Effects of nutrition on the fertility of lactating dairy cattle

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ABSTRACT

This meta-analysis of 39 experiments containing 118 treatments explored the effects of diet interventions in early lactation on the proportion of dairy cows pregnant to artificial insemination (AI; pregnancy to AI) and on calving to pregnancy interval. It also identified factors that may explain variation in these responses. The objectives were to identify effects of diet on reproduction, rather than differences between specific dietary interventions. The examination of calving to pregnancy interval used the more traditional method of analyzing differences between a treatment and the reference treatment used for comparison within a given experiment. The systematic review identified fewer experiments ($n = 39$) than had been expected. Four different multivariable models including the random effect of experiment were used to examine the effects of CPM-Dairy (version 3.08) estimated diet and production variables on proportion pregnant to AI. These models examined (1) output of products, (2) balance or duodenal availability of nutrients, (3) intake of nutrients, or (4) percentage of nutrients in the diet. The multivariable models identified positive associations between estimated increased fatty acid intake [incidence rate ratio (IRR) = 1.0003 ± 0.0001 g/d; $\pm$ standard error], starch intake (IRR = 1.061 ± 0.029 kg/d), metabolizable energy balance (IRR = 1.004 ± 0.002 MJ/d), and duodenal C14:0 (IRR = 1.008 ± 0.004 g/d) availability with the proportion of cows pregnant to AI, whereas rapidly fermentable sugar intake (IRR = 0.813 ± 0.054 kg/d), percentage of sugar in the diet (IRR = $0.960 \pm 0.015\%$), and milk protein yield (IRR = 0.922 ± 0.022 g/100 g per day) were associated with a reduced proportion of cows pregnant to AI. There was no multivariable model developed to assess variables

associated with calving to pregnancy interval but, univariably, increased metabolizable energy balance was associated with a shorter calving to pregnancy interval whereas increased milk production was associated with longer time to pregnancy. Increased intake of some AA, particularly threonine and lysine, were associated with a longer calving to pregnancy interval. It is clear nutritional management around calving can influence reproductive success. The importance of dietary fats and increased energy and protein balances in early lactation for improved fertility outcomes is supported and suggests that starch and sugars may have different effects on the proportion of cows that are pregnant to AI. This work also highlighted a need for further focused field studies exploring the roles of specific fatty acids, AA, phosphorus, and carbohydrates on reproduction.

Key words: carbohydrate, fats, fertility, protein

INTRODUCTION

Poor reproductive performance of lactating dairy cattle is a complex disorder that reflects associations with intensification of production and increased milk production (Butler, 2000; Lucy, 2001; Lean et al., 2008; Berry et al., 2014). However, it is difficult to determine a causal basis for the decrease in fertility, as genetics and environment have changed markedly over the last decades. Although some of the decline in fertility observed in the past is associated with genetic selection for greater milk production, the heritability of reproductive disorders is weak (Pryce et al., 1997; Berry et al., 2014). This suggests that much of the decline previously observed was associated with changes in nutrition, housing, and herd management and interactions of these environmental changes with genetics.

Nutritional influences on fertility have been examined and frequently reviewed (Butler, 2000; Bisinotto et al., 2012; Friggens et al., 2013), but difficulties and inconsistencies in study design occur. Studies must have large numbers of experimental units to identify biologically and economically important differences

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in proportion of cows pregnant (Lean et al., 2016). Nutritional influences during the transition period (± 4 wk of calving) may be of particular importance (Overton and Waldron, 2004), but it is clear that the effect of diet on fertility during this period is complex and multifactorial. Further, confounding is present in nutritional experiments, as the addition of one nutrient inherently alters concentrations of other nutrients in the diet (Lean et al., 2012). Nutrients may also interact to alter availability, uptake, or production of other nutrients and influence energy partitioning and future milk production beyond the period of immediate intervention (Block, 1984; van Saun et al., 1993; Degaris et al., 2008; Lean et al., 2014). Therefore, although experiments examining single nutritional interventions are essential to develop understandings of dietary components on reproductive outcomes, responses can be difficult to evaluate due to the potential for confounding. Meta-analysis using published literature provides the opportunity to combine studies and overcome the confounding caused by changes in single diets. It also allows the use of existing data to address hypotheses that could not be addressed previously, increases study power, and provides measures of the variation, or heterogeneity, of results among a group of experiments (Lean et al., 2009).

The effects of some nutritional interventions on pregnancy and time to pregnancy have been examined using meta-analysis (de Veth et al., 2009; Rabiee et al., 2010; Lean et al., 2012; Rodney et al., 2015). These studies used randomized controlled experiments that evaluated either the addition of fats (de Veth et al., 2009; Rodney et al., 2015), organic trace minerals (Rabiee et al., 2010), or protein (Lean et al., 2012) on reproductive outcomes. Few experiments have been identified that examine the effects of carbohydrate fractions on fertility, which has previously limited the ability to examine these specific dietary components using meta-analysis. Consequently, the effects of carbohydrate fractions were of interest in the current study.

The concept underpinning the current study is that reproductive failure in a group of cows is, in part, a metabolic disorder reflecting the inability of diets to supply adequate intakes, concentrations, or ratios of nutrients that are required for optimal reproductive performance. Our objective was to use carefully described dietary information from the available literature to explore the effects of the diet during the transition period on measures of pregnancy and calving to pregnancy interval as well as identifying factors that may explain variation in these responses. We hypothesized that dietary formulation and intake of nutrients during the transition period affect the probability of pregnancy to AI and interval from calving to pregnancy in dairy

cows. Previous meta-analyses examining the effects of nutritional interventions during transition on reproduction identified surprisingly few papers that were suitable for inclusion (Lean et al., 2012; Rodney et al., 2015). The current study of the proportion pregnant to AI differs from previously conducted meta-analyses in that the proportion pregnant for each treatment represents a single observation, whereas previous studies have examined the difference in proportion pregnant between a reference and treatment group. The former approach allows a focus on the effects of overall diet on reproduction rather than differences between specific interventions. As such, all nutrients and potential interactions among these could be examined, irrespective of whether they were the intended intervention of a particular diet. The examination of calving to pregnancy interval used the more traditional method of using the difference between treatment and a reference group for each variable in analyses.

MATERIALS AND METHODS

Literature Search

A systematic review across 3 databases (PubMed, Web of Science CABI, and Google Scholar) and references in papers was used to identify experiments exploring nutritional interventions during transition and fertility outcomes that were published in English between 1970 and 2015 in a peer-reviewed journal, conference proceedings, or as an accepted thesis. Combinations of the following search terms were used: cow, cattle, dairy, fertility, pregnancy, conception, reproduction, b-vitamin, β -carotene, biotin, calcium, CLA, cobalt, conjugated linoleic acid, copper, cottonseed, energy, fat, fiber/fibre, manganese, magnesium, minerals, molybdenum, omega-3, organic minerals, phosphorous, protein, selenium, soy, soybean meal, vitamin A, vitamin ADE, vitamin D, vitamin E, Zinpro, and zinc. The intent of searching "Zinpro" was to identify studies conducted on commercial mineral mixes. Results were ordered by relevance according to the database and articles were assessed until we found 500 chronological papers that, from the title, appeared unrelated to the topic and unworthy of further review.

Inclusion and Exclusion Criteria

For clarity of reporting these results, individual nutritional treatments will be referred to as treatments and manuscripts or published papers that report one or more treatments will be referred to as experiments. For calving to pregnancy interval, the difference between 2 treatments is used and is referred to as a comparison.

Treatments were suitable for inclusion if they were from randomized controlled experiments using *Bos taurus* dairy cows in their first or later lactation during the dry or lactating period (i.e., nulliparous nonlactating heifers were excluded). Experiments had to evaluate the effect of individually feeding cows during the transition period (up to 4 wk pre- or postpartum) and describe the diet in enough detail that it could be evaluated using CPM-Dairy (version 3.08; Cornell-Penn-Miner, <https://cahpwww.vet.upenn.edu/archives/9-Purchase-CPM-3.0.8.1.html>). Experiments reporting both pre- and postcalving interventions were identified in the systematic review; however, the low number of experiments reporting precalving diets meant analysis was confined to postcalving treatments only. The number of cows in each treatment and, for continuous variables, measures of variance or *P*-values that allowed an estimate of standard deviation to be derived must have been reported. Fertility measures must have been reported as (1) proportion of cows pregnant to first AI, second AI, or within 28 d of breeding season, before 126 DIM, or within the lactation (labeled throughout the manuscript as proportion pregnant to AI); or (2) calving to pregnancy interval or days open (labeled throughout the manuscript as calving to pregnancy interval). These fertility measures are detailed for each experiment in Table 1. The numerator for the proportion of cows pregnant was the cows pregnant and the denominator was the number of cows in the group with experiments reporting pregnancy to a single service (Table 1). Appropriate experimental design including details of randomization, suitable analysis, and elimination of bias or confounding was also evaluated.

