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A Meta-analysis of Ruminal Outflow of Nitrogen Fractions in Dairy Cows

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Abstract

Data from the scientific literature and statistical methods were used to estimate the magnitude and significance of the effects of the amount and source of dietary crude protein (CP) supplements on the supply of N fractions passing to the small intestine of lactating dairy cows. The passage of total N and nonammonia N from the rumen was influenced by the source of CP in the control diet and only marginally by the source of ruminally un-degradable protein (RUP) in the dietary treatment. Even though NH_3N did not appear to limit growth of the microbes, overall flow of microbial N from the rumen was depressed when RUP partially replaced other sources of CP in the diet. This response tended to be affected by the source of RUP in the dietary treatment. The passage of nonammonia, nonmicrobial N to the duodenum increased when cows consumed diets that contained RUP supplements. The magnitude of this response, however, was distinctly altered by the source of CP in the control diet with which the RUP treatment was compared. In addition, the proportion and source of total CP supplied by RUP in the treatment diet tended to modulate the response. Feeding RUP resulted in a significant increase in the ruminal outflow of total and essential amino acids but the magnitude of this response depended on the source of RUP. Feeding some RUP sources quantitatively improved the delivery of methionine and lysine to the small intestine. Therefore, variability exists in the ruminal outflow of N fractions when different sources of RUP are fed to cows. A portion of this variation is explained by the source of CP in the control diet, the source of RUP in the dietary treatment, the amino acid composition of the dietary CP, and the CP percentage of the diet.

Keywords: Rumen un-degradable protein; Amino acids; Meta-analysis; Dairy cow

Abbreviations

AA: Amino acids; ARNB: Apparent rumen nitrogen balance; BM: Blood meal; CORN: Corn protein sources of reduced ruminal protein degradability (corn gluten meal, brewers dried or wet grains); CP: crude protein; CPS: Control protein source; DIM: Days in milk; DM: Dry matter; DMI: Dry matter intake; EAA: Essential amino acids; FM: Fish meal; Lys: Lysine; MCP: Microbial crude protein; Met: Methionine; MIX: Mixtures of marine, animal, and plant RUP sources; MN: Microbial nitrogen; MP: Metabolizable protein; N: Nitrogen; n: Number of treatment comparisons; NAN: Nonammonia nitrogen; NANMN: Nonammonia nonmicrobial nitrogen; NEAA: Nonessential amino acids; NH_3N: Ammonia nitrogen; NI: Total nitrogen intake; NRC: National Research Council; NSC: Nonstructural carbohydrates; OM: Organic matter; OMTRD: Organic matter truly digested in rumen; PLANT: Plant protein sources other than soybean meal (canola meal, cottonseed meal, sunflower meal, whole soybeans, or whole horse beans); PRUP: Percentage of supplemental RUP in the treatment diet; Qb: Variation between groups of treatment comparisons; RDP: Rumen degradable protein; RDPI: Intake of RDP; REML: Restricted maximum likehood algorithm; RMSPE: Root mean squared prediction error; RUP: rumen un-degradable protein; RUPS: Rumen un-degradable protein source; SBM: soybean meal; SOY: Heated soybean meal, extruded soybean meal, whole roasted soybeans; TAA: Total amino acid flow; TN: Total nitrogen flow; VPS: Extruded cottonseed meal, whole roasted horse beans, heated alfalfa meal, feather meal.

Introduction

Supplying dairy cows with the proper quantity and pattern of essential amino acids (EAA) in Metabolizable Protein (MP) is required for maximizing their productivity and efficiency of protein utilization for milk production. Research indicates that Microbial Crude Protein (MCP) can supply the largest proportion of Amino Acids (AA) needed by well-fed dairy cows that are producing up to about 30 kg of milk daily [1]. As milk production increases, however, the resulting higher demand for EAA must be supplied from dietary CP that escapes ruminal fermentation [2].

The approach most frequently considered for improving the quantity and profile of EAA that reach the small intestine of dairy cows is to feed CP supplements high in Rumen Un-degradable Protein (RUP) [2-4]. Recent comprehensive integrations of published data from studies conducted to assess milk and milk protein responses to RUP feeding, however, show that the productive benefits of such an approach are rather small [5] and are overestimated by current protein systems [6].

Most research with fistulated dairy cows has focused on replacing soybean meal with heat-treated oilseeds and oilseed meals (mainly soybeans and soybean meal), byproducts of corn processing (mainly corn gluten meal and brewers dried or wet grains), and byproducts of animal and fish processing (mainly blood meal and fish meal; see Table 1 for references). The effects of these RUP supplements on the passage of N fractions from the rumen of lactating dairy cows have been reviewed previously [1,64,65]. Collectively, these reviews indicate that replacing soybean meal with RUP sources frequently depressed the ruminal outflow of Microbial Nitrogen (MN), enhanced the flow

from the rumen of Nonammonia Nonmicrobial Nitrogen (NANMN), did not affect the passage of total Nonmmonia Nitrogen (NAN) to the small intestine, and failed to increase consistently the ruminal outflow of EAA, Lysine (Lys), and Methionine (Met).

Item	*n*	Minimum	Maximum	Median	Mean	SD
DIM, d	210	16	250	107	111	65
DMI, kg/d	224	10.8	26.8	19.6	19.5	3.4
Forage, % of diet DM[1]	224	5.7	100	50	49.7	12
CP, % of diet DM	222	11.3	23.1	17.1	17	1.7
Protein source, % of diet CP						
Soybean meal	126	0.5	61.7	28.9	28.2	14
Heated soybean meal[2]	20	11.3	60.3	40.8	35.2	13
Extruded soybean meal	3	18.1	24.1	18.1	20.1	3.4
Corn gluten meal	24	2.9	56.4	22.6	25.4	17
Fish meal	19	6.3	34.4	15.2	16.1	7.2
Blood meal	48	1.1	58	10.6	13.7	12
N intake, g/d	224	264	855	527	531	121
Flow to small intestine, g/d						
Total N	208	198	930	545	537	127
NAN	207	173	858	507	502	117
Microbial N	224	100	484	259	271	81
NANMN[3]	221	61	576	234	237	87
Endogenous N[4]	224	20.5	50.9	37.2	37.1	6.5
Total AA	106	917	4113	2778	2610	630
EAA[5]	104	440	1970	1281	1218	302
Lysine	108	58	264	167	168	48
Methionine	103	16	83	50	50	15
OMTDR[6]**, kg/d**	177	5.1	15.4	9.2	9.3	2

Table 1: Descriptive statistics of the N flow data set[1]

[1]From Ipharraguerre and Clark (5); All data were taken or calculated from data reported in 57 scientific papers (7-63); Contributions of supplemental protein to dietary CP were estimated using the ingredient composition of experimental diets and the CP content of the protein sources either reported in the corresponding study or tabulated by NRC (2); [2]Non-enzymatically browned soybean meal; [3]Nonammonia nonmicrobial N; [4]All data were calculated as DMI (kg/d) x 1.9 according to NRC (2); [5]Essential amino acids; [6]Organic matter truly digested in the rumen.

