Analysis of production responses to changing crude protein levels in lactating dairy cow diets when evaluated in continuous or change-over experimental designs¹

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ABSTRACT

The objective of this study was to evaluate by meta-analysis the effect of experimental design on the production response functions obtained when changing crude protein (CP) levels in lactating dairy cow diets. The final database of studies meeting the selection criteria contained 55 publications with 23 classified as using a continuous (80 diets) and 34 classified as using a change-over (173 diets) experimental design (2 publications reported results from trials using both designs). Mixed model, weighted analysis of covariance was conducted on production measures in response to CP including the continuous covariates year of publication and average days in milk (DIM) and the discrete classification covariate of experimental design. The model was evaluated for curvilinearity in the response to CP, variance heterogeneity, and coincidence in the response between experimental designs, with $P < 0.05$ indicating significant effects and $P < 0.15$ indicating trends. On average, cows in experiments using continuous designs had a lower initial DIM, were on treatment longer, and produced a greater yield of milk and milk protein than cows in experiments using change-over designs. Production responses to increasing CP were increased dry matter intake (DMI), milk and component yield, and feed efficiency (milk Mcal/kg of DMI), and decreased milk N:intake N. Response in milk yield and feed efficiency to increased CP interacted with experimental design where continuous experiments had greater milk yield and feed efficiency response at higher levels of CP. Interaction between CP and design effects

on yield of milk protein and fat or milk N:intake N did not approach significance. The database is limited by the lower number of continuous studies and the differences in average DIM between designs; nevertheless, it is concluded that DMI, milk protein yield, milk fat yield, and milk N:intake N responses to CP did not depend on experimental design. Response of milk yield and feed efficiency to CP interacted with experimental design; however, prospective research on the influence of experimental design is required to test these results. **Key words:** crude protein, milk production, experimental design

INTRODUCTION

When designing experiments to test different dietary treatments fed to dairy cows, the researcher is required to balance many design parameters that could potentially affect the inferences made after the experiment has been concluded. Many decisions at this stage of experimental planning are made in an attempt to balance finite resources with obtaining sufficient statistical power to detect biologically or economically relevant treatment differences. Due to the relatively high cost of conducting feeding experiments with dairy cows and the practical significance of small treatment differences when extrapolated to a commercial scale, change-over designs are very attractive experimental designs that provide a good balance to both of these considerations. The power of these designs derive from the characteristic that treatment differences are compared within cows, because cows contribute a high amount of variance external to the treatments under examination. As a result, fewer cows are required to obtain a desired level of power than continuous experimental designs (i.e., randomized complete blocks, and so on) because treatment comparisons are made based on between-cow information in these designs.

Power calculations, replication, blocking, covariate periods, and so on are statistical devices that are used to increase the precision of an experiment (Cochran and

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Cox, 1957). Inaccuracy in response estimates cannot be corrected by means of these approaches. Using change-over designs in nutrition studies has been proposed to result in inaccuracies due to the previous nutritional condition influencing the measurements in the current period (Morris, 1999). Previous planes of nutrition are known to possibly result in significant productive effects after that plane of nutrition has been adjusted (Wilson and Osbourn, 1960; Ferrell et al., 1986). However, the extent to which these effects may persist during subsequent periods and are of a magnitude that could be classified as carry-over effects in a properly implemented change-over design is not well studied.

In one of the first publications to emphasize the implications of separating the direct from carry-over effects, Cochran et al. (1941) observed that failing to adjust for carry-over effects when different planes of feeding were provided to dairy cows resulted in an underestimation of treatment differences by ~11%. In a series of experiments, Kreuzer and Kirchgeßner observed that, in some cases and for some responses, effects of previous treatments were still present after 21 d on a different diet (Kreuzer and Kirchgeßner, 1985a,b; Kirchgeßner and Kreuzer, 1985). Cows respond to fluctuations in nutrient intake by coordinating body tissue and milk synthesis to maintain homeostasis and homeorhesis (Bauman and Currie, 1980); this coordination appears to occur rapidly for both short-term (Bjerre-Harpøth et al., 2012; Yoder et al., 2013; Ferraretto et al., 2014) and intermediate-term (Gross et al., 2011) fluctuations in nutrient intake. It is unclear the extent to which these responses are sustainable under conditions of long-term feeding. However, timing, duration, and CP level may all be important and animals to which dietary CP level is changed may fail to respond completely under some cases of longer term differences in CP concentration (Law et al., 2009).

