Ontario Pork Research Final Report (18-02) ONTARIO PORK **Executive Summary**
Reporting Date: September 18, 2023

Introduction: The NRC (2012) based estimated methionine requirements for gestating and lactating sows from very few studies, with a focus on the retention of methionine in maternal, fetal, and milk proteins. These estimates do not take into account the metabolism of the modern sow (many studies are over 50 years old) or the 'non-protein' roles of amino acids, which can use up a significant proportion of the amino acid. Methionine is one such essential amino acid that has over 50 non-protein roles in the body.

Objectives: (original objectives from project proposal)

1. Determine the dietary methionine required to maximize whole-body protein retention (sow and products of conception or milk)
2. Determine the dietary methionine required to optimize methionine use for non-protein roles.
3. Determine effect of dietary methionine supply on maternal and fetal piglet hoof health.

Materials and Methods: Two animal studies were completed with 70 and 75 gestating and lactating gilts, respectively. Animals were assigned to diets with increasing methionine supply. Nitrogen balances were completed at various points in gestation and lactation to assess protein retention in the gilt + products of conceptus (gestation) or the gilt + milk (lactation). Blood samples were collected routinely to measure the concentration of non-protein compounds that depend on methionine supply. The dietary methionine level that optimized each outcome was assessed statistically.

Results and Discussion:

1. To maximize nitrogen (protein) retention in gestation, standardized ileal digestible (SID) methionine should be supplied at 0.17, 0.19, 0.16, and 0.23 % between d 38-41, 53-56, 87-90, and 109-112 of gestation, respectively. Therefore, the current feeding recommendations by the NRC (2012) underestimate methionine requirements of gestating sows.
2. In early lactation, the methionine feeding level required to maximize milk production is possibly below 0.18% SID methionine. In peak lactation, milk production is maximized around the current feeding recommendations of 0.26% SID methionine. Therefore, precision feeding programs can be generated for both gestation and lactation diets to more closely match (daily) estimated methionine requirements and reduce nitrogen losses to the environment.
3. Optimizing the concentrations of products of methionine used for non-protein roles in the body is less clear, but it appears that greater methionine feeding levels are required versus to maximize protein retention.
4. Maternal and fetal piglet hoof health were not assessed, since we found these measurements to be too variable. Instead, a more comprehensive assessment of piglet tissues for accumulation of methionine-dependent non-protein compounds was completed. It seems that as milk protein output increases, the concentration of creatine in piglet plasma decreases, indicating competition between protein and non-protein roles of methionine in the lactating sow.

Conclusions:

Therefore, feeding recommendations for methionine for gestating and lactating sows should be updated to maximize protein retention. Further work is required to determine the optimal methionine feeding level for non-protein fates.

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Introduction: The NRC (2012) based estimated methionine requirements for gestating and lactating sows from very few studies, with a focus on the retention of methionine in maternal, fetal, and milk proteins. These estimates do not take into account the metabolism of the modern sow (many studies are over 50 years old) or the 'non-protein' roles of amino acids, which can use up a significant proportion of the amino acid. For example, methionine is the primary methyl donor in the body and is necessary for the synthesis of creatine (energy source) and over 50 additional non-protein roles in the body. It is possible that we should consider methionine use for these non-protein roles in addition to protein retention in nutrient requirement recommendations for sows.

Objectives: (original objectives from project proposal)

1. Determine the dietary methionine required to maximize whole-body protein retention (sow and products of conception or milk)
2. Determine the dietary methionine required to optimize methionine use for non-protein roles.
3. Determine effect of dietary methionine supply on maternal and fetal piglet hoof health.

Materials and Methods:

Gestation (Obj1): Seventy gestating gilts (166±13 kg initial body weight at d31 of gestation) were used to determine the dietary standardized ileal digestible (**SID**) Methionine (**Met**) content required to optimize whole-body nitrogen (**N**) retention versus the appearance of biomolecules derived via Met metabolism. Seven days prior to nitrogen balance measurements between d 38 and 41, d 53 and 56, d 87 and 90, and d 109 and 112 of gestation (periods 1, 2, 3, and 4, respectively), gilts were moved to individual stalls, and assigned to 1 of 7 dietary treatments that provided between 50 and 150% of estimated SID Met requirements (n = 10) in a cross-over design. All other indispensable amino acids were supplied at least 20% above estimated requirements (NRC, 2012). Nitrogen balances consisted of total urine collection and fecal grab sampling and blood samples were collected on days 41, 56, 90, and 109 after a 24-hr fasting period for analyses of plasma amino acids, homocysteine (**Hcys**), and glutathione (**GSH**) **(Obj2)**. Contrast statements were used to determine linear and quadratic effects of dietary SID Met levels. Linear and quadratic broken-line and polynomial quadratic models were used to determine the optimum inclusion of SID Met for whole-body N retention and plasma concentrations of methionine-derived compounds.

