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Effect of *N*-acetyl-L-methionine supplementation on lactation performance and plasma variables in mid-lactating dairy cows

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ABSTRACT

The objective of current study was to investigate the effect of *N*-acetyl-L-methionine (NALM) supplementation on lactation performance and plasma variables in mid-lactating dairy cows. Forty-eight multiparous cows were blocked into 12 groups based on parity, days in milk, and milk production and were randomly assigned to 1 of the 4 treatments: 0, 15, 30, or 60 g/d of NALM per cow to supplement the basal diet. The experiment was conducted over a 13-wk period, with the first week as adaptation. The yield of milk, fat-corrected milk, and milk lactose was increased quadratically, and energy-corrected milk yield tended to increase with increased NALM supplementation in a quadratic manner. The dry matter intake, milk protein yield, milk fat yield, contents of milk composition (protein, fat, lactose, total solids, and milk urea nitrogen), feed efficiency, and body weight change were not affected by NALM supplementation. In addition, plasma methionine concentration was increased quadratically, and proline, total nonessential AA, and total AA concentrations were significantly higher in the 30 g/d group compared with that of the control group. However, other AA and total essential AA concentrations were not affected with supplementation of NALM. Adding NALM increased concentrations of total protein and globulin in plasma, but decreased plasma urea nitrogen concentration in a quadratic manner. Meanwhile, plasma malonaldehyde concentration decreased linearly as doses of NALM addition increased. Our results suggested that the supplementation of NALM improved milk yield and protein synthesis in the liver, and lowered lipid peroxidation in mid-lactating dairy cows.

Key words: methionine derivative, *N*-acetyl-L-methionine, mid-lactating cow, lactation performance

INTRODUCTION

Methionine is considered as the first limiting AA for milk yield and milk protein synthesis in high-producing dairy cows (NRC, 2001). Increasing metabolizable Met can effectively improve lactation performance in dairy cows (Rulquin et al., 1993). Various kinds of rumen-protected Met products have been developed, such as physically coated Met crystals, Met analogs [2-hydroxy-4-(methylthio)-butanoic acid and 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester], and Met derivatives, which were widely used in dairy feed (Schwab and Ordway, 2003). Many studies have been focused on the effects of physically coated Met crystals and Met analogs on milk yield and milk components in dairy cows, but limited information is available about the effect of Met derivatives on lactation performance in dairy cows. The Met derivative is an inexpensive alternative relative to physically protected Met and Met analogs. In general, Met derivatives were formed by a chemical blocking group that has been added to the α-amino group in the Met molecule or in which the acyl-group has been modified (Schwab and Ordway, 2003). Some Met derivatives have been studied, such as isopropyl-DL-methionine, *N*-stearoyl-DL-methionine, and *N*-oleoyl-DL-methionine (Loerch and Oke, 1989).

As a kind of Met derivative, *N*-acetyl-methionine is produced by acetylation with methionine and excess acetic acid. *N*-Acetyl-methionine has 2 isomers: *N*-acetyl-L-methionine (**NALM**) and *N*-acetyl-D-methionine. The NALM had been proved to be a good Met source for rats, chickens, and humans, improving their daily gain and nitrogen utilization efficiency (Boggs et al., 1975; Baker, 1979; Stegink et al., 1980). However, studies on the effect of NALM in dairy cows are limited. It is known that aminoacylase-1 can hydrolyze NALM to the acetyl group and free Met crystals (Lindner et al., 2008). Boggs (1978) found that the concentration of aminoacylase-1 in the small intestine was very high. The pH in the small intestine of steer was in the range of 7.0 to 8.0 (Kern et al., 1974), which was considered

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to be the optimum pH range of aminoacylase-1 activity (Iyu and Svedas, 1982). Moreover, our previous study showed that the in vitro ruminal insoluble rate of NALM in the rumen fluid was 58.9% on average (unpublished). The results of these studies suggest that NALM has low degradability in the rumen and high absorptivity in the small intestine for ruminants, indicating that NALM may be a good Met source for dairy cows.