Although it is likely that the above generalizations are correct, they do not recognize that there is a lack of consistency in the response to the feeding of RUP sources. More importantly, there is a substantial degree of variation in the magnitude of reported RUP effects on the ruminal outflow of N fractions. These conclusions can be drawn from the compiled data shown in Table 1. The effect on the ruminal outflow of MN across RUP sources in this data set ranged from a significant reduction of about 36% [28] to a significant increase of about 22% [31]. Furthermore, for the compiled data there exists considerable variation even within individual sources of RUP. For example, when roasted soybeans were used to provide RUP in the diet the impact on the ruminal outflow of MN ranged from a reduction of about 10% that was not significant [14] to a significant increase of about 17% [35]. These inconsistent findings suggest that several variables moderate the effect of RUP supplements on the ruminal outflow of N fractions. Clark et al. [1] proposed that some of the most important variables in this regard are 1) the proportion of dietary CP that is supplied from RUP supplements, 2) the ruminal degradability of protein supplied from the control diet, 3) the type and amount of feed consumed by cows, and 4) the availability of energy, NH_3N, AA, and peptides in the rumen. For these reasons, employing a nonquantitative approach to summarize results from published experiments designed to investigate the effect of the amount and source of protein on the ruminal outflow of N may lead to biased generalizations. Therefore, the objective of this paper was to expand previous work [5] that used published data from the scientific literature and appropriate statistical methods to provide estimates of the magnitude and significance of the effects of the amount and degradability of dietary CP supplements on the supply of N fractions passing to the small intestine of lactating dairy cows. Data reported herein expands with additional treatment comparisons and complements results previously published in an invited paper that utilized only soybean meal as the control treatment [5].

Materials and Methods

A comprehensive literature survey was conducted to create a data set from peer-reviewed published studies that were designed to investigate the flow of N to the small intestine of lactating dairy cows (Table 1). Studies were selected based on factors discussed by Broderick and Merchen [66], Titgemeyer [67], and Firkins et al. [68]. The selection criteria included 1) lactating dairy cows fed ad libitum, 2) adequate description of methodology employed, 3) digesta samples collected from the omasum or duodenum, 4) use of external markers for estimating digesta flow, 5) adequate sampling frequency to account for diurnal variation in digesta flow (i.e., a minimum of eight samples over 48 hours), and 6) completeness of reported data (means and associated standard errors or deviations). The use of 2,6-daminopimelic acid as a microbial marker may result in biased estimations of MCP flow [66]; therefore, studies were excluded when this marker was used and N flows were outside the range of errors reported in Table 1.

The relationship between N availability and the ruminal outflow of N was subjected to multivariate regression analysis according to procedures outlined by St-Pierre [69]. Independent variables that were used as indicators of N availability included measured N intake, measured NH_3N concentration in the ruminal fluid, estimated intake of RDP (RDPI) and apparent rumen N balance (ARNB). The RDPI was calculated as N intake – NANMN flow (expressed as g/day) whereas ARNB was estimated as N intake – [(total N flow – endogenous N flow) – NH_3N flow] (expressed as g/day). In all cases,

endogenous N flow (g/day) was estimated as DMI (kg/day) x 1.9 as described in reference (2). Dependent variables that were considered in the analyses included measured flows of total N, NAN, MN, NANMN, total AA, EAA, Lys, and Met. A mixed model approach was used based on the assumption of random variation for the effect of study [69]. The general form of the statistical model used was:

$$Y_{ij} = \beta_o + S_i + \beta_1 X_{ij} + b_i X_{ij} + \varepsilon_{ij}$$

where:

Y_{ij} = the expected value for the dependent variable Y at level j of the independent variable X in the study i,

β_o = overall intercept (fixed effect),

S_i = effect of study i (random effect),

β_1 = overall slope that results from regressing Y on X across all studies (fixed effect),

X_{ij} = observed value j of the independent variable X in the study i,

b_i = effect of study i on the slope that results from regressing Y on X in study i (random effect), and

ε_{ij} = unexplained error (random).

Variable	Abbreviation	Definition
Control supplemental CP source	SBM	Soybean meal was the only or major source of supplemental protein
	PLANT	Plant protein sources other than soybean meal were the major source of supplemental protein (canola meal, cottonseed meal, sunflower meal, whole soybeans, or whole horse beans)
	CASEIN	Casein was the major source of supplemental protein
	RUP	A source of rumen un-degradable protein was included in the control diet (corn gluten meal or fish meal)
Supplemental rumen un-degradable protein source	SOY	Soybean protein sources of reduced ruminal protein degradability (heated soybean meal, extruded soybean meal, or whole roasted soybeans)
	CORN	Corn protein sources of reduced ruminal protein degradability (corn gluten meal, brewers dried grains, or brewers wet grains)
	MIX	Mix of protein sources of reduced ruminal protein degradability (mixes of protein sources of marine, animal, and (or) plant origin)
	FM	Fish meal
	BM	Blood meal
	VPS	Various protein sources of reduced protein degradability in the rumen not included in other categories (extruded cotton meal, whole roasted horse beans, heated alfalfa meal, feather meal)
Percentage of supplemental rumen un-degradable protein	< 25	Supplemental rumen un-degradable protein source(s) supplied less than 25% of the diet CP
	25-35	Supplemental rumen un-degradable protein source(s) supplied from 25.1 to 35.0% of the diet CP
	35-45	Supplemental rumen un-degradable protein source(s) supplied from 35.1 to 45.0% of the diet CP
	45-55	Supplemental rumen un-degradable protein source(s) supplied from 45.1 to 55.0% of the diet CP
	> 55	Supplemental rumen un-degradable protein source(s) supplied more than 55.1% of the diet CP
Total N intake	< 350	N intake was less than 350 g/d for the experimental diet
	350-450	N intake ranged from 351 to 450 g/d for the experimental diet
	450-550	N intake ranged from 451 to 550 g/d for the experimental diet
	550-650	N intake ranged from 551 to 650 g/d for the experimental diet
	> 650	N intake exceeded 651 g/d for the experimental diet

