Casein infusion rate influences feed intake differently depending on metabolizable protein balance in dairy cows: A multilevel meta-analysis

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ABSTRACT

The effects of casein infusion have been investigated extensively in ruminant species. Its effect on responses in dry matter intake (DMI) has been reviewed and indicated no significant effect. The literature reviewed in the current meta-analysis is more extensive and limited to dairy cows fed ad libitum. A total of 51 studies were included in the meta-analysis and data were fitted to a multilevel model adjusting for the correlated nature of some studies. The effect size was the mean difference calculated by subtracting the means for the control from the casein-infused group. Overall, casein infusion [average of 333 g of dry matter (DM)/d; range: 91 to 1,092 g of DM/d] tended to increase responses in DMI by 0.18 kg/d ($n = 48$ studies; 3 outliers). However, an interaction was observed between the casein infusion rate (IR) and the initial metabolizable protein (MP) balance [i.e., supply minus requirements (NRC, 2001)]. When control cows were in negative MP balance ($n = 27$ studies), responses in DMI averaged 0.28 kg/d at mean MP balance (-264 g/d) and casein IR (336 g/d), and a 100 g/d increment in the casein IR from its mean increased further responses by 0.14 kg/d (MP balance being constant), compared with cows not infused with casein. In contrast, when control cows were in positive MP balance ($n = 22$ studies; 2 outliers), responses in DMI averaged -0.20 kg/d at mean casein IR (339 g/d), and a 100 g/d increment in the casein IR from its mean further decreased responses by 0.33 kg/d, compared with cows not infused with casein. Responses in milk true protein yield at mean casein IR were greater (109 vs. 65 g/d) for cows in negative vs. positive MP balance, respectively, and the influence of the casein IR on responses was significant only for cows in negative MP balance. A 100 g/d increment in the casein IR from its

mean increased further responses in milk true protein yield by 25 g/d, compared with cows not infused with casein. Responses in blood urea concentration increased in casein studies ($+0.59$ mM) and the influence of the casein IR was greatest for cows in positive MP balance (0.26 vs. 0.11 mM per 100 g/d increment). Responses in DMI were also correlated negatively with responses in blood urea concentration only for cows in positive MP balance. Together, these results suggest an association between satiety and deamination and oxidation of AA supplied in excess of requirements for cows in positive MP balance. Therefore, casein stimulated appetite in cows fed MP-deficient diets possibly via the supply of orexigenic AA or through a pull effect in response to an increased metabolic demand. Conversely, casein induced satiety in cows fed diets supplying MP in excess of requirements. Not precluding other factors involved in satiety (e.g., insulin, gut peptides), casein could have increased the supply of AA (e.g., Ser, Thr, Tyr), which might depress appetite at the brain level or increase the deamination and the oxidation of AA in oversupply in agreement with the hepatic oxidation theory.

Key words: dairy cow, meta-analysis, casein, dry matter intake, hepatic oxidation theory

INTRODUCTION

Dry matter intake regulation in ruminants is a complex issue that involves physical and chemical characteristics of dietary ingredients (e.g., NDF), and brain sensitivity to humoral signals (Ketelaars and Tolkamp, 1992; Allen, 2000; Roche et al., 2008). Some AA also appear to have the potential to regulate DMI. Feed intake was decreased in cows fed MP-deficient diets, but the supply of rumen-protected AA diminished (Lys and Met) or even corrected (His) the difference in DMI (Lee et al., 2012). Conversely, i.v. injections of nonphysiological levels of Gly, Ala, and Lys decreased feed intake in ruminants (Baile and Forbes, 1974), and Ser, Thr, and Tyr were identified as centrally acting anorexigenic signals in dairy cows (Laeger et al., 2012).

Received September 21, 2015.

Accepted December 18, 2015.

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In addition, the accumulated evidence is persuasive that the liver plays an important role in satiety. In laboratory species, the infusion of substances oxidizable in the liver (e.g., glucose) depressed food intake, whereas the infusion of substances not oxidized in the liver had no effect on food intake, even though they could be metabolized in the liver (Forbes, 1992). In a review, Friedman (1997) reported that (1) the liver ATP concentration was important in the regulation of food intake because the portal infusion of a phosphate-trapping agent (L-ethionine) triggered feeding in rats; and (2) the liver energy status was relayed to the brain feeding centers via the hepatic branch of the vagal nerve because vagotomy prevented the eating response. Therefore, the liver may play a role in the regulation of food intake because it is sensitive to anaplerotic fuels (e.g., glucogenic AA, glycerol, lactate, propionate) increasing the concentration in ATP.

The involvement of substrate oxidation in DMI regulation is a relatively new concept in dairy cattle and was termed the hepatic oxidation theory (**HOT**) of the control of food intake (Allen et al., 2009). Application of this concept to dairy cows is appealing because it integrates the effects of various fuels (e.g., nonesterified fatty acids, glucogenic AA) and physiological states on feeding behavior (Allen et al., 2005; Allen, 2014). However, the demonstration of the HOT concept in ruminants presents a challenge in feeding experiments because ruminal fermentation affects the type and pattern of absorbed fuels (Allen et al., 2009). Moreover, responses in DMI can be subtle and difficult to demonstrate in dairy cows because most of the studies are conducted on a limited number of animals and lack sufficient statistical power.

In contrast to feeding studies, abomasal or duodenal infusion of a protein source has a minimal effect on rumen metabolism and dietary characteristics besides total energy and MP supply (Faverdin, 1999; Chamberlain and Yeo, 2003). Therefore, a review based on infusion studies appears ideal to evaluate responses in DMI to a known increment in MP. Casein and other protein sources have been infused over the last 4 decades in dairy cows, and so far little or no effect on DMI was reported (see review: Faverdin, 1999; Allen, 2000; Patton et al., 2015). However, conclusions from these reviews need to be revisited with updated literature, using new statistical tools available for meta-analysis.