Fagundes et al. (2018) found that NALM supplementation could increase milk fat yield and milk fat content, FCM, and ECM in early lactating cows when those cows were fed a MP-adequate diet. In addition, NALM was found to be a superior reactive oxygen species scavenger in a study on mice (Kouno et al., 2016), suggesting that NALM may have a positive effect on relieving oxidative stress in the body, and be beneficial for the health of dairy cows. These results suggested that NALM is a potential Met replacement and antioxidant in lactating cows. It is generally thought that cows have severe oxidative stress during the transition period and early-lactating stage (Putman et al., 2018). However, in mid-lactating cows with high production, issues associated with oxidative stress remained, which may due to the cellular metabolism changes and shifted toward oxidative phosphorylation when cows were recovering from the negative energy balance (Gabai et al., 2004). In mid-lactating cows, although oxidative stress is less critical than that of early-lactating cows, but energy and protein consumed for immune responses and free radicals scavenging reduce the nutrient availability for milk synthesis, which finally reduced their performance and persistency in rest of the lactation cycle (Mohebbi-Fani et al., 2016).

Thus, we hypothesized that NALM supplementation can improve the lactation performance and anti-oxidative capacity of mid-lactating dairy cows. Therefore, the objective of this study was to investigate the effect of NALM supplementation on feed intake, lactation performance, and plasma variables in mid-lactating dairy cows.

MATERIALS AND METHODS

The experiment was conducted at the experimental station of the Institute of Dairy Science, Zhejiang University (Hangzhou, China), and the Zhejiang University Institutional Animal Care and Use Committee approved all procedures involving animals.

Cows and Experimental Design

In a randomized block design, 48 mid-lactating cows (BW = 719 ± 66.4 kg, DIM = 167.6 ± 13; ±SD) were selected and assigned to 12 blocks based on parity, DIM, and milk yield and then randomly allocated into 1 of the 4 treatments: 0, 15, 30, or 60 g/d of NALM per cow to supplement a basal diet. The NALM (with 99% purity) was provided by CJ CheilJedang Corp. (Shanghai, China) and contained a Met concentration of 78.4%. In vitro ruminal insoluble rate of NALM in the rumen fluid was 58.9% on average in our previous study (unpublished). The appropriate amount of NALM to be supplemented was 30 g/d according to the recommendation of CJ CheilJedang Corp. In the current study, we included half (low dose, 15 g/d) and double (high dose, 60 g/d) the recommended dose to test if the NALM supplementation would have a linear or a quadratic effect (or both) on lactation performance. All cows were housed in a tiestall barn and were fed and milked 3 times at 0700, 1400, and 1930 h. All cows had free access to water and were given ad libitum access to the allowed 5% orts during the experiment. The NALM was top-dressed onto TMR diets. The experiment lasted for 13 wk, with the first week for adaptation during which NALM was supplemented.

Sampling and Analysis

The DMI was recorded for 2 consecutive days (d 6 and 7), and the samples of TMR and orts were collected on the same day (d 7) every other week throughout the trial. All of the samples were analyzed for DM (105°C for 5 h), CP (method 988.05; AOAC, 1990), ether extract (method 920.39; AOAC, 1990), crude ash (method 942.05; AOAC, 1990), and ADF (method 973.18; AOAC, 1990). The NDF content was analyzed using the methods of Van Soest et al. (1991) with the addition of sodium sulfite and amylase. An ANKOM^{2000} fiber analyzer (Ankom Technology Corp., Macedon, NY) was used to extract and filter NDF and ADF. The value for NE_L and absorbable Met and Lys in the experimental diet was estimated based on the Cornell Net Carbohydrate and Protein System model using the CPM Dairy 3.0 (Tedeschi et al., 2008). The ingredients and nutrient composition of the experimental diet are listed in Table 1. The BW was estimated at the beginning and end of the experiment with the calculated daily gain based on the method described by Yan et al. (2009).

Milk yield was recorded on d 6 and 7 weekly, and milk samples were collected on d 7 using milk-sampling devices (Waikato MilkingSystems NZ Ltd., Waikato, Hamilton, New Zealand). One 50-mL aliquot of the composited milk sample was collected at each milking of the sampling day, proportional to the yield (4:3:3, composite), with bronopol tablets added (milk preservative, D & F Control Systems, San Ramon, CA). Then milk sample was stored at 4°C for future analysis