In a comprehensive statistical analysis, Huhtanen and Hetta (2012) concluded that change-over designs were as accurate as continuous studies when expected treatment differences were not extreme. On average, the studies entering that analysis were conducted in 1988–1993 with 17.2 to 18.3 kg of DMI and 24.5 to 25.9 kg of milk yield and thus represent a different population of cows and nutrient demand than would be generally applicable for current and future research. The feeding protocols for the studies that entered the analysis by Huhtanen and Hetta were stated to primarily have been based on allocating a fixed amount of concentrates and allowing ad libitum consumption of forages, typically grass silage. A frequent response observed when CP% is increased is an increase in DMI (Rico-Gomez and Faverdin, 2001; Huhtanen and Hristov, 2009); given the fixed rate of concentrate feeding

in the previous analysis, it is questionable whether the responses would be similar when forage and concentrates are both offered for ad libitum consumption in a fixed proportion as is the case with a TMR. In addition, the response functions presented (Huhtanen and Hetta, 2012) were linear in nature and without accounting for potential diminishing or negative returns as has often been shown in nutrition. In consideration of these questions and to obtain a better understanding of the implications that choice of experimental design has on the inferences on protein nutrition, the literature was analyzed through a meta-analysis with the objective of evaluating the effect of experimental design on the response functions obtained when feeding different levels of CP.

MATERIALS AND METHODS

To accomplish this objective, a database of studies that had the principal objective of evaluating the effects of changing the level of CP on the productive responses in lactating dairy cows was established. The following criteria were used to allow inclusion in the database: (1) published since 1990 in the *Journal of Dairy Science*, (2) conducted in North America with the predominant forage sources coming from corn silage or alfalfa, (3) the experimental objective of the trial was to evaluate different levels of CP in early- to mid-lactation ($0 < \text{starting DIM} < 200$), (4) experimental design was described (including the number of animals per treatment or total, DIM, period length, adaptation period length), and (5) diet CP content, DMI, milk yield, and milk components were reported. Date range, journal, trial location, and forage source inclusion criteria were used in an attempt to reduce the heterogeneity within the population of studies that entered into the analysis. Experimental designs were classified as continuous when treatment diets were fed to cows for the entire treatment period (not considering the dietary change that occurs at the initiation of the study) or change-over when 2 or more dietary CP concentrations were fed over the course of the study.

The scope of the meta-analysis was purposely restricted to where CP levels changed so as to have an independent variable that is essentially always determined from chemical analyses and reported in the publication, therefore not dependent on book values or model predictions (as opposed to, for example, MP, which could be predicted to change due to altering rumen degradability or due to incidental changes through changes in energy formulation or DMI) and because of an hypothesized sensitivity in productive and adaptive responses to CP alterations. Experiments were coded as trials when ≥ 2 CP levels were fed in a

study. Several trials included treatments with 2 levels of CP: 1 treatment diet was a control level of CP, and the second level of CP included 2 or more treatment diets containing ingredients of differing degradability. For these trials, the control CP diet was not duplicated across the different ingredient comparisons; instead, the comparison with solvent soybean meal was the only one that was retained and used for the analysis. For instances where 2 or more factors were being studied, the main effect categories that included multiple levels of CP were coded as separate trials (e.g., a 2×2 factorial would be 2 trials).

Data extracted from the publications were year of publication, cow breed, design, objective of the study, number of cows per treatment or cow-periods per treatment, reported DIM at the start of the study, adaptation time, number of periods, length of the periods, average lactation number, BW, DMI, milk yield, protein % and yield, fat % and yield, lactose % and yield, MUN, reported CP and NDF concentrations for the complete diet, types of forage fed, forage concentrations, forage CP concentration, associated standard errors, whether milk protein was crude or true protein, and whether a fixed effect or mixed model was used in the statistical analysis. Milk protein was assumed to be CP unless it was stated to be true protein, and was converted to true protein (NRC, 2001) for analysis. Milk energy was calculated (NRC, 2001) as milk yield $\times$ (0.0929 $\times$ fat % + 0.0547 $\times$ CP % + 0.0395 $\times$ lactose %). If milk component yield was not reported, it was calculated from the milk yield and reported component concentration. Results were screened for potential reporting errors and 3 studies were rejected because components calculated from milk yield and concentrations were substantially different from reported component yields (10–25% different; all others were <10% different: average = 1%).