The current meta-analysis reviewed the effect of casein infusion on responses in DMI in cows fed ad libitum with a special emphasis on the influence of the casein infusion rate on responses in DMI when the initial balance of MP was either negative or positive. It was hypothesized that the rate of casein infusion nega-

tively influences responses in DMI when cows are in positive MP balance due to increased oxidation of AA in oversupply, in line with the HOT concept. Therefore, the objectives of the current meta-analysis were to (1) report a new methodological approach, taking into account the hierarchical nature of studies in the data set, (2) determine the effect of CN on responses in DMI in cows fed ad libitum, and (3) explore the influence of the CN infusion rate and other factors that could explain part of the heterogeneity.

MATERIALS AND METHODS

Data Sources

The Scopus online database was searched in February 2015 for studies where casein was infused postruminally in dairy cows with the terms: “casein or caseinate”, “abomasal or duodenal”, and “cow* or dairy”. To be included in the meta-analysis, the studies were required to (1) include a casein (**CAS**) treatment consisting of CN infused postruminally; (2) include a control (**CTL**) treatment consisting of water or saline also infused postruminally (plus a substrate if infused at the same rate in the CAS treatment); (3) report the sample size (*n*) and a measure of variance; (4) feed the forage or complete ration ad libitum; (5) report information on feed intake, feed ingredients, or ration composition; and (6) be published in English or French.

Reasons for the exclusion of studies were (1) use of CN hydrolysate (Seymour et al., 1990) or formaldehyde-treated CN (Broderick et al., 1972), (2) composition of diet not being similar in CTL and CAS treatments (Derrig et al., 1974; Rogers et al., 1984; Cant et al., 1991), (3) unsuitable randomization (Istasse et al., 1986), (4) absence of negative or suitable controls (Choung and Chamberlain, 1993c; Røjen et al., 2013), and (5) extremely low level of estimated MP supply in control cows (<800 g/d, Rogers et al., 1979). In Schwab et al. (1992), DL-Met was not infused with CN, but the DL-Met infusion was accepted as the negative control because DL-Met was not first-limiting compared with L-Lys. The final data set contained 51 studies from 26 manuscripts. A summary description of studies included in the data set is given in Table 1.

Data Extraction and Calculations

The current meta-analysis was conducted on mean DMI in CTL and CAS treatment groups. The mean DMI in CAS treatment did not include the DM from the casein infusate. Animal and dietary variables were evaluated for their influence on responses in DMI to ca-

Table 1. Characteristics of studies included in the data set

Authors and year	Details ¹	Study ²	Exp ²	Lab ²	Casein IR ³ (g/d)	DMI ⁴ (kg/d)		MP balance	Stage	Forage
						Control	Casein			
Vik-Mo et al., 1974	Exp 3	1	1	1	300	13.5	12.7	Positive	Mid/late	Grass/legume
Vik-Mo et al., 1974	Exp 3	2	1	1	600	13.5	12.4	Positive	Mid/late	Grass/legume
Vik-Mo et al., 1974	Exp 3	3	1	1	1,200	13.5	11.8	Positive	Mid/late	Grass/legume
Clark et al., 1977	No glucose	4	2	2	422	18.5	18.7	Positive	Mid/late	Grass/legume
Clark et al., 1977	Glucose	5	2	2	422	18.1	18.6	Positive	Mid/late	Grass/legume
Ørskov et al., 1977	Exp 1	6	3	3	300	10.9	11.1	Negative	Early	Grass/legume
Cohick et al., 1986		7	4	2	395	18.7	19.0	Positive	Early	Corn
Seymour et al., 1990	Soy	8	5	4	400	17.9	17.1	Positive	Mid/late	Corn
Seymour et al., 1990	Soy-gluten	9	5	4	400	17.1	17.2	Positive	Mid/late	Corn
Choung and Chamberlain, 1992a	No additive	10	6	5	230	14.2	14.9	Positive	Early	Grass/legume
Choung and Chamberlain, 1992a	Enzyme	11	6	5	230	14.6	14.8	Positive	Early	Grass/legume
Choung and Chamberlain, 1992b		12	7	5	230	13.8	14.4	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1992c		13	8	5	230	14.3	14.9	Positive	Mid/late	Grass/legume
Choung and Chamberlain, 1992c	Exp 1	14	9	6	400	20.4	20.3	Positive	Early	Corn
Schwab et al., 1992	Peak	15	9	6	400	21.1	21.9	Negative	Early	Corn
Schwab et al., 1992	Early	16	9	6	333	21.2	21.8	Negative	Mid/late	Corn
Schwab et al., 1992	Mid	17	9	6	266	18.6	17.8	Positive	Mid/late	Corn
Schwab et al., 1992	Late	18	10	5	110	13.6	13.7	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1993a		19	10	5	220	13.6	13.7	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1993a		20	10	5	330	13.6	14.3	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1993b	LA 0	21	11	5	230	14.4	14.4	Positive	Mid/late	Grass/legume
Choung and Chamberlain, 1993b	LA 50	22	11	5	230	13.6	13.4	Positive	Mid/late	Grass/legume
Choung and Chamberlain, 1993b	LA 100	23	11	5	230	12.8	13.1	Positive	Mid/late	Grass/legume
Dhiman et al., 1993	Exp 1; Trt 3	24	12	7	1,000	18.4	19.6	Negative	Early	Grass/legume
Choung and Chamberlain, 1995a	Exp 1	25	13	5	180	12.7	13.1	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1995a	Exp 2	26	14	5	230	11.0	11.3	Positive	Mid/late	Grass/legume
Choung and Chamberlain, 1995b	Exp 1	27	15	5	100	13.9	14.2	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1995b	Exp 1	28	15	5	200	13.9	14.4	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1995b	Exp 1	29	15	5	400	13.9	14.4	Negative	Mid/late	Grass/legume
Choung and Chamberlain, 1995b	Exp 2	30	16	5	160	13.6	14.2	Positive	Mid/late	Grass/legume
Choung and Chamberlain, 1995b	Exp 2	31	16	5	320	13.6	13.4	Positive	Mid/late	Grass/legume
Vicini et al., 1995	Pre-bST	32	17	8	454	21.1	20.7	Negative	Early	Corn
Vicini et al., 1995	bST	33	17	8	454	21.8	22.4	Negative	Early	Corn
Grinari et al., 1997	-INS	34	18	9	500	20.0	19.6	Positive	Mid/late	Grass/legume
Grinari et al., 1997	+INS	35	18	9	500	14.0	18.4	Negative	Mid/late	Grass/legume
Huhtanen et al., 1997	FA	36	19	10	400	18.6	18.2	Negative	Early	Grass/legume
Huhtanen et al., 1997	LAB	37	19	10	400	17.6	18.1	Negative	Early	Grass/legume
Mackle et al., 1999	No BCAA	38	20	9	600	24.8	23.7	Positive	Mid/late	Grass/legume
Mackle et al., 1999	BCAA	39	20	9	600	24.3	24.1	Positive	Mid/late	Grass/legume
Abramson et al., 2002		40	21	11	350	13.1	13.8	Negative	Mid/late	Corn
Abramson et al., 2002		41	21	11	700	13.1	13.8	Negative	Mid/late	Corn
Khalili and Huhtanen (2002)	Duodenum only	42	22	10	300	15.1	15.7	Negative	Mid/late	Grass/legume
Khalili and Huhtanen (2002)	Rumen/duodenum	43	22	10	300	15.7	16.3	Negative	Mid/late	Grass/legume
Vanhatalo et al., 2003a	No glucose	44	23	10	300	19.8	19.3	Negative	Early	Grass/legume
Vanhatalo et al., 2003a	Glucose	45	23	10	300	19.5	18.6	Negative	Early	Grass/legume
Vanhatalo et al., 2003b		46	24	10	300	16.4	16.4	Negative	Mid/late	Grass/legume
Pelling and Reynolds, 2008		47	25	12	790	23.7	23.5	Positive	Mid/late	Corn