Table 1. Ingredients and nutrient composition of the experimental diet

Item	Value	Supplementary *N*-acetyl-L-methionine (NALM), g/d			
		0	15	30	60
Ingredient, %, DM					
Corn	12.58				
Flaked corn	9.75				
Soybean meal	13.32				
Cottonseed meal	4.66				
Beet pulp	8.05				
Concentrate feed[1]	3.71				
Silage corn	17.66				
Alfalfa hay	12.89				
Oat hay	13.03				
Beer grain	4.34				
Chemical composition, %, DM					
CP	15.19				
NDF	31.68				
ADF	19.80				
Crude ash	6.69				
Ether extract	5.54				
NE_L,[2] Mcal/kg of DM	1.64				
Metabolizable Lys,[2] % of MP	6.55				
Metabolizable Met,[2] % of MP	2.01				
Lys/Met,[2] % of MP	3.26				
Metabolizable Lys,[2] g/kg of DM	7.55				
Metabolizable Met,[2] g/kg of DM	2.32				
Absorbed Met from NALM,[3] g/d		0	6.86	13.71	27.43
Total absorbed Met,[4] g/d		57.16	65.74	73.26	85.99
Absorbed Lys,[4] g/d		186.03	191.62	193.81	190.56
Lys/Met[4]		3.25	2.91	2.65	2.22

[1]Formulated to provide (per kilogram of DM): 150 to 180 g of salt, 250,000 IU of vitamin A, 50,000 IU of vitamin D_3, 1,100 IU of vitamin E, 3,000 mg of Zn, 17 mg of Se, 36 mg of I, 600 mg of Fe, 8 mg of Co, 630 mg of Mn, and 650 mg of Cu, water ≤100 g.

[2]All values were estimated using the CPM dairy nutritional model (Tedeschi et al., 2008).

[3]The value absorbed Met from NALM = addition of NALM (g/d) × 99% × 58.9% × 78.4%; 99% is the purity of the NALM product, 58.9% is the degradation rate in vitro study (unpublished), and 78.4% is the Met content in the NALM.

[4]Total absorbed Met (g/d) = metabolizable Met (g/kg of DM) × DMI (kg/d, Table 2) + absorbed Met from NALM (g/d); absorbed Lys (g/d) = metabolizable Lys (g/kg of DM) × DMI (kg/d, Table 2); Lys/Met = absorbed Lys (g/d)/total absorbed Met (g/d).

of milk compositions (protein, fat, lactose, MUN, and TS), with infrared analysis with a spectrophotometer (Foss-4000, Foss Electric A/S, Hillerod, Denmark).

Blood samples from the coccygeal vein of each cow at 3 h after the morning feeding were collected on d 7 of wk 6 and 12. The samples were collected using lithium-heparin-containing vacuum tubes (5 mL, Becton Dickinson, Franklin Lakes, NJ), centrifuged at 3,000 × *g* for 15 min at 4°C to collect the plasma, and stored at −20°C until analysis. Plasma samples were analyzed using an Auto Analyzer 7020 instrument (Hitachi High-Technologies Corporation, Tokyo, Japan) with colorimetric commercial kits (Ningbo Medical System Biotechnology Co. Ltd., Ningbo, China) to determine total protein, albumin, globulin, cholesterol, cholinesterase, bilirubin, creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glucose, and BUN according to a method previously described by Wang et al. (2015). The malondialdehyde (**MDA**; #MB-4750A), superoxide dismutase (#MB-4749A), glutathione peroxidase (#MB-4751A), glutathione reductase (#MB-4797A), catalase (#MB-4796A), and total antioxidant capacity (#MB-4798A) were determined using the ELISA method by Jiangsu MeiBiao Biological Technology Co. Ltd., Jiangsu, China (Zhao et al., 2017). The plasma samples from wk 12 were used to determine plasma AA concentrations using an Automatic AA Analyzer (Hitachi High-Technologies Corporation) according to the method described by Wang et al. (2016).

