J. Dairy Sci. 102:1247–1256
https://doi.org/10.3168/jds.2018-14970

The effect of by-product inclusion and concentrate feeding rate on milk production and composition, pasture dry matter intake, and nitrogen excretion of mid-late lactation spring-calving cows grazing a perennial ryegrass-based pasture

S. A. Condren,[1] A. K. Kelly,[1]* M. B. Lynch,[1] T. M. Boland,[1] S. J. Whelan,[2] C. Grace,[1] G. Rajauria,[1] and K. M. Pierce[1]
[1]School of Agriculture and Food Science, University College Dublin Lyons Farm, Lyons Estate, Celbridge, Naas, Co. Kildare, W23 ENY2, Ireland
[2]Institute of Technology Carlow, Wexford Campus, Summerhill, Wexford, Y35 KA07, Ireland

ABSTRACT

Interest is growing in the use of by-products as economical sources of nutrients that complement grazed grass, particularly at times when grass supply is insufficient to meet the nutritional demands of lactating dairy cattle. The objective of this research was to assess the effect of the amount of by-product inclusion and concentrate feeding rate on pasture dry matter intake, milk production and composition, and N excretion from spring-calving cows grazing summer pasture during mid-late lactation. Forty-eight Holstein Friesian dairy cows were randomly assigned to 1 of 4 dietary treatments in a 2 × 2 factorial design. Cows were grazed in one group on a perennial ryegrass-based sward, with pelleted concentrates offered twice daily during milking over a 63-d experimental period. The dietary treatments were 3 kg of concentrate containing 35% by-products; 6 kg of concentrate containing 35% by-products; 3 kg of concentrate containing 95% by-products; and 6 kg of concentrate containing 95% by-products on a fresh matter basis. The by-products used were soybean hulls, palm kernel expeller, and maize dried distillers grains with solubles, included in equal proportions on a dry matter basis. Pasture dry matter intake (14.5 kg/d) was not affected by the amount of by-product inclusion or feeding rate. By-product inclusion had no effect on milk yield (27.1 kg/d) or milk solids (MS) yield (2.0 kg/d). Cows offered 6 kg of concentrate had a greater milk (+1.6 kg/d) and MS (+0.13 kg/d) yield, consumed more N (+0.08 kg/d), and excreted a lower proportion of N in the milk (0.25 vs. 0.27) and feces (0.39 vs. 0.41) and a higher proportion in the urine (0.39 vs. 0.32) compared with cows offered 3 kg of by-product-based concentrate. In conclusion, by-products can be included at up to 95% of the concentrate fed to cows grazing pasture without affecting pasture dry matter intake, milk production or composition, or N excretion. Cows offered 6 kg of concentrates produced more milk and MS than cows offered 3 kg but had higher urinary N excretion. Economics of this yield response will depend on milk and concentrate prices.

Key words: dairy cow, by-product, grazing, milk production, nitrogen excretion

INTRODUCTION

In the temperate regions, pasture-based milk production systems have a competitive economic advantage over other production systems, as grazed grass is widely accepted as the cheaper source of feed (Finneran et al., 2010). However, grass quality and growth are seasonal and feed supplementation is often required to manage deficits in pasture supply (McEvoy et al., 2008; Reid et al., 2015) or to increase overall DMI to support milk production (Bargo et al., 2003). Additionally, when land availability or value is a limiting factor to increasing milk production in pasture-based dairy systems (Ruelle et al., 2018), the strategic use of supplementary grain or pelleted concentrates can increase milk production per cow and per hectare and thus increase overall farm profitability (Bargo et al., 2003).

Traditionally, concentrates were offered to dairy cows in the form of cereal grains and residues of oilseed crops, particularly soybean meal (Wilkinson, 2011). However, competition from the food ingredient and bioethanol industries, combined with concerns about the environmental impact of these feeds, has brought the long-term sustainability of this practice into question. The ability of ruminant animals to turn human inedible feeds such as grass and by-products into human edible food of high biological value is likely to become

---

Received April 24, 2018.
Accepted September 18, 2018.
*Corresponding author: alan.kelly@ucd.ie

of greater significance as the world population increases in the future (Wilkinson, 2011).

By-products are secondary products obtained during harvest or processing of a principal commodity (Grasser et al., 1995). The use of by-products in animal diets is becoming more prevalent, both as a strategy to reduce dependence on cereals and oilseeds, and due to a simultaneous increase in their availability with the growth of the bio-ethanol industry (Bocquier and González-García, 2010). Soybean hulls (**SH**), palm kernel expeller (**PKE**) and maize dried distillers grains (**DDGS**) are by-products commonly used in Ireland and the combined yearly imports of these by-products has increased by 32% since 2011 (Irish Grain and Feed Association, personal communication, Deirdre Webb, 2018).

The use of imported animal feed (including by-products) can lead to greater nutrient loading within the farm system as large portions of imported N and P are excreted onto pasture, in urine and feces (O'Brien et al., 2014). Ireland is obligated to comply with strict environmental regulations such as the European Union Good Agricultural Practice for the Protection of Waters Regulations (S.I., 2014) which aims to reduce N and P loss to the environment. Therefore, it is important to establish the effect, if any, on the quantity and pattern of N excretion from increasing feeding rates or the levels of by-products such as SH, PKE, and DDGS in the concentrate. In a companion study, as the portion of by-product in concentrates increased from 35 to 95% when cows were offered 6 kg/d in early lactation, Whelan et al. (2017) reported no effect on milk production, rumen fermentation, or excretion of N. However, research on the use of by-products with dairy cows grazing in a pasture-based system is limited (Whelan et al., 2017), particularly the potential interaction between the amount of by-product inclusion and concentrate feeding level. It is also not clear what effect, if any, offering by-products at different levels of inclusion would have on nutrient partitioning in the mid-late lactation dairy cow, which is important because the partitioning of nutrients to tissue deposition increases with the progression of lactation (Doyle et al., 2005). The objective of this study was to assess the effect of varying the amount of by-product (SH, PKE, and DDGS) inclusion and concentrate feeding rates on pasture DMI, milk production and composition, rumen fermentation parameters, BCS, BW, and N excretion by mid-late lactation spring-calving dairy cows grazing pasture. It was hypothesized that by-product inclusion would have no effect on animal performance and that increasing feeding rate would increase milk production.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Design

All procedures described in this experiment were approved by the Animal Research Ethics Committee at University College Dublin and conducted under experimental license from the Health Products Regulatory Authority (S.I. No. 543 of 2012). Each person who carried out procedures on the cows during the course of this experiment was licensed to do so by means of individual authorization from the Health Products Regulatory Authority.