Lactation (Obj1): Seventy-five primiparous Yorkshire × Landrace and Yorkshire sows were used to determine the effect of increasing dietary standardized ileal digestible (SID) methionine (Met) intake on whole-body nitrogen (N) utilization during lactation. Upon farrowing (190.2±18.0kg initial body weight), sows were assigned to 1 of 7 dietary SID Met levels (n=~10), ranging from 70% to 130% of estimated SID Met requirements (0.182 to 0.338% SID Met) via the addition of DL-Met. All other indispensable amino acids were supplied above estimated requirements (1.19% SID lysine, 19.74% crude protein) and additional cysteine was supplied to minimize Met utilization for transsulfuration. Litters were standardized to 13±2 piglets within 48 hours of birth and feed was offered according to a standard feeding curve (NRC, 2012). The N balance periods were between days 5 to 9 (early; N1) and 17 to 21 (peak; N2) of lactation where total urine was collected via urinary catheters and feces was grab-sampled; titanium dioxide was used as an indigestible marker for determining apparent total tract N digestibility. Blood samples were collected on days 9 and 21 from sows and piglets to assess plasma concentrations of methionine and methionine metabolites **(Obj2)**. Contrast statements were used to determine linear and quadratic effects of dietary SID Met levels.

Objective 3 - Maternal and fetal piglet hoof health were not assessed, since we found these measurements to be too variable. Instead, a more comprehensive assessment of piglet tissues for accumulation of methionine-dependent non-protein compounds was completed.

Results and Discussion:

Gestation: To maximize nitrogen (protein) retention in gestation, standardized ileal digestible (SID) methionine should be supplied at 0.17, 0.19, 0.16, and 0.23 % between d 38-41, 53-56, 87-90, and 109-112 of gestation, respectively. Therefore, the current feeding recommendations by the NRC (2012) underestimate methionine requirements of gestating sows (Tables 1 and 2). The plasma concentrations of methionine-derived non-protein compounds were less clear-cut. Some indicated an optimal methionine feeding level of at least that required to optimize nitrogen retention. Therefore, to maximize both nitrogen (protein) retention and the concentration of methionine-derived compounds, likely a higher methionine feeding level is required. We now are conducting additional studies with funds independent of the current research project, to quantify the use of methionine for protein synthesis versus synthesis of non-protein compounds (e.g., creatine).

Lactation: In early lactation (between day 5 and 9), there was no effect of methionine feeding level on total nitrogen retention or nitrogen retention in milk (Table 3). This means that methionine requirements for protein retention were already met at the lowest methionine feeding level when milk production was low. In peak lactation (between day 17 and 21), both total nitrogen retention and nitrogen retention in milk increased with dietary methionine supply (linear; $P < 0.05$). Therefore, it appears that the optimal feeding level for methionine in peak lactation is above the current NRC, 2012 recommendations (Table 3). As for the gestating gilt, results for plasma concentrations of methionine-derived non-protein compounds were less clear-cut, with minimal responses observed in the sow plasma. One interesting observation was that in piglet plasma (after 21 days of lactation), there was a linear reduction in creatine concentration with increasing methionine supply to the sow, but a tendency for greater creatine concentration in piglet muscle tissue. The consequences for the piglets were not obvious since piglet average daily gain was improved when sows were fed more methionine. Thus, we will conduct additional studies with funds independent of the current research project to quantify the use of methionine for protein synthesis versus synthesis of non-protein compounds in lactation as well.

Conclusions: Therefore, feeding recommendations for methionine for gestating and lactating sows should be updated to maximize protein retention. Further work is required to determine the optimal methionine feeding level for non-protein fates.