Experimental characteristics and reported production were summarized to describe the studies in the database with designs compared using a *t*-test (Proc Mixed), nonparametrically using a Wilcoxon test (Proc NPAR1WAY), and variance heterogeneity between designs was assessed using a Levene test for equality of variance (Proc GLM, version 9.4, SAS, SAS Institute Inc., Cary, NC). Quantitative mixed modeling (St-Pierre, 2001) with analysis of covariance was used with random effects of trial and a heterogeneous residual variance between designs. Treatment mean responses were weighted by the reciprocal of the variance (St-Pierre, 2001) and centered around 1 by experimental design and by whether a fixed or mixed model was used for statistical analysis in the studies. In instances where a SE was not reported or a value was calculated (e.g., calculating protein yield from protein concentration and milk yield), the SE was imputed to the median

reported SE for the response. Feed efficiency (**FE**; Mcal milk/kg of DMI) was weighted by the weight value for milk yield and ratio of N in milk true protein to N intake (**MTPN:NI**; g of milk true protein N/100 g of N intake) was weighted by the weight value for milk protein yield. Fixed effects of trial average DIM and year of publication were included as covariates. A quadratic model was evaluated to test for a curvilinear response function in the mixed procedure of SAS using REML estimation:

$$Y_{ijk} = B_o + B_y [Year_j - 2015] + B_d [DIM_j - 130] + B_1 CP_{ijk} + B_2 CP_{ijk}^2 + B_{oi} z_i + B_{1i} z_i \times CP_{ijk} + B_{2i} z_i \times CP_{ijk}^2 + b_{oij} + b_{1ij} CP_{ijk} + e_{ijk},$$

where i = continuous or change-over designs; j = 1, ..., n_i trial within design; k = 1, ..., n_{ij} observations within trial within design; z_i = 1 or -1 for continuous or change-over trials, respectively; CP_{ijk} = diet CP concentration; Y_{ijk} = dependent, continuous response variable; B_o = overall fixed effect intercept; B_y = fixed effect covariate for year of publication of trial j ; B_d = fixed effect covariate for average DIM for cows in trial j ; B_1 = overall fixed effect linear slope parameter estimate; B_2 = overall fixed effect quadratic slope parameter estimate; B_{oi} = fixed effect deviation in the predicted intercept due to classification within design i ; B_{1i} = fixed effect deviation in the linear slope parameter estimate due to classification within design i ; B_{2i} = fixed effect deviation in the quadratic slope parameter estimate due to classification within design i ; b_{oij} = random effect of trial j within design i on the intercept; b_{1ij} = random effect of trial j within design i on the linear regression coefficient; e_{ijk} = residual error within design i with

$$e_{ijk} \sim iid \ N \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_\gamma^2 \\ \sigma_\delta^2 \end{pmatrix} \right]$$

and

$$\begin{bmatrix} b_{oij} \\ b_{1ij} \end{bmatrix} \sim iid \ N \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_{b_{oij}}^2 & \sigma_{b_{oij}b_{1ij}} \\ \sigma_{b_{1ij}b_{oij}} & \sigma_{b_{1ij}}^2 \end{pmatrix} \right],$$

where σ_γ^2 and σ_δ^2 are the error variances associated continuous or change-over trials, respectively. Residual analysis for outliers, influence, and normality was conducted. Model evaluation was conducted as follows: (1) the most parsimonious random effect specification was

based on minimizing the Bayesian information criterion; (2) the appropriateness of the quadratic function was tested based on a test of the quadratic parameters by an F test according to the hypothesis that $B_2 + B_{2i} z_i = 0$; (3) to evaluate the effect of experimental design on the response function, a test of coincidence for the slope of the response function was conducted based on an F test for the sequential (type 1) sums of squares of the fixed effect deviations according to the simultaneous hypothesis that $B_{1i} = B_{2i} = 0$. This test is an omnibus test for differences in slope at any level of CP. For instances in which coincidence was rejected for a quadratic function ($P < 0.15$), the first derivatives of the response functions were analyzed statistically and tested for differences between designs using Proc PLM for representative levels of CP and differences reported in the graphs for those levels of CP. For graphical representations of the predicted response functions, adjusted response values ($\mathbf{Y}_{\text{adjusted}}$) were presented (St-Pierre, 2001); additionally, R^2 was calculated for the weighted fixed effect model conditional on the presence of the random effects and the fixed effect covariates publication year and DIM, which is calculated from $\mathbf{Y}_{\text{adjusted}}$ and the 2-dimensional prediction relating $\mathbf{Y}_{\text{adjusted}}$ to CP. Significant differences were declared at $P < 0.05$ and trends toward significance at $P < 0.15$.