Continued

Table 1 (Continued). Characteristics of studies included in the data set

Authors and year	Details ¹	Study ²	Exp ²	Lab ²	Casein IR ³ (g/d)	DMI ⁴ (kg/d)		Categorical moderators ⁵		
						Control	Casein	MP balance	Stage	Forage

Choi et al., 2013
Larsen et al., 2014
Larsen et al., 2014
Larsen et al., 2014

4 DIM
15 DIM
29 DIM

48
49
50
51

26
27
27
27

13
14
14
14

270
696
490
212

14.7
14.3
18.8
22.1

15.4
14.9
17.8
19.9

Positive
Negative
Negative
Negative

Mid/late
Early
Early
Early

Grass/legume
Corn
Corn
Corn

¹LA = lactic acid; INS = insulin; FA = formic acid; LAB = lactic acid bacteria; BCAA = branched-chain AA.
²Hierarchical structure of studies in the data set. Studies were nested within experiments (Exp) and experiments were nested within laboratories (Lab; refer to the text).
³Casein infusion rate (IR; as-is basis).
⁴DMI (kg/d) for each treatment group.
⁵Negative or positive MP balance (supply minus requirements in control cows; NRC, 2001). Stage of lactation at start of experiment = early (≤ 100 DIM) or mid/late (> 100 DIM).
Type of forage = grass/legume- versus corn silage-based diets.

sein infusion. Continuous variables included rate of CN infusion (kg of DM/d), ration parameters, and nutritional balances (Allen, 2000; Hristov et al., 2004). Unless indicated otherwise in the manuscript, the amount of CN infused was assumed to be reported on an as-is basis and to contain 91.0% DM (NRC, 2001). The CTL and CAS rations were reconstituted as described in Martineau et al. (2011). The following parameters from the control ration were included in the data set: CP (% DM), RDP (% DM and % CP), proportion of concentrate (%), NDF (% DM), forage NDF (% DM; **fNDF**), ADF (% DM), ether extract (% DM; **EE**), NFC (% DM), TDN (% DM), and NE_L (Mcal/kg).

Nutritional balances (dietary supply minus requirements; NRC, 2001) of control cows in RDP (kg of DM/d), MP (kg of DM/d), and NE_L (Mcal/d) were determined using the following default values if necessary: DIM = 100 d; milk NPN concentration = 5%; milk fat = 3.42%; milk lactose = 4.60%. Body weight was missing in 16 studies and assumed to be 602 or 564 kg for North American cows or other, respectively (Huhtanen and Hristov, 2009).

Three categorical variables were tested: (1) stage of lactation at the start of the experiment (early ≤ 100 DIM; mid/late > 100 DIM), (2) type of forage (grass/legume: grass/legume/mixed hay or silage or straw; corn silage), and (3) MP balance (negative or positive) of control cows. Stage of lactation was missing in Clark et al. (1977), but the information was obtained from Istasse et al. (1986). Stage of lactation was not reported in Vik-Mo et al. (1974) and Choi et al. (2013), but cows were assumed to be in mid/late lactation based on milk yield (≤ 18 kg/d). Note that MP balance was tested as a categorical variable only if the interaction between MP balance values and the CN infusion rate was significant (see below).

Dispersion Measures. Dispersion measures were converted into standard deviation when aggregating data. For studies reporting standard error of the mean (or standard error), standard deviation was calculated by multiplying standard error of the mean by $\sqrt{n}$; and for studies reporting the standard error of the difference between any 2 means, standard deviation was calculated by multiplying standard error of the difference by $\sqrt{n}/\sqrt{2}$. A mixed procedure was used in 6 studies and the same conversion factors were applied. In experiments using a factorial arrangement of treatments, the dispersion measure of the interaction term was selected to calculate standard deviation.

Effect Size and Statistical Weight. The effect size for statistical analyses was the mean difference (**MD**) in DMI (kg/d):

$$\text{Effect size: MD} = \text{CAS} - \text{CTL},$$

calculated with the *escalc* function of the metafor package (version 1.9–5) in R (version 3.1.2, R Foundation for Statistical Computing, Vienna, Austria; R Development Core Team. 2008). In metafor, more precise studies with a larger sample size or low SD have more of an effect on results. The statistical weight was the inverse of variance corrected by the addition of σ^2 (Viechtbauer, 2010).