Knowledge Transfer:

Podcast: **The importance of methionine for lactating sows**; SwineIT; Swine Nutrition Blackbelt: by PhD candidate, Cierra Kozole. https://www.youtube.com/watch?v=1pCCkH9-0qI

ASAS Midwest March, 2023 Abstract (attached): **The effect of increasing standardized ileal digestible methionine intake during lactation on whole-body nitrogen utilization of primiparous sows**

C. Kozole[1], J. K. Htoo[2], C. J. Munoz Alfonso[1], and L. Huber[1]

ASAS Midwest March, 2023 Abstract (attached): **The effect of increasing standardized ileal digestible methionine intake on whole-body nitrogen retention and plasma amino acids, homocysteine, and glutathione of gilts in late gestation**

C. J. Munoz Alfonso[1], C. Kozole[1], J. K. Htoo[2], and L. Huber[1]

ASAS Annual Meeting, July, 2022 Abstract: **The effect of increasing standardized ileal digestible methionine intake on whole-body nitrogen retention of gilts during late gestation**

C. J. Munoz Alfonso, J. K. Htoo, and L. Huber

Scientific publications will be forthcoming. We will aim to publish in the Journal of Animal Science. The PhD students (C. Kozole and C. J. Munoz Alfonso) are still completing their programs, therefore, KTT activities will continue beyond the end of this research contract.

Table 1. Nitrogen utilization in gestating gilts fed diets containing 0.08%, 0.11%, 0.13%, 0.16%, 0.19%, 0.21%, or 0.24 % standardized ileal digestible Met between d 38 and 41, d 53 and 56, or d 87 and 90 of gestation.

	Standardized ileal digestible Met, %							SEM[1]	*P*- value[2]	
	0.08	0.11	0.13	0.16	0.19	0.21	0.24		linear	quadratic
Period 1 (d 38 to 41)										
N intake, g/d	46.14	48.41	47.29	46.96	50.18	47.83	47.72			
Urinary N excretion, g/d	13.85	12.42	14.83	14.06	13.95	12.05	13.77	2.34	0.865	0.808
Fecal N excretion, g/d	11.47	11.63	11.72	14.42	14.14	11.91	12.99	0.77	0.082	0.076
Total N excretion, g/d	20.82	24.19	26.55	28.48	27.80	23.59	26.91	2.10	0.113	0.051
Whole-body N retention, g/d	22.79	24.04	20.74	18.45	22.4	24.17	20.88	2.07	0.730	0.417
Period 2 (d 53 to 56)										
N intake, g/d	46.14	48.41	47.29	46.96	50.18	47.83	47.72			
Urinary N excretion, g/d	13.48	10.52	14.22	13.36	10.04	11.19	14.19	2.04	0.935	0.428
Fecal N excretion, g/d	9.86	12.74	12.12	13.20	13.17	11.27	10.90	0.65	0.667	<.0001
Total N excretion, g/d	24.05	23.60	26.13	26.74	23.00	22.46	24.88	1.68	0.683	0.438
Whole-body N retention, g/d	22.09	24.80	21.16	20.19	27.18	25.37	22.84	1.68	0.195	0.930
Period 3 (d 87 to 90)										
N intake, g/d	46.14	48.41	47.29	46.96	50.18	47.83	47.72			
Urinary N excretion, g/d	12.31	9.55	9.72	11.98	10.46	8.55	8.73	1.46	0.138	0.814
Fecal N excretion, g/d	7.37	11.14	11.00	11.03	14.92	12.41	11.37	1.19	0.005	0.013
Total N excretion, g/d	19.75	20.72	20.64	23.07	25.40	20.93	20.05	1.87	0.555	0.082
Whole-body N retention, g/d	26.40	27.68	26.65	23.86	24.78	26.90	27.67	1.87	0.970	0.250

[1] Maximum value for the standard error of the means.

[2]*P*-values for linear or quadratic contrast effects of the treatments.

Table 2. Nitrogen utilization in gestating gilts fed diets containing 0.10%, 0.13%, 0.17%, 0.20%, 0.23%, 0.27,%, or 0.30% standardized ileal digestible Met between d 109 and 112 of gestation

	Standardized ileal digestible Met, %							SEM[1]	*P*- value[2]	
	0.10	0.13	0.17	0.20	0.23	0.27	0.30		linear	quadratic
Period 4 (d 109 to 112)										
N intake, g/d	71.51	70.30	73.97	70.53	72.39	72.49	74.56			
Urinary N excretion, g/d	19.47	10.38	11.89	11.61	13.11	13.21	17.68	2.02	0.874	<.0001
Fecal N excretion, g/d	22.86	18.74	20.20	17.50	15.83	15.72	16.09	0.79	<.0001	0.011
Total N excretion, g/d	42.49	29.29	31.31	29.36	29.12	28.18	34.61	2.42	0.006	<.0001
Whole-body N retention, g/d	29.02	41.02	42.66	41.17	43.28	44.31	39.95	2.42	<.0001	<.0001

[1] Maximum value for the standard error of the means.