Table 2: Categorical variables used in the meta-analysis of the ruminal outflow of N fractions

Treatment means were weighted by the reciprocal of the variance of the means to account for unequal replications and heterogeneous variances across studies [70]. Models were tested using a single independent variable and its squared term or were expanded to accommodate multiple independent variables, their squared term, and all possible two-way interactions. For models involving multiple variables, interactions and squared terms were sequentially removed from the model until the remaining highest order term in the model was significant ($P<0.05$). In all cases, an unstructured covariance model and the restricted maximum likelihood (REML) algorithm were used to estimate model parameters. Final models were selected based on the Bayesian Information Criterion, parameter significance, magnitude of the root mean squared prediction error (RMSPE), and residual analysis. Weighted residuals were inspected for abnormal patterns by calculating mean and linear biases as described by St-

Pierre [71]. All computations were carried out using the MIXED, REG, and UNIVARIATE procedures of SAS [72].

The impact of the source of N on the passage of N fractions to the small intestine of lactating dairy cows was examined using the data set described in Table 1 according to meta-analytic techniques described by Curtis and Wang [73] and Hedges et al. [74]. This approach has been used in other disciplines, including medical, physical, behavioral, and, more recently, ecological sciences, to eliminate biased generalizations with remarkable success [74,75]. Results for treatment comparisons from compiled studies were expressed as the natural log of the response ratio, here defined as the ratio between the reported responses to the feeding of high RUP diets (treatment diets) and of control diets. This index of the magnitude of the response was used for analyses [74]. The approach taken in this meta-analysis consisted of identifying the source of variation in the magnitude of the response among treatment comparisons from data compiled from the scientific literature and determining whether particular variables of interest elicited quantitatively different effects. For this purpose, variation in the log response ratio was analyzed by stratifying treatment comparisons into groups described by four categorical variables. These categorical variables were the supplemental CP source in the control diet, the supplemental RUP source in the experimental diet, the percentage of dietary CP supplied as supplemental RUP, and N intake (Table 2).

Total variation or heterogeneity in the log response ratio was partitioned into variation between groups (Q_b) of treatment comparisons (i.e., true variation in results across comparisons) and variation within groups of treatment comparisons (i.e., sampling variation in the estimate of each comparison; Hedges et al. [74]. For each categorical variable, between-group variation (Q_b) was tested across all compiled data for each parameter of interest (i.e., flows of total N, NAN, MN, NANMN, total AA, EAA, Lys, and Met). If a significant Q_b was detected for a categorical variable, the data set was subdivided according to the subgroups of this variable and the first step repeated. This iteration was continued until the number of categorical variables bearing a significant Q_b was reduced to one or zero [73].

Y	X_1	X_2	Intercept	SE	b_1	SE	b_2	SE	RMSPE	Mean bias	Linear bias
TN	NI		163**	32.7	0.70**	0.06			26.5	0.1	0.02
NAN	NI		147**	35.4	0.68**	0.06			26.6	0.7	0.01
MN	NH_3N		247**	17.5	1.62	1.15			19.6	1.4	0.05*
MN	NI		103**	32.7	0.31**	0.06			21.3	2	0.06**
MN	ARNB		267**	10	-0.13	0.08			14	1.2	0.42*
MN	RDPI		155**	16.6	0.38**	0.06			21.1	1.8	0.06**
MN	NI	OMTDR	76	36.5*	0.15*	0.07	11.7**	3.37	14.3	1.1	0.04**
MN	RDPI	OMTDR	140**	29.3	0.19**	0.05	8.15*	3.41	17.9	1.2	0.04*
MN	ARNB	OMTDR	115**	25	-0.21**	0.06	17.2**	2.73	14	0	0.02
NANMN	NI		3.7	22.9	0.43**	0.04			28.3	0.16	0.03
TAA	NI	NI x NI	-476	437	8.72**	1.93	-0.005**	0	158	-1.3	0.04
EAA	NI		649**	188	1.15**	0.31			66	0	0.02
LYS	NI		110**	25.6	0.11**	0.04			11.2	0.1	0.04
MET	NI	NI x NI	-11	12	0.18**	0.05	-0.0001*	0	3.6	0.02	0.04

Table 3: Regression analysis of the relationship between N availability and the ruminal outflow of N in dairy cows[1]; [1]RMSPE = root mean squared prediction error, TN = total N flow (g/d), NAN = nonammonia N flow (g/d), MN = microbial N flow (g/d), NANMN = nonammonia nonmicrobial N flow (g/d), TAA = total amino acid flow (g/d), EAA = essential amino acid flow (g/d), LYS = Lys flow (g/d), MET =Met flow (g/d), NI = total N intake (g/d), NH3N = ammonia N concentration in the rumen (mg/dl), ARNB = apparent rumen N balance (g/d), RDPI = RDP intake (g/d), OMTDR = OM truly digested in the rumen (kg/d); *P<0.05; **P<0.01.

Subsequently, means and 95% confidence intervals were calculated. Means were considered to be significantly different (P<0.05) from one another if their confidence intervals did not overlap and to be different from zero whenever their confidence interval did not encompass zero [76]. Results from studies with more than one treatment comparison were assumed to be independent and entered individually in the data set. Variation in the log response ratio across treatment comparisons was considered random and was estimated using a mixed model approach [75]. Each log response ratio was weighted by the reciprocal of the mixed model variance (i.e., total variance), which gives greater weight to treatment comparisons from studies that used larger sample size and whose estimates had greater precision (i.e., smaller standard

error or standard deviation; Hedges et al. [74]. The MIXED procedure of SAS [72] was used to estimate the variance (REML algorithm) of the log response ratio and procedures developed by Wang and Bushman [75] for SAS [72] were adapted to conduct the remaining analyses. Findings are reported as the mean percentage change (i.e., [(response ratio – 1) x 100]) resulting from the feeding of high RUP treatment diets. Therefore, if there was no difference in the feeding of the control and high RUP treatment diets then the response to the feeding of the control and high RUP treatment diets were the same. In this case, the response ratio would be equal to one and the mean percentage change would be zero. If the response was greater when feeding the high RUP treatment diets, then the response ratio would be >1 and the mean percentage change would be positive. Conversely, if the response was less when the high RUP treatment diets were fed then the response ratio would be <1 and the mean percentage change would be negative.