Forty-eight dairy cows (*Bos taurus*, strain Holstein Friesian) were selected from the spring calving dairy herd at University College Dublin Lyons Farm, Celbridge, Kildare, Ireland (53°17′56″N, 6°32′18″W). The experiment was designed as a 2 × 2 factorial with cows blocked according to parity and randomly assigned to 1 of 4 pasture-based dietary treatments ($n = 12$). Treatments were balanced for DIM (138 ± 49.1), predicted 305-d milk solid (fat + protein, **MS**) yield (639 ± 189.5 kg), BCS (3 ± 0.8), and pre-experimental milk yield (31.9 ± 10.25 kg). The experiment was conducted for 63 d from mid-July to mid-September 2015.

Diets consisted of a predominantly perennial ryegrass (*Lolium perenne*) pasture (with a small amount of white clover (*Trifolium repens*) and 1 of 2 supplementary concentrates offered at 2 feeding rates (fresh weight): 3 kg of concentrate containing 35% by-products (**BP35 3 kg**); 6 kg of concentrate containing 35% by-products (**BP35 6 kg**); 3 kg of concentrate containing 95% by-products (**BP95 3 kg**) and 6 kg of concentrate containing 95% by-products (**BP95 6 kg**). The by-products used were SH, PKE, and DDGS, which were in equal proportions on a DM basis. The supplementary concentrates were formulated to be isonitrogenous (16% CP) and the ingredient inclusion levels offered during this experiment are presented in Table 1 and were dispensed in the milking parlor, using the Feedrite automatic system linked to cow electronic identification (Dairymaster, Kerry, Ireland) and were manufactured by Gain Feeds (Portlaoise, Ireland).

Cows were grazed in a single group and were offered a fresh allocation (8 kg of DM/cow) of pasture twice daily (16 kg of DM/d, total, above 4 cm). Grazing area was allocated based on pre-grazing herbage mass. Briefly, an area (0.25 m^2) was cut using a handheld shears (Gardena Accu 90; Gardena GmbH, Ulm, DE) to a height of 4 cm at 4 random, representative locations throughout the paddock and for each 0.25 m^2 of grass was then collected and weighed; a sample of pasture was taken for determination of DM and routine chemical analysis. The mean pre-grazing herbage mass was 861 ± 512.1 kg of DM/ha. The postgrazing sward heights were also measured daily, and a total of 50 measurements were taken across each grazing area using a rising pasture plate meter with a steel plate (plate diameter of 355 mm and area density of 3.2 kg/m^2; Jenquip, Feilding, New Zealand). Average postgrazing sward surface height was 5.6 ± 1.63 cm.

Concentrate samples were collected weekly for DM and chemical analysis (Table 1). The pasture offered during this experiment was estimated (by visual assessment) to contain 97% perennial ryegrass and 3% white clover. Cows had ad libitum access to fresh water.

Data and Sample Collection

Cows were milked twice daily at 0700 and 1600 h. Measurement of milk yield and composite milk sample collection was facilitated using the Weighall milk metering and sampling system (Dairymaster). Milk samples were taken from one successive morning and evening milking each week and pooled on a per cow basis according to yield.

Individual cow BW were measured once weekly using electronic scales (Dairymaster) as the cows exited the milking parlor after the morning milking and again after evening milking through the automatic cow-drafting unit (Dairymaster) and the mean was calculated for each cow per day. Body condition score was assessed by a single fully trained operator, using a scale of 1 to 5 with 0.25 increments according to Edmonson et al. (1989), following evening milking each week.

Blood samples were collected by jugular venipuncture weekly, to coincide with milk and rumen fluid sample collection and BCS assessment, into a 4-mL gray-top Vacutainer (REF 368921; BD, Plymouth, UK), immediately stored on ice before being centrifuged at 2,100 × g for 20 min at 4°C for extraction of plasma. These samples were stored at −20°C pending analysis.

A sample of rumen fluid was collected after evening milking each week using the Flora Rumen Scoop esophageal sampler (Prof-Products, Ontario, Canada) as per Whelan et al. (2017). The ruminal pH was measured immediately with an Orion 3 Star pH Benchtop meter (Thermo Fisher Scientific, Waltham, MA) and the sample was then strained through 4 layers of cheesecloth and a 4-mL sub-sample collected using an automatic pipette and mixed with 1 mL of 50% wt/vol trichloroacetic acid, cooled on ice, and later stored at −20°C for VFA and NH_3N analysis.

N Partitioning Study

An N partitioning study was conducted during wk 6 (165 ± 49 DIM) of the 9-wk experiment. Pasture

Table 1. Chemical composition of concentrates and pasture and ingredient inclusion (%) level of concentrates fed during the experiment[1]

Item	BP35	BP95	Pasture
Chemical composition (% of DM unless stated)			
DM (% of fresh weight)	90.4	92.0	16.9
Ash	6.9	7.5	9.2
CP	16.1	15.9	21.1
NDF	27.2	50.0	42.1
ADF	14.6	29.4	20.3
Water-soluble carbohydrates	0.0	0.0	8.2
Ether extract	2.5	5.6	3.1
Gross energy (MJ/kg of DM)	16.9	17.7	17.0
Ingredient inclusion level of concentrates (%)			
Barley	45.0	0.0	
Soybean meal	12.0	0.0	
Distillers dried grain	11.6	31.0	
Palm kernel expeller	11.6	31.0	
Soybean hulls	11.6	31.0	
Molasses	5.0	5.0	
Calcined magnesite	0.8	0.8	
Salt	0.7	0.7	
Palm oil	0.6	0.6	
Lime flour	0.5	0.2	
Monocalcium diphosphate	0.3	0.0	
Vitamin and mineral premix[2]	0.5	0.5	

[1] BP35 = concentrate containing 35% by-products; BP95 = concentrate containing 95% by-products.

