		12.9		18.5	
Phosphorus[2] (g/kg of DM)			5.5		5.5		5.5	

[1]Concentrates FB0, FB-Low, and FB-High contained 0, 349, and 698 g of field beans (FB) per kilogram, respectively.
[2]Estimated from composition of individual concentrate ingredients.

increasing levels of FB inclusion ($P = 0.054$; Table 4), with this overall linear effect significant ($P = 0.020$). None of the BW parameters examined (average BW, end-of-study BW, nadir BW, BW loss to nadir, and days to nadir BW) were affected by treatment ($P > 0.05$). Both BCS ($P = 0.046$) and BW ($P < 0.001$) varied with time (Figures 3 and 4, respectively), but we found no treatment × time interactions for either ($P < 0.05$). Plasma glucose and BHB concentrations were unaffected by treatment ($P > 0.05$), although both increased with time post-calving (Figure 5a, $P = 0.010$; Figure 5b, $P = 0.002$, respectively). Plasma NEFA concentrations were significantly higher with FB0 than with FB-High ($P = 0.022$); however, plasma urea concentrations followed the reverse trend ($P < 0.001$). Plasma NEFA concentrations decreased with time post-calving (Figure 5c, $P < 0.001$), but plasma urea concentrations increased (Figure 5d, $P < 0.001$). We detected no treatment × time interactions for any of the blood metabolites examined ($P > 0.05$). With increasing FB inclusion levels, analysis revealed a tendency for linear decrease in plasma glucose concentrations ($P = 0.067$), whereas plasma NEFA concentrations showed a linear decrease ($P = 0.005$). Plasma urea levels increased linearly with FB inclusion ($P < 0.001$).

Fecal scores differed between wk 6 and 12 post-calving (overall SEM = 0.048, $P = 0.012$), but treatment had no effect on mean fecal scores (probability of having a score of 1, 2, or ≥ 3: 0.08, 0.47, and 0.45 for FB0; 0.05, 0.42, and 0.53 for FB-Low; 0.07, 0.45, and 0.48 for FB-High: overall SEM = 0.061, $P = 0.878$). We found no interaction between treatment and measurement period (SEM = 0.084, $P = 0.783$). The digestibility of ADF decreased ($P = 0.035$: linear, $P = 0.013$) from FB0 to FB-High, but none of the other digestibility coefficients were affected by FB inclusion level (Table 5). Neither total N intake, digestible N intake, N output in feces, urine, manure, or milk, nor any of the N utilization coefficients examined were affected by treatment ($P > 0.05$; Table 6). Treatment had no effect ($P > 0.05$) on GE intake, digestible energy (DE) intake, ME intake, energy output in feces, urine, CH_4, heat, or milk, or any of the energy utilization coefficients examined (Table 7). Total CH_4 production, CH_4 as a proportion of DMI, OM intake, and milk production were unaffected by treatment ($P > 0.05$; Table 7). Similarly, CH_4-E, as a proportion of GE or ME intake, was unaffected by treatment ($P > 0.05$). There was a linear trend for ME/GE ($P = 0.071$) to decrease with increasing FB inclusion in the diet.

Vaginal mucus scores decreased with week (2, 4, and 6) post-calving (overall SEM = 0.042, $P < 0.001$), but treatment had no effect on mean mucus scores (prob-

Table 3. Effects of field bean (FB) inclusion level in dairy cow concentrates on mean DMI and milk production parameters over the 20-wk experimental period, and on mean milk fatty acid (FA) concentrations (mean of 2 sampling occasions)

	Treatment				*P*-value			
Item	FB0	FB-Low	FB-High	SEM	Treatment	Time	Treatment × time	Linear
Total DMI (kg/d)	21.9	21.6	22.1	0.50	0.497	<0.001	0.722	0.611
Milk yield (kg/d)	35.7	33.2	33.9	0.89	0.272	<0.001	0.723	0.539
Milk fat (g/kg)	42.8[a]	42.5[ab]	41.3[b]	0.42	0.031	<0.001	0.297	0.012
Milk protein (g/kg)	33.8[a]	33.6[a]	32.2[b]	0.33	<0.001	<0.001	0.460	<0.001
Milk lactose (g/kg)	48.3	48.2	47.9	0.112	0.113	<0.001	0.883	0.046
Milk fat yield (kg/d)	1.52[a]	1.39[b]	1.39[b]	0.025	<0.001	<0.001	0.248	0.002
Milk protein yield (kg/d)	1.20[a]	1.11[b]	1.09[b]	0.026	0.035	<0.001	0.712	0.020
Milk fat + protein yield (kg/d)	2.71[a]	2.49[b]	2.47[b]	0.050	0.007	<0.001	0.196	0.006
Milk FA (g/100 g of total FAME identified)								
Total SFA[1]	73.9[a]	75.4[b]	75.2[b]	0.42	0.028	0.908	0.448	0.138
Total MUFA[2]	22.8[b]	21.4[a]	21.5[a]	0.37	0.016	0.197	0.551	0.016
Total PUFA[3]	3.3	3.2	3.3	0.07	0.495	<0.001	0.240	0.948
C14:0	12.9	12.8	12.6	0.18	0.328	0.060	0.144	0.138
C16:0	35.8[a]	38.4[b]	39.4[b]	0.57	<0.001	0.002	0.571	<0.001
C18:0	9.8[b]	9.2[ab]	8.4[a]	0.27	0.002	<0.001	0.835	<0.001
C18:1 *cis*-9	18.2[b]	17.0[a]	17.1[a]	0.35	0.022	0.912	0.512	0.020
C18:2	1.77	1.72	1.81	0.04	0.292	<0.001	0.387	0.565
C18:2 *cis*-9,*trans*-11 (CLA)	0.54[b]	0.45[a]	0.40[a]	0.019	<0.001	0.133	0.751	<0.001
C18:3n-3	0.49[a]	0.52[b]	0.56[c]	0.015	0.006	<0.001	<0.001	0.002

[a–c]Means with the same superscript within a row do not differ significantly ($P > 0.05$).