RESULTS AND DISCUSSION

Database Description

Characteristics of the trials entering the analysis are reported in Table 1. Twenty-three publications included 80 diets that reported results of continuous trials, and 34 publications included 173 diets reporting results from change-over trials (2 publications reported results from trials using both designs). On average, the trials of the 2 designs were published at the same time, with the same CP and BW. Production characteristics of trials entering the analysis are presented in Table 2 and in Figure 1 for DMI and milk yield. As expected due to the range of time, cows, and basal diets entering the analysis, a considerable amount of variability existed in the database. The 2 primary differences observed at this stage of analysis were the starting DIM of cows in the 2 different designs and the duration of time on treatment. Days on the same diet averaged 84 d for cows in continuous designs, whereas cows in change-over designs averaged 22 d on the same diet; this difference was expected based on the nature of these designs. Cows in continuous designs started on trial significantly earlier than cows in change-over designs, which was consistent with the database used by Huhtanen and Hetta (2012) in their analysis, although

the magnitude of this difference is greater for this database. It was expected that cows in change-over trials would have a predominance of starting DIM after peak milk so that the lactation curve would have a lower rate of change than before peak milk; what was somewhat unexpected was the lack of a greater proportion of continuous experiments also beginning at this stage of lactation. Over 50% of studies started within a month of calving for cows in continuous designs, whereas this occurred in less than 10% of change-over studies. Thus, the majority of studies using continuous designs would include the time of peak milk production and possibly also enhanced responsiveness to nutritional manipulation (Wu and Satter, 2000; Law et al., 2009; Caccamo et al., 2012). The effect that this characteristic has on the inferences about experimental design is not known, although productive yield and measures of efficiency were greater for trials that used continuous designs compared with change-over designs (Table 2). Attempts to account for an interaction between DIM and design were unsuccessful for all production response variables; however, average DIM was retained in the analysis as a covariate. Therefore, although response to CP will be interpreted with respect to differences in experimental design, the differences in the DIM for these populations of cows must be borne in mind.

Meta-Analysis of Responses to CP

Parameter estimates for the mixed model meta-analysis are shown in Table 3. Results for DMI and milk yield are shown in Figure 2. Similar to Huhtanen and Hetta (2012), insufficient statistical evidence was found to reject coincidence of the response functions for DMI ($P > 0.382$). Even after accounting for the effects of trial on DMI, which accounted for a significant proportion of the variation, a substantial proportion was still left unassigned, which is unsurprising given the number of factors affecting DMI (Allen, 2000). A time-dependent DMI response to changing CP has been described previously (Faverdin et al., 2003), in which the response in DMI was greater and followed a more dose-dependent relationship with increasing protein when studies were conducted for more than 1 mo than when conducted for less than 1 mo. Such a differential response due to experimental design was not statistically significant in this analysis.

Milk yield increased when CP increased, although the magnitude of this response depended on the experimental design of the trial ($P < 0.007$); in change-over trials the milk yield response increased quadratically with CP, whereas in continuous trials the response to additional CP was linear. At CP greater than 15.8% ($P < 0.05$), milk yield response to additional CP was lower

Table 1. Summary characteristics of studies entering the analysis designed to evaluate the responses to changes in dietary CP with continuous or change-over experimental designs

Design	n	Mean ¹	SD ²	Minimum	Median ³	Maximum
Year published ⁴						
Continuous	23	2000	8	1990	1999	2015
Change-over	34	2000	7	1990	2001	2015
% CP ⁵						
Continuous	80	17.1	2.2	12.0	16.9	23.8
Change-over	173	16.7	2.2	11.3	16.9	23.9
n per treatment ^{5,6}						
Continuous	80	10.6 ^b	3.6 ^b	4	11 ^b	29
Change-over	173	14.3 ^a	8.0 ^a	4	15 ^a	32
BW ⁷						
Continuous	22	607	39	507	613	660
Change-over	38	618	49	532	610	745
Days on the same diet ⁷						
Continuous	25	84 ^a	48 ^a	18	84 ^a	210
Change-over	40	22 ^b	5 ^b	10	21 ^b	35
Starting DIM ⁷						
Continuous	25	57 ^b	54	1	28 ^b	186
Change-over	40	88 ^a	45	21	97 ^a	179
Average DIM ⁷						
Continuous	25	99 ^b	51	23	80 ^b	208
Change-over	40	132 ^a	47	45	143 ^a	211

¹Significant differences occur between unadjusted responses with different superscripts (^{a,b} $P < 0.05$) estimated by mean separation with a *t*-test.