Statistical Analysis

The effects of NALM on DMI, lactation performance, plasma variables of dairy cows were analyzed using the MIXED procedure in the SAS software version 2000

Table 2. Effect of supplementation of *N*-acetyl-L-methionine (NALM) on the feed intake and lactation performance in mid-lactating Holstein dairy cows

	Supplementary NALM, g/d					*P*-value[1]			
Item	0	15	30	60	SEM	T	L	Q	T × W
DMI, kg/d	24.64	25.38	25.67	25.24	0.55	0.59	0.50	0.22	0.07
Yield,[2] kg/d									
Milk	31.51[b]	31.91[ab]	33.23[a]	31.43[b]	0.64	0.16	0.96	0.04	0.15
FCM	33.96[b]	34.31[ab]	35.98[a]	33.64[b]	0.76	0.17	0.83	0.05	<0.01
ECM	35.44[b]	35.63[ab]	37.38[a]	34.99[b]	0.77	0.18	0.77	0.06	<0.01
Fat	1.25	1.26	1.33	1.23	0.04	0.40	0.77	0.14	<0.01
Protein	1.11	1.10	1.14	1.10	0.02	0.43	0.93	0.29	0.06
Lactose	1.57[b]	1.59[ab]	1.66[a]	1.56[b]	0.03	0.10	0.77	0.03	0.25
Milk composition, %									
Fat	4.00	3.98	4.05	3.95	0.13	0.96	0.80	0.75	<0.01
Protein	3.51	3.46	3.49	3.48	0.03	0.70	0.74	0.59	0.21
Lactose	5.00	4.99	5.00	4.99	0.03	0.99	0.85	0.91	0.22
TS	13.16	13.03	13.16	12.97	0.09	0.35	0.22	0.75	0.10
MUN, mg/dL	15.39	15.72	15.92	15.12	0.35	0.38	0.49	0.12	0.12
Feed efficiency[3]									
Milk/DMI	1.27	1.24	1.31	1.25	0.03	0.33	0.76	0.35	0.63
FCM/DMI	1.36[ab]	1.33[ab]	1.40[a]	1.31[b]	0.03	0.09	0.29	0.29	0.08
ECM/DMI	1.43	1.38	1.45	1.37	0.03	0.13	0.33	0.50	0.08
BW, kg									
Initial	701	714	737	715	16.9	0.49	0.53	0.19	
End	727	742	758	742	20.9	0.78	0.60	0.37	
BW gain, kg/d	0.24	0.22	0.31	0.28	0.10	0.93	0.65	0.88	

[a,b]Means within the same row with different superscripts differ ($P < 0.05$).

[1]T = treatment; L = linear; Q = quadratic; T × W = interaction between treatment and week.

[2]FCM = milk yield × 0.432 + milk fat yield × 16.216; ECM = 0.3246 × milk yield + 13.86 × milk fat yield + 7.04 × milk protein yield (Orth, 1992).

[3]Milk/DMI = kg of milk yield/kg of DMI; FCM/DMI = kg of FCM yield/kg of DMI; ECM/DMI = kg of ECM yield/kg of DMI. Milk yield and feed intake were measured at the same time (d 6 and 7, every 2 wk).

(SAS Institute Inc., Cary, NC), with covariance type AR (1) for repeated measures analysis. A randomized block design with repeated measures was used for the analyses, with the week, treatment, interaction of treatment × week, and block as the main effects and the cow within the diet plan as a random effect. Plasma AA were also analyzed using the MIXED procedure in SAS, with the treatment and block as the fixed effects. The linear and quadratic effects of the treatment on all the variables were tested with orthogonal polynomials accounting for unequal spacing of NALM supplement levels. The results were listed as least squares means and were separated using the PDIFF option when the fixed effects were significant. $P \leq 0.05$ indicated statistical significance, and $0.05 < P \leq 0.10$ indicated trends.

RESULTS AND DISCUSSION

The contents of Lys and Met in MP were 6.55 and 2.01% in the basal diet, respectively (Table 1). The Lys/Met ratio were 3.25:1, 2.91:1, 2.65:1, and 2.22:1 in rations supplemented with 0, 15, 30, and 60 g/d of NALM, respectively (Table 1). The yield of milk ($P = 0.04$), FCM ($P = 0.05$), ECM ($P = 0.06$), and milk lactose ($P = 0.03$) increased or tended to increase quadratically by NALM supplementation (Table 2). In wk 4, 5, 6, 8, 9, 10, 11, and 12, the milk yield of the 30 g/d group was higher or tended to be higher than that of 0 g/d group (Figure 1). In addition, the feed efficiency (FCM/DMI) in the 30 g/d group was higher than that of the 60 g/d group ($P = 0.09$, Table 2). However, the DMI, yield of protein and fat, milk composition (protein, fat, lactose, TS, and MUN), feed efficiency (milk/DMI and ECM/DMI), and BW change were not affected when cows were supplemented with NALM ($P > 0.10$, Table 2).