Data and Diet Extraction

Data were extracted from each published paper, including authors, year of publication, reference (including journal) of the publication, title of the publication, parity of cows (first or multiple lactations), and number of cows in treatment and reference groups. As stated, reproductive variables examined were defined as proportion of cows pregnant to AI and calving to pregnancy intervals for each treatment. A summary of experiments and treatments included in the analysis is provided in Table 1.

To extract and model dietary information, data from accepted experiments were entered into CPM-Dairy (version 3.08) following the standard operating procedure described in Rabiee et al. (2012). Ration composition and intake in the experiments were entered using ingredients selected from the standard CPM-Dairy feed bank and calibrated to the specifications described in the experiment. Preference was given to revising nutri-

ent compositions of forages. No nutrient content revisions beyond expected typical biological variation were identified. Once calibrated for the reference treatments, no further corrections were made to the analyses of individual components during modeling of the treatment diet. It was assumed that the same forages were used in all treatments of a given experiment, and any tested difference in the comparison of diet analyses within an experiment would likely reflect the distribution of errors around feed sampling and analysis. These data were combined with information on the cattle, including breed, parity, BCS, and BW, and production, housing, and environment from the paper to predict diet composition and outcomes. Body weight was estimated for 5 studies and BCS for 9 studies. Individual dietary components and production factors were extracted. Throughout the current paper, the international system of units was used; a full list of variables assessed and units used are presented in Table 2.

Statistical Analysis

All statistical analyses were conducted using Stata (Intercooled Stata v.13 and v.14, Stata Corp., College Station, TX). Following initial exploration of the data, a Poisson regression indicated that the data were overdispersed and use of a negative binomial model for data analysis was indicated. Negative binomial metaregression analyses (NBREG) were used to explore the effect of each predictive variable (e.g., dietary component or output) extracted on proportion of cows pregnant, and the contribution of each observation was weighted by the number of cows on the treatment. The unit of interest was each individual treatment within study. Variance in the proportion of pregnant cows was examined by generalized linear latent and mixed models (GLLAMM; Skrondal and Rabe-Hesketh, 2004; Grilli and Rampichini, 2006). The variance attributable to treatment was small (<3% of total variance) and the variance attributable to experiment was approximately 30% of the total variance. Consequently, the random effect of experiment, but not treatment, was included in the statistical models developed. Variables with univariable $P < 0.20$ in the negative binomial analysis were assessed in population averaging, multivariable models (XTNBREG) with backward elimination, in which the random effect of experiment was accounted for. The multivariable models were developed to understand responses further and control for confounding among variables.

The population averaging model is an equal within-panel (experiment) robust correlation model for the dispersion parameter (δ_i). As described by Hausman et al. (1984) for a random-effects overdispersion model,

Table 1. Summary of experiments included in the meta-analysis

Reference	N	Dietary intervention ¹	Duration of treatment (d)	Proportion pregnant to AI ² (%)	Calving to pregnancy interval (d)
Ambrose et al., 2006	59	Sunflower seed (0.1% α -linolenic acid)	From 28 d before to 32 d after AI	32 ^A	—
Barton et al., 1996 ³	62	Flaxseed (56.7% α -linolenic acid)	1 to 100 d postpartum (or 120 d postpartum if not pregnant)	48	—
	32	13% CP	Throughout lactation	41 ^A	71 ^F
Bruckental et al., 1986	32	20% CP		44	81
	12	Basal diet (9.2% CP)		—	79 ^G
	12	Soybean meal (14.3% CP)		—	110
	12	Soybean meal (18.0% CP)		—	88
	12	Urea (14.3% CP/kg of CP)		—	94
	12	Urea phosphate		—	93
Bruckental et al., 1989	78	Low soybean meal (17% CP) – multiparous	0 to 168 d postpartum	49 ^B	—
	66	High soybean meal (21% CP) – multiparous		42	—
	70	Fish meal (21% CP) – multiparous		50	—
	30	Low soybean meal (17% CP) – primiparous	0 to 112 d postpartum	47	—
	29	High soybean meal (21% CP) – primiparous		45	—
	28	Fish meal (21% CP) – primiparous		57	—
Canfield et al., 1990	15	Moderate protein (16% CP) – primiparous	0 d postpartum to 20 d after first AI	47 ^A	—
	16	High protein (19% CP) – primiparous		31	—
	16	Moderate protein (16% CP) – multiparous		50	—
	16	High protein (19% CP) – multiparous		31	—
Carroll et al., 1988 ³	29	13% CP	5 to 100 d postpartum (or 126 d if not pregnant)	62 ^A	72 ^F
	28	20% CP	Fed for 100 d starting at 5 $\pm$ 2 DIM	54	82
Carroll et al., 1990	23	Control		50 ^A	—
	23	5% Added prilled long-chain fatty acids		50	—
Carroll et al., 1994	9	Control (0% fish meal)	12 to 125 d postpartum	67 ^A	—
	9	Fish meal (3.5%)		33	—
Castañeda-Gutiérrez et al., 2005	16	CSFA (approximately 1%)	2 wk prepartum to 9 wk postpartum	19 ^C	160 ^H
	16	CLA isomers (approximately 0.2%) + CSFA (approximately 0.9%)		31	135
	15	CLA isomers (approximately 0.3%) + CSFA (approximately 0.8%)		33	151
Chester-Jones et al., 2013	25	67% Inorganic sulfate and 33% AA complex	Fed for the entire lactation and some cows remained on treatment for subsequent lactation	28 ^A	159 ^F
	24	67% Inorganic sulfate and 33% polysaccharide complex		71	114
de Feu et al., 2009	24	100% Polysaccharide complex		29	135
	26	100% Inorganic sulfate		27	139
	20	Standard TMR		50 ^A	117 ^G
Edwards et al., 1980	20	High-quality TMR		21	120
	18	13% CP	Entire lactation	44 ^A	123 ^F
	18	15% CP		39	141
	18	17% CP		33	139
Ferguson et al., 1990	52	Control (0 kg of fat) – herd 1	1 to 150 d postpartum	33 ^A	105 ^F
	44	0.5 kg of fat – herd 1		57	99
	34	Control (0 kg of fat) – herd 2		38	93
	27	0.5 kg of fat – herd 2		63	88
	33	Control (0 kg of fat) – herd 3		52	95
	24	0.5 kg of fat – herd 3		67	82

Continued

Table 1 (Continued). Summary of experiments included in the meta-analysis

Reference	N	Dietary intervention ¹	Duration of treatment (d)	Proportion pregnant to AI ² (%)	Calving to pregnancy interval (d)
Ferris et al., 2010	40	Low phosphorous diet (4.4 g of P/kg of DM in winter and 3.6 g of P/kg of DM in summer) – yr 1	From parturition for up to 4 lactations)	38 ^A	—
	40	High phosphorous diet (7.0 g of P/kg of DM in winter and 6.8 g of P/kg of DM in summer) – yr 1		40	—
	50	Low phosphorous diet (4.4 g of P/kg of DM in winter and 3.6 g of P/kg of DM in summer) – yr 2		32	—
	50	High phosphorous diet (7.0 g of P/kg of DM in winter and 6.8 g of P/kg of DM in summer) – yr 2		46	—
	50	Low phosphorous diet (4.4 g of P/kg of DM in winter and 3.6 g of P/kg of DM in summer) – yr 3		32	—
	50	High phosphorous diet (7.0 g of P/kg of DM in winter and 6.8 g of P/kg of DM in summer) – yr 3		26	—
	50	Low phosphorous diet (4.4 g of P/kg of DM in winter and 3.6 g of P/kg of DM in summer) – yr 4		24	—
	50	High phosphorous diet (7.0 g of P/kg of DM in winter and 6.8 g of P/kg of DM in summer) – yr 4		8	—
Garcia Bojalil et al., 1998	11	0% CSFA and 11.1% DM ruminally degradable intake protein		27 ^A	80 ^F
	11	2.2% CSFA and 11.1% DM ruminally degradable intake protein		45	83
	10	0% CSFA and 15.7% of DM ruminally degradable intake protein	0 to 120 d postpartum	40	73
	11	2.2% CSFA and 15.7% DM ruminally degradable intake protein		45	86
Garnsworthy et al., 2009	14	Low starch (9.8% starch)		21 ^A	66 ^G
	11	High starch (18.2% starch)		9	81
Gilmore et al., 2011	27	Low protein and methionine (14% CP, 40 g/d methionine)		33 ^A	134 ^G
	27	Recommended protein (17% CP)		29	142
Holter et al., 1992	18	Control		33 ^A	—
	19	Lined whole cottonseed (15%)		47	—
	54	Lined whole cottonseed and CSFA at ~3%		42	—
Howard et al., 1987 ³	47	Moderate protein (15% CP)		—	80 ^F
	36	High protein (20% CP)	0 to 112 d postpartum	—	80
Hutchinson et al., 2011	33	CSFA (60 g/d)		39 ^A	111 ^G
	9	Lipid-encapsulated CLA (80 g/d)		52	109
Lucy et al., 1991	8	Unbalanced basal diet formulated to be deficient in nutrients supplied by 2.9 kg of long bemudagrass hay offered separately		33 ^D	—
	8	Balanced basal diet with long bemudagrass hay offered separately		50	—
	9	0% CSFA, bemudagrass hay chopped and fed with TMR		33	—
	9	2.2% CSFA, bemudagrass hay chopped and fed with TMR		67	—
	8	Alfalfa hay chopped and mixed with TMR		100 ^R	—
	9	Alfalfa hay cubed and mixed with TMR		33	—
Lucy et al., 1992	9	Control (0% CSFA) no niacin	15 to 98 d postpartum	44 ^A	63 ^F
	8	CSFA (3.0%) no niacin		25	63
	9	Control (0% CSFA) niacin added		44	70
	8	CSFA (3.0%) niacin added		0 ^R	0