In the figures developed from the meta-analysis, symbols represent the mean percentage change, the bars represent the 95% confidence interval, and the number in parenthesis is the number of treatment comparisons. If the 95% confidence interval bar does not overlap zero, then the mean percentage change is different from zero (P<0.05). If the 95% confidence interval bars for the RUP supplements do not overlap, then they are significantly different (P<0.05) from each other.

Results and discussion

Ruminal outflow of total N and NAN

Regression analysis of compiled data revealed a significant linear relationship between N intake and the passage of total N and NAN (Table 3) to the small intestine of lactating cows fed unrestricted amounts of feeds. Similar results were reported in earlier reviews of studies with dairy cows [1] and steers [77]. Naturally, because of the extensive transformations that N undergoes in the rumen it should be expected that the amount of N consumed by dairy cows dictates only a part of the variation in the passage of N and NAN to the lower gastrointestinal tract.

For all data compiled, meta-analysis indicated that the feeding of RUP supplements in experimental diets to dairy cows increased the ruminal outflow of total N by 9% when compared with CP sources of higher ruminal degradability in control diets (Figure 1). This effect was affected by the source of RUP in the dietary treatment (P<0.05) and the source of CP in the control diet (P<0.001; Table 4). Ruminal outflow of total N did not increase when cows were fed dietary treatments that contained blood meal (BM) or fish meal (FM), but increased significantly (mean increase 8 to 14%) when they consumed diets containing mixtures of marine, animal, and plant RUP sources (MIX) or corn protein of reduced ruminal protein degradability (CORN = corn gluten meal, brewers dried or wet grains; SOY = heated soybean meal, extruded soybean meal, whole roasted soybeans; VPS = extruded cottonseed meal, whole roasted horse beans, heated alfalfa meal, feather meal). Examination of variation within the source of RUP, however, revealed an effect (Q_b = 12.25, P<0.002) of the source of CP in the control diet for experiments in which SOY were investigated as the RUP supplement. In these experiments, the inclusion of soybean meal (SBM) in the control diet negated the significant increase in the ruminal outflow of total N that occurred when CASEIN and (PLANT) sources other than SBM (canola meal, cottonseed meal, sunflower meal, whole soybeans, or whole horse beans) were used as protein supplements in the control diet. It should be noted that as the data set was divided, not every protein supplement in each categorical variable was represented. For example, RUP sources were not included in control diets from studies designed to evaluate protected soy-protein products. In addition, the small number of comparisons using CASEIN and PLANT in the control diet resulted in large confidence intervals, providing little power to draw statistical inferences about the relative magnitude of their mean effect.

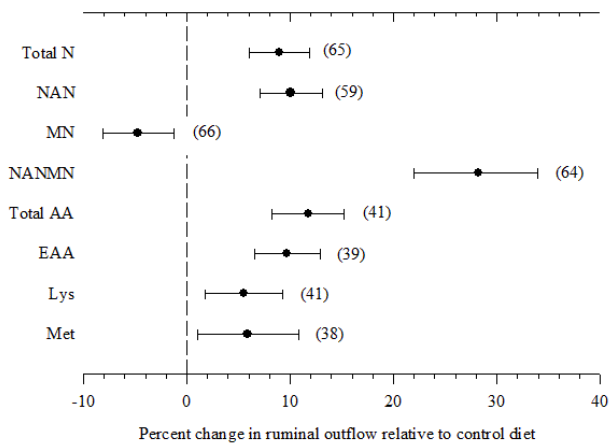

Figure 1: Overall effect of RUP supplements on the flow of N fractions from the rumen of lactating dairy cows fed high RUP diets compared with control diets. Means ± 95% confidence interval (n) are shown. See Table 3 for definition of N fractions.

The use of CASEIN as the source of CP in the control diet resulted in the largest mean increase in passage of total N to the duodenum when feeding RUP dietary treatments. Additionally, a significant increase in the ruminal outflow of N was found when SBM (5%) and PLANT (11%), but not RUP (2.6%), were fed in the control diets.

Ruminal outflow, g/d	n[2]	CPS	RUPS	PRUP	TNI
Total N	65	21.58***	11.49*	5.65	2.31
NAN	59	13.06**	10.02	6.11	2.51
MN	66	5.17	9.96†	6.64	5.81
NANMN	64	14.92**	9.29†	8.43†	4.07
Total AA	41	4.83	12.19*	5.26	7.46
EAA	39	2.31	7.8	1.27	5.12
Lysine	41	8.17*	14.19**	2.53	6.88
Methionine	38	11.19**	9.89†	2.71	5.04

Table 4: Between group variation (Q_b) for the log response ratio across four categorical variables[1]

[1]CPS = control protein source, PRUP = percentage of supplemental RUP in the treatment diet, RUPS = RUP source, TNI = total N intake. Definitions and levels of categorical variables are described in Table 2; 2Total number of treatment comparisons used in the meta-analysis; †P<0.10; *P<0.05; **P<0.01; ***P<0.001.

Therefore, the composition of the control diet with which the RUP treatment is compared has a significant effect on the response obtained when RUP is fed to lactating dairy cows. The analysis of each of these control subgroups (i.e., CASEIN, RUP, SBM, and PLANT) for other significant categorical divisions (e.g., N intake) showed no additional significant heterogeneity between groups (Q_b; data not shown).

The flow of NAN from the rumen of dairy cows was increased 10% (overall effect) by the feeding of diets in which RUP supplements replaced a portion of other sources of supplemental CP (Figure 1). As was observed for the flow of total N, the passage of NAN from the rumen was strongly influenced ($P<0.01$) by the source of CP in the control diet and only marginally ($P<0.10$) by the source of RUP in the dietary treatment (Table 4). Further partitioning of the data set showed no additional significant effects.

The highly significant influence that the source of CP in the control diet had on the passage of N and NAN to the duodenum when feeding RUP supplements (Table 4), demonstrates that increases in the ruminal outflow of these N fractions were partly determined by the ruminal degradability of the major source of supplemental CP in the control diet. Therefore, the likelihood of enhancing the flow of total N and NAN from the rumen by providing a portion of dietary CP from RUP supplements increases when the ruminal degradability of the major source of supplemental CP in the control diet is high (e.g., CASEIN, PLANT). These observations support discussion provided in literature surveys by Clark et al. [1] and Stern et al. [64].