Forest Plot. A forest plot was constructed using standardized mean difference (SMD) values calculated with the *escalc* function of metafor by dividing MD values by their pooled standard deviation (SD_{pooled}) adjusted for the positive bias associated with small samples (Hedges and Olkin, 1985):

$$SD_{\text{pooled}} = \sqrt{[SD_1^2(n_1 - 1) + SD_2^2(n_2 - 1)] / [n_1 + n_2 - 2]},$$

$$SMD = MD / SD_{\text{pooled}} \times \left\{ 1 - 3 / [4 \times (n_1 + n_2) - 9] \right\},$$

where n_1 and n_2 refer to the number of replica for CAS and CTL treatments, respectively. Therefore, SMD values in the forest plot took into account the variability in each study.

Statistical Models

Multilevel Model. Use of the metafor package in R has been described by Appuhamy et al. (2013) and Bougouin et al. (2014). The principles behind the metafor package are the same as the methodology proposed by St-Pierre (2001). In contrast with previous meta-analyses in R, a multilevel model was used to recognize the hierarchical nature of studies included in the data set (Konstantopoulos, 2011; Viechtbauer, 2014). For example, the current meta-analysis included effect sizes created from (1) graded CN infusion rates compared with a common control (e.g., Vik-Mo et al., 1974), (2) repeated measurements on the same cows throughout lactation (Larsen et al., 2014), (3) studies from a factorial arrangement of treatments (e.g., Clark et al., 1977), and (4) studies conducted either by the same authors or at the same research center (e.g., Hannah Research Institute, UK). Indeed, the underlying true effects are expected to be more similar for such studies, in violation of the principle that studies should be statistically independent in a meta-analytic review (Wang and Bushman, 2007).

To account for this form of dependency or correlation among studies, 2 grouping variables were created to cluster studies originating from the same experiment and experiments originating from the same laboratory (Table 1). The random effects of study, experiment,

and laboratory were specified as a list of one-sided formulas in the random argument of the *rma.mv* function of metafor. The REML method was used and the effect sizes with the same level within each grouping variable received the same random effect; otherwise, effect sizes were assumed to be independent (Viechtbauer, 2010). The variance component (σ^2) was divided into 3 parts to indicate where most of the variation lied, that is, between (1) studies nested within experiments, (2) experiments nested within laboratories, and (3) laboratories.

Global Effect Size and Meta-Regression. The global effect size summarizes the effect of CN infusion on responses in DMI across studies under the assumption of perfect control conditions. In a meta-regression, the true effect size is a function of both the fixed effect of moderators and the random effects of study and clustering variables plus a certain amount of residual heterogeneity. Meta-regressions explain part of the heterogeneity, improve conclusions, and may identify important new research topics.

All continuous moderators were centered on their mean. Centering moderators does not change the regression coefficients, but the intercept will be the global effect size estimate at the mean value of all the moderators included in the model (and not at the zero value of moderators if not centered). Centering moderators allows the interpretation of regression coefficients in terms of change in the response in DMI (kg/d) for a 1 unit change in a moderator from its mean (all other moderators being constant).

The *mod* argument of the *rma.mv* function of metafor (REML method) was used to test the meta-regressions. In a first step, the influence of each moderator was evaluated separately; however, for a continuous moderator, the meta-regression included the quadratic term and the interaction between each categorical moderator (i.e., stage of lactation and type of forage) and the continuous moderator. The meta-regression was reduced by removing not significant ($P > 0.10$) variables. In a second step, a multivariate meta-regression included all significant ($P \leq 0.10$) moderators and interaction terms, and the infusion rate of CN which was forced into the meta-regression because it was the variable of interest. A backward stepwise selection procedure was used to eliminate (one at a time) not significant variables and the multivariate meta-regression was further reduced to the smallest number of relevant variables (including the CN infusion rate).

Meta-regressions were compared using the log-likelihood ratio test (LRT; $P \leq 0.10$) of the *anova* function of metafor or the Akaike information criterion (AIC) of the *fitstats* function of metafor. The LRT test was used to compare hierarchical meta-regressions with different number of variables (maximum likelihood

method). Alternatively, the AIC was used to compare meta-regressions with the same number of moderator and the same studies.

Outlier Management. Sensitivity analyses were conducted to detect effect sizes exerting substantial influence on results (Viechtbauer and Cheung, 2010). Graphical assessment of all relationships (response and absolute values) was carried out with Minitab (version 16.2.1, 2010, Minitab Inc., State College, PA). The *influence* function of metafor was also used to detect outliers based on the *rma* function of metafor (not yet available with *rma.mv*). Outliers were removed from the data set if they induced a qualitative change on coefficient terms or *P*-values. Outliers were not removed permanently from the data set because their effect could vary depending on moderators included in the meta-regression.

Tests for Residual Heterogeneity. A significant heterogeneity statistic indicates that the variability in the observed effect sizes (and not accounted for by the moderators in a meta-regression) is larger than ex-

pected based on the sampling variability and the given covariances among the sampling errors (Hedges and Olkin, 1985; Viechtbauer, 2015).

Animal Care and Use Committee approval was not required for this meta-analytic review because all data were obtained from the literature.

RESULTS AND DISCUSSION

The current meta-analysis was conducted on studies where sodium, calcium, or lactic CN was infused postruminally in lactating dairy cows fed ad libitum. A total of 51 studies met the inclusion criteria either from Europe ($n = 32$), North America ($n = 16$), or other continents ($n = 3$). The rate of CN infusion (g/d; as-is basis), DMI (kg/d) for CTL and CAS treatments, and the corresponding SMD value with its 95% of CI (whiskers) are presented in a forest plot along with the author(s) and year arranged in a chronological order (Figure 1). Approximately 60% of studies showed a positive effect of CN infusion on DMI, but this effect

Figure 1. Forest plot showing mean DMI (kg/d) in the control and casein treatments along with the standardized mean difference (boxes) and 95% of CI (whiskers). The dotted line represents a zero standardized mean difference. LA = lactic acid; INS = insulin; FA = formic acid; LAB = lactic acid bacteria; Duo = duodenum; BCAA = branched-chain AA.