[2]*P*-values for linear or quadratic contrast effects of the treatments.

Table 3. Nitrogen utilization between days 5 and 9 (early) and days 17 and 21 (peak) of lactation in first-parity sow fed diets with increasing methionine contents.

	Methionine level[1]								*P* – value[2]	
Item	70%	80%	90%	100%	110%	120%	130%	SEM[3]	Linear	Quadratic
Early lactation (d 5-9)										
No. of sows	9	10	9	6	9	9	10			
Litter growth rate, kg/d	2.20	2.46	2.39	2.73	2.65	2.33	2.58	0.21	0.135	0.154
Piglet average daily gain, g	177	213	190	225	211	189	204	12.7	0.291	0.036
N intake, g/d	136.1	140.7	154.7	147.4	160.9	138.2	137.7	8.4	0.864	0.011
Total N excretion, g/d	65.0	67.4	73.2	69.8	77.9	64.3	57.7	6.3	0.334	0.011
Fecal N, g/d	21.1	20.1	25.0	24.0	26.4	20.1	20.0	2.4	0.857	0.019
Urinary N, g/d	43.8	47.3	48.6	46.1	51.6	43.5	38.1	5.1	0.244	0.032
N retention, g/d[4]	71.3	72.9	81.1	77.4	83.4	73.0	80.4	6.6	0.274	0.371
N absorbed, g/d[5]	115.5	120.4	129.4	123.2	134.7	117.7	117.9	7.3	0.810	0.034
True milk protein N, g/d	63.9	68.5	69.0	72.3	80.0	68.6	71.7	7.1	0.228	0.108
Maternal N retention, g/d[6]	2.4	4.4	12.1	5.1	3.4	4.4	8.7	10.4	0.722	0.374
Urine production, kg/d	9.1	7.0	9.8	6.2	8.4	7.1	8.1	3.1	0.471	0.626
N retained, % of intake	53.0	51.9	52.8	51.7	51.9	52.7	58.1	3.4	0.223	0.166
N retained, % of absorbed	62.7	60.6	62.4	61.5	62.1	61.9	67.4	3.7	0.252	0.244
Peak Lactation (d17-21)										
No. of sows	11	10	9	10	11	9	11			
Litter growth rate, kg/d	2.46	2.65	2.64	3.09	2.75	2.96	2.73	0.14	0.029	0.031
Piglet average daily gain, g	210	234	214	262	237	248	229	12	0.065	0.035
N intake, g/d	205.5	205.8	198.3	204.5	209.2	213.5	210.2	5.7	0.078	0.338
Total N excretion, g/d	87.3	97.4	91.3	86.1	86.0	89.9	78.7	6.2	0.087	0.325
Fecal N, g/d	36.9	36.8	35.4	35.7	38.2	37.1	33.5	1.7	0.345	0.346
Urinary N, g/d	50.5	60.6	55.9	50.4	47.8	52.8	45.3	5.4	0.093	0.403
N retention, g/d	117.9	108.7	107.2	118.7	123.2	124.1	131.4	8.0	0.008	0.157
N absorbed, g/d	168.7	169.1	162.9	169.0	170.9	176.6	177.0	5.4	0.024	0.164
True milk protein N, g/d	80.2	84.7	83.7	108.2	82.1	106.6	90.0	6.5	0.013	0.112
Maternal N retention, g/d	37.7	24.0	23.5	10.5	41.1	17.5	41.4	11.0	0.884	0.031
Urine production, kg/d	10.3	8.7	7.8	10.0	9.0	10.3	10.2	1.2	0.492	0.254
N retained, % of intake	57.5	52.7	53.3	57.5	58.8	57.8	62.5	3.2	0.025	0.155
N retained, % of absorbed	70.1	63.9	64.6	69.6	71.8	69.8	74.2	3.6	0.041	0.204

[1] Methionine supply relative to estimated requirements for gilts over a 21-day lactation period with a litter size of 14 and piglet average daily gain of 235g/d (NRC, 2012).

[2] Probability values for linear and quadratic contrasts.

[3] Maximum value of the standard error of the mean.

[4] N intake – N excreted in feces – N excreted in urine.

[5] N intake – N excreted in feces.
[6] N retention – true milk protein N output.