Continued

Table 1 (Continued). Summary of experiments included in the meta-analysis

Reference	N	Dietary intervention ¹	Duration of treatment (d)	Proportion pregnant to AI ² (%)	Calving to pregnancy interval (d)
Markus et al., 1996	11	Control – multiparous	~23 d postpartum for 16 wk	73 ^D	163 ^G
	10	Tallow (2.7%) – multiparous		90	124
	10	Whole sunflower seeds (7.1%) – multiparous		90	172
	6	Control – primiparous		100 ^R	115
Mikula et al., 2011	6	Tallow (2.7%) – primiparous	14 d prepartum to parturition, parturition to 120 d	50	112
	6	Whole sunflower seeds (7.1%) – primiparous		67	155
	12	Maize (2.5 kg/d)		75 ^A	115 ^F
	12	Triticale then maize (2.5 kg/d of triticale from 14 d prepartum to parturition then 2.5 kg/d of maize to 120 d postpartum)		75	104
Moallem et al., 2010	12	Maize then triticale (2.5 kg/d of maize from 14 d prepartum to parturition then 2.5 kg/d of triticale to 120 d postpartum)	21 to 100 d postpartum	33	156
	12	Triticale (2.5 kg/d)		42	130
	20	CSFA (approximately 0.2%)		35 ^A	88 ^F
	20	Encapsulated lipid supplement (approximately 0.2%)		35	82
Monssavi et al., 2008	5	Control (0% fish meal)	21 d prepartum to 35 d postpartum	—	99 ^F
Petit and Twagirumungu, 2006	5	Fish meal (3.5% prepartum, 1.95% postpartum)	6 wk prepartum to 120 d postpartum. If cows were diagnosed pregnant, the diet was fed for 50 d of gestation	—	111
	36	Whole flaxseed (10.6%)		44 ^A	—
	34	CSFA (3.9%)		56	—
	40	Micronized soybeans (18.05%)		40	—
Rueggsegger and Schultz, 1985 ³	27	Soybean meal (20%)	10 to 115 d postpartum	—	115 ^G
Salfer et al., 1995	20	Heat-treated whole soybean (25%)	14 d prepartum to 151 d postpartum	—	109
	16	Control (0% PHT pre- and postpartum)		—	88 ^F
	16	1% PHT at d 14 to parturition, 2% PHT 1 to 151 DIM		—	102
	16	0% PHT at d 14 to parturition, 2% PHT 1 to 151 DIM		—	95
Senatore et al., 1996	40	Single diet	0 to 100 d postpartum	43 ^A	—
Siciliano-Jones et al., 2008	125	Minerals provided in sulfate form	3 wk prepartum through to 35 wk of lactation	40 ^A	115 ^F
	125	Minerals (Zn, Mn, and Cu) supplied as AA complexes		28	124
Sklan et al., 1989	54	Control	0 to 170 d postpartum	28 ^A	—
Sklan et al., 1994	54	Calcium soaps of fatty acids (2.4%)	0 to 120 d postpartum	43	—
	29	Control (0% CSFA) – multiparous		41 ^E	—
	33	CSFA (2.5%) – multiparous		33	—
	19	Control (0% CSFA) – primiparous		74	—
Soltan, 2010	21	CSFA (2.5%) – primiparous	3 wk prepartum to 12 wk postpartum	33	—
	60	Single diet		44 ^B	—
Son et al., 1996	17	Tallow (3%) and low supplementary escape protein	14 to 84 d postpartum	24 ^A	—
	15	Tallow (3%) and high supplementary escape protein		67	—
	15	Control (tallow 0%, replaced with corn) and high supplementary escape protein		27	—
	15	Control (tallow 0%, replaced with corn) and low supplementary escape protein		40	—
Son et al., 2000	11	Control	1 to 98 d postpartum	36 ^A	—
	14	CSFA		36	—

Continued

Table 1 (Continued). Summary of experiments included in the meta-analysis

Reference	N	Dietary intervention ¹	Duration of treatment (d)	Proportion pregnant to AI ² (%)	Calving to pregnancy interval (d)
Toni et al., 2007	90	Inorganic minerals	60 d prepartum to 200 d postpartum	19 ^A	111 ^F
Westwood et al., 2000	90	AA complexes of Zn, Mn, and Cu	21 d prepartum to 150 d postpartum	21	112
	40	High degradable (19.3% CP, 15% RUP; 85% RDP)	21 d prepartum to 150 d postpartum	45 ^A	102 ^G
Wu et al., 2000	42	Low degradable (19.3% CP, 35% RUP; 65% RDP)	From beginning to end of lactation	67	84
	26	0.31% P		88 ^A	78 ^F
		0.40% P		44	106
		0.49% P		33	112

¹CSFA = calcium salts of fatty acids; PHT = partially hydrogenated tallow.

²Measures that contributed to proportion pregnant to AI were reported as conception to first service (^A); conception to first 2 services or pregnant after 28 d of breeding season (^B); pregnancy before 126 DIM (^C); pregnancy within the lactation (^D); or pregnancy to first service (^E). Diets that had 0 or 100% conception or pregnancy destabilized the model and were removed from analysis of this measure (^F). Measures that contributed to calving to pregnancy interval were days open (^F); days from calving to conception (^G); or days from calving to pregnancy (^H).

³Breed of cattle was described as Holstein, Holstein-Friesian, or Friesian except for Barton et al. (1996; 50% Holstein and 50% Jersey); Carroll et al. (1988; 36 Holstein, 14 Jersey, 7 Guernsey); Rueggsegger and Schultz (1985; 56 Holstein, 4 Brown Swiss); and Howard et al. (1987; 109 Holstein, 47 Ayrshire).

δ_i is allowed to vary randomly across groups; namely, it is assumed that $1/(1 + \delta_i) \sim \text{Beta}(r, s)$, where r, s are parameters for δ_i . The joint probability of the counts for the i th group is

$$\Pr(Y_{i1} = y_{i1}, \dots, Y_{in_i} = y_{in_i} | X_i) = \int_0^\infty \prod_{t=1}^{n_i} \Pr(Y_{it} = y_{it} | x_{it}, \delta_i) f(\delta_i) d\delta_i = \frac{\Gamma(r+s) \Gamma\left(r + \sum_{t=1}^{n_i} \lambda_{it}\right) \Gamma\left(s + \sum_{t=1}^{n_i} y_{it}\right)}{\Gamma(r) \Gamma(s) \Gamma\left(r + s + \sum_{t=1}^{n_i} \lambda_{it} + \sum_{t=1}^{n_i} y_{it}\right)} \prod_{t=1}^{n_i} \frac{\Gamma(\lambda_{it} + y_{it})}{\Gamma(\lambda_{it}) \Gamma(y_{it} + 1)},$$

for $X_i = (x_{i1}, \dots, x_{in_i})$ and where f is the probability density function for δ_i . The Y_{it} is the count of the t th observation in the i th group, n is the observed event count for unit i during the time period t , λ_{it} is the Poisson parameter, and Γ is the gamma function. The resulting log-likelihood is

$$\ln L = \sum_{i=1}^n \omega_i \left\{ -\ln \Gamma(r) - \ln \Gamma(s) - \ln \Gamma\left(r + s + \sum_{k=1}^{n_i} \lambda_{ik} + \sum_{k=1}^{n_i} y_{ik}\right) + \sum_{t=1}^{n_i} [\ln \Gamma(\lambda_{it} + y_{it}) - \ln \Gamma(\lambda_{it}) - \ln \Gamma(y_{it} + 1)] \right\},$$

where $\lambda_{it} = \exp(x_{it}\beta + \text{offset}_{it})$ for estimates $k = 1 \dots n_i$ and ω_i is the weight for the i th group. Four models were examined: (1) output of products, (2) balance or duodenal availability of nutrients, (3) intake of nutrients, or (4) percentage of nutrients in the diet. Statistical significance was accepted if $P < 0.05$. Diets ($n = 3$) that resulted in 0 or 100% pregnancy (Lucy et al., 1991, 1992; Markus et al., 1996) created instability in the models and were removed from analyses. Collinearity among variables was explored for all models developed using the Collin function in Stata that provides the variance inflation index and condition number. Collinearity, as indicated by a condition index > 50 (data not provided) for starting models in the multivariable analysis for CPM-estimated metabolizable AA and duodenal fatty acids, was present and model coefficients were unstable during backward stepping procedures. If more than one strongly collinear variable qualified for entry into a model, one variable was chosen based on biological significance, rather than solely on P -values. Plausible quadratic relationships for variables were evaluated. Because of collinearity observed among CPM-predicted fatty acid intakes that had individual positive associations with the proportion pregnant,