The plasma concentration of Met ($P = 0.04$) were increased quadratically by NALM supplementation, and Pro ($P = 0.08$), total NEAA ($P = 0.05$), and total AA ($P < 0.01$) concentrations of 30 g/d group was higher than that of cows in the 0 g/d group (Table 3). The total EAA and other AA concentrations were not affected by NALM supplementation ($P > 0.10$, Table 3). Supplementation with NALM increased or tended to increase concentrations of total protein ($P = 0.06$) and globulin ($P = 0.05$) in the plasma in a quadratic way (Table 4), but the BUN ($P = 0.08$, quadratic) and MDA ($P = 0.05$, linear) concentrations in plasma decreased

Table 3. Effect of supplementation of *N*-acetyl-L-methionine (NALM) on plasma AA concentrations in mid-lactating Holstein dairy cows[1]

	Supplementary NALM, g/d					*P*-value[3]		
Item,[2] μmol/L	0	15	30	60	SEM	T	L	Q
EAA								
Arginine	47.26	47.15	45.16	45.97	2.15	0.89	0.61	0.69
Histidine	56.88	55.56	55.83	58.48	3.94	0.95	0.71	0.67
Isoleucine	91.64	102.37	96.33	104.96	5.85	0.39	0.19	0.86
Leucine	145.32	150.90	153.44	152.21	7.35	0.89	0.54	0.58
Lysine	76.29	77.50	81.72	76.84	6.23	0.93	0.93	0.57
Methionine	31.14^{b}	30.83^{b}	36.73^{a}	28.81^{b}	1.98	0.06	0.54	0.04
Phenylalanine	42.99	44.40	41.74	42.88	2.55	0.91	0.83	0.91
Threonine	81.68	82.71	83.21	72.30	4.40	0.23	0.11	0.27
Valine	230.09	221.26	257.68	231.32	13.62	0.31	0.68	0.31
Total EAA	779.18	790.75	821.66	787.14	22.22	0.58	0.77	0.22
NEAA								
Alanine	157.37	159.37	173.87	166.20	9.38	0.63	0.41	0.43
Aspartate	6.62	6.96	6.36	6.71	0.42	0.79	0.93	0.84
Glutamate	26.13	27.48	30.99	27.19	2.18	0.45	0.70	0.16
Glycine	379.58	352.74	365.18	379.06	19.94	0.73	0.79	0.40
Proline	74.44^{b}	72.99^{b}	91.99^{a}	83.12^{ab}	5.41	0.08	0.12	0.20
Serine	95.22	93.73	104.38	99.06	6.13	0.65	0.49	0.55
Total NEAA	722.76^{b}	706.37^{b}	785.81^{a}	765.66^{ab}	21.84	0.05	0.06	0.41
Total AA	1,505.90^{bc}	1,491.86^{c}	1,614.90^{a}	1,572.63^{ab}	26.82	<0.01	0.02	0.12

[a–c]Means within the same row with different superscripts differ ($P < 0.05$).

[1]Amino acid concentrations were measured in wk 12 of the experiment.

[2]Total EAA = arginine + histidine + isoleucine + leucine + lysine + methionine + phenylalanine + threonine + valine; total NEAA = alanine + aspartate + glutamate + glycine + proline + serine; total AA = total EAA + total NEAA.

[3]T = treatment; L = linear; Q = quadratic.