²Significant variance heterogeneity was detected between designs with different superscripts (^{a,b} $P < 0.05$) estimated using Levene's test.

³Significant differences occur between unadjusted responses with different superscripts (^{a,b} $P < 0.05$) estimated nonparametrically using Wilcoxon's test.

⁴The level of observation is publication.

⁵The level of observation is treatment.

⁶n per treatment is the number of experimental units fed each treatment; for continuous studies this is usually the number of animals in a trial divided by the number of treatments, whereas for change-over studies this is usually the number of cow-periods on trial.

⁷The level of observation is experiment.

in change-over trials than in continuous trials; at CP below this level, no difference was found in the slope of the response function. In contrast, the analysis by Huhtanen and Hetta (2012) did not identify a differential response in milk yield to increased protein supplementation. The ability of the body to compensate by repartitioning nutrients during times of altered nutrient availability has been documented previously, although under what would be perceived as more extreme conditions than are present in this database (Botts et al., 1979; Gross et al., 2011). It is possible that the cow would buffer the response to changes in shorter term nutrient availability, as would be present in change-over trials, and thus the full effect of changes in CP is not realized as they would be under conditions of longer term changes in nutrient availability. The direction of changing CP may also be an important factor affecting the response; Law et al. (2009) found that decreasing CP from 17.3 to 14.4% in mid lactation had no effect of milk production, whereas increasing CP from 14.4 to 17.3% resulted in a transient improvement in milk yield for cows on the 17.3% CP diet.

It is unclear what effects the proliferation of change-over designs ($>2\times$ as many diets in this database) and a potential lower response compared with continuous designs may have on practical nutritional recommendations. For example, it has been shown (Lee et al., 2012) that MP allowable milk is under-predicted by the NRC (2001); an artificially low production response to CP derived from a predominance of change-over designs could potentially contribute to this observation. However, given the aforementioned differences in DIM, differences from the results of the previously published meta-analysis, and the lack of differential response in milk components that will be discussed subsequently, these results should be tested prospectively under a controlled experimental setting.

In contrast to the design dependent differences in the milk yield response to CP, response functions for milk protein and fat yields were not different between designs (Table 3; Figure 3). These results are consistent with the analysis conducted by Huhtanen and Hetta (2012), although the magnitudes of the overall responses to additional CP are lower in this analysis than they were for

the previous analysis. The time course of milk protein and fat responses to dietary treatment have been investigated previously under different conditions and adjusted to the new dietary conditions often within 3 wk (Gross et al., 2011; Rico and Harvatine, 2013), which was the median period length for change-over studies in this analysis. Responses to changes in the postruminal supply of protein (Broderick et al., 1970) or individual AA (Bequette et al., 2000; Cant et al., 2001) has also occurred rapidly, consistent with the coincidence of the milk protein yield responses between designs. Milk protein percent response to CP was significantly affected by experimental design ($P < 0.027$); however, very little variation was accounted for by the CP content of the diet ($R^2 < 0.15$) after accounting for the random effect of trial. Fat content tended to respond differently between designs ($P < 0.105$), although as was the case for protein percent, little variation was explained by the CP content of the diet ($R^2 < 0.20$). Of all the production responses to CP supplementation evaluated in the analysis by Huhtanen and Hetta (2012), milk fat concentration response was the only one that tended to differ between designs; increasing diet CP in continuous trials tended to decrease milk fat concentration to a

greater extent than what was observed in change-over trials. In this analysis, milk fat content did not respond to increases in CP in continuous trials, whereas milk fat content was increased with increasing CP in change-over trials, but again, CP accounted for little variability in this analysis.