Table 2. Summary of mean, SD, minimum, and maximum values observed in the experiments and estimated by CPM¹; all estimates are based on 118 observations, except for BW (n = 113) and BCS (n = 109)

Variable	Mean	SD	Minimum	Maximum
BW (kg)	588.5	38.6	488	663
BCS (1–5)	2.8	0.3	2.3	3.7
Milk production (kg/d)	33.4	6.1	19.1	52.9
Milk protein (%)	3.1	0.2	2.7	3.7
Milk protein yield (kg/d)	1.0	0.2	0.6	1.6
Milk fat (%)	3.5	0.4	2.3	4.3
Milk fat yield (kg/d)	1.2	0.2	0.6	1.7
DMI (kg/d)	20.6	2.7	10.2	25.1
ME balance (MJ/d)	7.1	26.0	–58.5	84
MP balance (g/d)	–102.9	291.9	–799	712.3
CP (%)	18.0	2.4	10.8	25.6
CP eaten (kg/d)	3.7	0.7	1.7	6.0
RUP (% CP)	36.2	5.5	20.4	50.4
RUP eaten (kg/d)	1.4	0.4	0.6	2.2
RDP (% CP)	63.8	5.5	49.6	79.6
RDP eaten (kg/d)	2.4	0.5	1.1	4.3
Soluble protein (% CP)	34.1	6.5	13.0	57.9
Soluble protein eaten (kg/d)	1.3	0.4	0.5	3.1
Urea cost (mJ/d)	0.8	2.0	0	11.6
Methionine (g/d)	45.4	6.7	24.5	58.7
Lysine (g/d)	147.3	24.0	62.5	188.9
Arginine (g/d)	143.0	24.4	59.5	198
Threonine (g/d)	108.0	16.2	49.8	136.9
Leucine (g/d)	184.0	32.5	104.5	260.4
Isoleucine (g/d)	111.8	17.2	54.9	139.9
Valine (g/d)	126.6	21.2	60.2	179.6
Histidine (g/d)	58.2	10.2	26.9	79.7
Phenylalanine (g/d)	114.9	18.7	57.5	148.9
Tryptophan (g/d)	34.5	5.9	15	51.2
Total EAA (g/d)	1,073.7	167.3	515.3	1,345.1
Ether extract (%)	4.8	1.6	1.8	8.6
Long-chain fatty acids (%)	3.8	1.5	1.3	7.5
C12:0 intake (g/d)	6.5	6.3	0	37.5
C14:0 intake (g/d)	7.8	6.5	1.0	31.4
C16:0 intake (g/d)	164.3	98.1	36.4	482.2
C16:1 intake (g/d)	5.1	6.6	0.8	41.9
C18:0 intake (g/d)	35.4	48.1	5.0	367.8
C18:1 <i>trans</i> intake (g/d)	1.9	5.8	0	40.2
C18:1 <i>cis</i> intake (g/d)	160.6	93.5	9.6	494.6
C18:2 intake (g/d)	322.0	164.5	92.8	876.7
C18:3 intake (g/d)	65.2	61.5	16.1	531.2
C other intake ² (g/d)	17.9	10.4	6.4	64.4
C12:0 duodenal availability (g/d)	6.5	6.3	0	37.5
C14:0 duodenal availability (g/d)	7.8	6.5	1.0	31.4
C16:0 duodenal availability (g/d)	178.4	94.1	9.8	486.5
C16:1 duodenal availability (g/d)	4.3	0.9	2.2	8.8
C18:0 duodenal availability (g/d)	456.6	147.0	175.5	869.2
C18:1 <i>trans</i> duodenal availability (g/d)	71.6	52.7	12.2	315.3
C18:1 <i>cis</i> duodenal availability (g/d)	44.2	34.9	5.3	206.7
C18:2 duodenal availability (g/d)	39.6	23.9	6.1	170.9
C18:3 duodenal availability (g/d)	3.9	4.0	0.5	27.2
C other duodenal availability ² (g/d)	52.1	8.5	26.1	84.9
Sugar (%)	5.1	2.3	2.1	13
Starch (%)	26.2	7.6	8.9	47.9
NDF (%)	31.9	4.7	14.4	46.1
Physically effective NDF (%)	23.2	3.9	5.7	31.8
NFC (%)	40.8	5.6	27.8	56.5
Fermentable carbohydrate total intake (kg/d)	8.7	1.2	4.8	11.2
NDF intake (kg/d)	2.1	0.5	0.2	3.5
Starch intake (kg/d)	4.3	1.3	1.5	8.5
Soluble fiber intake (kg/d)	1.2	0.5	0.3	2.5
Sugar intake (kg/d)	1.0	0.5	0.2	2.6
Calcium (%)	0.9	0.3	0.3	1.5
Phosphorous (%)	0.5	0.1	0.3	1.0
Magnesium (%)	0.3	0.1	0.2	0.5

¹CPM-Dairy (version 3.08; Cornell-Penn-Miner, <https://cahpwww.vet.upenn.edu/archives/9-Purchase-CPM-3.0.8.1.html>).

²The C other are fatty acids other than those listed in Table 2 and with more than 12 carbon atoms.

these were combined (for those individual fatty acids that had $P < 0.20$) into positive fatty acids intake (including C14:0, C16:0, C16:1, C18:0, C18:1 *trans*, C18:1 *cis*, and other fatty acids intake) and positive fatty acids duodenal availability (including C14:0, C16:0, C16:1, C18:0, C18:1 *cis*, and other fatty acids duodenal; Table 3). These variables were tested in intake and balance or duodenal availability multivariable models, respectively. Similarly, CPM-predicted metabolizable AA were combined and assessed as a single variable as these were also collinear. Model fit during development of the final model was evaluated using the Wald statistic. No final models had a condition index >10 and mean variance inflation factors were <4 (data not shown).

Dietary or production variables were used as predictors to explore their associations with calving to pregnancy interval. For those analyses, the difference in interval to pregnancy between a given treatment and the reference treatment within an experiment was the response of interest. Random effects standardized mean difference (SMD), also called effect size (ES) meta-analysis, was used following the method of DerSimonian and Laird (1986) as outlined by Rodney et al. (2015). If the experiment reported separate estimates of the measure of variance [standard error (SE) or standard deviation (SD)] for each treatment, these were recorded as such. Many experiments reported a common SE or SD, and these estimates were used for the multiple treatments. In experiments in which SE was reported, an SD was derived before analysis. Some experiments reported only exact P -values and these were used to estimate SD, or, in experiments in which nonsignificance was indicated without an exact P -value, $P = 0.50$ was used to determine the related SD. To explore sources of heterogeneity of response arising from diet, for each dietary variable the differences between a treatment and the reference treatment within an experiment were calculated and a random effects meta-regression analysis (METAREG; Higgins and Thompson, 2002) was used to screen individual variables. For fat, starch, or energy and mineral interventions, the reference treatment was defined as the diet containing less of the ingredient of interest, or the most commonly used variety of the ingredient based on the a priori hypothesis that increasing fat, starch, or energy or mineral intake would reduce calving to pregnancy interval. Alternatively, increasing the amount of CP or degradability of protein in the diet has negative effects on fertility (Lean et al., 2012). Consequently, the reference treatment for experiments using protein comparisons was classified as that with the most CP or RDP inclusion. The individual relative risk was used for each diet as the outcome and the associated SE was

used as the measure of variance. A smaller number of comparisons were available for inclusion in our analysis as a result of the fewer diets identified reporting calving to pregnancy interval and the unit of interest being the difference between a treatment and the reference treatment for each comparison. As a result, a multivariable analysis was not conducted for calving to pregnancy interval. A random-effects weighted mean difference (WMD) between the treatment group and reference group is provided for the interval to pregnancy, with the weighting reflecting the inverse of the variance of the treatments included according to the *nostandard* method in the metan model of Stata to allow an interpretation of treatment effects in familiar units (d) rather than ES. A P -value of <0.05 was accepted as being significant and $P > 0.05$ and < 0.1 were identified as trends.