[1]Sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0.

[2]Sum of C14:1, C15:1, C16:1, C17:1, C18:1 *cis*-9, C18:1 *cis*-11, C20:1 *cis*-11, C22:1 *cis*-13, and C24:1 *cis*-15.

[3]Sum of C18:2, C18:2 *cis*-9,*trans*-11, C18:3n-3, C18:3n-6, C20:2 *cis*-11,14, C20:3n-3, C20:3n-6, C20:4n-6, C20:5n-3, C22:2 *cis*-13,16, C22:5n-3, and C22:6n-3.

ability of having a score of 0, 1, or 2–3: 0.70, 0.13, and 0.17 for FB0; 0.71, 0.13, and 0.16 for FB-Low; 0.67, 0.16, and 0.17 for FB-High; overall SEM = 0.047, $P = 0.97$). Analysis revealed no interaction between treatment and measurement week (SEM = 0.072, $P = 0.476$). Treatment had no effect on the proportion of cows showing commencement of luteal activity before d 42 post-calving, days to commencement of luteal activity, or peak progesterone content at commencement of luteal activity ($P > 0.05$; Table 8). Treatment did not affect conception to first service, conception to first and second service, days to conception, or the proportion of cows pregnant at the end of the breeding season ($P > 0.05$). The proportion of cows with at least one incident of digestive upset, mastitis, or lameness, as well as mean locomotion score, were unaffected by treatment (Table 8; $P > 0.05$).

DISCUSSION

Average daily FB intakes for FB-Low and FB-High were estimated as 4.1 and 8.4 kg/cow, respectively, with the latter substantially higher than FB intake levels used in any previous study. At the maximum inclusion level adopted (FB-High, 698 g of FB per kilogram of concentrate), FB replaced all of the soybean meal, rapeseed meal, maize gluten feed, and wheat in the concentrate, compared with the control treatment (FB0). Although it was not possible to achieve a common starch level with each of the concentrates (291, 309, and 338 g per kilogram of DM for FB0, FB-Low, and FB-High concentrates, respectively), all had a similar CP content, resulting in a total diet CP content of 180 g per kilogram of DM across the 3 diets. This total diet CP content was somewhat higher than planned, yet it remains typical of many grass silage–based diets offered in the United Kingdom.

Cow Performance

The potential negative effects of antinutritional factors within FB on DM intake are often cited by nutritionists as a reason for low inclusion levels in dairy cow diets. In addition, preference trials (Hutson and van Mourik, 1981) have suggested that FB may be unpalatable. However, the current study clearly demonstrated that FB intakes of up to 8.4 kg/cow per day had no negative effects on DM intakes. This is in agreement