Table 2. Description of cow- and ration-associated factors separated by MP balance category¹

Item	Negative MP balance (n = 27)				Positive MP balance (n = 24)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Casein infusion rate (g of DM/d)	336	175.4	91	910	368	212.4	146	1,092
Cow-associated factor ²								
DIM (d)	100	56.7	4	210	119	43.6	28	203
DMI (kg/d)	16.4	3.36	10.9	22.1	16.6	3.87	11.0	24.8
Milk yield (kg/d)	24.7	9.10	12.1	43.4	21.7	6.89	10.7	37.4
Milk fat (%)	4.3	0.69	2.8	5.3	3.9	0.79	3.0	5.4
True milk protein (%)	3.0	0.31	2.5	4.2	3.0	0.20	2.5	3.5
Milk lactose (%)	4.7	0.20	4.4	5.1	4.7	0.08	4.6	4.8
Blood urea (mM)	3.6**	0.94	2.0	5.5	6.0**	3.40	2.0	10.3
Ration-associated factor ³								
MP supply (g/d)	1,453*	397.4	867	2,382	1,733*	511.0	1,143	2,924
MP requirements (g/d)	1,718	501.3	1,116	2,656	1,595	387.6	930	2,277
MP balance (g/d)	-264***	198.3	-751	-21.3	139***	178.9	17	647
NE _L (Mcal/kg of DM)	1.52**	0.081	1.26	1.70	1.58**	0.075	1.48	1.74
NE _L supplied (Mcal/d)	25	5.3	17	35	26	6.1	17	38
NE _L required (Mcal/d)	27†	5.9	19	39	25†	4.4	18	34
NE _L balance (Mcal/d)	-2.5***	2.76	-9.0	3.3	1.7***	2.84	-2.3	8.0
Concentrate (%)	35†	14.9	0	66	42†	13.3	26	64
CP (% of DM)	15.3**	1.35	13.1	18.1	16.5**	1.95	13.5	23.4
RDP (% of DM)	11.0	1.32	9.6	13.7	10.7	2.02	6.6	16.8
RUP (% of DM)	4.23***	1.108	2.69	6.40	5.81***	1.196	3.69	7.62
RDP (% of CP)	72***	6.4	60	83	65***	7.8	48	74
RDP balance (g/d)	211	255.2	-75.1	775	92	339.6	-578	1,112
NDF (% of DM)	40†	5.0	30	47	37†	6.7	28	48
Forage NDF (% of DM)	33	7.2	22	46	30	7.6	18	40
ADF (% of DM)	25†	5.7	17	41	23†	4.2	16	29
Ether extract (% of DM)	2.8**	0.66	1.8	4.0	3.3**	0.48	2.7	4.2
NFC (% of DM)	38	5.0	29	46	39	6.5	22	48
TDN (% of DM)	66*	4.1	54	73	69*	3.8	61	74

¹Mean, SD, minimum (min), and maximum (max) values are reported for each parameter and separated by MP balance category (refer to Table 1). All values were calculated for the control treatment, except the CN infusion rate. Means between negative and positive MP balances were separated by Tukey-Kramer test (1-way ANOVA analysis).

²Number of studies = 27 and 24 for negative and positive MP balance, respectively, except for DIM (n = 24 and 17 studies), milk lactose % (n = 21 and 15 studies), and blood urea concentration (n = 16 and 10 studies).

³Information on feed ingredient composition was not reported in 7 studies. Information on ration composition was not reported in 1 study but was calculated from feed ingredient composition. Supply in MP and balances in RDP, MP, and NE_L were calculated according to NRC (2001).

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; † $P \leq 0.10$.

was significant only in few studies based on SMD 95% CI limits.

Description of the Data Set

Twice as many studies were found for mid/late versus early lactating cows (35 vs. 16), and most cows in early lactation were in negative MP balance (Pearson chi-square, $P = 0.03$; Table 1). Similarly, twice as many studies were found for cows fed grass/legume- versus corn silage-based diets, but the meta-design was balanced (Pearson chi-square, $P = 0.51$).

The studies reviewed in the current meta-analysis covered a large variety of feeding conditions. Inclusion percentages of grass/legume forages, corn silage, grain, and protein sources averaged $62 \pm 14.6\%$ (range: 34 to 100%), $12 \pm 20.8\%$ (range: 0 to 74%), $27 \pm 12.5\%$ (range: 0 to 57%), and $8 \pm 6.6\%$ (range: 0 to 36%), respectively. All-forage diets were fed in Dhiman et al.

(1993) and Khalili and Huhtanen (2002), and protein sources were not fed in Ørskov et al. (1977), Choung and Chamberlain (1993a), Huhtanen et al. (1997), and Abramson et al. (2002). Added urea supplied 10% of total N intake in Vik-Mo et al. (1974) and about 30% of total N intake in Ørskov et al. (1977) and Abramson et al. (2002).

In Table 2, cow- and ration-associated parameters are summarized by MP balance category (negative or positive). The infusion rate of CN averaged 351 ± 192.4 g of DM/d and was greater than 500 g of DM/d in 8 studies (Table 1); therefore, more research is required to increase the accuracy of results at high infusion rate of CN. Cow-associated factors did not differ between MP balance categories, except for blood urea concentration, which was 67% higher ($P = 0.01$) for cows in positive MP balance (Table 2). Estimated MP requirements did not differ between MP balance categories, but estimated MP supply was 16% lower ($P = 0.03$) for cows

in negative MP balance. Supply in NE_L did not differ between MP balance categories, but NE_L requirements tended ($P = 0.07$) to be 11% greater for cows in negative MP balance (Table 2). Although the meta-design was balanced between types of forage and balances in MP, some differences (i.e., NDF, ADF, and TDN) were related to the inclusion of 3 all-forage studies in the negative MP balance category (Table 2).