RESULTS

Literature Review and Assessment

The detailed systematic review identified more than 60,000 results across the 3 databases. If a reason for exclusion could be clearly identified in the title of a paper, the experiment was excluded during the screening phase. Such exclusions included experiments not in English, experiments that used *Bos taurus indicus*, crossbred cows, or nulliparous nonlactating cows, experiments unrelated to cattle or transition nutrition, abstracts, and reviews. Some experiments contained a single treatment, whereas others reported 2 or more treatments, which were assessed separately. Of the 334 experiments that remained for eligibility, the main reason treatments were excluded from the meta-analysis was that they were not completely randomized experiments with a single treatment assigned per cow (i.e., were reviews, case studies, or Latin square or crossover designs) that assessed nutrition and reproduction during the transition period (65 experiments). Studies that included changes in nutritional intervention during the transition period that could not be adequately quantified from the data reported, including those that used an injectable intervention (160 experiments) and those that used *Bos taurus indicus* crossbred, beef, or only nulliparous cows (36 experiments), or did not report the reproductive variables specified for inclusion (e.g., ovulation or reproductive hormone concentrations) or unit of interest was the oocyte or conceptus (34 experiments) were excluded. Some of the treatments excluded after assessment for eligibility examined valid interventions, but the experiment contained a lack of detail about the diet or feed intake was not accurately measured (e.g., pasture or group feeding), making the

Table 3. Univariable effects of production and dietary factors affecting pregnancy to AI and calving to pregnancy interval¹

Item (values on DM basis)	Pregnancy per AI			Calving to pregnancy interval		
	Incidence rate ratio (SE)	95% CI	P-value	Model coefficient effect size ² (SE)	95% CI	P-value
Milk production (kg/d)	0.99 (0.006)	0.981 to 1.004	0.199	0.07 (0.024)	0.024 to 0.121	0.004
Milk protein (%)	0.75 (0.148)	0.513 to 1.108	0.150	0.59 (0.485)	−0.393 to 1.568	0.233
Milk protein yield (100 g/d)	0.996 (0.002)	0.992 to 1.0002	0.066	0.05 (0.026)	−0.006 to 0.097	0.082
Milk fat (%)	0.86 (0.071)	0.730 to 1.010	0.066	−0.27 (0.233)	−0.742 to 0.198	0.249
Milk fat yield (kg/d)	0.996 (0.002)	0.993 to 0.999	0.009	0.14 (0.137)	−0.140 to 0.412	0.325
DMI (kg/d)	1.00 (0.014)	0.975 to 1.029	0.909	0.09 (0.075)	−0.067 to 0.237	0.266
ME balance (MJ/d)	1.01 (0.001)	1.002 to 1.008	0.001	−0.01 (0.005)	−0.021 to −0.002	0.020
MP balance (g/d)	1.00 (0.0001)	0.999994 to 1.001	0.055	0.0001 (0.0002)	−0.0003 to 0.001	0.614
CP (%)	0.99 (0.015)	0.962 to 1.019	0.507	0.04 (0.022)	−0.004 to 0.084	0.077
CP intake (kg/d)	0.98 (0.050)	0.888 to 1.086	0.727	0.23 (0.115)	−0.006 to 0.459	0.056
RUP (% CP)	1.01 (0.007)	0.998 to 1.023	0.115	0.002 (0.007)	−0.011 to 0.015	0.783
RUP intake (kg/d)	1.078 (0.109)	0.882 to 1.312	0.470	0.15 (0.130)	−0.113 to 0.415	0.254
RDP (% CP)	0.99 (0.006)	0.977 to 1.002	0.114	−0.002 (0.007)	−0.015 to 0.011	0.781
RDP intake (kg/d)	0.92 (0.070)	0.796 to 1.070	0.289	0.16 (0.142)	−0.122 to 0.450	0.254
Soluble protein (% CP)	0.998 (0.006)	0.986 to 1.009	0.707	−0.003 (0.006)	−0.014 to 0.009	0.646
Soluble protein intake (kg/d)	0.96 (0.084)	0.811 to 1.142	0.657	0.01 (0.143)	−0.276 to 0.304	0.923
Urea cost (MJ/d)	1.03 (0.017)	0.996 to 1.061	0.082	−0.002 (0.035)	−0.073 to 0.069	0.953
Methionine intake (g/d)	0.995 (0.005)	0.985 to 1.006	0.357	0.02 (0.010)	−0.004 to 0.037	0.105
Lysine intake (g/d)	0.999 (0.002)	0.996 to 1.002	0.482	0.01 (0.003)	−0.001 to 0.012	0.082
Arginine intake (g/d)	0.9998 (0.002)	0.997 to 1.003	0.903	0.004 (0.002)	−0.001 to 0.008	0.109
Threonine intake (g/d)	0.998 (0.002)	0.994 to 1.003	0.508	0.01 (0.005)	−0.0004 to 0.019	0.061
Leucine intake (g/d)	0.999 (0.001)	0.997 to 1.001	0.424	0.003 (0.002)	−0.001 to 0.007	0.138
Isoleucine intake (g/d)	0.999 (0.002)	0.994 to 1.003	0.570	0.01 (0.004)	−0.003 to 0.014	0.162
Valine intake (g/d)	0.999 (0.002)	0.996 to 1.003	0.781	0.01 (0.003)	−0.002 to 0.011	0.155
Histidine intake (g/d)	0.998 (0.004)	0.990 to 1.005	0.564	0.01 (0.006)	−0.003 to 0.023	0.114
Phenylalanine intake (g/d)	0.999 (0.002)	0.995 to 1.003	0.576	0.01 (0.003)	−0.001 to 0.012	0.116
Tryptophan intake (g/d)	0.99 (0.006)	0.980 to 1.004	0.186	0.01 (0.012)	−0.010 to 0.038	0.254
Total EAA intake (g/d)	0.9998 (0.0002)	0.9994 to 1.0003	0.536	−0.003 (0.004)	−0.001 to 0.001	0.448
Ether extract (%)	1.05 (0.025)	1.001 to 1.097	0.047	−0.02 (0.047)	−0.110 to 0.078	0.734
Long-chain fatty acids (%)	1.05 (0.026)	1.002 to 1.103	0.039	−0.02 (0.051)	−0.124 to 0.082	0.678
C12:0 intake (g/d)	0.998 (0.006)	0.986 to 1.009	0.687	−0.02 (0.047)	−0.110 to 0.081	0.760
C14:0 intake (g/d)	1.02 (0.005)	1.007 to 1.026	0.001	0.01 (0.013)	−0.020 to 0.034	0.593
C16:0 intake (g/d)	1.00 (0.0003)	0.9999 to 1.001	0.116	−0.0003 (0.001)	−0.002 to 0.001	0.645
C16:1 intake (g/d)	1.01 (0.004)	1.005 to 1.023	0.001	0.01 (0.014)	−0.018 to 0.038	0.490
C18:0 intake (g/d)	1.00 (0.001)	1.001 to 1.003	0.001	−0.001 (0.001)	−0.003 to 0.001	0.367
C18:1 <i>trans</i> intake (g/d)	1.01 (0.005)	1.001 to 1.021	0.024	0.0003 (0.021)	−0.042 to 0.042	0.987
C18:1 <i>cis</i> intake (g/d)	1.00 (0.0004)	1.0004 to 1.002	0.003	0.00003 (0.001)	−0.002 to 0.002	0.972
C18:2 intake (g/d)	1.00 (0.0002)	0.9996 to 1.00049	0.933	0.0001 (0.001)	−0.001 to 0.002	0.850
C18:3 intake (g/d)	0.9999 (0.00048)	0.999 to 1.001	0.788	−0.002 (0.005)	−0.012 to 0.009	0.764
C other intake (g/d)	1.01 (0.003)	1.003 to 1.014	0.004	−0.001 (0.004)	−0.009 to 0.007	0.767
C12:0 duodenal availability (g/d)	0.998 (0.006)	0.986 to 1.009	0.688	−0.01 (0.047)	−0.109 to 0.083	0.789
C14:0 duodenal availability (g/d)	1.02 (0.005)	1.007 to 1.026	0.001	0.01 (0.013)	−0.020 to 0.034	0.593
C16:0 duodenal availability (g/d)	1.00 (0.0004)	0.9999 to 1.001	0.102	−0.0003 (0.001)	−0.002 to 0.001	0.659
C16:1 duodenal availability (g/d)	1.08 (0.038)	1.011 to 1.158	0.023	−0.05 (0.121)	−0.292 to 0.196	0.693
C18:0 duodenal availability (g/d)	1.00 (0.0002)	1.000003 to 1.001	0.048	−0.0003 (0.001)	−0.001 to 0.001	0.642
C18:1 <i>trans</i> duodenal availability (g/d)	1.00 (0.001)	0.999 to 1.002	0.601	0.001 (0.002)	−0.003 to 0.005	0.610
C18:1 <i>cis</i> duodenal availability (g/d)	1.00 (0.001)	1.00004 to 1.004	0.045	−0.00001 (0.002)	−0.004 to 0.004	0.995
C18:2 duodenal availability (g/d)	1.00 (0.002)	0.997 to 1.005	0.511	−0.0004 (0.002)	−0.005 to 0.004	0.834
C18:3 duodenal availability (g/d)	1.00 (0.009)	0.986 to 1.020	0.763	−0.004 (0.016)	−0.037 to 0.028	0.780
C other duodenal availability (g/d)	1.01 (0.004)	0.999 to 1.015	0.087	−0.003 (0.005)	−0.014 to 0.008	0.606
Sugar (%)	0.95 (0.013)	0.929 to 0.981	0.001	−0.01 (0.064)	−0.136 to 0.123	0.919
Starch (%)	1.02 (0.005)	1.006 to 1.025	0.001	−0.01 (0.011)	−0.032 to 0.012	0.358
NDF (%)	0.99 (0.009)	0.974 to 1.011	0.400	0.01 (0.020)	−0.029 to 0.052	0.555
Physically effective NDF (%)	0.97 (0.011)	0.945 to 0.989	0.003	−0.03 (0.036)	−0.097 to 0.047	0.487
NFC (%)	1.01 (0.008)	0.992 to 1.022	0.399	−0.03 (0.015)	−0.055 to 0.006	0.113
Total fermentable carbohydrate intake (kg/d)	0.97 (0.032)	0.907 to 1.032	0.311	−0.07 (0.097)	−0.267 to 0.124	0.464
NDF intake (kg/d)	0.82 (0.071)	0.694 to 0.972	0.022	0.21 (0.227)	−0.245 to 0.672	0.353
Starch intake (kg/d)	1.09 (0.034)	1.023 to 1.155	0.007	−0.05 (0.071)	−0.194 to 0.091	0.469
Soluble fiber intake (kg/d)	0.81 (0.054)	0.715 to 0.927	0.002	−0.06 (0.235)	−0.532 to 0.418	0.809
Sugar (kg/d)	0.79 (0.053)	0.689 to 0.896	0.000	−0.07 (0.310)	−0.698 to 0.555	0.818
Calcium (%)	1.16 (0.161)	0.881 to 1.519	0.294	−0.50 (0.514)	−1.535 to 0.541	0.339