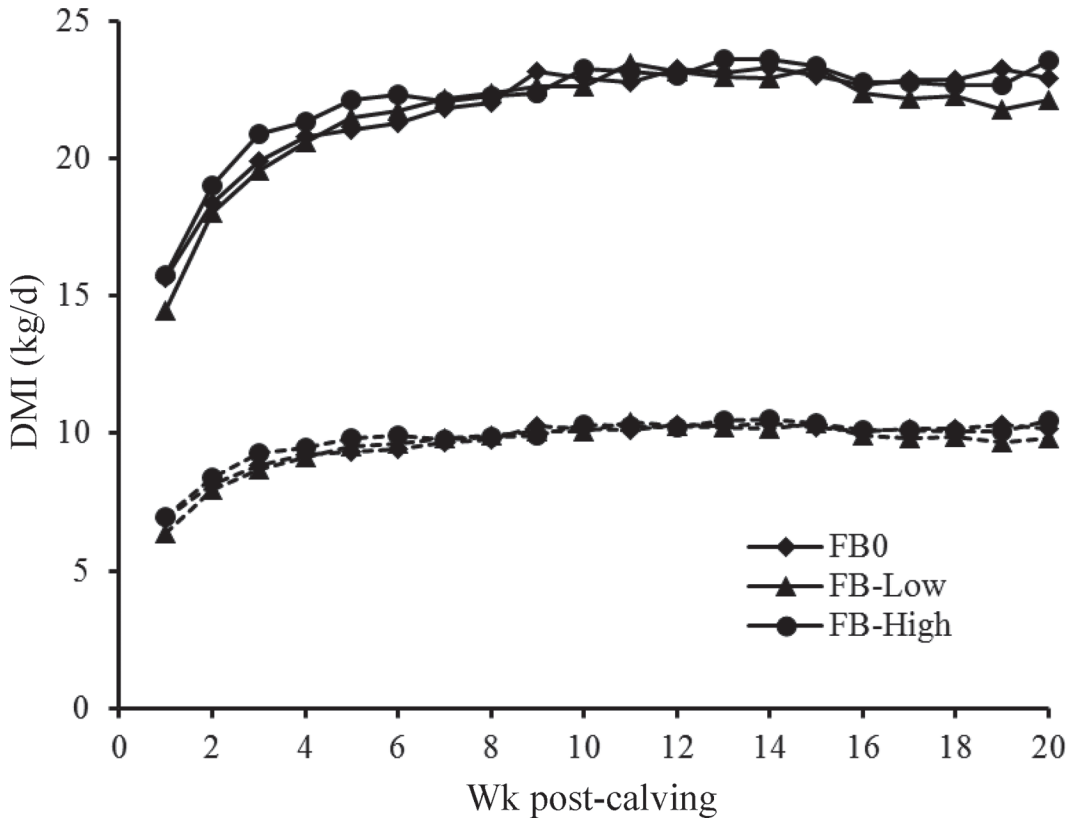

Figure 1. Silage DMI (dashed lines) and total DMI (solid lines) responses of dairy cows over the first 20 wk of lactation, to concentrates containing a range of field bean (FB) inclusion levels (FB0, FB-Low, or FB-High). Silage DMI: SEM = 0.22; treatment $P = 0.497$; time $P < 0.001$; treatment × time $P = 0.722$. Total DMI: SEM = 0.50; treatment $P = 0.497$; time $P < 0.001$; treatment × time $P = 0.722$.

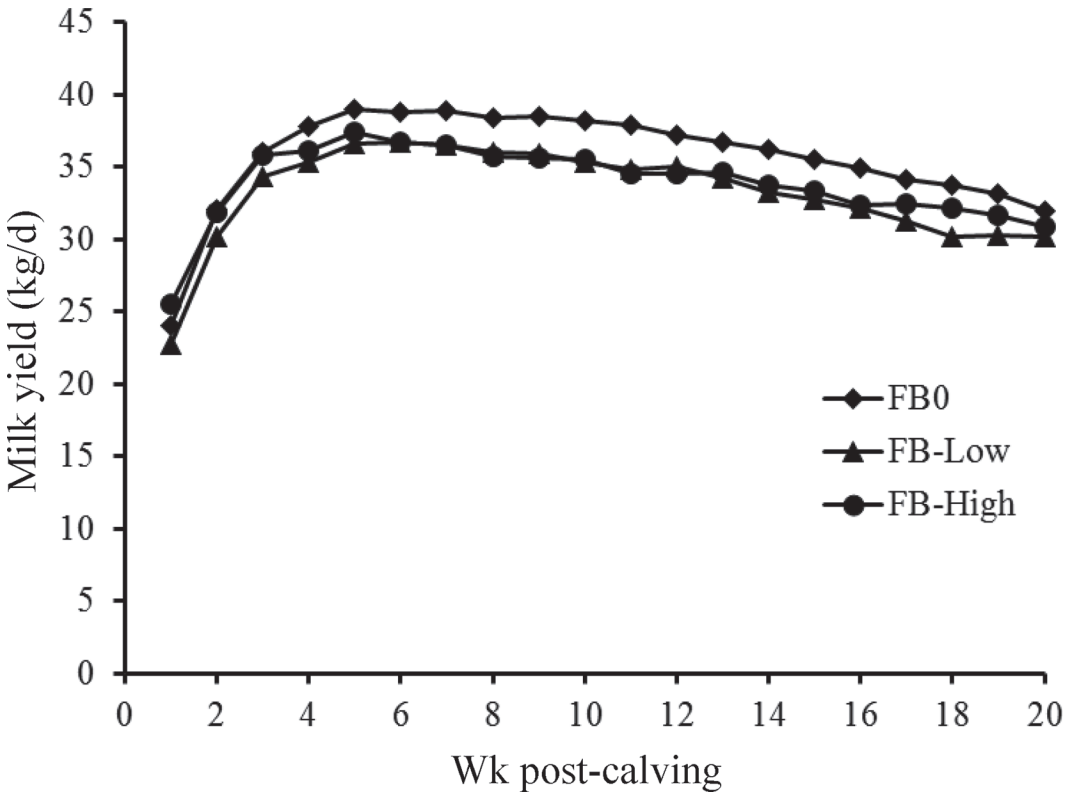

Figure 2. Milk yield response of dairy cows to concentrates containing a range of field bean (FB) inclusion levels (FB0, FB-Low, or FB-High) over the first 20 wk of lactation. SEM = 0.89; treatment $P = 0.272$; time $P < 0.001$; treatment × time $P = 0.723$.

with most published studies (Ingalls and McKirdy, 1974; Tufarelli et al., 2012; Ramin et al., 2017; Johnston et al., 2019), albeit the maximum daily FB intake in these studies was 5.0 kg/cow per day, but the diets offered and concentrate feeding strategies adopted also differed considerably from those in the current study. In contrast, Puhakka et al. (2016) found intakes to be reduced when FB replaced rapeseed meal in dairy cow diets. Those authors suggested that this may have been due to a reduction in NDF digestibility, a poorer amino acid profile with FB, and a "pull effect" caused by the higher milk yield with the rapeseed meal treatment. Despite reductions in ADF digestibility with FB inclusion in the current study, this had no effect on DMI.

In common with DMI, milk yield was unaffected by FB inclusion. However, the reduction in both milk

Table 4. Effects of field bean (FB) inclusion level in dairy cow concentrates on BCS, BW, and plasma metabolite concentrations

	Treatment				*P*-value			
Item	FB0	FB-Low	FB-High	SEM	Treatment	Time	Treatment × time	Linear
Average BCS	2.49	2.53	2.51	0.024	0.259	0.046	0.248	0.371
End-of-study BCS	2.42	2.53	2.57	0.047	0.054			0.020
Average BW (kg)	598	600	599	8.0	0.899	<0.001	0.760	0.646
End-of-study BW (kg)	614	621	617	9.6	0.870			0.779
Nadir BW (kg)	570	565	569	8.1	0.902			0.963
Loss to nadir BW (kg)	23	20	30	4.8	0.276			0.270
Days to nadir BW	50	35	46	11.2	0.608			0.775
Plasma metabolites								
Glucose (mmol/L)	3.81	3.73	3.71	0.036	0.153	0.010	0.954	0.067
BHB (mmol/L)	0.51	0.51	0.48	0.018	0.419	0.002	0.123	0.206
NEFA[1] (mEq/L)	0.43[a]	0.39[ab]	0.36[b]	0.020	0.022	<0.001	0.633	0.005
Urea (mmol/L)	4.63[a]	5.28[b]	5.79[c]	0.103	<0.001	<0.001	0.567	<0.001

[a–c]Means with the same superscript within a row do not differ significantly ($P > 0.05$).