Ruminal outflow of MN

In most ruminant production systems, MCP that exits the rumen contributes the largest proportion of the protein supply to the host animal. In a summary of 152 treatment means, MN provided from 39 to 89% of the NAN flow that reached the duodenum of lactating dairy cows (mean = 59%; Clark et al. [1], although under some circumstances this proportion may approximate 100% [78]. During the last 30 years, extensive research has been conducted to quantify the outflow of MCP from the rumen of dairy cows. A salient characteristic of these estimates is their highly variable nature, which sometimes has resulted in conflicting outcomes. In part, this is explained by the technical difficulties that are encountered when measuring MCP production in vivo [66, 79,80] and the numerous factors that modulate the extent of this process [1,2,4,64,81-84]. Ultimately, the amount of MCP that exits the rumen is a function of the rate of microbial growth, the efficiency of this process (generally expressed as g of MN/ kg of OM fermented in the rumen), and the dilution rate of ruminal contents [4].

Changes in the availability of N can alter the growth and the efficiency of growth of ruminal microorganisms both in vivo and *in vitro*. In this regard, it would be expected that a deficiency of available N, rather than a surplus, would have a negative impact on the outflow of MN from the rumen. Satter and Slyter [85] demonstrated that 2 to 5 mg of NH_3N/dl of ruminal fluid is the minimum amount of N required to maximize microbial growth in continuous culture fermenters. In the literature, it is commonly assumed that this concentration range represents the lower limit for evaluating the adequacy of N availability for MCP synthesis in vivo. Using data from four experiments, however, Clark et al. [1] showed that the availability of NH_3N and the outflow of MN from the rumen of dairy cows fed various protein supplements were not significantly correlated when the concentration of NH_3N exceeded 2 mg/dl of rumen fluid. Similarly, the relationship between these two variables was not evident for the compiled data set used in this report in which the concentration of NH_3N and the ruminal outflow of MN ranged from 1.4 to 32 mg/dl and from 100 to 484 g/day, respectively (Table 3). In addition, using N intake, ARNB, or RDPI as independent variables failed to improve RMSPE and all equations resulted in biased predictions (Table 3).

Ørskov [78] postulated that the concentration of NH_3N that is needed to optimize microbial growth depends on the digestibility of the organic matter (OM) consumed by the animal. Clark et al. [1] noted that above 2 mg of NH_3N per dl of rumen fluid, changes in MN flow through the forestomachs were better explained by fluctuations in the amount of OM fermented in the rumen than by NH_3N concentration. This is consistent with the notion that the amount of OM that is degraded in the rumen (i.e., energy supply) largely dictates the rate of microbial growth. As the amount of OM fermented in the rumen increases, the quantity of N taken up by ruminal microorganisms is expected to rise [86]. This increased requirement for N would demand a timely increase in the ruminal influx of N in order to sustain MCP synthesis and minimize the extent of energy-spilling fermentation. A higher rate of N entry into the rumen may be achieved by enhancing N intake through increases in dry matter intake (DMI), the content of dietary CP in the dry matter (DM), CP degradability in the rumen, or N recycling.

An appraisal of these likely events was attempted by adding the reported amount of OM truly digested in the rumen (OMTDR) to the regression equations having N intake, RDPI, and ARNB as independent variables. When N intake and OMTDR were combined, the best-fit model predicted a linear positive response in the ruminal outflow of MN (Table 3). Although this approach improved the RMSPE when compared with the model having N intake as the sole independent variable, weighted residuals still were biased (Table 3). Similar findings were obtained when OMTDR was added to the RDPI model (Table 3). When OMTDR was added to the ARNB model, the resulting prediction of the passage of MN to the small intestine increased linearly as the amount of OMTDR and the apparent deficiency of available N in the rumen (i.e., negative ARNB) became larger (Table 3). This combination of variables (ARNB and OMTDR) resulted in the smallest RMSPE and removed much of the linear bias from the weighted residuals. Based on data reviewed by the NRC (2), these findings could be attributed to the improvement of the efficiency of microbial growth that occurs when the balance of N in the rumen of dairy cows turns negative. These findings also suggest that, as the ARNB became progressively more negative while the amount of OMTDR remained constant or increased, more endogenous N must have been recycled into the rumen to permit microbial growth and fermentation. This suggestion is supported by studies with steers [87] and dairy cows [88] in which it was estimated that the amount and proportion of endogenous urea N used by ruminal microbes increased when the availability of N in the rumen, but not of energy, became increasingly deficient. Although available data do not allow speculating about the maximum or optimum amount of N recycling in lactating dairy cows, it appears that N recycling can compensate for sizable deficits of N in the forestomachs (~ 100 g/day; Marini et al. [89]. It is not logical to expect that N recycling can sustain indefinitely the synthesis of MCP in the rumen. However, it seems that under situations as varied as those represented by these studies the passage of MN to small intestine of lactating cows was not limited by the ruminal availability of NH_3N.

Even though NH_3N did not appear to limit growth of the microbes, microbial N flow from the rumen was depressed (overall effect = –4.8%) when RUP supplements partially replaced other sources of CP in the diet of lactating dairy cows (Figure 1) and this response tended to be affected ($P < 0.10$) by the source of RUP in the dietary treatment (Table 4). When cows consumed dietary treatments containing CORN, FM, BM, or MIX the ruminal outflow of MN decreased by 5.0, 9.0, 10.2, and 12.2%, respectively, but this negative effect was significant only for the MIX (Figure 2). In contrast, when cows consumed diets containing SOY or VPS changes in the passage of MN to the small intestine were not different from zero nor from each other (Figure 2). Estimation of the mean response for the source of CP in the control diet (i.e., CASEIN, SBM, PLANT, and RUP) showed that the magnitude of the effect of RUP treatments on the ruminal outflow of MN was not dependent on the protein supplement in the control diet (data not shown). It should be noted that the limited number of published comparisons that were available to be compiled resulted in large confidence intervals and little power to draw statistical inferences about the relative magnitude of mean effects. Division of the data set followed by further examination of variation did not reveal additional significant effects (data not shown).