Given the different response functions for milk yield between designs, a significant difference in the response functions for FE (Figure 4A; $P < 0.001$) was observed. The difference between designs in the marginal response in FE to CP was only significant when CP was greater than 16.1%; at CP below this level, no difference was found in the slope of the response function. Above 16.1%, FE increased in continuous designs, whereas a plateauing effect was found for change-over designs. This design-dependent response at higher levels of CP could be due to partitioning consumed energy toward body tissue repletion from what would have likely have been a lower level of CP in many change-over trials (Botts et al., 1979). Phuong et al. (2013) did not detect an association between energy efficiency and digestible CP intake in their meta-analysis, although the analysis did not account for the experimental design in the statistical model; Huhtanen and Hetta (2012) did not

Table 2. Summary production statistics for studies entering the analysis designed to evaluate the responses to changes in dietary CP with continuous or change-over experimental designs

Design	n	Mean ¹	SD ²	Minimum	Median ³	Maximum
DMI, kg/d						
Continuous	80	22.1 ^b	2.7 ^x	16.2	22.5 ^b	27.6
Change-over	173	23.0 ^a	2.3 ^y	17.8	23.3 ^a	30.2
Milk, kg/d						
Continuous	80	36.1 ^a	5.9	18.8	36.3 ^a	49.3
Change-over	173	34.0 ^b	5.6	23.6	34.7 ^b	47.7
Protein, g/d						
Continuous	80	1,020 ^a	164 ^y	492	1,014 ^a	1,390
Change-over	173	967 ^b	179 ^x	651	947 ^b	1,488
Fat, g/d						
Continuous	80	1,211	235	620	1,186	1,708
Change-over	169	1,177	229	744	1,180	1,860
Protein, %						
Continuous	80	2.83	0.17	2.51	2.81	3.35
Change-over	173	2.84	0.18	2.20	2.84	3.27
Fat, %						
Continuous	80	3.37 ^b	0.47 ^a	2.30	3.36 ^b	4.59
Change-over	169	3.48 ^a	0.35 ^b	2.20	3.51 ^a	4.32
Milk Mcal ⁴ /kg of DMI						
Continuous	80	1.10 ^a	0.16	0.78	1.07 ^a	1.64
Change-over	169	1.01 ^b	0.15	0.73	1.03 ^b	1.50
g of milk protein N/100 g of N intake						
Continuous	80	26.95 ^a	4.88	14.62	27.25 ^a	37.76
Change-over	173	25.00 ^b	4.85	15.15	24.55 ^b	40.85

¹Significant differences occur between unadjusted responses with different superscripts (^{a,b} $P < 0.05$) estimated by mean separation with a *t*-test.

²Significant variance heterogeneity was detected between designs with different superscripts (^{a,b} $P < 0.05$; ^{x,y} $P < 0.15$) estimated using Levene's test.

³Significant differences occur between unadjusted responses with different superscripts (^{a,b} $P < 0.05$) estimated nonparametrically using Wilcoxon's test.

⁴Calculated milk Mcal = milk yield $\times$ (0.0929 $\times$ fat % + 0.0547 $\times$ CP % + 0.0395 $\times$ lactose %); NRC, 2001.

Figure 1. Observed DMI (A) and milk yield (B) for CP feeding studies designed as continuous or change-over designs included in the analysis where treatments with a common basal diet are connected by lines. Size of the symbols for continuous (○) and change-over (□) are proportional to the weight of the observation in the analysis. Trial represented in gray was removed as an outlier from all analyses.

report the results for FE in response to CP supplementation; however, for increased concentrate supplementation, no differential effect by design was present.

Consistent with previous analyses (Huhtanen and Hristov, 2009), the MTPN:NI is decreased with in-

creasing N intake in this analysis. Response functions of MTPN:NI did not differ between designs ($P < 0.234$; Figure 4B). In contrast to the analysis by Phuong et al. (2013) where energy and N efficiency were positively associated, FE and MTPN:NI response functions

Table 3. Parameter estimates for the productive response to changes in dietary CP for continuous or change-over experimental designs