[1]NEFA = nonesterified fatty acids.

fat and milk protein concentrations with FB inclusion meant that yields of fat, protein, and fat plus protein were lower with both FB-Low and FB-High, compared with the FB0 treatment. Literature evidence concerning the effects of FB inclusion on milk production and milk composition is inconsistent. For example, both Puhakka et al. (2016) and Ramin et al. (2017), in studies involving maximum FB intakes of 3.7 and 2.6 kg of DM per day, respectively, observed reductions in milk yield when rapeseed meal was replaced by FB. Milk protein content was reduced in the former study, but milk fat content was unaffected by FB inclusion in either study. In contrast, at an FB intake level of 4.7 kg/d (Johnston et al., 2019), neither milk yield nor milk fat content were affected by FB inclusion level, and milk protein content tended to decrease ($P = 0.08$). In several on-farm studies conducted in Italy (Comellini et al., 2009; Volpelli et al., 2010; Tufarelli et al., 2012; Volpelli et al., 2012), the inclusion of FB in dairy cow diets had no effect on either milk yield or milk fat and protein content, although in a further study (Mordenti et al., 2007) milk yield was reduced but milk fat content was increased with FB inclusion. However, FB inclusion levels in these studies were low, normally less than 2.0 kg/cow per day.

Energy intake is a key driver of milk protein content, yet the reduction in milk protein content with the FB-Low treatment is unlikely to be driven by energy supply, given the similar intakes across all treatments and the absence of treatment effects on any of the BW or BCS score parameters. Indeed, plasma NEFA concentrations decreased with FB inclusion; however, there was a trend for end-of-study BCS to increase with FB inclusion, suggesting an improved energy balance with FB-High. Rather, it is more likely that this reduction was caused by a deficit of specific amino acids. For example, FB is lower in both lysine and methionine than are soybean meal or rapeseed meal (Ewing, 1997), with both of these amino acids important for milk protein synthesis (Schwab et al., 1976). When the rations were evaluated using Feed into Milk, the UK dairy cow rationing system (Thomas, 2004), lysine supply relative to requirements were "adequate" for all 3 treatments, whereas for methionine the FB0 ration was classified as "borderline," with both the FB-Low and FB-High rations classified as "poor." When Puhakka et al. (2016) replaced rapeseed meal with FB, total concentrations of essential amino acids and plasma concentrations of lysine and methionine were reduced; these authors suggested that this was likely partly responsible for the

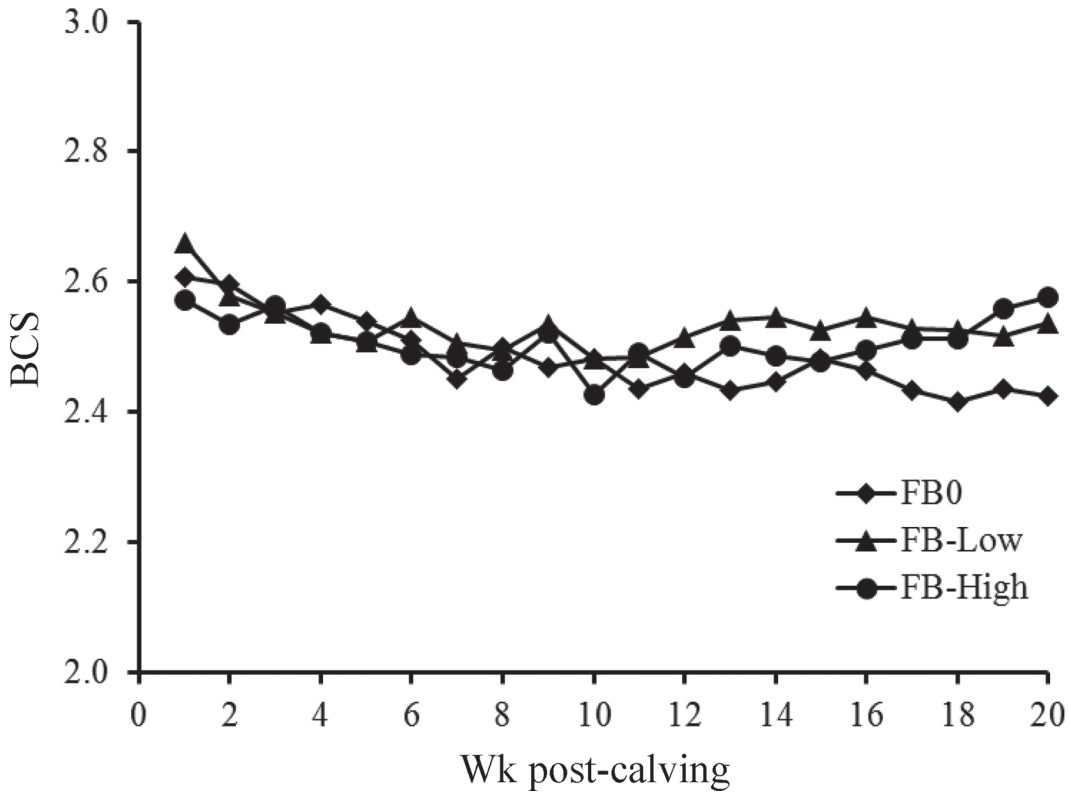

Figure 3. Body condition score (BCS) response of dairy cows to concentrates containing a range of field bean (FB) inclusion levels (FB0, FB-Low, or FB-High) over the first 20 wk of lactation. SEM = 0.024; treatment $P = 0.259$; time $P < 0.046$; treatment × time $P = 0.248$.