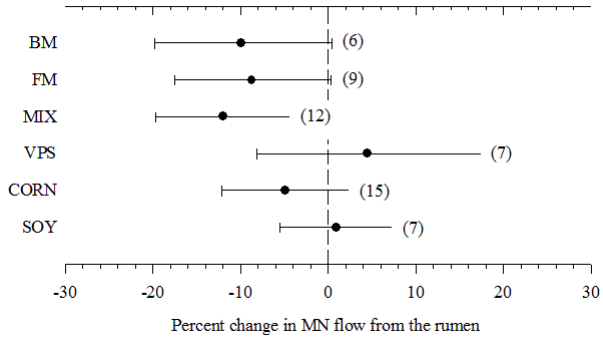

Figure 2: Effect of the source of RUP in the treatment diet on MN flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

Despite these limitations, results show that the ruminal outflow of MN was decreased the most by RUP supplements that have the lowest rate and extent of protein degradation in the rumen [2]. This observation is in agreement with the notion that replacing rumen degradable protein (RDP) with RUP in the diet of dairy cows can depress the growth or the efficiency of growth of ruminal bacteria because of a shortage of energy [1,79], AA, peptides, or NH_3N [1,83,84] in the rumen. Based on results for the effect of N availability on the ruminal outflow of MN, however, it seems reasonable to speculate that for the data compiled, the reduction in the ruminal output of MN in response to the feeding of RUP supplements was not caused by a deficiency of NH_3N in the rumen. Furthermore, data summarized by Hoover and Stokes [83] and findings reported in this paper suggest that within the range of results compiled, the rate at which AA and peptides were released from protein supplements of low ruminal degradability might have hampered the production of MCP in the rumen.

Ruminal outflow of NANMN

Using data from five studies designed to investigate the effect of N intake on the ruminal outflow of N fractions, Clark et al. [1] reported a linear correlation ($r^2 = 0.61$) between N intake and the delivery of NANMN to the small intestine of dairy cows. The authors postulated that as N intake increased a depression in ruminal protein degradability might have occurred, which enhanced the delivery of undegraded feed CP to the duodenum. When data from a wider variety of studies were pooled (Table 1), a significant linear relationship was also detected between the amount of N consumed by dairy cows and the passage of NANMN to small intestine (Table 3). Furthermore, abnormal patterns were not detected in the plot of weighted residuals (Table 3), which supports the proposal by Clark et al. [1].

Although N intake contributes to variation in the ruminal outflow of NANMN, other factors discussed more extensively in other literature surveys [1,2,4,78,90,91] play an important role in modulating the amount of feed CP that reaches the small intestine of dairy cows. Source of supplemental CP fed to dairy cows is another factor suggested to influence the outflow of NANMN from the rumen.

Across the entire data set (Table 1), the passage of NANMN to the duodenum increased significantly (overall effect = 28.8%) when dairy cows consumed diets that contained RUP supplements (Figure 1). The magnitude of this response, however, was distinctly altered ($P < 0.01$) by the source of CP in the control diet with which the RUP dietary treatment was compared (Table 4). In addition, the proportion of total CP supplied in the treatment diet from RUP and the source of RUP tended ($P < 0.10$) to modulate the response.

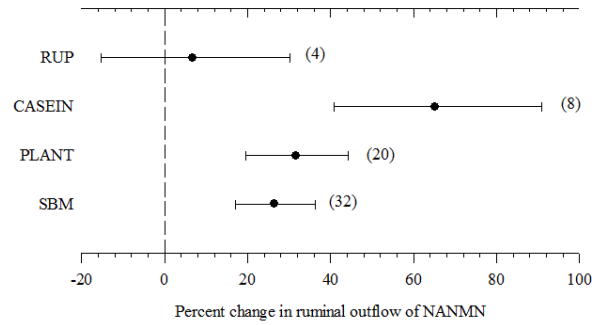

Figure 3: Effect of the source of CP in the control diet on NANMN flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

As shown in Figure 3, replacing CASEIN in the control diet with RUP supplements elicited the largest mean increase (67.4%) in the ruminal outflow of NANMN. Intermediate increments that also were significantly different from zero were found when SBM and PLANT were the protein supplements in the control diets compared with the RUP treatments (22.9 and 30.9%, respectively; Figure 3). In contrast, when RUP supplements provided a portion of the CP in the control diet with which the RUP treatments were compared then feeding high RUP diets failed to enhance significantly (6.2%) the ruminal outflow of

NANMN (Figure 3). The small number of comparisons in which CASEIN and RUP supplements were used to formulate the control diet limited statistical inferences about the relative magnitude of their mean effects.

Estimation of the mean response for each RUP source in treatment diets showed that all supplements, except for FM (9.6%), significantly increased (from 24 to 40%) the amount of NANMN delivered to the small intestine of dairy cows (Figure 4). Caution should be exercised in interpreting these data owing to the highly significant effect that was caused by the source of CP in the control diet with which the RUP treatment was compared, as previously indicated. Indeed, FM was used in the control diet for two of the four comparisons between control diets that contained RUP supplements (i.e., RUP) and RUP dietary treatments, which depressed values obtained for ruminal outflow of NANMN when control and treatment diets were compared.

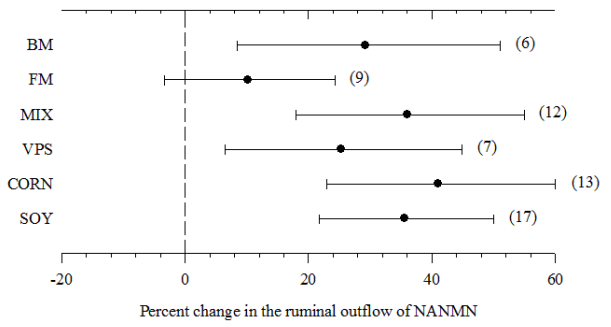

Figure 4: Effect of the source of RUP in the treatment diet on NANMN flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

Collectively, findings support conclusions reported in previous literature surveys [1,65] that some RUP supplements increased quantitatively the supply of NANMN to the small intestine of dairy cows. Results also demonstrate that the magnitude of this increase was strongly affected by the source of CP in the control diets, suggesting that the ruminal degradability of the major source of CP in the control diet partly modulates the magnitude of change in the ruminal outflow of NANMN in response to feeding RUP supplements to dairy cows. Unfortunately, sufficient data are not available to allow detecting differences among RUP sources.