Continued

Table 3 (Continued). Univariable effects of production and dietary factors affecting pregnancy to AI and calving to pregnancy interval¹

Item (values on DM basis)	Pregnancy per AI			Calving to pregnancy interval		
	Incidence rate ratio (SE)	95% CI	P-value	Model coefficient effect size ² (SE)	95% CI	P-value
Phosphorous (%)	1.96 (0.577)	1.099 to 3.487	0.023	2.10 (1.185)	−0.295 to 4.495	0.084
Magnesium (%)	1.09 (0.649)	0.338 to 3.505	0.887	3.27 (2.449)	−1.678 to 8.221	0.189

¹Factors associated with proportion pregnant to service were examined using a negative binomial regression while factors associated with calving to pregnancy interval were assessed using a random effects standardized mean difference (SMD) (effect size) meta-analysis and meta-regression. Differences between a treatment and the reference treatment within experiment for each production and dietary variable were calculated for use in the meta-regression.

²Standard deviation = 28.7 d.

diets unsuitable for extraction and inclusion in the current study.

The systematic review identified 39 experiments containing 118 individual treatments that were suitable for inclusion in our analyses of proportion pregnant and calving to pregnancy interval. Of these, 35 experiments with 109 treatments reported proportion of pregnant cows and 23 experiments containing 42 comparisons reported calving to pregnancy interval. The treatments examined a range of dietary interventions with varying inclusion rates and timings of intervention. Few experiments provided details on prepartum diets; hence, the meta-analysis focused on postpartum, early-lactation diets. A further 3 experiments (Colazo et al., 2009; Nowak et al., 2013; Badiel et al., 2014) provided details of precalving diets only and were not included in this analysis. If an experiment reported multiple precalving treatments and a common postcalving diet, the postcalving treatment was used for the multiple groups, but these were included in the analysis as separate treatments. Descriptive statistics of dietary components and production outputs is provided in Table 2.

Reproduction Outcomes

The systematic review identified 35 experiments, reporting 109 treatments that provided details of the proportion pregnant. The mean proportion pregnant was 0.44 (SD = 0.16). Because of the complex nature

of, and likely collinearity between, components of the diets, dietary variables and production outputs were classified as reporting the (2) output of nutrient including production, (2) balance or duodenal availability of nutrient, (3) intake amount, or (4) percentage of nutrient in the diet, and these were explored as 4 separate models. Univariable analyses of the associations between each variable and fertility outcomes are detailed in Table 3 and multivariable models including the random effect of experiment for factors affecting the proportion pregnant are described in Table 4.

Proportion Pregnant to AI

(1) Outputs. In the univariable model (Table 3), milk fat yield (0.996 ± 0.002 kg/d; $\pm$ SE) was negatively associated ($P = 0.009$) and milk fat percentage (0.86 ± 0.071) and milk protein yield (0.996 ± 0.002 kg/d) tended to be negatively associated with pregnancy to AI ($P = 0.066$). Although milk production (kg/d) and milk protein percentage were not associated ($P \geq 0.15$) with pregnancy to AI (Table 3), they were both included in the multivariable models (Table 4). We noted a tendency for urea cost (MJ/d) to be positively associated (1.03 ± 0.017 , $P = 0.082$) with pregnancy to AI (Table 3). The multivariable model, accounting for the random effect of experiment, resulted in a negative association of milk protein yield (0.922 ± 0.022 100 g/d; $P = 0.001$) with pregnancy to AI (Table 4).

Table 4. Multivariable negative binomial regression models assessing associations between dietary components or production factors and pregnancy to AI¹

Model	Covariable	Incidence rate ratio (SE)	95% CI	P-value
Outputs	Milk protein yield (100 g/d)	0.992 (0.022)	0.881 to 0.965	0.001
Balance and duodenal availability	ME balance (MJ/d)	1.004 (0.002)	1.001 to 1.008	0.017
	C14:0 duodenal (g/d)	1.008 (0.004)	1.0005 to 1.015	0.037
	Fatty acid intake (g/d)	1.0003 (0.0001)	1.000 to 1.001	0.050
Intake	Starch (kg/d)	1.061 (0.029)	1.006 to 1.118	0.029
	Sugar (kg/d)	0.813 (0.054)	0.713 to 0.927	0.002
	Sugar (% DM)	0.960 (0.015)	0.930 to 0.990	0.010

¹Models accounted for the random effect of experiment.

(2) Nutrient Balance and Duodenal Availability. The nutrient balance and duodenal availability model was used to analyze associations of CPM-estimated ME balance (MJ/d), MP balance (g/d), and duodenal availabilities of several individual fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1 *cis*, and other, g/d; Table 3) with pregnancy to AI. The negative association for CPM-estimated metabolizable EAA availabilities (g/d) with pregnancy to AI were also considered in the development of the multivariable model. When examined in the multivariable model, CPM-estimated ME balance (1.004 ± 0.002 MJ/d) and duodenal C14:0 (1.008 ± 0.004 g/d) availability remained in the model, both having positive relationships with pregnancy to AI ($P = 0.017$ and 0.037 , respectively, Table 4). The combined positive association between duodenal fatty acid availability (g/d) was also considered for inclusion in this model, but resulted in poorer model fit than the final model that included only C14:0 (g/d).

(3) Intake. The intake model identified positive associations between CPM-estimated individual fatty acid intakes (C14:0, C16:0, C16:1, C18:0, C18:1 *trans*, C18:1 *cis*, and other intake, g/d) or starch intake (kg/d) with pregnancy to AI (Table 3). The CPM-estimated NDF (0.82 ± 0.071 kg/d), soluble fiber (0.81 ± 0.054 kg/d), and sugar intakes (0.79 ± 0.053 kg/d) were negatively associated with pregnancy to AI ($P = 0.022$, 0.002 , and 0.001 , respectively; Table 3). The multivariable model included combined positive fatty acids intake (g/d), starch intake (kg/d), and sugar intake (kg/d). Fatty acid intake (1.0003 ± 0.0001 g/d) and starch intakes (1.061 ± 0.029 kg/d) were positively associated with pregnancy to AI ($P = 0.05$ and 0.029 , respectively), whereas increasing sugar intake (0.813 ± 0.54 kg/d) was associated with decreased pregnancy to AI ($P = 0.002$, Table 4).