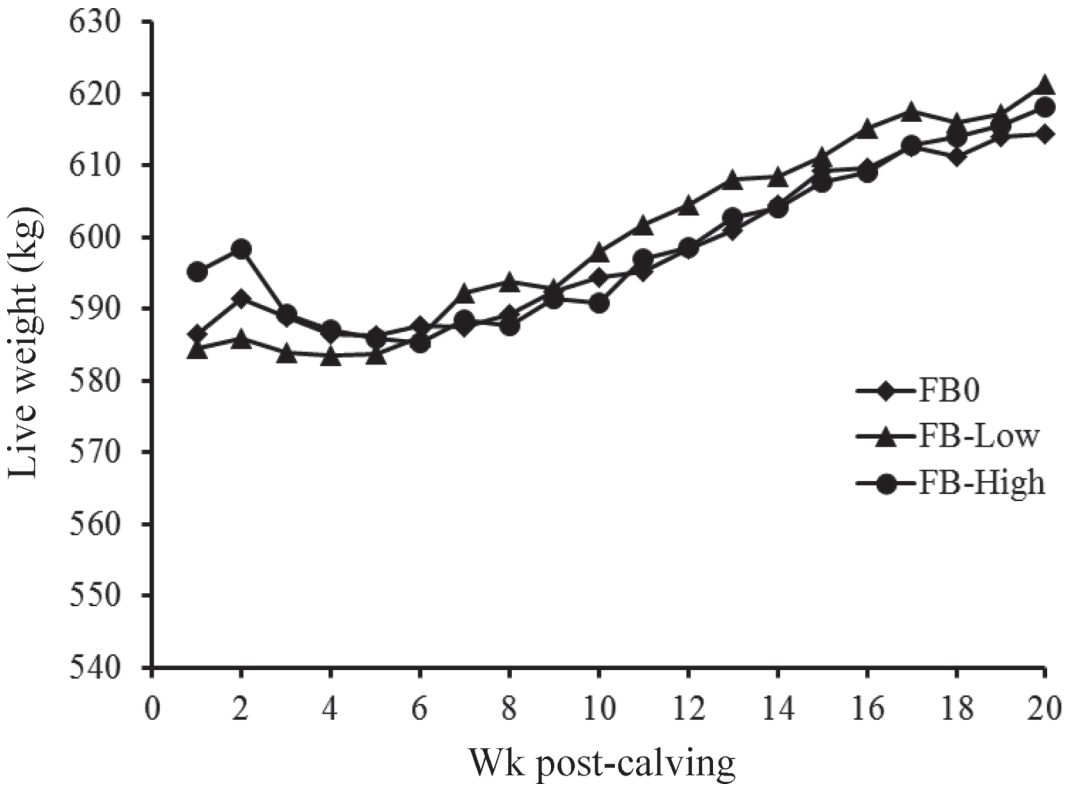

Figure 4. Body weight response of dairy cows to concentrates containing a range of field bean (FB) inclusion levels (FB0, FB-Low, or FB-High) over the first 20 wk of lactation. SEM = 8.0; treatment $P = 0.899$; time $P < 0.001$; treatment × time $P = 0.760$.

reduction in milk protein content and milk protein yield that they observed.

In the current study, FB replaced or partially replaced both maize starch (less degradable than FB starch) and wheat starch (more degradable than FB starch) in the concentrate (Offner et al., 2003), likely affecting rumen fermentation patterns and the associated reduction in milk fat content with the FB-High treatment. This reduction was in contrast to most previous studies; we propose that with the FB-High treatment, a reduction in rumen acetate production occurred due to the higher-concentrate starch and slightly lower fiber content, and this may have contributed to a reduction in de novo synthesis of milk fat. Possible adverse effects of diet on rumen function are supported in part by the lower ADF digestibility observed with this treatment. In addition, we postulate that altered rumen fermentation and biohydrogenation pathways, leading to inhibition of mammary lipid synthesis by specific fatty acid intermediates, as proposed by Bauman and Griinari (2001) and reviewed more recently by others (e.g., Harvatine et al., 2009), may also have contributed to this reduction. In addition, some evidence suggests that dietary methionine promotes milk fat synthesis (Hao et al., 2018), and, as such, the likely deficit of methionine in the FB diets in the present study may have contributed to the reduction in milk fat concentrations.

Effects of FB inclusion on the fatty acid profile of milk do not appear to have been examined previously; we found that FB inclusion in the current study increased the degree of saturation of the milk produced. This appears to have been largely driven by increasing concentrations of C16:0 in milk, with FB particularly high in C16:0 fatty acids (Grela and Gunter, 1995), with Moate et al. (2008) estimating that 42% of C16:0 is derived directly from the diet. Similarly, decreasing concentrations of C18:1 with FB inclusion may reflect the higher concentration of C18:1 in soybean compared with FB (Grela and Gunter, 1995).