[2] Vitamin and mineral premix contained 33.9% Ca, 500 mg of Co/kg, 7,400 mg of Cu/kg, 2,000 mg of I/kg, 130 mg of Se/kg, 10,000 mg of Mg/kg, 25,000 mg of Zn/kg, 1,600,000 IU of vitamin A/kg, 400,000 IU of vitamin D_3/kg, and 2,000 mg of vitamin E/kg.

DMI and diet DM digestibility were estimated for a period of 6 d to facilitate calculation of N intake and feces N excretion. Determination of pasture DMI was achieved using the *n*-alkane technique of Dove and Mayes (2006). Individual animals were dosed with a paper bolus impregnated with 500 mg of the *n*-alkane *n*-dotriacontane (C32) for a period of 12 d following morning and evening milking, and for the last 6 d, samples of pasture, concentrate, feces, and milk were collected. Pasture samples from the pasture allocation for morning and evening were collected using a handheld shears as previously described. Pasture samples were then oven-dried at 55°C for 48 h and pooled per study period.

Feces samples were collected per rectum for the last 6 d of the study, and following morning and evening milking, samples were placed immediately into a forced-air oven and dried at 55°C until a constant weight was reached. Dried feces were later pooled per cow for analysis.

Milk samples were collected daily during morning and evening milking and pooled in proportion to yield for each period, a subsample drawn off and analyzed in a commercial laboratory for fat, protein, casein, lactose, urea, and somatic cell concentration.

Sample Analyses

Dried samples of pasture, concentrate, and feces were ground in a hammer mill fitted with a 1-mm screen (Lab Mill, Christy Turner, Suffolk, UK). Ash concentrations were determined by complete combustion in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 550°C for 5 h (AOAC International, 2005a; method 942.05). Nitrogen concentrations were determined using a Leco FP 528 instrument (Leco Corp., St. Joseph, MI; AOAC International, 2005b; method 990.03). Neutral detergent fiber and ADF were determined by the method of Van Soest et al. (1991) adopted for use in the Ankom 220 Fiber Analyzer (Ankom Technology, Macedon, NY). Samples of concentrate were analyzed with a thermo-stable α-amylase and 20 g of $NaSO_3$ was added to neutral detergent solution, whereas grass and feces samples were analyzed with neutral detergent solution only. Neutral detergent fiber and ADF are expressed inclusive of residual ash. Gross energy of concentrates and pasture was determined by bomb calorimeter (Parr 1281 Bomb Calorimeter, Parr Instrument Company, Moline, IL). The ether extract was determined using Soxtex instrument (Tecator, Hoganas, Sweden) and light petroleum ether. In vitro DM digestibility of pasture and concentrates offered was determined using a modification of Tilley and Terry (1963) for use in the Ankom Daisy (Ankom Technology). Concentrations of milk fat, protein, and lactose (and all other milk quality parameters) were determined in a commercial milk laboratory (Independent Milk Laboratories, Cavan, Ireland) using mid-infrared spectrophotometry (CombiFoss 5000, Foss Analytical A/S, Hillerød, Denmark).

The VFA and NH_3N concentrations in rumen fluid were analyzed according to Whelan et al. (2012). Samples were allowed to thaw for 16 h at 4°C and were centrifuged at 2,100 × *g* for 10 min at 4°C before analysis. To calculate DMI, *n*-alkanes were extracted from pasture, concentrate, and feces samples according to the method of Dove and Mayes (2006). Following extraction, samples were analyzed for concentrations of *n*-alkanes by GC, using the method previously reported by Whelan et al. (2017).

Statistical Analysis

Data were checked for normality and homogeneity of variance by histograms, QQ-plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS (version 9.1.3, SAS Institute Inc., Cary, NC). Data that were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. The transformed data were used to calculate *P*-values. The corresponding least squares means and standard error of the nontransformed data are presented in the results for clarity. Animal performance, milk quality, and rumen function parameters (ruminal pH, rumen NH_3, and VFA concentration) were analyzed using repeated measures ANOVA (MIXED procedure), with terms for by-product treatment, concentrate feeding strategy, and week of experiment. Biologically significant interactions between the fixed effects were also tested and included in the final model if statistically significant ($P < 0.05$). Days in milk was used as a covariate, week of experiment was used as the repeated unit, block was included as the random factor, and cow was the most suitable subject. The type of variance-covariance structure used was chosen depending on the magnitude of the Akaike information criterion for models run under compound symmetry, unstructured, autoregressive, heterogeneous first order autoregressive, or Toeplitz variance-covariance structures. The model with the least Akaike information criterion value was selected. For analyzing the N balance data, a mixed model ANOVA was used with terms for by-product treatment concentrate feeding strategy and their interactions. Where interactions were not significant, this term was excluded from the model. Days in milk were used as a covariate in the final model and block was included as the random factor. Differences between means were determined by F-tests using type III sums of squares. The PDIFF option and the Tukey test were applied as appropriate to evaluate pairwise comparisons. A probability of $P < 0.05$ was selected as the level of significance, and statistical tendencies were reported when $P < 0.10$.

RESULTS

DMI, Milk Production, and Composition and Nutrient Digestibility

Dry matter intake and milk production and composition results are presented in Table 2. Total DMI was higher (+2.91 kg, $P < 0.01$) in cows offered 6 kg of concentrate compared with cows offered 3 kg, with no effect of the amount of by-product inclusion on total DMI ($P = 0.11$). Cows offered 6 kg of concentrate produced more milk (+1.62 kg, $P < 0.01$) than those offered 3 kg, but no effect was observed on the amount of by-product inclusion ($P = 0.77$) on milk production and no by-product inclusion or concentrate feeding rate interaction ($P = 0.64$) was observed.