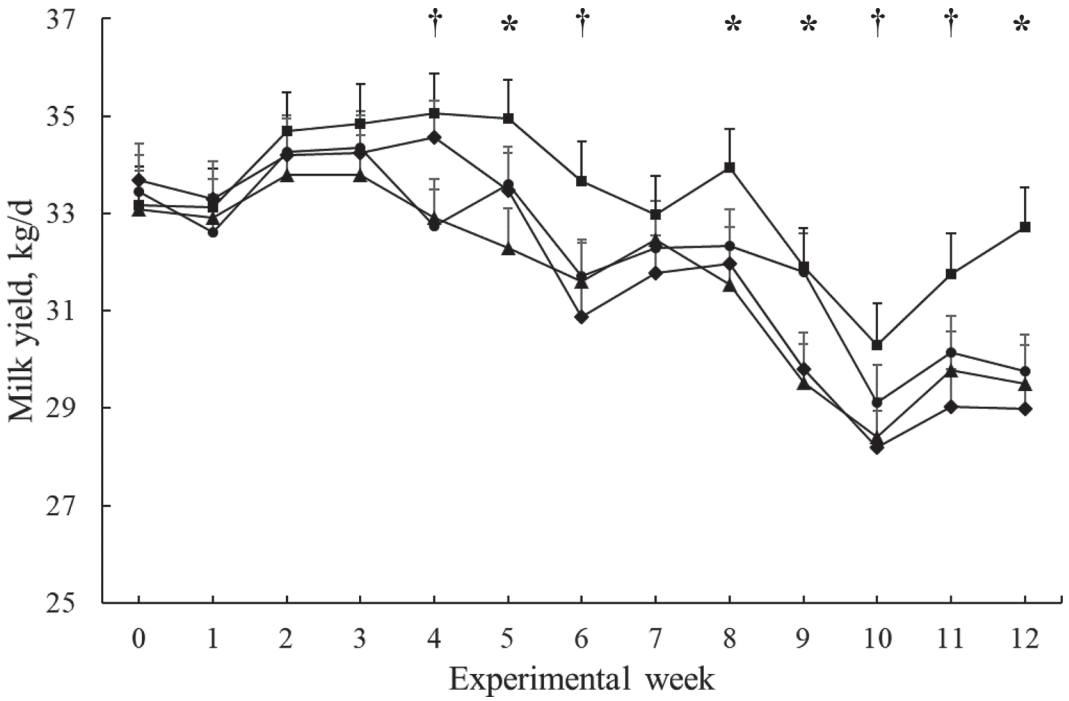

Figure 1. Change in milk yield of mid-lactating cows fed basal diet with supplementation of *N*-acetyl-L-methionine (NALM) at 0 (▲), 15 (●), 30 (■), or 60 (◆) g/d. Bars indicate SEM. The † and * indicate a significant effect of NALM between group 0 (▲) and 30 (■) at $P < 0.10$ and $P < 0.05$, respectively.

with NALM supplementation (Table 4). The addition of NALM did not affect albumin, cholesterol, cholinesterase, bilirubin, creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glucose, superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, and total antioxidant capacity in plasma ($P > 0.10$, Table 4).

To our knowledge, limited studies were conducted to investigate the effects of dietary NALM in dairy cows. A recent publication on early lactating dairy cows found that FCM and ECM increased when cows were fed NALM under an MP-adequate diet condition (Fagundes et al., 2018). Consistent with Fagundes et al. (2018), we found that FCM and ECM were higher in cows fed NALM with 30 g/d, compared with those of the control cows. In addition, milk yield and lactose yield increased with NALM supplementation of 30 g/d. The increase of FCM, ECM, and lactose yield in NALM-fed animals may be attributed to the higher milk yield in these animals compared with those of cows that were not fed NALM, as milk composition was not affected by NALM addition. Moreover, when cows were fed with 30 g/d of NALM, milk yields were greater in wk 4, 5, 6, 8, 9, 10, 11, and 12, compared with those of the control cows, which might be due to the adaptation periods of the cows to the feed additive. These results indicated that, in addition to early lactating stage (Fagundes et al., 2018), feeding NALM toward cows fed MP-sufficient diet throughout the whole lactating period, might increase milk production of lactating cows in the whole cycle.

It is worth noting that the milk yield, ECM, and FCM increased quadratically by NALM supplementation, but milk yield of cows fed 60 g/d of NALM was lower than that in cows with 30 g/d of NALM. This phenomenon could be associated with the lower Met concentration in plasma of cows fed 60 g/d of NALM, as plasma Met is positively associated with milk yield (Wang et al., 2010). Moreover, BUN concentration can be an indicator of microbial protein synthesis (Moharrery, 2010). Part of ammonia that is not used for microbial protein synthesis and absorbed from the rumen would be used to produce urea in the liver, which consumes energy (Moharrery, 2010; Gonzaga Neto et al., 2015). The lower BUN concentration of cows fed 15 and 30 g/d of NALM indicated less energy for BUN synthesis in the liver. In the meantime, quadratically increased total protein concentration in plasma in NALM-added cows suggests a quadratic change in hepatic energy consumption. Thus, the energy saved by BUN synthesis may be used to synthesize proteins in the liver when cows are fed 15 and 30 g/d of NALM, contributing to the quadratic change in milk yield following NALM addition.