It is possible that the reduction in cow performance observed with FB inclusion could be avoided by supplementing the diet with specific limiting amino acids. Nevertheless, cow performance data need to be considered within the context of the total diet CP in the study (180 g/kg of DM), a level not unusual with early-lactation dairy cows offered grass silage–based diets in the United Kingdom and Ireland due to the often variable composition of grass silage on offer. However, it is possible that greater differences between treatments might have been observed with lower-protein diets as,

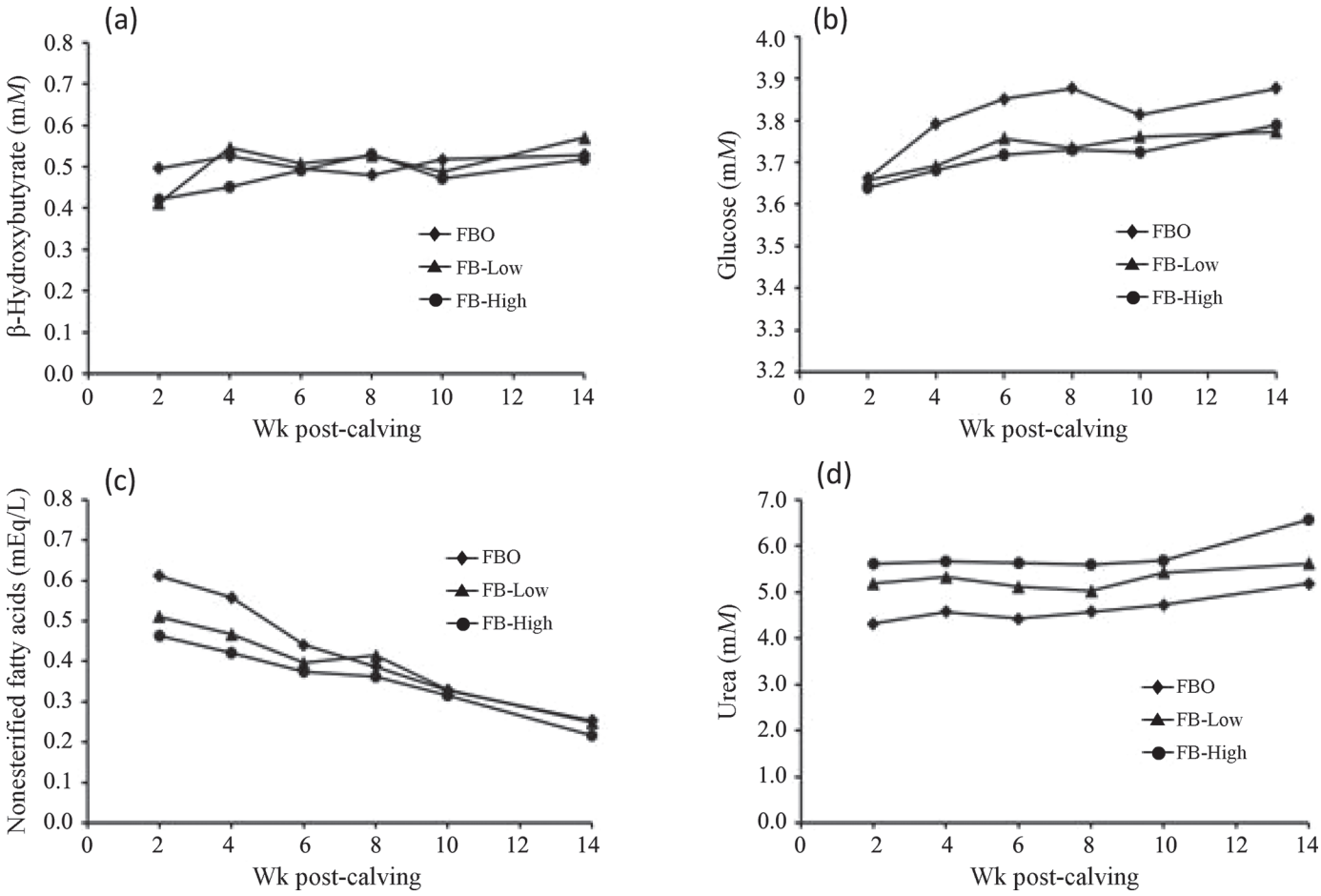

Figure 5. Effect of field bean (FB) inclusion level in the diet (FB0, FB-Low, or FB-High) on plasma chemical concentrations among dairy cows. (a) β-Hydroxybutyrate: SEM = 0.018; treatment $P = 0.419$; time $P = 0.002$; treatment × time $P = 0123$. (b) Glucose: SEM = 0.036: treatment $P = 0.153$; time $P = 0.010$; treatment × time $P = 0.954$. (c) Nonesterified fatty acids: SEM = 0.020; treatment $P = 0.022$; time $P < 0.001$; treatment × time $P = 0.633$. (d) Urea: SEM = 0.103; treatment $P < 0.001$; time $P < 0.001$; treatment × time $P = 0.567$.

in general, responses to protein source and quality tend to be greater at lower diet protein levels. Nevertheless, Puhakka et al. (2016) observed no interaction between diet protein level (154 vs. 190 g/kg of DM) and milk production when rapeseed meal was partially replaced with FB.