An interaction ($P = 0.01$) was observed between the amount of by-product inclusion and concentrate feeding rate for milk fat yield, whereby the cows offered 6 kg of BP35 produced more milk fat (+0.06 kg, $P = 0.01$) than those offered 6 kg of BP95. At the 3 kg feeding rate, however, there was no difference in milk fat yield ($P = 0.88$) between the BP35 and BP95. Milk fat yield was higher when cows were offered 6 kg of BP35, compared with 3 kg of BP35 (+0.11 kg, $P < 0.01$), with no difference between feeding rates ($P = 0.22$) for BP95.

An interaction ($P < 0.01$) was observed between the amount of by-product inclusion and concentrate feeding rate for milk protein concentration. Cows offered 6 kg of BP35 had an increased protein percentage (+0.12%, $P = 0.01$) compared with those offered 6 kg of BP95, although the protein concentration of the cows offered 3 kg of BP35 did not differ ($P = 0.24$) from the cows offered 3 kg of BP95. Cows offered 6 kg of BP35 had the same protein percentage ($P = 0.13$) as those offered 3 kg of BP35. However, cows offered 6 kg of BP95 had a decreased protein concentration (- 0.11%, $P = 0.01$) compared with those offered 3 kg of BP95.

An interaction ($P < 0.01$) was also observed between the amount of by-product inclusion and concentrate feeding rate for milk protein yield, with cows offered 6 kg of BP35 producing more milk protein (+0.05 kg, $P = 0.01$) than those offered 6 kg of BP95. At the 3 kg feeding rate, however, cows offered BP35 had a lower milk protein yield (−0.03 kg, $P = 0.03$) than those offered BP95. Also, cows offered 6 kg of BP35 produced more milk protein (+0.09 kg, $P < 0.01$) than those offered 3 kg of BP35. However, no difference was observed between feeding rates when cows were offered BP95.

An interaction ($P = 0.01$) was observed between the amount of by-product inclusion and concentrate feeding rate for MS yield, with cows offered 6 kg of BP35 producing more MS than those offered 6 kg of BP95 (+0.10 kg, $P = 0.01$). However, cows offered 3 kg of BP35 had the same MS yield as those offered 3 kg of

Table 2. The effect of the amount of by-product inclusion and concentrate feeding rate on DMI and milk production variables[1]

	Treatment					P-value		
Item	BP35 3 kg	BP35 6 kg	BP95 3 kg	BP95 6 kg	SEM	By-product	Feeding rate	Interaction
DMI (kg/d)								
Pasture	14.14	14.38	14.72	14.84	0.501	0.21	0.55	0.72
Concentrate	2.72^{a}	5.42^{b}	2.99^{a}	5.52^{b}	0.117	0.13	<0.01	0.47
Total	16.62^{a}	19.80^{b}	17.71^{a}	20.36^{b}	0.507	0.11	<0.01	0.61
Milk production (kg/d)								
Milk yield	26.27^{a}	27.95^{b}	26.40^{a}	27.97^{b}	0.248	0.77	<0.01	0.64
Fat	1.03^{a}	1.14^{b}	1.05^{ac}	1.08^{c}	0.013	0.09	<0.01	0.01
Protein	0.89^{a}	0.98^{b}	0.92^{c}	0.93^{c}	0.010	0.70	<0.01	<0.01
Milk solids yield	1.92^{a}	2.11^{b}	1.97^{ac}	2.01^{c}	0.021	0.22	<0.01	0.01
Lactose	1.14^{a}	1.23^{b}	1.16^{a}	1.22^{b}	0.013	0.59	<0.01	0.98
Milk quality (%)								
Fat	3.94^{a}	4.06^{b}	3.91^{a}	3.90^{a}	0.038	0.01	0.13	0.09
Protein	3.40^{ab}	3.46^{a}	3.45^{a}	3.34^{b}	0.020	0.12	0.30	<0.01
Casein	2.61^{ab}	2.66^{b}	2.66^{b}	2.57^{a}	0.017	0.23	0.33	<0.01
Lactose	4.33^{a}	4.34^{b}	4.32^{a}	4.34^{b}	0.009	0.38	0.04	0.33
Urea	0.03	0.03	0.03	0.03	0.001	0.75	0.13	0.62
Somatic cells ($\times 10^3$ cells/mL)	79.70	60.46	53.97	75.05	8.021	0.49	0.91	0.01

$^{a–c}$Means with different superscripts differ ($P < 0.05$).

[1] BP35 3 kg = 3 kg of concentrate containing 35% by-products; BP35 6 kg = 6 kg of concentrate containing 35% by-products; BP95 3 kg = 3 kg of concentrate containing 95% by-products; BP95 6 kg = 6 kg of concentrate containing 95% by-products.

Table 3. The effect of the amount of by-product inclusion and concentrate feeding rate on nutrient digestibility[1]

Nutrient digestibility (g/100 g of intake)	By-product inclusion level				Concentrate feeding rate			
	BP35	BP95	SEM	*P*-value	3 kg	6 kg	SEM	*P*-value
Ash	30.55	34.99	1.828	0.10	27.75	37.70	1.828	0.01
NDF	52.71	48.21	1.249	0.02	49.37	51.56	1.249	0.22
ADF	43.43	34.23	1.662	0.01	37.48	40.20	1.662	0.26

[1] BP35 = concentrate containing 35% by-products; BP95 = concentrate containing 95% by-products.

BP95 ($P = 0.30$). Also, cows offered 6 kg of BP35 had a higher MS yield than those offered 3 kg of BP35 (+0.19 kg, $P < 0.01$) but no difference was observed between feeding rates in cows offered BP95 ($P = 0.50$). Cows offered 6 kg of concentrates had a higher lactose concentration (+0.02, $P = 0.04$) and lactose yield (+0.08 kg, $P < 0.01$) than those offered 3 kg, with no effect of the amount by-product inclusion ($P = 0.38$, $P = 0.59$, respectively).