(4) Diet Percentage. In the univariable model, positive associations of the CPM-estimated dietary percentage of ether extract, long-chain fatty acids, starch, and P, and negative associations of the dietary percentage of physically effective NDF and sugar were identified with pregnancy to AI (Table 3). Sugar percentage alone remained in the multivariable model, having a negative association with pregnancy to AI (0.960 ± 0.015 , $P = 0.01$, Table 4).

Calving to Pregnancy Interval

Twenty-three experiments containing 42 comparisons reported calving to pregnancy interval. The meta-analysis indicated that interventions designed to improve reproductive performance during the transition period tended to numerically reduce the calving to pregnancy

interval (SMD = -0.05 ; WMD = 0.71 d less; 95% CI = -3.40 to 1.97 d), and this result was homogeneous [I^2 (transformation of the square root of the χ^2 heterogeneity statistic divided by its df describing the proportion of total variation in treatment estimates due to heterogeneity) = 0.0% ; Figure 1]. As expected from previous meta-analyses (Lean et al., 2012; Rodney et al., 2015), diets that increased the amount of fat (WMD = 1.64 less d; 95% CI = -5.28 to 2.01 d) or decreased the amount or degradability of protein in the diet (WMD 7.41 d less; 95% CI = -13.18 to -1.63) were associated with a shorter calving to pregnancy interval (Figure 1), although this was only a tendency for the diets reporting interventions using fats. Increasing dietary starch or energy density tended to increase calving to pregnancy interval, although only 3 diets examined the latter intervention and the confidence interval was large (95% CI = -0.14 to 0.70 ; Figure 1). We noted mixed associations with mineral-based interventions increasing the calving to pregnancy interval (WMD = 6.77 d increased; 95% CI = 0.82 to 12.54), reflecting varying responses between and within the same experiment. When examined using meta-regression, an increased CPM-estimated difference in ME balance (MJ/d) between the treatment and reference treatments was associated with shorter calving to pregnancy interval (ES = -0.01 ± 0.005 per MJ difference in ME balance per day; $P = 0.02$), whereas an increased difference in milk production (kg/d) was associated with longer calving to pregnancy interval (ES = 0.07 ± 0.024 per kg difference in milk production; $P = 0.004$; Table 3). An increase of 10 MJ/d of difference in ME balance would result in -0.1 ES or 2.87 d shorter interval to pregnancy based on the SD of 28.7 d for pregnancy interval. Increased differences between the treatment and reference treatments in CP percentage (ES = 0.04 ± 0.022 per percentage difference in CP), CP intake (ES = 0.23 ± 0.115 per kg/d difference in CP intake), P percentage (ES = 2.10 ± 1.185 difference in P intake), and milk protein yield (ES = 0.05 ± 0.026 per 100 g/d difference in milk protein yield) tended to be associated ($P < 0.09$) with longer calving to pregnancy interval (Table 3). Increased CPM-estimated metabolizable AA intake, particularly of lysine (ES = 0.01 ± 0.003 per g/d difference in lysine intake) and threonine (ES = 0.01 ± 0.005 per g/d difference in threonine intake), tended to be associated with increased calving to pregnancy interval ($P = 0.082$ and 0.061 , respectively), as did the difference in Mg (ES = 3.27 ± 2.449 ; $P = 0.189$) percentage of the treatment and control diets, whereas the differences in NFC percentage (ES = -0.03 ± 0.015 , $P = 0.113$) tended to be associated with decreased interval (Table 3).

Figure 1. Forest plot of individual standardized mean difference (SMD), 95% CI, and weights for comparisons of calving to pregnancy interval of cows fed different nutritional interventions during the early lactation period (weighted mean differences are provided in results). For fat, starch or energy, and mineral comparisons, the reference treatment was the one with less of the ingredient of interest. For protein-based comparisons, the reference treatment was the one with more CP or RDP. Estimates were made of the SMD using a random effects method (DerSimonian and Laird, 1986). The weights that each comparison contributed are in the right-hand column and are indicated by the size of the box. The larger the box, the greater the contribution of that comparison to the overall estimate. The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in days to pregnancy, whereas points to the right of the line indicate an increase. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effect size. The overall pooled effects size and 95% CI are indicated by the diamonds at the bottom of each fat group. The overall effect was highly homogeneous, as indicated by the I^2 of 0.00% (transformation of the square root of the χ^2 heterogeneity statistic divided by its df, describing the proportion of total variation in treatment estimates due to heterogeneity), indicating little error attributable to measurement error.

DISCUSSION

This study provides a quantitative evaluation of dietary factors and production outputs that are associated with the probability of pregnancy to service and time to pregnancy from 118 diets contained within 39 experiments. The dietary data were extracted rigorously according to previously described methods (Rabiee et al., 2012) using feed data from the experiments themselves and from the CPM-Dairy (v. 3.08) feedbank and calibrating these according to tested data from the experiments. Notwithstanding the rigor applied to diet extraction, and the choice of the CPM model (as it is the most extensively tested; Bateman et al., 2001; Moate et al., 2004, 2006; Tedeschi et al., 2008), limitations in the estimates of dietary components exist. These include limitations or errors of the model, variations in composition and timing of the diets fed, and feed analysis estimations. It is anticipated that these errors are likely to be nondifferential; that is, neither favoring nor supporting the key hypotheses. Under these conditions, errors in estimation are likely to drive hypotheses toward the null and, therefore, our findings are likely to be robust. Although interventions in the prepartum and early lactation period can have long lasting effects on metabolism and fertility, the proximity of dietary changes, for example of protein, to the time of conception or pregnancy can also be crucial. We focused on nutritional interventions fed during the early postpartum period, but it is worth noting that all but 3 of the diets of Canfield et al. (1990) were fed not just in this early lactation but for at least 30 d into the breeding period. The choice of statistical models used was determined by the use of count data; that is, the proportion of cows pregnant. The decision was made to use a negative binomial, random effects model based on the clustering of treatments within experiments, and overdispersion of the data (Neuhaus et al., 1991). The term overdispersion reflects an evaluation that the variance estimated is larger than the expected count (Rabe-Hesketh and Skrondal, 2008).

Extensive literature has observed that cows with greater milk production generally have poorer fertility (Spalding et al., 1975; Lean et al., 1989; Butler, 2000; Westwood et al., 2002; Pryce et al., 2004), and that genetic selection for increased production can reduce fertility (Hageman et al., 1991; Buckley et al., 2000; Horan et al., 2005; Pollott and Coffey, 2008). Whereas genetic differences were not examined in this data set, we identified associations between increased milk fat (kg/d) and protein production (kg/d) with reductions in the proportion pregnant, actual milk yield (kg/d), and milk protein yield (100 g/d) with longer calving to pregnancy interval (Table 3). Milk protein yield (100

g/d) remained in this final model (Table 4), having a greater significance than in the univariable model. The multivariable model accounted for the random effect of experiment, which was an important factor influencing relationships in proportion pregnant, estimated as contributing approximately 30% of the variance in this outcome in our study. However, protein yield in very early lactation (first 3 wk of lactation) was positively associated with the proportion of first services that resulted in pregnancy (Rodney et al., 2016), and others identified positive associations between milk protein percentage and improved reproductive performance (Buckley et al., 2003; Madouasse et al., 2010; Morton et al., 2016). These findings, overall, highlight possible differences between experiments conducted at the level of the individual and those at the group level. As milk protein production increases in a group of cows, it may be expected that nutrient intakes will need to be more closely aligned with nutrient losses, whereas the individual within the herd with greater production may have better phenotypic adaptation to the environment allowing greater milk protein yield and percentage.

The availability of nutrients that can be allocated to reproduction is not just determined by immediate diet (i.e., the immediate intake of nutrients as DMI), but also by endogenous body tissue reserves, reflected in labile BW and BCS (Lean and Rabiee, 2006). Hence, DMI before and after calving is a key determinant of exogenous nutrient availability. The irreversible loss of nutrients in milk production and use of nutrients for maintenance and growth diminishes the nutrient pool available for reproduction (Baldwin et al., 1987; Friggens et al., 2013). The difference between dietary intake and expenditure determines the nutrient balance, and if a negative balance occurs endogenous reserves are depleted. Many studies have examined the effects of estimated negative energy balance on fertility (Wathes et al., 2007). The length and severity of a negative energy balance at the onset of lactation is largely determined by DMI around calving (Villa-Godoy et al., 1988) and milk yield (Westwood et al., 2002). Estimated energy balance (MJ/d) was, as anticipated, positively associated with improved proportion pregnant and shorter calving to pregnancy interval (Tables 2 and 3). A better energy balance during the first 3 to 4 wk of lactation reduces the interval to first ovulation and increases the probability of pregnancy at the following breeding (Butler and Smith, 1989). Excessively low or high BCS at calving, or extreme losses of BW or BCS in early lactation, are usually associated with impaired reproductive outcomes (Heuer et al., 1999; Pryce et al., 2001; Buckley et al., 2003; López-Gatius et al., 2003). Body weight and BCS change are often used as proxies for energy balance, although they may more correctly

be interpreted as a reflection of nutrient balances of protein, minerals, specific fatty acids and vitamins, as well as energy.