Table 5. Effects of field bean (FB) inclusion level in dairy cow concentrates on DMI, milk yield, and total ration digestibility coefficients

	Treatment				*P*-value	
Item	FB0	FB-Low	FB-High	SEM	Treatment	Linear
Total DMI (kg/d)	21.2	23.4	20.7	1.73	0.537	0.830
Milk yield (kg/d)	30.2	31.0	31.2	3.00	0.967	0.811
Digestibility coefficients (g/g)						
DM	0.784	0.775	0.771	0.0094	0.609	0.341
OM	0.800	0.791	0.787	0.0098	0.602	0.339
Nitrogen	0.716	0.694	0.708	0.0186	0.517	0.685
Gross energy	0.777	0.767	0.764	0.0089	0.548	0.303
ADF	0.732[a]	0.671[ab]	0.644[b]	0.0286	0.035	0.013
NDF	0.699	0.659	0.666	0.0273	0.340	0.256

[a,b]Means with the same superscript within a row do not differ significantly ($P > 0.05$).

Table 6. Effect of field bean (FB) inclusion level in dairy cow concentrates on nitrogen utilization parameters

	Treatment				*P*-value	
Item	FB0	FB-Low	FB-High	SEM	Treatment	Linear
N intake and output (g/d)						
Total N intake	582	642	569	49.7	0.560	0.851
Digestible N intake	420	444	405	34.7	0.729	0.772
Feces N	163	198	164	17.9	0.332	0.967
Urine N	199	182	185	23.8	0.868	0.868
Manure N	362	380	349	34.43	0.817	0.799
Milk N	139	163	140	16.0	0.505	0.961
N utilization (g/g)						
Feces N/N intake	0.284	0.306	0.292	0.0132	0.517	0.685
Urine N/N intake	0.339	0.287	0.332	0.0316	0.477	0.880
Manure N/N intake	0.623	0.593	0.624	0.0276	0.673	0.983
Milk N/N intake	0.241	0.255	0.245	0.0175	0.843	0.872
Feces N/manure N	0.464	0.516	0.470	0.0285	0.403	0.886
Urine N/manure N	0.536	0.484	0.530	0.0285	0.403	0.886

Nutrient Utilization

Field beans contain several antinutritional substances, some of which are known to have negative effects on rumen function and digestion ability (Newton and Hill, 1983; Dixon and Hosking, 1992). For example, the FB variety Fuego is a colored-flower variety, which is known to be a tannin-containing type. However, fecal scores—a simple proxy for rumen function—were unaffected by treatment in this study. In addition, with the exception of ADF, no digestibility coefficients for the other parameters examined were affected by FB inclusion. In our previously published work, we found fiber digestibility to be unaffected with an FB intake similar to that used with the FB-Low treatment (Johnston et al., 2019), but the reduction in ADF digestibility with the highest FB treatment in the current study is similar to the reduction in NDF digestibility observed by Puhakka et al. (2016). Puhakka et al. (2016) attributed this to the readily fermentable FB carbohydrate and

Table 7. Effects of field bean (FB) inclusion level in dairy cow concentrates on energy utilization parameters and methane production

	Treatment				*P*-value	
Item[1]	FB0	FB-Low	FB-High	SEM	Treatment	Linear
Energy intake and output (MJ/d)						
GE intake	392	425	375	31.7	0.548	0.719
Fecal energy	87	99	88	8.1	0.542	0.885
DE intake	305	327	287	24.6	0.546	0.610
Methane energy	28	29	30	3.0	0.890	0.641
Urinary energy	13	13	13	1.0	0.889	0.921
Milk energy	102	105	102	9.6	0.964	0.994
ME intake	264	284	243	21.4	0.446	0.513
Heat production	151	158	165	15.1	0.803	0.519
Retained energy	11	21	−23	18.0	0.240	0.204
Energy utilization (MJ/MJ)						
DE/GE	0.770	0.768	0.765	0.0092	0.518	0.292
ME/GE	0.675	0.667	0.650	0.0088	0.169	0.071
Heat production/ME	0.564	0.573	0.679	0.0495	0.243	0.136
Milk energy/ME	0.384	0.372	0.419	0.0171	0.188	0.182
Retained energy/ME	0.052	0.055	−0.097	0.0621	0.195	0.123
CH_4 production						
CH_4 (g/d)	512	527	549	54.4	0.890	0.641
CH_4/feed intake or milk yield (g/kg)						
CH_4/DMI	23.8	22.8	26.4	1.51	0.270	0.258
CH_4/OM intake	25.7	24.5	28.4	1.63	0.281	0.274
CH_4/milk yield	16.8	17.2	17.8	1.21	0.850	0.850
CH_4/ECM yield	15.5	15.6	16.7	0.823	0.506	0.298
CH_4-E/energy intake (MJ/MJ)						
CH_4-E/GE intake	0.071	0.069	0.081	0.0067	0.241	0.205
CH_4-E/ME intake	0.106	0.104	0.124	0.0103	0.150	0.113

[1]GE = gross energy; DE = digestible energy; CH_4-E = methane energy.

Table 8. Effects of field bean (FB) inclusion level in dairy cow concentrates on fertility parameters and dairy cow health (95% CI in parentheses)