Nutrient digestibility figures are presented in Table 3. Digestibility of NDF (+4.5 g/kg of DMI, $P = 0.02$) and ADF (+9.2 g/kg of DMI, $P = 0.01$) was higher in cows offered BP35 than cows offered BP95, with no effect of the amount of by-product inclusion on ash digestibility ($P = 0.10$). Cows offered 6 kg of concentrate had a higher ash digestibility than cows offered 3 kg (+10.0 g/kg, $P = 0.01$), with no effect of concentrate feeding rate on digestibility of NDF or ADF ($P = 0.22$ and $P = 0.26$, respectively).

BW, BCS, and Blood Glucose

Amount of by-product inclusion ($P = 0.36$, Table 4) and concentrate feeding rate ($P = 1.00$) had no effect on BCS change. Body weight change was not affected by the amount of by-product inclusion ($P = 0.97$). However, cows offered 6 kg of concentrate tended to lose less BW (−14.6 kg) than those offered 3 kg (−21.7 kg, $P = 0.09$).

The amount of by-product inclusion ($P = 0.22$) and concentrate feeding rate ($P = 0.20$), as well as the interaction ($P = 0.96$), had no effect on blood glucose concentrations.

N Partitioning and Rumen Fermentation

The amount of by-product inclusion did not affect N intake ($P = 0.14$, Table 5) or the proportion of N excreted in the feces ($P = 0.62$) or urine ($P = 0.19$). Cows offered BP35 had a tendency toward a higher proportion of N in the milk ($P = 0.08$) than those offered BP95. Cows offered 6 kg of concentrate had a higher N intake ($P < 0.01$) and excreted a lower proportion of N in the milk ($P = 0.03$) and feces ($P < 0.01$) and a higher proportion in the urine ($P < 0.01$) than cows offered 3 kg of concentrate.

An interaction ($P = 0.01$, Table 6) was observed between the amount of by-product inclusion and concentrate feeding rate for ruminal pH, with cows offered 6 kg of BP35 having a higher ruminal pH than those offered 6 kg of BP95 ($P < 0.01$), whereas cows offered 3 kg of BP35 had the same ruminal pH as those offered 3 kg of BP95. Increasing feeding rate reduced ruminal pH at the BP95 inclusion ($P < 0.01$), with no difference between feeding rates at the BP35 inclusion level ($P = 0.12$). An interaction ($P < 0.01$) was also observed between the amount of by-product inclusion and week of experiment for ruminal pH, with cows offered BP35

Table 4. The effect of the amount of by-product inclusion and concentrate feeding rate on BW and BCS[1]

Item	By-product inclusion level				Concentrate feeding rate			
	BP35	BP95	SEM	*P*-value	3 kg	6 kg	SEM	*P*-value
BW (kg)								
BW start	648	658	11.6	0.56	651	655	11.6	0.80
BW end	634	640	11.6	0.73	630	644	11.6	0.39
BW change	−18.1	−18.2	2.88	0.97	−21.7	−14.6	2.88	0.09
BCS								
BCS start	2.84	2.86	0.027	0.61	2.85	2.85	0.027	1.00
BCS end	2.86	2.84	0.026	0.55	2.85	2.85	0.026	0.95
BCS change	0.01	−0.03	0.029	0.36	−0.01	−0.01	0.029	1.00

[1] BP35 = concentrate containing 35% by-products; BP95 = concentrate containing 95% by-products.

Table 5. The effect of the amount of by-product inclusion and concentrate feeding rate on partitioning of N[1]

	By-product inclusion level				Concentrate feeding rate			
Item	BP35	BP95	SEM	*P*-value	3 kg	6 kg	SEM	*P*-value
N (kg/d)								
Intake	0.536	0.560	0.011	0.14	0.509	0.587	0.011	<0.01
Milk	0.143	0.142	0.003	0.78	0.138	0.147	0.003	0.06
Feces	0.207	0.214	0.005	0.32	0.209	0.212	0.005	0.77
Urine	0.187	0.204	0.007	0.11	0.162	0.229	0.007	<0.01
Proportion of N								
Milk	0.270	0.252	0.007	0.08	0.273	0.250	0.007	0.03
Feces	0.388	0.382	0.008	0.62	0.411	0.359	0.008	<0.01
Urine	0.342	0.366	0.012	0.19	0.316	0.392	0.012	<0.01

[1] BP35 = concentrate containing 35% by-products; BP95 = concentrate containing 95% by-products.

having a higher ruminal pH than those offered BP95 in wk 3, 5, 6, 7, 8, and 10, with no difference observed in the other weeks of the experiment.

Cows fed BP35 had a higher rumen NH_3 concentration (+0.25 mmol/L, $P = 0.01$) than those fed BP95, with no effect of feeding rate observed ($P = 0.24$). Total VFA concentrations were greater ($P = 0.01$) for cows on the higher feed rate (6 kg of concentrate, total VFA = 125 mmol/L) compared with those on the lower level of supplementation (3 kg of concentrate, total VFA = 121 mmol/L). On the higher by-product concentrate BP95, VFA concentrations were greater than the BP35 ($P = 0.03$; BP 95, total VFA = 124 mmol/L versus BP35, total VFA = 120 mmol/L). An interaction ($P = 0.02$) was observed between the amount of by-product inclusion and concentrate feeding rate for propionate concentration, with cows offered 6 kg of BP95 having the higher levels compared with the other diets. The acetate:propionate ratio followed a similar trend ($P = 0.07$) with lowest ratio observed for cows offered 6 kg of BP95 compared with the other diets. Butyrate concentrations were higher ($P = 0.01$) for cows on the 6 kg feeding rate (15.95 mmol/L) compared with cows on the 3 kg feeding rate (15.02 mmol/L). Valerate production was higher ($P = 0.04$) for cows offered 6 kg of BP95 compared with cows on all other diets, lower for those on 3 kg of BP95 than cows on the BP35 diet offered 6 kg ($P < 0.05$), with the BP35 at the 2 feeding levels intermediate.