Design	Intercept ¹			Linear			Quadratic			Fit ²		Coincidence ³
	B_o	SE	P	B_1	SE	P	B_2	SE	P	CV	R ²	P
DMI, kg/d												
Overall	22.24	0.649	<0.001	0.182	2.26E-02	<0.001	— ⁴	—	—		0.352	0.382
Continuous	22.47	1.140	<0.001	0.135	5.88E-02	0.026	—	—	—	0.034	0.156	
Change-over	22.61	0.691	<0.001	0.191	2.43E-02	<0.001	—	—	—	0.019	0.507	
SED ⁵	1.16			0.064			—					
P	0.909			0.382			—					
Milk, kg/d												
Continuous	31.94	2.13	<0.001	0.681	0.110	<0.001	—	—	—	0.039	0.730	0.007
Change-over	29.43	3.75	<0.001	1.397	0.432	0.002	−3.02E−02	1.31E−02	0.023	0.019	0.738	
SED	4.06			0.445			1.31E−02					
P	0.538			0.110			0.023					
Protein, g/d												
Overall	1,018	47	<0.001	15.87	1.82	<0.001					0.791	0.739
Continuous	989	73	<0.001	16.92	3.64	<0.001	—	—	—	0.035	0.734	
Change-over	1,034	53	<0.001	15.51	2.09	<0.001	—	—	—	0.023	0.836	
SED	79			4.20								
P	0.566			0.739								
Fat, g/d												
Overall	1,230	54	<0.001	20.43	1.98	<0.001	—	—	—		0.618	0.287
Continuous	1,131	87	<0.001	24.81	4.40	<0.001	—	—	—	0.046	0.627	
Change-over	1,269	59	<0.001	19.54	2.18	<0.001	—	—	—	0.031	0.653	
SED	89			4.91			—					
P	0.124			0.287			—					
Protein, %												
Continuous	3.72	0.44	<0.001	−8.64E−02	4.94E−02	0.086	2.36E−03	1.39E−03	0.096	0.027	0.086	0.027
Change-over	2.44	0.23	<0.001	5.00E−02	2.71E−02	0.069	−1.31E−03	8.13E−04	0.110	0.017	0.125	
SED	0.49			5.63E−02			1.61E−03					
P	0.011			0.018			0.025					
Fat, %												
Continuous	3.67	0.22	<0.001	−4.32E−03	0.012	0.712	—	—	—	0.046	0.006	0.105
Change-over	3.36	0.13	<0.001	0.016	0.005	0.001	—	—	—	0.026	0.178	
SED	0.23			1.26E−02			—					
P	0.174			0.105			—					
Milk Mcal ⁶ /kg of DMI												
Continuous	0.969	0.060	<0.001	0.0153	0.0031	<0.001	—	—	—	0.038	0.602	<0.001
Change-over	0.635	0.132	<0.001	0.0640	0.0154	<0.001	−1.73E−03	4.71E−04	<0.001	0.025	0.430	
SED	0.140			0.0158			4.71E−04					
P	0.018			0.003			<0.001					
g of milk protein N/100 g of N intake												
Overall	67.73	2.96	<0.001	−3.197	0.334	<0.001	5.69E−02	1.00E−02	<0.001		0.944	0.238
Continuous	75.37	7.00	<0.001	−4.110	0.780	<0.001	8.20E−02	2.22E−02	<0.001	0.029	0.925	
Change-over	61.82	3.82	<0.001	−2.600	0.442	<0.001	3.79E−02	1.33E−02	0.005	0.019	0.957	
SED	7.88			0.895			2.58E−02					
P	0.090			0.096			0.092					

¹Intercept for the adjusted response to CP, where year = 2015 and average DIM = 130.²CV = square root of the model mean square error/average. R² = 1 − error sum of squares/total sum of squares, conditional on the presence of year, average DIM, and the random effect of trial in the model. A heterogeneous variance model was used where residual error was different between designs as determined by improved model fit statistics, otherwise a pooled error term was used.³Statistical test of whether the slope of the response functions for the design types were different at any level of CP.⁴Quadratic effects were not significant where indicated ($P > 0.15$).⁵Standard error of the difference.⁶Calculated milk Mcal = milk yield × (0.0929 × fat % + 0.0547 × CP % + 0.0395 × lactose %); NRC, 2001.

Figure 2. (A) Adjusted DMI response function to increases in dietary CP concentration did not depend on the experimental design ($P_{\text{coincident response}} > 0.382$; $R^2_{\text{Continuous}} = 0.156$, $R^2_{\text{Change-over}} = 0.507$). (B) Adjusted milk yield response function to increases in dietary CP concentration depended on the experimental design ($P_{\text{coincident response}} < 0.007$; $R^2_{\text{Continuous}} = 0.730$, $R^2_{\text{Change-over}} = 0.738$). Marginal responses to additional dietary CP differed ($*P < 0.05$) when CP ≥ 15.8 . Responses were adjusted to year = 2015, average DIM = 130, and for the random effects of experiment. Size of the symbols for continuous ($\circ$) and change-over ($\square$) are proportional to the weight of the observation in the analysis. Shaded bands are 95% confidence intervals.