In terms of plasma AA profiles, the Met concentration in plasma of the 30 g/d group was higher than that

Table 4. Effect of supplementation of *N*-acetyl-L-methionine (NALM) on the plasma physiological and biochemical variables in mid-lactating Holstein dairy cows[1]

	Supplementary NALM, g/d					*P*-value[3]			
Item[2]	0	15	30	60	SEM	T	L	Q	T × W
Total protein, g/L	76.59[b]	77.85[ab]	79.63[a]	76.40[b]	1.23	0.25	0.87	0.06	0.28
Albumin (A), g/L	26.89	26.61	26.20	27.21	0.46	0.47	0.59	0.16	0.34
Globulin (G), g/L	49.67[b]	50.94[ab]	53.43[a]	49.10[b]	1.51	0.21	0.78	0.05	0.06
A/G	0.54[ab]	0.53[ab]	0.50[b]	0.56[a]	0.02	0.23	0.49	0.07	0.04
Cholesterol, mmol/L	6.55	6.68	6.01	6.68	0.35	0.50	0.96	0.33	0.70
Cholinesterase, U/L	102.11	105.61	104.11	100.75	2.94	0.66	0.56	0.33	0.53
Bilirubin, μmol/L	1.28	1.26	1.18	1.33	0.06	0.43	0.62	0.17	0.40
Creatinine, μmol/L	70.04	70.05	65.73	68.26	2.05	0.41	0.41	0.33	0.43
ALP, U/L	36.73	40.10	40.80	38.39	4.20	0.90	0.86	0.48	0.62
AST, U/L	94.62	101.10	87.10	103.36	6.62	0.32	0.52	0.35	0.34
ALT, U/L	25.02	25.99	24.89	23.96	1.50	0.82	0.48	0.67	0.34
Glucose, mmol/L	3.33	3.32	3.37	3.31	0.06	0.90	0.90	0.63	0.59
BUN, mmol/L	6.03[ab]	5.84[ab]	5.61[b]	6.16[a]	0.21	0.31	0.62	0.08	0.12
TAOC, U/mL	17.83	18.28	17.31	18.38	0.73	0.72	0.72	0.58	0.13
SOD, pg/mL	330.55	305.87	330.22	307.20	10.04	0.16	0.26	0.90	0.10
MDA, nmol/mL	16.86[a]	16.63[a]	16.19[ab]	15.14[b]	0.59	0.20	0.05	0.52	0.36
GSH-Px, ng/L	787.81	805.20	798.43	827.96	27.65	0.77	0.34	0.89	0.32
Catalase, ng/L	40.88	41.98	37.90	41.89	1.40	0.17	0.87	0.18	0.84
GR, ng/L	321.39	330.30	316.33	318.95	13.09	0.88	0.73	0.96	0.15

[a,b]Means within the same row with different superscripts differ ($P < 0.05$).

[1]Plasma variables were measured in wk 6 and 12 of the experiment.

[2]ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; TAOC = total antioxidant capacity; SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; GR = glutathione reductase.

[3]T = treatment; L = linear; Q = quadratic; T × W = interaction between treatment and week.

of control group, which is consistent with the results of study in humans (Stegink et al., 1980, 1982) and the results of Fagundes et al. (2018). Increased plasma concentration of Met can improve the AA supply and optimize the AA balance to the mammary gland for milk production (Wang et al., 2010). This might be the reason why plasma concentrations of Pro, total NEAA, and total AA in cows fed 30 g/d of NALM were higher than those of cows in the other groups. Similarly, we observed a quadratic increase in plasma Met concentration following increased NALM addition (i.e., plasma Met concentration was lower in cows fed 60 g/d of NALM than animals with 30 g/d of NALM). When AA were excessive in plasma, the liver removed these AA for tissue protein synthesis and gluconeogenesis to avoid plasma hyperaminoacidemia (Lapierre et al., 2007), which finally reduces utilization efficiency of absorbed AA (Ruiz et al., 2002; Fox and Tedeschi, 2003). Thus, the lower plasma Met concentration in cows with 60 g/d of NALM could be associated with higher metabolism efficiency in the liver of dairy cows under high NALM addition conditions.