DISCUSSION

The objective of this study was to investigate the effect of varying the amount of by-product inclusion and concentrate feeding rates on pasture DMI, milk production and composition, rumen fermentation parameters, BCS, BW and N excretion in mid-late lactation spring calving dairy cows grazing a perennial ryegrass-based pasture. The hypotheses of this experiment were that increasing the amount of by-product (SH, PKE, and

Table 6. The effect of the amount of by-product inclusion and concentrate feeding rate on rumen fermentation and blood metabolites[1]

	Treatment					*P*-value		
Item	BP35 3 kg	BP35 6 kg	BP95 3 kg	BP95 6 kg	SEM	By-product	Feeding rate	Interaction
Rumen fermentation (mmol/L)								
Acetate	75.39	75.48	77.00	78.78	0.976	0.2	0.33	0.40
Propionate	24.71	25.36	25.16	27.63	0.404	0.001	0.005	0.02
Butyrate	15.16^{bc}	15.71^{ac}	14.87^{b}	16.19^{a}	0.213	0.77	0.002	0.14
Valerate	1.54^{bc}	1.60^{c}	1.50^{b}	1.70^{a}	0.040	0.47	0.003	0.03
Isovalerate	1.39^{bc}	1.36^{bc}	1.35^{b}	1.46^{a}	0.03	0.48	0.29	0.04
Isobutyrate	1.39	1.32	1.35	1.34	0.030	0.74	0.33	0.43
Acetate:propionate	3.10^{b}	3.09^{b}	3.09^{b}	2.85^{a}	0.039	0.06	0.002	0.07
Rumen NH_3N	3.35^{a}	3.41^{a}	3.06^{b}	3.21^{b}	0.091	0.01	0.24	0.61
Rumen pH	6.42^{a}	6.35^{a}	6.36^{a}	6.13^{b}	0.023	<0.01	<0.01	0.01
Total VFA	120	122	121	127	1.5	0.03	<0.01	0.13
Blood metabolite (mmol/L)								
Glucose	3.39	3.42	3.36	3.39	0.020	0.22	0.19	0.96

[a–c] Means within a row with different superscripts differ ($P < 0.05$).

[1] BP35 3 kg = 3 kg of concentrate containing 35% by-products; BP35 6 kg = 6 kg of concentrate containing 35% by-products; BP95 3 kg = 3 kg of concentrate containing 95% by-products; BP95 6 kg = 6 kg of concentrate containing 95% by-products.

DDGS) inclusion in the diet would have no effect on animal performance and increasing feeding rate increased milk production. These hypotheses were accepted.

DMI, Milk Production, and Composition and Nutrient Digestibility

Pasture DMI intake was similar among all treatments, regardless of concentrate feeding rate or the amount of by-product inclusion, consistent with Reid et al. (2015), where cows were at a similar stage of lactation and offered similar levels of concentrate. This is important because well-managed pasture has a relatively high nutritive value and pasture substitution would reduce any benefits of increased concentrate allocation on milk production or weight gain. Previous studies suggest that stage of lactation (Stockdale, 2000) and cow BW affect substitution rate of pasture, but the effect was found to be inconsistent within the feeding range of 2 to 6 kg of DM/d (Peyraud and Delaby, 2001).

Indeed, offering cows an additional 3 kg of concentrate corresponded to a marginal improvement in milk yield of 1.62 kg/d (or 0.54 kg of milk/kg of concentrate) and greater MS (fat and protein) of 0.09 kg/d. This was consistent with the research of Sairanen et al. (2006) and Ramsbottom et al. (2015) who reported responses to concentrates supplementation in pasture fed cows of 0.57 and 0.67 kg of milk/kg of concentrate, in mid-lactation and throughout a full lactation, respectively. However, the overall response rate was below that reported by Kennedy et al. (2003), who found an additional response to concentrates of 1.1 kg of milk/kg of concentrate, when concentrate was increased from 3 to 6 kg in grass-fed dairy cows at 110 DIM. Retrospective calculations of the energy balance in the cows during that experiment have shown that those offered 3 kg of concentrate were marginally energy deficient, whereas those offered 6 kg of concentrate were marginally positive for energy balance. Therefore, it would not have been possible to stimulate greater levels of milk production without the provision of additional energy. It is possible that a combination of advanced stage of lactation and apparent limitation in the total amount of energy consumed by the cows offered 6 kg/d concentrate resulted in the low response to supplementary concentrates observed in the present study.

Crucially, this low response rate of the dairy cows to increased concentrate supplementation demonstrates the need for close monitoring of animal performance and costs of production. At the time of this study, replacing barley and soybean meal with SH, PKE, and DDGS reduced the cost of the BP95 concentrate by approximately €0.03/kg (approximately $0.035/kg) compared with BP35, which offers an opportunity for cost saving at the farm level. By contrast, increasing the feeding rate of concentrates from 3 to 6 kg incurred an additional cost of €0.70 (approximately $0.82) per cow per d. The value of the extra milk produced equated to approximately €0.43 (approximately $0.50) at the average milk price for July to September 2015. Therefore, the response to concentrates observed was found to be uneconomical at the time of this experiment.

In Ireland, dairy farmers are typically paid on the basis of fat and protein concentration in the milk. Therefore, it is important that any changes in dietary regimen do not affect negatively on these milk constituents. Dietary starch, NDF, and CP intake are known to affect both milk protein (Rius et al., 2010) and milk fat (van Knegsel et al., 2007) synthesis. At the 6 kg feeding rate, cows offered BP35 would have consumed 1.35 kg more starch and 1.30 kg less NDF than the cows offered BP95, resulting in an overall improvement in milk protein concentration and MS yield. This is contrary to the findings of Whelan et al. (2017), who found no effect of the amount of by-product inclusion on MS yields when cows were offered BP35 or BP95 at a 6 kg feeding rate, which were the same by-products as were fed in this study. However, cows in that study were at an earlier stage of lactation (64 ± 24 DIM) and consumed a greater quantity of pasture that contained less CP, NDF, and ADF. This would have increased the relative importance of pasture as a carbohydrate source in the experiment of Whelan et al. (2017), reducing any potential effects of the greater NDF intake at BP95 level. The contribution of pasture to the diet at the 3 kg feeding rate may also help explain the lack of difference between BP35 and BP95 for milk fat and protein concentration. At this feeding rate, the difference in NDF intake between BP35 and BP95 was just 0.6 kg/d with pasture contributing to 0.85 of the diet, greatly reducing any effects of supplementary concentrate type on milk fat and protein yield. Thus, where high quality pasture makes up a significant portion of the total DMI, it is possible to substitute barley and soybean meal with PKE, DDGS, and SH with no effect on milk quality or production.