Effect of CN on Responses in DMI (All Studies)

Global Effect Size. The effect of CN infusion on responses in DMI differed whether the meta-analysis was conducted on all studies or after the removal of 3 outlying studies from Vik-Mo et al. (1974). Before removal of outliers, the heterogeneity was highly significant ($Q = 110$; $P < 0.001$) and 85% of the total heterogeneity was observed at the laboratory level (data not shown). After removal of outliers, the global effect size estimate indicated that the infusion of CN (mean = 333 g of DM/d) tended ($P = 0.10$) to increase responses in DMI by 0.18 kg/d. The heterogeneity was reduced by half ($Q = 60$; $P = 0.10$), mostly at the laboratory level (data not shown). The forest plot clearly showed that the studies from Vik-Mo et al. (1974) appeared discordant compared with the other studies in the data set (Figure 1). Our results differ from those reported in Faverdin (1999) and Allen (2000), but the current review is more exhaustive (e.g., all the research conducted at the Hannah Research Institute is included). Clark (1975) reported that CN infusion increased DMI in Spechter (1972), but data from this early study were not available.

Meta-Regressions. Interactions between categorical and continuous moderators were not significant. When analyzed separately, stage of lactation and type of forage had no influence on responses in DMI, but several continuous moderators did. The infusion rate of CN had no influence on responses in DMI. Fiber and energy moderators had an opposite influence on

responses in DMI: the influence of NDF, fNDF, and ADF was positive ($P \leq 0.001$, data not shown) and that of EE, NFC, TDN, and NE_L was negative ($P \leq 0.022$, data not shown).

A quadratic relationship was observed for the influence of MP balance (kg/d) and NE_L balance (Mcal/d) on responses in DMI:

$$\text{Responses in DMI (kg/d)} = -0.26 (\pm 0.513)$$

$$\times \text{MP balance} - 3.15 (\pm 1.22) \times \text{MP balance}$$

$$\times \text{MP balance} + 0.27 (\pm 0.255)$$

$$(\text{AIC} = 108.0; \sigma^2 = 0.414; n = 51);$$

$$\text{Responses in DMI (kg/d)} = -0.07 (\pm 0.041)$$

$$\times \text{NE}_L \text{ balance} - 0.015 (\pm 0.0089) \times \text{NE}_L \text{ balance}$$

$$\times \text{NE}_L \text{ balance} + 0.25 (\pm 0.236)$$

$$(\text{AIC} = 106.6; \sigma^2 = 0.276; n = 51).$$

The P -value of the quadratic term was 0.010 and 0.098 for the former and latter, respectively. The quadratic relationship suggested a positive slope when balance values were negative, and a negative slope when balance values were positive. Collectively, these results suggest that responses in DMI were influenced by the composition of the diet and the nutritional status of control cows.

Multivariate Meta-Regressions. Although the AIC value indicated a better fit for the meta-regression with NE_L balance, multivariate meta-regressions were developed to the balance in MP because the quadratic term was highly significant as shown above. It was anticipated that some moderators could capture the variability associated with MP balance $\times$ MP balance.

Two multivariate meta-regressions were tested: (1) rate of CN infusion $\times$ MP balance and (2) rate of CN infusion, MP balance, and MP balance $\times$ MP balance. The multivariate meta-regressions also included NDF,

Table 3. Correlation coefficients showing the relationships between the casein infusion rate (IR; kg of DM/d), the dietary contents (% DM) in NDF, forage NDF (fNDF), ADF, ether extract (EE), NFC, TDN, and in NE_L (Mcal/kg); and the nutritional balances (supply minus requirements; NRC, 2001) in MP (kg of DM/d) and NE_L (Mcal/d) in control cows

Item	NDF	fNDF	ADF	EE	NFC	TDN	NE_L	MP balance	NE_L balance
Casein IR	-0.49	-0.46	-0.28	0.33	0.29	0.20	0.20	-0.02	0.09
NDF		0.88	0.80	-0.60	-0.91	-0.84	-0.72	-0.21	-0.28
fNDF			0.85	-0.70	-0.77	-0.84	-0.73	-0.09	-0.26
ADF				-0.58	-0.81	-0.90	-0.77	-0.18	-0.34
EE					0.38	0.71	0.68	0.24	0.36
NFC						0.85	0.69	0.14	0.24
TDN							0.91	0.20	0.34
NE_L								0.15	0.23
MP balance									0.83

Table 4. Meta-regressions showing the influence of moderators on responses in DMI (kg/d) to CN infusion in dairy cows fed ad libitum¹

Item ²	Effect size		Heterogeneity					Outliers ³
	Mean $\pm$ SE	P-value	Lab	Exp	Study	QE-test (df)	P-value	
All studies (n = 51)								
Meta-regression 1								
Intercept	0.01 $\pm$ 0.095	0.94	0.02 (14)	0.00 (27)	0.02 (51)	52 (df = 46)	0.26	
NE _L (Mcal/kg)	-4.13 $\pm$ 1.097	<0.01						
MP balance (kg/d)	0.14 $\pm$ 0.404	0.73						
Casein IR (kg of DM/d)	-0.51 $\pm$ 0.472	0.28						
MP balance $\times$ casein IR	-5.04 $\pm$ 2.328	0.03						
Meta-regression 2 ⁴								
Intercept (negative MP balance)	0.39 $\pm$ 0.141	<0.01	0.04 (14)	0.00 (27)	0.00 (51)	50 (df = 47)	0.34	
Intercept (positive MP balance)	-0.27 $\pm$ 0.125	0.03						
MP balance (negative): casein IR	1.00 $\pm$ 0.673	0.14						
MP balance (positive): casein IR	-2.00 $\pm$ 0.462	<0.01						
1) Studies with negative MP balance (n = 27)								
Meta-regression 3								
Intercept	0.28 $\pm$ 0.113	0.01	0.00 (9)	0.00 (15)	0.00 (27)	18 (df = 24)	0.82	
MP balance (kg/d)	1.78 $\pm$ 0.605	<0.01						
Casein IR (kg of DM/d)	1.40 $\pm$ 0.698	0.04						
2) Studies with positive MP balance (n = 24)								
Meta-regression 4 ⁵								
Intercept	-0.20 $\pm$ 0.117	0.08	0.01 (8)	0.00 (14)	0.00 (22)	17 (df = 20)	0.65	1, 3
Casein IR (kg of DM/d)	-3.34 $\pm$ 0.809	<0.01						

¹Each meta-regression adjusted data for the random effects of studies nested within experiments (Exp), experiments nested within laboratories (Lab), and laboratories (refer to the text and Table 1). Continuous moderators were centered on their mean (from the control treatment). The variance and the number of levels (in parentheses) are reported for Lab, Exp, and Study. The residual heterogeneity statistic (QE-test) and its P-value are reported for each model.