Item	Treatment			SEM	*P*-value
	FB0	FB-Low	FB-High		
Pre-d 42					
Proportion of cows showing luteal activity	0.75 (0.54–0.88)	0.67 (0.44–0.83)	0.79 (0.58–0.91)		0.632
Days to commencement of luteal activity	22.1	21.6	22.1	2.25	0.980
Peak progesterone content at commencement of luteal activity (ng/mL)	31.3	26.9	32.5	2.74	0.353
Conception to first service (proportion)	0.45 (0.27–0.65)	0.33 (0.16–0.55)	0.30 (0.15–0.51)		0.511
Conception to first and second service (proportion)	0.62 (0.42–0.79)	0.57 (0.36–0.75)	0.50 (0.31–0.69)		0.681
Days to conception	84	102	90	9.5	0.412
Cows pregnant at end of breeding season (proportion)	0.91 (0.70–0.98)	0.80 (0.57–0.92)	0.74 (0.53–0.88)		0.307
Proportion of cows with at least one incidence of illness					
Digestive upset	0.17 (0.06–0.44)	0.35 (0.17–0.70)	0.08 (0.02–0.32)		0.117
Mastitis	0.10 (0.03–0.32)	0.07 (0.02–0.27)	0.08 (0.02–0.29)		0.889
Lameness	0.29 (0.14–0.61)	0.35 (0.17–0.69)	0.37 (0.19–0.72)		0.876
Mean locomotion score	2.5	2.5	2.4	7.30	0.593

associated fermentation products, decreasing rumen pH and having a negative effect on cellulolytic bacteria activity, rather than to the presence of antinutritional factors. Puhakka et al. (2016) also found the apparent digestibility of DM, OM, N, and starch to increase with FB inclusion, but they attributed this to the lower proportion of silage in the diets of cows offered FB, rather than to FB inclusion per se. Thus it appears that any effects of FB inclusion on digestibility are likely to be caused by other dietary factors, rather than by the presence of antinutritional factors. This is supported by the findings of Melicharová et al. (2009), who found cow performance to be unaffected when FB varieties containing either high or low levels of ANF were offered.

Although our earlier work (Johnston et al., 2019) indicated a reduction in the ratio of milk N to N intake with FB inclusion, a similar effect was not observed in the nutrient utilization portion of the current study, in agreement with other previous findings (Puhakka et al., 2016; Ramin et al., 2017). Nevertheless, the nutrient utilization phase in the current study involved only 4 cows per treatment and was undertaken at the end of the 20-wk feeding study. When N use efficiency over the entire study period is examined using treatment mean intake and milk production data (with milk N calculated as milk protein/6.38), the ratio of milk N to N intake was 0.30, 0.28, and 0.27 for FB-0, FB-Low, and FB-High, respectively. The decreasing N use efficiency with increasing FB inclusion levels was reflected in an increase in plasma urea N levels, in agreement with Volpelli et al. (2012), the trend ($P = 0.078$) observed in our previous work (Johnston et al., 2019), and the increase in milk urea concentrations observed by Puhakka et al. (2016) when the rapeseed component of the diet was replaced by FB. In contrast, other studies (Comellini et al., 2009; Volpelli et al., 2010; Tufarelli et al., 2012) have found both blood and milk urea N concentrations to be either reduced or unchanged with FB inclusion. Protein in FB has a higher degradability in the rumen compared with protein from soybean meal or rapeseed meal (Ewing, 1997), and in general blood and milk urea levels increase when FB replaces a less-degradable protein source.

None of the energy utilization parameters examined were affected by FB inclusion level, which was largely in agreement with our earlier study (Johnston et al., 2019), except that these latter authors inexplicably found a quadratic effect of FB inclusion on urine E output. Similarly, none of the CH_4 production parameters examined were affected by FB inclusion, in agreement with previous indications (Johnston et al., 2019). However, Ramin et al. (2017) found that ratios of CH_4 to DMI and CH_4 to ECM were unaffected when rapeseed

meal was replaced by FB, whereas total CH_4 production tended to be reduced, which they attributed in part to the higher fat content of rapeseed meal. Despite evidence suggesting that CH_4 production may be reduced when ruminants are offered legume forages (Lüscher et al., 2014), there appears to be little evidence that this is the case with grain legumes, in line with the findings of the current study. Our results demonstrate enteric CH_4 emissions to be unaffected by FB inclusion, but the effects of replacing imported protein ingredients with locally grown FB, across the life cycle of milk, needs to be examined.

Fertility and Health

Negative energy balance can have a detrimental effect on reproductive performance (Beam and Butler, 1999; Butler, 2003), but BCS and BW data in the current study suggest that energy balance differed little between treatments. However, the presence of phytoestrogens in many legumes species (for example, daidzein and genistein; Kaufman et al., 1997), including some FB cultivars, is often highlighted as a cause for concern in relation to dairy cow fertility. Phytoestrogens, compounds that are produced naturally in many plant species, may have adverse effects on ovarian function, including possibly inhibited luteinizing hormone surge, altered development of or lack of dominant follicles, abnormal follicular waves, early embryonic death, and repeat breeding (Mostrom and Evans, 2018). Although these compounds have been shown to suppress the secretion of luteinizing hormone (Zdunczyk et al., 2005) and to lower progesterone concentrations (Piotrowska et al., 2006), neither the time to onset of cyclicity, the proportion of cows cycling within 42 d of calving, nor the peak progesterone concentrations during the first estrus cycle were affected by diet in the present study. While actual conception rates within the study must be considered within the context of the number of cows on the study, we found no evidence that reproductive outcomes were affected by FB inclusion level. Vaginal mucus scores, assessed as an indicator of immune status, did not differ across treatments, suggesting that FB treatment has no effect on immune status. In addition, none of the health parameters examined were affected by FB inclusion in the diet, which again suggests that immune function was unaffected by treatment.

CONCLUSIONS

Including FB (*Vicia faba.* var. Fuego) in dairy cow diets at up to 8.4 kg/cow per day had no detrimental effects on feed intake, milk production, body tissue reserves, fertility performance, cow health, or diet digestibility. The reduction in milk fat content with the highest FB treatment was likely due to the effect on rumen function of the high starch levels with this treatment, and the reduction in milk protein levels with FB inclusion was likely due to a deficit in specific limiting amino acids. Methane production was unaffected by FB inclusion.

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