BW, BCS, and Blood Glucose

Aside from increasing milk production, concentrate supplementation may also be used by dairy farmers as a strategy to replenish the body reserves lost by the cow during early lactation. At the 6 kg feeding rate, however, Whelan et al. (2017) reported no effect of increasing by-product inclusion level on either BW or BCS change. This is consistent with the findings of the current study and the calculated energy balance for dairy cows offered 6 kg of supplementary concentrate.

In addition, the blood glucose concentrations of the cows offered 3 kg of concentrate were within the normal range proposed by Mee and Nolan (1994), and there was no change in BCS with this group, suggesting that these cows also had adequate nutrition, consistent with Law et al. (2009) and Reid et al. (2015).

N Partitioning and Rumen Fermentation

There is increased focus on the sustainability of dairy production systems and one of the principal concerns in high input pasture-based dairy systems is the loss of imported N into ground and surface water, and atmosphere, both of which can be related to N voided in the paddock by the dairy cow. The BP35 and BP95 diets were formulated to be isonitrogenous and intakes of pasture and concentrate were similar between treatments. Therefore, the amount of by-product inclusion did not affect the amount or partitioning of N excreted, which is consistent with the results of Whelan et al. (2017). Cows offered 6 kg of concentrate had a higher N intake; however, N excretion in the feces was similar to that of cows consuming 3 kg of concentrates. This demonstrates the poor relationship between N intake and N excreted in the feces as reported in previous experiments (Mulligan et al., 2004; Whelan et al., 2012). Although cows that were offered the higher level of concentrates had a tendency for a higher milk N level, the majority of the extra N consumed was entreated to the urine, indicating that N was supplied in excess of requirements. Dietary protein intake is the most important factor determining milk N efficiency and urinary N losses, which has significant environmental consequences, as urinary N has a greater potential for volatilization and leaching than fecal N (Guliński et al., 2016). In a grazing scenario, excess dietary N excreted as urea in the urine is concentrated in localized patches and then subsequently lost to surface and groundwater via leaching and N_2O emissions (de Klein et al., 2010).

Rumen NH_3N was increased when cows consumed a starch-based concentrate (BP35) compared with a fiber-based concentrate (BP95). This is consistent with the work of Khalili and Sairanen (2000), who concluded that the rapid fermentation of starch in the barley-based diet did not improve the utilization of grass N in the rumen. There was no effect of concentrate type on ruminal pH at the 3 kg feeding level, which is consistent with previous research (Khalili and Sairanen, 2000); however, cows in the current study that were fed BP35 had a higher ruminal pH than those offered BP95 at the 6 kg feeding level. Sun and Oba (2014) found no differences in ruminal pH of dairy cows fed either a barley or DDGS-based concentrate, although this experiment was carried out with cows on a grass silage-based diet. Ruminal pH was greater than 6.0 at all stages during this experiment, and for the majority of the time, cows offered BP35 had a higher ruminal pH than that of the cows offered BP95. Ruminal production of VFA is primarily responsible for reduced ruminal pH (Kolver and De Veth, 2002), and in this study ruminal pH was lowered and total VFA concentrations were greater with the higher concentrate feeding rates at the BP95 inclusion level, and were both unchanged with different feeding rates at the BP35 inclusion level.

CONCLUSIONS

This research shows that by-products (SH, PKE, and DDGS) can be included at up to 95% of the concentrate fed to cows grazing pasture without affecting pasture DMI, milk production or composition, or N excretion. Cows offered 6 kg of concentrates produced more milk and MS than cows offered 3 kg but had higher urinary N excretion. The economics of this yield and MS response will depend on milk and concentrate prices.

ACKNOWLEDGMENTS

This work was funded by the Irish Government under the National Development Plan 2007-2013 through the Department of Agriculture Food and the Marine Research Stimulus Fund:RSF 11/S/122: FEFAN.

REFERENCES

AOAC International. 2005a. 942.05. Ash in Animal Feed. Official Methods of Analysis, 18th ed. AOAC International, Gaithersburg, MD.

AOAC International. 2005b. 990.03. Crude Protein in Animal Feed. Official Methods of Analysis, 18th ed. AOAC International, Gaithersburg, MD.

Bargo, F., L. Muller, E. Kolver, and J. Delahoy. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. J. Dairy Sci. 86:1–42. https://doi.org/10.3168/jds.S0022-0302(03)73581-4.

Bocquier, F., and E. González-García. 2010. Sustainability of ruminant agriculture in the new context: Feeding strategies and features of animal adaptability into the necessary holistic approach. Animal 4:1258–1273. https://doi.org/10.1017/S1751731110001023.

de Klein, C., R. Monaghan, S. Ledgard, and M. Shepherd. 2010. A system's perspective on the effectiveness of measures to mitigate the environmental impacts of nitrogen losses from pastoral dairy farming. Paper presented at the Proceedings of the Australasian Dairy Science Symposium.

Dove, H., and R. W. Mayes. 2006. Protocol for the analysis of *n*-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nat. Protoc. 1:1680. https://doi.org/10.1038/nprot.2006.225.

Doyle, P., S. Francis, and C. Stockdale. 2005. Associative effects between feeds when concentrate supplements are fed to grazing dairy cows: A review of likely impacts on metabolisable energy supply. Aust. J. Agric. Res. 56:1315–1329. https://doi.org/10.1071/AR05087.

Edmonson, A., I. Lean, L. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Finneran, E., P. Crosson, P. O'Kiely, L. Shalloo, D. Forristal, and M. Wallace. 2010. Simulation modelling of the cost of producing and utilising feeds for ruminants on Irish farms. J. Farm Manage. 14:95–116.