Examination of the variation within the percentage of dietary CP supplied from RUP was not possible because the effect of the source of CP in the control diet was confounded with other categorical variables. For example, all comparisons that involved the use of RUP sources in the control diet only used dietary treatments in which RUP supplements provided less than 25% of the dietary CP. Clark et al. [1] summarized reports in which RUP supplements were compared with SBM as sources of supplemental CP for dairy cows. They noted that the ruminal outflow of NANMN was increased when RUP sources provided 35% or more of the total CP intake. When data were analyzed separately from studies in which control diets that contained SBM were compared with high RUP treatments, a significant effect (Q_b = 14.27, P < 0.006) for the percentage of dietary CP supplied by RUP was detected. As reported by Ipharraguerre and Clark [5], replacing SBM with RUP sources in the experimental diet significantly increased the escape of NANMN from the rumen at all percentages of SBM replacement. Although confidence intervals are too large to draw statistical inferences, it appears that the magnitude of the response to increasing rates of substitution of RUP sources for SBM is not linear.

Ruminal outflow of total and EAA

Regression analysis of compiled data revealed a curvilinear (quadratic) relationship between N intake and the passage of total AA to small intestine (Table 3). According to this relationship, and within the range of the data compiled, decreasing returns in the amount of EAA and nonessential amino acids (NEAA) that exited the forestomachs were obtained as the amount of N consumed by lactating cows increased. One could speculate that this happened because increasing proportions of AA of feed origin were wastefully degraded in the rumen as the input of dietary N progressively exceeded microbial needs. However, the quadratic nature of the relationship appears to be determined, at least in part, by the comparatively low AA flows (from 917 to 1300 g/day) reported in two studies (15,56). Examination of the residual plot (Table 3) and the distribution of weighted residuals (data not shown), however, did not justify the elimination of these studies from the data set. Alternatively, more biologically sound models (i.e., non-linear functions) may better fit the data, but attempts of this kind were not taken because they would be outside the scope of this paper. For these reasons, caution is recommended when interpreting the discussed relationship between the ruminal outflow of total AA and N intake.

When examining the relationship between N intake and the flow of EAA at the duodenum, a linear model of first order provided the best fit for the pooled data (Table 3). If for the range of available data it is assumed that the true underlying relationships between N intake and the passage of total AA and EAA to the small intestine are as indicated above, it would seem reasonable to suggest that ruminal microorganisms preferentially metabolized NEAA when the availability of amino N in the rumen increased because of higher N intake. This suggestion is supported by results from Velle et al. [92], who after infusing mixtures of EAA and NEAA into the rumen of dairy cows observed that the infusions containing solely NEAA were more rapidly (34 vs. 26% h^{-1}) and extensively (87 vs. 78% after 8 hours) degraded.

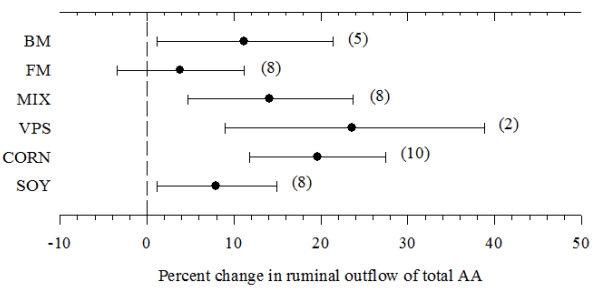

Figure 5: Effect of the source of RUP in the treatment diet on total AA flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

Even though these observations are compelling, the aforementioned findings suggest that within the range of data collected in this paper, it is unlikely that the supply of EAA to the small intestine can be selectively modified sufficiently to affect milk and milk protein production through manipulation of the amount of N consumed by dairy cows without considering the source of CP fed to the cows.

Results of the meta-analysis indicate that feeding RUP supplements to dairy cows resulted in a significant overall increase (11.7%) in the ruminal outflow of total AA (Figure 1), but the magnitude of this response depended on the RUP treatments (P<0.05; Table 4). All RUP supplements significantly enhanced (from 8 to 23%) the ruminal escape of total AA with the exception of FM (3.5%; Figure 5). Further analysis of variation within the source of RUP showed no additional significant effects (data not shown).

Across all comparisons, the ruminal outflow of EAA was significantly increased (9.6%) when cows were fed diets that contained RUP supplements (Figure 1). As shown in Table 4, variation in this response was homogenous (P>0.10) across source of CP in the control diet, source and percentage of RUP, and amount of N intake. For instance, all RUP treatments elicited a positive percentage change in the intestinal supply of EAA, although this effect was not significantly different from zero for FM (Figure 6). Likewise, the magnitude of this response was not altered by the source of CP in the control diet (data not shown).

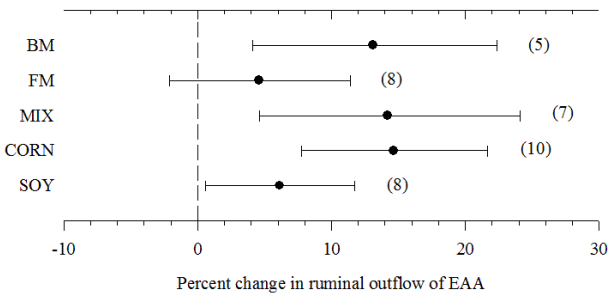

Figure 6: Effect of the source of RUP in the treatment diet on EAA flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

In a previous review, Santos et al. [65] concluded that when sources of RUP replaced SBM in the diet of dairy cows the ruminal outflow of EAA was not consistently increased. The authors attributed the inconsistent outcomes to greater flows of MN for diets containing SBM than for diets high in RUP. Results from our meta-analysis show that the flow to small intestine of EAA was quantitatively improved when various sources of RUP were used to replace a portion of different sources of CP in diets fed to dairy cows. Additionally, results indicate that this response was homogenous across treatment comparisons compiled for the meta-analysis. Therefore, it appears that the mean increase in the ruminal outflow of total and essential AA of NANMN origin elicited by most RUP supplements was greater than their detrimental effect on the ruminal outflow of MN.

Ruminal outflow of Lys and Met

Research suggests that Lys and Met are the two EAA that most frequently limit the synthesis of milk and milk protein or both in the mammary gland of dairy cows fed a broad variety of diets [2,93-95]. It is also recognized that a curvilinear relationship exists between the amount of Lys and Met that reaches the absorption sites in the small intestine and the output of milk protein [2,93,94]. Thus, it might be possible to formulate diets with the ideal quantities and proportions of Lys and Met so as to maximize milk and milk protein yields and minimize the amount of MP required by dairy cows. A prerequisite for achieving that goal in commercial settings is the identification of practical and economical alternatives for increasing the flow of these EAA to and absorption from the gastrointestinal tract.