Although less well explored than ME balance, MP balance is also important for successful reproduction. In our study, improved early-lactation MP balance tended ($P = 0.055$) to increase the proportion pregnant (Table 3). Rodney et al. (2016) found that increased prepartum MP balance slightly decreased the proportion of first services that resulted in pregnancy, highlighting the difference in pre- and postpartum metabolism. Notably, the prepartum MP balance (Rodney et al., 2016) was markedly positive, exceeding 400 g/d on average. In contrast, van Saun et al. (1993) found greater milk protein percentage and a trend to fewer services per conception in cows fed more RUP before calving; those cows did not gain weight, suggesting a lesser MP balance than those of Rodney et al. (2016). Increasing CP intake may increase nutrient loss via increased milk production and have negative effects on fertility (Lean et al., 2012), in association with higher urea nitrogen concentrations in blood (Westwood et al., 1998). Increasing CP content of the diet does not necessarily increase MP availability, but decreasing the degradability of protein or increasing the fermentability of the diet may be more effective in increasing MP availability.

Alterations in dietary protein may not simply affect MP balance, but also specific AA composition and supply of metabolizable AA. Specific roles for AA in reproductive performance are not well defined. Lysine and methionine have been suggested to be the most co-limiting AA for production, and supplementation of these may increase milk yield, but results are inconsistent (Rogers et al., 1989; Doepel et al., 2004; Ardalan et al., 2010). Supplementation of lactating cows with rumen-protected methionine or lysine has had positive or negligible effects on reproductive outcomes (Rogers et al., 1989; Ardalan et al., 2010). The current study focused on diets in the early lactation period (Table 1), whereas other studies (Rogers et al., 1989; Ardalan et al., 2010) supplemented cows later in lactation (from 4 wk). It is notable that the combined EAA (g/d) reduced the proportion of cows pregnant in a univariable model when the effect of experiment was considered (incidence rate ratio = 0.9996 ± 0.0002 ; $P = 0.045$). Limitations in these data, including a lack of specifically detailed AA profiles of the feeds, may have in part prevented stronger relationships from being observed. Given the importance of protein and the need to increase understandings of roles of specific AA, this should be an area for further investigation.

The tendency for increased protein intake, dietary percentage, or degradability to be associated with reduced calving to pregnancy interval (Figure 1) is

consistent with other studies (Folman et al., 1981; Carroll et al., 1994; Butler, 2000; Westwood et al., 2002; Lean et al., 2012). Butler (1998) reviewed the means by which feeding excess protein can be detrimental. The tendency ($P = 0.084$) for CPM-estimated urea cost (MJ/d) to be positively associated with the proportion of cows pregnant was surprising, and a similarly positive relationship between estimated urea costs and fertility was identified in a meta-analysis examining the effects of feeding fats on fertility (Rodney et al., 2015). This relationship may indicate interactions between dietary components, in particular between CPM-estimated microbial protein production and rumen-available fats, rather than simply the effect of protein content of the diet per se. Optimal levels of proteins and nitrogen in diets containing additional fat need to be reviewed.

Microbial lipolysis and biohydrogenation in the rumen ensure that intake of fatty acids and those available for absorption in the duodenum differ; hence, these issues were explored separately. Fats not only provide an energy source but also are essential precursors for steroid hormones, and the beneficial effects of fat have been observed independently of the provision of energy (Staples et al., 1998). We noted a strong correlation between individual fatty acids and substantial instability identified, as indicated by marked changes in coefficients, in the development of multivariable models when stepwise removal of individual fatty acids was conducted (data not shown). In the current study, intakes of many of the fats (C14:0, C16:1, C18:0, C18:1 *trans*, C18:1 *cis*, and other, g/d) were univariably associated with an increased proportion of cows pregnant (Table 3). These were combined into a single variable for the multivariable intake analysis to account for the collinearity among the fats. Other fatty acids include those other than those listed in Table 2 that contain more than 12 carbon atoms and include fatty acids with more than 18 carbon atoms, but not C18:3. Consequently, we found no association between C18:3 identified with the proportion of cows pregnant, but fatty acids present in fish oil, docosahexaenoic acid, docosapentaenoic acid, and eicosapentaenoic acid are included in the other fatty acids associated with the proportion of cows pregnant. Strong correlation and collinearity among individual fatty acids was also identified in the balance and duodenal availability model and, whereas a positive association between duodenal myristic acid (C14:0; g/d) and proportion pregnant was identified, this finding may indicate a positive effect of fats on pregnancy, in general, rather than a specific effect of myristic acid intake per se. The positive effects of feeding fats on reproductive outcomes, both proportion pregnant and calving to pregnancy interval (Tables 2 and 3), observed in our study are consistent

with a previous meta-analysis (Rodney et al., 2015) that found feeding fats improved (27% increase in relative risk) the proportion of cows pregnant and tended to decrease calving to pregnancy interval. In particular, positive effects of CLA on fertility have been identified (de Veth et al., 2009); however, the limited number of diets that fed CLA in this data set and outputs from CPM-Dairy precluded individual investigation, and further examination of this area may be beneficial.

Carbohydrates are important sources of energy for cows, as well as for rumen microorganisms and generally increase the efficiency of protein utilization and microbial protein production (Hoover and Stokes, 1991; Aldrich et al., 1993; Hristov et al., 2005). However, increased concentrations of rapidly fermentable carbohydrates can increase the risk of acidosis (Plaizier et al., 2008). The type of carbohydrate also influences the risk of acidosis, with sugars posing a greater risk than starches (Golder et al., 2012). Consequently, carbohydrates have positive and negative effects on fertility. The positive associations of starch percentage and intake (kg/d), possibly because of a slower fermentation rate than sugars, and negative associations of soluble fiber and sugar percentage and intake (kg/d) with proportion pregnant were identified in univariable analyses (Tables 2 and 3). We also found a negative association between increased CPM-estimated physically effective NDF intake (kg/d) and proportion pregnant in the univariable analysis. The only variable that remained in the multivariable models was the effect of sugar intake (kg/d), which was negatively associated with the proportion of cows pregnant. Ruminant acidosis may decrease fertility by reducing feed intake, producing a metabolic acidosis leading to detrimental alteration of uterine environment, stimulating inflammation that induces prostaglandin release, and resulting in luteolysis in a process analogous to that of mastitis (Moore et al., 1991; Zebeli et al., 2015).

The positive univariable association between P percentage and the proportion of cows pregnant is consistent with studies in beef cattle that found improved fertility outcomes with increased dietary P concentration (Hart and Michell, 1965). However, studies in dairy cattle have not identified an influence of dietary P on reproduction (Carstairs et al., 1980; Wu and Satter, 2000; Wu et al., 2000), possibly because of the body's ability to provide P from skeletal stores or because a smaller study power did not allow a rigorous assessment. Ferris et al. (2010) examined cattle over 4 lactations in a study designed to examine long-term depletion of P stores, and found numerical, yet non-statistical improvements in several fertility parameters, although this work lacked the study power to examine these robustly.

CONCLUSIONS

This study highlights several important findings for future research on the effects of transition nutrition on fertility. It confirms that nutritional management of cows during the transition period can have substantial effects on reproductive success, and this finding is consistent with previous meta-analytical studies in this area. The number of well-described diets that were able to be included in the analysis was limited, and less than expected. This lack of large numbers of suitable treatments and the complexity of responses of the cattle to these treatments strongly suggest a crucial need for further focused field experiments exploring the roles of nutrition on reproduction and interactions among dietary components. Overall, this study confirmed earlier findings that excessive protein intake can impair fertility, but that a positive MP balance is consistent with better fertility. However, it may be necessary to increase protein intake when feeding fats, and other work suggests a need to control the MP balance before calving. The role of specific metabolizable AA needs further study. This study confirms the positive effects of feeding fats and highlights a need for detailed studies in this area. We also, critically, identified potential effects of specific carbohydrate fractions, especially sugar (kg/d), starch (kg/d), and physically effective NDF (kg/d) on reproductive outcomes. The importance of increased energy and protein balances in early lactation for improved fertility outcomes was supported.

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