Figure 3. (A) Increases in milk protein yield to additional dietary CP % did not depend on the experimental design ($P_{\text{coincident response}} > 0.739$; $R^2_{\text{Continuous}} = 0.734$, $R^2_{\text{Change-over}} = 0.836$). (B) Increases in milk fat yield to additional dietary CP % did not depend on the experimental design ($P_{\text{coincident response}} > 0.287$; $R^2_{\text{Continuous}} = 0.627$, $R^2_{\text{Change-over}} = 0.653$). Responses were adjusted to year = 2015, average DIM = 130, and for the random effects of experiment. Size of the symbols for continuous ($\circ$) and change-over ($\square$) are proportional to the weight of the observation in the analysis. Shaded bands are 95% confidence intervals.

Figure 4. (A) Increases in feed efficiency [milk Mcal/kg of DMI; milk Mcal = milk yield $\times$ (0.0929 $\times$ fat % + 0.0547 $\times$ CP % + 0.0395 $\times$ lactose %); NRC, 2001] to additional dietary CP % depended on the experimental design ($P_{\text{coincident response}} < 0.001$; $R^2_{\text{Continuous}} = 0.602$, $R^2_{\text{Change-over}} = 0.430$). Marginal responses to additional dietary CP differ ($P < 0.05$; *) when CP, % $\geq 16.10\%$. (B) Reduced ratio of milk true protein N to N intake (g of milk N/100 g of N intake) to additional dietary CP % did not depend on the experimental design ($P_{\text{coincident response}} < 0.238$; $R^2_{\text{Continuous}} = 0.925$, $R^2_{\text{Change-over}} = 0.957$). Responses were adjusted to year = 2015, average DIM = 130, and for the random effects of experiment. Size of the symbols for continuous (○) and change-over (□) are proportional to the weight of the observation in the analysis. Shaded bands are 95% confidence intervals.

were negatively associated when CP was varied in this analysis. Whereas decreasing CP intake is a very powerful strategy to increase MTPN:NI, optimizing the total ration to improve FE and MTPN:NI simultaneously would prove extremely beneficial as economic and environmental pressures drive dairies to continue to make more milk components with lower inputs in an environmentally responsible manner.

Overall, response functions obtained from either continuous or change-over designs were generally similar for the studies in this database, which is consistent with the analysis reported previously that used a significantly different database (Huhtanen and Hetta, 2012). Important exceptions to this generalization was the significant difference in response function for milk yield and FE in this analysis, whereas milk protein and fat content tended to have different response functions between designs though accounting for little variability. In the study of Cochran et al. (1941), in which the design and analysis of balanced change-over designs was proposed, it is important to note that the treatment effects on milk yield were underestimated when the effect of carry-over was not included in the statistical

model. This was observed even though the researchers employed 2 orthogonal Latin squares that were balanced for the carry-over effects of previous treatment. Simply designing the treatment sequences to be balanced for carry-over was insufficient to reduce the effect of previous treatment on the results and eliminate this source of bias. It is unclear what proportion of the change-over studies entering this analysis, or in the literature generally, modeled the effects of carry-over in the statistical analysis. Although many reported that the change-over designs employed were balanced for the effects of previous treatment, few appeared to account for the possible effect of previous treatment in the statistical model. Based on the results of this analysis and to reduce the probability of biased estimates of direct treatment effects when experiments are designed as balanced change-overs (Jones and Kenward, 2003), future studies using change-over designs should consider specifically accounting for the variance due to the effects of previous treatment in the statistical model, testing these effects for significance, and reporting the outcome of this analysis (as, for example, in Tomlinson et al., 1994).

CONCLUSIONS

Analysis of recent literature on feeding different levels of CP would support a differential response in milk yield and FE between studies conducted using change-over designs compared with designs with continuous feeding. In contrast, a common DMI, milk protein yield, milk fat yield, and milk N:N intake response profile between experimental designs could not be rejected. The population of cows entering the analysis was not consistent between experimental designs, with the continuous designs using a higher proportion of early lactation cows and change-over designs using a higher proportion of postpeak cows. Due to greater efficiency of change-over designs, future research would benefit from more formal, prospective comparisons between experimental designs with a consistent population of cows and diets between designs to test the results of this literature analysis. Nevertheless, researchers must remain cognizant of the potential for the choice of experimental design affecting the production response inferences when the level of CP is altered.

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