For the compiled data, the relationship between N intake and the ruminal outflow of Lys and Met was described best by a linear and a quadratic function, respectively (Table 3). In part, this could be ascribed to differences in the dynamics of the transactions that these EAA undergo in the rumen. For instance, the rate and extent of Met disappearance from ruminal fluid was reported to be significantly lower than for Lys in dairy cows (96,97), sheep [98], and in vitro [99,100]. It is possible, therefore, that as the ruminal influx of Met and Lys increased because of higher N intake, proportionally more Met than Lys might have exited the rumen. This hypothesis has been confirmed in experiments in which Met and Lys were individually infused at various doses into the rumen of dairy cows [96,97]. Additionally, Scheifinger et al. [100] found that in vitro cultures of NSC-fermenting bacteria that are normally present in the rumen (members of the genera *Megasphera, Eubacterium*, and *Streptococcus*) produced Met but not Lys. These authors reasoned that when conditions in the rumen support the growth of bacteria of these genera (e.g., high availability of NSC), the overall rate of Met utilization by ruminal microorganism might decline. Because in most of the studies considered dairy cows were fed medium to high levels of cereal grains, it could be speculated that microbial populations prevalent in the rumen of those cows might have favored the escape, rather than the utilization, of Met as the concentration of this AA increased in the rumen in response to higher N intake. Results suggest that N intake might have different effects on the profile of EAA that reaches the small intestine of dairy cows by altering in different ways the ruminal metabolism of individual AA.

Previous regression analyses [2,101] revealed that most of the variation in the profile of EAA that exit the rumen is accounted for by the content of individual EAA in RUP and the proportional contribution of RUP to total protein reaching the small intestine. Using this data set, the meta-analysis revealed that the RUP treatment (P<0.01) and the source of CP in the control diet (P < 0.05) altered (Table 4) the overall increase (5.4%) in the ruminal escape of Lys that arose from feeding high RUP diets to dairy cows (Figure 1). When RUP treatments were compared with control diets that contained PLANT the flow of Lys to the lower gastrointestinal tract was increased by about 16.5%. The magnitude of this response was considerably smaller, and not different from zero, when RUP treatments were compared with control diets that contained either SBM (3.4%) or RUP supplements (6.5%; data not shown). High RUP diets that contained CORN (– 7.5%) or VPS (– 7.4%) decreased the amount of Lys that passed from the rumen and high RUP diets that contained BM (18.1%), MIX (5.9%), FM (5.1%), and SOY (3.1%) increased passage of Lys to the small intestine, but this effect was only significant for BM (Figure 7). Further partitioning of the data set did

not reveal additional significant effects. These results suggest that the percentage of Lys in dietary DM, the ruminal degradability of protein supplied by the RUP treatment, and the source of CP in the control diet determine, at least in part, the amount of Lys that reaches the small intestine of dairy cows.

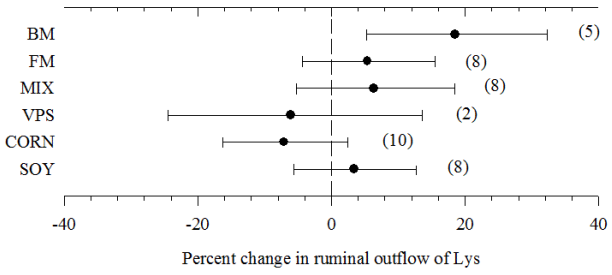

Figure 7: Effect of the source of RUP in the treatment diet on Lys flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

In comparison with CP supplements of higher ruminal degradability, the feeding of RUP sources to dairy cows resulted in greater (overall effect = 5.8%) flow of Met to the duodenum (Figure 1). This effect was largely influenced ($P<0.01$) by the source of CP in the control diet, and to a lesser extent, by the source of RUP in the dietary treatment ($P<0.10$; Table 4). Across all comparisons, the ruminal outflow of Met significantly increased when high RUP dietary treatments were compared with control diets that contained PLANT (14%) or RUP supplements (i.e., RUP, 21.8%; data not shown). The influence that the control diets that contained RUP supplements had on the magnitude of the response to RUP treatments was largely determined by results from a dose response study [8]. In that study increasing the supply of RUP from about 9% (control diet) to about 54% (treatment diet) of the dietary CP increased Met flow to duodenum by about 55%.

Among the various RUP dietary treatments, when CORN and FM partially replaced protein sources of higher ruminal degradability in the diet of dairy cows the escape of Met from the rumen increased (21.4 and 8.2%, respectively); however, the positive effect was significant only for CORN (Figure 8). It should be noted that in five of the nine comparisons involving CORN, these RUP supplements supplied more than 45% of the dietary CP. In contrast, in seven of the eight comparisons involving FM, this byproduct supplied less than 25% of the dietary CP. Despite these differences, the effect of the proportion of dietary CP provided from the RUP sources on the ruminal outflow of Met was not significant for the compiled data (Table 4). Examination of subgroups for the source of CP in the control diet (i.e., CASEIN, SBM, PLANT, and RUP) and the source of RUP in the treatment diets (i.e., BM, CORN, FM, MIX, SOY, and VPS) showed no additional significant effects (data not shown).

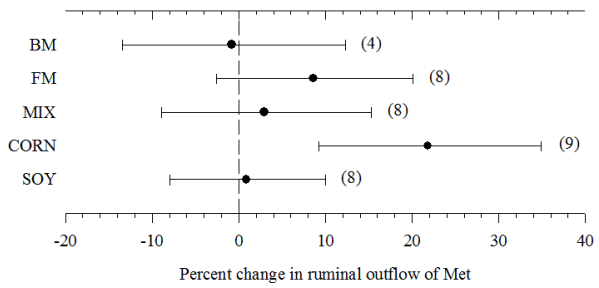

Figure 8: Effect of the source of RUP in the treatment diet on Met flow from the rumen of lactating dairy cows fed RUP treatment diets. Means ± 95% confidence interval (n) are shown. Variable abbreviations are described in Table 2.

Outcomes of this meta-analysis indicate that byproducts of corn processing and FM were the most effective sources of RUP for enhancing the delivery of Met to the small intestine of dairy cows. This response, however, was more consistent for corn proteins of reduced ruminal degradability. In contrast, the least effective sources of RUP for improving the intestinal supply of Met were protected soy proteins and BM. Therefore, it appears that the content of Met and the ruminal degradability of protein supplied from the RUP treatment and the source of CP in the control diet partly dictate the amount of Met that reaches the lower gastrointestinal tract of dairy cows.

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