In contrast to other studies with traditional rumen-protect Met products (Batistel et al., 2017; Sun et al., 2017), the protein content, fat content, milk protein, and fat yield were not improved by supplementation of NALM in this study. The content of Lys in MP in this study was 6.55%, which was lower than the ideal content for maximum lactating performance (7.2%, NRC, 2001). Previous studies have found that milk protein did not respond to Met addition alone but increased when Lys and Met were introduced together (Wang et al., 2010; Awawdeh, 2016). This is because the ratio of Lys/Met is important regulator in milk protein synthesis, with the most optimized ratio at 3.0:1 (NRC, 2001; Wang et al., 2010). Thus, the limited effect of NALM on milk protein and fat in this study may be attributed to the ratio of Lys/Met in the diet supplemented with NALM. Further investigation is needed to study the effect of NALM in milk components when the Lys and Lys/Met ratio meet the optimized needs of dairy cows. Another possibility is that a part of free Met from NALM is used efficiently by the liver to be involved in protein anabolism in the liver (Finkelstein, 1990), as the total protein and globulin concentrations in plasma were higher in the 30 g/d group than that of control group.

Although cows with 30 g/d of NALM seemed to be the optimal for milk yield under the current conditions, the Lys/Met ratio of diet in these cows was 2.65:1, which is not the optimized ratio reported for milk yield (NRC, 2001). In some studies, milk yield is also the greatest when dietary Lys/Met ratio is at 2.69:1 (Doepel et al., 2004; Van Amburgh et al., 2015). A higher milk yield but not milk protein yield is found in the experiment of Fagundes et al. (2018), in which the Lys/Met ratio is 2.71:1. Thus, future study should be conducted to investigate the bio-availability of NALM in depth.

In general, MDA is one of the major aldehydes formed after lipid hydroperoxide breakdown (Mateos et al., 2005), which is a toxic substance for cells and tissues. Therefore, some studies believe that the MDA concentration in plasma is one of the commonly applied parameters for lipid peroxidation and oxidative stress in biomedical science (Nielsen et al., 1997; Suttnar et al., 2001; Del Rio et al., 2005). We found the plasma MDA concentration decreased linearly with NALM supplementation, indicating that lipid peroxidation or oxidative stress decreased when cows were fed with NALM. Although the metabolic status was relatively stable in mid-lactating dairy cows, oxidative stress is still an important issue for cows at this stage, and may cause substantial tissue damage and render cows more susceptible to various health disorders (Mohebbi-Fani et al., 2016). The linearly decreased MDA concentration in plasma with NALM supplementation indicated that lipid peroxidation or oxidative stress decreased as the dose of NALM increased, and it is beneficial for the healthy condition of mid-lactating dairy cows. In this experiment, the linearly reduced MDA with NALM addition could be attributed to 2 reasons. First, NALM is a kind of antioxidant that can scavenge reactive oxygen species and reduced oxidative stress (Kouno et al., 2016), leading to lower plasma MDA content in cows with NALM than the control cows. In addition, methionine may increase hepatic synthesis of taurine and glutathione, which are antioxidants (Zhou et al., 2016). When cows were fed with 60 g/d of NALM, the excessive plasma methionine would be removed by the liver for taurine and glutathione synthesis, which may further improve the antioxidant capacity in these cows. Thus, the cows with 60 g/d of NALM had lower plasma methionine concentration, but their plasma MDA concentrations were still lower compared with the animals with 30 g/d of NALM. In future study, more information should be determined to evaluate the effect of NALM on oxidative stress status of dairy cows in a systematic way.

CONCLUSIONS

Under the current experimental conditions, NALM supplementation improved milk yield in a quadratic manner, possibly by reducing urea synthesis quadratically to save energy for protein synthesis and ultimately increase milk production, and improve the balance of Met and available AA to mammary gland in mid-

lactating cows. Moreover, NALM supplementation improved the antioxidative capacity of mid-lactating cows. The optimized dose of NALM supplementation is about 30 g/d for mid-lactating cows. The effect of NALM on lactation performance and health status in lactating dairy cows should be further investigated at different stages and the AA balance should be further investigated.

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