Grasser, L., J. Fadel, I. Garnett, and E. DePeters. 1995. Quantity and economic importance of nine selected by-products used in California dairy rations. J. Dairy Sci. 78:962–971. https://doi.org/10.3168/jds.S0022-0302(95)76711-X.

Guliński, P., E. Salamończyk, and K. Młynek. 2016. Improving nitrogen use efficiency of dairy cows in relation to urea in milk–A review. Anim. Sci. Pap. Rep. 34:5–24.

Kennedy, J., P. Dillon, L. Delaby, P. Faverdin, G. Stakelum, and M. Rath. 2003. Effect of genetic merit and concentrate supplementation on grass intake and milk production with Holstein Friesian dairy cows. J. Dairy Sci. 86:610–621. https://doi.org/10.3168/jds.S0022-0302(03)73639-X.

Khalili, H., and A. Sairanen. 2000. Effect of concentrate type on rumen fermentation and milk production of cows at pasture. Anim. Feed Sci. Technol. 84:199–212. https://doi.org/10.1016/S0377-8401(00)00130-9.

Kolver, E., and M. De Veth. 2002. Prediction of ruminal pH from pasture-based diets. J. Dairy Sci. 85:1255–1266. https://doi.org/10.3168/jds.S0022-0302(02)74190-8.

Law, R., F. Young, D. Patterson, D. Kilpatrick, A. Wylie, and C. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92:1001–1012. https://doi.org/10.3168/jds.2008-1155.

McEvoy, M., E. Kennedy, J. Murphy, T. Boland, L. Delaby, and M. O'Donovan. 2008. The effect of herbage allowance and concentrate supplementation on milk production performance and dry matter intake of spring-calving dairy cows in early lactation. J. Dairy Sci. 91:1258–1269. https://doi.org/10.3168/jds.2007-0710.

Mee, J. F., and M. Nolan. 1994. Summer mini-metabolic profile of 50 spring-calving dairy herds. Pages 40–41 in Moorepark Research and Development Division Research Report, Teagasc, Moorepark, Ireland.

Mulligan, F., P. Dillon, J. Callan, M. Rath, and F. O'Mara. 2004. Supplementary concentrate type affects nitrogen excretion of grazing dairy cows. J. Dairy Sci. 87:3451–3460. https://doi.org/10.3168/jds.S0022-0302(04)73480-3.

O'Brien, D., P. Brennan, J. Humphreys, E. Ruane, and L. Shalloo. 2014. An appraisal of carbon footprint of milk from commercial grass-based dairy farms in Ireland according to a certified life cycle assessment methodology. Int. J. Life Cycle Assess. 19:1469–1481. https://doi.org/10.1007/s11367-014-0755-9.

Peyraud, J. L., and L. Delaby. 2001. Ideal concentrate feeds for grazing dairy cows-responses to supplementation in interaction with grazing management and grass quality. Recent Advances in Animal Nutrition 35:203–220.

Ramsbottom, G., B. Horan, D. Berry, and J. Roche. 2015. Factors associated with the financial performance of spring-calving, pasture-based dairy farms. J. Dairy Sci. 98:3526–3540. https://doi.org/10.3168/jds.2014-8516.

Reid, M., M. O'Donovan, J. Murphy, C. Fleming, E. Kennedy, and E. Lewis. 2015. The effect of high and low levels of supplementation on milk production, nitrogen utilization efficiency, and milk protein fractions in late-lactation dairy cows. J. Dairy Sci. 98:5529–5544. https://doi.org/10.3168/jds.2014-9016.

Rius, A., J. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M. McGilliard, B. Bequette, R. Akers, and M. Hanigan. 2010. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. J. Dairy Sci. 93:3114–3127. https://doi.org/10.3168/jds.2009-2743.

Ruelle, E., L. Delaby, M. Wallace, and L. Shalloo. 2018. Using models to establish the financially optimum strategy for Irish dairy farms. J. Dairy Sci. 101:614–623. https://doi.org/10.3168/jds.2017-12948.

Sairanen, A., H. Khalili, and P. Virkajärvi. 2006. Concentrate supplementation responses of the pasture-fed dairy cow. Livest. Sci. 104:292–302. https://doi.org/10.1016/j.livsci.2006.04.009.

S.I. 2014. S. I. 31. European Union (Good Agricultural Practice for Protection of Waters) Regulations 2014. In Statutory Instruments No. 31 of 2014.

Stockdale, C. 2000. Levels of pasture substitution when concentrates are fed to grazing dairy cows in northern Victoria. Aust. J. Exp. Agric. 40:913–921. https://doi.org/10.1071/EA00034.

Sun, Y., and M. Oba. 2014. Effects of feeding a high-fiber byproduct feedstuff as a substitute for barley grain on rumen fermentation and productivity of dairy cows in early lactation. J. Dairy Sci. 97:1594–1602. https://doi.org/10.3168/jds.2013-7068.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18:104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x.

van Knegsel, A., H. Van den Brand, J. Dijkstra, and B. Kemp. 2007. Effects of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation. Theriogenology 68:S274–S280. https://doi.org/10.1016/j.theriogenology.2007.04.043.

Van Soest, P. J., J. Robertson, and B. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Whelan, S., W. Carey, T. Boland, M. Lynch, A. Kelly, G. Rajauria, and K. Pierce. 2017. The effect of by-product inclusion level on milk production, nutrient digestibility and excretion, and rumen fermentation parameters in lactating dairy cows offered a pasture-based diet. J. Dairy Sci. 100:1055–1062. https://doi.org/10.3168/jds.2016-11600.

Whelan, S., K. Pierce, C. McCarney, B. Flynn, and F. Mulligan. 2012. Effect of supplementary concentrate type on nitrogen partitioning in early lactation dairy cows offered perennial ryegrass-based pasture. J. Dairy Sci. 95:4468–4477. https://doi.org/10.3168/jds.2011-4689.

Wilkinson, J. 2011. Re-defining efficiency of feed use by livestock. Animal 5:1014–1022. https://doi.org/10.1017/S175173111100005X.