²Meta-regressions were conducted on all studies and on studies where control cows were in negative or positive MP balance according to NRC (2001; refer to Table 1). MP balance = supply minus requirements in kilograms per day (DM basis).

³Studies (refer to Table 1) deemed outliers and not included in the model.

⁴Intercepts were different: $P < 0.001$. MP balance category $\times$ casein infusion rate (IR) had a P -value < 0.001 .

⁵MP balance was not included in meta-regression 4 because $P = 0.20$. Average casein IR = 0.339 kg of DM/d (n = 22 studies).

EE, and NE_L. Among significant fiber moderators, NDF was chosen over fNDF because its AIC value was lowest, and among significant energy moderators, NE_L was chosen in lieu of NFC and TDN because its correlation with NDF was lowest ($r = -0.72$; Table 3). Moderators EE and NDF were not significant and deleted from both multivariate meta-regressions. The AIC and QE-test values indicated that the first multivariate meta-regression explained more variability compared with the second: 99.3 and 51.9 versus 100.8 and 53.7, respectively. The first multivariate meta-regression could not be reduced to less number of relevant variables based on LRT analyses and is presented as meta-regression 1 in Table 4.

Effect of CN on Responses in DMI (by MP Balance Categories)

Meta-Regression 2. An interaction was observed ($P = 0.03$) between MP balance values and the CN infusion rate (meta-regression 1, Table 4). Therefore, a new meta-regression was developed to test the interac-

tion between the rate of CN infusion and the categories of MP balance (negative or positive; meta-regression 2, Table 4). Again, the interaction was significant ($P < 0.001$; Table 4) and the relationship is depicted in Figure 2, taking into account the relative statistical weight of each study. Note that the removal of the apparent outlier (study 3; Table 1) was tested and did not qualitatively change the results (data not shown).

Studies with Negative MP Balance. The global effect size estimate indicated that the infusion of CN (mean = 336 g of DM/d) increased ($P < 0.001$) responses in DMI by 0.39 kg/d (data not shown). Responses in DMI averaged 0.28 kg/d at mean CN infusion rate and a 1 unit (kg of DM/d) increase in the CN infusion rate increased ($P = 0.044$) further responses by 1.4 kg/d (MP balance being constant), compared with cows not infused with CN (meta-regression 3; Table 4).

In the current meta-analysis, responses in milk true protein yield (MTPY) to CN infusion differed ($P = 0.003$) between negative and positive MP balance. Responses in MTPY averaged 109 and 65 g/d at mean infusion rate of CN for cows in negative and positive MP

balance, respectively. For cows in negative MP balance, 1 unit (kg/d) increase in the CN infusion rate increased ($P < 0.001$) further responses by 252 g/d, compared with cows not infused with CN. In contrast, the infusion rate had no influence on responses in MTPY for cows in positive MP balance (data not shown). Cows were in negative MP balance in Khalili and Huhtanen (2002) and Faverdin et al. (2003). In these studies, a MP supply not only increased milk protein yield and DMI by modified the feeding behavior. In Khalili and Huhtanen (2002), CN increased DMI (+4%), the eating time per meal (+17%), and the total chewing time (+6%). In Faverdin et al. (2003), partially hydrolyzed isolates of soybean protein enriched with AA increased DMI (+9%), decreased the number of meals per day (−13%), but cows ate more DM per meal (+27%) and per unit of time (+16%). Taken together, these results suggest that cows in negative MP balance are able to adapt their feeding behavior to face the increased energy demand (i.e., a pull effect on DMI). Alternatively, Lee et al. (2012) hypothesized that milk yield is increased as a consequence of increased DMI. Milk yield and DMI were decreased in MP-deficient diets and the supply of rumen-protected AA diminished (Lys and Met) or even corrected (His) the difference in DMI and

milk yield (Lee et al., 2012). Therefore, CN infusion might also increase DMI by supplying orexigenic AA in dairy cows.

Studies with Positive MP Balance. The global effect size estimate was not different from zero. Responses in DMI averaged −0.2 kg/d at mean CN infusion rate (339 g of DM/d) and a 1 unit (kg of DM/d) increase in CN infusion rate decreased ($P < 0.001$) further responses by 3.34 kg/d, compared with cows not infused with CN (meta-regression 4; Table 4).

Factors inducing satiety have been reviewed (Ingvarsen and Andersen, 2000; Allen et al., 2009; Allen, 2014). Because CN infusion has minimal effect on rumen metabolism, satiety could involve brain sensitivity to humoral signals (e.g., gut peptides, pancreatic hormones), but results are not yet conclusive in dairy cows (Relling and Reynolds, 2008; Relling et al., 2010). Insulin and glucagon are considered important satiety hormones in nonruminants (Woods et al., 2006), but the role of insulin on the regulation of DMI is less clear in dairy cows. Many AA, especially the branched-chain AA, are insulin secretagogues in ruminants (Kuhara et al., 1991). Accordingly, CN increased plasma concentration of branched-chain AA, but with no effect on DMI (Huhtanen et al., 1997; Miettinen and Huhtanen,

Figure 2. Graph showing the influence of the CN infusion rate (kg of DM/d; centered values) on responses in DMI (kg/d) as a function of MP balance (meta-regression 2 in Table 4). Positive MP balance: straight bold line and closed circles; negative MP balance: dashed bold line and open circles. Each response in DMI is drawn proportional to its statistical weight (i.e., the inverse of the corresponding SE). Study 35 (refer to Table 1) is not shown for scaling reasons.

Figure 3. Graph showing the variation in MP supply (g of DM/d) against the amount of MP (g of DM/d) infused by CN. The variation in MP supply was the difference in dietary MP supply (casein minus control treatments) plus the amount of MP infused by CN.

1997). Some AA might also regulate DMI by exerting a central feed intake signaling in dairy cows. Laeger et al. (2012) compared plasma and cerebrospinal fluid (CSF) concentrations of AA and other metabolites of dairy cows before and after a 50% feed restriction. Plasma concentration of Ser, Thr, and Tyr did not change, but CSF concentration was reduced by 20, 24, and 31%, respectively. The authors identified these AA as potential anorexigenic signals and concluded that the decrease in CSF concentration could be an adaptational response to improve appetite when the energy balance is negative. Jordi et al. (2013) administered isomolar doses of AA individually by gavage to rats and found that Arg, Lys, and Glu had a negative effect on food intake. The role of these AA in cows remains to be evaluated in further studies as abomasal or i.v. infusion of Arg (Vicini et al., 1988; Doepel and Lapierre, 2011) and Lys (Robinson et al., 2000) did not alter DMI. Laeger et al. (2012) reported similar CSF concentrations in Arg and Lys before and after feed restriction, but the drop in Glu (−9%) did not reach significance.

Amino acids might also regulate DMI via the HOT concept (Allen and Piantoni, 2013; Allen, 2014). Deamination and oxidation of AA can increase when an oversupply in AA or an imbalance (deficiency of a limiting AA) is present. Most AA can be oxidized in the trichloroacetic acid cycle (via acetyl CoA or cycle intermediates) and generate ATP in the liver (Allen et al., 2009; Allen and Piantoni, 2013). Broad evi-

dence in nonruminant species suggests that oxidation of metabolic fuels, including glucogenic AA, increases the liver energy charge (ATP concentration), which in turn decreases the discharge rate of the hepatic branch of the vagal nerve and signals brain's feeding centers to inhibit feed intake (Friedman, 1997). In dairy cows, the HOT concept might also apply and could explain the depression in feed intake observed during the peripartum period because of the "flood" of readily oxidizable nonesterified fatty acids to the liver (Allen et al., 2009). The HOT concept in dairy cows remains to be clearly demonstrated, but a recent study indicated that metabolic oxidative processes could be associated with satiety in dairy cows because time periods in which cows did not eat coincided with postprandial increases in whole-body net fat and carbohydrate oxidation rates (Derno et al., 2013).

If the HOT concept is correct, glucogenic AA should be oxidized at a greater extent when negative responses in DMI are observed (i.e., responses in DMI and in blood urea concentration should be negatively correlated). The correlation between responses in DMI and in blood urea concentration was negative only for cows in positive MP balance ($r = -0.74$; $P = 0.02$; data not shown). The influence of the CN infusion rate on responses in blood urea concentration was also analyzed. Responses in blood urea concentration averaged 0.59 mM at mean CN infusion rate (351 g of DM/d) and responses tended ($P = 0.08$) to differ for cows in

negative and positive MP balance. For cows in negative MP balance, a 1 unit (kg of DM/d) increase in the CN infusion rate from its mean increased ($P = 0.03$) further responses in blood urea concentration by 1.1 mM, compared with cows not infused with CN. For cows in positive MP balance, the same increment in CN infusion rate increased ($P < 0.001$) further responses by 2.6 mM, compared with cows not infused with CN (data not shown). Collectively, our observations for cows in positive MP balance suggest a link between the lack of response in MTPY, the increased synthesis of urea and the negative response in DMI. This link lends to support the HOT concept for the regulation of DMI in dairy cows when the supply of MP exceeds requirements for production and maintenance.

Effect of Variation in DMI Responses

Assuming that CN contains 92.7% CP on a DM basis (NRC, 2001) and has a true intestinal protein digestibility of 100% (Huhtanen and Hristov, 2010), the amount of MP infused ranged from 84 to 1,012 g/d. Because cows on the CAS treatment did not eat the same amount of DM as cows on the control treatment (range -2.2 up to 4.4 kg of DMI/d; Table 1), this creates a deficit (or surplus) in estimated MP that should be deducted from (or added to) the amount of MP infused by CN. In the data set, the variation in MP supply (or adjusted MP supply) ranged from -66 to 953 g/d (y-axis in Figure 3). Although the correlation between the variation in MP supply and MP infused by CN was high ($r = 0.87$), values were quite discordant in some studies (e.g., study 35). Therefore, future meta-analyses reviewing the effects of CN should test the influence of the variation in MP supply and not the rate of CN infusion itself, except for responses in DMI (due to a circular argument).

CONCLUSIONS

Overall, the average postruminal supply of 333 g of CN/d (DM basis) tended to increase DMI by 0.18 kg/d in cows fed ad libitum. However, a significant interaction was present between the infusion rate of CN and the initial MP balance (supply minus requirements). Casein infusion increased DMI by 0.39 kg/d only in cows in negative MP balance. The rate of CN infusion had a positive influence on responses in DMI and in MTPY in MP-deficient diets, but had a negative influence on responses in DMI and no influence on responses in MTPY when MP was supplied in excess of requirements. The rate of CN infusion had a greater influence on responses in blood urea concentration when cows were in positive MP balance. Taken together, these re-

sults suggest an increased deamination and oxidation of AA provided in excess of requirements and not directed toward MTPY. Notwithstanding other factors that could induce satiety (e.g., supply of anorexigenic AA), our results lend to support the HOT concept for the control of food intake. To test the influence of supplemental MP presented to the intestine, the amount of MP infused by CN should be corrected by the difference in estimated dietary MP supply.

ACKNOWLEDGMENTS

The authors gratefully thank Steve Méthot (Dairy and Swine Research and Development Centre; Sherbrooke, QC, Canada) and Robin White (National Animal Nutrition Program, University of Kentucky, Lexington, KY) for their statistical advice and contribution for this manuscript. Appreciation is also extended to Dairy Farmers of Canada (Ottawa) and Agriculture and Agri-Food Canada (Ottawa) for the financial support and to Valacta (Sainte-Anne-de-Bellevue, Canada).

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